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Abstracts from the 10th World Congress for Microcirculation

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PLENARY LECTURES

PL1
Imaging the microcirculation in infections

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Using spinning disk microscopy has allowed us to track pathogens and immune cells in the microcirculation. Observing the liver microvasculature revealed numerous immune speed bumps that slowed, delayed and even prevented bacteria from disseminating to other organs. For example, when Borrelia burgdorferi enters the vasculature they are immediately caught by the liver vascular macrophage, the Kupffer cells and are engulfed and antigens are presented on CD1d to resident vascular immune cells including invariant Natural Killer T cells (iNKT cells). These iNKT cells receive messages and rapidly make gamma interferon to help with immunity. Absence of these events leads to massive dissemination of borrelia especially to joints. In the joints the iNKT cells live closely apposed but outside the vasculature and prevent borrelia from infiltrating. They appear to kill the borrelia as they try to breach the barrier and enter tissues. Staphylococcus aureus is able to survive in the vasculature inside Kupffer cells avoiding neutrophils and proliferating intracellularly. Oxidants but not proteases limit but do not eradicate this pathogen inside Kupffer cells. The bacteria cause the Kupffer cells to lyse and the bacteria emerge where neutrophils are now poised and ready to catch bacteria via neutrophil extracellular traps (NETs). The NETs are able to catch the bacteria but some may escape and reach kidneys. We have also noted that as long as the S.aureus remains hidden in Kupffer cells it is protected from antibiotics so we are designing novel ways of delivering anti-microbial agents into Kupffer cells. Some bacteria like Streptococcus pneumonia evades the Kupffer cells but are caught in spleen by different specialized macrophage and neutrophils and also adhere to the endothelium in lung vasculature. In the lung we have a resident population of vascular neutrophils that constantly patrol the lung and can detect and phagocytose bacteria attached to endothelium suggesting some communication inter-cellular communication. Clearly much anti-microbial activity occurs in the vasculature prior to dissemination of bacteria into tissues.

PL2
Mapping oxygen in the brain of awake resting mice

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The brain is extremely sensitive to hypoxia. Yet, the physiological values of oxygen concentration in the brain remain elusive because high resolution measurements have only been performed during anesthesia, which affects two main parameters modulating tissue oxygenation, i.e. neuronal activity and cerebral blood flow. Using the recent finding that measurements of capillary erythrocyte-associated transients i.e. micron-scale fluctuations of oxygen partial pressure (Po_2) associated with individual erythrocytes, can be used to infer Po_2 in nearby neuropil, we non-invasively mapped Po_2 and blood flow parameters in the brain of awake, resting mice. We find region- and layer-specific differences in tissue Po_2, hematocrit and blood flow. Tissue Po_2 at rest is 23 mm Hg on average, ranges from few to 50 mm Hg, but doubles with isoflurane anesthesia. This study emphasizes the importance of measurements in physiological conditions to determine the safe margin of brain oxygenation and to model brain metabolism.

PL3
Angiogenesis revisited: Endothelial cell metabolism as a target?
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Angiogenesis, the growth of new blood vessels, plays a crucial role in numerous diseases, including cancer. Anti-angiogenesis therapies have been developed to starve cancer cells from nutrients. Clinically approved anti-angiogenic drugs prolong the survival of cancer patients, but their success is limited by intrinsic refractoriness and acquired resistance. New strategies are thus needed to block tumor angiogenesis via alternative mechanisms. We recently reported that PFKFB3-driven glycolysis regulates the endothelial tip cell function during vessel sprouting, even capable of overruling the potent pro-stalk activity of Notch, and that its loss in endothelial cells causes vascular hypobranching defects. Moreover, partial and transient reduction of glycolysis by blocking PFKFB3 reduced pathological angiogenesis in several disease models. Ongoing studies explore the role of lipid and amino acid metabolism in vessel sprouting, and assess the therapeutic potential of targeting these metabolic pathways for anti-angiogenic therapy.

SYMPOSIUM

S1-1
Pericyte-containing retinovessels: The yin-yang of their physiology and pathobiology
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Pericyte-containing microvessels constitute the largest portion of the vascular system. Although present in nearly all tissues, this component of the microvasculature is likely to be particularly important in the retina where the density of abluminal pericytes is greater than anywhere else. The multitude of pericytes appears essential for the highly decentralized and autoregulated control of blood flow that occurs in the retina. Pericyte-containing microvessels are not only important in retinal physiology, but also in pathobiology. For example, pericyte loss is an early hallmark of the major sight-threatening disorder, diabetic retinopathy. In our studies, we focus on the role of ion channels in the physiology and pathobiology of pericyte-containing capillaries and pre-capillary arterioles, which together constitute the most decentralized operational unit in the retinal vasculature. We discovered that there is physiological specialization within the capillary/arteriole unit. For example, capillaries possess an abundance of functional ATP-sensitive potassium (K_{ATP}) channels, but only a paucity of voltage-dependent calcium channels (VDCCs); on the other hand, the converse is the case for pre-capillary arterioles. This specialization is likely to enhance the decentralized regulation of retinal blood flow. Importantly, physiological differences within the retinal microvasculature have important pathobiological implications. Namely, the presence of K_{ATP} channels and absence of VDCCs almost completely accounts for why pericyte-containing retinal capillaries are far more vulnerable than arterioles to pathophysiological conditions of the diabetic retina such as hypoxia, ischemia, oxidative stress and purinergic vasotoxicity. Thus, the concept is emerging that the pathophysiology of pericyte-containing retinovessels is closely linked with their physiology.

S1-2
Pacemaker role of pericytes in the microvasculature of visceral organs
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The importance of pericytes in regulating blood flow has been revealed in several vascular beds, but their functions in the microcirculation of visceral organs remains to be determined. In visceral organs that undergo considerable distension upon filling, the microvasculature appears to display intrinsic contractile properties. Submucosal venules in the urinary bladder generate spontaneous constrictions triggered upon the firing of spontaneous Ca^{2+} transients and transient depolarisations. These events are initiated within venular pericytes upon the spontaneous Ca^{2+} release from sarcoplasmic reticulum (SR) and the opening of Ca^{2+}-activated chloride channels that trigger Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels (VDCC). These L-type VDCC play a critical role in maintaining synchrony within venular pericytes. In the stomach myenteric layer, highly-synchronous spontaneous Ca^{2+} transients are evident in both arteriolar smooth muscle cells (SMCs) and capillary pericytes of the microvasculature tree. Spontaneous Ca^{2+} transients initiated in capillary pericytes appear to spread to their neighbouring arteriolar SMCs. Capillary Ca^{2+} transients primarily rely on spontaneous SR Ca^{2+} release, but also require Ca^{2+} influx through T-type VDCC for their synchrony. The opening of T-type VDCC may also contribute to the Ca^{2+} transients that propagate into arteriolar SMCs. Thus, pericytes in the visceral microvasculature may act as the originators of synchronous spontaneous Ca^{2+} transients that regulate contractility of "upstream" arterioles and "downstream" venules so that the microcirculation blood flow meets tissue demand. Pericytes in the microvasculature

appear to function as voltage-dependent coupled Ca^{2+} oscillators, their synchrony depending on various VDCC types in a microvasculature bed-dependent manner.

S1-3
The evolving role of renal pericytes
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Pericytes have increasingly been the subject of much interest in the renal field, with particular attention focusing on their key role as regulators of medullary blood flow (MBF), their ability to coordinate tubular and vascular function via tubulovascular cross-talk mechanisms, and most recently, their role in the pathogenesis of renal diseases such as fibrosis and associated forms of chronic disease. Pericyte-mediated regulation of vasa recta diameter provides compelling evidence to support regulated MBF, a notoriously controversial subject. Coordination of tubular and vascular function by pericytes, particularly in salt-sensitive animal models, provides important mechanistic information regarding the physiological workings of the kidney in health and disease. Moreover, the emergent role of renal pericytes in vessel rarefication during fibrosis promises to reveal novel therapeutic targets to counteract devascularization, disease progression and loss of kidney function.

S1-4
How myocytes and pericytes integrate Ca^{2+} signalling and tone in ureteric microvascular networks *in situ*
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The microcirculation is the site of gas and nutrient exchange. Control of central or local signals acting on the myocytes, pericytes and endothelial cells within it, is essential for health. Due to technical problems of accessibility, the mechanisms controlling Ca^{2+} signalling and contractility of myocytes and pericytes in different sections of microvascular networks *in situ* have not been investigated. We aimed to investigate Ca^{2+} signalling and functional responses, in a microcirculatory network *in situ*. Using live confocal imaging of ureteric microvascular networks, we have studied the architecture, morphology, Ca^{2+} signalling and contractility of myocytes and pericytes in response to central and local vasoconstrictors and vasodilators. In myocytes and pericytes, Ca^{2+} signals induced by different agonists appear as Ca^{2+} wave-like oscillations and arise from Ca^{2+} release from the sarcoplasmic reticulum mediated exclusively by inositol 1,4,5-trisphosphate receptor channels. The responses in pericytes are less oscillatory, slower and longer-lasting than those in myocytes. Myocytes and pericytes are electrically coupled, transmitting Ca^{2+} signals between arteriolar and venular networks dependent on gap junctions. Endothelial Ca^{2+} signalling inhibits intracellular Ca^{2+} oscillations in myocytes and pericytes via L-arginine/NO/cGMP/PKG. There are physiological differences in the contractile responses of pericytes and myocyte to vasoactive molecules, which suggest that these two types of microvascular cells could have different functional roles in the regulation of local blood flow.

S2-1
Neuronal VEGF endocytosis triggers the programmed regression of hyaloid vessels
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Vascular development is a dynamic and stepwise process consisting of molecularly and morphologically different angiogenic processes. Hyaloid vasculature is a temporary circulatory system in fetal eyes, which immediately degenerates after birth as retinal vessels grow. Failure to regress these vessels leads to an ocular pathology called persistent fetal vasculature, which causes severe intraocular hemorrhage and impairs visual function. However, it is poorly understood how this degeneration program starts at birth. Here, we show striking upregulation of VEGFR2 occurs in retinal neurons just after birth via activation of the Distal-Multipotent-Mesodermal-Enhancer (DMME), known as a hemangioblast-specific enhancer of VEGFR2. Lack of neuronal VEGFR2 interrupts this program resulting in massive hyaloid vessels that persist even after postnatal day 10. This abnormality is caused by excessive VEGF proteins in the vitreous cavity due to impaired neuronal endocytosis of VEGF, recently described to account for neuronal avascularity during postnatal 1st week (Okabe et al. Cell 2014). Taken together our data indicate neuronal VEGFR2 determines the timing of transition from the fetal to the neonatal as well as from the neonatal to the adult circulatory systems in retina.

S2-2
Apelin/APJ system regulates parallel juxtapositional alignment of arteries and veins
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Alignment of arteries, veins and nerves is observed in adult peripheral tissues. This alignment is considered to have some physiological function. Some lines of evidence suggest that

the signals from blood vessels and the nervous system may together determine the appropriate patterning and branching of the vascular network. Although intimate associations and functional interactions between nerve and artery have been documented in vascular remodeling, the mechanisms that determine the alignment of veins and arteries have not been elucidated. Arteries and veins were previously identified by their morphological appearance. Venous-specific EphB4 is a receptor for arterial-specific EphrinB2; this system regulates distinct boundaries between arteries and veins by repulsive effects. Repulsion is one of mechanism to maintain distance between arteries and veins; however, how arteries and veins show juxtapositioning has not yet been elucidated. Apelin is an endogenous ligand for the APJ receptor. We previously showed that the apelin/APJ system is involved in maturation of blood vessels by regulating caliber size modification and permeability during angiogenesis. Recently, we found the role of apelin-APJ signaling for A-V alignment. In this symposium, we will show how apelin/APJ system regulates A-V alignment and the role of A-V alignment for homeostasis.

S2-3
Calcium imaging of endothelial cells helps understanding of angiogenic sprouting and tip-stalk determination

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Angiogenic sprouting is mainly regulated by VEGF-dependent signalling. Tip cells continuously responding to VEGF are thought to express Dll4, thereby activating Notch expressed on stalk cells to suppress the expression of VEGFR2. By probing VEGFR2 responses *in vivo* using zebrafish, we here demonstrated that VEGF signalling is essential for locating sprouting cells of intersomitic vessels from dorsal aorta (DA) and that stalk cells can respond to VEGF even after tip-stalk arrangement is determined. We first established transgenic zebrafish, Tg(flk1:Gal4DBD) and crossed them with Tg(uas:GCamp7) to obtain Tg fish expressing GCamp7 exclusively in endothelial cells (ECs), EC- Ca^{2+} monitoring fish. Among ECs of the DA, those exhibiting most frequent Ca^{2+} increases became tip cells of intersomitic vessels. When embryos of EC-Ca^{2+} monitoring fish were treated with VEGFR2 inhibitor, Ca^{2+} responses were completely inhibited in ECs of the DA, indicating thatthis Tg fish can be used for indirectly monitoring VEGF2 activity. We noticed that stalk cells exhibited Ca^{2+} responses as well as tip cells, suggesting that VEGFR2 was not completely suppressed by Dll4/Notch-mediated signalling in stalk cells. Furthermore, forced expression of sFlt1 resulted in lack of Ca^{2+} responses of ECs of the DA. Dll4 depletion led to an increase of the number of ECs in the somatic boundaries. Collectively, these data indicate the essential role for VEGF in angiogenic sprouting and suggest the basal lateral inhibition of ECs in the DA mediated by Dll4-Notch signalling to determine where ECs sprout to form intersomitic vessels.

S2-4
Roles of signaling and transcriptional networks during endothelial-to-mesenchymal transition

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Endothelial cells undergo differentiation into mesenchymal cells during various physiological and pathological processes including heart valve formation and cancer progression, respectively. However, the molecular mechanisms that regulate such endothelial-to-mesenchymal transition (EndMT) remain to be elucidated. Here we show that TGF-beta plays important roles during mesenchymal differentiation of endothelial cells. By addition of TGF-beta2, MS-1 endothelial cells underwent mesenchymal transition characterized by re-organization of actin stress fiber and increased expression of various mesenchymal markers such as SMA. We found that TGF-beta2-induced EndMT of MS-1 cells is dependent on the activation of Rho signals. Whereas activation of Rho signals via TGF-beta-induced non-Smad signals has been implicated in EMT, we found that Arhgef5, a guanine nucleotide exchange factor, is induced by Smad signals and contributes to the TGF-beta2-induced SMA expression in MS-1 cells. We also found that TGF-beta2 induces the expression of myocardin-related transcription factor-A (MRTF-A) in a Smad-dependent fashion and its nuclear accumulation in MS-1 cells and that MRTF-A is required and sufficient for TGF-beta2-induced SMA expression. These results indicate that activation of Smad signals by TGF-beta2 have dual effects on the activation of Rho signals and MRTF-A leading to the mesenchymal transition of MS-1 endothelial cells. Taken together, these findings suggest that TGF-beta2 activates multiple transcriptional and signaling networks during mesenchymal transition of endothelial cells.

S3-2
Patrolling monocytes in atherosclerotic arteries

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Atherosclerosis is an inflammatory disease of large arteries. Apoe−/− mice develop macrophage-rich atherosclerotic

lesions throughout the arterial tree. Lesional macrophages are thought to be derived from blood monocytes, but monocyte transmigration into atherosclerotic lesions has never been observed. Here, we report a new model of multiphoton intravital microscopy of lesions in the external carotid artery of live mice. A cardiac-triggered and automatically frame-selected and registered acquisition system on a Leica SP5 multiphoton microscope platform delivers movies of a 456 × 196 micron field of view, with a final frame rate of 20 frames per minute. Using Cx3cr1GFP/+ mice in which Ly-6Clow monocytes are green fluorescent, we find these monocytes patrolling on the endothelium with and against the direction of flow and also in the circumferential direction. The monocytes appear polarized and use alpha4 integrins for adhesion. The migration speed of these patrolling monocytes ranges from 0.5 to 10 microns/s. Net displacement ranges from nearly zero to about 150 microns, with confinement ratios ranging from zero (returning to origin) to 1 (straight line) with typical values between 0.2 and 0.4. The dwell fraction (no detectable cell movement between frames) ranges from zero to 0.8 with typical values between 0.2 and 0.5. In one movie, we observed 14 monocytes intensely scrutinizing a hot spot on the endothelium overlying atherosclerotic plaque. This hot spot patrolling may be a precursor to transendothelial migration. Experiments are under way to uncover the underlying molecular mechanisms.

S3-3
Dendritic cell migration through afferent lymphatic vessels

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Afferent lymphatic vessels are intimately linked with the induction of immune responses, since they mediate the transport of antigen, inflammatory mediators, and leukocytes from peripheral tissues to draining lymph nodes. In comparison to leukocyte migration through blood vessels, trafficking via lymphatic vessels is much less well characterized. Antigen-presenting dendritic cells (DCs) represent an important cell type migrating via this route and are key for the induction of protective immunity as well as for the maintenance of immunological tolerance. Our group has recently established an intravital microscopy (IVM) model in the murine ear skin to image DC migration into and within afferent lymphatic vessels. Performing IVM we found that DCs actively migrate and patrol within initial lymphatic capillaries and are only passively propagated by lymph, in direction of the draining lymph node, once they reach larger collecting vessels. We could identify a first molecule involved in intralymphatic DC migration, namely the Rho-associated protein kinase (ROCK). In our ongoing work we are further elucidating the mechanism and the functional significance of leukocyte migration through afferent lymphatic vessels. Our recent IVM findings reveal that the LEC-expressed chemokine CCL21 orchestrates intralymphatic DC migration in downstream direction of the draining lymph node and indicate a novel role for lymph flow in establishing an intraluminal haptotactic CCL21 gradient.

S3-4
Interactions between mast cells, MHC class II positive cells and eosinophils by the adult and aged lymphatic vessels

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In current study we investigated the interactions between mesenteric lymphatic vessels (MLVs), mast cells (MCs), eosinophils, and immune cells in adult (9-month) and aged (24-month) Fisher-344 rats. MCs are known to inhabit in greater density the tissue near MLVs. Acute inflammatory-like activation induces MC degranulation with subsequent recruitment of eosinophils and of MHC class II+ cells towards MLVs. Aging permanently decreases contractility of MLVs and at the same time influences mesenteric MCs by inducing their chronic basal activation. As a result of latter, the number of intact MCs available to react acutely to inflammatory stimuli diminished with age. Such leads to decreased traffic/activation of eosinophils and MHC class II+ cells that are attracted to aged MLVs. Such findings are correlated with the results of comparative analysis of inflammatory cytokines profiles in blood and mesenteric tissues in adult and aged animals with or without induction of acute peritonitis by intraperitoneal injection of lipopolysaccharide (LPS) in 10 mg/kg. We propose that a key component of aging-associated alterations of the mesenteric lymphatic vessels/mast cells/eosinophils/inflammatory cells system is the increased basal activation of resident MCs in aged mesentery that maintain a low grade of chronic inflammation. These aging-associated changes in basal status of MCs limit their ability to effectively develop immune response to acute inflammation, by recruiting/activating enough MHC class II+ cells and eosinophils. NIH AG-030578 & DK099161.

S4-1
Reendothelialization process by resident endothelial cells of the pial artery after the damage through a photochemical reaction

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Since endothelial damage and subsequent exposure of subendothelial tissue in cerebral artery quickly causes luminal platelet thrombus, urgent endothelial repair is critical to minimize ischemic damage, especially after rupture of atherosclerotic plaque. Reendothelialization is also essential to recover normal vascular tone controlled by vasodilatory mediators, such as nitric oxide. Furthermore, loss of endothelial tight junction results in breakdown of blood-brain barrier causing vasogenic edema. Restoration of endothelial function in the cerebral microvessels is, therefore, an urgent process for the brain. Origin of cells responsible for reendothelialization is controversial. Some believe in the presence of "Endothelial Precursor Cell (EPC)" coming from bone marrow, spleen, fat tissue or vascular niche, others are skeptical of the presence of such precursor cells. To elucidate the repair process and to identify cells responsible for reconstructing endothelium, we injured endothelial cells (EC) of the middle cerebral artery in a 350 micro-m long segment by photochemical reaction of Rose Bengal with laser beam illuminated through a cranial window. CAG-enhanced GFP transgenic mouse was utilized to visualize various vessel and blood components. Continual microscopic observation for the next 4 days revealed that elongation, migration and proliferation of preexisting ECs at the edges are the main mechanisms of reendothelialization. Mononuclear cells isolated from the bone marrow and transplanted through the tail vein never became endothelial cells, even though they may help repair process by paracrine. Therapies specifically targeting endothelial repair and protection are now under development and would have clinical benefit.

S4-2
Cilostazol inhibits leukocyte-endothelial cell interactions in murine microvessels after transient bilateral common carotid artery occlusion

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Leukocyte behavior in the cerebral microvasculature following vessel occlusion has not been fully elucidated. The purpose of this study was to investigate the effects of cilostazol on leukocyte behavior (rolling and adhesion) in murine cerebral microvessels following transient bilateral carotid artery occlusion using intravital fluorescence microscopy. Four groups of mice were assigned: a sham group ($n = 16$); an ischemia (induced by 15-min occlusion of bilateral common carotid arteries) and reperfusion (I/R) group ($n = 13$); I/R+cilostazol (I/R+CZ3 mg/kg) group (I/R after oral administration of cilostazol at 3 mg/kg) ($n = 8$); and I/R+cilostazol (I/R+CZ30 mg/kg) group (I/R after oral administration of cilostazol at 30 mg/kg) ($n = 12$). Leukocytes labeled with 0.05% acridine orange were administered intravenously and their behavior was investigated at 3 and 6 h after reperfusion. Numbers of rolling or adherent leukocytes were expressed as the count per square millimeter per 30 s. Numbers of rolling and adherent leukocytes at 3 and 6 h after reperfusion were significantly higher in the I/R group than in the sham or I/R+CZ30 mg/kg groups in both pial veins ($p < 0.05$) and pial arteries ($p < 0.05$). Cilostazol (30 mg/kg) inhibited leukocyte-endothelial interactions following cerebral ischemia and reperfusion.

S4-3
The effects of methylene blue on autophagy and apoptosis in MRI-defined normal tissue, ischemic penumbra and ischemic core

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Background and Purpose: Methylene blue (MB) USP, which has energy-enhancing and antioxidant properties, is currently used to treat methemoglobinemia and cyanide poisoning in humans. We recently showed that MB administration reduces infarct volume and behavioral deficits in rat models of ischemic stroke and traumatic brain injury. This study reports the underlying molecular mechanisms of MB neuroprotection following transient ischemic stroke in rats.
Methods: Rats were subjected to transient ischemic stroke. Multimodal MRI during the acute phase and at 24 hrs was used to define three regions of interest (ROIs): (i) the perfusion-diffusion mismatch salvaged by reperfusion, (ii) the perfusion-diffusion mismatch not salvaged by reperfusion, and (iii) the ischemic core. These ROIs were extracted for western blot analyses of autophagic and apoptotic markers.
Results: The major findings were: (i) MB treatment reduced infarct volume and behavioral deficits, (ii) MB improved CBF to perfusion-diffusion mismatch tissue after reperfusion and minimized harmful hyperperfusion 24 hours after stroke, (iii) MB inhibited apoptosis and enhanced autophagy

in the perfusion-diffusion mismatch tissue, (iv) MB inhibited apoptotic signaling cascades (p53-Bax-Bcl2-Caspase3), and (v) MB enhanced autophagic signaling cascades (p53-AMPK-TSC2-mTOR).
Conclusions: MB induced neuroprotection, at least in part, by enhancing autophagy and reducing apoptosis in perfusion-diffusion mismatch tissue following ischemic stroke.

S4-4
Ameliorating effects of Chinese herb compound preparation on cerebral microcirculatory disturbances and neuronal injuries after ischemia-reperfusion

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Background: Stroke is the second leading cause of mortality, with long-term physical and cognitive disabilities. Cerebral microcirculatory disturbances accompanied by neuronal injury play a key role thereof. Cerebralcare Granule (CG) is a Chinese herb compound preparation that has been used for treatment of cerebrovascular related diseases. However, the effect of CG on ischemia-reperfusion (I/R) induced cerebral injury is unclear.
Methods: Global or focal cerebral I/R was conducted by clamping bilateral carotid arteries for 30 minutes in gerbils or middle cerebral artery occlusion for 1 hour in rat, respectively, followed by reperfusion for indicated periods. CG (0.4 or 0.8 g/kg) was administrated orally 90 min before ischemia for pre-treatment, or given daily by gavage after 3 hrs of reperfusion for post-treatment until the end of the experiments.
Results: Cerebral microcirculatory disturbances, assessed by optical microcirculation observation system, were restrained by both pre- and post-treatment with CG, resulting in an increase of RBC velocity and blood flow and a decrease of leukocyte adhesion on, ROS production in, and albumin leakage out of cerebral venules. Moreover, blood brain barrier disruption, brain edema and infarction, together with microvascular ultrastructural damages such as capillary occlusion and perivascular vacuole, were all ameliorated by CG. In parallel, CG improved neurological deficits and neuronal injury in cortex as well as hippocampus after I/R.
Conclusion: The present study demonstrated that CG was able to ameliorate I/R-induced cerebral damage, suggesting a potential role of Chinese herb compound preparation in clinical treatment of ischemic stroke.

S5-1
Evolving concepts in the regulation of endothelial barrier permeability. Tonic modulation of endothelial barrier functions and inflammatory cell trafficking

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The normal endothelial barrier is maintained in a low permeability state by the balance of pro and anti-inflammatory mechanisms. Recent discoveries have greatly expanded our knowledge of endogenous mechanisms acting to stabilize the endothelial barrier and reduce inflammatory cell infiltration. For example sphingosine-1-phosphate (S1P) derived mainly from red cells in the normal vasculature acts continuously to stabilize the vascular barrier by strengthening endothelial cell junctions and stabilizing the endothelial glycocalyx. S1P also regulates immune functions including trafficking of lymphocytes. The symposium, Endogenous mediators of endothelial barrier stability: basic mechanisms and implications in human vascular disease and recovery, also focuses on the actions of atrial natriuretic peptide (ANP), and cAMP dependent pathways to regulate the endothelial barrier and immune cell trafficking. Additional topics include the contrast between physiological actions of ANP to increase albumin permeability to regulate an expanded plasma volume and the anti-inflammatory actions of ANP to attenuate responses to histamine and reduce neutrophil attachment and transmigration across an endothelial monolayer. New observations of normal and inflammatory states modulated by cAMP dependent Epac1 and Epac2 will be evaluated. The inflammatory and anti-inflammatory mechanisms of immune cells in the ischemic mice brain model will also be shown and evidence of the neuroprotective effects of the S1P1 receptor modulator fingolimod in this mouse model and in in human stroke patients presented. The Symposium provides an opportunity to compare and contrast the roles of endogenous mechanisms to modulate both vascular barrier stability and immune cell trafficking. FEC research supported by HL 28607.

S5-2

Distinct cell-specific protective actions of atrial- and C-type Natriuretic peptides in *acute* postischemic microcirculatory inflammation

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Atrial- and C-type natriuretic peptides (ANP, CNP) are released from cardiac atria and vascular endothelium, respectively. They bind to their specific cyclic GMP (cGMP)-forming guanylyl cyclase receptors A and B. cGMP-dependent protein kinase I (cGKI) was demonstrated to stabilize mast cells (MC). MC degranulation is involved in postischemic reperfusion (I/R) injury. It is unknown whether and how ANP and/or CNP modify I/R-induced MC degranulation and microvascular permeability. Here we combined intravital microscopy of mouse cremaster microcirculation [3] with experiments on cultured MCs to address these questions.

I/R (vs sham), in wild type mice, stimulated degranulation of resident perivascular MC and FITC-dextran extravasation indicating vascular leakage. Local ANP superfusion during I/R significantly attenuated vascular leakage but not MC degranulation. CNP, however, decreased both MC degranulation and vascular leakage. These CNP actions were reproduced with the cGKI-stimulator 8-Br-cGMP. The protective CNP effects were independent of endothelial cGKI, since they were preserved in mice with endothelial cGKI deletion. Local CNP mRNA expression was not changed during I/R. CNP, but not ANP, provoked cGMP formation in human MCs (HMC-1.1 line, Dr. Butterfield) and primary cultured murine bone marrow MCs. This was associated with phosphorylation of the cytoskeleton-associated vasoactive stimulated phosphoprotein at Ser-237.

Our study demonstrates distinct cell-specific effects of NPs. CNP stabilizes resident MCs and thereby reduces postischemic vascular leakage. ANP prevents vascular leakage via endothelial actions. Our future studies will attempt to dissect the MC signaling pathways and the possible clinical implication.

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S5-3

Atrial Natriuretic Peptide (ANP) down-regulates neutrophil recruitment on inflamed endothelium by reducing PMN deformability, while adhesive function is maintained

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Atrial natriuretic peptide (ANP) attenuates ischemia/reperfusion-induced renal injury in rats in part by reducing neutrophil (PMN) activation and accumulation at sites of inflammation. There is interest in ANP's capacity to increase blood flow/exchange area, and attenuate endothelial barrier function when provided as a therapeutic to treat acute inflammation. To delve into the mechanism of action of ANP on PMN recruitment, human PMN were perfused over IL-1B-stimulated umbilical vein endothelial cell (HUVEC) monolayers in a microfluidic lab-chip. PMN rolling, firm adhesion, and transendothelial migration were reduced by up to 50% compared to controls ($p < 0.01$) in a dose dependent manner following pretreatment with ANP (1–10 nM). Capture of PMN via selectin mediated tether formation did not convert to shear resistant integrin dependent arrest due to formation of long tethers that abruptly ruptured as shear was ramped from 2 to 20 dynes/cm^2. ANP inhibition did not involve down regulation in expression of VCAM, ICAM, or E-selectin on HUVEC, nor diminished function of L-selectin/B2-integrin on PMN. Rheological analysis by micropipette of PMN deformability revealed that ANP does not change cortical tension but decreased the ratio of cortical tension/viscosity by ~40%, which accounted for a concomitant increase in hydrodynamic drag force and a reduction in the footprint of PMN adhesion on endothelium. We conclude that ANP enhances PMN rigidity that diminishes their recruitment efficiency on inflamed vasculature by effectively increasing the rupture force on selectin and integrin bond formation.

S5-4

cAMP dependent pathways: New insights from Epac knockout mice

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The second messenger cAMP is a central modulator of key processes related to fluid balance, like renal water retention, aldosterone synthesis, and micro-vascular permeability.

Cyclic AMP generally tightens the endothelial micro-vascular barrier through increased endothelial cell adherence, but indirect actions via modulation of perivascular inflammation and thrombocyte function can also contribute. Classically, PKA (cAMP-dependent protein kinase) has been considered the major mediator of the cAMP-induced vascular effects. The discovery of cAMP-activated Rap GDP exchange factors 3 (Epac1) and 4 (Epac2) together with the development of selective Epac activators has prompted *in vitro* investigations, which suggest that Epac1 may contribute to the cAMP-induced narrowing of the inter-endothelial slit. To study the *in vivo* role of Epac in the control of microvascular permeability we produced Epac1−/− and Epac2−/− Bl6 mice. We found that the basal micro-vascular permeability for albumin was increased in intact Epac1−/− mice, but not in Epac2−/− mice. The Epac1−/− mice had enhanced microvascular permeability also for gadomer-17 and the low MW probe dotarem. The functional findings were in line with electron microscope observation of wider inter-endothelial slits with less junctional material in the Epac1−/− micro-vasculature. We found also that the Epac1−/− mice had increased diuresis, with about 20% increased glomerular filtration rate. In conclusion, Epac1 may have as major role to limit loss of fluid from the vascular compartment, both via renal clearance and general trans-vascular flux.

S5-5
Regulation of cerebral post-ischemic inflammation by DAMPs and immune cells

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Inflammation is an essential step for the pathology of ischemic stroke. Because there is no pathogen in brain, the inflammation is triggered by some endogenous molecules (DAMPs: danger associated molecular patterns). High mobility group box 1 (HMGB1) and peroxiredoxin (Prx) have been recently identified as DAMPs in the ischemic brain. HMGB1 exaggerates the disruption of blood brain barrier; on the other hand, Prx activates infiltrating immune cells and induces the inflammatory cytokines production through TLR2 and TLR4 signaling pathway. Among various inflammatory cytokines, IL-23 induces IL-17 producing T cells, and which results in secondary progression of ischemic brain injury. FTY720 (fingolimod) inhibits T cells infiltration and is expected to be used as a new neuroprotective agent for ischemic stroke. Both the extracellular release of Prx and the infiltration of immune cells reach the peak within 1–3 days after the onset of ischemic stroke and thereafter they decrease. This will lead to the resolution of post-ischemic inflammation. Indeed, the gene expression profile of infiltrating immune cells in the late phase shows the phenotype for anti-inflammation and tissue repair. The novel neuroprotective strategy for ischemic stroke can be developed by clarifying the detailed mechanisms to turn off the inflammatory response.

S6-1
Angiogenesis revisited: Endothelial cell metabolism as a target?

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Angiogenesis, the growth of new blood vessels, plays a crucial role in numerous diseases, including cancer. Anti-angiogenesis therapies have been developed to starve cancer cells from nutrients. Clinically approved anti-angiogenic drugs prolong the survival of cancer patients, but their success is limited by intrinsic refractoriness and acquired resistance. New strategies are thus needed to block tumor angiogenesis via alternative mechanisms. We recently reported that PFKFB3-driven glycolysis regulates the endothelial tip cell function during vessel sprouting, even capable of overruling the potent pro-stalk activity of Notch, and that its loss in endothelial cells causes vascular hypobranching defects. Moreover, partial and transient reduction of glycolysis by blocking PFKFB3 reduced pathological angiogenesis in several disease models. Ongoing studies explore the role of lipid and amino acid metabolism in vessel sprouting, and assess the therapeutic potential of targeting these metabolic pathways for anti-angiogenic therapy.

S6-2
Looking back 30 years of discovery of the EPR effect of nanomedicine for treatment, imaging and next generation PDT for cancer: Problems, solutions and prospects

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Tumor is unique in vascular architectures, excessive production of vascular-mediators, and extravasation of macromolecules from vascular blood-bed into interstitium of tumor tissue. This phenomenon was named EPR (enhanced permeability and retention) effect in solid tumor in 1986. Then we investigated the EPR-effect more detail. We found many mediators are involved for EPR-effect, e.g. bradykinin, NO, and others. It is now considered that EPR is the foremost important element for cancer-selective

drug-delivery. However, in actual *in-vivo* setting of cancer with great diversity, there are cases with poor EPR-effect, and some tumors remain inaccessible to drug-delivery, resulting in therapeutic failure.

Notwithstanding this we found that EPR effect can be augmented 2–3 fold by nitroglycerin, or ACE-inhibitors, and angiotensin-induced hypertension. Consequently, the tumor-delivery of nanomedicine can be enhanced. Another property of EPR effect is it is also observed in metastatic cancers. Since few drug is effective against metastatic tumors, this notion is a great advantage of nanomedicine with EPR.

In traditional photodynamic therapy (PDT), most photo-sensitizers (PS) developed are low MW, so little tumor selective accumulation can be seen. We prepared polymer-conjugated -PS, zinc-protoporphyrin having MW > 50 KDa, that showed tumor selective accumulation as revealed by fluorescent image of autochthonous cancers. After single iv dose of this followed by 2–3 xenon light irradiations, most tumors regressed. Thus, nanoprobes with EPR-effect seem to result remarkable therapeutic effect. All in all, further enhancement of EPR by vascular modulators can be extended to next generation cancer treatment utilizing the EPR-effect-driven tumor-delivery.

S6-3
Molecular Targeting of Tumor Vasculatures

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Tumor vessels are excessively malformed, leaky, and branched in structure, but paradoxically they are poorly perfused due to high interstitial fluid pressure, which lead to moderate hypoxia. These features are mainly caused by abnormally high VEGF-A and Ang2 levels in the tumor microenvironment, and blockade of VEGF-A and/or Ang2 has been shown to suppress effectively the pathological characteristics of tumor vessels. We recently developed a novel Ang2 binding and Tie2 activating antibody (ABTAA), and found that enhanced drug delivery into the core of growing tumor can be achieved by ABTAA though suppression of vascular destabilization and subsequent enhanced blood flow and oxygenation. Thus, Tie2 agonist is a potential therapeutic option to suppress solid tumor growth and metastasis through enhanced drug delivery, particularly in advanced cases. Nevertheless, such anti-angiogenic therapies against tumor progression still seem not so effective, selective and limited in clinics. To overcome these limitations, we have uncovered the role of RhoJ, a new endothelial-enriched Rho GTPase, during tumor progression. RhoJ blockade offers a double assault on tumor vessels by both suppressing tumor angiogenesis and disrupting the preformed tumor vessels, through the activation of the RhoA-ROCK (Rho kinase) signaling pathway in tumor endothelial cells, consequently leading to functional failure of tumor vasculatures. These results identify RhoJ blockade as a selective and effective therapeutic strategy for targeting tumor vasculature with minimal side effects.

S6-4
CO-sensitive membrane receptors regulating metabolic systems for regulating cancer proliferation and chemoresistance

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CO derived from heme oxygenase benefits maintenance of hepatic sinusoidal perfusion. Liver constitutes a major organ for heme degradation and CO generation, while significance of such microenvironments for metastatic cancer growth (e.g. colon cancer) remain largely unknown. Progesterone receptor membrane component 1 (PGRMC1) is a heme-containing protein also known as the sigma-2 receptor. Although PGRMC1 interacts with epidermal growth factor receptor (EGFR) and cytochromes P450 and is implicated in cancer proliferation and chemoresistance, the structural basis of its function is unknown. Here we determined the crystal structure of the PGRMC1 cytosolic domain at 1.95 Å. The heme iron is five-coordinated by Tyr113 alone, while those found in related cytochrome b5 proteins are six-coordinated by two histidine residues. PGRMC1 dimerizes by stacking interactions of two heme molecules protruding from each monomer, and this heme-mediated dimerization is essential for its function. CO at physiological concentrations interferes with PGRMC1 dimerization by forming a six-coordinate CO-Fe(II)-Tyr113 complex. Inhibition of PGRMC1 dimerization prevents its interaction with EGFR and cytochromes P450, limits cancer proliferation and confers chemosensitivity to anticancer drugs. PGRMC1-knocked down HCT116 cell xenografts in superimmunodeficient NOG mice exhibit lesser growth of metastasis than the wild-type xenografts. While it remains unknown if the tenuous balance between heme and CO in the host tissues determines extension of cancer metastasis, this study suggests that CO is an anti-cancer gas mediator that inhibits PGRMC1 dimerization, serving as a new therapeutic device for cancer.

S6-5
Overcoming obesity-induced tumor progression and resistance to anti-angiogenic therapy

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Today, the majority of cancer patients are overweight or obese at the time of diagnosis. Excess body weight associates with more aggressive disease. However, how it contributes to the progression of the disease and failure of anti-cancer therapy was not well understood. Here we found that diet-induced obesity significantly hinders the effects of anti-vascular endothelial growth factor (VEGF) therapy on tumor growth and metastasis of breast cancers. We revealed that obesity associates with increased numbers of intra-tumor adipocytes, and that these adipocyte-rich regions are hypoxic and over-express inflammatory cytokines as well as alternative angiogenic factors. Genetical or pharmacological blockade of these factors abrogated obesity-induced resistance to anti-VEGF therapy in both primary and metastasis sites indicating that the obesity-associated inflammation induces resistance to anti-VEGF therapy. Interestingly, the anti-diabetic drug metformin could also reduce expression of these factors and restore tumor sensitivity to anti-VEGF therapy.

We also discovered that in pancreatic cancers, obesity induces tumor-associated macrophage (TAM) infiltration, accelerates tumor growth and increases metastasis via VEGF receptor-1 (VEGFR-1) pathway. Systemically, VEGFR-1 inhibition during obesity reduces weight gain but caused hyperinsulinemia. Combining metformin with VEGFR-1 inhibition not only prevented this metabolic alteration, but also further decreased tumor growth via increased vascular perfusion with concomitant increase in anti-tumor cytotoxic lymphocytes infiltration.

Collectively, obesity fuels tumor progression and resistance to anti-angiogenic therapy via multiple mechanisms i.e., production of angiogenic and inflammatory factors. Targeting these factors and/or addition of an anti-diabetic drug may be a potential strategy to potentiate cancer therapy in the obese setting.

S7-2
The lung glycocalyx in pressure-dependent albumin transport and permeability

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Introduction: The lung vascular glycocalyx participates in permeability by creating a filter over the cell junction and caveolea and also by mechanosensing. Using the isolated perfused rat lung, we observed an increase in whole lung filtration coefficient (Kf) in response to increased left atrial pressure; Kf could be partially blocked by LNAME and heparanase. However, a portion of the increased Kf response was not blocked by LNAME or heparanase. We investigated the possibility of a second pressure-dependent mechanism that may involve the glycocalyx.

Methods: Using rat and human lung microvascular endothelial cells cultured on porous membranes, acute changes in hydrostatic pressure were induced. Pressures of 15 and 30 cm H2O were used at durations of 30 and 60 minutes. Protein concentrations in cell lysates from pressure-exposed cells were significantly higher than controls. Western blots for BSA were performed. In addition, a rat model of hypertensive pulmonary edema was used to assess whole lung albumin content.

Results: We found time- and pressure-dependent effects on BSA uptake in both RLMVEC and HLMVEC. Heparanase treatment increased albumin uptake in both control and pressure-treated cells. Lung tissue from rats subjected to acute increase in vascular pressure also showed increase in BSA content.

Summary: Removal of RLMVEC heparan sulfate increases pressure-induced albumin uptake. Breakdown of the glycocalyx is expected to increase albumin transcytosis, contribute to tissue oncotic pressure and result in increased extravascular lung water. The role of heparan sulfate proteoglycans in albumin transcytosis is under investigation.

S7-3
The endothelial glycocalyx as a barrier to leukocyte adhesion and its stabilization with low molecular weight heparin

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The endothelial cell (EC) glycocalyx consists of a layer of proteoglycans and glycosaminogylcans (GAGs), which in conjunction with adsorbed proteins, form a barrier between blood and the EC. Shedding of the glycocalyx in response to cytokines (e.g. TNF-alpha) and chemoattractants (e.g. fMLP) increases diffusion of solutes through the ESL and exposes ligands that facilitate WBC-EC adhesion. The mechanical properties of the ESL also affect capillary hemodynamics. Decreased shear stresses on the EC surface result in an unfurling of the ESL, manifest by an increased thickness of the layer, which may increase resistance to flow in capillaries by 25% in the low flow state. Thus, a means of stabilizing the ESL to resist structural changes during inflammation and reduced flows is highly desirable. To that end, the infusion of low molecular weight heparin (LMWH) was explored as a

means of mitigating shedding of the ESL during the inflammatory process. WBC-EC adhesion in response to fMLP was observed in post-capillary venules of mesentery (rat) following infusion of varied concentrations of LMWH. High concentrations of LMWH (1.6 mg/kg) resulted in diminished shedding of glycans, an attenuation of fMLP induced WBC adhesion, and a gathering of GAGs on the EC. These results appear to result from the ability of LMWH to scavenge heparanase secreted by activated ECs, and ligation of components of the glycocalyx. Thus, the mitigation of pro-inflammatory conditions by LMWH observed in sepsis and ischemia/reperfusion, may be due, in part, to its stabilization of the EC glycocalyx.

S7-4
Genetic deletion of endothelial hyaluronan induces albuminuria and progressive glomerulopathy

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We tested the hypothesis that the glomerular endothelial glycocalyx constitutes a primary barrier against albumin filtration and development of renal injury. To this end we constructed mice with a VE-cadherin Cre-recombinase inducible deletion of endothelial hyaluronan synthase 2 (has2-cKO). Eight week old has2-cKO and ctrl mice were injected intraperitoneal with tamoxifen and followed for 12 weeks. Endothelial cationic ferritin coverage was reduced in has2-cKO mice and during the 12 weeks these mice developed systemic edema with a maximum urinary albumin excretion at 8 weeks (median ACR has2-cKO 114.5 [99.1–126.3] vs. ctrl 28.7 [25.9–36.6]), without changes in blood pressure and heart rate. While up to 4 weeks glomerular capillary hypertrophic changes are most eminent, from week 8 this progressed into microaneurisms, diffuse glomerular rarefaction and eventually loss of capillaries. From week 8, podocytic changes were accompanied by a significant reduction in synaptopodin expression (has2-cKO 7.5 $\pm$ 0.8 vs. ctrl 9.2 $\pm$ 0.2 AU) and a reduction, in nephrin expression at 12 weeks (has2-cKO 6.4 $\pm$ 2.5 vs. ctrl 9.1 $\pm$ 0.3 AU), accompanied with focal podocyte foot process effacement and increased GBM thickness. Together, these data point toward a role for hyaluronan in stabilization of endothelial surface glycocalyx and loss of hyaluronan results in destabilization and loss of glomerular capillary organization and albuminuria. Financial Support by the Dutch Kidney Foundation (grants C08.2265 & C09.03).

S8-1
Coronary microvascular remodeling and dysfunction in ischemic heart disease

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The major factors determining myocardial perfusion and oxygen delivery have been elucidated over the past several decades, and this knowledge has been incorporated into the management of patients with ischemic heart disease. The basic understanding of the fluid mechanical behavior of a coronary artery stenosis has also been translated to the cardiac catheterization laboratory where measurements of coronary pressure distal to a stenosis and coronary flow are routinely obtained. In recent years, the contribution of coronary microvascular dysfunction to a variety of cardiovascular diseases, including ischemic heart disease, is being increasingly recognised. Studies in dogs and swine support the concept that coronary microvascular dysfunction is a key feature of ischemic heart disease, and indicate that the coronary microvasculature is an important therapeutic target for the treatment of ischemic heart disease.

S8-2
Coronary microvascular remodeling: Linking experimental findings in animals with observations in patients

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The coronary microvascular bed adapts to meet local oxygen requirements of the myocardium. The translation of experimental findings on structural adaptations of individual vessels in animals to functional changes observed in humans with coronary artery disease remains challenging. Microvascular adaptations to long-standing epicardial coronary artery disease include the formation and remodeling of collateral vessels but also structural and functional changes in coronary resistance vessels. Depending on the local hemodynamic, mechanical and pharmacologic environment, coronary microvessels can remodel inward or outward. Measurements in humans obtained during catheterization have

demonstrated an increased hyperemic microvascular resistance downstream of an epicardial stenosis and an impaired regulatory capacity of the microcirculation after revascularization. In these functional observations, collateral contributions and microvascular compression by heart contraction are pertinent factors for quantifying coronary microvascular resistance. Principally, the structure of the microvascular network forms the substrate for local remodeling, and we performed high-resolution 3D visualization and analysis of the intramural coronary arterial tree, including collateralization, in the human and canine heart to establish a link to functional observations in patients.

S8-3
Coronary microvascular remodeling-model approaches

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It is of basic and clinical importance to investigate coronary vascular remodeling in response to heart diseases (e.g., RV and LV hypertrophy and congestive heart failure-CHF) as well as during normal growth and aging. We have reconstructed the entire coronary arterial tree from the casting and histological measurements in swine models and performed the hemodynamic analysis. The compensatory remodeling in RV and LV hypertrophy of young porcine increases coronary vessel number (a remarkable increase in RV hypertrophy), but maintains vascular hierarchy and uniform wall shear stress in perfusion arterioles. The increase in ventricle wall stress during CHF causes coronary vascular rarefaction to increase the vascular flow resistance which in turn compromises the perfusion of the heart. On the other hand, based on the micro-computed tomography measurements of coronary arterial trees in mice at different ages (1 week to 8 months), we showed a constant exponent, but age-dependent coefficients in the length-volume scaling law during normal growth and aging. In particular, a significant decrease or increase of the coefficient in the length-volume scaling law was found to be a good index of positive remodeling or diffuse disease, respectively, in epicardial coronary arteries.

S8-4
Coronary functional remodeling in Takotsubo cardiomyopathy

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Takotsubo cardiomyopathy (TCM) is characterized by transient systolic ballooning of the apex in the absence of epicardial coronary disease. Although the exact mechanism of TCM is still unknown, we have evidence suggesting that remodeling of coronary control mechanisms produces the pathology. Previously we found that mice null for Kv1.5 channels (Kv1.5−/−) had impaired metabolic dilation (functional remodeling) vs. wild types during increased cardiac work. Because this type of demand-induced ischemia precipitates the phenotype of apical ballooning in humans, we hypothesized that Kv1.5−/− mice would develop TCM when subjected to a chronic metabolic challenge. Transaortic constriction (TAC) was performed to increase afterload in Kv1.5−/− and WT male mice. Ten days after TAC the constriction was released (de-banding). Cardiac function was measured at 1–6 weeks after de-banding using high frequency ultrasound (Vevo-770).

Results: more than 50% of Kv 1.5−/− mice developed apical ballooning 3–7 days after TAC, along with impaired flow reserve. M-mode echocardiography revealed apical ballooning. The apex was significantly dilated compared to the base in Kv1.5−/− mice. During systole the base contracted, but the apex dilated, which contrasts to the WT, in which uniform contraction was observed in the base and apex. One week after de-banding apical ballooning was absent in Kv 1.5−/− and 6 weeks later the ejection fraction approximated that before TAC. We conclude that functional remodeling in the coupling of myocardial blood flow to cardiac work leads to Takotsubo cardiomyopathy.

S9-1
Mechanisms for enhanced endothelium-derived hyperpolarizing factor-mediated responses

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The endothelium plays an important role in modulating vascular tone by synthesizing and releasing endothelium-derived relaxing factors (EDRFs), including vasodilator prostaglandins, nitric oxide (NO) and endothelium-derived hyperpolarizing factors (EDHFs). The contribution of EDRFs to endothelium-dependent vasodilatation markedly varies depending on the vessel size with the physiological balance between NO and EDHF; NO plays dominant roles in conduit arteries and EDHF in resistance vessels. Thus, EDHF rather than NO plays a crucial role in microcirculation where blood pressure and organ perfusion are mainly regulated. Indeed, accumulating evidence has demonstrated the critical roles of EDHF in modulating blood pressure and organ perfusion in general and coronary autoregulation and metabolic dilatation in particular. Regarding the mechanisms for enhanced EDHF-mediated responses in microcirculation, we have

recently demonstrated that caveolin-1 functionally suppresses endothelial NO synthase in small vessels, switching its function from NO to EDHF generation in mice and that relaxation responses of vascular smooth muscle cells to hydrogen peroxide/EDHF are enhanced through protein kinase G1a-mediated mechanism. In addition, we have recently demonstrated that genetic disruption of the physiological balance between NO and EDHF toward NO dominance in mice causes reduced EDHF-mediated relaxations in microcirculation *ex vivo* and reduced survival after cardiac pressure-overload induced by transverse aortic constriction, associated with accelerated left ventricular systolic dysfunction, reduced coronary flow reserve and enhanced myocardial hypoxia in mice *in vivo*. These findings shed new light on the significance of maintaining EDHF-mediated relaxations in microcirculation to develop a novel therapeutic strategy.

S9-2
Carbon monoxide regulates directional biotransformation of glucose via protein arginine methylation

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Stress-inducible heme oxygenase-1/carbon monoxide (CO) system protects cells and tissues against oxidative stress, while the gas-responsive direct signaling mechanisms for the protection remain unknown. Since macromolecules possessing metal-centered prosthetic groups such as enzymes in metabolic enzymes might serve as targets for covalent binding of molecular oxygen or CO, we have recently attempted to mine the gas-responsive enzymes through reading out alterations in metabolites using metabolome analyses based on capillary electrophoresis assisted by mass spectrometry (CE-MS) in varied experimental models where O_2 or CO is largely altered. Metabolomics screening using human monoblastic leukemia cell line U937 allowed us to show that CO-sensitive methylation of PFKFB3, a regulatory enzyme producing fructose 2,6-bisphosphate (F-2,6-BP), serves as an allosteric activator of phosphofructokinase-1, a rate-limiting glycolytic enzyme. In the U937 cells, PFKFB3 is constitutively arginine di-methylation at R131 and R134 under support of protein arginine methyltransferase-1. Either induction of HO-1 or exposure to CO reduces the methylation level of PFKFB3 in varied cancer cells to suppress F2,6-BP, limiting glycolytic flux and shifting glucose utilization toward pentose phosphate pathway. The regulation of PFKFB3 methylation status depends on inhibitory effects of the heme-containing cystathionine beta-synthase that modulates remethylation metabolism. Loss of PFKFB3 methylation increases NADPH and reduced form of glutathione to ensure resistance against oxidative stress for cancer cell survival. These results suggest that the methylation status of PFKFB3, a target undergoing CO/CBS-responsive arginine methylation is determinant triggering directional glucose utilization for supporting anti-oxidative capacity.

S9-3
Significance of nitric oxide synthases in the cardiovascular system: Lessons from triple nitric oxide synthases null mice

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Nitric oxide (NO) is synthesized by three distinct isoforms of NO synthases (nNOS, iNOS, and eNOS), all of which are expressed in the cardiovascular system. Due to the absence of specific non-selective NOSs inhibitors, the ultimate roles of entire NOSs in the cardiovascular system still remain to be fully elucidated. We addressed this point in mice lacking all three NOSs, and revealed the following three findings. First, the triple NOSs null mice spontaneously developed acute myocardial infarction with a shorter survival, whereas they exhibited markedly smaller cerebral infarct size after middle cerebral artery occlusion with a longer survival, suggesting the different role of NOSs in myocardial and cerebral infarction. Second, we indicated an involvement of NOSs in the mechanism of connection between remote organs, as evidenced by the facts that bone marrow transplantation from the triple NOSs null mice resulted in accelerated vascular lesion formation after carotid artery ligation (the bone marrow-vascular connection), and that subtotal nephrectomy in the triple NOSs null mice led to a very early onset of acute myocardial infarction (the reno-cardiac connection). Third, large clinical studies have reported that cardiac left ventricular hypertrophy (LVH) is a risk factor for cardiovascular death not only in hypertensive patients, but also in normotensive subjects; however, the underlying mechanisms remain unknown. We demonstrated that reduced NO production was associated with LVH and cardiovascular death in normotensive humans as well as in the triple NOSs null mice. These results provide novel insights into the significance of NOSs in the cardiovascular system.

S9-4
NO the gate-keeper of endothelial function
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Endothelium-dependent dilatations are due mainly to the release of nitric oxide (NO) which is formed by the constitutive endothelial NO synthase. NO diffuses to the underlying vascular smooth muscle where it mainly, but not solely, stimulates soluble guanylyl cyclase with the resulting production of cyclic GMP. In addition to its direct action on vascular smooth muscle, NO indirectly favors vasodilatation by controlling the release and the action of endothelium-derived contracting factors (EDCF). Thus, NO exerts a long term inhibitory effect on the production of vasoconstrictor prostanoids (in particular prostacyclin activating TP receptors on the vascular smooth muscle cells) and endothelin-1 (activating ET-A receptors on the vascular smooth muscle cells). These endothelium-derived vasoconstrictors only emerge and contribute to pathophysiology (e.g. diabetes, hypertension) when the release of NO is curtailed by endothelial dysfunction. However, NO inhibits also the release of endothelium-derived hyperpolarizing (EDH) factors (potassium ions, hydrogen peroxide) and signals (myo-endothelial coupling). As a consequence, EDH-mediated dilatations are more prominent in smaller blood vessels where the release of NO is less or in larger arteries when it becomes dysfunctional. Hence, NO is not only a major direct vasodilator mediator, but also a key regulator of other endothelium-dependent responses.

S10-1
Rapid determination of mitochondrial size, shape, position, density and motility in live fully-differentiated vascular smooth muscle cells reveal changes in hypertension and age
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Mitochondrial structure is central to the function of both the organelle and cells. Mitochondria in cultured cells may be immobile rod-shaped structures or may move, fuse and divide in rapidly reshaping branching networks. However, the precise arrangement of mitochondria in fully-differentiated (native) vascular cells is poorly understood because complex, difficult to visualize, mitochondrial structures exist. We developed methods to visualize and measure size, shape, density, distribution and motility of the entire mitochondrial complement in live, native cells or intact tissue. The electrical signatures were used to resolve each mitochondrion's structure and position by imaging fluctuations in fluorescence intensity of membrane potential sensitive dyes ("flickers"). Each organelle was mapped using a pixel-by-pixel covariance analysis of the time derivative of flickers; a method we call flicker-assisted localization microscopy (FaLM). In normotensive animals, mitochondria are spherical or short rod-shaped structures. In hypertension, mitochondria are larger and have a greater length-to-width ratio than controls. The organelles comprise more of the cell volume and are more densely clustered in hypertension. Sites of mitochondrial clusters are associated with larger Ca^{2+} signals. In aged animals a subpopulation (~5%) of highly-elongated mitochondria (length-to-width ratio > 3) exists which were not observed in younger animals. Mitochondria in smooth muscle from older animals are immobile while directed and Brownian-like mitochondrial motility occurred in younger animals. In conclusion, vascular disease and age may alter size, shape, density, position, clustering and motility of mitochondria. FaLM is a convenient method to determine the structure of mitochondria even when the organelles are hidden in dense clusters.

S10-2
Mitochondrial mechanisms in cerebral vascular control: Shared signaling pathways with preconditioning
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Mitochondrial initiated events protect the neurovascular unit, composed of vascular cells, neurons, and astroglia, against lethal stresses via a process called preconditioning and also acutely promotes changes in cerebrovascular tone through shared signaling pathways. Activation of the adenosine triphosphate (ATP)-dependent potassium channels on the inner mitochondrial membrane (mitoKATP channels), with pharmacological agents such as BMS-191095 and diazoxide, is a specific and reproducible way to induce protection of neurons, astroglia, and cerebral vascular endothelium. Through the opening of mitoKATP channels, mitochondrial depolarization leads to activation of protein kinases and transient increases in cytosolic calcium (Ca2 +) levels that activate terminal mechanisms such as nitric oxide production and increased catalase levels that protect the neurovascular unit against lethal stress. Release of reactive oxygen species (ROS) from mitochondria has similar protective effects. Signaling elements of the preconditioning pathways also are involved in the regulation of vascular tone but with an unusual twist. Activation of mitoKATP channels in cerebral arteries causes vasodilation, with cell-specific

contributions from endothelium (nitric oxide), vascular smooth muscle (calcium sparks), and nerves (nitric oxide). Pre-existing chronic conditions, such as insulin resistance or diabetes, prevent preconditioning and impair relaxation to mitochondrial centered responses in cerebral arteries. Surprisingly, mitochondrial mechanisms following transient ischemic stress protect cerebral vascular endothelium and promote the restoration of blood flow. Therefore, mitochondria may represent the elusive link between blood flow and metabolism under normal conditions as well as providing an important, but underutilized target in attenuating vascular dysfunction and brain injury in stroke patients.

S10-3
Mitochondrial thioredoxin reductase and its importance for vascular homeostasis

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Increasing evidence is emerging that links an enhanced production of reactive oxygen species <ROS> by endothelial cells <ECs> to the development of various debilitating vascular diseases.

ROS act as cellular signaling molecules and can directly inactivate endothelium-derived nitric oxide. The mitochondrial thioredoxin system, which is composed of NADPH, thioredoxin reductase 2 < Txnrd2 > , and thioredoxin 2, is pivotal in the defense against oxidative stress by regulating the protein dithiol/disulfide balance through its disulfide reductase activity. Here, we analyzed the impact of an EC-specific deletion of Txnrd2 on vascular homeostasis in a mouse model. Interestingly, mice displayed renal abnormalities, including thickening of the Bowmans capsule, glomerular fibrin deposits and scarring. Furthermore, deletion of Txnrd2 in the endothelium resulted in thickening of the wall of skeletal and renal arterioles, as well as cell deposits within the lumen of these vessels.

These observations, all indicative of endothelial dysfunction, were accompanied by hypertension as well as a pro-inflammatory endothelial phenotype, observed as increased leukocyte adhesion and transmigration. These data illustrate the importance of maintaining a delicate balance of mitochondrial ROS levels within the ECs to prevent endothelial dysfunction.

S10-4
The role of endothelial mitochondrial damage in microvascular rarefaction and fibrosis

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Microvascular rarefaction, a loss of microvascular density, is a hallmark of the long-term complications of hypertension and diabetes, and a major player in the pathogenesis of post-ischemic fibrosis. Ischemic injury to the microcirculation is increasingly being recognized as the major factor in the pathogenesis of renal and cardiac fibrosis. The loss of capillary perfusion results in chronic tissue hypoxia, causing further capillary and tissue damage, inflammation, and fibrosis. Mitochondrial swelling and loss of cristae membranes represent an early event in endothelial cells during ischemia, and the loss of cristae membranes limits the ability of these cells to regenerate ATP upon reperfusion. Loss of ATP causes separation of endothelial tight junctions, detachment of endothelial cells from the basement membrane, resulting in increased capillary permeability, interstitial edema, inflammation, and fibrosis. Recent studies have shown that a mitochondria-targeted peptide (SS-31/Bendavia) protects mitochondria structure in capillary endothelial cells during ischemia, and prevents endothelial cell swelling, cell detachment, and cell death. As a result, SS-31 protects endothelial barrier function, prevents capillary rarefaction, and blocks post-ischemic fibrosis. Other preclinical studies demonstrate the ability of SS-31 to reverse microvascular rarefaction in the kidney and the heart in chronic renal hypertension, and protect the retinal choroidal microvasculature and prevent retinal pigment epithelium degeneration in diabetes associated with high fat feeding. Thus endothelial protection represents a novel therapeutic target for mitigating chronic complications associated with ischemia, hypertension, and diabetes, and Bendavia is currently being evaluated in multiple Phase 2 clinical trials.

HYBRID SYMPOSIUM

HS1-1
Patrolling monocytes in vascular homeostasis

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Nonclassical patrolling monocytes are characterized by their unique ability to actively patrol the vascular endothelium under homeostatic and inflammatory conditions. Patrolling monocyte subsets ($CX3CR1^{high}Ly6C^{-}$ in mouse, and $CX3CR1^{high}CD14^{dim}CD16^{+}$ in humans) are distinct from the classical monocyte subsets ($CCR2^{high}Ly6C^{+}$ in mouse,

and CCR2^{high}CD14^{+}CD16^{-} in humans) and exhibit unique functions in the vasculature and inflammatory disease. This subset is regulated by the transcription factor Nr4a1, and we will discuss mechanisms for regulation of development of this subset by Nr4a1. Patrolling monocytes function in a number of disease settings by removing damaged cells and debris from the vasculature, wound healing, and resolving inflammation in damaged tissues. This presentation will discuss functions of patrolling monocytes, particularly in the settings of atherosclerosis and cancer.

HS1-2
Fate and function of neutrophils in sterile injury

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Neutrophils are recruited to sites of sterile injury in dramatic fashion and not dissimilar to what is observed at a site of infection. However, why they are drawn there and what they do when they get there remains unclear. Using a simple model of thermal injury in the liver, we tracked neutrophils in the injury site over 24 hours to investigate repair. Peak neutrophil levels were noted at 4 hours dissipating at 8–12 hours, leaving very few by 24 hours. Early recruitment was entirely dependent on endogenous chemokines and formylated peptides and required platelets to facilitate neutrophil entry into the injury. They crawled within the sinusoids, but once neutrophils penetrated the site of injury, they appeared to crawl along the outside of collapsed blood vessels but inside what appeared to be channels or sleeves. PECAM-1 antibody, infused following injury, failed to penetrate and label endothelial cells in the collapsed vessels within the injury site but rather identified endothelial cells in patent vessels outside of, and leading up to, the site of injury. Removal of neutrophils delayed clearance of cellular debris, reduced the number of these channels, reduced the number of locally replicating cells, affected collagen deposition and affected the healing process. By 12–24 hours, when most of the neutrophils had disappeared, many monocytes infiltrated the area, but there was never any overt uptake of neutrophils by monocytes. Neutrophil disappearance occurred regardless of the presence of monocytes. Although some neutrophils did leave the injury site and re-entered the vasculature, their fate remains to be determined.

HS1-3
Tracking the origins of tumor-infiltrating monocytes using KikGR and Fucci technologies

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Solid tumors contain large numbers of myeloid cells, including monocytes and monocyte-derived macrophages that promote tumor progression. These tumor-infiltrating monocytes are supplied from myeloid cell pools in the bone marrow and spleen. Recently, using mice expressing the photoconvertible protein Kikume Green-Red (KikGR) or expressing fluorescent ubiquitination-based cell-cycle indicator (Fucci) proteins, we developed methods for tracking intertissue migration and monitoring hematopoiesis, respectively. The KikGR approach allowed us to label and track the redistribution of monocytes from the BM and spleen to the tumor via the circulation, whereas the Fucci approach allowed us to determine the extent of monocytopoiesis and the relative age of monocytes in different myeloid cell pools. Analysis of KikGR data by mathematical modeling allowed us to determine migration probability and residence time for specific monocyte populations. We used these approaches to show that during tumor development the bone marrow dramatically accelerates production of monocytes, rapidly transferring many of these newly formed cells to a reservoir in the spleen. However, these spleen monocytes were less able than their bone marrow counterparts to enter the tumor and made only a minor contribution to the tumor-infiltrating monocyte population. KikGR and Fucci mice offer powerful, quantitative means for tracking intertissue cell migration and monitoring cell proliferation under steady-state conditions. Future studies might use these approaches to investigate the contributions of specific myeloid cell populations to other inflammatory diseases.

HS1-4
Recruitment of monocytes and macrophages to the site of sterile injury

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Monocytes and macrophages are classified as mononuclear phagocytes that reside and accumulate in the healthy and

injured tissue, contribute to homeostasis and tissue repair. There are at least two subsets of monocytes: pro-inflammatory ($CCR2^{hi}CX3CR1^{low}$) and non-classical ($CCR2^{low}CX3CR1^{hi}$) monocytes. Using intravital microscopy and mice with fluorescent reporters for different subsets of monocytes, we were able to track the dynamic spectrum of monocytes that enter into a site of sterile hepatic injury from circulation *in vivo*. We observed that the $CCR2^{hi}CX3CR1^{low}$ monocytes were recruited to the injured area, forming a ring around the injured area. These monocytes converted into $CCR2^{low}CX3CR1^{hi}$ within 72 hours, but did not differentiate into F4/80 + macrophages at this time point. On the other hand, we have observed a distinct accumulation of F4/80 + macrophages within the injury site very quickly within hours post-injury. Further analyses suggested that resident macrophages from the peritoneal cavity are able to respond to signals derived from the necrotic hepatocytes and rapidly occupy the injured area without entering into the circulation. In addition, we have found that peritoneal macrophages in the sterile injury adopt an alternatively-activated phenotype and create a favorable microenvironment for tissue repair. Our results provide insights into the dynamics and functions of monocytes/macrophages in the context of sterile inflammation and tissue repair.

HS1-5
Immune suppression after stroke

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Stroke is a leading cause of morbidity and mortality worldwide. Despite its recognised debilitating neurological deficits, the major cause of death after stroke is infection. In fact, bacterial infection is the most frequent medical complication after stroke, and it is now increasingly accepted that stroke results in impairment of the immune system, and this contributes to the associated life-threatening sequelae of overwhelming infection. We were the first to directly image and describe the activities of the peripheral immune system in living mice after stroke. In this study, we found the behaviour and function of invariant natural killer T (iNKT) cells, which are important in the host antibacterial defense, were impair in an adrenergic-mediated manner after stroke. Recently, we performed a pilot human study and revealed similar stroke-induced regulation of iNKT cells in stroke patients. Based on these findings, we proposed a more selective modulation of the immune system following stroke could be beneficial. In particular, selecting facets of the immune system to target would allow the protective and regenerative properties of the immune response to remain intact while blunting the pro-inflammatory response generated towards the injured brain. Therefore, we tested the capacity of post-stroke administration of novel iNKT cell-targeting drugs in modifying iNKT cell activity in such a way as to restore immune system function and thereby limit bacterial infection after stroke. Identifying a new and better targeted approach to reduce bacterial infection in stroke patients will bypass the growing problem of antibiotic resistance, ultimately improving patient outcomes.

HS2-1
The exquisite control of endothelial function by TRPV4 channels

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Endothelial cells (ECs) line all blood vessels, and are one of the most numerous (> 1 trillion) cell types in the human body, with the majority of ECs constituting capillary beds. Intracellular Ca^{2+} in ECs has a central role in the elaboration of vasodilatory influences, the control of barrier function and angiogenesis. The bipolar milieu experienced by ECs—blood on one side and smooth muscle or the tissue parenchyma on the other side—as well as their exceptional thinness to length (0.5 vs. 75 μm) poses a critical dilemma: How does EC calcium signaling effectively and safely control diverse functions in a cell type with such a unique architecture?

Here, I will provide evidence that EC TRPV4 channels are uniquely suited for this function. When open, Ca^{2+} permeates TRPV4 at 10 times the rate that it passes through classical voltage-dependent calcium channels. To safely govern EC function, while minimizing Ca^{2+} overload, these channels are low in number and function at exceedingly low open probability to drive vasodilation. Moreover, spatial control of EC TRPV4 channel function exists in both cerebral and systemic (mesenteric) vascular beds. EC-dependent vasodilators (such as acetylcholine) activate TRPV4 channels only at EC projections to the smooth muscle—termed myoendothelial projections or "MEPs" (*Science Signaling*, 2014). At MEPs, TRPV4 channels are clustered in 4 channel metastructures and exhibit cooperative opening, features which depend on intracellular Ca^{2+}, protein kinase C and the anchoring protein, AKAP150 (Science, 2012; *Science Signaling*, 2014).

Remarkably—given the apparent similarity in structural and functional organization—TRPV4 channels in cerebral ECs contrast with those in systemic ECs, with an approximately 20-fold lower open probability. This difference in activity appears to be caused by the engagement of an autocrine negative feedback module consisting of tonic EC nitric oxide production leading to the suppression of TRPV4

channel activity, an effect that extends to TRPV4 channels in ECs of brain capillaries. In conclusion, TRPV4 channels by virtue of the exceedingly high Ca^{2+} influx rate coupled with spatially and temporally controlled low activity are perfectly poised to exert local control over endothelial function. Supported by the NIH, Totman Medical Research Trust, and Fondation Leducq.

HS2-2
Cerebrovascular protective effects of TRPA1 channels

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Lipid peroxidation metabolites generated by ROS activate Ca2 + influx through transient receptor potential ankyrin 1 (TRPA1) channels in the endothelium of cerebral arteries to cause dilation. Oxidative degradation of membrane lipids is increased in the vascular wall during a number of cardiovascular diseases, including hypertension. We hypothesized that TRPA1 activity is enhanced in cerebral artery endothelial cells during hypertension and that augmented activity of the channel under these conditions is protective against hemorrhagic stroke. Hypertension was induced in control and endothelial cell specific TRPA1 knockout mice (eTRPA1−/−) by infusion of angiotensin II (1200 ng/kg/min, s.c.) and high salt (8%) diet. After 2 weeks of this treatment, the nitric oxide synthase inhibitor L-NAME (120 mg/kg/day) was added to the drinking water to further increase mean arterial pressure (MAP). MAP was monitored by radiotelemetery and did not differ between groups under any conditions. All mice suffered fatal stroke. The maximal survival time of eTRPA1−/− mice (22 days) was significantly less than that of control (52 days) littermates (n = 19–23). Histopathology revealed that eTRPA1−/− mice develop more intracerebral hemorrhage (ICH) lesions than control mice. However, the volume of each lesion was smaller in eTRPA1−/− mice. Together these data provide evidence that TRPA1 channels present in the cerebral endothelium are protective against hemorrhagic stroke associated with hypertension. R01HL091905.

HS2-3
TRPV4 sparklets in arteriolar smooth muscle

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In arterial smooth muscle, L-type $Ca_v1.2$ channels play a critical role in multiple physiological processes including excitability, contraction, and gene expression. Long openings and re-openings of $Ca_v1.2$ channels in arterial myocytes produce Ca^{2+} sparklets that increase Ca^{2+} influx and vascular tone. These stuttering persistent Ca^{2+} sparklets arise from the subcellular interactions between Cav1.2 channels, the scaffolding protein AKAP150, and associated proteins at only a few sub-sarcolemmal regions in resistance arteries. During my talk, we will present the results of new experiments involving the use of optogenetic approaches to investigate the mechanisms leading to subcellular variations in Ca^{2+} sparklet activity in arteriolar smooth muscle. These findings will form the basis for a new model for the local control and amplification Ca^{2+} influx via Cav1.2 channels in resistance artery smooth muscle.

HS2-4
A physiological role for TRPV4 sparklets

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Increases in global calcium within vascular smooth muscle cells (SMCs) are crucial for myogenic tone development and maintenance in resistance arterioles, while calcium changes in endothelial cells (ECs) modulate SMC contractility. Focal calcium influx through TRPV4 channels on ECs, termed sparklets, has been linked to activation of intermediate-conductance calcium-activated potassium channels (IK_{Ca}), hyperpolarization and vasodilation. We examined how these channels can be activated physiologically by altering intraluminal pressure in isolated, pressurized rat cremaster arterioles. ECs were loaded with Oregon Green 488 BAPTA-1 and imaged using confocal microscopy at 3 Hz. Intraluminal pressure was varied between 5 and 80 mmHg, and the frequency of calcium events determined at each pressure. In separate experiments, myogenic tone-pressure response curves were repeated in the presence of an inhibitor of nitric oxide synthase (L-NAME), and then inhibitors of K_{Ca} channels (TRAM-34, and/or apamin). At low intraluminal pressure, ECs spontaneously generated calcium events at a frequency of ~5 events/min which were partially inhibited in the presence of RN1734, a TRPV4 channel antagonist. Spontaneous events significantly decreased from ~5 to ~2 events/min when intraluminal pressure was raised. At low pressures, inhibition of IK_{Ca}, but not SK_{Ca}, resulted in a significant increase in myogenic tone. Immunohistochemical staining for IK_{Ca} and SK_{Ca} channels occurred in myoendothelial projections and in SMCs. At low pressures EC spontaneous calcium events activate IK_{Ca}, which supress myogenic tone by generating hyperpolarization. These data support a role for pressure-dependent TRPV4-mediated spontaneous EC calcium activity in the modulation of myogenic tone.

HS2-5
Extracellular histones activate local and propagating endothelial calcium signals

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Histone exposure to endothelial cells causes Ca^{2+} overload and cell death, but the specific cellular ion channels involved have not been identified. We studied the spatial and temporal characteristics of endothelial Ca^{2+} signals after exposure of 3rd order mesenteric arteries to purified histones (Roche). We utilized transgenic mice with endothelial cell-specific expression of a Ca^{2+} biosensor (GCaMP5) to perform spinning disc confocal imaging of endothelial Ca^{2+} signals without interference from vascular smooth muscle or other cell types. We found that histones induce two distinct types of endothelial Ca^{2+} signals. First, at low concentrations (1 μg/mL), histones trigger local Ca^{2+} events that are brief and contained within an endothelial microdomain. At higher concentrations (10 μg/mL), histones trigger much larger, longer duration, calcium waves that propagate within and between endothelial cells. In addition, we found that these two Ca^{2+} signals may involve different ion channels. Histones increase Ca^{2+} signals even in the presence of cyclopiazonic acid (CPA), arguing against a role for IP3-receptor mediated Ca^{2+} events. Treatment with 10 nM GSK219, a transient receptor potential cation V4 channel (TRPV4) antagonist, suppresses the localized Ca^{2+} events induced by 1 μg/mL histones, while the propagating Ca^{2+} events seen at 10 μg/mL are not inhibited by GSK219 (10 or 100 nM). The data demonstrate that extracellular histones are robust activators of endothelial Ca^{2+} signals through TRPV4 dependent and TRPV4 independent pathways.

HS3-1
Cross-talk between antioxidant genes and microRNAs in blood vessel formation

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Heme oxygenase-1 (Hmox1), a major antioxidant gene, mitigates response against oxidative stress, but also participates in blood vessel formation. We showed that Hmox1 strongly affects microRNA biogenesis and differentiation of skeletal myoblasts (Kozakowska et al., ARS, 2012). Interaction between Hmox1 and miR-378 influences also growth and angiogenesis in non-small cell lung carcinoma (Skrzypek et al., ARS, 2013). Here we investigated the role miR-378 in revascularization after hind limb ischemia.After ischemia blood flow restoration was strongly inhibited in miR-378 KO mice (kindly provided by Dr. Eric Olson, UT Southwestern, Dallas, USA) in comparison to WT animals. miR-378 is highly expressed in skeletal myoblasts and the muscles, but not in endothelial cells. Interestingly, bone marrow-derived miR-378-deficient proangiogenic cells showed impaired proliferation, migration and ability to form tubes on Matrigel comparing to WT cells. Upon ischemia miR-378 is downregulated in WT muscles, followed by upregulation at day 21. In miR-378-deficient ischemic muscles potent inflammatory response, assessed by cellular infiltration, expression of Hmox1 and inflammatory cytokines was observed. Interestingly, expression of VEGF and Ang-2 was upregulated, while Ang-1 tended to be diminished in muscles of miR-378 KO mice. Number of capillaries was not, however, different in WT and miR-378 KO mice, but arterioles were less abundant in miR-378 KO animals. Interestingly, intramuscular AAV-miR-378 gene therapy accelerated additionally revascularization in WT animals, while it was ineffective in miR-378 KO mice.In sum, lack of miR-378 appears to influence ischemic revascularization affecting local and systemic inflammation. Supported by OPUS 2012/07/B/NZ1/02881 and MAESTRO 2012/06/A/NZ1/00004 grants.

HS3-2
Role of CLIC proteins in the regulation of pulmonary vascular inflammation and angiogenesis

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Choride Intercellular Channel (CLIC) proteins are homologous to glutathione-S-transferases and exist as both soluble and integral membrane proteins where they act as chloride channels and interact with a number of signalling and structural proteins. CLIC proteins are important regulators of vascular development and tumor angiogenesis.

We have shown that increased expression of CLIC1 and CLIC4 accompanies abnormal angiogenesis and inflammation in pulmonary arterial hypertension (PAH), while global deletion of CLIC4 protects against the disease. CLIC4 compromises pulmonary endothelial barrier function and enhances angiogenesis, whereas CLIC4 gene silencing has an inhibitory effect. The mechanism of CLIC4 effects involves p65-mediated activation of nuclear factor-κB, followed by stabilization of hypoxia-inducible factor-1α and increased downstream production of vascular endothelial growth factor and endothelin-1. An unbiased proteomic screen and cytokine microarray analysis of CLIC4-overexpressing human pulmonary artery endothelial show increased

expression of proteins involved in NFkB signalling, including RANTES, kindlin-3, interleukin 6 and matrix proteoglycans. CLIC4 induces leukocyte adhesion and migration in response to two pathologically relevant factors, hypoxia and TNF-α, while CLIC4 gene silencing attenuates inflammatory responses in *in vitro* and *in vivo*.

Conclusions: Increased CLIC4 expression is an early manifestation and mediator of endothelial dysfunction in pulmonary hypertension. Our study implicates CLIC4 in the regulation of angiogenic and inflammatory responses in pulmonary vascular endothelium.

HS3-3

The intrinsic system that governs angiogenesis and stress resistance of vascular endothelium

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Cancer and atherosclerosis are 2 major diseases associated with aging. Cancers stimulate ECs and make neo-vessels to gain oxygen and nutrient, whereas the loss of integrity vascular endothelium is the primary cause of atherosclerosis. We are now focusing on the intrinsic system that governs angiogenesis and stress resistance of vascular endothelium. We have isolated vasohibin-1 (VASH1) as an angiogenesis inhibitor produced by ECs. There are two transcripts of VASH1: full length VASH1A consisting of 7 exons and the splicing variant VASH1B consisting of 4 exons. Both VASH1A and VASH1B were induced in ECs by the VEGF stimulation and exhibited anti-angiogenic activity. However, when the expression of endogenous VASH1A of VASH1B was modulated, the knockdown of VASH1A induced premature senescence of ECs whereas the knockdown of VASH1B reduced the senescent phenotype. Conversely, the overexpression of VASH1A made ECs resistant to stress-induced cell death, whereas the overexpression of VASH1B caused EC death. Thus, their effects on endothelial cell survival/death are distinct and adjective. We applied VASH1A and VASH1B to anti-angiogenic cancer therapy. VASH1A reduced the tumor vascular density but remaining tumor vessels were stabilized and functioning, whereas those treated with VASH1B were immature and non-functioning. When VASH1A and VASH1B gene combined, the proper combination of VASH1A and VASH1B resulted in the better outcome of anti-angiogenic cancer therapy. The level of expressions of VASH1A and VASH1B may change during aging. Especially, the expression of VASH1A declines during replicative senescence of ECs. This decreased expression of VASH1A may relate to age-related vascular diseases.

HS3-4

Gene expression analysis in small arteries of spontaneously hypertensive rats. Evidence for ER stress

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Small arteries are known to develop functional and structural alterations in hypertension. However, the mechanisms of this remodeling are not fully understood. In this study we analyzed gene expression associated with the development of hypertension in mesenteric arteries of spontaneously hypertensive rats (SHR). Three sublines of SHR and Wistar Kyoto rats (WKY) as control were studied at 6 weeks and 5 months of age. miRNA and mRNA microarrays were performed and analyzed with bioinformatical tools such as Ingenuity® Pathway Analysis (IPA). Principal component analysis showed a clear separation in both miRNA and mRNA expression levels between both ages studied, demonstrating a strong age-related expression. At miRNA level, IPA identified differences between SHR and WKY related to metabolic diseases, cellular growth, and proliferation. The mRNAs differentially expressed in SHR were related to oxidative stress, cellular movement and proliferation. The most strongly upregulated gene was thrombospondin 4 (Thbs4), a protein involved in the endoplasmic reticulum (ER) stress response by activating the transcription factor 6α (Atf6α). Both Atf6α and its downstream targets were also differentially expressed in SHR vs WKY. These mRNAs were confirmed at the protein level by western blot. These data revealed a number of genes and miRNAs in mesenteric arteries of SHR, which had not been related to hypertension previously. We also identified a link between the ER stress response and hypertension.

HS3-5

VEGF knockdown in muscle improves recovery of blood flow after ischaemia

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Lower limb ischaemia is a common morbidity factor associated with some chronic diseases, such as diabetes. In

most of these conditions, Vascular Endothelial Growth Factor (VEGF) signaling is altered relative to physiological baseline, which causes severe impairments in blood flow. We used a transgenic mouse approach to test the hypothesis that lack of VEGF-A hinders arteriolargenesis (the growth of new arterioles). We have produced transgenic mice (termed ACLL) where VEGF-A is specifically knocked out in skeletal muscle cells. ACLL mice were given doxycycline (+dox) or sucrose-only (-dox) in the drinking water for 10 weeks. They were then subjected to unilateral hindlimb ischaemia and speckle imaging was used to monitor blood flow in the hindpaws. VEGF tissue specific inducible knockout showed a significantly improved ischaemic/contralateral blood flow ratio when compared to the control cohort at both early (3–7 days) and late (days 14–21 days, $p < 0.01$ for all days, repeated measures test). At the end of the experiment, the ischemic adductor muscle was sectioned and stained for endothelial cells (IB4) and alpha-smooth muscle actin. There was no difference in capillary density between the two groups; however, arteriole density was significantly increased in VEGF knockout mice. This data suggests that VEGF of non-endothelial origin hinders arteriolargenesis and, hence, functional control of blood flow.

HS4-1
Early insights linking muscle metabolism, vascular control and regulation of physiological angiogenesis

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Olga was born in Czechoslovakia and studied medicine at Charles University, after which she joined the distinguished muscle physiologist Ernest Gutmann at the Czech Academy of Sciences. Olga developed an interest in muscle blood flow and its regulation, and conducted important experiments that underpinned her subsequent contributions to understanding physiological control of angiogenesis. Her main interest was on the role of various factors connected with increased blood flow (the monograph "Muscle blood flow" was published in 1973), and supplemented traditional approaches such as drop counter estimates of perfusion following muscle stimulation with novel methods such as X-ray microanalysis of muscle cryosections to determine the time course of metabolic vasodilators. Drawing on her medical training, she provided impressive insights into microvascular physiology. A major advance was in understanding the local mechanisms regulating growth of capillaries in skeletal and cardiac muscle, showing that mechanical factors (especially increased shear stress) in conjunction with growth factors play a powerful role (the hugely influential monograph "Angiogenesis" appeared in 1986). This process is fundamental to muscle performance following endurance training, and she championed its application to different clinical situations. Based on *in vivo* ischaemic models and electrical stimulation, she promoted therapeutic approaches to ameliorate peripheral vascular disease. This has proved of great benefit to patients with intermittent claudication, and also in other cases of poor muscle blood flow (e.g. hypertension, stroke, heart failure). The talk will outline some of her seminal contributions to the field, and illustrate where these have influenced subsequent developments in microvascular physiology.

HS4-2
Methods for the investigation of flowmotion

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Vasomotion is the rhythmic oscillation of vascular tone that occurs in arterial vessels. This results in the fluctuation of microvascular perfusion (flowmotion) in many tissues *in vivo*. Flowmotion enables greater delivery of nutrients to a tissue, however it has only recently become apparent that alterations in flowmotion may have other important physiological roles such as linking endothelial dysfunction to insulin resistance and diabetes. Vasomotion can be measured in intact tissues and isolated vessels with microscopy, but microvascular flowmotion *in vivo* is harder to observe and quantitate. In humans, intravital videomicroscopy can be used to observe the nailfold microcirculation but has limited use in other tissues. Laser Doppler Flowmetry (LDF) using skin surface probes has been the most commonly used technique to observe microvascular flowmotion in humans. LDF can also be used to measure flowmotion in other tissues but this involves invasive needle probes to be inserted into the tissue of interest with the concomitant risk of disrupting normal blood flow patterns and thus has limited use to mostly skin. A method based on contrast-enhanced ultrasound (CEU) using gas-filled microbubbles is potentially a new minimally-invasive technique to assess microvascular flowmotion in a number of tissues. Developments in ultrasound technology have allowed real-time measurement of microvascular volume that like LDF can be analysed to determine microvascular flowmotion rhythms. Application of these techniques (LDF and CEU) will allow microvascular flowmotion to be investigated in a number of pathological states and may provide a new understanding of the origin and consequences of metabolic diseases.

HS4-3
Flow motion dynamics of blood flow and oxygenation

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Tissue blood perfusion is influenced by neural, humoral and local control mechanisms. The role of low frequency, periodic flow motion imparted by these control mechanisms on blood flux within the microvasculature is much debated and the spatio-temporal relationship between blood flow motion and tissue oxygenation unclear. Spectral analysis over the range (0.0095–2 Hz) of the component frequencies of the blood flow (BF) and oxygenation ((oxyHb, deoxyHb, totalHb and SO2) signals obtained in skin using a combined laser Doppler and white light spectroscopy probe (Moor Instruments, UK) reveals a significant positive correlation between the contributions in the three lower frequency bands [endothelial (0.0095–0.02 Hz), sympathetic (0.02–0.06 Hz) and myogenic (0.06–0.15 Hz)] to the BF and oxygenation signals. However, differences in the relative contribution of the component frequencies to flow motion activity, as evidenced by a relatively small contribution of respiratory and cardiac activities to the oxygenation signals, suggests a significant dissociation between these oscillators. Exploration of the dynamic characteristics of flow motion using frequency coherence demonstrates considerable concordance within the endothelial (0.83[0.07] [mean(SD)]) and neurogenic (0.54[0.16]) components of BF and SO2 suggesting that they are modulated in a similar manner. These relationships are diminished and the intrinsic variability in flow motion signals lost in individuals with cardiovascular and metabolic disease. Whether these phenomena are causally related and represent a reduced homeostatic capacity to respond to changing local or systemic demands under pathophysiological conditions remains contentious.

HS4-5
Mapping oxygen in the brain of awake mice

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Although much progress has been made in the field of brain metabolism, several basic parameters of brain physiology remain to be determined. One such parameter is the optimal range of brain oxygen concentrations in physiological conditions that avoid the serious confounds of anesthesia, stress or tissue damage due to invasive recording techniques. We have reported the possibility of high-resolution mapping of oxygen in the living brain, using a two-photon lifetime microscopy (2PLM) and the phosphorescent oxygen sensor PtP-C343. Using this approach, we then showed that we can detect capillary erythrocyte-associated transients, i.e. Po2 fluctuations associated with individual erythrocytes. Interestingly, Po2 between two erythrocytes is at equilibrium with Po2 in the nearby neuropil and can thus be used to infer Po2 in the nearby neural tissue. Here, I will present the Po2 maps of two brain regions recorded in real physiological conditions, i.e. in the awake unstressed resting mouse.

HS4-6
Metabolic regulation: Insights from simulation approaches

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In vascular network adaptation, each vessel reacts according to a common set of genetically determined responses or "rules" to local conditions and to signals transmitted from other vessels of the network. Single vessel reactions, in turn, modify the functional properties of the vascular bed at large. Resulting complex interrelations in the vascular network require computational approaches to quantitatively analyze adaptive stimuli and vascular responses.

In metabolic regulation, vascular reactions to local oxygenation are assumed to match blood supply to tissue demand via negative feedback regulation: Low oxygen levels evoke vasodilatation, and thus an increase of blood flow and oxygen supply, by increasing (decreasing) availability of vasodilatory (vasoconstricting) metabolic signal substances with decreasing oxygen partial pressure. Here, a methodological approach combining *in vivo* microvascular network characterization with theoretical modeling of vessel diameter adaptation was used to address principles of metabolic vessel diameter regulation. The following hypotheses were derived: (1) In addition to oxygen dependent signaling, metabolic negative feedback regulation can be achieved by signal substances produced independently of local oxygenation (i.e. reflecting the pure presence of cells) due to the "dilution effect". (ii) Vasodilator signaling but not vasoconstrictor signaling can prevent shunt perfusion when the signal is conducted upstream to feeding arterioles. (iii) In steady state requiring low perfusion heterogeneity, metabolic signaling from a perivascular tissue sleeve is optimal providing strong negative feedback. During transient increases of tissue oxygen demand, however, RBC derived vasodilators or vasoconstrictors entering flowing blood may support the perfusion amplification needed by evoking positive feedback effects.

HS5-1
Endothelial-smooth muscle cell interactions in the regulation of vascular tone

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Evidence from high-resolution imaging has shown that the 3 dimensional structure of arterioles varies considerably during vasomotor responses. By comparing fully dilated arterioles to those with myogenic tone and then further constricted to α_1-adrenoceptor agonists it is clear that both the endothelial cells (ECs) and smooth muscle cells (SMCs) change shape. How this influences the endothelium-dependent regulation of vascular tone is not yet known. Direct EC-SMC contact sites occur through holes in the internal elastic lamina (IEL) and basement membrane (BM). Where myoendothelial gap junctions occur, current and small molecules such as Ca^{2+}/IP_3 can pass between the different cell types. The projection of the ECs towards the SMCs, either within or fully through the IEL, serves as a signaling microdomain, with high levels of expression of key proteins such as intermediate-conductance K_{Ca} channels and transient receptor potential channels. Therefore, if these contact sites are altered, this may influence endothelium-dependent responses, especially those secondary to hyperpolarization of ECs. Further to this, the shape of the IEL changes as vasoconstrictors are added to myogenically-active arterioles. The corrugated shape effectively presents a larger surface area for the basolateral EC membrane to remain attached to the basement membrane, but also forces the nucleus of ECs into the lumen and alters the orientation of the cell organelles relative to each other. How this might affect the spatio-temporal responses to agonists acting at ECs will be discussed.

HS5-2
Structural and cellular mechanisms underlying adaptive and pathological vascular responses to mechanical forces

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The distribution of extracellular matrix proteins (ECM) within the walls of resistance vessels is complex both in variety of proteins and structural arrangement. For example, elastin exists as discrete fibers varying in orientation across the adventitia and media as well as in more sheet-like structure of the internal elastic lamina (IEL). These structural patterns likely sub-serve specific functional properties, including mechanosensing, control of forces, cellular positioning, and communication between cells. Current knowledge of developmental, or age-related, characteristics of elastin, and other ECM proteins, largely comes from studies of conduit vessels with resistance artery data lacking. The present studies examined, in rat, developmental (from day 3 of life onward) changes in the expression of vessel wall (small mesenteric and cerebral arteries) ECM proteins and placed this in context of vessel structure and function. Using qPCR elastin, collagens 1 and 3 and lysyloxidase mRNA peaked at approximately postnatal day 11 in both vasculatures before declining at later times. During the postnatal period, the IEL elastin component appeared to accumulate/mature progressing from a punctate and fibrous appearance into a more continuous sheet. Similarly, in mesenteric vessels adventitial elastin fibers continued to be laid down with age. With respect to function, endothelial-dependent hyperpolarization-mediated responses were absent in both vessels at day 3 but evident by day 19. During this period ion channel mRNA expression was altered with increased expression of both small and large conductance Ca2 + activated K+ channels. Collectively these data show marked postnatal remodeling and maturation of small artery structure and function.

HS5-3
Mechanosensitive $G_{q/11}$-protein Coupled Receptors Mediate Myogenic Vasoconstriction

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Myogenic vasoconstriction which is also known as Bayliss effect is mediated by vascular smooth muscle cells (VSMCs) of small resistance arteries sensing mechanical stretch. During the last three decades several proteins have been proposed to be mechanosensors in VSMCs. Our previous studies have highlighted an agonist-independent mechanical activation of $G_{q/11}$-protein coupled receptors ($G_{q/11}$PCRs) in VSMCs of resistance arteries. In particular, angiotensin II AT_1 receptors (AT_1Rs) emerged as mechanosensors mediating myogenic tone. Moreover, we found that the AT_{1B} receptor isoform was more mechanosensitive than the AT_{1A} receptor isoform. Interestingly, cysteinyl leukotriene 1 receptors ($CysLT_1$) were up-regulated in AT_1Rs-deficient arteries as an essential backup strategy to compensate for the loss of AT_1Rs. Up-regulation of $CysLT_1$Rs resulted in increased myogenic tone at low intraluminal pressures which resulted in hyperactivity of AT_1R-deficient arteries. Only at high intraluminal pressures myogenic tone was reduced reflecting the loss of AT_1Rs. $CysLT_1$Rs were involved in myogenic vasoconstriction of wild type arteries as well. Simultaneous blockade of AT_1Rs and $CysLT_1$Rs in wild type arteries caused reduction of myogenic tone of more than 60% just as application of the selective $G_{q/11}$-protein inhibitor YM-254890. Our findings suggest that AT_1Rs and $CysLT_1$Rs are crucial mechanosensors in resistance arteries mediating

60% of myogenic vasoconstriction via the $G_{q/11}$-protein pathway without the involvement of endogenous agonists.

HS5-4
Cytoskeletal reorganization: A fundamental process linked to vascular smooth muscle contraction

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The actin cytoskeleton is a key structural and functional element of vascular smooth muscle cells, like other eukaryotic cells. Accumulating evidence indicates that actin cytoskeletal structure of differentiated, contractile vascular smooth muscle cells is dynamic and actively remodelled during contraction and relaxation. Light fluorescence microscopy and biochemical analysis indicates that contraction evoked by agonist treatment or mechanical stimulation is accompanied by, and dependent on, F-actin polymerization from a pool of free G-actin monomers that constitute approximately 20–30% of total actin content. Conversely, relaxation involves depolymerization and an increase in G-actin content. The mechanisms responsible for this dynamic reorganization of the cytoskeleton are yet to be fully identified, but roles for Rho-associated kinase, protein kinases C, A and G, cofilins, heat shock proteins, vasodilator-stimulated phosphoprotein and integrin adhesion proteins, such as vinculin, paxillin, p130CAS, N-WASP and Arp2/3 are indicated. That force generation is affected by manipulation of the actin cytoskeleton with latrunculins, cytochalasin and jasplakinolide, and by abnormal cytoskeletal remodelling in disease, is consistent with the view that dynamic cytoskeletal reorganization is a fundamental process of vascular smooth muscle contractility.

HS5-5
Continuous serelaxin infusion alters circumferential wall stiffness but not myogenic tone of mesenteric resistance arteries in spontaneously hypertensive rats

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Vascular stiffness and dysfunction are strongly associated with cardiovascular disease. The peptide hormone serelaxin (human relaxin-2) has beneficial effects in acute heart failure which are attributed to its vascular actions. Previous studies indicate that 3–5 days serelaxin treatment reduces myogenic tone but has little effect on arterial stiffness. Moreover, there are conflicting reports on the optimum duration of treatment because most studies are in healthy animals. Therefore we investigated the vascular actions of serelaxin in spontaneously hypertensive rats (SHRs). Male Wistar Kyoto (WKY) rats and SHRs (25 weeks old) were treated with serelaxin (13.33 μg/kg/hour) or placebo (20 mM sodium acetate) subcutaneously for 10 days. Mesenteric artery passive mechanical wall properties and myogenic tone were analysed using pressure myography. Inner and outer diameters and volume compliance were significantly reduced in SHRs, but there was no significant difference in circumferential wall stiffness between the two rat strains. Serelaxin treatment significantly reduced circumferential stiffness in SHRs but not WKYs, and this was associated with outward remodelling. Elastase incubation revealed that this difference in circumferential stiffness was not due to elastin. Myogenic tone was not altered in the mesenteric arteries of SHRs, and 10 days of serelaxin treatment did not reduce myogenic tone in either WKYs or SHRs. In summary, 10 days of serelaxin treatment in SHRs has minimal effects on myogenic tone but reduces circumferential stiffness. We suggest that a longer duration of serelaxin treatment may be necessary to induce vascular remodelling.

HS6-1
Coupling of angiogenesis and osteogenesis in bone

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The mammalian skeletal system harbours a hierarchical system of mesenchymal stem cells, osteoprogenitors and osteoblasts sustaining lifelong bone formation. Osteogenesis is indispensable for the homeostatic renewal of bone as well as regenerative fracture healing, but these processes frequently decline in ageing organisms leading to loss of bone mass and increased fracture incidence. There is evidence indicating that the growth of blood vessels in bone and osteogenesis are coupled, but relatively little is known about the underlying cellular and molecular mechanisms. Here we identify a new capillary subtype in the murine skeletal system with distinct morphological, molecular and functional properties. These vessels are found in specific locations, mediate growth of the bone vasculature, generate distinct metabolic and molecular microenvironments, maintain perivascular osteoprogenitors, and couple angiogenesis to osteogenesis. The abundance of these vessels and associated osteoprogenitors was strongly reduced in bone from aged animals, which was pharmacologically reversible to restore bone mass.

HS6-2

BMP2 regulates both osteogenesis and angiogenesis during postnatal bone repair

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Distraction osteogenesis (DO) is a surgical procedure that promotes new bone formation by mechanically lengthening the bone. DO stimulates a robust angiogenic response occurring both in regenerating bone tissue and in the surrounding muscle needed for bone regeneration. Prior studies showed that vascular smooth muscle cells are the primary source of BMP2 expression during bone regeneration leading to the hypothesis that vessels are the source of BMP2 that promotes skeletal regeneration. To test this hypothesis BMP2 was conditionally deleted in smooth muscle cells using transgenic mice with a tamoxifen inducible Cre driven by the alpha-smooth muscle actin promoter crossed to a BMP2 Floxed mice. After surgery mice received tamoxifen or vehicle throughout the experimental period. Skeletal and vascular tissues were characterized with microCT using barium perfusion to visualize the vessels. qRT-PCR analysis of bone and vessel mRNAs were used in conjunction with histological analysis to follow new bone and vessel formation. Conditional deletion led to decreased BMP2 expression in surrounding muscle but not within the distraction gap. Although the BMP2 conditional deletion showed increased gene expression for osteogenesis it did not translate into increased skeletal tissues but showed decreased bone formation and a failure to heal the osteotomy. Multiple markers for angiogenesis, VEGFR2, Pecam1, and Ve-Cadherin, were strikingly decreased ~ 45%, 60%, and 85% in the musculature while contrast enhanced microCT studies showed new vessel formation in muscle failed to develop when BMP2 expression was deleted. These results show BMP2 is a morphogenetic signal for both osteogenesis and angiogenesis.

HS6-3

The molecular signature of the stroma response in prostate cancer-induced osteoblastic bone metastasis highlights expansion of hematopoietic and prostate epithelial stem cell niches

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The supportive cellular and extra-cellular tumor microenvironment is considered to be critically important for tumor progression and therapy resistance. Interactions between cancer cells and the tissue-specific stroma is critical for primary and metastatic tumor progression. Osteotropic cancer cells preferentially colonise the bone/bone marrow, where they can alter the physiological balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Osteotropic prostate cancer generally elicits an osteoblastic response (osteoinduction), while in breast and lung cancer the osteolytic bone phenotype prevails (osteolysis). The exact molecular mechanisms of tumor-bone interactions leading to osteoinduction or osteolysis have, however, remained largely elusive.In this study we have used xenograft models of experimentally-induced bone metastasis (human tumor xenografts in immunodeficient mice) and exploited the divergence between human and mouse RNA sequences to allow the dissection of the stroma (mouse) from the cancer cell (human) transcriptome. Using osteoinductive human prostate cancer cells (VCaP, C4-2B) we generated the osteoblastic bone metastasis-associated stroma transcriptome, indicative of the stromal reaction in osteosclerotic bone lesions. After subtraction of the genes shared by inflammation, wound healing and desmoplastic responses a curated gene signature of the bone marrow/bone-specific stroma response to prostate cancer-induced, osteoblastic bone metastasis was generated.A robust induction of genes involved in osteogenesis and angiogenesis dominates the osteoblastic bone metastasis-associated stroma transcriptome. Our data show an amplification of hematopoietic and prostate epithelial stem cell niche components. These components may function to reinforce the bone metastatic niches, thus providing the specific growth support for human osteo-inductive prostate cancer cells at skeletal sites.

HS6-4
Novel approaches to investigating tumour-endothelial interactions

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Tumour cell colonisation of bone marrow is a critical step in the formation of bone metastasis in breast and prostate cancer. Tumour initiating cells (TIC) have recently been identified in solid tumours, including prostate. Integrin av is associated with human prostate and up-regulated in TICs. This study investigates whether prostate cancer metastasis responds to the integrin antagonist (GLPG0187) treatment using the novel dorsal window skinfold chamber *in vivo* model with engrafted metatarsal. We have previously shown that metatarsals rapidly re-vascularise with the host vasculature by day 5–7. Prostate cancer (PC3-GFP) cells adhere to the endothelium and/or to metatarsal matrix 3 days after injection and the number of cells in and surrounding the bone is maintained or increased during the study.

PC3 cells (1 × 105) were injected via the intra-cardiac following which the integrin antagonist (GLPG0187, 100 mg/kg/day, ip) or control (PBS) was administered. Metatarsel recordings were made every 24 hour up to 4 weeks. Tissue was then resected & processed for uCT, multi-photon analysis and histology. Functionalin vitroassays were used to determine the response to GLPG0187 on cell proliferation, viability and migration. Atomic force microscopy was used to determine the effects of GLPG0187 on tumour cell:endothelial cell adhesion.

GLPG0187 significantly ($p = 0.05$) reduced tumour cell number throughout the experimental duration by 52%, and inhibited angiogenesis *in vivo*. *In vitro* GLPG0187 reduced cell proliferation ($p = 0.01$) and migration ($p = 0.01$) with no effect on viability; tumour:endothelial cell adhesion was also reduced ($p = 0.05$) as demonstrated using AFM.

HS6-5
The importance of the perivascular niche in the early stage of breast cancer bone colonization

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Advanced breast cancer is frequently associated with metastasis and the most common metastatic site is the skeleton. During dissemination to bone, breast cancer cells locate in a putative "metastatic niche" and this microenvironment plays a key role in the colonisation, dormancy and proliferation of the cancer cells in bone. Although the precise cellular composition of the niche remains to be established, it has been suggested to be broadly overlapping with hematopoietic stem cell and peri-vascular bone niches. Using *in vivo* models of bone metastasis, we mimicked the early steps of bone metastasis and investigate the relationship between the different cellular population of the niche and the breast cancer cells labelled with fluorescent membrane dyes. By multiphoton and confocal microscopy, we were able to detect single tumour cells within the bone microenvironment following injection in immunocompromised mice. Breast cancer cells homed preferentially in the trabecular region of the long bone, which is particularly rich in osteoblasts and highly vascularised. Moreover, we were able to visualise tumour cells in close proximity to a particular vessel subtype using an immunofluorescence protocol with antibodies against endomucin and CD31. The data obtained evidenced the direct association between vessels, osteoblast and breast cancer cells during the early steps of bone metastasis. Our results highlighted the importance of the perivascular niche in the firsts stages of skeleton colonisation, underlining the possibility of new therapeutic approaches targeting this component of the niche.

HS7-1
Developmental aspects of a life course approach to healthy ageing

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There is increased awareness that the process of human ageing commences as early as conception with the inheritance of a specific genome, and it does not cease until death. Epidemiological studies have demonstrated that environmental influences during pre-conception, intrauterine and early postnatal life are able to modify gene expression, largely by epigenetic processes, with corresponding changes in form and function which establish the later predisposition of the individual to age-related system decline. The biological process through which these changes in gene expression are established is known as "developmental plasticity". This process is ubiquitous throughout the animal world, and it provides the mechanism whereby a given genotype can develop into a range of phenotypes which are best adapted to the environment that they are likely to meet once development is completed, a concept formalised as a predictive adaptive response. Recent changes in the environment, with urbanisation, sedentary lifestyle, poor nutrition, smoking and exposure to environmental pollutants and chemicals, increasingly make such phenotypes maladaptive, with consequent effects on noncommunicable disease prevalence. Developmentally-induced adaptive responses are usually thought to operate on Darwinian fitness, i.e. survival to the time of successful reproduction, rather than on ageing per se. New

concepts challenge this view, and this talk will discuss this, using cardiovascular and musculoskeletal ageing as examples, along with some common underlying mechanisms. It will note the potential for translational public health strategies to promote healthy ageing by adopting a developmental perspective.

HS7-2
Gestational xenobiotic exposures: Microvascular implications for the past, present, and future

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Air pollution particulate matter and engineered nanomaterials (ENM) encompass the broad definition of xenobiotic particles. While the effects of perinatal air pollution exposure have been thoroughly investigated, the outcomes associated with ENM exposure are still in their infancy. As the potential uses, and therefore exposures, increase exponentially so does the need for thorough safety assessments. Up to this point, the majority of research in the field of cardiovascular nanotoxicology has focused on the coronary and vascular reactions to pulmonary exposures in young adult, healthy, male models; however, as unintentional and intentional exposures grow and diversify, the non-pulmonary risks to under-represented populations becomes more evident. The development of the maternal-fetal circulation during successful gestation is one of the most unique, complex, dynamic, and acutely demanding physiological systems. Perturbations to this system could lead to devastating consequences to the mother and/or developing fetus. Fetal development in a hostile gestational environment may lead to systemic alterations which may facilitate adult disease, a fundamental postulate of the Barker Hypothesis. Recently we have initiated the evaluation of gestational ENM exposures. Through evaluations of *in vivo* arteriolar function prior to conception and maternal, fetal, and progeny isolated arterioles, we have demonstrated endothelial dysfunction at each stage with possible mitochondrial, epigenetic, and inflammatory mechanisms. A better understanding of the mechanisms associated with the multigenerational effects, may allow pregnant women to reap the benefits associated with these developing technologies or better assist in proper regulation of ENM applications. Financial Support: NIH-K99-ES024783 (PAS).

HS7-3
Heterogeneity of coronary vasculature and its complex development

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The mechanisms of heart development are quite complex. Rough shape of heart is formed during the organogenesis phase, besides heart proceeds with delicate and sophisticated changes in its shape during the late gestational stage. Recently, it is focused that coronary vessels are composed of many types of cell. Both endothelial cells and smooth muscle cells show heterogeneities in their origins. Previously we discovered novel cell sources, which came from craniofacial region to the coronary smooth muscle cells (Arima et. al., Nature Commun. 2012) and other group also reported sinus venosus endothelium, proepicardium and endocardial cells contributed to the coronary endothelial cells.

Different cell sources coordinate through the late embryonic phase through myocardial compaction, remodeling of the coronary vessels, and maturation of valves. The concept of developmental origin of health and disease (DOHaD) is thought to affect to the growth of these important events. However those mechanisms are not fully understood.

Here we developed the mouse model of low birth weight by calorie restriction during mid to late gestation periods. We compared the heart and the coronary vasculature using the multi-photon microscopy.

I will introduce about the heterogeneity of the coronary vasculature and the impact of malnutrition to the proper development of heart and coronary circulation.

HS7-4
Retinal vascular imaging in early life: Insights into processes and risk of cardiovascular disease

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The origins of cardiovascular disease (CVD) may be traced to vascular and metabolic processes in early life. Because the blood vessels in the eye can be seen and imaged non-invasively using modern retinal cameras, they offer a unique perspective of the human circulation and therefore, retinal scans of these blood vessels may be useful for understanding the early changes and mechanisms of major CVD, including stroke, diabetes and hypertension. Epidemiological and clinical studies have shown that retinal vascular imaging, measuring a range of retinal vascular changes, are predictive of CVD before they become clinically symptomatic. For example, narrower retinal arteries are associated with subsequent development of hypertension, while wider retinal

veins are associated with the development of stroke and CVD deaths, independent of a person's traditional risk profile. More recently, retinal vascular imaging has been applied in children and adolescents to examine the use of this technology in understanding CVD risk in early life. These studies show that the same retinal vascular changes linked with stroke, hypertension and CVD were associated in children with lower birth weight, shorter gestational age, hypertension, overweight/obesity, and type 1 diabetes. Thus, retinal vascular imaging supports the view that many CVD risk factors are already associated with structural and functional changes in the retinal microvasculature in early life, and the microcirculation may be a site for pre-clinical process underlying the development of CVD in adulthood. Thus, retinal vascular imaging may offer the potential to risk stratify a person's lifetime probability of developing CVD.

HS8-1
Contributions of platelets to inflammation and neutrophil recruitment in the acutely inflamed glomerulus

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In acute glomerulonephritis, platelets are recruited to the glomerulus where they contribute to glomerular inflammation by promoting glomerular neutrophil recruitment. However, the mechanisms of platelet recruitment to the glomerulus have not been determined. Therefore the aim of this study was to examine the mechanisms whereby platelets promote leukocyte recruitment and inflammation in the glomerulus. Intravital microscopy was used to examine the glomerular microcirculation in kidneys of mice undergoing glomerular inflammation induced by an antibody against the glomerular basement membrane (anti-GBM Ab). Neutrophils recruited to the glomerulus were visualised by fluorescent dyes. To visualise platelets, platelets were either isolated from a donor mouse, labelled with CFSE, and transfused into recipient mice or, in confocal microscopy experiments, directly visualised using a platelet-specific antibody. Platelet recruitment was initiated within 5 minutes of administration of anti-GBM antibody. This was unaltered by inhibition of GPIb alpha, but was prevented by the absence of platelet GPVI. Fibrinogen was deposited in glomerular capillaries during the inflammatory response, and inhibition of alpha IIb beta 3, fibrinogen and ICAM-1 inhibited platelet recruitment. Confocal intravital microscopic analysis of endogenous platelets revealed that under inflammatory conditions, platelets are retained in glomerular capillaries for longer periods, interacting with either the endothelium or with neutrophils present in the glomerular capillaries. These data indicate that platelet recruitment to the glomerulus in the anti-GBM antibody-induced model of acute glomerular inflammation is dependent on the combined actions of GPVI and the alpha IIb beta 3/fibrinogen/ICAM-1 pathway.

HS8-2
Platelet-leukocyte interdependence in the inflamed microcirculation

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Inflammation in the microcirculation has long been known to involve interactions between leukocytes and endothelial cells, including leukocyte rolling, adhesion and transendothelial migration. However, platelets are traditionally viewed in the context of hemostasis and thrombosis. In recent years, increasing evidence suggests that platelets are also important mediators of inflammation in the microcirculation. In a variety of models, a close temporal and spatial correlation is evident between platelet and leukocyte recruitment in the inflamed microcirculation. Under some conditions, there is evidence of interdependence of recruitment of platelets and leukocytes during microvascular inflammation: platelets are required for efficient leukocyte recruitment and leukocytes are required for efficient platelet recruitment. Adhesive interactions between platelets and leukocytes facilitate interdependent recruitment of the two cell types; P-selectin, P-selectin glycoprotein ligand-1, and beta-2 integrins are among the molecular mechanisms involved. In a mouse model of corneal epithelial wound injury, our group generated evidence that platelets and their interactions with leukocytes are necessary for efficient wound healing responses. Examples of microvascular platelet-leukocyte interdependence in other experimental models support the notion that platelets, via their interactions with leukocytes, contribute to a variety of inflammatory responses and suggest that these interactions may provide the basis for new therapies in selected inflammatory diseases.

HS8-3
Platelets are rapid responders to bacteremia

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Platelets are normally thought of as critical to hemostasis and thrombosis. However there is growing evidence that platelets interact with various immune cells to help capture and/or eradicate bacteria, particularly in the mainstream of blood. For example, introduction of bacteria into blood results in

very rapid capture by Kupffer cells that then phagocytose and kill the bacteria. We observed that Kupffer cells are constantly probed by platelets. These cells continuously touch down on Kupffer cells and then return back into the mainstream of blood. Following the capture of bacteria by Kupffer cells, the platelets touch down and remain adherent to Kupffer cells, rapidly inducing further platelet recruitment leading to encapsulation of the bacteria by platelets. This leads to more efficient clearance of the bacteria from the blood. If the bacteremia is sufficiently severe, neutrophils are also recruited to adhere to the vessel wall and then platelets bind to neutrophils inducing the release of large web like structures called NETs (neutrophil extracellular traps). The NETs adhere to the vessel wall and help trap circulating bacteria. The NETs are covered with potent proteases including elastase and also cytotoxic histones. Administration of DNAse degrades the DNA portion of NETs releasing entrapped bacteria. Interestingly DNAse does not reduce histones that appear to be attached to the vasculature via von Willebrand factor and only partially reduce elastase and proteolytic activity of the NETs. Overall, platelets appear to contribute to reduction of bacteria in blood by interacting with various immune cells and inducing anti-microbial processes.

HS8-4
Platelet abnormalities in inflammatory bowel disease

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Patients with inflammatory bowel disease (IBD) exhibit a 3-fold higher risk for development of thromboembolism than the general population. This increased risk for thrombus development is accompanied by abnormalities in coagulation, fibrinolysis and in the number and function of platelets. These features can be recapitulated in different animal models of experimental IBD, such as dextran sodium sulfate (DSS) or T-cell transfer induced colitis. Within the inflamed bowel, there is evidence for enhanced platelet adhesion in the microvasculature and for accelerated thrombus development, although the latter response is also evidenced in distant organs. Experimental IBD is also associated with thrombocytosis, the appearance of increased numbers of active and immature platelets, an unchanged platelet life-span, and elevated levels of circulating platelet-leukocyte aggregates. Several pro-inflammatory cytokines, including tumor necrosis factor-α, interleukin-1β, and interleukin-6 (IL-6), exhibit significantly elevated plasma levels in human and experimental IBD, and have been implicated in the enhanced microvascular thrombosis detected in experimental IBD. Of the cytokines studied to date, IL-6 appears to recapitulate nearly all of the platelet abnormalities observed in human and experimental IBD. This cytokine is a potent stimulant of thrombopoiesis and it reproduces the changes in platelet number and function elicited by IBD when infused at concentrations that mimic the cytokine level detected in animal models. Furthermore, immunologic or genetic blockade of IL-6 is associated with significantly blunted platelet responses and attenuated thrombus development. These findings in animal models raise hope for the development of novel therapeutic strategies to reduce thrombosis-related mortality in patients with IBD.

HS8-5
Uridine triphosphates analogues as inhibitors of platelet aggregation

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Background: Platelets express two ADP receptors namely P2Y1 and P2Y12 that regulate ADP and other agonists-induced platelet aggregation. P2Y1 receptor activation causes platelet shape change while P2Y12 receptor activation induces platelet aggregation. The aim of the present study was to characterise the effects of uridine triphosphate (UTP) and its analogues on ADP-induced platelet aggregation.
Methods: The experiments were performed on platelet rich plasma freshly isolated from blood donated by healthy human volunteers.
Results: Both UTP and S-UTP inhibited ADP-induced platelet aggregation in a conc.-dependent manner, S-UTP being more potent. The IC50 values against ADP (10 microM)-induced platelet aggregation were 32 and 0.36 microM for UTP and S-UTP, respectively. Likewise, both nucleotides potently antagonised collagen (2 microg/ml)- and epinephrine (10 microM)-induced platelet aggregation. However, both UTP and S-UTP had no effect on ADP- and MRS2365 (P2Y1 receptor agonist)-induced platelet shape change suggesting their inactivity at P2Y1 receptors.
Conclusion: The novel data demonstrate that UTP and S-UTP are potent P2Y12 receptor antagonists and inhibit agonist-induced platelet aggregation.

HS9-1
Measuring the chemistry in tissues and individual cells using mass spectrometry

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In the postgenomic era, one expects the suite of chemical players in a tissue or cell to be known and their functions

uncovered. However, many cell-to-cell signaling molecules remain poorly characterized and for those that are known, their localization and dynamics are oftentimes unknown. A suite of mass spectrometry-based approaches are described that allow the investigation of individual endocrine cells and tissues; these approaches include capillary electrophoresis coupled to mass spectrometry and direct mass spectrometric-based profiling and imaging.[1–3]. Using these mass spectrometry tools, new serotonin-related compounds, the cellular redox state, and literally hundreds of new neuropeptides/hormones have been characterized in well-defined neuronal networks, and in several cases, the functional roles of these molecules described. Imaging mass spectrometry and dynamic sampling of the extracellular environment are used for elucidating novel cell to cell signaling molecules in a range of model systems. Several additional examples of neuropeptide and hormone discovery are described across a range of metazoan life.

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HS9-2
Development of an imaging mass spectrometry technique for visualizing localized cellular signaling mediators in tissues

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In vivo concentrations of cellular signaling mediators such as inflammatory mediators are normally maintained at very low levels due to their strong ability to induce a biological response. The production, diffusion, and decomposition of such mediators are spatio-temporally regulated. Therefore, in order to understand biochemical basis of disease progression and develop new therapeutic strategies, it is important to understand the spatiotemporal dynamics of the signaling mediators *in vivo*, during the progression of disorders, *e.g.*, chronic inflammatory diseases; however, the lack of effective imaging technology has made it difficult to determine their localizations *in vivo*.

Such characterization requires technical breakthroughs, including molecular imaging methods that are sensitive enough to detect low levels of metabolites in the heterogeneous tissue regions in diseased organs. We and other groups have attempted to fill this technical gap by developing highly sensitive imaging mass spectrometry (IMS) technologies. To date, we have established two key techniques toward this goal, including.

(i) a sample preparation procedure that has eliminated the problem of the postmortem degradation of labile metabolites, and.

(ii) on-tissue derivatization of metabolites, which can enhance analyte ionization efficiency.

In this talk, I will review recent progress in the development of these technologies as well as how the highly sensitive IMS technique has contributed to increasing understanding of the biochemical basis of disease mechanisms, discovery of new diagnostic markers, and development of new therapies.

HS9-3
Visualization of metabolites localization at the micro-region using imaging mass spectrometry

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Imaging mass spectrometry (IMS) is becoming a powerful technology to evaluate the distribution of the metabolites or the administered drugs within the biological tissues. This technology enables to visualize the localization of multiple key molecules at a spatial resolution of $>$ 10 micrometer level in the case of MALDI-IMS. The value of a spatial resolution normally depends on the pretreatment of matrix coating to ionize the target molecules, because a size of matrix crystals influences the resolution of molecular images. Therefore, to produce a homogeneous thin layer of small crystals from matrix, coating method using solid to vapor-phase transition has been also reported. The display of metabolites localization at the high spatial resolution provides insight into the altered metabolic level at the micro-region. For instance, it enables to evaluate the influx/exchange of key compounds into peripheral tissue and the different metabolic profiling between normal/disease regions in tissue section. Superposition of visualized molecular images to optical microscopic images could help to further appreciate the metabolic process at the micro-region of interest. Thus the IMS technology is a significant approach to elucidate the altered metabolic profiling including information of localized metabolites. Here I would like to introduce useful IMS experiments analyzed by a general MALDI-TOF MS and an atmospheric MALDI-MS with a microscopy and show the effectiveness of this attractive technology.

HS9-4
Microscopic imaging mass spectrometry reveals a host-dependent mechanism for ammonia detoxification in the tumor-bearing liver of superimmunodeficient NOG mice

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Glutaminolysis in proliferating cancer cells is known to play a crucial role in maintaining their energy metabolism by using glutamine as a carbon resource for their survival under hypoglycemic conditions. A notion that glutaminolysis yields ammonia as a cytotoxic gas led us to hypothesize that cancer possesses mechanisms to protect against ammonia toxicity *in vivo*. In the model of hepatic metastasis of human-derived colon cancer HCT116 xenografts in super-immunodeficient NOG mice, we revealed that the liver serves as a niche that helps absorb ammonia derived from metastatic cancer foci by triglycerides and degrade it through urea cycle in the host tissue. In the tumor-bearing livers, tissue contents of urea were significantly elevated as compared with those in the controls. Microscopic imaging mass spectrometry (MIMS) revealed that ammonium adducts of triglycerides are localized in the host liver to greater extents than those in the tumor foci. The distribution of urea was unable to be visualized by MIMS, because the molecule can hardly be ionized under atmospheric MALDI conditions. However, surface-enhanced Raman scattering (SERS) imaging allowed us to visualize distribution of urea, demonstrating that urea-derived signals are significantly greater in the host liver than in the tumor regions. These results suggest that tumor burdens detoxification of ammonia derived from cancer foci to the host metabolic systems.

HS9-5
Quantitative mass spectrometry imaging and profiling of neurotransmitters, neuropeptides and drugs directly in tissue sections

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There is a great need to image and quantify the distribution of drugs and their metabolites, as well as endogenous compounds in tissue sections from different organs. Current neuroimaging techniques have limited ability to directly identify and quantify e.g., neurotransmitters from brain tissue sections. We present MALDI-mass spectrometry imaging (MSI) protocols for the quantification of drugs, neurotransmitters and neuropeptides directly in tissue sections at near cellular spatial resolution. The concentrations of drugs, neuropeptides and neurotransmitters are determined using either external standard curves, and by using labeled compounds (deuterated analogues) as internal standards. After selecting regions of interest on the tissue section our in-house developed software msIQuant automatically calculates the concentration based on the standard curve. Changes in absolute neurotransmitter concentrations were mapped in Parkinson's disease animal models and in response to drug treatments, demonstrating the powerful application of mass spectrometry imaging in neuroscience. Our approach facilitates data processing and provides better reproducibility and may be considered as an effective tool to quantify drugs and endogenous compounds in tissue regions of interest.

The work was supported by Swedish Research Council (Medicine and Health, Natural and Engineering Sciences, and Research Infrastructure).

HS10-2
Sprouting and splitting in organ vascular development

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Intussusceptive angiogenesis, known also as splitting angiogenesis is an alternative way to the sprouting mode of blood vessel formation. The formation, expansion and remodeling of the vasculature is an interplay between both angiogenic processes.

We employed the zebrafish model to demonstrate the interplay between sprouting (SA) and intussusceptive angiogenesis (IA).

During early zebrafish development and fin regeneration in adult animals the formation of the primitive capillary plexus has been initiated by SA. Further vascular growth and remodeling thus occurs by IA. IA is characterized by formation of multiple transluminal endothelial pillars. Their expansion contributes to rapid enchantment of the capillary surface area. Subsequent elongation and fusion of serried pillars splits the vascular segments and prune small capillaries that remodel the disorganized vascular meshwork into the typical tree-like arrangement. IA is also important in creation and restauration after damage of the zebrafish fin specific angioarchitecture.

The main driving force for IA seems to be the blood flow with particular shear stress geometry on the site of pillar

formation. Induction or downgrade of the IA has been achieved by pharmacologically and genetically blood flow manipulations. Computational modeling based on the obtained hemodynamically profiles could predict exactly the path of future pillar formation followed by segregation of new vascular segments and supplying vessels.

The obtained data demonstrated how the interplay between growth factor gradients and hemodynamically conditions enforce sprouting and intussusception to work hand in hand and create complex vascular structures.

HS10-3
Formation and maintenance of microvascular networks by angiogenesis, remodeling and pruning: An integrative model

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To meet the functional demands of a tissue efficiently, the microcirculation must include a dense meshwork of small vessels providing a large surface area for transport and short diffusion distances from capillaries to tissue, together with a hierarchical network of larger vessels that provide convective blood delivery to all parts of the tissue. This structure must also be capable of responding to changing functional demands. The question arises how are these requirements met by a structure that is generated without a predetermined spatial pattern, through the stochastic processes of angiogenesis? Here, a dynamic theoretical model based on experimental data is used to simulate the interacting processes of sprouting angiogenesis, structural remodeling and pruning. At each time step, the spatial oxygen field resulting from the current vascular configuration is calculated. A growth factor is assumed to be generated in hypoxic regions and to diffuse through the tissue, stimulating sprouting angiogenesis when its level exceeds a certain threshold. When sprouts meet other vessels, they are assumed to form connections allowing flow. Flowing segments are subject to structural adaptation of their diameters in response to local metabolic and hemodynamic stimuli, including the possibility of pruning of redundant vessels. It is shown that these mechanisms acting in parallel are capable of generating well-organized functional networks. Effects of defects in these mechanisms on vascular network properties are predicted. These results provide an integrative view of the processes underlying vascular network formation in normal and pathological conditions.

HS10-4
Relationship between microvascular blood flow and angiogenic factors in pre-eclampsia

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Microvascular dysfunction occurs in preeclampsia with reduced tissue perfusion precedes the onset of the disease. Angiogenic imbalance may play a role in preeclampsia. We hypothesized that an imbalance in pro- and anti-atherogenic factors affects microvascular function in pre-eclampsia and correlates with reduced tissue blood flow. Strain gauge plethysmography was used to measure maternal tissue blood flow and was correlated with circulating angiogenic factors; soluble fms-like tyrosine kinase 1 (sFlt-1), soluble endoglin (sEng) and placental growth factor (PlGF). Tissue blood flow was significantly reduced in the pre-eclampsia group compared to normal pregnancy ($p < 0.0001$). Serum sFlt-1 and sEng were significantly increased in pre-eclampsia compared to normal pregnancy ($p < 0.0001$). In contrast, serum PlGF was significantly reduced in pre-eclampsia compared to normal pregnancy ($p < 0.0001$). There was a strong inverse correlation between microvascular blood flow and sFlt-1 and sEng in the normal pregnancy and pre-eclampsia groups ($p < 0.0001$). There was a positive correlation between microvascular blood flow and PIGF in normal pregnancy and pre-eclampsia ($p < 0.0001$). Blood flow also showed a strong correlation with the sFlt-1: PIGF ratio in normal pregnancy and pre-eclampsia ($p < 0.001$). The data shows that the anti-angiogenic factors studied correlate inversely with microvascular perfusion during both normal pregnancy and pre-eclampsia. In pre-eclamptic patients, the elevated levels of these anti-angiogenic factors inversely correlate with microvascular blood flow whilst they exist at lower levels in patients with normal pregnancies and are associated with normal blood tissue perfusion.

HS10-5

Arterial-venous identity specification in pre-vascularized engineered implants requires perivascular cell recruitment and is impaired in diabetes

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Re-vascularization approaches have the potential to regenerate ischemic tissues, holding promise for translational therapies. However, most re-vascularization attempts fail due to the lack of a mature vasculature. A hierarchical vascular network, consisting of specific arterio-venous (AV) types, is essential for sustaining vascular function. We define factors that control AV specification in pre-vascularized engineered implants in health and diabetes (a disease characterized by vascular dysfunction) by using the only pre-vascularized engineered tissue described to yield vessels with specific AV identities. Hypothesis: AV specification is dependent on perivascular cell (PVC) recruitment and is impaired in diabetes. Using microvessel fragments isolated from EphrinB2-GFP (arterial-reporter) mice and implanted into engineered constructs, we show for the first time that preventing PVC recruitment by blocking PDGFRb resulted in lack of proper AV identity (ubiquitous EphrinB2 expression and absence of vessel network hierarchy). Controls exhibited mature networks with arterial ($EphrinB2^{+}$) and venous ($EphrinB2^{-}$) vessels. Analysis of endothelial cells co-cultured with PVCs of arterial or venous origin point to endothelial-PVC cell-cell Notch signaling involvement in arterial specification. No differences in the percentage of vessel perfusion or shear stress between microvessels in control or PDGFRb-blocked implants were observed.Lack of proper AV identity in the absence of PVCs (PDGFRb-blocked) was comparable to microvessels in constructs implanted into Type I diabetic mice (streptozotocin-injected). With regards to underlying mechanisms, qPCR showed significantly reduced expression levels of genes involved in the Notch signaling pathway, suggesting that an inability of vessels to acquire proper AV identity contributes to microvascular dysfunction in diabetes.

HS11-1

Hydrogen sulfide-nitric oxide stimulation of VEGF ischemic vascular remodeling

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Ischemic vascular remodeling acts to resolve deprivation of tissue blood flow and perfusion, and remains an unrealized therapeutic goal for multiple cardiovascular disease conditions. Work from our laboratory has revealed that members of the gasotransmitter family, hydrogen sulfide and nitric oxide, play critical roles in regulating ischemic vascular remodeling through arteriogenesis and angiogenesis stimulation involving VEGF dependent activity. Specifically, we show that chronic tissue ischemia augments expression and activity of the H2S generating enzyme cystathionine gamma lyase (CSE) that subsequently increases ischemic tissue NO bioavailability leading to increased monocyte recruitment and tissue VEGF expression. Genetic deficiency of CSE significantly blunts ischemia mediated increased NO bioavailability, monocyte recruitment, VEGF expression and vascular remodeling responses. Moreover, H2S and NO bioavailability are blunted in animals older than 18 months of age, which may be a contributing factor to diminished ischemic vascular remodeling during aging. These data reveal a novel and unrecognized role for H2S and NO in controlling molecular responses necessary for ischemic vascular remodeling that may be of therapeutic benefit.

HS11-2

Unveiling the cellular and molecular mechanism underlying vascular development by fluorescence-based bio-imaging in zebrafish

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Vascular networks develop through two distinct processes; vasculogenesis and angiogenesis. Vasculogenesis is defined as the formation of primitive vascular plexus, while angiogenesis refers to the subsequent growth and expansion of developed blood vessels. Formation of functional vasculature also requires lumen formation, arterial venous specification and mural cell coverage. However, the cellular and molecular mechanisms of vascular development *in vivo* remain largely unknown, because a method of addressing these questions has not been established. To overcome this problem, we have adopted fluorescence-based bio-imaging techniques using zebrafish as a model animal.

To investigate the cellular and molecular mechanisms of vascular development, we have developed the transgenic

zebrafish lines in which endothelial cells express various types of fluorescence-based biosensors. Those have enabled us to simultaneously visualize cellular structure, including cytoskeleton and cellular signaling, including activity of various signaling molecules and transcription factors. By performing live imaging of these transgenic lines, we successfully visualized cell-cycle progression of endothelial cells during vascular development, delineated the signaling pathways underlying the endothelial cell migration during angiogenesis and demonstrated a crucial role of β-catenin-mediated transcription in the development of venous vessels. We further analyzed mural cell dynamics *in vivo* by establishing the transgenic zebrafish lines that express fluorescence proteins specifically in the mural cells. In this symposium, we will introduce how fluorescence-based bio-imaging technique can be exploited for vascular biology research.

HS11-3
Perivascular cell dynamics in the vasculatures of the eye

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Embryologically, the retina is an avascular neuroepithelium. Vascular lineage cells appear at the optic nerve head at 14 weeks gestation, before differentiation, proliferation and transformation into solid vascular cords. Angiogenic tips are already ensheathed by both astrocytes (blood-retinal-barrier induction) and pericytes (vessel stability & blood flow regulation) during retinal vessel formation. Pericyte loss has been shown as the earliest indicator of diabetic vascular instability. To determine the contribution of pericytes to vessel stability during normal development and in the kitten model of retinopathy of prematurity (ROP), we established the desmin ensheathment ratio [DER - relative occurrence of desmin (pericytes) to lectin (endothelium)], and found a DER of <0.9 indicates an actively remodeling or unstable vascular bed. Further, DER is low when the retinal vasculature is responsive to the expression and withdrawal of $VEGF^{165}$. The pericyte/endothelial ratio (PER - number of pericytes/capillary length) was reduced in aging rat retinal vasculature. Astrocyte loss during proliferative ROP exemplifies astrocyte susceptibility to hypoxia-induced cell death. Where astrocytic ensheathment of blood vessels is lost & the glia limitans (formed by astrocytes & Muller cells) is breached, pathological, pre-retinal neovascularization can occur. We also showed a very low PER for the human choriocapillaris as compared to the intra-retinal capillaries. Choroidal vessels also lack astrocytic ensheathment, display leaky barrier properties and demonstrate markedly different abilities to autoregulate blood flow compared to the retina. Taken together, these studies demonstrate the importance of perivascular cell dynamics in determining functional characteristics of the various vascular beds in the mammalian eye.

HS11-4
Adaptation of the coronary microcirculation in aging: Is regeneration possible?

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In aged rats and humans, coronary blood flow reserve (CFR) is significantly decreased and may contribute to the increased risk for CVD as people age. Adipose-derived stromal vascular fraction (SVF) cells have shown an innate capacity for angiogenesis and regeneration in the periphery. We wanted to assess the therapeutic potential of SVF to improve poor CFR in aged rats and evaluate the effect on underlying ROS signaling mechanisms. SVF was isolated from fat pads of young (4 mos, ySVF) or old (24 mos, oSVF) rats through mincing, enzyme digestion, and removal of buoyant adipocytes. SVF from either age group was plated on Vicryl and cultured for 14 days before implantation on epicardial surface of old rats. Control groups included sham-control surgery (no patch) or an epicardial patch with dead cells as a control intervention group. After 6 weeks, microspheres were injected during baseline and dobutamine infusion to evaluate CFR (hyperemic BF/baseline BF). Coronary arterioles were isolated, pressurized and evaluated for endothelium-dependent and -independent responses. CFR in the apical region of the LV was increased by 35 $\pm$ 7% following treatment with a ySVF epicardial patch compared to all other groups. Flow-dependent vasodilation was significantly increased in arterioles isolated from ySVF-treated hearts compared to untreated old females, oSVF and control patch groups. Additionally, vasodilation to NO was increased in arterioles from ySVF-treated hearts. Discussion: These results suggest that this adipose-derived cell-based therapy could improve age-related declines in the reactivity of the coronary microvasculature, possibly through the perturbation of the NO-signaling pathway.

HS11-5
Angioregulatory peptide responses to physical deconditioning

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Temporal expression of positive and negative angiogenic factors in response to detraining is poorly understood. Expression of anti-angiogenic peptides (thrombospondin-1, TSP-1; and endostatin) and pro-angiogenic factors (vascular endothelial growth factor, VEGF; matrix metalloproteinases-2 and -9), was examined in response to skeletal muscle detraining in C57BL/6 mice. Mice voluntarily exercised for 21 days, and then basal and acute response to exercise were evaluated in hindlimb muscles following 1-, 7-, 14- and 28-days detraining (D1, D7, D14, D28, respectively, $n = 12$/group). In D1 mice, evidence of training was seen by greater muscle capillary-to-fiber ratio (C:F), increased maximal running time, and elevated basal expression of VEGF and MMP-9 ($p < 0.05$) compared to controls. In D7 mice, C:F levels were similar to control levels, but basal VEGF and TSP-1 were both elevated ($p < 0.05$). In D14 and D28 mice, TSP-1 expression was similar to controls, but endostatin tended to decrease compared to controls. The response of VEGF to acute exercise was blunted with training, and remained blunted in 2 of the 3 hindlimb muscles after 28 days of detraining. These data suggest that TSP-1 may be an important mediator for the regression of skeletal muscle capillaries occurring with physical deconditioning, and highlight the observation that capillary regression can occur even when VEGF is elevated is skeletal muscle. These data indicate that we must better understand the time course and responses of both positive and negative anigogenic regulators before a complete understanding of the molecular responses underpinning skeletal muscle angiogenesis will be gained.

HS12-1
RGS5 integrates angiotensin II and PPAR vascular signaling to regulate blood pressure during pregnancy

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Pregnancy induces extensive adaptation of maternal cardiovascular physiology. Despite increased cardiac output and plasma volume, blood pressure decreases due to reduced peripheral resistance. The renin-angiotensin system is crucial for these adaptations. Paradoxically, healthy pregnant women are resistant to pressor effects of infused angiotensin II (AngII), in contrast to women with preeclampsia. The mechanisms contributing to reduced peripheral resistance of pregnancy remain poorly understood. We hypothesized that AngII signaling could be regulated at a post-receptor level by Regulator of G protein signaling (RGS) molecules; these are intracellular GTPase-activating (GAP) proteins for heterotrimeric G proteins which tightly control GPCR activation including ATR1. Indeed, RGS5 is a major regulator of hemodynamic adaptation during pregnancy. In normal pregnancy, RGS5 levels increase with plasma volume expansion and antagonize AngII signaling. In contrast, loss of RGS5 in pregnant mice increases AngII sensitivity, causing vascular dysfunction and gestational hypertension. Further challenge by increasing AngII levels results in preeclampsia-like symptoms, namely, more severe hypertension, proteinuria, placental pathology and reduced birth weight. In humans, RGS5 expression in myometrial arteries is suppressed in hypertensive/preeclamptic pregnancies indicating a similar regulatory role. Importantly, ablation of a single RGS5 allele in mice is sufficient to trigger pregnancy-induced hypertension in previously asymptomatic individuals. Upregulation of RGS5 expression via peroxisome proliferator activated receptor (PPAR) activation normalizes vascular function and blood pressure in pregnant heterozygote null mice. These findings highlight a key role of RGS5 at the interface between AngII and PPAR signaling, and its modulation is a promising therapeutic strategy for pregnancy-related hypertension.

HS12-2
Inflammasome activity is essential for one kidney/deoxycorticosterone acetate/salt-induced hypertension in mice

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Inflammasomes are enzyme complexes that facilitate caspase-1-mediated processing of the pro-inflammatory cytokines interleukin(IL)-1β and IL-18. Hypertension is associated with renal inflammation, but little is known about the role of inflammasomes in this condition. We investigated whether hypertension in mice is associated with increased inflammasome expression/activation in the kidney, and if inhibition of inflammasome activity reduces blood pressure (BP), markers of renal inflammation and fibrosis. Wild-type and inflammasome-deficient ASC$^{-/-}$ mice were uninephrectomised, treated with deoxycorticosterone acetate, and given saline to drink (1K/DOCA/salt). Some wild-type mice were further treated with a novel inflammasome inhibitor, MCC950 (or vehicle), after induction of hypertension. Normotensive control mice were uninephrectomised and received placebo and water. BP was measured by tail cuff while renal expression levels of inflammasome subunits, inflammatory markers and collagen were assessed by qPCR, immunoblotting and Picrosirius red staining. 1K/DOCA/salt-induced hypertension was associated with increased renal mRNA expression (fold-change vs. control; $p < 0.05$) of inflammasome subunits NLRP3 (2.3 ± 0.2), ASC (2.8 ± 0.6) and pro-caspase-1 (2.6 ± 0.5), and the cytokine, pro-IL-1β (4.0 ± 0.8), as well as protein levels of active caspase-1 (1.6 ± 0.2) and mature IL-1β (2.1 ± 0.3). ASC$^{-/-}$ mice displayed blunted hypertensive responses to 1K/DOCA/salt (140 ± 3 vs. 155 ± 8 mmHg in wild-types; $p < 0.05$), and were protected from increases in renal expression of IL-6, IL-17A, CCL2, ICAM-1 and VCAM-1, and accumulation of collagen. Like ASC-deficiency, treatment with MCC950 reduced BP and renal inflammation in 1K/DOCA/salt-treated mice. In conclusion, renal inflammation, fibrosis and hypertension induced by 1K/DOCA/salt-treatment require inflammasome activity. These findings highlight the inflammasome/IL-1β pathway as a potential therapeutic target in hypertension.

HS12-3
Further insight into vascular Kv7 channel function

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The past decade has seen voltage-dependent potassium channels encoded by KCNQ genes (Kv7 channels) established as key regulators of arterial diameter, as well as master players in endogenous vasodilatations for a range of receptor agonists. This presentation will provide an overview of this field of research and highlights how recent studies have provided considerable insight into the factors that regulate Kv7 channel activity. In particular, the molecular architecture of the arterial channel will be described and role of certain intracellular signals in altering Kv7 activity will be elaborated. Most specifically, the involvement of G-protein beta gamma subunits, will be discussed in depth. Overall, the talk will provide both a retrospective and future perspective of this dynamic area of vascular biology.

HS12-4
Serelaxin reduces endothelium-derived vasoconstrictor prostanoids in mesenteric arteries of spontaneously hypertensive rats

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Introduction: It is established that endothelial dysfunction in spontaneously hypertensive rats (SHRs) involves upregulation of cyclooxygenase (COX)-derived vasoconstrictor prostanoids. Recently, we demonstrated that serelaxin treatment increases vasodilator prostanoid production and augments endothelial function in healthy rats.
Aim: To investigate the effect of serelaxin on COX-derived prostanoids in mesenteric arteries of SHR.
Methods: Male Wistar Kyoto rats (WKY) and SHRs were subcutaneously infused with either placebo (20 mM sodium acetate) or serelaxin (13.3 ug/kg/h) using osomotic

minipumps for 3 days. Endothelial (acetylcholine, ACh) and vascular smooth muscle (sodium nitroprusside, SNP) function was assessed via wire myography. The COX inhibitor indomethacin (1 uM) was used to investigate the mechanisms of serelaxin action on prostanoid pathways.

Results: In SHRthe sensitivity to ACh was significantly reduced but there wasno effect on SNP-evoked relaxation, indicating endothelial dysfunction. Treatment with serelaxin reversed endothelial dysfunction. To directly assess the constrictor effect of COX-derived prostanoids, ACh-evoked contraction was determined after blockade of NOS and EDH. In resting mesenteric arteries, ACh-induced contraction was significantly increased in SHRs compared with WKYs. Serelaxin treatment of SHRs significantly reduced ACh-induced contraction. The contractile response to ACh was abolished by indomethacin, thromboxane receptor (TP) antagonist or endothelial denudation. In SHRs there is upregulation of endothelium-derived prostanoids which target the TP to produce contraction, and this pathway is inhibited by serelaxin treatment.

Conclusion: Serelaxin treatment restores endothelial vasodilator function in SHRs and attenuates endothelium-dependent contraction. This, in part, involves a reduction in the production of COX-derived vasoconstrictor prostanoids and/or their associated signalling pathways.

HS12-5

Fundamental role for the KCNE4 ancillary subunit in Kv7.4 regulation of arterial tone

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Kv7 channels are important determinants of vascular reactivity, however little is known about the regulation of these channels in smooth muscle. The KCNE ancillary subunits (KCNE1-5) are known to dramatically alter Kv7 channel expression and function; however they are yet to be studied in the vasculature. The aim of this study was to investigate the expression of the KCNE subunits in different rodent blood vessels and determine whether they had a functional impact on Kv7 channels in the vasculature.QPCR analysis of different rat arteries found that the KCNE4 isoform predominated and proximity ligation experiments showed KCNE4 co-localised with Kv7.4 and Kv7.5 in mesenteric artery myocytes. In HEK cells expressing Kv7.4, co-expression of KCNE4 increased membrane expression of Kv7.4 and dramatically increased and altered Kv7.4 current properties. Knock down of KCNE4 in rat mesenteric arteries made the vessels more sensitive to methoxamine, which coincided with the effect of the Kv7 blocker, linopirdine, being attenuated in these vessels. Current clamp experiments identified a more depolarised membrane potential in myocytes with KCNE4 knocked down and impaired responses to the Kv7 activator S-1. When KCNE4 expression was reduced, less Kv7.4 expression was found in the membrane of the mesenteric artery myocytes. These data show that KCNE4 is consistently expressed in a variety of arteries, and knockdown of the expression product leads to reduced Kv7.4 membrane abundance, a depolarised membrane potential and an augmented response to vasoconstrictors.

HS12-6

Natriuretic peptides in the treatment of pulmonary hypertension: PDE2 inhibition augments their therapeutic capacity

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Treatment of pulmonary hypertension (PH) remains sub-optimal, largely due to lack of selective pulmonary vasodilators, and inadequate reversal of right ventricular hypertrophy (RVH). Phosphodiesterase (PDE) 5 inhibition is a first-line treatment for PH. It prevents the breakdown of cGMP, a key second messenger in pulmonary vasodilation. We identified a pulmonary-specific, synergistic relationship between PDE5 inhibition and the cGMP-elevating natriuretic peptides (NPs). PDE2 has not been investigated in PH, yet it is an ideal therapeutic candidate. PDE2 hydrolyses both cGMP and cAMP (enhanced by prostacyclin therapy in PH) and, importantly, is stimulated by cGMP. We hypothesised that by enhancing NPs and inhibiting PDE2 the pathogenesis of PH would be mitigated. In mouse models of PH, prophylactic treatment with either the neutral endopeptidase inhibitor, ecadotril (60 mg/kg/day; increases NP bioavailability), the PDE2 inhibitor, BAY 60-7550 (BAY, 10 mg/kg/day), or both, lowered right ventricular systolic pressure (RVSP) to values comparable with controls. In established PH, neither ecadotril, nor BAY was sufficient to reverse PH. However, when combined, ecadotril/BAY attenuated both RVSP and RVH. Relaxation in response to atrial NP (ANP) in isolated pulmonary arteries of chronically hypoxic rats, was enhanced after BAY incubation (EC_{50}: -8.4 ± 0.17 vs. $-8.7 \pm 0.16, p < 0.05$). In cultured pulmonary artery smooth muscle cells, proliferation (as % control) was decreased in response to ANP ($79.9 \pm 6.3\%$, $p < 0.05$) and BAY ($81.7 \pm 3.6\%$, $p < 0.05$); this was augmented by combined

ANP/BAY (56.3 ± 4.2%, $p < 0.001$). Dual treatment with BAY/prostacyclin analogue showed similar additive effects. In conclusion, enhancing NP bioavailability, in addition to PDE2 inhibition is a viable treatment strategy for PH.

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YOUNG INVESTIGATORS SYMPOSIUM

YIS-1

VEGF-A_{165}b ameliorates vascular dysfunction in diabetic retinopathy

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Aim: Diabetic retinopathy (DR) is associated with upregulated vascular endothelial growth factor (VEGF), particularly pro-angiogenic VEGF-A_{165}a. This is responsible for pathological neovascularisation and tight-junction (TJ) breakdown, contributing to DR and diabetic macular oedema (DME). Anti-angiogenic isoforms, such as VEGF-A_{165}b, are down-regulated in diabetes, potentially contributing to vascular dysfunction. This study assesses whether VEGF-A_{165}b can abrogate blood-retina-barrier and outer-retinal barrier (ORB) breakdown and prevent subsequent leakage.

Methods: Retinal pigment epithelial (1°RPE) cells were treated with 2.5 nM VEGF-A_{165}a, low glucose (5 mM) and high glucose (25 mM) media ± 2.5 nM VEGF-A_{165}b and were assayed for TJ integrity through occludin and ZO1 expression and trans-epithelial electrode resistance (TEER). *In vivo* retinal dysfunction was measured in streptozotocin-induced (STZ, 50 mg/kg) diabetic rats after 1 and 8 weeks of diabetes ±VEGF-A_{165}b (10 ng/μL intravitreally and biweekly 20 ng/g i.p respectively). Evans' blue extravasation and IB4 immunofluorescence assessed vascular leakage and vascular remodelling respectively.

Results: Occludin expression decreased and paracellular flux increased in 1°RPE cultured in 25 mM glucose and partially restored with VEGF-A_{165}b treatment. VEGF-A_{165}b restored VEGF-A_{165}a-induced reduction in occludin and ZO1 expression and increased paracellular flux. VEGF-A_{165}b reduced Evans' blue extravasation at both 1 ($p < 0.05$) and 8 ($p < 0.001$) weeks post STZ-injection relative to vehicle-treated diabetic rats. VEGF-A_{165}b also reduced vascular density ($p < 0.001$), and upregulated VE-cadherin and occludin expression relative to vehicle-treated diabetic rats.

Conclusion: VEGF-A_{165}b restores paracellular resistance *in vitro* and restores vascular density and solute flux *in vivo*, demonstrating that VEGF-A_{165}b prevents ORB breakdown and may be a therapeutic target in treating DR and DME.

YIS-2

Exercise training ameliorates microvascular deterioration and VEGF signaling downregulation in aging rat brain

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During advancing age, reduction of basal blood flow and microvascular loss in the brain contributes tissue perfusion insufficiency. Capillary loss in aged tissues appears to be related to downregulation of vascular endothelial growth factor (VEGF) signaling. Regular exercise has been reported to have beneficial effects to brain health in aging individuals. Therefore, the present study aimed to investigate effect of exercise training on age-induced cerebromicrovascular alteration with modulation of VEGF signaling. Male Wistar rats were divided into 3 groups; sedentary-young (4 months), sedentary-aged (22 months) and exercised-aged (22 months). Exercise program included swimming training 5 days/week for 8 weeks. *In situ* study of brain microvascular networks was performed to determine regional blood flow (BF) (by Doppler flowmetry) and microvascular vascularity (MV) (using a laser scanning confocal fluorescent microscopy). Level of VEGF, VEGFR2, Akt and PI3K in isolated brain microvessels were determined by immunoassay. MV and BF was significantly lower in the sedentary-aged rats compared with the sedentary-young rats, whereas that in the exercised-aged rat was significantly higher than the sedentary-aged rats. The protein level of VEGF and VEGFR2 were significantly lower in the sedentary-aged rats compared with the sedentary-young rats, whereas those in the exercised-aged rats were significantly higher than those in the sedentary-aged rats. The expression of phosphorylated Akt and PI3K corresponded to the alterations in the VEGF and VEGFR2 levels. These findings suggest that exercise training ameliorates cerebromicrovascular deterioration and VEGF signaling downregulation during aging.

YIS-3

Vascular effects on astrocytes Ca2+ dynamics in cerebral cortex

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Recent *in vivo* evidence conducted in anesthetized or slightly sedated animals questions the direct role of astrocyte intracellular Ca2+ in mediating functional hyperemia as spatial and temporal profile of astrocyte Ca2+ transients are poorly associated with the onset of vascular responses. Our

objective was to uncover the spatiotemporal basis of the communication between astrocytes and the vasculature in fully awake, behaving mice. A craniotomy over the barrel cortex with the dura removed was performed. A custom build two-photon microscopy was used to image the vasculature and astrocytes Ca2+ from either C56Bl/6 mice or GLAST-cre-LSL-GCaMP3 mice or TEK-cre-LSL-ArchT3 mice. We found that 5-second whiskers' stimulation induced fast vasodilatory responses in penetrating arterioles while generated a delayed onset of endfoot and cell-wide Ca2+ transients. Interestingly, the onset of these Ca2+ transients was typically observed at the peak of the sensory induced vasodilation and toward the end of the vibrissae stimulation. Thus, we tested if the vasculature was communicating back to the astrocytes and consequently modulating astrocytes Ca2+ . We showed that manipulation of the vasculature via pharmacology or using optogenetic tools induced vascular changes that were followed by an alteration in astrocyte Ca2+ signals. Our data redefine the uni-directional communication between astrocytes and the vasculature. We introduce a potential role of the vasculature as the modulator of astrocytes Ca2+ transients and propose that astrocytes act as responders to changes in blood flow.

YIS-4

Large-area surface-enhanced raman spectroscopy imaging as a novel method to visualize alterations in small molecular metabolites in ischemic brain tissues

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While imaging mass spectroscopy is a powerful method to visualize metabolites, it requires matrix deposition to ionize target molecules. On the other hand, surface-enhanced Raman scattering (SERS) can provide information on the structure of the molecules without labeling and ionization, serving as a finger-print method. Here, we report a novel large-area SERS devise, named "*gold nano-coral*" (GNC), which is capable of detecting a region of energy failure of the brain tissue. Until now, application of SERS for tissue-imaging has been limited because of technical constraints to fabricate a SERS substrate ensuring hot-spot formation uniformly over a large area with ease. To overcome this hurdle, we utilized the boehmite nanostructure that is easily achieved by immersing the aluminum film in boiling water. Sharp geometry of boehmite served as an efficient template for the gold deposition ensuring strong enhancement of SERS signals. This simple, reproducible and rapid method makes it possible to produce a SERS substrate with a size of a square-centimeter order, the dimension necessary to accommodate most tissue samples. GNC substrate enabled the large-area SERS imaging to visualize an ischemic core of mouse brain tissue without labeling for the first time. Furthermore, we attempted to detect carbon monoxide (CO) that is known to occur abundantly in normal brain and to decrease in the ischemic core as a regulator of neurovascular units. The experiments *in vitro* showed that CO generates the notable Raman peak at 2150 cm^{-1}, suggesting that GNC-enhanced SERS microscopy serves a potentially powerful method to visualize the gas.

YIS-5

Dynamics of angiogenesis and blood flow in mouse long bone

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Angiogenesis and osteogenesis are coupled coherent processes occurring during the development of skeletal system. Distinct capillary subtypes have been identified to play an important role in coupling of angiogenesis and osteogenesis. These blood vessels show presence of specialized structures like loops and bulges, but their involvement in mediating non-sprouting angiogenesis is not known. Here, using a novel intra vital imaging technique, we describe the mechanism of blood vessel growth in the mice long bones. The arrangement of blood vessels describes a peculiar blood flow pattern, which attributes the heterogenic phenotypes in capillaries. Blood flow regulates formation of specialized structures in the vascular front through Notch signalling to couple angiogenesis with osteogenesis during early development. Therefore in the aged mice when the blood flow is severely down, activating Notch signalling in endothelial cells could promote formation of these angiogenic structures in blood vessels to promote neo-osteogenesis and thus restore bone mass.

YIS-6

Clonidine restores pressor responsiveness to phenylephrine and angiotensin II in ovine sepsis

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Objectives: In sepsis, prolonged, sympathetic overstimulation may lead to vasopressor-refractory hypotension. We therefore examined the effects of the α_2-adrenergic agonist clonidine on mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA) and pressor responsiveness to phenylephrine (PE) and angiotensin II (Ang II) during hypotensive sepsis in conscious sheep.

Methods: Sepsis was induced by intravenous infusion of *Escherichia coli* (2.8×10^9 bolus + 1.26×10^9 colony forming units i.v) for 32 h. Pressor responses to increasing doses of PE and Ang II were measured at baseline, and at 24, 28 and 32 hour of sepsis. Sheep were treated with clonidine (1 μg/kg/h, $n = 6$) or saline-vehicle ($n = 6$) from 24 to 32 hour of sepsis.

Measurements and Main Results: Sepsis was characterized by hypotension (~12 mmHg), increased heart rate (HR) (~80 bpm), increased RSNA (~70%) and blunted pressor responses to PE and Ang II. In vehicle-treated sheep, MAP progressively declined from 25 to 32 h of sepsis (73 ± 3–66 ± 3 mmHg, $p = 0.013$), while the elevations in HR and RSNA and reduced pressor responsiveness to vasopressors persisted. Clonidine-treatment prevented the further decline in MAP, substantially reduced HR and RSNA and restored pressor responsiveness to both PE and Ang II towards pre-septic levels.

Conclusions: Administration of clonidine during hypotensive sepsis reduced RSNA, restored vascular sensitivity to both PE and Ang II and resulted in better preservation of arterial pressure. Considering these findings, a clinical trial for the use of clonidine in the treatment of persistent vasopressor-refractory hypotension in patients with septic shock would be worthwhile.

YIS-7

Complex signalling pathways determine the role of Kv7 channels in relaxations of the rat mesenteric artery

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The Kv7 family of voltage gated potassium channels have been established as important contributors to vasorelaxations in various vascular beds. In particular, Kv7 channels have been shown to contribute to Gs-coupled, cyclic AMP dependent vasorelaxation, therefore this study delved further into the mechanisms involved in this process. In isometric tension studies, relaxations in rat mesenteric arteries to the beta adrenoceptor agonist isoproterenol produced relaxations that were sensitive to the pan-Kv7 channel blocker linopirdine, but not the Kv7.1 selective blocker HMR1556. Further studies showed that isoproterenol mediated relaxations were attenuated in the presence of H89 (protein kinase A inhibitor) and ESI-09 (exchange protein activated by cAMP (EPAC) inhibitor) but not by gallein (beta gamma subunit inhibitor). Relaxations to the cell permeable EPAC-selective activator 8-pCPT-2-O-Me-cAMP-AM were also attenuated in the presence of linopirdine but not by gallein. EPAC mediated relaxations were also attenuated in the presence of paxilline (BKCa channel inhibitor), an effect which was additive to that of linopirdine. Furthermore, both 8-Br-cAMP and 8-pCPT-2-O-Me-cAMP-AM enhanced currents from overexpressed Kv7.4 channels, the isoform which is particularly crucial for vascular control. Whilst gallein attenuated 8-Br-cAMP enhancement of these currents, it had no effect on 8-pCPT-2-O-Me-cAMP-AM mediated increases in Kv7.4 current. These findings demonstrate the complex signalling pathways which couple to Kv7 channels in the rat mesenteric artery.

POSTERS
ANGIOGENESIS/LYMPHANGIOGENESIS/ MICROVASCULAR REMODELING/INJURY & REPAIR

P1
Tetrahydrocurcumin induced tumor vascular normalization via inhibition of vascular endothelial growth factor expression in cervical cancer xenografts in nude mice

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In solid tumors, antiangiogenic, specifically anti-VEGF, treatments can "normalize" their vasculature, reducing hypoxia and creating a window of opportunity for concurrent chemotherapy; thus, we investigated the effect tetrahydrocurcumin (THC), a major metabolite of curcumin, on tumor vascular normalization in cervical cancer xenografts in nude mice. Female BALB/c nude mice were used and divided into control and cervical cancer cells (CaSki)-implanted groups (CaSki group). Cervical cancer cells were subcutaneously injected in nude mice to establish subcutaneous tumors. One month after the injection, mice were orally administered vehicle or 100, 300, and 500 mg/kg of THC daily for 30 consecutive days. One month after the administrations, tumor microvasculature was observed under confocal microscope. The microvascular density (MVD) was evaluated using the CD31 expression. Vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1alpha (HIF-1alpha) expression were also detected by immunohistochemistry. Confocal fluorescent microscopy revealed that the implanted CaSki cells increased MVD and changed the microvascular network, including the appearance of dilatation, tortuosity and hyper-permeability. All doses of THC treatment decreased MVD and all pathological features. This indicates that THC treatment leads to a process of vascular normalization. The increment of MVD in CaSki group was significantly decreased by THC treatment. The CaSki group also showed a significantly increased VEGF, and HIF-1alpha expression, which were down-regulated after THC administration at all doses. Conclusively, THC exhibits tumor vascular normalization activity in CaSki-implanted nude mice model. This effect is likely to be mediated by the down-regulation of HIF-1alpha and VEGF expression.

P2
VEGF knockdown in muscle improves recovery of blood flow after ischaemia

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Lower limb ischaemia is a common morbidity factor associated with some chronic diseases, such as diabetes. In most of these conditions, Vascular Endothelial Growth Factor (VEGF) signaling is altered relative to physiological baseline, which causes severe impairments in blood flow. We used a transgenic mouse approach to test the hypothesis that lack of VEGF-A hinders arteriolargenesis (the growth of new arterioles). We have produced transgenic mice (termed ACLL) where VEGF-A is specifically knocked out in skeletal muscle cells. ACLL mice were given doxycycline (+dox) or sucrose-only (−dox) in the drinking water for 10 weeks. They were then subjected to unilateral hindlimb ischaemia and speckle imaging was used to monitor blood flow in the hindpaws. VEGF tissue specific inducible knockout showed a significantly improved ischaemic/contralateral blood flow ratio when compared to the control cohort at both early (3–7 days) and late (days 14–21 days, $p < 0.01$ for all days, repeated measures test). At the end of the experiment, the ischemic adductor muscle was sectioned and stained for endothelial cells (IB4) and alpha-smooth muscle actin. There was no difference in capillary density between the two groups; however, arteriole density was significantly increased in VEGF knockout mice. This data suggests that VEGF of non-endothelial origin hinders arteriolargenesis and, hence, functional control of blood flow.

P3
Cyanidin attenuates tumor chemotherapy-induced neurotoxicity via inhibition of ROS-mediated DNA damage and apoptosis in PC12 cells

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Objectives: Cisplatin-based chemotherapy in clinic is severly limited by its adverse effect, including neurotoxicity. Oxidative damage contributes to cisplatin-induced neurotoxicity, but the mechanism remains unclearly. Cyanidin a natural flavonoid compound exhibits powerful antioxidant activity. Hence, we investigated the protective effects of cyanidin on

PC-12 cells against cisplatin-induced neurotoxicity and explored the underlying mechanisms.
Methods: MTT assay, flow cytometry analysis, TUNEL-DAPI co-staining, caspase activity, detection of ROS accumulation and western blotting assay were all employed to detect the protective mechanism.
Results: The results showed that cisplatin-induced cytotoxicity was completely reversed by cyanidin through inhibition of PC-12 cell apoptosis, as proved by the attenuation of Sub-G1 peak, PARP cleavage and caspases-3 activation. Mechanistically, cyanidin significantly inhibited reactive oxygen species (ROS)-induced DNA damage in cisplatin-treated PC-12 cells.
Conclusions: Our findings revealed that cyanidin as an apoptotic inhibitor effectively blocked cisplatin-induced neurotoxicity through inhibition of ROS-mediated DNA damage and apoptosis, predicating its therapeutic potential in prevention of chemotherapy-induced neurotoxicity.

P4
Minimally invasive surgery joint local cooling lavage protects rats brain from ICH-induced inflammation injury and apoptosis

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Objectives: Hypothermia treatment is one of the neuroprotective strategies that improve neurological outcomes effectively after brain damage. Minimally invasive surgery (MIS) has been a more and more important treatment of intracerebral hemorrhage (ICH). Herein we evaluated the neuroprotective effect and mechanism of MIS joint local cooling lavage (LCL) treatment on intracerebral hemorrhage (ICH) via detecting the inflammatory responses, oxidative injury and neuronal apoptosis around the hematoma cavity in rats.
Methods: ICH model was established by type IV collagenase caudatum infusion. The rats were treated with MIS 6 h after injection then were lavaged by normothermic (37 centigrade) and hypothermic (33 centigrade) normal saline (NS) in brain separately.
Results: The results showed that the MIS joint LCL treatment significantly suppressed IHC-induced inflammation injury and apoptosis in Rats, as convinced by the decline of active-caspase-3 and TUNEL-positive cells, followed by the decrese of IL-1beta and LDH and increase of IL-10 and SOD.
Conclusions: This study demonstrated that the strategy of using MIS joint LCL may achieve enhanced neuroprotection against ICH-induced inflammation injury and apoptosis in Rats with potential clinic application.

P5
Microsomal prostaglandin E synthase-1 up-regulates COX-2 derived PGE2 in endothelial cell under hypoxia condition in mouse ischemic hind limb model

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Angiogenesis is a process involved in several physiological events including embryonic development, female reproductive cycle placentation and wound repair. It was reported that Cyclooxygenase 2 (COX-2) derived Prostaglandin E2 (PGE2) induce angiogenesis, especially in tumor angiogenesis. But the precise of the mechanism of COX-2 derived PGE2 on angiogenesis recovery from ischemic condition is not well understood. We evaluated this phenomenon by using model of acute hind limb ischemia of microsomal prostaglandin E synthase-1 deficient mice (mPGES-1KO) and wild type mice (WT).In order to confirm whether COX-2 involved in ischemic recovery, aspirin and (JTE522) were administrated after surgical treatment.Compared to control mice, mice treated with Aspirin treated and selective COX-2 inhibitor (JTE522) treated mice were significantly suppressed blood flow recovery ($p < 0.05$). Furthermore, mPGES-1KO significantly suppressed blood flow recovery compared to WT ($p < 0.05$). Immunohistochemical analysis showed that CD31 and COX-2 positive cells in ischemic muscle were diminished in aspirin treated mice, JTE522 treated mice and mPGES-1KO. In contrast, there was no difference in not-ischemic muscle. Expression of COX-2 and PGE2 secretion on cultured human umbilical vein endothelial cells and was enhanced by hypoxia condition (1% O^2) compared to normoxia condition (20% O^2) and that was suppressed by COX-2 inhibitor (NS398) treatment.These results suggested that mPGES-1 up-regulates COX-2 derived PGE2 in endothelial cell under hypoxic condition and that induced recovery from ischemia.

P6

The role of limbal lymphatics in corneal fluid homeostasis and proper pathogen clearance

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Cornea maintains its clarity despite of constant exposure to external pathogens. Once pathogens invade corneal stroma, rapid clearance of intrastromal pathogens is important for inflammation resolution, avoiding vision-threatening stromal edema and irreversible scar formation. Here, we demonstrated that corneal lymphatic vessels are essential for eliminating pathogens from cornea and maintaining properly hydrated status of cornea. We discovered that corneal lymphangiogenesis did not occur during development and even under inflammation in angiopoietin-2 (Ang2)-deficient mice, which are already reported to have systemic lymphatic defects. In these mice, transport of intrastromal pathogens to draining lymph nodes (DLNs) and maintenance of corneal fluid homeostasis were impaired. Subsequently, chronic corneal inflammation with severe fibrosis and thickening occurred in aged Ang2-deficient mice. These findings led us to develop a novel inducible corneal lymphatic vessel ablation model. We generated LYVE-1-Cre/iDTR double transgenic mice and were able to selectively ablate corneal lymphatic vessels in diphtheria toxin-inducible manner. In agreement with Ang2-deficient mice, corneal lymphatic vessel-ablated mice presented impaired intrastromal pathogen clearance resulting in severe keratitis. Intriguingly, acute loss of corneal lymphatic vessels caused corneal stromal edema even though the intraocular pressure was not elevated. These observations suggest that corneal lymphatic vessels are essential for maintaining dehydrated clear cornea and proper pathogen clearance through the absorption of stromal fluid and transport of intrastromal pathogens to DLNs.

P7

Role of TP signaling in enhancement of lymphangiogenesis in diaphragms during endotoxin induced petitonitis in mice

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It is said that lymphangiogenesis occurs in tissues duing wound healing, and tumor metastasis. Recent studies suggest that lymphangiogenesis is also found during development of inflammation, and up-regulated by inducible COX-2. The objective of the present study was to estimate lymphatic vessels in a diaphragm which are essential for draining peritoneal fluid, and to evaluate the role of thromboxane A2-TP signaling during enhancement of the lymphangiogenesis in diaphragms of peritonitis mice. Male C57/BL6 mice (8–10 weeks old) were used. Peritonitis was induced by injections of lipopolysaccharide (LPS, Sigma, E coli, 0111-B4, 20mcg/mouse/2 days) into peritoneal cavities of mice from 1 day to 14 days. As a parameter of lymphangiogesesis, we evaluated lymphatic microvessels using whole-mounted diaphragm tissues. Lymphatics were stained with antibodies against Lyve-1. These lymphatic vessels were increased time-dependently. A week after LPS applications, gene expressions of COX-2 and VEGF-C/D in the diaphragm were up-regulated with the increased lymphangiogenesis in the diaphragm. In addition, thromboxane synthase (TXS) was up-regulated in the diaphragm of peritonitis mice, and lymphangiogenesis was suppressed in the diaphragm in TP knock out mice. *In vitro*, TP agonist (U-46619) treatment increased VEGF-C/D expression in macrophages and T-cells isolated from WT mice in a dose-dependent manner.Our results revealed that lymphangiogenesis is up-regulated by TXA2-TP signaling possibly via induction of VEGF-C/D. These results suggest that TP signaling may become a therapeutic target in controlling lymphangiogenesis.

P8

Increased mouse mesenchymal stem cells homing and neovascularization in LPS-induced inflammation in aged rats after exercise training

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In aging, the increased oxidative stress in the physiological environment causes a reduction in number and function of stem cells necessary for neovascularization. Besides that, the decreased levels of stromal cell-derived factor-1 (SDF-1) and vascular endothelial growth factor (VEGF), theessential molecules for neovascularization process, are significantly reported in aging. Recently, studies have shown about antioxidant effect of exercise training that is able to increase neovascularization in the aged. Therefore, we studied the effect of exercise training whether it can enhance the efficacy of therapeutic mesenchymal stem cells (MSCs) transplantation in lipopolysaccharide (LPS)-induced inflammation in

aged rat model or not.Male Wistar rats (20–22 months old) were divided into four groups: Aged + LPS, Aged + LPS+ Exercise, Aged + LPS+ MSC, and Aged + LPS + Exercise + MSC. Exercise training program included swimming training for 5 days/week for 8 weeks. LPS was injected subcutaneously with the concentration of 1 mg/1 mL in normal saline. After that, the rats were individually kept in cages for 7 days until the day of the experiment. Then the capillary vascularity (%CV), skin blood perfusion (BF), number of MSC homing, SDF-1 and VEGF expressions were determined. The results showed that exercise training was able to increase BF, %CV, and MSC homing in LPS-induced inflammation area. Moreover, the findings were also demonstrated thatthe efficacy of therapeutic MSC transplantation associated with increase in SDF-1 and VEGF expression in the model of LPS-induced inflammation in aged rats. This knowledge will be beneficial in future cell therapy applications.

P9
Mechanistic effects of spinal cord injury on splanchnic vascular functions and roles of regenerative medicine

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The aim of this study was to determine the effects of spinal cord section on blood flow and sympathetic tone in order to understand the influence of spinal cord injury on regional vascular functions. Carotid, renal, celiac, hindquarter and mesenteric flows in conscious rats were observed using an implanted electromagnetic flowmeter under anesthesia with thiamylal sodium. Arterial pressure (AP) was measured in the terminal aorta. The spinal cord was transected at thoracic 1 (Th1) under ether anesthesia. One hour later, when the rat had recovered consciousness and arterial pressure had partially recovered, hexamethomium (C6) was intravenously injected for ganglionic blockade. In a neuraxis-intact state, sympathetic tone (AP/regional flow) was decreased significantly by ganglionic blockade in the carotid, renal, celiac and hindquarter beds but not in the mesenteric bed. Celiac and hindquarter tones in spinal control Wistar rats and spinal spontaneously hypertensive rats were not decreased significantly by C6, while carotid, renal, and mesenteric tones were significantly decreased. Total flow rate in those vascular beds decreased markedly after spinal transection. The results suggest that (1) sympathetic tones in the celiac and hindquarter beds are supraspinal in origin, but those in the carotid, renal and mesenteric beds are in spinal origin, (2) mesenteric tone in control and spontaneously hypertensive rats is suppressed reflexively in a neuraxis-intact state, and (3) hepatic collateral circulation is damaged by the decrease of venous return and central venous pressure due to spinal transection. Application of human iPS cells to spinal cord injury is essential for medical treatment.

P10
The role of interleukin-7 in lymphatic vessel function and its therapeutic potential for the treatment of lymphedema and chronic skin inflammation

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Interleukin-7 (IL-7) is a cytokine known for its role in T and B cell homeostasis and lymph node organogenesis. Our lab has recently identified a novel role for IL-7 in lymphatic vessel biology. Lymphatic endothelial cells were shown to express IL-7 and its receptor chains. IL-7 was able to activate human lymphatic endothelial cells *in vitro* and to induce lymphangiogenesis *in vivo*. Furthermore, the lymphatic network and function were greatly affected in IL-7Ralpha-/- and IL-7 transgenic mice. Functional drainage assays revealed that genetic overexpression of IL-7 or exogenous application of recombinant IL-7/anti-IL-7 complexes to WT mice markedly improved the drainage of a lymphatic specific dye from dermal lymphatics. Moreover, the use of bone marrow chimeras indicated that the drainage enhancing effect of IL-7 was dependent on IL-7 receptor expression in stromal cells rather than hematopoietic cells. Intravital imaging experiments revealed that the pumping rate of collecting lymphatics remained unchanged in IL-7Ralpha-/- mice compared to WT mice, whereas increased leakage of dye from lymphatic capillaries was observed. We are currently investigating the mechanism by which IL-7 enhances drainage by performing intravital contractility studies and *ex vivo* analyses of the lymphatic capillaries and collectors in IL-7Ralpha-/- mice. Furthermore, we are exploring whether the drainage enhancing effect of IL-7 can be used to treat diseases characterized by lymphatic drainage dysfunction, such as lymphedema and chronic skin inflammation. To this end we have created a murine IL-7-Fc fusion protein to extend the half-life of IL-7 in mice, making it suitable for therapy experiments.

P11
Glycation of vitronectin inhibits VEGF-induced angiogenesis by uncoupling VEGF receptor-2-alphav beta3 integrin cross-talk

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Glycation of vessel wall proteins is thought to play an important role in the pathogenesis of vascular complications in diabetes mellitus. However, no previous study has implicated glycated vitronectin (VN) in the control of vascular endothelial growth factor (VEGF) signaling. To explore whether the glycation of VN affects angiogenic signaling and to understand the molecular mechanisms involved, we synthesized glycated VN by incubating VN with methylglyoxal (MGO) *in vitro* and identified the formation of glycated VN by an LC-ESI-MS/MS based method. We tested the hypothesis that glycation of VN down-regulates VEGF receptor-2 (VEGFR-2) activation by uncoupling the interaction between VEGFR-2 and alphav beta3. Unmodified and MGO-glycated VN were used as substrates for human umbilical vein cells (HUVECs). The effects of glycated VN on VEGF signaling in HUVECs were investigated. The glycation of VN inhibited VEGF-induced phosphorylation of VEGFR-2 and the intracellular signaling pathway downstream of VEGFR-2. Glycated VN inhibited the binding of VEGFR-2 to beta3 integrin and inhibited the phosphorylation of beta3 integrin. Furthermore, glycation of VN significantly decreased VEGF-induced migration of HUVECs *in vitro* and vessel outgrowth in an *ex vivo* angiogenesis model. Collectively, these data indicate that the glycation of VN inhibits VEGF-induced VEGFR-2 activation by uncoupling VEGFR-2-alphav beta3 integrin cross-talk. The glycation of VN causes a reduction in the migration of endothelial cells and vessel outgrowth. This may provide a mechanism for the failure of collateral sprouting in diabetic microangiopathy.

P12
Pre-treatment effects of low-dose simvastatin on wound healing in diabetic mice

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Non-healing diabetic ulcer with high risk of amputation is the major problem for diabetic patients. Previous studies have shown that simvastatin at therapeutic dose can increase angiogenesis, and improve wound healing in diabetic and non-diabetic patients. This study aims to evaluate whether the pre-treatment of low-dose simvastatin supplementation can improve wound healing associated with angiogenesis in a diabetic mouse model. Balb/c nude mice were divided into three groups including control (CON), diabetic (DM, streptozotocin (STZ) 45 mg/kg i.p. daily for 5 days), and diabetic pre-treated with low-dose simvastatin (DM+SIM) groups. Seven days before wounding, DM+SIM were started to have a single dose of simvastatin administration (oral, 0.25 mg/kg/day). Ten weeks after the diabetic induction, all mice were created bilateral full-thickness excisional skin wounds on the back (0.6×0.6 cm^2). On day 14 post-wound, the percentage of wound closure (%WC), the percentage of capillary vascularity (%CV), and the neutrophil infiltrations were determined by using Image Pro-Plus, confocal fluorescence microscopy and H&E staining, respectively. Tissue vascular endothelial growth factor (VEGF) was detected by ELISA at day 7 and 14 post-wound. On day 14, the %WC and %CV in CON and DM+SIM were significantly increased than DM. The number of neutrophil infiltration in CON and DM+SIM were significantly decreased than DM. The VEGF levels in CON and DM+SIM were significantly higher than DM on day 7 without difference on day 14. In conclusion, the present study demonstrated that the pre-treatment of low-dose simvastatin could increase angiogenesis, reduced inflammation, and improve wound healing in diabetic mice.

P13
Host vascular invasion within newly induced glomeruli in engineered renal tisseus

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In renal development, glomerulus vascular development mechanism was considered to originate from circulation endothelial precursors. On the other hand, networks of endothelial cells formed new vessels within engineered tissues *in vivo*. Usually, role of vessels is oxygen and nutrition supply, but the glomerulus vascular role is wastes filtration from circulating blood. In this study, we examined the mechanism of glomerulus vascular induction by tissue engineering technology. Here, we tried to fabricate renal tissue at the femoral arteriovenous and examined the origin of newly induced vessel and glomerulus vessel within transplanted tissues. Firstly, the condition of glomerulus induction within engineered renal tissue without endothelial cells was determined. Spheroid formation gave induction of ureteric bud cells self-organization and newly vascularization after transplantation. Two weeks after transplantation, we obtained clearly glomeruli vascular originated from host

within transplanted tissues. Next, we will try to examine the effects of endothelial cell-networks within engineered renal tissues for glomerulus induction.Acknowledgments This study was supported by Creation of innovation centers for advanced interdisciplinary research areas Program in the Project for Developing Innovation Systems "Cell Sheet Tissue Engineering Center (CSTEC)" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.Conflicts of interestShimizu Tatsuya is a stakeholder of CellSeed Inc. Tokyo Women's Medical University is receiving research funding from CellSeed Inc. Teruo Okano is a founder and a member of the board of CellSeed Inc., which has licenses for certain cell sheet-related technologies and patents from Tokyo Women's Medical University.

P14

RhoJ is an effective and selective target of antiangiogenic cancer therapy

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Current anticancer therapy targeting angiogenesis is limited by its cytostatic nature as well as systemic side effects. To overcome these limitations, we have uncovered the role of RhoJ, a Rho GTPase in endothelial cells, during tumor progression. Since we observed that, compared with in normal vasculature, RhoJ was highly expressed in vasculature of various tumor models, such as implanted Lewis lung carcinoma and B16F10 melanoma cells and a spontaneous breast cancer model, MMTV-PyMT, we assumed that targeting RhoJ could be a novel therapeutic target. Conventional or conditional deletion of RhoJ presented dual effects on tumor vessels both by inhibiting new vessel formation and by disrupting the integrity of tumor vessels. *In vitro* study using siRNA showed that the RhoA-ROCK (Rho kinase) signaling pathway in human umbilical vein endothelial cells was observed, which consequently resulted in a functional failure of endothelial cells, suggesting the mechanism of RhoJ targeting. Moreover, synergistic effects were observed when RhoJ inhibitor was employed in concert with a cytotoxic chemotherapeutic agent (Cisplatin), angiogenesis-inhibiting agent (VEGF-trap), or vascular-disrupting agent (Combretastatin-A4-Phosphate). Conclusively, these results suggest RhoJ blockade as a selective and effective therapeutic strategy for targeting tumor angiogenesis with minimal side effects.

P15

Neocollateral formation supplements collateral remodeling after acute arteriolar occlusion in the chick chorioallantoic membrane

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Introduction: Collateral circulation provides protection against ischemic injury in vascular obstructive diseases. However, processes and mechanisms to establish functional collateral flow have not yet been fully elucidated. Using continuous intravital microscopy in the *ex ovo* chick chorioallantoic membrane (CAM) model, this study aims to investigate vascular reactions to acute arteriolar occlusion and to quantify the hemodynamic and metabolic stimuli involved.

Methods: White leghorn chicken eggs were transferred on embryonic day 3 (E3) into petri dishes. On E12, CAM microcirculation was observed and recorded using time-lapse microscopy. A selected arteriole was occluded using laser irradiation. Before and after this occlusion, blood flow velocities in selected vessels were measured (spatial correlation) and hemoglobin oxygen saturation was determined (hyperspectral analysis).

Results: Immediately after the arteriolar occlusion, native collaterals were recruited from pre-existing arteriolo-arteriolar anastomoses. In cases with more than one native collaterals, many of them initially showed outward remodeling after occlusion, but underwent regression at a later stage resulting in only few dominating collaterals. With a delay of several hours after occlusion, additional collaterals were generated via neocollateral formation in yet underperfused regions around the occlusion site. Typically, a capillary pathway, usually the one connecting two arteriolar side branches closest to the occlusion site, underwent arterialization and eventually formed a functional microvascular collateral establishing significant collateral flow.

Conclusions: Neocollateral formation supplements collateral remodeling after acute arteriolar occlusion in the *ex ovo* chick chorioallantoic membrane. Our data suggest that this neocollateral formation might be initiated by metabolic signals from the tissue.

P16

The effects of cell arrangement on vessel diameter in a microfluidic angiogenesis model

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Introduction: Integration of capillaries into 3D tissues is one of the key challenges in tissue engineering. Blood vessels are essential for cell survival, because they deliver nutrients and oxygen. Vascular structure is hierarchical from arteries to capillaries *in vivo*, but it is unclear how to construct such vascular networks *in vitro*. Here, we investigated the interactions between endothelial cells and pericytes with different cell arrangement using a microfluidic culture system for tissue engineering of hierarchical vascular networks.
Methods: The microfluidic system used in this study had two medium channels, which were separated by 1,300-micrometer gel region. Human umbilical vein endothelial cells (HUVECs) were seeded in one of the medium channels, while mesenchymal stem cells (MSCs) were seeded in the same or other channels, which were named "same-side seeding" and "other-side seeding" conditions, respectively. The diameter of constructed microvessels was evaluated on days 10, 14 and 21 in each culture condition.
Results and Discussion: We succeeded in creating stable capillary networks covered by pericytes that were differentiated from MSCs. On day 10, the vessel diameter in the position near the root of vascular structures was 27 micrometer in the condition of other-side seeding, which was larger than that in same-side seeding. The large vessels in other-side seeding gradually became smaller during day 10–21, while that in same-side seeding was < 10 micrometer during the period. These results suggest remodeling of the constructed capillaries in other-side seeding. This culture system is useful to further investigate the mechanism to control vessel diameter *in vitro*.

P17

Role of microsomal prostaglandin E synthase (mPGES)-1 in hepatic ischemia/reperfusion injury

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Background: Prostaglandin E2 (PGE2) contributes to hepatic ischemia/reperfusion (I/R) injury, however, a role of microsomal prostaglandin E synthase (mPGES)-1, which is the synthase of PGE2, is still unknown. The aim of this study was to examine the role of mPGES-1 in hepatic I/R injury.
Methods: Male mPGES-1 knockout (mPGES-1-/-) mice and their wild-type counterparts (WT) mice were subjected to 60 min of 70% hepatic warm ischemia followed by reperfusion.
Results: Compared with WT mice, ALT levels in mPGES-1-/- mice at 6 hour and 24 hour after reperfusion were reduced. The mRNA levels of TNF-a, IL-1b, IL-6, IFNg in mPGES-1-/- mice were lower than those in WT mice. The numbers of accumulated Gr-1-positive neutrophils at 24 hour were decreased in mPGES-1-/- mice. Among the subtypes of PGE2 receptors (EPs), hepatic I/R upregulated the expression of EP2 and EP4 in WT livers, and those levels were approximately decreased by 50% in mPGES-1-/- livers. Treatment of WT mice with an EP2 antagonist, but not with an EP4 antagonist, decreased the levels of ALT, TNF-a, IL-1b, IL-6, IFNg at 24 hour after reperfusion in comparison with vehicle.
Conclusions: These results indicate that mPGES-1-derived PGE2 enhances the production of pro-inflammatory cytokines and recruitment of neutrophils into the livers through EP2 receptors during hepatic I/R injury.

P18

Notch pathway targets proangiogenic regulator Sox17 to restrict angiogenesis

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The Notch pathway stabilizes sprouting angiogenesis by favoring stalk cells over tip cells at the vascular front. Because tip and stalk cells have different properties in morphology and function, their transcriptional regulation remains to be distinguished. Transcription factor Sox17 is specifically expressed in endothelial cells, but its expression and role at the vascular front remain largely unknown.To specify the role of Sox17 and its relationship with the Notch pathway in sprouting angiogenesis.Endothelial-specific Sox17 deletion reduces sprouting angiogenesis in mouse embryonic and postnatal vascular development, whereas Sox17 overexpression increases it. Sox17 promotes endothelial migration by destabilizing endothelial junctions and rearranging cytoskeletal structure and upregulates expression of several genes preferentially expressed in tip cells. Interestingly, Sox17 expression is suppressed in stalk cells in which Notch signaling is relatively high. Notch activation by overexpressing Notch intracellular domain reduces Sox17 expression both in primary endothelial cells and in retinal angiogenesis, whereas Notch inhibition by delta like ligand 4 (Dll4) blockade increases it. The Notch pathway regulates Sox17 expression mainly at the posttranscriptional level.

Furthermore, endothelial Sox17 ablation rescues vascular network from excessive tip cell formation and hyperbranching under Notch inhibition in developmental and tumor angiogenesis.Our findings demonstrate that the Notch pathway restricts sprouting angiogenesis by reducing theexpression of proangiogenic regulator Sox17.

P19

Angiogenesis and lymphangiogenesis in MALT lymphoma in stomach, liver: Significance of VASH2

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We established a low-grade MALT lymphoma model in C57BL/6 mouse infected with Helicobacter heilmannii obtained from cynomolgus monkey. We have reported the significance of VEGF and its receptors, Flt-1, Flk-1, Flt-4 in the MALT lymphoma by immunohistochemistry. Recently, vasohibin-2 (VASH2) has been identified as a new molecule acting as a stimulant of angiogenesis. Thus, the present study was designed to identify the localization of VASH2 in the MALT lymphoma in comparison with vascular endothelial growth factor (VEGF) A and C and its receptors. In addition, the effect of axitinib, one of the tyrosine kinase inhibitors, on the VASH2 and VEGF in the MALT Lymphoma was investigated. Nine months after the infection, small lymphocyte aggregates mostly composed of B cells were observed in the portal area of the liver as well as the gastric MALT lymphoma in ~50% of the infected mice. PCR and *in situ* hybridization analysis showed the existence of H. heilmannii not only in the fundic mucosa but in the liver. VASH2 immunoreactivity was found in the mesenchymal cells surrounding the irregular microvascular network in the MALT lymphoma. The localization of VEGFA and C were also observed in the mesenchymal cells but the pattern of the distribution was different. The administration of axitinib to the infected mice induced significant suppression of the MALT lymphoma, while the VASH2 immunoreactivity increased. In conclusion, VASH2 were shown to exist in the gastric and hepatic MALT lymphoma and suggested to have some function in the tumor microvasculature.

P20

Arterial-venous identity specification in pre-vascularized engineered implants requires perivascular cell recruitment and is impaired in diabetes

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Re-vascularization approaches have the potential to regenerate ischemic tissues, holding promise for translational therapies. However, most re-vascularization attempts fail due to the lack of a mature vasculature. A hierarchical vascular network, consisting of specific arterio-venous (AV) types, is essential for sustaining vascular function. We define factors that control AV specification in pre-vascularized engineered implants in health and diabetes (a disease characterized by vascular dysfunction) by using the only pre-vascularized engineered tissue described to yield vessels with specific AV identities.

Hypothesis: AV specification is dependent on perivascular cell (PVC) recruitment and is impaired in diabetes.Using microvessel fragments isolated from EphrinB2-GFP (arterial-reporter) mice and implanted into engineered constructs, we show for the first time that preventing PVC recruitment by blocking PDGFRb resulted in lack of proper AV identity (ubiquitous EphrinB2 expression and absence of vessel network hierarchy). Controls exhibited mature networks with arterial ($EphrinB2^{+}$) and venous ($EphrinB2^{-}$) vessels. Analysis of endothelial cells co-cultured with PVCs of arterial or venous origin point to endothelial-PVC cell-cell Notch signaling involvement in arterial specification. No differences in the percentage of vessel perfusion or shear stress between microvessels in control or PDGFRb-blocked implants were observed.Lack of proper AV identity in the absence of PVCs (PDGFRb-blocked) was comparable to microvessels in constructs implanted into Type I diabetic mice (streptozotocin-injected). With regards to underlying mechanisms, qPCR showed significantly reduced expression levels of genes involved in the Notch signaling pathway, suggesting that an inability of vessels to acquire proper AV identity contributes to microvascular dysfunction in diabetes.

P21

Mass transport from bloodstream to biosensors: Impact of convection and diffusion

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Implantable biosensors have tremendous clinical potential. While the fabrication of robust sensors has progressed, the foreign body response to implanted sensors still leads to their failure to accurately report changes in blood and tissue concentrations of target analytes. Hydrogel biosensors can encourage tissue integration and formation of functional microvasculature to improve mass transport to the sensing elements. Solid and porous poly-hydroxyethyl-methacrylate (pHEMA) was constructed as a platform into which various biosensing capabilities could be embedded. These biosensors were implanted into the brains of mice, and into the skin of rat dorsum, rat hindlimb, and pig dorsum. Sensors were optically interrogated through overlying tissues. Up to 1 year after implantation, sensors were explanted with adjacent tissue. Quantitative histological analysis of microvascular density and cytology of biopsied tissue was performed. In rats, the tissue within 50 μm of the porous pHEMA (80 μm pores) had a microvessel density of 469 $\pm$ 91 microvessels/mm^2. For solid pHEMA density was 242 $\pm$ 62 microvessels/mm^2. As a result, the median diffusion distance from any sensing element to the nearest microvessel in solid pHEMA was 132 $\pm$ 57 μm and increased over time. Conversely, porous pHEMA reduced the median diffusion distance from 97 $\pm$ 48 μm at 1 week to 36 $\pm$ 16 μm at 1 month; $p < 0.05$). Tissue integration resulted in cell densities that mimicked control tissue with somewhat elevated populations of various macrophage phenotypes. Sensor functionality was further demonstrated in clinically relevant models, including hindlimb and CNS ischemia models. Efficiency of mass transport to the biosensors was increased both by enhanced microvascular convection and by reduced diffusion distances.

P22

FRG1 and its interacting partner EEF1A: putative angiogenic regulators

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FSHD region gene 1 (FRG1) over-expression in mice, frogs and worms has been associated with muscular and vascular abnormalities. FRG1 knockdown in Xenopus resulted in decreased angiogenesis and reduced expression of the angiogenic regulator DAB2. Our initial study in HEK cell line showed that FRG1 can affect cell migration. We hypothesized that FRG1 and its interacting partners have role in angiogenesis and tumor progression.

We used HeLa, HepG2, PC3, HEK, and HUVEC as *in vitro* model for various experiments. We did Co-immunoprecipitation with FRG1 antibody to find out interacting proteins. Binding proteins were identified by Mass spectrometry. Results were validated by co-immunoprecipitation and reverse co-immunoprecipitation followed by western blotting. Immuno-flurescence was done to find out co-localization of FRG1 and its interacting protein.

We identified EEF1A as an interacting partner of FRG1. EEF1A exists in two closely related variant forms, EEF1A1 and EEF1A2, which are encoded by separate loci. EEF1A1 is almost ubiquitously expressed, whereas EEF1A2 expression is limited to neurons, heart and muscle. Our immuno-florescence studies using transformed and tumor cell lines indicated that FRG1 co-localized with both the isoforms with enhanced co-localization at the peri-nuclear region. This co-localization was further validated by co-immunoprecipitation. This interaction was found to be actin-independent as evidenced by no change in co-localization after disruption of actin. Real time PCR showed that ectopic change in FRG1 expression altered EEF1A1 transcript levels positively.

We conclude that, FRG1 interacts with EEF1A (an oncogene). This interaction might be which might be important for its non-canonical functions in angiogenesis.

P23

Differential effects of cAMP/PKA and cAMP/Epac signalling on *in vitro* angiogenesis: Role of Rho GTPases

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Background: cAMP mediates its effects via activation of its two well characterised effectors i.e. PKA and Epac. The aim of the present study was to analyse the effects of these cAMP effectors on angiogenesis *in vitro*.

Methods: The study was carried out on cultured HUVEC. Angiogenesis was analysed by endothelial cell tube formation and 3-D spheroid assay. cAMP analogues, 8-pCPT-2-O-Me-cAMP (200 microM) and N6-Benzoyl-cAMP (50 microM) were used to activate Epac or PKA, respectively.

Results: Specific activation of either PKA or Epac induced HUVEC proliferation and migration (wound healing) which was accompanied by enhanced phosphorylation of Akt and

p42/44 MAPK which were abrogated by inhibitors of both Akt and p42/44 MAPK. Accordingly, specific activation of PKA induced endothelial cell tube formation and promoted sprouting of spheroid in 3-D collagen gels. However, activation of Epac abrogated endothelial cell tube formation and VEGF-induced sprouting. Furthermore, Epac activation attenuated VEGFR2 phosphorylation. Although activation of both PKA and Epac induced Rac1 activation, however, both have differential effects on RhoA activity. PKA antagonised RhoA activity, while Epac caused an activation of RhoA. Inhibition of either RhoA activity or downstream Rho kinase resulted in increased HUVEC tube formation and sprouting and attenuated the anti-angiogenic effect of Epac activation. Similarly, specific activation of RhoA abrogated PKA-induced angiogenesis. The data was further confirmed by over expression of constitutive active and dominant negative RhoA using lentivirus based vectors.

Conclusion: cAMP/PKA and cAMP/Epac signalling pathways have differential effects on *in vitro* angiogenesis. PKA activation promotes while Epac activation antagonises angiogenesis.

P24
Physiological role of anti-angiogenic VEGF isoforms

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Vascular endothelial growth factor (VEGF-A) has been widely described to be involved in diseases associated with angiogenesis – cancer, diabetes, age related macular degeneration, and many others. It has also been implicated in other disorders including chronic pain, lung fibrosis, infertility and kidney failure. In 2001 at the World Congress on Microcirculation we identified a new isofom of VEGF-A, termed VEGF-A_{165}b, which acted as an anti-angiogenic compound and counteracted the previously described VEGF-A isoforms. Since 2001, we and others have identified physiological roles for VEGF-A_{165}b in the kidney, spinal cord, sensory nervous system, brain, pituitary, muscle, mammary gland, ovary, lung, testis, gut, vascular system and skin. It is now clear that the anti-angiogenic isoforms are as critical to human health as the pro-angiogenic isoforms. Manipulation of the expression of these isoforms has now been identified as potential therapeutic approach in a number of different conditions including pain, blindness, cancer, infertility, lung disease and renal failure. The mechanisms underlying VEGF isoforms control include growth factor, environmental impacts such as hypoxia, and genetic factors. Here we show that manipulation of VEGF-A_{165}b expression is a potential therapeutic approach in eye disease, and investigate its potential for other diseases as widespread as heart disease, cancer, pain and kidney failure.

P25
VEGF-A_{165}b ameliorates vascular dysfunction in diabetic retinopathy

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Aim: Diabetic retinopathy (DR) is associated with upregulated vascular endothelial growth factor (VEGF), particularly pro-angiogenic VEGF-A_{165}a. This is responsible for pathological neovascularisation and tight-junction (TJ) breakdown, contributing to DR and diabetic macular oedema (DME). Anti-angiogenic isoforms, such as VEGF-A_{165}b, are down-regulated in diabetes, potentially contributing to vascular dysfunction. This study assesses whether VEGF-A_{165}b can abrogate blood-retina-barrier and outer-retinal barrier (ORB) breakdown and prevent subsequent leakage.

Methods: Retinal pigment epithelial (1°RPE) cells were treated with 2.5 nM VEGF-A_{165}a, low glucose (5 mM) and high glucose (25 mM) media ± 2.5 nM VEGF-A_{165}b and were assayed for TJ integrity through occludin and ZO1 expression and trans-epithelial electrode resistance (TEER). *In vivo* retinal dysfunction was measured in streptozotocin-induced (STZ, 50 mg/kg) diabetic rats after 1 and 8 weeks of diabetes ±VEGF-A_{165}b (10 ng/μL intravitreally and biweekly 20 ng/g i.p respectively). Evans' blue extravasation and IB4 immunofluorescence assessed vascular leakage and vascular remodelling respectively.

Results: Occludin expression decreased and paracellular flux increased in 1°RPE cultured in 25 mM glucose and partially restored with VEGF-A_{165}b treatment. VEGF-A_{165}b restored VEGF-A_{165}a-induced reduction in occludin and ZO1 expression and increased paracellular flux. VEGF-A_{165}b reduced Evans' blue extravasation at both 1 ($p < 0.05$) and 8 ($p < 0.001$) weeks post STZ-injection relative to vehicle-treated diabetic rats. VEGF-A_{165}b also reduced vascular density ($p < 0.001$), and upregulated VE-cadherin and occludin expression relative to vehicle-treated diabetic rats.

Conclusion: VEGF-A_{165}b restores paracellular resistance *in vitro* and restores vascular density and solute flux *in vivo*, demonstrating that VEGF-A_{165}b prevents ORB breakdown and may be a therapeutic target in treating DR and DME.

P26

Effects of shuangdan mingmu capsule on the expression of retinal VEGF and VEGFR protein activity in rats with diabetic retinopathy

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Objectives: Shuangdanmingmu capsule (SDMM) is a patented Chinese medicine composed by Glossy privet fruit, Eclipta alba, Dogwood, Chinese yam, Poria cocos, Rhizoma alismatis, Pseudo-ginseng, etc. It has been widely used to treat fundus lesions, decreased vision of DR patients in clinic. Clinical studies reported that SDMM was safe and effective in promoting blood circulation, such as lowering platelet adherence and improving hemorrheologic indexes. Here, we observe the effects of Shuangdan Mingmu Capsule on the expression of retinal VEGF and VEGFR protein activity in rats with diabetic retinopathy.

Methods: 40 SD rats were randomly divided into 10 rats normal in group, 30 rats in model group. A rat of diabetic retinopathy was induced by intravenous injection of STZ. Then the model rats were randomly divide into three groups: model control group, shuangdan mingmu group, positive control group. The normal group and model control group was given normal saline by gavage. The above 2 groups were intragastric administration once a day. Shuangdan Mingmu group was given the solution of Shuangdan Mingmu capsule by gavage and Saline by intravitreal injection every 10 days for 1 times. Positive control group was given calcium dobesilate by gavage, 1 times a day and recombinant human endostatin by intravitreal injection every 10 days for 1 times. The effects of Shuangdan Mingmu Capsule on the expression of rat retinal VEGF and VEGFR protein with diabetic retinopathy was tested by western blot after 8 weeks.

Results: (i) Compared with the normal group, the expression of VEGF and VEGFR protein in model control group was increased ($p < 0.01$); (ii) Compared with the model control group, the expression of VEGF and VEGFR protein in Shuangdan Mingmu group and positive control group was decreased ($p < 0.01$); (iii) Compared with Shuangdan Mingmu group and positive control group, the expression of VEGF and VEGFR protein didn't have statistically significant ($p > 0.05$).

Conclusions: Our results suggest that Shuangdan Mingmu capsule can reduce the production of retinal VEGF and VEGFR protein in rats with diabetic retinopathy, thus the capsule may inhibit retinal neovascularization.

ATHEROSCLEROSIS/THROMBOSIS/PLATELETS

P27

The clinical efficacy and immunomodulatory mechanism of Yi Qi Tong Yang soup treating chronic immune thrombocytopenia

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For investigating the clinical efficacy of Yi Qi Tong Yang soup treating Chronic Immune Thrombocytopenia(CITP), and analysing the immune status of CITP patients before and after treatment, we have studied 27 healthy controls and 31 patients treated with Yi Qi Tong Yang soup for more than 6 months. Then, we evaluated the clinical efficacy and detected the expression of PAIgG, PAC3 and the cell count of Th1, Th2, Treg, Th17 and Th22 by flow cytometry. After treatment, PLT count of CITP patients was increased (from 33. 77 $\pm$ 20. 69 to 52.61 $\pm$ 65.23, $p < 0.05$). The total effective was 58.06%. Compared with the healthy control group, the expression of PAIgG and PAC3 was significantly increased before treatment ($p < 0.01$). After treatment, there was no difference for the expression of PAC3 compared with control, and there was an decrease for PAIgG but no reseaching the normal level. In effective cases, the cell count of Th1 have decreased and Th2 have increased after treatment ($p < 0.05$). The cell count of Treg and Th22 have significantly decreased compared with control group, while Th17 cells significantly increased ($p < 0.01$). After treatment, there was no difference for Treg cell compared with the healthy control group, and significantly increased for Th17 cell but no reseaching the normal level, but there was no change for Th22 cell. This study showed that Yi Qi Tong Yang soup could promote platelet increase and regulate the immune disorder status in CITP patients.

P28

Laser-induced thrombus formation in angiotensin II type 1 and type 2 receptor-knockout murine brain microvasculature observed on intravital fluorescence microscopy

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Objective: The purpose of this study was to observe the effect of angiotensin II type 1 (AT1) and type 2 (AT2) receptor deficiency on the process of laser-induced thrombus formation and platelet behavior in murine brain microvasculature using intravital fluorescence microscopy.

Methods: C57BL/6J mice (control group, $n = 10$), AT1 receptor-knockout mice (AT1KO group, $n = 8$) and AT2 receptor-knockout mice (AT2KO group, $n = 11$) were anesthetized with chloral hydrate and inserted a catheter in their cervical vein. A cranial window was prepared in the parietal region. Platelets were labeled *in vivo* by intravenous administration of carboxylfluorescein succinimidylester. Laser irradiation (1000 mA, DPSS laser 532 nm, TS-KL/S2; Sankei) was spotted for 4 seconds on pial arteries to induce thrombus formation. Labeled platelets and thrombus were observed continuously with a fluorescence microscope.
Results: After laser irradiation to the pial artery, the complete occlusion rate was 62% (18/29 vessels) in the control group, 33% (8/24 vessels, $p = 0.037$ vs. control group) in the AT1KO group and 43% (12/28 vessels, $p = 0.146$ vs. control group). Thirty minutes after laser irradiation, the area of platelet thrombus was $345 \pm 226\ \mu m^2$ in the control group, $183 \pm 122\ \mu m^2$ in the AT1KO group ($p = 0.007$ vs. control group) and $553 \pm 308\ \mu m^2$ in the AT2KO group ($p = 0.003$ vs. control group).
Conclusion: The present study suggested that the laser-induced thrombus formation in murine pial arteries was inhibited by AT1 receptor deficiency, and was promoted by AT2 receptor deficiency.

P29
Inhibitory effect of caffeic acid on ADP-induced thrombus formation and platelet activation involves mitogen-activated protein kinases

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Caffeic acid (CA), one of the active constituents of Radix Salvia miltiorrhizae, exhibits antioxidant and anti-inflammatory activities. However, few studies have assessed the ability of CA to inhibit platelet mediated thrombus generation *in vivo*. In this study, we investigated the antithrombotic effect of CA in mouse cerebral venules using intravital microscopy. The antiplatelet activity of CA in ADP stimulated mouse platelets *in vitro* was also examined in attempt to explore the underlying mechanism. Our results demonstrated that CA (5 mg/kg) significantly inhibited thrombus formation *in vivo*. *In vitro*, CA (5–100 μM) inhibited ADP-induced platelet aggregation, P-selectin expression, ATP release, Ca2 + mobilization, and integrin GPIIbIIIa activation. Additionally, CA attenuated p38, ERK, and JNK activation, and enhanced cAMP levels. Taken together, these data provide evidence for the inhibition of CA on platelet-mediated thrombosis *in vivo*, which is, at least partly, mediated by interference in phosphorylation of ERK, p38, and JNK leading to elevation of cAMP and down-regulation of P-selectin expression and GPIIbIIIa activation. These results suggest that CA may have potential for the treatment of aberrant platelet activation-related diseases.

P30
Uridine triphosphates analogues as inhibitors of platelet aggregation

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Background: Platelets express two ADP receptors namely P2Y1 and P2Y12 that regulate ADP and other agonists-induced platelet aggregation. P2Y1 receptor activation causes platelet shape change while P2Y12 receptor activation induces platelet aggregation. The aim of the present study was to characterise the effects of uridine triphosphate (UTP) and its analogues on ADP-induced platelet aggregation.
Methods: The experiments were performed on platelet rich plasma freshly isolated from blood donated by healthy human volunteers.
Results: Both UTP and S-UTP inhibited ADP-induced platelet aggregation in a conc.-dependent manner, S-UTP being more potent. The IC50 values against ADP (10 microM)-induced platelet aggregation were 32 and 0.36 microM for UTP and S-UTP, respectively. Likewise, both nucleotides potently antagonised collagen (2 microg/mL)- and epinephrine (10 microM)-induced platelet aggregation. However, both UTP and S-UTP had no effect on ADP- and MRS2365 (P2Y1 receptor agonist)-induced platelet shape change suggesting their inactivity at P2Y1 receptors.
Conclusion: The novel data demonstrate that UTP and S-UTP are potent P2Y12 receptor antagonists and inhibit agonist-induced platelet aggregation.

CANCER METABOLISM AND MICROCIRCULATION

P31

Induction of DNA damage-mediated cell cycle arrest in human glioma cells by caudatin, a natural cytostatic reagent

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Objectives: Caudatin, one of the species of C-21 steroidal glycosides mainly isolated from the root of Cynanchum bungei Decne, exhibits potent anticancer activities. However, the mechanism remains poorly defined. In the present study, the growth inhibitory effect and mechanism of caudatin on human glioma cells were evaluated *in vitro*.
Methods: Several methods of cell biology and molecular biology *in vitro* were employed.
Results: The results revealed that caudatin time- and dose-dependently inhibited U251 and U87 cells growth. Flow cytometry analysis indicated that caudatin-induced growth inhibition against U251 and U87 cells was mainly achieved by induction of G0/G1 and S phase cell cycle arrest through triggering DNA damage, as convinced by the up-regulation of p53, p21 and histone phosphorylation, as well as the down-regulation of cyclin D1. Moreover, caudatin treatment also triggered the activation of ERK and inactivation of AKT pathway. LY294002 (an AKT inhibitor) addition enhanced caudation-induced AKT inhibition, indicating that caudatin inhibited U251 cells growth with an AKT-dependent manner.
Conclusions: Our findings indicate that caudatin may act as a novel cytostatic reagent against human glioma cells through induction of DNA damage-mediated cell cycle arrest with involvement of modulating MAPKs and AKT pathways.

P32

Monoolein suppresses tumor growth and angiogenesis in human cervical cancer xenografts in nude mice

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Background and Objective: Monoolein, a biosurfactant has been demonstrated the anti-proliferative activity against cervical cancer cells lines and leukemia cells lines *in vitro*. The present study, examined the effect of monoolein (MO) on tumor growth and angiogenesis in human cervical carcinoma cell line (HeLa) implanted athymic nude mice.
Materials and methods: HeLa cells were subcutaneously injected into the dorsal of BALB/c-nude mice to form xenograft tumors. Mice were treated and untreated with MO (HeLa-MO, 200 mg/kg body weight) and vehicle (HeLa-vehicle, 0.05% ethanol), respectively. When the tumor size reached ~ 50–100 mm^3, mice were administered with MO and vehicle once daily by peritumoral injections for 30 days. Tumor size was measured and tumor inhibition rate was calculated. The tumor blood flow was analyzed using the Laser Doppler flowmeter. At the end of study, tumor microvasculature was observed under intravital fluorescence video-microscopic technique and microvascular density was analyzed using digital image processing software. Immunohistochemical analysis of CD31 expression was performed.
Results: Monoolein showed significant tumor growth suppression and tumor inhibition rate was 59.20%. The tumor blood flow and microvascular density were significantly reduced in HeLa-MO group compared with those of HeLa-vehicle group ($p < 0.001$), respectively. CD 31 expression was significantly attenuated compared with those of HeLa-vehicle group ($p < 0.001$).
Conclusion: Our findings demonstrate the effectiveness of Monoolein as an angiogenesis inhibitor in suppressing tumor growth during tumor progression in HeLa-cells implanted nude mice.
Key word: Monoolein, HeLa cells, tumor growth, tumor blood flow, angiogenesis inhibitor

P33

Arctigenin, an antiausterity antitumor agent, increases intra-tumor blood circulation through vascular re-modeling *in vivo*

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Background: Arctigenin, abundantly contained in burdock fruit, a Kampo medicine is preferentially cytotoxic during glucose-deprivation and is under clinical evaluation for anticancer activity. While arctigenin monotherapy exerted antitumor activity in various xenograft models, we previously observed arctigenin had synergistic effect with

conventional cytotoxic antitumor agents on tumor growth. We postulated that arctigenin might improve tumor perfusion through vascular re-modeling enhancing the intratumor accumulation of these antitumor agents. In this study, we evaluated alterations in tumor perfusion *in vivo* by using dynamic contrast-enhanced magnetic resonance (MR) imaging.

Materials and Methods: Nude mice were transplanted with a human pancreatic cancer cell line (SUIT-2) subcutaneously. After reaching tumor size of ~30 mm^3, tumor bearing mice were divided into two groups, arctigenin-treated and control groups. Mice were fed arctigenin containing (0.5%) or control diet. Four weeks after the start of arctigenin treatment, we sequentially acquired T1-weighted images of the tumor before, during, and after i.v. bolus administration of Gadolinium-DTPA by using a 9.4 tesla Bruker scanner. We then calculated percent increase in MR signals in the tumor during 90–120 seconds after Gadolinium-DTPA administration as an indicator of tumor perfusion.

Results: Arctigenin treatment reduced tumor volume to 84% compared to control. Arctigenin-treated tumor exhibited 18.1 $\pm$ 13.7% increase in MR signals compared to the baseline values, whereas untreated tumors showed marginal increase of 2.6 $\pm$ 3.4% ($p < 0.05$, *t*-test).

Conclusion: Our results strongly suggested that arctigenin treatment increased intra-tumor blood circulation.

P34

Acanthus ebracteatus Vahl could inhibit tumor growth and tumor angiogenesis associated with inhibition of hypoxia-inducible factor-1 regulatory pathway in human cervical carcinoma cell implanted nude mice

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Cervical cancer is the malignant cancer which occurs within epithelial layers of cervical mucosa. It is clearly that persistent infection with high risk human papilloma viruses (HPVs) is required for the development and maintenance of cervical cancer. During the last decade, the antitumor potential of Thai medicinal plants, Acanthus ebracteatus Vahl. (AE) has been reported. Our previous study firstly indicated that aqueous crude extract of AE has anti-angiogenic effect on human cervical carcinoma cell implanted nude mice. In the present study, the anti-angiogenesis of AE was further performed to define whether its mechanism(s) related to tumor biomarkers, HIF-1alpha, and NF-kappa-B or not. Inbred female BALB/c-nude mice (20–25 g) were divided into 3 groups; control (MEM medium injection [CON]), HPV-mice (injected subcutaneously at dorsal skinwith CaSki cells [1 $\times$ 107cells/200 microliters]), and AE-treated HPV-mice (HPV+AE, 3000 mg/kg BW by gavage daily starting at 4-week after CaSki cells injection). 7, 14, 21, and 28 days after treatment, the tumor size was measured by caliper, capillary vascularity (%CV) were determined using laser scanning confocal microscopy, and tumor tissue HIF-1alpha, NF- kappa B, and VEGF expressions were determined by immunohistochemistry. The results showed that the tumor size and % CV of HPV+AE were decreased significantly compared to HPV mice at 21 and 28 days. Moreover, the VEGF, HIF-1-alpha, and NF-kappa B expressions were decreased significantly compared to HPV-groups at both 7 and 14 days. It demonstrated that AE treatment seems to have the beneficial mechanisms to prevent tumor angiogenesis and tumor growth through HIF-1 blockade.

P35

Arctigenin enhances the chemosensitivity to chemotherapeutic agents through microcirculatory changes

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Cancer microenvironment is characterized by severe hypoxia and nutrient deprivation, especially. Tumor hypoxia is mainly caused by poor blood supply due to structurally and functionally immature vasculature despite vigorous angiogenesis. Severe tumor hypoxia and glucose deprivation promote tumor progression and affects tumor behavior especially resistance to chemotherapeutic agents. We have sought agents that are toxic to cancer cells during hypoxic and/or nutrient deprivation and discovered several candidate compounds, including arctigenin. Arctigenin was found to attenuate cancer cells tolerance to glucose deprivation and showed antitumor activity in mouse xenograft models. GBS-01 is an orally available extract prepared from Arctii Fructus and contains arctigenin and its glycoside, arctiin at 10% in total. Clinical evaluation of monotherapy with GBS-01 for

anticancer activity is on going among patients with fluoropyrimidine-refractory pancreatic cancer. In the present study, we evaluated combination use of GBS-01 with various chemotherapeutic agents in mouse xenograft model. Several chemotherapeutic agents, including gemcitabine, 5-FU, CPT-11, and oxaliplatin exerted the synergistic effect. However, arctigenin did not show synergistic effect with any chemotherapeutic agents in *in vitro* cytotoxicity assay. These results indicate that arctigenin exert a synergistic effect by modulating tumor tissue. Actually, arctigenin treatment decreased tumor hypoxia evidenced by HRE-luciferase construct-transfected tumor cells. We also evaluate changes in structure of tumor blood vessels after the treatment with GBS-01 using immunohistochemistry of microvessel and scanning electron microscopy.

P36
Mechanical properties of human bone marrow endothelial cells and their adhesive interaction with breast cancer cells measured by atomic force microscopy

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Force spectroscopy using atomic force microscopy (AFM) is a sensitive and quantitative method to analyze cell elasticity and adhesion properties. The purpose of our study was to characterize cell elasticity in human bone marrow endothelial cells (HBME) and their adhesion to human breast cancer cells. Mechanical properties of two HBME lines, HBME-1 and HBMEC-60, were determined by AFM nano-indentation. Cell elasticity (i.e. Young's modulus) was significantly increased in confluent vs. non-confluent HBME-1 (6.79 ± 2.58 vs. 4.73 ± 1.78 kPa) and HBMEC-60 (4.25 ± 1.14 vs 2.03 ± 1.09 kPa). In adhesion experiments, a single breast cancer cell, MDA-MB-231 (MB231) was attached to the AFM cantilever and brought into contact with a confluent HBME monolayer for different contact periods. The force required to separate cells was analyzed as a measure of cell-cell adhesion. Adhesive interaction between the MB231 and HBME cells increased progressively as cell-cell contact time was increased from 0.5 to 300 section. Studies of the ligand-receptor involvement showed that at 30-section of contact time, adhesion was decreased with treatment using function-blocking antibodies to anti-integrin beta 1 (−27%), anti-Thomsen-Friedenreich-antigen (TF-Ag, −20%), or when antibodies were combined (−38%); but increased with integrin beta 1 function-activating antibody (+48%). In summary, HBME stiffness is influenced by confluency, cell-cell contact time enhances adhesion between MB231 and HBME and adhesion is mediated, in part, by integrin beta 1 and TF-Ag. Further characterization of the molecules responsible for the adhesive interaction may provide new therapeutic targets for preventing metastasis. (Supp. 1I01BX000609 VA BLR&D Service and Development-VVG; NIH/NCI R01CA160461-VVG). R01CA160461-VVG).

P37
The importance of the perivascular niche in the early stage of breast cancer bone colonisation

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Advanced breast cancer is frequently associated with metastasis and the most common metastatic site is the skeleton. During dissemination to bone, breast cancer cells locate in a putative "metastatic niche" and this microenvironment plays a key role in the colonisation, dormancy and proliferation of the cancer cells in bone. Although the precise cellular composition of the niche remains to be established, it has been suggested to be broadly overlapping with hematopoietic stem cell and peri-vascular bone niches. Using *in vivo* models of bone metastasis, we mimicked the early steps of bone metastasis and investigate the relationship between the different cellular population of the niche and the breast cancer cells labelled with fluorescent membrane dyes. By multiphoton and confocal microscopy, we were able to detect single tumour cells within the bone microenvironment following injection in immunocompromised mice. Breast cancer cells homed preferentially in the trabecular region of the long bone, which is particularly rich in osteoblasts and highly vascularised. Moreover, we were able to visualise tumour cells in close proximity to a particular vessel subtype using an immunofluorescence protocol with antibodies against endomucin and CD31. The data obtained evidenced the direct association between vessels, osteoblast and breast cancer cells during the early steps of bone metastasis. Our results highlighted the importance of the perivascular niche in the first stages of skeleton colonisation, underlining the possibility of new therapeutic approaches targeting this component of the niche.

P38

LKB1/AMPK regulates autophagy-mediated MMP-9 expression to promote cancer cell development during microenvironmental stress

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Overcoming terrible tumor microenvironment as a metabolic stress is a crucial step for further malignant alteration of cancer cells. However, the underlying mechanisms of adaptation under microenvironmental stress are not fully understood. Liver kinase B1 (LKB1)/AMP-activated kinase (AMPK) is the major cellular energy sensor and master regulator of metabolic homeostasis. Nutrients starvation conditions that decrease intracellular ATP levels below a certain level promote AMPK activation by LKB1. A previous study suggested that LKB1/AMPK pathway promotes cell survival during glucose deprivation through the maintenance of intracellular NADPH level to protect metabolic stress and oxidative stress, possibly because of the function of LKB1/AMPK in cancer progression. However, other roles by which LKB1/AMPK involves in the contribution to malignant development are poorly understood. We showed that activation of LKB1/AMPK pathway, during glucose deprivation stress, induces the transcriptional activation and the expression of matrix metalloproteinase-9 (MMP-9) protein. We also found that migration capability of cancer cells are also enhanced under glucose restriction conditions. Most intriguingly, LKB1/AMPK-dependent selective autophagycally degradation of Keap1 and significant phosphorylation of p62 were observed under glucose deprivation, leading to cause the remarkable induction of nuclear factor erythoid 2-related factor 2 (Nrf2), a transcription factor known as a master regulator against oxidative stress. In addition, glucose deprivation-mediated Nrf2 induction stimulated transcriptional activation of MMP-9 through Nrf2 binding sites in its promoter region. Our data clearly established that LKB1/AMPK signal has played a prominent role in adaptation against microenviromental stress, resulting in further cancer development.

CELL SIGNALING: PROTEINS, PATHWAYS, AND MECHANISMS

P39

Curcumin antagonizes beta-amyloid-induced neurotoxicity in PC12 cells, from rational design to signaling pathways

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Objectives: Progressive accumulation of beta-amyloid (A-beta) will form the senile plaques, caused oxidative damage and neuronal cell death, which was accepted as the major pathological mechanism to the Alzheimer's disease (AD). Hence, inhibition of A-beta-induced oxidative damage and neuronal cell apoptosis by agents with potential antioxidant properities represents one of the most effective strategies in combating human AD. Curcumin (Cur) a natural extraction from curcuma longa has potential of pharmacological efficacy, including the benefit to antagonize A-beta-induced neurotoxicity. However, the molecular mechanism remains elusive. The present study aim to evaluate the protective effect of curcumin against A-beta-induced cytotoxicity and apoptosis in PC12 cells and investigate the underlying mechanism.

Methods: Several methods of cell biology and molecular biology *in vitro* were employed.

Results: The results showed that curcumin markedly reduced A-beta-induced cytotoxicity by inhibition of mitochondria-mediated apoptosis through regulation of Bcl-2 family. The PARP cleavage, caspases activation and ROS-mediated DNA damage induced by A-beta were all significantly blocked by curcumin. Moreover, regulation of p38MAPK and AKT pathways both contributed to this protective potency.

Conclusions: Our findings suggested that curcumin could effectively suppressed A-beta-induced cytotoxicity and apoptosis by inhibition of ROS-mediated oxidative damage and regulation of ERK pathway, which validated its therapeutic potential in chemoprevention and chemotherapy of A-beta-induced neurotoxicity.

P40

Gene expression analysis in small arteries of spontaneously hypertensive rats: Evidence for ER stress

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Small arteries are known to develop functional and structural alterations in hypertension. However, the mechanisms of this remodeling are not fully understood. In this study we analyzed gene expression associated with the development of hypertension in mesenteric arteries of spontaneously hypertensive rats (SHR). Three sublines of SHR and Wistar Kyoto rats (WKY) as control were studied at 6 weeks and 5 months of age. miRNA and mRNA microarrays were performed and analyzed with bioinformatical tools such as Ingenuity® Pathway Analysis (IPA). Principal component analysis showed a clear separation in both miRNA and mRNA expression levels between both ages studied, demonstrating a strong age-related expression. At miRNA level, IPA identified differences between SHR and WKY related to metabolic diseases, cellular growth, and proliferation. The mRNAs differentially expressed in SHR were related to oxidative stress, cellular movement and proliferation. The most strongly upregulated gene was thrombospondin 4 (Thbs4), a protein involved in the endoplasmic reticulum (ER) stress response by activating the transcription factor 6α (Atf6α). Both Atf6α and its downstream targets were also differentially expressed in SHR vs. WKY. These mRNAs were confirmed at the protein level by western blot. These data revealed a number of genes and miRNAs in mesenteric arteries of SHR, which had not been related to hypertension previously. We also identified a link between the ER stress response and hypertension.

P41

Repression of autophagy in gastric epithelial cells infected with H. pylori induces CD44 expression through the accumulation of CagA oncoprotein

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Objective: *Helicobacter pylori* effector protein CagA acts as an oncoprotein to be identified in relation to human gastric carcinogenesis. Translocated CagA was degraded by autophagy. CagA-degrading autophagy was induced by VacA via binding to low-density lipoprotein receptor-related protein-1 (LRP1). However, the autophagy was suppressed specifically in CD44v9-positive gastric cancer stem-like cells, resulting in intracellular CagA accumulation. The present study was conducted to examine LRP1 signals to the autophagy and to the CD44 expression.

Methods: Autophagy was assessed by autophagic flux assay. The participation of LRP1 in autophagy was assessed using an *lrp1*-siRNA. Expression of Lamp1, lysosomal membrane protein, was assessed by western blotting and Chip assay.

Results: Lamp1 expressions were significantly increased during the autophagy. Lamp1 expressions were repressed by treatment with *lrp1*-siRNA. Binding of nuclear-translocated LRP1 to the LAMP1 promoter region was enhanced during the autophagy. Specific knockdown of *lamp1* decreased the formation of autophagolysosome, leading to the accumulation of intracellular CagA. These results suggested that LRP1 functions as a transcriptional factor for LAMP1 and induces autophagic degradation of CagA by promoting autophagolysosome formation. Additionally, LRP1-binding protein which binds to LRP1 and suppresses transcription of LAMP1 was identified. In the cells overexpressing the LRP1-binding protein, autophagy was suppressed and CagA accumulated. The accumulation of intracellular CagA caused increased CD44 expression in LRP1-binding protein overexpressing cells.

Conclusion: Overexpression of the LRP1-binding protein suppressed autophagy, resulting in intracellular CagA accumulation. Accumulation of intracellular CagA enhanced the CD44 expression, a marker for cancer stem-like cells.

P42
Chemoprevention of astragalus in lung adenocarcinoma

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Lung cancer is the most common malignancy, and also a major cause of cancer-related mortality worldwide. Although the progression of lung cancer has been inhibited by chemotherapeutic agents, overall survival of patients still remains unpleasant in lung cancer prognosis. The side effects of chemotherapy and drug resistance mainly contribute to the high mortality of lung cancer. Therefore, the application of low-toxicity and great effectiveness agents, which derived from diet, fruits or plants, would provide a novel strategy for lung cancer prevention. *Astragalus membranaceus* (Fisch.) Bge, the commonly used Chinese herbal medicines, could improve body homeostasis by antioxidant and anti-inflammation. However, the efficacy of Astragalus in cancer prevention and the mechanism underlying still remains unclear. In our study, we found that the water extraction of Astragalus could significantly reduce the incidence of lung cancer in B[a]P-induced lung adenocarcinoma animal model. Further study demonstrated that Astragaloside IV, the major compound in Astragalus, could induce tumor apoptosis by upregulating the expression of Bax, and decreasing that of Bcl-2 in A549 cells. Other than that, it could inhibit the migration and invasion of lung adenocarcinoma cells via inducing the expression of E-cadherin while downregulating the expression of N-cadherin, Nanog, Snail and Vimentin. Moreover, the significant decreased protein expression of B-catenin implied that the activation of Wnt/B-catenin pathways by Astragaloside IV would be the mechanism responsible for the tumor inhibition. In summary, the water extraction of Astragalus could significantly decrease the incidence of lung cancer through activating the Wnt/B-catenin pathways.

P43
Caudatin induces caspase-dependent apoptosis in human glomia cells with involvement of mitochondrial dysfunction

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Objectives: Caudatin, one of the species of C-21 steroidal glycosides mainly isolated from the root of Cynanchum bungei Decne, exhibits potent anticancer activities. However, the mechanism remains poorly defined. In the present study, the growth inhibitory effect and mechanism of caudatin on human glioma cells were evaluated *in vitro*.

Methods: Several methods of cell biology and molecular biology *in vitro* were employed.

Results: The results revealed that caudatin treatment significantly induced cell growth inhibition of U251 and U87 cells in a time- and dose-dependent manner. Flow cytometry analysis showed that caudatin-induced apoptosis was confirmed by the increasing of Sub-G1 peak through caspase activation. Moreover, caudatin treatment resulted in mitochondrial dysfunction of U251 cells, as convinced by dissipation of mitochondrial membrane potential through regulation of the Bcl-2 Family. Z-VAD-fmk (caspase inhibitor) distinct weaken caudation-induced apoptosis and caspase activation, further confirmed that caudatin induced U251 cells apoptosis in a caspase-dependent manner.

Conclusions: Our findings indicate that caudatin may act as a potential anticancer agent against human glioma cells through induction of caspase-dependent apoptosis in human glomia cells with involvement of mitochondrial dysfunction.

CEREBRAL CIRCULATION

P44
Enhanced neuroprotective effects against ischemic brain injury by intranasal delivery of granulocyte colony-stimulating factor in rats

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Objectives: Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth factor with strong neuroprotective properties. However, it has limited capacity to cross the blood-brain barrier and thus potentially limiting its protective capacity. Recent studies demonstrated that intranasal drug administration is a promising way in delivering neuroprotective agents to the central nervous system. The current study therefore aimed at determining whether intranasal administration of G-CSF increases its delivery to the brain and its neuroprotective effect against ischemic brain injury.

Methods: Transient focal cerebral ischemia in rat was induced with middle cerebral artery occlusion.

Results: Our resulted showed that intranasal administration is 8–12 times more effective than subcutaneous injection in delivering G-CSF to cerebrospinal fluid and brain parenchyma. Intranasal delivery enhanced the protective effects of

G-CSF against ischemic injury in rats, indicated by decreased infarct volume and increased recovery of neurological function. The neuroprotective mechanisms of G-CSF involved enhanced upregulation of HO-1 and reduced calcium overload following ischemia. Intranasal G-CSF application also promoted angiogenesis and neurogenesis following brain ischemia.

Conclusions: G-CSF is a legitimate neuroprotective agent and intranasal administration of G-CSF is more effective in delivery and neuroprotection and could be a practical approach in clinic.

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Pre-reperfusion of curcumin could protect blood-brain barrier against I/R Injury associated with Nrf2, NF-kappa-B, and caspase-3 expressions in Transient MCAO Rat Model

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Background: Oxidation, inflammation, and apoptosis are three critical factors for the pathogenic mechanism of cerebral ischemia/reperfusion (I/R) damage. Curcumin has been elucidated to exhibit substantial biological properties via anti-oxidation, anti-inflammation and anti-apoptosis effects, however, its molecular mechanism against cerebral I/R injury remains unclear.

Objective: To investigate the effects of curcumin on cerebral I/R injury associated with water content, infarction volume, blood-brain barrier (BBB) disruption and the expression of NF-kappa-B, caspases-3, and Nrf2.

Methods: The middle cerebral artery occlusion (MCAO, 1-hour occlusion and 24-hour reperfusion) was performed in male Wistar rats ($n = 64$) as representing cerebral I/R injury model. In MCAO+CUR group, rats were received curcumin administration (300 mg/kg BW, ip.) at 30-min after occlusion. The same operated procedures were performed in SHAM rats without MCAO occlusion. At 24-hour post-operation, all of these parameters including neurological deficit scores, BBB disruption, water content, and infarction volume were determined. Brain tissue NF-kappa-B, caspases-3, and Nrf2 were assayed by immunohistochemistry.

Results: Compared with SHAM group, the BBB disruption, neurological deficit scores, brain water content and infarction volume were severely demonstrated in MCAO group. NF-kappa-B and caspases-3 were enhanced in MCAO group. However, in MCAO+CUR group, the upregulated Nrf2, an anti-oxidation related protein, collaborating with the decline of other biomarkers were significantly observed.

Conclusion: The protective effects of curcumin against cerebral I/R injury are attributed by its anti-oxidation, anti-inflammation and anti-apoptosis where as its mechanisms involved the elevation of Nrf2 and the down-regulation of NF-kappa-B and caspases-3.

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Exercise training ameliorates microvascular deterioration and VEGF signaling downregulation in aging rat brain

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During advancing age, reduction of basal blood flow and microvascular loss in the brain contributes tissue perfusion insufficiency. Capillary loss in aged tissues appears to be related to downregulation of vascular endothelial growth factor (VEGF) signaling. Regular exercise has been reported to have beneficial effects to brain health in aging individuals. Therefore, the present study aimed to investigate effect of exercise training on age-induced cerebromicrovascular alteration with modulation of VEGF signaling. Male Wistar rats were divided into 3 groups; sedentary-young (4 months), sedentary-aged (22 months) and exercised-aged (22 months). Exercise program included swimming training 5 days/week for 8 weeks. *In situ* study of brain microvascular networks was performed to determine regional blood flow (BF) (by Doppler flowmetry) and microvascular vascularity (MV) (using a laser scanning confocal fluorescent microscopy). Level of VEGF, VEGFR2, Akt and PI3K in isolated brain microvessels were determined by immunoassay. MV and BF was significantly lower in the sedentary-aged rats compared with the sedentary-young rats, whereas that in the exercised-aged rat was significantly higher than the sedentary-aged rats. The protein level of VEGF and VEGFR2 were significantly lower in the sedentary-aged rats compared with the sedentary-young rats, whereas those in the exercised-aged rats were significantly higher than those in the sedentary-aged rats. The expression of phosphorylated Akt and PI3K corresponded to the alterations in the VEGF and VEGFR2 levels. These findings suggest that exercise training ameliorates cerebromicrovascular deterioration and VEGF signaling downregulation during aging.

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Interstitial transport in the rodent brain

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Alzheimer's disease is associated with failure of amyloid-beta removal, causing its deposition along the brain vasculature. We visualized the removal of tracers from the rodent brain. We injected a mix of FITC-dextran (MW 500 kD) and TR-dextran (MW 3 kD) mix into the cisterna magna (CM) or striatum for 30 mins and followed their fate by confocal microscopy and an imaging cryomicrotome, with additional labeling of vessels. After infusion in the striatum, dyes spread in the parenchyma, and found their way through the interstitium to the closest lateral ventricle. From there, they reached the ventricular system, cisterns and subarachnoid space (SAS). The small dextran was also taken up by parenchymal cells and the choroid plexus. Dyes occasionally associated with arteries around the injection site. Following CM infusion, dyes dispersed throughout the SAS, the cisterns and along PVS. The large dye was confined to the SAS and PVS of cortical vessels, whereas the small dye also crossed the pia mater. Neither dye was detected in the ventricular system. Dyes were extruded from the SAS via the cribriform plate into the nose. These data reveal a flow of interstitial fluid from the parenchyma to the ventricular system, from where dyes reach the SAS with the cerebrospinal fluid. From the SAS, dyes disperse along perivascular spaces. This might be a consequence of mixing by pulsations. Disturbances in this transport pathway could influence the physiological drainage of Amyloid-beta, contributing to the pathophysiology of Alzheimer's disease.

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Abnormal ROK activity contributes to the dysfunctional myogenic response of cerebral arteries of type 2 diabetic Goto-Kakizaki rats

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The myogenic response of cerebral resistance arteries involving constriction and relaxation to increased and decreased intravascular pressure, respectively, is a key physiological mechanism controlling blood flow to the brain. Previous studies have shown that this mechanism is dysfunctional in animal models of type 2 diabetes (T2D), but the underlying defects remain to be defined. Pressure-dependent activation of Rho-associated kinase (ROK) leading to phosphorylation of the myosin targeting subunit 1 (MYPT1), suppression of MLCP activity, and augmentation of myosin light chain (LC20) phosphorylation is a key mechanism in myogenic force generation. Here we tested the hypothesis that inappropriate ROK-mediated phosphorylation of MYPT1 contributes to the dysfunctional myogenic response of endothelium-denuded posterior cerebral arteries of T2D Goto-Kakizaki (GK) rats. Vessels of GK and Wistar (WR) control were studied by pressure myography in the presence or absence of ROK inhibitor (H1152, 0.5 MicroM) and western blotting was employed to quantify LC20, MYPT1-T697 & -T855 phosphorylation at 10, 60 and 120 mmHg and ROK2 expression. Enhanced basal myogenic tone was observed in GK vessels that was abolished by H1152. LC20 and MYPT1-T855 phosphorylation were elevated at 10 mmHg, and no evidence of a pressure-dependent change was detected in GK vessels. H1152 reversed the enhanced basal phosphorylation of LC20 and MYPT1. Our findings suggest that the myogenic response of cerebral arteries of T2D GK rats is dysfunctional due to an elevated basal and a lack of pressure-dependent ROK-mediated phosphorylation of MYPT1.

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Microcirculatory disturbance after subarachnoid hemorrhage

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Purpose: The mechanism of brain damage immediately after subarachnoid hemorrhage (SAH) is not well understood. Platelet-leukocyte-endothelial cell interactions were observed in venules 2 hours after SAH 1). We investigated cerebral microcirculation immediately after SAH.

Methods: Femoral artery and vein were cannulated in mice. Q-dot 655 nanocrystal (Q21021MP; Invitrogen) or rhodamine-6G was injected from the cannulated femoral vein, after a craniotomy at the parietal bone without cutting dura matter. SAH was induced at a prone position by using the endovascular perforation model 2). We measured diameter in precapillary arterioles and blood cell flow in arterioles and capillaries with a line scan method using two-photon laser scanning microscopy at a depth of about 100 micrometer 3,4), at the time when SAH was induced at the skull base and 1 hour after SAH.

Results: When SAH occurred, the cell flows decreased or disappeared in precapillary arterioles and capillaries, and gradually recovered. The velocities of blood cells also

decreased in capillaries 1 hour after SAH, and rolling and adherent leukocytes block the blood cell flow in capillaries. Administration of a P-selectin-blocking antibody increased the flow velocities of blood cells significantly compared to non-P-selectin treated group.

Conclusion: The cerebral blood flow decreased in precapillary arterioles and capillaries, immediately after the SAH was induced at the skull base.

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P50

Vasoreactivity of intracortical penetrating arteries of the cerebral cortex in response to cortical spreading depression and hypercapnia in anesthetized mice

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Cortical spreading depression (CSD) induces marked hyperemia after a transient drop of regional cerebral blood flow (rCBF), accompanied with pial arterial constriction and subsequent dilation. To further understand the microcirculatory response associated with CSD, we examined the temporal diametric changes in intracortical penetrating arteries. In urethane-anesthetized and artificially ventilated Tie 2-GFP mice having fluorescence in endothelial cells ($n = 14$), the diameter of single penetrating arteries at various depths (0, 150 and 300 μm) was measured using two-photon microscopy, with simultaneous recording of rCBF by means of laser Doppler flowmetry during repeated passages of CSD induced by application of KCl. The first CSD elicited marked constriction ($-26 \pm 23\%$) and subsequent dilation ($+22 \pm 19\%$) throughout the arteries, which corresponded temporally to a transient drop and subsequent increase of rCBF. Second or later CSD elicited marked dilation ($+40 \pm 24\%$) with smaller or no constriction throughout the arteries, which corresponded temporally to the increase of rCBF. The extent of vasodilation was negatively correlated with the basal diameter of the identical artery ($r = -0.553$). Inhalation of 5% CO_2 caused increase of rCBF ($30 \pm 17\%$) and general dilation ($+14 \pm 11\%$) which was negatively correlated with the basal diameter ($r = -0.449$) before CSD induction. However, these responses were significantly suppressed ($14 \pm 8\%$ and $+2 \pm 6\%$, respectively) after CSD passage with loss of the correlation ($r = 0.032$). These results indicate that CSD-induced rCBF changes are affected by changes in the diameter of intracortical penetrating arteries, showing a significant negative correlation to basal diameter despite elimination of responsiveness to hypercapnia.

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Dynamics of red blood cells in intraparenchymal capillaries and arterial diameter during cortical spreading depression observed with high-speed camera confocal fluorescence microscope in anesthetized mice

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Cortical spreading depression (CSD) involves mass depolarization of neurons and glial cells, followed by sustained suppression of spontaneous neuronal activity, and dramatically affects metabolism and circulation. To further understand the microcirculatory response during CSD, we observed and analyzed the temporal changes of red blood cell (RBC) velocity and its distribution in individual capillaries by two-dimensional spatial analysis. In urethane-anesthetized and artificially ventilated Tie 2-GFP mice having fluorescently labeled endothelial cells ($n = 17$), intraparenchymal images (50 μm from the surface) were obtained using a high-speed camera laser-scanning confocal fluorescence microscope at 125 frames/s for 1 min. The velocity of FITC-labeled RBCs in each capillary was automatically evaluated with our original Matlab domain software (KEIO-IS2). Application of KCl elicited repeated CSD episodes. During the first CSD passage, RBC velocity in capillaries was significantly decreased ($-24 \pm 35\%$), which temporally corresponded to marked arterial constriction ($-22 \pm 18\%$), evaluated from the identical images, and a transient drop of regional cerebral blood flow (rCBF), measured with laser Doppler flowmetry. During passage of subsequent CSD waves, RBC velocity was significantly raised ($+16 \pm 42\%$) prior to arterial dilation and increase of rCBF. Just after CSD passage, RBC velocity returned to around baseline, despite arterial dilation ($+17 \pm 14\%$) and hyperperfusion ($24 \pm 20\%$ compared to pre-KCl level). RBC velocity was somewhat increased ($6 \pm 36\%$) during the CSD

interval period, with slight arterial constriction (6 ± 10%) and decreased rCBF (20 ± 9%) (post-CSD oligemia). These results suggest that a local control mechanism of capillary perfusion, which does not necessarily involve net rCBF changes, may mediate the CSD-induced microcirculatory response.

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Implications of alphaV-beta3 integrin signalling in the regulation of Ca2 + waves and myogenic tone in cerebral arteries

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The myogenic response is central to blood flow regulation in the brain. Its induction is tied to elevated cytosolic [Ca2+], a response primarily driven by voltage-gated Ca2 + channels and secondarily by Ca2+ wave production. While the signaling events leading to the former are well studied, those driving Ca2+ waves remain uncertain. We postulated that alphaV-beta3 integrin signaling is integral to the generation of pressure-induced Ca2+ waves and cerebral arterial tone. This hypothesis was tested in rat cerebral arteries using the synergistic strengths of pressure myography, rapid Ca2+ imaging and western blot analysis. GRGDSP, a peptide that preferentially blocks alphaV-beta3 integrin, attenuated myogenic tone in a manner consistent with SR Ca2+ release playing a modest role. The RGD peptide was subsequently shown to impair Ca2+ wave generation and MLC20 phosphorylation, the latter of which was attributed to the modulation of MLCK and MLCP via MYPT1-T855 phosphorylation. Subsequent experiments revealed that elevated pressure enhanced PLCgamma1 phosphorylation in a RGD dependent manner and that phospholipase C inhibition attenuated Ca2+ wave generation. Direct inhibition of IP3Rs also impaired Ca2+ wave generation, myogenic tone and MLC20 phosphorylation, partly through the T-855 phosphorylation site of MYPT1. Our investigation reveals a hitherto unknown role for alphaV-beta3 integrin as a cerebral arterial pressure sensor. The membrane receptor facilitates Ca2+ wave generation through a signaling cascade involving PLCgamma1, IP3 production and IP3R activation. These discrete asynchronous Ca2+ events facilitate MLC20 phosphorylation and myogenic tone by influencing both MLCK and MLCP activity.

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Smooth muscle/endothelial KIR channels tune electrical communication in cerebral arteries

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Global blood flow control is enabled by the conduction of charge along the arterial wall. The distance over which electrical phenomena spread is governed by several factors, among which ion channels play a crucial role. In this study, we determined the role of inward rectifying K+ channels (KIR) in setting membrane conductance and controlling charge distribution along the arterial wall. Middle cerebral arteries (100 micron diameter) from hamster were first studied with a standard conduction protocol. A focal KCl stimulus elicited a constrictor response that conducted robustly, with a decay constant of ~0.5 mircon/100 microns vessel length. Blockade of KIR by Ba2 + facilitated decay equivalent to ~1 mircon/100 microns vessel length; selective inhibition of other K+ channels had no effect. Patch clamp electrophysiology and Q-PRC highlighted the presence of a KIR current in smooth muscle comprised of KIR2.1/2.2 subunits. Incorporating this current into an electrical model revealed that this conductance was too small to account for the change in conduction decay; consequently another KIR current must be present. Electrophysiology and Q-PCR confirmed a second KIR current in the endothelium. Computational modeling subsequently confirmed that simultaneous inhibition of both currents had a greater effect on conduction decay, the result of a sizable voltage shift increasing feedback from voltage dependent- and Ca2 + activated K+ channels. In summary, our observations indicate that KIR channels are present in endothelial and smooth muscle cells, and together these K+ conductances synergistically tune electrical communication. Supported by CIHR.

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Novel intact *ex vivo* preparation of pressurized intracerebral arterioles and capillaries reveals conducted upstream vasodilation following application of neurovascular coupling agents onto capillaries

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We previously developed a unique approach to isolate, pressurize and study mouse intracerebral parenchymal

arterioles (PA) (Dabertrand *Circ Res* 2012, *PNAS* 2015). We extended this approach by pressurizing PA with a capillary ramification left intact and sealed by compressing its extremity with a glass micropipette. We then compared the vascular reactivity in PA and capillary plus PA (CAPA) preparations isolated from the same animals ($n = 7$). Pressurization of the entire tree induced vasoconstriction (i.e. myogenic tone) in the PA segment (38.6%) as in PA without capillaries (37.9%). Bath elevation of extracellular $[K^+]_e$ concentration from 3 to 10 mM, known to activate strong inward rectifier K^+ (K_{ir}) channels, caused almost a maximal dilation of arterioles in PA and CAPA preparations, 74.3% and 68.9%, respectively. Similarly, the IK and SK channel agonist, NS309 (1 microM), strongly, rapidly, and reversibly dilated PA, with and without capillaries (84.9% and 88.1%), suggesting that PA endothelial function is intact. We then investigated the possibility of retrograde signaling from capillaries to upstream arterioles by local application of vasoactive substances. Pressure ejection of NS309 onto capillaries had no effect on PA. However application of 10 mM $[K^+]_e$ to the capillaries led to a rapid dilatory response (lag time ~2 s) in the upstream PA (53.4%; $n = 6$), which was abolished by the K_{ir} inhibitor Ba^{2+}. Prostaglandin E2 also induced upstream, Ba^{2+}-sensitive arteriolar dilation (38.6%; $n = 5$; lag time ~8 s). These data support the concept that capillary K_{ir} channels act as K^+ sensors to initiate retrograde electrical signals to dilate upstream arterioles.

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Retrograde regenerative electrical signaling through capillary K_{IR} channels regulates blood flow into the brain

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Capillaries are in close proximity to every neuron in the brain to rapidly deliver nutrients. We tested the hypothesis that the capillary network acts as a sensory web to detect neuronal activity through activation of strong inward rectifier K^+ ($K_{IR}2$) channels by external K^+. To investigate this, ion channel currents were measured in freshly isolated brain capillary endothelial cells (cECs) using the patch clamp technique, and hemodynamics were observed *in vivo*.

cECs possessed K_{IR} currents sensitive to 100 microM Ba^{2+} and 20 microM ML133. Unlike ECs from arteries, small- and intermediate-conductance Ca^{2+}-activated K^+ (SK and IK) channels were absent from cECs. Picospritzing 10 mM K^+ onto capillaries *in vivo* evoked hyperemia within seconds, measured as increased capillary red blood cell flux and velocity, which was sensitive to 100 microM Ba^{2+}.

$K_{IR}2$ channels are robustly activated by external K^+ and membrane hyperpolarization and, therefore, are ideally suited to sense small changes in external K^+, a byproduct of neural activity,and to translate this into a regenerative hyperpolarizing signal (1). This electrical signal would travel upstream to parenchymal arterioles and pial arteries to induce vasodilation. The absence of SK and IK channels (which have prominent roles in all arteriolar ECs investigated) in cECs increases the efficiency of K_{IR}-mediated electrical signaling by increasing membrane resistance. Overall, this mechanism would rapidly translate neural activity into functional hyperemia.

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Reference: 1. Longden & Nelson (2015) *Microcirculation* 22 (3): 183–196.

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Focal cerebral ischemic stroke results in endothelial BK_{Ca} expression and altered function, with no change in TRPV4 function, in middle cerebral artery

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Large conductance calcium-activated potassium channels (BK_{Ca}) are absent in healthy intact artery endothelium, but have a stress-induced expression following chronic hypoxia and cell isolation/culture. BK_{Ca} can form complexes with transient receptor potential vanilloid type 4 channels (TRPV4) that are implicated in control of vessel tone and in some models, stroke etiology. Confocal-immunohistochemistry determined BK_{Ca}-alpha and -beta1, and TRPV4 distribution in adult male SD rat middle cerebral artery (MCA) in acute endothelin-induced stroke and chronic hypoxia; and hypoxia following stroke, and in human cerebral pial arterioles from terminal cerebral stroke and control subjects. MCA pressure myography and BK_{Ca} and TRPV4 activators and blockers determined channel function. BK_{Ca}-alpha and -beta1 were absent in endothelium of MCA

from untreated and saline-treated control rats, but present in stroke, hypoxia and hypoxia following stroke. Smooth muscle BK_{Ca}-alpha and -beta1 were present in control and upregulated in stroke, hypoxia and hypoxia following stroke. Endothelial TRPV4 were present in control and upregulated in stroke and hypoxia, but unchanged in hypoxia following stroke. Smooth muscle TRPV4 were present in control, and unchanged in hypoxia; with reduced expression in stroke and hypoxia following stroke. Endothelial BK_{Ca}-alpha was absent in control human pial arterioles, present in stroke; and upregulated in smooth muscle of stroke. In rat MCA, basal BK_{Ca} regulating myogenic tone is increased in stroke; whereas TRPV4 function is unchanged. Intact MCA endothelium can be induced to express functional BK_{Ca}, and thus targeting of BK_{Ca} and its related signaling pathways is a rational approach to correct altered cerebral perfusion.

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Intranasal delivery of calcitonin gene-related peptide enhances arterial NO-cGMP pathway and reduces cerebral vasospasm after experimental subarachnoid hemorrhage

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Calcitonin gene-related peptide (CGRP)is quickly consumed from the vessel wall after subarachnoid hemorrhage (SAH). The purpose of this study is to investigate whether intranasal delivery of CGRP stimulates NO generation, leading to synergistic effects of vasodilation in rats after SAH. Adult Wistar rats were randomly divided into four groups: sham-operated, SAH, SAH treated with intranasal saline, and SAH treated with intranasal CGRP. Compared with sham group, SAH group demonstrated a decreased BA diameter and an increased thickness of BA wall. Intranasal CGRP treatment significantly attenuated the vasospasm while intranasal saline failed to do so. Nitrate reductase assay demonstrated that rats in SAH and intranasal saline groups showed decreased NO contents. Intranasal CGRP treatment partially restored NO contents, accompanied by an increased content of arterial cGMP. The levels of arterial eNOS mRNA was decreased in both SAH group and intransal saline treated SAH groups, and eNOS mRNA was partially recovered in intranasal CGRP+SAH group. It was concluded that impaired NO-cGMP pathway may lead to the cerebral vasospasm after SAH; and intranasal delivery of CGRP protects the brain against cerebral vasospasm via stimulating NO product and activity.

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Three-dimensional microcirculation imaging with fluorescence red blood cells in anesthetized rat cerebral cortex

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To characterize microcirculatory behaviors of the red blood cells (RBCs) in the multiple brain capillaries simultaneously, we developed an animal model in which the RBCs express green fluorescent protein (GFP). The flowing RBCs were visualized with two-photon microscopy excited at 880 nm through a cranial window with intact dura, made over the somatosensory cortex in the isoflurane-anesthetized rats. Three dimensional imaging was conducted in an either time domain (XYt scan) or a space domain (XYZ scan). For a XYt scan imaging, an image (256 by 256 pixels) was sequentially acquired at a rate of 50 frame per sec, whereas a z-stack image (512 by 512 pixels) was acquired with a step size of 2 μm up to a depth of 400 μm from the cortical surface for a XYZ scan imaging. We observed that the RBCs elongated during a passage through a narrow capillary, and an occasional stop of the RBC flows. With a combination of a red fluorescent marker in the blood plasma, the concurrent fluorescence imaging of the blood plasma and RBCs successfully showed a redistribution of the RBCs across the capillaries under resting conditions. However, the regulatory mechanism in switching at a fork in the capillary networks remains unclear. The local cellular activity and/or hemodynamic factors should be further determined to unveil the regulatory mechanisms of the spatiotemporal microvascular flow.

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Preliminary study on mitophagy and its role after ischemic injury in rats

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Background: Mitochondria dysfunction is implicated in diverse conditions. Mitochondrial dynamics has attracted increasing attention as to its relationship with mitochondria autophagy (mitophagy). However, mitochondrial fission and its role in clearance of injured mitochondria in ischemic injury, have not been elucidated yet.

Methods: Hypoxic/ischemic (H/I) injury was induced both by permanent middle cerebral artery occlusion (pMCAO) in rats or by oxygen glucose deprivation (OGD) in PC12 cells. To assess mitophagy and their role in ischemic injury, various approaches were used, including behavior test, TTC-staining, electron microcopy, western blot and immunofluorescence staining.
Results: We found that H/I led to fragmentation of mitochondria and induction of mitophagy. Inhibition of Drp1 by Mdivi-1 or siRNA resulted in accumulation of damaged mitochondria through selectively blocking mitophagy without affecting non-selective autophagy. Mdivi-1 increased infarct volume and aggravated neurological deficits. We further demonstrated inhibiting Drp1 contributed to mitochondria-mediated ROS generation, cyt-c release and caspase-3 activation.
Conclusion: Taken together, we proved that Drp1-dependent mitophagy was triggered after H/I, which was involved in removal of damaged mitochondria. Thus, Drp1 related pathway involved in selective removal of dysfunctional mitochondria is proposed as an efficient target for treatment of cerebral ischemia.

ION CHANNELS AND TRANSPORTERS

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Activation of the small-conductance calcium-activated potassium (SK) channels in freshly isolated coronary arterial endothelial cells by shear stress

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We examined the effects of laminar shear stress on ion channel activation in freshly isolated bovine coronary endothelial cells (BCEC). BCEC were seeded in glass capillary of 1 × 1 mm size and the roof at the end 1–2 mm was removed. BCEC attachment was allowed for 1–2 hours in culture medium and the tubes were mounted in a chamber on the stage of an inverted microscope. The capillary tube was connected to a precision peristaltic pump. Ionic currents in the BCEC exposed at the end of the capillary tube were studied using patch clamp techniques in whole-cell configuration. BCEC had very little K^+ currents at baseline. Exposure to shear stress (10 dynes/cm^2) resulted in a 4-fold increase in K^+ currents, most of which were SK currents inhibited by apamin (200 nM). The SK (apamin-sensitive) currents in BCEC were also activated by exposure to isoproterenol (1 μM) or to the TRPV4 activator, GSK1016790A (150 nM). Sucrose density gradient fractionation showed both SK3 and TRPV4 channels are targeted to the caveolin-rich low buoyant density fraction where SK3 and TRPV4 can be co-immunoprecipitated. Shear stress-mediated vasodilation in isolated mouse coronary arteries was significantly blunted after pre-incubation with apamin (200 nM) or with the TRPV4 inhibitor, HC-067047 (200 nM). Shear stress-mediated Ca^{2+} increase in BCEC was also abolished by treatment with HC-067047. These results indicate that TRPV4-SK3 form a signaling complex to mediate vasodilation in response to shear stress.

P61
TRPV1-mediated Ca^{2+} influx and constriction of the meningeal vasculature

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TRPV1 channels play an important role in pain sensation and are activated by capsaicin, heat, acidic pH and inflammatory agents such as histamine and bradykinin. Although extensively studied in sensory nerves, the role of TRPV1 channels in other organ systems, including the vasculature, has yet to be fully understood. Here, we examined the hypothesis that activation of smooth muscle (SM) TRPV1 channels causes increased Ca^{2+} influx and constriction of the middle meningeal artery (MMA). Consistent with this hypothesis, capsaicin caused a sustained and repeatable constriction (EC_{50} ~100 nM) of isolated pressurized MMA. Interestingly, capsaicin did not constrict small diameter cerebral or mesenteric arteries. Capsaicin-induced MMA constriction was abolished by the TRPV1 antagonist capsazepine and SB366791, removal of extracellular Ca^{2+}, and reduced by ~ 34% by the voltage-dependent calcium channel (VDCC) blocker diltiazem. Capsaicin also caused a significant and sustained increase in intracellular Ca^{2+} in MMA SM freshly isolated from transgenic (acta2-GCaMP5-mCherry) mice expressing the ratiometric genetically-encoded calcium indicator, GCaMP5-mCherry, driven by the SM-specific promoter acta2. Further, unitary optical SM TRPV1 Ca^{2+} signals (TRPV1 sparklets) were observed using confocal microscopy in MMA slit open and loaded with the Ca^{2+} indicator dye, Fluo-4. Here were demonstrate the functional presence of TRPV1 channels in SM of MMA, but not mesenteric or cerebral arteries. Calcium influx triggered by SM TRPV1 channel activation causes profound constriction of MMA. Supported by Totman Research Trust, P. Martin Endowment, NIH P01HL095488, P30RR032135 & P30GM103498 and AHA 14SDG20150027.

P62
Complex signalling pathways determine the role of Kv7 channels in relaxations of the rat mesenteric artery

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The Kv7 family of voltage gated potassium channels have been established as important contributors to vasorelaxations in various vascular beds. In particular, Kv7 channels have been shown to contribute to Gs-coupled, cyclic AMP dependent vasorelaxation, therefore this study delved further into the mechanisms involved in this process. In isometric tension studies, relaxations in rat mesenteric arteries to the beta adrenoceptor agonist isoproterenol produced relaxations that were sensitive to the pan-Kv7 channel blocker linopirdine, but not the Kv7.1 selective blocker HMR1556. Further studies showed that isoproterenol mediated relaxations were attenuated in the presence of H89 (protein kinase A inhibitor) and ESI-09 (exchange protein activated by cAMP (EPAC) inhibitor) but not by gallein (beta gamma subunit inhibitor). Relaxations to the cell permeable EPAC-selective activator 8-pCPT-2-O-Me-cAMP-AM were also attenuated in the presence of linopirdine but not by gallein. EPAC mediated relaxations were also attenuated in the presence of paxilline (BKCa channel inhibitor), an effect which was additive to that of linopirdine. Furthermore, both 8-Br-cAMP and 8-pCPT-2-O-Me-cAMP-AM enhanced currents from overexpressed Kv7.4 channels, the isoform which is particularly crucial for vascular control. Whilst gallein attenuated 8-Br-cAMP enhancement of these currents, it had no effect on 8-pCPT-2-O-Me-cAMP-AM mediated increases in Kv7.4 current. These findings demonstrate the complex signalling pathways which couple to Kv7 channels in the rat mesenteric artery.

P63
Activators of KCa channels enhance endothelium-dependent modulation of nerve-evoked constriction in rat mesenteric arteries

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The sympathetic nervous system and the vascular endothelium act in concert to regulate arterial diameter and thus blood flow and pressure. Vasoconstriction triggers a negative feedback response whereby activation of endothelial small (SKCa) and intermediate (IKCa) conductance calcium-activated potassium channels and/or release of endothelium-derived (NO) limit further reductions in vessel diameter. Thus, we have investigated whether small molecule activators of SKCa and IKCa channels can enhance endothelial modulation of nerve-evoked vasoconstriction in the rat perfused mesenteric bed. N-cyclohexyl-N-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-4-pyrimid-iamine (CyPPA) and naphtho[1,2-d]thiazol-2-ylamine (SKA-31), activators of SKCa and IKCa channels respectively, each caused concentration-dependent, reversible attenuation of nerve-evoked vasoconstriction without altering basal perfusion pressure. Block of NO signaling significantly enhanced nerve-mediated vasoconstriction and prevented the actions of CyPPA but did not significantly affect responses to SKA-31. In contrast, inhibition of transient receptor potential C3 (TRPC3) channels prevented the actions of SKA-31 but was without effect on responses to CyPPA. Selectivity of CyPPA and SKA-31 for SKCa and IKCa channels was demonstrated using apamin and 1-[(2-chlorophenyl) diphenyl methyl]-1H-pyrazole (TRAM-34) respectively.This data indicates the different functional roles of SKCa and IKCa channels in endothelium-dependent inhibition of nerve-evoked vasoconstriction of mesenteric arteries; SKCa channels appear to be involved in NO-mediated attenuation of vasoconstriction whereas activation of IKCa channels is linked to an NO-independent pathway. The ability of KCa channel activators to suppress nerve-evoked constriction supports the proposal that these channels may provide novel targets for drugs to overcome the endothelial dysfunction and increased sympathetic outflow associated with the development of hypertension.

P64
Investigation of the functional role of TRPC3 and TRPV4 in endothelium-dependent modulation of tone in rat mesenteric arteries

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Transient receptor potential (TRP) channels contribute to endothelial cytosolic calcium signaling. Roles for both transient receptor potential C3 (TRPC3) and vallinoid type 4 (TRPV4) in agonist-evoked endothelium-dependent vascular relaxation have been proposed. Thus, in this study

we have investigated the functional contribution of these channels to endothelium-dependent modulation of phenylephrine- and nerve-evoked increases in tone and to acetylcholine-evoked relaxation in rat mesenteric arteries. Using an immunohistochemical approach, TRPC3 and TRPV4 antibodies showed low level diffuse and punctate labeling in endothelial cells and absence in smooth muscle cells. 1-[4-[(2,3,3-Trichloro-1-oxo-2-propen-1-yl)amino]phenyl]-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (Pyr3), a selective inhibitor of TRPC3 channels enhanced nerve- and phenylephrine-induced increases in tone in endothelium-intact arteries but was without effect on acetylcholine-evoked relaxations. 1-(4-chloro-2-nitrophenyl) sulfonyl-4-benzylpiperazine (RN 1747), an agonist at TRPV4 channels, did not alter vascular tone when applied alone but did enhance endothelium-dependent relaxations to acetylcholine, an effect which was blocked by 3-([1,4′-bipiperidin]-1′-ylmethyl)-7-bromo-N- (1-phenylcyclopropyl)-2-[3-(trifluoromethyl)phenyl]-4-quinolinecarboxamide (GSK 2193874) a selective inhibitor of TRPV4 channels. However, GSK 2193874 alone did not alter nerve- of phenylephrine-induced responses or acetylcholine-evoked relaxations. Our findings indicate that TRPC3 channels are involved in endothelium-dependent modulation of smooth muscle contraction but do not appear to contribute to acetylcholine-evoked vasorelaxation. In contrast, inhibition of TRPV4 channels does not appear to alter vascular tone but activation of these channels does enhance vasorelaxation to acetylcholine. Thus, despite showing a similar pattern of endothelial localization, TRPC3 and TRPV4 make distinct contributions to regulation of arterial diameter in rat mesenteric arteries.Supported by HSFC and FoMD 75th Anniversary Award.

CORONARY CIRCULATION

P65
Micro channel array flow analyze research of elderly hypertension erythrocyte hemorheology

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Fund Project: China-Japan Cooperative Research Projects (No.XYW01-20100526):

Objective: Apply micro channel array flow analyzer (MC-FAN) to observe erythrocyte rheology, erythrocyte aggregation and erythrocyte deformability changing characteristics of elderly hypertension patients.

Methods: Testing 109 confirmed elderly hypertension patients, average age 65.81 ± 3.90, 21 elderly healthy, average age 66.54 ± 4.02. Apply micro channel array flow analyzer (MC-FAN) to observe erythrocyte rheology, and compare other laboratory relevant indexes between the two groups. Relevant analysis and multiple regression analysis were used to analyze between transiting times and other indexes.

Results: 1. The MC-FAN TTs of elderly hypertension patients was 33.86 ± 8.35 s. The MC-FAN TTs of elderly healthy was 28.25 ± 9.28 s, the comparison of statistics has significant extended ($p < 0.05$). 2. Erythrocyte aggregation index of elderly hypertension patients compare with elderly healthy, the comparison of statistics has significant extended ($p < 0.05$). 3. Elderly hypertension patients' micro channel array flow transiting times were respectively significant negative correlated with integrated erythrocyte deformation index. Transiting time was significant positive correlated with erythrocyte aggregation index, and was significant positive correlated with body mass index.

Conclusion: 1 Compared with the elderly healthy, the MC-FAN TTs of elderly hypertensive patients were significantly extended; the blood flow speed of elderly hypertensive patients were significantly decreased. 2 Elderly hypertension patients' micro channel array flow transiting times were respectively significant negative correlated with integrated erythrocyte deformation index, were significant positive correlated with erythrocyte aggregation index, red blood cell distribution width standard deviation, waistline and body mass index.

Keywords: Elderly hypertension; erythrocyte rheology; Erythrocyte deformability; Erythrocyte aggregation

P66
The effect of panax notoginseng component eluting stent on intimal hyperplasia in porcine coronary artery

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Objective: To observe and evaluate the effect of traditional Chinese medicine panax notoginseng component eluting stent (NES) on porcine coronary artery intimal hyperplasia.

Method: NES is a cobalt alloy stent using polymer materials as the carrier to control drug release. Eighteen healthy miniature porcines were randomly divided into NES group and siro1imus eluting stent (SES) group and bare metal stent (BMS) group. The same stent was implanted respectively into the anterior decending branch, left circumflex artery and right coronary artery. 30 days later, the porcine coronary artery angiography were examined, the blood vessels of implanted stent were extracted and measured.

Results: Optical coherence tomography (OCT) morphology analysis showed that mean lumen diameter, mean lumen area in NES and SES group were significantly larger than that in BMS stent group ($p < 0.05$); compared with BMS stent group, lumen area stenosis percentage in NES stent group area decreased by 32.87% (47.12%:31.63%), lumen diameter stenosis percentage in NES stent group decreased by 32.18% (43.20%:29.30%). Histopathological examination showed that there were different degree of hyperplasia in vascular intimas of NES and SES and BMS group, especially in BMS stent group. Scanning electron microscopy showed stents were covered completely with endothelium in BMS group, there were incomplete endothelial cover and accumulative white blood cells and acidophilic granulocyte in NES group and SES group.
Conclusion: NES can effectively inhibit neointimal proliferation and biological compatibility is favorable, which have potential clinical application prospect.

P67
H_2O_2-induced coronary collateral arterioles compensates NO-mediated small arteriolar endothelial dysfunction during coronary occlusion in diabetic dogs *in vivo*

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Background: It has been previously demonstrated that endothelial caveolin-1 play important roles of hydrogen peroxide (H_2O_2) production as an endothelium-derived hyperpolarizing (EDH) in mouse mesenteric arteries. We thus examined whether this mechanism is involved in the EDH-mediated responses as a compensatory mechanism of NO in diabetes mellitus (DM) during acute coronary occlusion.
Methods: Canine subepicardial coronary collateral small arteries (CSA> 100 μm) and arterioles (CA < 100 μm) were observed by a microscope. Experiments were performed during LAD occlusion (90 min) under the following conditions ($n = 6$ each); (i) control, (ii) DM and (iii) DM+K_{Ca}^{++} channel blockade (apamin+charybdotoxin). Myocardial levels of caveolin-1, H_2O_2 and eNOS were measured by ELISA.
Results: Although the levels of caveolin-1 in the LAD area were comparable between the control and DM groups, caveolin-1 levels were greater in coronary microvessels than in conduit arteries in the control group ($p < 0.01$). NO-mediated coronary vasodilatation of CSA by bradykinin significantly decreased in DM with the decrease of eNOS compared with control ($p < 0.05$), and were restored by compensation of EDH/H_2O_2 in CA ($p < 0.05$) with H_2O_2 production, and were diminished residual vasodilatation by K_{Ca}^{++}channel blockade.
Conclusions: NO-mediated vasodilatations of CSA during acute coronary occlusion are impaired in DM and are compensated by EDH/H2O2 of CA in dogs *in vivo*.

ENDOTHELIAL CELL BIOLOGY

P68
S-nitrosylation of VASP mediates the increase in microvascular permeability in response to pro-inflammatory agents

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Nitric oxide synthase (eNOS)-derived nitric oxide (NO) is a key factor in the regulation of microvascular permeability. Platelet-activating factor (PAF) increases permeability through a signaling cascade associated to eNOS. Recently, S-nitrosylation has been reported as a posttranslational modification induced by NO that depends on NO local concentration and eNOS location. We tested the hypothesis that pro-inflammatory agonists induce S-nitrosylation of vasodilator-stimulated phosphoprotein (VASP) as a mechanism to reduce We measured platelet activating factor (PAF) stimulated S-nitrosylation of VASP in cultured endothelial cells and in the mouse cremaster muscle *in vivo*. Tumor necrosis factor-alpha; (TNF-a) also increased VASP-S-nitrosylation. Deletion or depletion of eNOS in mice and endothelial cells abolished S-nitrosylation. PAF-induced S-nitrosylation of VASP associated with redistribution of VASP. To ascertain the importance of eNOS subcellular location in this process, we used ECV-304 cells transfected with cytosolic eNOS (GFPeNOSG2A) and plasma membrane eNOS (GFPeNOSCAAX). PAF induced S-nitrosylation of VASP in cells with cytosolic eNOS but not in cells wherein eNOS is anchored to the cell membrane. Using site-directed mutagenesis, we determined that the three cysteines present in VASP are important in the hyperpermeability response to PAF. Fondecyt 1130769.

P69
Myeloperoxidase modulates endothelial glycocalyx and influences vascular properties

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Background: It has been shown previously that myeloperoxidase (MPO), a highly cationic heme enzyme with pro-inflammatory properties, reduces the electrostatic repulsion between the negatively charged endothelial glycocalyx (EG) and neutrophils thereby mediating neutrophil-recruitment. We hypothesize that MPO can modulate the EG structure and affect vascular functions.

Materials and Methods: The murine cremaster-muscle model was used to characterize EG by intravital microscopy. The EG integrity was analyzed by determining the thickness of EG using the FITC-dextran exclusion technique. The carotid artery was used for systemic administration of the various substances. Local inflammation was induced by intrascrotal TNF-alpha injection, which triggers release of MPO from neutrophils.

Results: The EG thickness was reduced significantly in cremasteric capillaries after MPO treatment. A similar effect was also observed with catalytically inactive MPO and a cationic protein, protamine. To study this interaction further, heparin was administered in MPO-treated mice, which is known to release the immobilized MPO. The EG thickness observed was significantly higher than in mice treated only with MPO. Lastly, it was shown clearly that local inflammation does not induce a reduction in the thickness of EG in $MPO^{-/-}$ but in WT mice.

Conclusions: The results clearly show that systemically administered as well as neutrophil-secreted MPO modulates the EG thickness. This modulation seems to be independent of MPO's catalytic properties but dependent on its surface charge. This strengthens our hypothesis that MPO physically modulates the EG structure and this effect is reversed when the EG-bound MPO is removed.

P70
In vitro and *in vivo* confirmation of new concept of pulmonary blood flow-mediated CO_2 gas excretion in the lungs

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We studied physiological roles of flow through pulmonary arterioles in CO_2 gas exchange. We previously established human pulmonary arteriolar endothelial cells (HPAoEC). The cells demonstrated marked immunocytochemical staining of PECAM-1, VEGF R2, ACE-1, and CA type IV on their cell surface. Ten seconds shear stress stimulation caused the co-release of H^+ and ATP via the activation of F_1/F_O ATP synthase on the HPAoEC. F_1/F_O ATP synthase was immunocytochemically observed on the cell surface of non-permeabilized HPAoEC. Ten seconds shear stress stimulation also produced stress strength-dependent CO_2 gas excretion from the HPAoEC, which was significantly reduced by the inhibition of F_1/F_O ATP synthase or CA IV on the endothelial cell surface. Next, to further examine the validity of this concept in *in vivo* rabbit lungs, we investigated the effects of intra-mediastinal balloon catheterization-induced changes in pulmonary blood flow on the end-expiratory CO_2 gas pressure ($PECO_2$), the maximal velocity of the pulmonary artery, systemic arterial pressure, and heart rate of anesthetized rabbits. In the experiment in which small pulmonary arteries were subjected to stenosis, the $PECO_2$ fell rapidly. The flow-dependent changes in the $PECO_2$ were significantly reduced by the treatment with the F_1/F_O ATP synthase antibody. In conclusion, we have proposed a new concept of CO_2 exchange in the human lung, flow-mediated F_1/F_O ATP synthase-dependent H^+ secretion, resulting in the facilitation of a dehydration reaction involving HCO_3^- in plasma and the excretion of CO_2 gas from arteriolar endothelial cells.

GASBIOLOGY: O_2, NO, CO, H_2S, AND OTHER SMALL SIZE MEDIATORS

P71
Hydrogen sulfide-induced vasodilation involves activation of endothelial TRPV4 and BK channels in small mesenteric arteries

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Hydrogen sulfide (H_2S) is a recently described gaseous vasodilator produced within the vasculature by the enzymes cystothionine gama-lyase and 3-mercaptopyruvate. Previous data demonstrate that endothelial cells (EC) are the source of endogenous vascular H_2S production and are required for H_2S dilation. However, the signal transduction pathway activated by H_2S within EC has not been elucidated. Both TRPV4 and Large conductance Ca^{2+}-activated K^+ channels (BK channels) have shown in EC. Calcium influx through TRPV4 may result in activation of the BK channel. Thus, we hypothesized that H_2S-mediated vasodilation involves activation of TPRV4 and BK channels within the endothelium. In pressurized, phenylephrine-constricted mesenteric arteries, H_2S elicited a dose-dependent vasodilation blocked by inhibition of TRPV4 channels (GSK2193874A) or iberiotoxin

(100 nM) administered within the lumen. H_2S (1 microM) resulted in a TRPV4-dependent increase (1.8 fold) in localized calcium events in EC of pressurized arteries selectively loaded with Fluo-4 and Oregon Green. In pressurized EC tubes, H_2S (1 microM) and GSK101679A (30 nM) increased calcium events 1.8 and 1.5 fold, respectively. H_2S-induced an iberiotoxin-sensitive current measured using whole-cell patch clamp techniques in freshly dispersed EC. H_2S increased whole-cell K+ current from 10 pA/pF to 30 pA/pF at 150 mV. These results demonstrate that H_2S-mediated vasodilation involves activation of TRPV4 channels and BK channels within EC. Activation of TRPV4 channels may result in calcium events that result in the opening of endothelial BK channels, endothelial hyperpolarization, and a subsequent release of endothelial vasodilator factors.

P72
Deletion of heme oxygenase-2 exacerbates cerebral energy metabolism during acute focal brain ischemia

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Brain generates a micromolar order of carbon monoxide (CO) via heme oxygenase (HO) reactions. Previous study postulated that HO-2 generates CO in an O_2-dependent manner and reserve the capacity to dilate cerebral arterioles upon hypoxia through a mechanism involving the ability of CO to inhibit an H_2S producing system. By acting as an acute O_2 sensor within the neurovascular unit, HO-2 contributes to the maintenance of cerebral ATP levels against acute global hypoxia (Morikawa et al., PNAS, 109, 1293–1298). Here, we examined the hypothesis that the deletion of HO-2 exacerbates cerebral energy metabolism during acute focal brain ischemia. We conducted quantitative imaging mass spectrometry (Q-IMS) analysis for adenylates and lactate to decipher local cerebral responses of energy metabolism from wild-type (WT) and HO-2-null mice that underwent a 60-min occlusion of the left middle cerebral artery (MCAO). In ipsilateral hemispheres, MCAO caused elevation of AMP, ADP, and lactate, and depletion of ATP in both WT and HO-2 null mice. Ischemic core assigned by ATP contents (< 1.6 micro mol/g tissue) was larger in HO-2-null than WT mice. Striking differences were found in the contralateral hemispheres, where the levels of ADP, AMP and lactate in HO-2-null mice were higher than those in WT mice. These results suggest that the HO-2/CO system plays roles in protecting against ischemia not only at the ischemic core where the severe reduction of blood flow occurs, but it also protects even more effectively in the trans-hemispheric regions where only a subtle reduction of blood flow takes place.

P73
Hypoxia-inducible factors induce cystathionine β-synthase gene expression under hypoxia

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Cystathionine β-synthase (CBS) catalyzes homocysteine to cystathionine and produces hydrogen sulfide (H_2S) from cysteine. Recently, H_2S produced by CBS was shown to dilate blood vessels in mouse brain under acute hypoxia. (PNAS 2012 Morikawa et. al.) This mechanism is important to sustain blood flow and ATP level in mouse brain under acute hypoxia, but expression of CBS under sustained hypoxia have not been elucidated. To address this, we assessed the expression of H_2S producing enzymes under hypoxia. Interestingly, we found that CBS is induced under sustained hypoxia in U87-MG human glioblastoma, PC12 rat pheochromocytoma cells, and rat brain cortex and cerebellum. This induction was abrogated by HIF (hypoxia inducible factor) inhibitors or shRNA targeting HIFs, suggesting that CBS induction was HIF dependent. HIF binding sites on Human and rat CBS genes were identified by ChIP assay and luciferase reporter assay. Our findings suggest that induction of CBS may work to keep blood flow under sustained hypoxia and may work to adapt brain to hypoxia.

P74

Deletion of heme oxygenase-2 increases baseline microvascular flow in the murine cerebral cortex

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Heme oxygenase-2 (HO-2) generates carbon monoxide (CO) in an O2-dependent manner. We have previously found that mice with targeted deletion of HO-2 display impaired vasodilatory responses to hypoxia leading to an impaired ability to maintain ATP levels upon hypoxia (Morikawa *et al.*, PNAS, 109, 1293–1298, 2012). Here we hypothesized that HO-2 reserves the capacity to dilate precapillary arterioles at the baseline which makes it possible to induce proper compensatory vasodilatation upon hypoxia. We, therefore, examined baseline microvascular blood flow in the cortex. To this end, we used two-photon laser scanning microscopy to image the flow motion of erythrocytes (Kleinfeld *et al.*, 95, 15741–15746, 1998) using a thinned skulled preparation (Nakamura *et al.*, Acta Physiologica, 203, 187–196, 2011). We found that erythrocyte velocities of precapillary arterioles and those of capillaries in the HO-2 null mice were significantly increased than those in the wild type mice. On the other hand, there was no difference in vessel calibers at neither the levels of penetrating arterioles, precapillary arterioles nor capillaries between two groups. Furthermore, the profiling of 95 metabolites extracted from brain tissues showed that HO-2 deletion causes a hypermetabolic state. These results indicate that the targeted deletion of HO-2 in the brain leads to the lack of a physiologic lock by the constitutive CO, leading the futile elevation of the basal blood flow and basal metabolism.

IMAGING

P75

Possible implication of xanthine oxidase activation on the pathogenesis diabetic nephropathy

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Background and Aim: Endothelial dysfunction represents a predominant early feature of diabetes and makes diabetic patients prone to renal complications. Recent evidence has indicated possible role of xanthine oxidase (XO) in the pathogenesis of vascular dysfunction associated with diabetes. However, it is not clear whether XO activity is involved in pathogenesis of diabetic nephropathy (DN). We investigated the contribution of XO activation on the progression of mouse DN by selective XO inhibitors, Topiroxostat (Top) and Febuxostat (Feb).

Method: Male Ins2Akita heterozygote (Akita; 10 weeks old) mice were used. Wild-type (WT) mice were used for control. Akita mice were treated with Top (3 mg/kg/day), Feb (1 mg/kg/day) or Vehicle (Vehi) for 4 weeks. Serum uric acid and urinary albumin excretion (UAE) were measured. Glomerular pathological changes were also examined by light microscope and electron microscope. Glomerular permeability was assessed using 2 photon microscopy and fluorescent labeling albumin.

Result: Serum uric acid levels showed no significant difference between all groups. Akita+Top or Akita+Feb groups showed significant reduction of UAE in comparison with Akita+Vehi group. Mesangial expansion, glomerular collagen IV deposition, and glomerular endothelial injury (examined by lectin stain and transmission electron microscope) were ameliorated in Akita+Top or Akita+Feb group compared with Akita+Vehi group. Furthermore, glomerular permeability was deteriorated in Akita+Vehi group compared with WT group. These changes were ameliorated with addition of Top or Feb.

Conclusion: XO inhibitors preserved glomerular endothelial function and improved deteriorated glomerular permeability, indicating that XO activation is involved in pathogenesis of DN.

P76

Endothelial dysfunction as a marker of the acute pancreatitis severity

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Introduction: Endothelial dysfunction is one of the most important pathophysiological disorders in patients with acute pancreatitis (AP).

Aims and Methods: To study the endothelial function in patients with AP 46 patients were examined (22 women, mean age 43.3 $\pm$ 2.0 years.). The severity of AP was detected in accordance with the classification of Atlanta 2012. To investigate endothelial function we used wavelet analysis of

skin temperature (WAST) in the appropriate frequency ranges (0.0095–0.02 Hz) following response to the contralateral cold test [1, 2].

Results: Dysregulation of vascular tone during the cold test was observed in all patients with AP. The basal amplitudes of skin temperature oscillations in patients with AP were much lower than in healthy volunteers and progressively decreased according to disease severity ($p = 0.026$) indicating the severity of AP (Rs = −0.32; $p = 0.029$). The increase of amplitudes of skin temperature oscillations during the cold test was observed only in patients with severe AP. We consider it to be a diagnostic criteria of severe AP with 78% sensitivity, 54% specificity.

Conclusion: The WAST was used for assessing endothelial dysfunction in patients with AP. The degree of endothelial dysfunction is related to the disease severity.

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P77

Preliminary results from a novel implementation of a non-invasive laser speckle imaging (LSI) technique during free-flap breast reconstruction

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Inadequate tissue perfusion is a major cause of post-operative complications following breast reconstruction. This pilot observational study aims to assess the feasibility of using LSI in assessing flap tissue perfusion intra-operatively and to assess its potential clinical usefulness in mapping tissue blood flow to reduce post-operative complications.

Methods: The feasibility of performing LSI (moorFLPI-2) intra-operatively in nineteen breast reconstructions ($n = 16$, mean age = 50 years; range = 32–68 years) was assessed. The potential clinical usefulness of LSI images was assessed by comparing (i) tissue perfusion in four zones, clinically divided according to Holm's classification, when abdominal flap was isolated on pedicle; (ii) tissue perfusion after anastomosis and occurrence of post-operative complications.

Results: The feasibility of LSI intra-operatively was demonstrated. Areas of perfusion zones above an arbitrary tissue viability threshold (200 PU) were calculated (percentage of total zone (Z) area), these were Z1 (median: 81; 25th, 75th quartiles: 68.94)%; Z2 (67; 29.81)%; Z3 (51; 30.73)% and Z4 (1; 0.6)%,($p = 0.001$ for Z4 vs. all; $p = 0.003$ for Z1 vs. Z3; Wilcoxon). Clinically detectable low tissue perfusion after anastomosis was observed on LSI images, and the images provided additional information (not seen clinically) on the boundaries between areas of poorly perfused and well-perfused tissues.

Conclusion: Intra-operative use of LSI was feasible and our preliminary results suggested that LSI has the potential of aiding surgical decisions by early detection of poorly perfused tissue. This may reduce the resultant post-operative complications by avoiding use of poorly perfused tissue. Clinical trials are needed to investigate this further.

P78

Comparison of tissue viability imaging and laser speckle contrast imaging for assessment of microvascular function

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The skin has become the organ of choice for assessing microvascular function. Laser-Doppler flowmetry (LDF) may still be regarded as the gold standard, but modern camera-based techniques take advantage of both high spatial and temporal resolutions. In this study we used polarization spectroscopy imaging (TiVi) and Laser Speckle Contrast Imaging (LSCI), to measure microvascular responses in the forearm to iontophoresis of noradrenalin (NA) and sodium nitroprusside (SNP), local heating, arterial occlusion and post-occlusive hyperemia.Eighteen healthy subjects were studied. NA and SNP were given by a 10-minute, 0.02 mA current pulse. Heating was at 42 C for 40 min. Occlusion and subsequent hyperemia was induced using a pressure cuff (250 mmHg) both with and without prior exsanguination. Perfusion and RBC concentration were measured continuously using LSCI and TiVi. No change in perfusion (LSCI) was observed after iontophoresis of NA, while RBC concentration (TiVi) decreased by 7.3 ± 8.4% ($p = 0.02$). Both techniques measured significant vasodilatation after iontophoresis of SNP. Local heating increased perfusion (LSCI) by 224 ± 43% ($p < 0.001$) and RBC concentration (TiVi) by 140 ± 116% ($p < 0.001$). Unlike with LDF, we did not observe a neurally mediated peak with LSCI and TiVi in most test subjects. Significant decreases in perfusion ($p < 0.001$) and RBC concentration ($p = 0.004$) were seen after forearm occlusion. The post-occlusive hyperemic perfusion response

was significantly stronger after exsanguination ($p < 0.001$).These results indicate that LSCI and TiVi are valuable techniques for measuring the responses to vascular provocations.

P79
Assessment of venous stasis in the skin using polarization spectroscopy imaging

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Laser Doppler flowmetry is often used to measure changes in skin blood flow during arterial occlusion, but the vascular changes during venous occlusion are difficult to detect. The current study aimed to find out whether polarization spectroscopy imaging (TiVi) can more sensitively detect venous occlusion in the skin in human forearms.We studied 10 healthy volunteers. Arterial and venous occlusions were simulated by inflating a blood pressure cuff around the upper right arm. Changes in the concentration of red blood cells (RBC) were measured using TiVi, while skin perfusion and concentration of moving red blood cells (CMBC) were measured using LDF during exsanguination and subsequent arterial occlusion, postocclusive reactive hyperaemia, and graded increasing and decreasing venous stasis. During arterial occlusion there was a significant reduction in the mean concentration of RBC from baseline, as well as in perfusion and CMBC ($p < 0.008$). Venous occlusion resulted in a significant 28% increase in the concentration of RBC ($p = 0.002$), but no significant change in perfusion (mean change −14%) while CMBC decreased significantly by 24% ($p = 0.02$). With stepwise increasing occlusion pressures there was a significant rise in the TiVi index and reduction in perfusion ($p = 0.008$), while the reverse was seen when venous flow was gradually restored.The concentration of RBC measured with TiVi changes rapidly and consistently during both total and partial arterial and venous occlusions, while LDF was less consistent. This suggests that TiVi could be useful technique for assessment of the effects of venous stasis.

P80
Nanoscale nonlinear elasticity in blood vessels of living mammalians studied by atomic force microscopy

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By the improvement of nanomechnanical imaging *in vivo*, physiological processes of tissues on the level of organism, especially nanomechanical properties of blood vessel endothelium to primarily smooth muscle cells and elastic tissue fibers are urgent to exploring. In this study, we measure nanoscale nonlinear elasticity of vascular endothelial cell by biological atomic force microscopy in living mammalian bodies. Through the theoretical calculation, the results show that reversible stress-softening and stress-stiffening behaviors are coexist in different dimensional structures of blood vessels in the same region, arising from the coupling nanostructure of one-dimensional nematic order with two dimensional semiflexible random networks in elastic tissue fiber. The changes of nanostructure of vascular endothelium and internal elastic lamina in response to pharmaceutical stimulation further reveal the nanomechanical cooperation of elastic tissue fibers through different dimensional nanostructures. Our study provides a dynamic method to observe the variation of arterial nanomechanics and pathophysiology in real time.

P81
Visualization of *in vivo* renin activity and its application to study the pathogenetic mechanisms of diabetic nephropathy

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We have attempted to develop a novel technique to visualize tissue renin activity *in vivo* by using the fluorescence resonance energy transfer (FRET) system and multiphoton laser microscopy (MP-LM). By using this FRET system, we evaluated the renoprotective effects of renin inhibitor aliskiren in diabetic nephropathy. Diabetes was induced with streptozotocin (STZ) in C57B6/mice, and they were treated with aliskiren (Alis:25 mg/kg/day with osmotic pump) or angiotensin receptor blocker valsartan (Val: 15 mg/kg/day by gavage) for 4 weeks. Diabetic mice (DM) showed higher levels of urinary albumin excretion (UAE) (Control: 85 ± 15 μg/day, DM: 220 ± 25 μg/day, $p < 0.05$). Treatment with Alis or Val significantly lowered UAE (Alis: 120 ± 21 μg/day, Val: 135 ± 18 μg/day). Renin activity in living kidney, assessed by the FRET method, was markedly enhanced in the glomeruli of the DM group (1.7 ± 0.2 fold to Control, $p < 0.05$). Alis suppressed plasma and renal renin activity to the basal level. Glomerular permeability of macromolecules was also visualized *in vivo* using MP-LM with 70-kDa dextran. Glomerular permeability was significantly greater in DM compared with control and was ameliorated by Alis or Val treatment. We have successfully developed a novel *in vivo* imaging technique to visualize renin activity in living animal. Aliskiren inhibited both

plasma and tissue renin activities, ameliorated tissue oxidative stress and attenuated UAE in a diabetes model. The *in vivo* imaging technique with FRET technique will provide a novel approach to explore pathophysiology of kidney diseases.

P82
Quantification of myocardial blood flow in mice using contrast echocardiography

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Murine models are implemented widely in basic cardiovascular research, but there are limited, and often only qualitative, methods available to measure blood flow. To overcome this limitation we have adapted myocardial contrast echocardiography (MCE, a commonly used clinical and experimental technique) to measure myocardial blood flow (MBF) in the mouse heart. Lipid-shelled microbubbles were prepared by sonication of an aqueous lipid dispersion of polyoxyethylene-40-stearate and distearoyl phosphatidylcholine saturated with decafluorobutane gas. After preparation of contrast agent, 20–40 μL/min was infused i.v. (500000 bubbles min-1). MCE was performed with a multi-pulse contrast-specific pulse sequence designed to detect the non-linear microbubble signal at a low mechanical index (MI 0.2–0.25). Data were acquired after a high-MI (1.9) pulse sequence used to destroy microbubbles in the acoustic field. After destruction, replenishment curves were obtained and rate of signal increase (beta) and plateau intensity (A) were calculated by using y = a (1-e(-bt))) equation. Relative blood volume was calculated as the ratio of intensity in the interest (ROI) and left ventricular cavity signal intensity [RBV = A(ROI)/A(cavity)]. MBF was calculated as RBV × beta. Measurements of MBF using MCE were compared to those obtained from nuclide-labeled microspheres at different flow rates. The comparison of flows obtained using microspheres (independent variable) and MCE had a correlation coefficient of 0.98 ($p = 0.04$). The slope of the relationship was 1.395 suggesting that MCE overestimated MBF. However due to the high correlation, MCE after correction can provide an accurate non-invasive method for measuring MBF in the mouse heart.

P83
Microaneurysms in deep capillary plexus layer are associated with diabetic macular edema

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Purpose: To study the association between the location of microaneurysms (MAs) detected by En Face OCT angiography (AngioVue, Avanti OCT, Optovue) and macular edema detected by SD-OCT (Cirrus HD-OCT, Carl Zeiss Meditec) in patients with diabetic macular edema.
Methods: Retrospective chart review of 10 eyes from 9 patients with clinically significant macular edema who have undergone fundus examination including En Face OCT angiography (6 × 6 mm) and Macular Cube (512 × 128). Each images of superficial capillary plexus layer and deep capillary plexus layer were overlaid onto the image of topographic map (6 × 6 mm) using Photoshop, and the area of edema, number of MAs were measured by Image J software (NIH). The association between the location and number of MAs and macular edema were evaluated.
Results: Average number of MAs detected by En Face OCT angiography were 18 (10–28), and 76.5 ± 9.7% of MAs (7–27) were located in deep capillary plexus layer. Most MAs (93.6 ± 8.6%) were found in the area where retinal thickness was more than 400 μm, and there was a significant difference ($p < 0.01$). The macular volume and the number of MAs in deep capillary plexus layer in edema were also significantly correlated ($r = 0.78$, $p < 0.05$).
Conclusions: Our study demonstrated a novel association between the patterns of MAs detected by En Face OCT angiography and diabetic macular edema. The MAs located in deep capillary plexus layer might be responsible for pathogenesis of diabetic edema.

P84
Combined intra-operative thermal and laser speckle contrast imaging to assess bowel perfusion: A case study

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Introduction: Serious post-operative complications following colorectal resection surgery occur in up to 20% of the cases. Adequate perfusion of the anastomosis margins is a prerequisite for appropriate healing. In this study, the utility

of medical thermal imaging (TI) and laser speckle contrast imaging (LSCI) to detect expected perfusion differences in the bowel was evaluated.

Methods: Intra-operative TI and LSCI measurements of the bowel of a 54 year old man undergoing ileo-colic resection were performed. Off-line, three regions of interest (small bowel, large bowel, and de-vascularised bowel) were characterized in terms of temperature mean (TI, units °C) and flux mean (LSCI, perfusion units PU).

Results: The temperature mean values were 30.8°C (small bowel), 30.0°C (large bowel), and 28.1°C (de-vascularised bowel). Compared to the reference small bowel, the large and the de-vascularised bowel were 2.6% and 8.8% cooler, respectively. The flux mean values were 663 PU (small bowel), 467 PU (large bowel), and 172 PU (de-vascularised bowel). Compared to the reference small bowel, the large and the de-vascularised bowel were 29.5% and 74.0% less perfused, respectively.

Conclusion: This is the first reported study performing combined TI and LSCI measurements intra-operatively during colorectal resection surgery. Results show that both technologies are sensitive enough to detect the expected physiological differences in perfusion at different locations of the bowel. Differences assessed by laser speckle contrast imaging appear larger than those from thermal imaging.

P85

Bone and microvascular imaging by k-edge subtraction μCT using synchrotron lights with zirconia contrast medium

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Three-dimensional assessment of microvasculature in the presence of bone is of essential importance for full documentation of the roles of bone vascularity in bone structural changes. We developed subtraction μCT using synchrotron lights and a zirconia-based vascular contrast-casting agent (ZrCA). Its feasibility was examined by application to a rat bone defect model at early healing stages and a murine model of breast cancer bone metastasis.

Rats underwent a drill-hole surgery on a tibial diaphysis at 12 weeks of age. Five or 10 days later, ZrCA was injected via the abdominal aorta. After injection completion, the whole body was immersed into a cold-water bath to solidify the contrast medium, and the treated tibia was harvested and ethanol-fixed. Vascular cast tibial specimens were similarly prepared from mice several days after the intratibial injection of breast cancer cells at 5 weeks of age. The specimen was CT-scanned with X-rays of 17.9 and 18.1 keV, just below and above the ZrCA absorption k-edge, respectively, and scan data sets were reconstructed by 2D filtered back-projection. The vasculature was highlighted in the 18.1-keV CT image but not in the 17.9-keV CT image. The subtraction between these images allowed clear segmentation of microvasculature from bone structure. The degree of mineralization was also assessable.

In conclusion, k-edge subtraction μCT with using ZrCA and synchrotron lights is available for extraction of bone microvasculature, contributing to the understanding of the anatomical and functional relationships between bone structure and the associated vascularization.

P86

Enlargement of foveal avascular zone in diabetic patients evaluated by En Face OCT angiography

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Purpose: To evaluate the area of foveal avascular zone (FAZ) detected by En Face OCT angiography (AngioVue, Avanti OCT, Optovue) in healthy and diabetic eyes.

Methods: Retrospective chart review of patients who have undergone fundus examination including En Face OCT angiography. The eyes with proliferative diabetic retinopathy (DR) and history of laser photocoagulation were excluded. The area of FAZ was measured using Image J software (NIH). The area of FAZ in the superficial and deep plexus layer were evaluated.

Results: The patients were divided into 3 groups; (i) non-diabetes mellitus (DM) (32 eyes), (ii) DM without DR (42 eyes), (iii) DM with DR (39 eyes). There was no statistically significant difference in age among 3 groups. The area of FAZ in superficial layer was 0.26 ± 0.06 mm^2 in non-DM eyes, whereas, 0.35 ± 0.07 mm^2 in DM without DR eyes, and 0.38 ± 0.13 mm^2 in DR eyes. The diabetic eyes, regardless of the presence of DR, showed statistically significant enlargement of FAZ compared to non-DM eyes ($p < 0.01$). The area of FAZ in deep plexus layer was also significantly larger in the diabetic eyes compared to non-DM eyes (non-DM eyes; 0.37 ± 0.11 mm^2, DM without DR eyes; 0.54 ± 0.13 mm^2, DM with DR eyes; 0.59 ± 0.17 mm^2, $p < 0.01$).

Conclusions: Our data suggest that the diabetic eyes show the impairment of retinal microcirculation in the macula even before the retinopathy develops. En Face OCT angiography is useful in non-invasive screening for the detection of the early microcirculatory disturbance in diabetic patients.

P87

Three-dimensional characterization of microvessels in whole organs and small animals co-localized with labeled biomarkers by a fluorescent imaging cryomicrotome system (3D-FICS)

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Molecular imaging represents an active area of translational research that provides information about the molecular events underlying pathological processes and treatment effects. Serial cryo-sectioning combined with microscopy enables acquisition of high-resolution volumetric data to validate *in vivo* imaging modalities, for detailed structural information and functional insight by use of targeted molecular biomarkers.

We have previously developed a fully automated cryomicrotome for episcopic multispectral imaging of whole organs and biological specimens at resolutions between 2 and 40 μm over a large field of view (2–14 cm). By alternately sectioning and imaging, co-registered reflectance and fluorescence images of the frozen tissue block face are acquired by appropriate selection of optical filters for excitation and emission on a motorized filter wheel. The combined image stacks yield detailed 3D representations of arterial networks filled with fluorescent cast, co-localized with multicolored markers of blood flow (microspheres) or biological activity (e.g. fluorescent monocytes).

Recently, we designed an advanced system based on a commercial motorized cryostat that was modified to allow continuous sectioning and imaging controlled through custom automation software. This 3D-FICS is equipped with a hyperspectral tunable laser and fully tunable thin-film filters and achieves up to 20× higher light transmission with superior bandpass filter characteristics over a spectral range extending from 360 to 900 nm (optional NIR path to 2400 nm). Moreover, individual specimen slices can be collected for detailed microscopy and immuno-histochemical analysis. We show representative applications of these systems for organs and tissue samples from mice to humans in multidisciplinary research areas.

P88

Large-area surface-enhanced Raman spectroscopy imaging as a novel method to visualize alterations in small molecular metabolites in ischemic brain tissues

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While imaging mass spectroscopy is a powerful method to visualize metabolites, it requires matrix deposition to ionize target molecules. On the other hand, surface-enhanced Raman scattering (SERS) can provide information on the structure of the molecules without labeling and ionization, serving as a finger-print method. Here, we report a novel large-area SERS devise, named '*gold nano-coral*' (GNC), which is capable of detecting a region of energy failure of the brain tissue. Until now, application of SERS for tissue-imaging has been limited because of technical constraints to fabricate a SERS substrate ensuring hot-spot formation uniformly over a large area with ease. To overcome this hurdle, we utilized the boehmite nanostructure that is easily achieved by immersing the aluminum film in boiling water. Sharp geometry of boehmite served as an efficient template for the gold deposition ensuring strong enhancement of SERS signals. This simple, reproducible and rapid method makes it possible to produce a SERS substrate with a size of a square-centimeter order, the dimension necessary to accommodate most tissue samples. GNC substrate enabled the large-area SERS imaging to visualize an ischemic core of mouse brain tissue without labeling for the first time. Furthermore, we attempted to detect carbon monoxide (CO) that is known to occur abundantly in normal brain and to decrease in the ischemic core as a regulator of neurovascular units. The experiments *in vitro* showed that CO generates the notable Raman peak at 2150 cm^{-1}, suggesting that GNC-enhanced SERS microscopy serves a potentially powerful method to visualize the gas.

INFLAMMATION/LEUKOCYTE-ENDOTHELIUM INTERACTIONS/IMMUNE CELL TRAFFICKING

P89

Effect of nicotine on DSS-induced murine colitis in point of adhesion molecules on the microvascular endothelium

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Introduction: Adhesion molecules play important roles in inflammatory bowel disease (IBD) and modulation of adhesion molecules could be a new therapeutic target. Nicotine absorbed by smoking is reported to work protectively in some ulcerative colitis (UC) cases but still controversial. Although protective effect of nicotine on colonic carcinoma has been reported, not yet studied precisely in IBD. Our aim is to investigate the effect of nicotine on dextran sulfate sodium (DSS)-induced murine colitis especially in point of adhesion molecules.
Method: C57BL/6J mice were treated with DSS. Some mice were treated with nicotine for 7 days. Disease activities of colitis were assessed by DAI score. mRNA expressions of ICAM-1, VCAM-1 and MAdCAM-1 in colonic tissues were measured by qPCR. We also observed adhesion number of splenocyte on colonic submucosal vasculature of DSS treated mice with or without nicotine treatment under intra vital fluorescence microscope.
Result: Nicotine treatment significantly decreased DAI score induced by DSS and significantly attenuated the increased expression of VCAM-1 and MAdCAM-1 induced by DSS. In microscopic study, increased adhesion number induced by DSS was significantly decreased by nicotine treatment.Conclusion: Microvascular endothelial expression of adhesion molecules, especially MAdCAM-1 was significantly inhibited by simultaneous exposure to nicotine. This study showed that nicotine might have a protective effect on inflammation of colonic mucosa of IBD via modulation of MAdCAM-1.

P90

Monocytes interact with neutrophils in the glomerular microcirculation to promote acute glomerulonephritis

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In vivo imaging studies have shown that neutrophils and monocytes undergo retention in glomerular capillaries under resting conditions. Upon induction of glomerular inflammation, the duration of neutrophil and monocyte retention increases, a response associated with induction of glomerular injury. While the pathological role of neutrophils in glomerular pathology is well established, the contribution of intraglomerular monocytes has not been examined. Therefore, the aim of this study was to investigate the contribution of intravascular monocytes in acute experimental glomerulonephritis. Using multiphoton and spinning disk confocal intravital microscopy of the mouse kidney, leukocyte behaviour in glomeruli was examined following induction of glomerulonephritis induced by anti-glomerular basement membrane antibody. Depletion of monocytes resulted in attenuation of glomerular injury in this model, as determined by measurement of albuminuria. In addition, both glomerular neutrophil trafficking and the proportion of neutrophils generating reactive oxygen species were significantly reduced in monocyte-depleted mice, consistent with the hypothesis that intravascular monocytes promote neutrophil-dependent glomerular injury. To investigate whether neutrophils and monocytes interact in the glomerular microcirculation, spinning disk confocal microscopy was used to examine the behaviour of monocytes and neutrophils in the glomerulus. In the absence of inflammation, monocytes and neutrophils underwent interactions in glomerular capillaries, and inflammation caused the duration of these interactions to increase. Notably, neutrophils that interacted with monocytes persisted in the glomerulus for longer, and were more likely to generate reactive oxygen species. In conclusion, these data indicate an important role for monocytes in mediating neutrophil recruitment and activation in acute neutrophil-dependent glomerulonephritis.

P91

Nitric oxide activates ICAM-1 on the endothelium at the onset of the inflammatory response

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Endothelium plays a vital role on the inflammatory response through induction and expression of adhesion molecules on its surface after stimulation with pro-inflammatory cytokines. Nitric oxide (NO) has been described as a negative regulator of leukocyte adhesion at extended times of treatment with pro-inflammatory agonists. However, the fact that the same agonists induce leukocyte adhesion promote increase in permeability activating NO production at the onset of the process, prompt the question about whether NO induced by eNOS activates leukocyte adhesion at the beginning of the inflammatory response. We test the hypothesis that NO regulates ICAM-1 at the onset of the inflammatory response. As a model we used EAhy926 cells and TNF-alpha as agonist. eNOS activation and ICAM-1 levels were evaluated through western-blot, immunofluorescence and biotinylation. Leukocyte adhesion was measured by MPP (mieloperoxidase) activity after incubating leukocytes with monolayers of endothelial cells. We demonstrate that TNF-alpha induced an increase in leukocyte adhesion at the onset of the inflammatory response. This increase was dependent on NO production since eNOS inhibition with L-NMA, decreased the adhesion. The increase in leukocyte adhesion was correlated with eNOS phosphorylation and increase on ICAM-1 levels at cell surface and total levels. Increase in ICAM-1 expression was inhibited in presence of NAC (inhibitor of S-nitrosylation). These results demonstrate that TNF-alpha increases leukocyte adhesion dependent on eNOS activity. These results could have powerful applications on the inflammation field.

P92

Atrial natriuretic peptide (ANP) down-regulates neutrophil recruitment on inflamed endothelium by reducing PMN deformability, while adhesive function is maintained

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Atrial natriuretic peptide (ANP) attenuates ischemia/reperfusion-induced renal injury in rats in part by reducing neutrophil (PMN) activation and accumulation at sites of inflammation. There is interest in ANP's capacity to increase blood flow/exchange area, and attenuate endothelial barrier function when provided as a therapeutic to treat acute inflammation. To delve into the mechanism of action of ANP on PMN recruitment, human PMN were perfused over IL-1B-stimulated umbilical vein endothelial cell (HUVEC) monolayers in a microfluidic lab-chip. PMN rolling, firm adhesion, and transendothelial migration were reduced by up to 50% compared to controls ($p < 0.01$) in a dose dependent manner following pretreatment with ANP (1–10 nM). Capture of PMN via selectin mediated tether formation did not convert to shear resistant integrin dependent arrest due to formation of long tethers that abruptly ruptured as shear was ramped from 2 to 20 dynes/cm^2. ANP inhibition did not involve down regulation in expression of VCAM, ICAM, or E-selectin on HUVEC, nor diminished function of L-selectin/B2-integrin on PMN. Rheological analysis by micropipette of PMN deformability revealed that ANP does not change cortical tension but decreased the ratio of cortical tension/viscosity by ~40%, which accounted for a concomitant increase in hydrodynamic drag force and a reduction in the footprint of PMN adhesion on endothelium. We conclude that ANP enhances PMN rigidity that diminishes their recruitment efficiency on inflamed vasculature by effectively increasing the rupture force on selectin and integrin bond formation.

P93

Immune cell derived NGF links dysfunction of the immune and sympathetic nervous systems in obesity-related hypertension

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Hypertension in the obese is a rapidly increasing problem. It has both neurological and immunological antecedents, however the relationship between these is unclear. Here we used a mouse model of diet-induced obesity to demonstrate that immune cells, located within the vascular adventitia of arteries important for controlling blood pressure, produce nerve growth factor (NGF) and rapidly initiate growth of the perivascular sympathetic nerve plexus; which increases vascular tone and thereby elevates blood pressure. We also show that RAG1$^{-/-}$ mice lacking T and B cells do not develop hypertension when obesity is induced with a high fat

diet (HFD) and show that their sympathetic innervation and vascular tone remains normal, suggesting that neurogenic hypertension in the obese likely occurs independently of weight gain. Furthermore, we show that the adoptive transfer of wildtype CD3 + T cells restores the sympathetic hyperinnervation and associated hypertension whereas the transfer of CD3 + T cells deficient in NGF had no effect on sympathetic innervation or blood pressure. These results not only demonstrate the role of T cells in the genesis of obesity-related hypertension but also demonstrate the pivotal role of NGF in linking dysfunction of the immune and sympathetic nervous systems that characterize hypertension in the obese.

P94

Leukotriene B4 (LTB4) receptor type 1 (BLT1) attenuates acetaminophen-induced liver injury through inhibiting hepatic neutrophil activation

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Aims: Leukotriene B4 (LTB4) is a potent chemoattractant for neutrophils, and is essential for promotion of inflammation. Following acetaminophen (APAP) over-dose, neutrophils accumulate into the injured liver. However, the role of accumulated neutrophils in APAP-induced liver injury is controversial. In this study, we investigated the role of LTB4 receptor type 1 (BLT1) in APAP hepatotoxicity.
Methods: BLT1-knockout mice (BLT1-/-) or their wild-type counterparts (WT) were subjected to APAP over-dose (300 mg/kg) and neutrophil activation status was determined during liver injury.
Results: Compared with WT, BLT1-/- exhibited higher levels of ALT and necrotic area at 24 h, and lower survival rate (WT;100% vs. BLT1-/-;48.6% survival rate). The numbers of recruited Gr1-positive neutrophils into the BLT1-/- livers were larger than those in WT livers, which was associated with enhanced CXCL2 expression. The mRNA expression of TNF, IL-1, and MMP-9 in BLT-/- livers were enhanced. Neutrophils in WT livers showed up-regulation of Gr1 expression and priming for reactive oxygen during the injury. Treatment of WT mice with a BLT1-inhibitor ONO4057 also enhanced liver injury, accumulation of neutrophils, and mRNA levels of TNF and MMP-9 after APAP administration.
Conclusions: These results indicate that BLT1 signaling plays a role in APAP-induced liver injury through attenuating the accumulation of hepatic activated neutrophils.

P95

Immune suppression after stroke

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Stroke is a leading cause of morbidity and mortality worldwide. Despite its recognised debilitating neurological deficits, the major cause of death after stroke is infection. In fact, bacterial infection is the most frequent medical complication after stroke, and it is now increasingly accepted that stroke results in impairment of the immune system, and this contributes to the associated life-threatening sequelae of overwhelming infection. We were the first to directly image and describe the activities of the peripheral immune system in living mice after stroke. In this study, we found the behaviour and function of invariant natural killer T (iNKT) cells, which are important in the host antibacterial defense, were impair in an adrenergic-mediated manner after stroke. Recently, we performed a pilot human study and revealed similar stroke-induced regulation of iNKT cells in stroke patients. Based on these findings, we proposed a more selective modulation of the immune system following stroke could be beneficial. In particular, selecting facets of the immune system to target would allow the protective and regenerative properties of the immune response to remain intact while blunting the pro-inflammatory response generated towards the injured brain. Therefore, we tested the capacity of post-stroke administration of novel iNKT cell-targeting drugs in modifying iNKT cell activity in such a way as to restore immune system function and thereby limit bacterial infection after stroke. Identifying a new and better targeted approach to reduce bacterial infection in stroke patients will bypass the growing problem of antibiotic resistance, ultimately improving patient outcomes.

P96

Platelet-lymphocyte crosstalk: A key microvascular response to inflammation

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The inflammatory bowel diseases, Crohn's disease and ulcerative colitis, while primarily affecting the gastrointestinal tract, are associated with an increased risk for, and incidence of, systemic arterial and venous thromboembolism. Three important features of IBD are (i) thrombocytosis and the appearance of immature platelets; (ii) enhanced thrombus formation and (iii) increased leukocyte production/recruitment/activation. Platelet count and function are altered in a manner that favors homotypic and

heterotypic platelet aggregation and the formation of thrombi both within the colonic microvasculature and in tissues distant from the inflamed bowel. IL-6 appears capable of inducing all of the platelet alterations (thrombopoiesis, thrombocytosis, enhanced reactivity & aggregation) that accompanies human and experimental IBD. In different chronic (T-cell transfer, DSS cycling) and acute (DSS) models of experimental colitis, thrombus formation was induced in arterioles using the light/dye-method. Accelerated thrombus formation was demonstrated in arterioles of cremaster muscle, cecum, and brain during experimental colitis. This response was accompanied by thrombocytosis and an elevated level of immature platelets, which are known to be highly reactive. IL-6 deficient (IL-6-/-) mice and bone marrow chimeras (IL-6-/- marrow transplanted into wild type (WT) recipients IL-6-/-to WT) exhibit blunted thrombocytosis, reduced platelet hyper-reactivity responses, and an attenuation of thrombus formation during colitis. T cell immunoblockade with a CD3-directed antibody was also shown to attenuate the elevated platelet count, appearance of immature platelets and thrombus formation associated with colonic inflammation. Our findings links IL-6, lymphocytes and platelets in mediating thrombotic abnormalities associated with inflammation.

P97

The administration of antioxidants reduced the leukocytes-endothelial interaction induced by ultraviolet B irradiation in cutaneous microvasculature

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Objective: Ultraviolet B (UVB: wave length 280–315 nm) irradiation induces leukocyte-endothelial interaction via reactive oxygen production. The objective of the present study is to elucidate the physiological role of P-selectin and to evaluate effects of antioxidants on the leukocyte-endothelial interactions by UVB irradiation (UVBIR) using the mouse-dorsal skin chamber (DSC).

Methods: DSCs were surgically implanted to female hairless HR-1 mice. To elucidate the role of P-selectin on leukocyte adhesion after UVBIR, we prepared 2 experimental groups; anti P-selectin IgG treatment+UVBIR, vehicle IgG treatment +UVBIR. Antibody (40 μg per animal) was *i.v.* injected prior to UVBIR. To examine the effect of antioxidants on the leukocytes adhesion, mice were administrated vitamin E (VE) and green tea catechins (GTC) via feeding tube. Before and after UVBIR (240 mJ/cm^2) to the skin within the chamber, leukocytes were stained with rhodamine 6G, and the their behaviors within microvasculature were recorded and analyzed.

Results: The mean value of rolling leukocytes number was significantly higher in the venules at 6 hours after UVBIR than that in pre-UVBIR. This increase was suppressed when anti P-selectin antibody was injected prior to UVBIR. When VE or GTC was administrated prior to UVBIR, increase of rolling leukocytes number was also suppressed.

Conclusions: Our findings suggest that the administrations of antioxidants show the preventive effect on UVB induced leukocyte-endothelial interaction which is mainly mediated by P-selectin and its ligand.

INSTRUMENTATION, METHODOLOGY, AND EXPERIMENTAL MODELS

P98

Wavelet-analysis of skin temperature oscillations for revealing endothelial dysfunction in patients with type 2 diabetes

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The purpose of this work is to study the possibilities of wavelet analysis of skin temperature (WAST) for the diagnosis of impaired regulation of microvascular tone in patients with type 2 diabetes during the local heating test. A control group consisted of healthy male and female volunteers (n = 5 each), aged 34–47 years old. A group of patients with type 2 diabetes comprised thirteen people, seven men and six women, aged 36–51 years old. The mean disease duration was 7.4 years. Skin temperature oscillations, reflecting intrinsic myogenic activity (0.05. . .0.14 Hz), neurogenic factors (0.02. . .0.05 Hz) and endothelial activity (0.0095. . .0.02 Hz) increase greatly during local heating for healthy subjects. In the group of patients with type 2 diabetes, no statistically significant differences in the amplitudes in the endothelial range were observed. Relative changes in the oscillation amplitudes in patients with type 2 diabetes were markedly lower compared to the control group. Local heating is a fairly simple and effective test to evaluate the mechanisms involved in regulating the vascular tone, in particular, the endothelial dysfunction (ED). There are several reasons why this test has not found wide application in practice; however, when combined with an easy-to-implement technique WAST, it can be successfully used in routine clinical studies, especially for screening and early detection of ED. However, further validation is needed

to be performed while blocking endothelium-dependent pathways, e.g. by using microdialysis.

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P99

Evaluation of endothelial dysfunction in patients with metabolic syndrome based on the wavelet-analysis of skin temperature oscillations

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Endothelial dysfunction occurs frequently in patients diagnosed with metabolic syndrome (MS) and manifests, in particular, as a disorder of the endothelial factor of vascular tone regulation. This study aimed to explore changes in microvascular tone during a contralateral cold pressor test (CPT) and to compare the results obtained in healthy subjects and in MS patients. Wavelet analysis of low-amplitude skin temperature oscillations in the appropriate frequency ranges [1] was used as a method for characterization of different mechanisms of vascular tone regulation. The total of 30 adults with MS aged 27...50 years participated in this study. The control group included 20 healthy men and women aged 30...55 years. Vascular endothelial growth factor (VEGF) was used as a biochemical marker of endothelial dysfunction. Patients with MS had significant differences in all metabolic parameters compared to the control group; the levels of the VEGF were significantly higher. The responses to the CPT test in MS patients differed substantially from those of healthy subjects in the endothelial frequency range. A significant correlation (0.5, $p < 0.01$) between the relative change in the amplitude of temperature fluctuations and the VEGF was found. Vasodilation aberrations identified in the functional cold test confirmed the relationship between abnormal vascular reactivity and markers of endothelial dysfunction.

This work was supported by the Russian Science Foundation grant No. 14-15-00809.

1. Smirnova E., et al. Assessment of endothelial dysfunction in patients with impaired glucose tolerance during a cold pressor test. Diab Vasc Dis Res 2013; 10(6): p.489.

P100

Different limited femoral artery and balloon dilatation to establish the diabetes chronic lower limb ischemia rat model

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This study was aimed to establish diabetes chronic lower limb ischemia rat model.

Methods: 40 SD rats were used in the study. High-fat feed were given for 4 weeks. To establish high fat diabetic rat model, intraperitoneal injection with streptoxolocin (STZ) were used. Then rats were randomly divided into four groups. After balloon dilatat injure the right iliac and femoral artery, Proximal iliac artery sutured inadequaly with 7-0 Blood vessel suture, all branchs were ligated. In the first group, right distal femoral artery cut off. In the remaining three groups, different diameter super sliding guide wire were used respectively, after two different point thread ligation, the guide wire was taken out. Models of chronic lower limb ischemia were established. All the left limbs were taken for blank control. After 4 weeks high-fat feeded, rteriography and vascular numeration, capillary density, lumen area and pathology were observed.

Results: Postoperative blood glucose, blood lipid were significantly higher in rats. Compared with blank control limb, the trunk blood flow decreased significantly after surgery, the number of collateral vessels aand capillary density increased. And comparing different guide wire, the trunk blood flow were significantly restricted, compensatory collateral vessels increased in 0.014 and 0.018 guide wire group than in 0.035 guide wire group.

Conclusion: Balloon Dilatation and limited Femoral artery can result in diabetes chronic lower limb ischemia rat model used for experimental study.

P101

A cell culture microdevice with a continuous oxygen gradient for microvascular research *in vitro*

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Oxygen molecules diffuse into tissues via microcirculation and an oxygen gradient is formed between the vessels and tissues. Oxygen concentration is thought to be a determinant of cellular functions, but the only way to investigate the effect of oxygen concentration on cells is to use gas-controlled

incubators at a constant partial oxygen pressure (pO_2) level. This is because; currently, no technique exists for creating an oxygen gradient in cell culture dishes.

We developed a custom-built cell-culture microdevice, comprising an oxygen-permeable PDMS polymer to generate an oxygen gradient on the cell culture area in the device. An oxygen-sensing film, consisting of a mixture of PDMS and Pd-TCPP, allowed pO_2 distribution imaging on the culture layer during cell culture and quantifying the cellular oxygen consumption rate. Culture medium was continuously exchanged through multiple microchannels installed in the device to maintain the oxygen gradient for a long term without transient hyper-oxygenation.

We cultured endothelial cells under the oxygen gradient. VEGF mRNA expression increased according to the pO_2 gradient, especially in hypoxic area. Additionally, mouse primary hepatocytes were cultured under the oxygen gradient, and mRNA expression of PEPCK and GK significantly increased in culture areas corresponding to periportal and pericentral regions, respectively.

The oxygen gradient in the microdevice can be calculated mathematically and controlled by changing the sizes of gas channels; therefore, it could be expected to mimic microcirculatory oxygen gradient of various organs, and it may be useful for microcirculation research *in vitro*.

P102
The protective effect of panax quinquefoliu saponin and panax pseudo-ginseng components on gastric mucosal lesions induced by dual antiplatelet drugs in rat with myocardial infarction

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Objective: To observe the protective effect of Chinese medicine panax quinquefoliu saponin (PQS) and panax pseudo-ginseng (PPG) components on gastric mucosal lesions induced by clopidogrel and aspirin (dual antiplatelet drugs) in rat with acute myocardial infarction(AMI).

Method: Forty-eight Spragu-Dawley rats were established into AMI models. Succesful operation later, they were randomly divided into AMI model group, dual antiplatelet drugs for AMI group (DAAMI), dual antiplatelet drugs and Chinese medicine for AMI group (DACMAMI), 12 in each group, at the same time, 12 rats were as sham group (left anterior descending coronary artery was not tied). 28 days later, serum gastrin (GAS), nitric oxide (NO), maximum platelet aggregation rate (PAGT), 6-keto prostaglandin F1-alpha (6-keto-PGF1-alpha), thromboxane B2 (TXB2) and motilin (MTL) were measured. Histopathological and ultrastructural changes of gastric mucosa were observed.

Results: Compared with AMI model group, Guth grade and Whittle grade of gastric mucosa significantly increased in DAAMI group ($p < 0.01$). Compared with DAAMI group, Guth grade and Whittle grade of gastric mucosa significantly decreased in DACMAMI group ($p < 0.01$). Compared with AMI model group, MTL and 6-keto-PGF1-alpha contents significantly increased in DAAMI group, PAGT and GAS content significantly decreased ($p < 0.05$). Compared with DAAMI group, 6-keto-PGF1-alpha and GAS contents significantly increased in DACMAMI group ($p < 0.05$), ET-1 and MTL contents significantly decreased ($p < 0.05$).

Conclusions: PQS and PPG components have protective role on gastric mucosal lesions induced by antiplatelet drugs after acute myocardial infarction, whose mechanism could be related to increased GAS and NO contents and decreased ET-1 and MTL contents.

P103
Evaluation of laser speckle flowgraphy: Development of novel skin blood flow measurement technique

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Objective: Currently, there is no measurement device capable of comparing skin blood flow. Thus, we experimentally prepared a laser speckle flowgraphy (LSFG), and investigated whether or not it can accurately measure skin blood flow.

Methods: (i) Using the thermal diffusion method (TDM) established as a blood flow measurement method and LSFG, facial skin blood flow was measured in 130 healthy females, and the correlation between the values measured by the 2 methods was investigated. (ii) Using strain-gauge plethysmography (SPG), capable of measuring the absolute value of blood flow (mL/min/100 g), and LSFG, skin blood flow was measured on the ventral side of the left finger in 50 healthy adults, and the correlation between the values measured by the 2 methods was investigated.

Results: (i) An inverse proportion of the slope of the values with the TDM method to the skin blood flow has been clarified based on Grayson's theory. A significant inverse correlation was noted between the slope with the TDM method and MBR values. (ii) When skin blood flow was measured at the fingertip using SPG and LSFG, a significant

positive correlation was noted between the 2 measurement methods, suggesting that the MBR value with LSFG is a useful index of skin blood flow.

Conclusions: It is suggested that LSFG is a useful device for skin blood flow evaluation because it is capable of noninvasively and continuously measuring blood flow without limitation of the measurement region. Its application in medical care and esthetic fields is expected.

P104
Automated methodology for *ex vivo* measurement of vascular permeability

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Vascular barrier function is an area of much interest, yet there are few robust measurements of permeability in intact, *ex-vivo* arteries. The majority of the published literature on vascular permeability is based on methods utilizing cultured cells rather than intact preparations. We have developed a novel, automated system to quantify solute flux across isolated, pressurized, intact microvessels. We used a software-controlled, wireless, microfluidics pump system in conjunction with a custom-built pressure myograph chamber to obtain highly accurate volumetric samples at specific time points, allowing precise measurements of solute flux across the microvascular wall. Mouse, mesenteric resistance arteries (200 microns in diameter) were excised, cannulated on glass micropipettes, and maintained at 37 Celcius. The fluorescent tracer FITC-dextran (4 kDa MW) was perfused through the arteries for 5 minutes, before pressurizing to 80 mm Hg. Using an automatic step protocol programmed into the software, the bath was flushed and filled three times to remove residual FITC-dextran. Readings were then taken immediately following the wash protocol (time 0 reading) and then twice more at 15 minute intervals with three washes in between. The amount of FITC-dextran collected was corrected for the surface area of the vessel and the volume of sample collected. These values were expressed as [FITC-dextran absorbance (520 nm)]/(mm^2 x min × mL). The variability for repeat measures of vascular permeability was extremely low and we consistently observed a robust increase in permeability with positive controls. Using this automated system, vascular permeability can be accurately measured in intact, *ex-vivo* artery preparations.

P105
Quantification and imaging of regional vascular permeability and partial pressure of oxygen in tumor microcirculation

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Quantification of vascular permeability is important for cancer research because tumor neovessels have a higher vascular permeability than that of normal tissue. Previous studies have reported the mean permeability in the field of view (FOV) of a microscope or regional permeability by setting region of interests (ROIs) manually. However, tumor vascular permeability is spatially heterogeneous and local tissue hypoxia has been suggested to be an important factor for vascular permeability but existing methods cannot reflect them. In this study, we developed an imaging method to visualize the heterogeneous vascular permeability and partial pressure of oxygen (pO_2) simultaneously. We implanted tumors in window chambers on the back of mice and injected fluorescent dye via the tail vein. Three dimensional time-lapse images were acquired by confocal microscopy, and then vascular structure was extracted from the image and multiple ROIs were automatically set in the FOV. The vascular permeability at each point was quantified from fluorescent intensity change and the differences of the permeability in the entire vasculature was imaged as a pseudocolor image. After acquiring fluorescent intensity change, oxygen sensitive dye was injected for quantifying pO_2. Pulsed laser irradiation to excite the dye and phosphorescence analysis in the entire FOV successfully imaged the tissue pO_2. In conclusion we developed an imaging method to visualize heterogeneous vascular permeability and pO_2 simultaneously. Applying our method to tumor vasculature will contribute to cancer research.

P106
Clinical microvascular imaging: A Review of techniques

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Growing interest in the microcirculation has helped drive forward the development of perfusion imaging of the microvessels, bringing excellent opportunities for microvascular research. There is a wide variety of methods now available for clinical microvascular imaging, including capillaroscopy, infrared thermography, laser Doppler perfusion

imaging and laser speckle contrast imaging. Emerging technologies include imaging photoplethysmography, optical coherence tomography, photoacoustic tomography, hyperspectral imaging, and tissue viability imaging. This review highlights clinical applications and potential research areas for each of the 9 techniques from our review paper (Allen and Howell 2014). The rapid pace of technological advancement has delivered plenty of options for microvascular imaging in the early part of the twenty-first century. The coming years will deliver a better understanding of the clinical utility of all of these techniques, with consequent benefits for researchers, practitioners, and ultimately patients.

Reference: Allen J and Howell KJ. Clinical microvascular imaging: techniques and opportunities for clinical physiological measurements. *Physiological Measurement* 2014 35: R91-141.

P107
Photoplethysmography and its application to clinical physiological measurement: An overview

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Photoplethysmography (PPG) is a simple and low-cost optical technique that can detect blood volume changes in the microvascular bed (Allen 2007). The PPG waveform comprises a pulsatile ("AC") physiological waveform attributed to cardiac synchronous changes in the blood volume with each heart beat, and is superimposed on a slowly varying ("DC") baseline with various lower frequency components attributed to respiration, sympathetic nervous system activity and thermoregulation. PPG technology can provide valuable information about the cardiovascular system, and is used in a wide range of commercially available medical devices for measuring oxygen saturation, blood pressure and cardiac output, assessing autonomic function and detecting peripheral vascular diseases. There has also been a resurgence of interest in the technique in recent years, driven by the demand for low-cost and portable technology for the primary care and community based clinical settings, the wide availability of low-cost and small semiconductor components, and the advancement of computer-based pulse wave analysis techniques. This overview will summarize opportunities for PPG in clinical microvascular assessment.

Reference: Allen J. Photoplethysmography and its application in clinical physiological measurement. *Physiological Measurement* 2007 28:R1–39.

P108
Utility of combined fluorescence spectroscopy and tissue oxygen saturation measurements in systemic sclerosis: A pilot study

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Patients with systemic sclerosis (SSc) can experience significant morbidity and mortality, with connective tissue and microvascular changes being key features of the disease [1,2]. The development of methods to aid early diagnosis is very important and so the aim of this pilot study was to assess the classification performance of combined optical non-invasive skin fluorescence spectroscopy and tissue oxygen spectrophotometry measurements in SSc. Two groups, comprising 14 SSc patients and 9 control subjects, were included in the study. Fluorescence and tissue oxygen measurements were collected from 3 sites (chest, arm and leg). Fluorescence intensities at wavelengths attributed to collagen, elastin and L-tryptophan were extracted from each site Excitation Emission Matrix (EEM) and a Fluorescence Score formed using site averaging ((Elastin+Collagen)/Tryptophan, dimensionless). Tissue oxygen saturation values from the same three sites were also averaged (giving Tissue Oxygenation, %). Overall, patients with SSc had significantly increased Fluorescence Scores and significantly reduced Tissue Oxygenation when compared to healthy controls. An experimental linear cluster separation produced an overall classification accuracy of at least 95%. The results of this pilot study demonstrate the potential diagnostic utility of these two novel "optical biopsy" methods in patients with systemic sclerosis. Further validation work is being undertaken.

References: [1] ACR Preliminary criteria for Systemic Sclerosis. Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis. Arthritis Rheum 1980;23:581-90.

[2] Atlas of Capillaroscopy in Rheumatic Diseases. Cutolo M. Elsevier 2010.

P109

Photoplethysmography assessment of endothelial function in patients with Raynaud's phenomenon and systemic sclerosis: A pilot study

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Patients with systemic sclerosis (SSc) can experience significant morbidity and mortality [1], and with microangiopathy often present in the disease [2]. The development of methods to aid early diagnosis is very important and so the aim of this pilot study was to investigate the potential clinical utility of photoplethysmography (PPG) in assessing endothelial function in patients with systemic sclerosis and primary Raynaud's phenomenon (PRP). PPG finger pulse measurements were made at rest and following a 5 minute blood pressure cuff occlusion challenge to the arm, in 19 SSc, 19 PRP and 23 healthy control subjects. Endothelial function measures were obtained using novel pulse wave analysis and then compared for the three subject groups [3]. Endothelial function was significantly impaired in SSc, but with no difference between healthy controls and PRP. By using measures of endothelial function, PPG based methods differentiated SSc from control and PRP subjects with an accuracy of at least 81%.

References: [1] ACR Preliminary criteria for Systemic Sclerosis. Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis. Arthritis Rheum 1980 23:581–90.

[2] Atlas of Capillaroscopy in Rheumatic Diseases. Cutolo M. Elsevier 2010.

[3] McKay ND, Griffiths B, Di Maria C, Hedley S, Murray A, Allen J. Novel photoplethysmography cardiovascular assessments in patients with Raynaud's phenomenon and systemic sclerosis: a pilot study. Rheumatology (Oxford). 2014 53:1855–63.

P110

A proposed integral diagnosis system with D-dimer for siagnosis of non-overt and overt disseminated intravascular coagulation

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D-dimer (D-D) was shown as an important indicator for the diagnosis of overt disseminated intravascular coagulation (DIC) and non-overt disseminated intravascular coagulation (pre-DIC). However, its diagnostic cutoff value in the clinic is not clearly defined. To determine the diagnostic efficacy of D-dimer (D-D) for the diagnosis of DIC and pre-DIC, compared to or combined with other DIC coagulation indicators, D-D and other coagulation indicators were determined, and their diagnostic roles were further examined by binary logistic regression analysis. When D-D was more than 3.0 mg/L was used as the cutoff, the sum of diagnostic sensitivity and specificity reached to maximum for DIC and pre-DIC, while the sum of misdiagnosis and missed diagnosis rate were minimum. Combined two factors, D-Dwas more than 3.0 mg/L and fibrin degradation products (FDP) was more than 20 mg/L (pre-DIC was more than 10 mg/L), increased the sensitivity and specificity of diagnosis of DIC and pre-DIC. Logistic regression analysis revealed that the rise of FDP and D-D, the decline of antithrombin III (AT III) and blood platelet count (PLT) were risk factors for pre-DIC and DIC (P was less than0.05), with a relative risky order of FDP was more than D-D was more than PLT was more than AT III. Our study also suggested that D-D was more than 3.0 mg/L + FDP was more than 10 mg/L and AT III was less than 70% could be used as a criteria for pre-DIC diagnosis in clinical practice.

LYMPHATIC AND VENULAR FUNCTION

P111

Pharmacological modulators of intracellular calcium alter mesenteric lymphatic contractions

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Various Ca^{2+}-channel blockers produce the side effect of peripheral edema. We studied mesenteric collecting lymphatics isolated from SD rats, investigating responsiveness to L-type calcium channel blockade and exposure to sigma receptor agonists. Isolated lymphatics were mounted onto resistance-matched glass micropipettes, in a 37°C albumin physiological salt solution (APSS) bath with 1-2 cm H_2O luminal pressure. Responses to nifedipine (10^{-10}–10^{-6} M), diltiazem (10^{-9}–10^{-5} M) and the anxiolytic drug afobazole (50, 100 and 150 μM) were assessed. Video recordings were used to obtain contraction frequency (CF), end diastolic diameter (EDD), end systolic diameter (ESD), phasic contraction amplitude (AMP), tone % and ejection fraction (EF). Ca^{2+}-free APSS was used to obtain the maximal passive diameter (MaxD) used to normalize EDD, ESD and AMP. Expression of the sigma 1 receptor in lymphatics was verified by q-PCR and western blot. The L-type channel blockers, nifedipine and diltiazem, when applied directly to the APSS

bath, inhibited lymphatic pumping at concentrations of 10^{-7} and 10^{-6} M, respectively. Afobazole increased the ESD and decreased AMP and EF at all 3 concentrations, but did not alter CF. BD 1047 dihydrobromide and BD 1063 dihydrochloride, both sigma 1 receptor antagonists, blocked the increase in ESD and decreases in AMP and EF produced with 50 and 100 μM afobazole. Results indicate that nifedipine and diltiazem, used to treat angina and hypertension, and agonists of the sigma 1 receptor (afobazole) negatively impact lymphatic contractility. Impairment of lymphatic pump function may thus be the cause of edema observed in patients prescribed these medications.

P112

Estimation of pressure drop required for lymph flow through initial collecting lymphatics

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Lymphatic function is critical for maintaining interstitial fluid balance and is linked to multiple pathological conditions. While the smooth muscle contractile mechanisms responsible for fluid flow through collecting lymphatic vessels are well studied, how fluid flows through initial lymphatic networks remains poorly understood. The objective of this study was to estimate the upstream suction pressure needed for flow through an intact initial lymphatic network. Pressure drops were computed for real and idealized networks with varying branch orders using a segmental Newtonian flow model. Vessel geometries per branch order were based on measurements from adult Wistar rat mesenteric microvascular networks. Suction pressure estimates for a real network were predicted by calculations from idealized networks with complete bifurcation, uniform vessel length of 1000 microns, and uniform vessel diameters set equal to the average diameter of the first two branch orders of the realistic network. For two diameter cases (40 microns, 80 microns), pressures were estimated across ranges of viscosity (1 cp, 1.5 cp) and output velocity (2 mm/s, 4 mm/s). The suction pressures needed for network sizes of up to 10 branch orders ranged from 0.47 mmHg to 5.65 mmHg. The results offer valuable insight and support the possibility for suction pressures generated from cyclic smooth muscle contractions of upstream collecting lymphatics being sufficient for fluid flow through an initial lymphatic network.

P113

Supervised exercise training as an adjunct therapy for venous ulceration: Protocol for a randomised controlled feasibility trial in a National Healthcare Service

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Chronic venous insufficiency (CVI) affects 6.6–9.4% of the general population (increasing to 10–21% in adults > 50 years), with 1–2% developing venous ulceration. Treatment of venous ulcers places a huge financial burden on healthcare services. Compression garments (bandages or stockings) are the most common venous-ulceration treatment. However, recurrence rates are high and thus alternative therapies need to be pursued. Supervised exercise training (e.g. aerobic and resistance exercises) might be useful adjunct to compression in this patient group, because it is known to have favourable effects on lower-limb blood flow and vascular function. Hence we are conducting a feasibility study for a definitive, randomised controlled trial of a combined exercise and compression intervention in patients with newly-diagnosed venous ulcers.The main study aim is to identify the primary outcome and estimated sample size for the subsequent trial. This is a U.K.-based, two-centre, two-arm, parallel-group, NIHR-funded, randomised feasibility trial. Eighty patients with newly-diagnosed venous ulcers will be randomised 1:1 either to a 12-week exercise programme combined with compression stockings, or usual care. Assessed outcomes include cardiopulmonary fitness, health-related quality of life, ulceration size and microvascular function. Assessments will be repeated at the intervention end (12 weeks) and also 6 months and 1 year following randomisation. Intervention and associated healthcare costs will be calculated and the participants' experiences will be assessed. A definitive randomised trial will be considered feasible if an appropriate primary outcome variable is defined and if adherence to the exercise programme is $\geq 75\%$ in $\geq 67\%$ of the intervention group.

METABOLOMICS AND DISEASE

P114

Hypothermic intervention causes reciprocal changes in acetylated metabolites in neonatal hypoxia-ischemia

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Lines of clinical evidence support the therapeutic benefit of mild hypothermia in infants with intrapartum asphyxia. However, the exact molecular mechanisms whereby hypothermia leads to better outcomes remain unclear. To decipher multifold mechanisms responding to the hypothermic intervention, we employed a two-step approach; metabolomics to target metabolic pathways responding to lowering temperature, and quantitative imaging mass spectrometry (Q-IMS) to reveal spatial alterations in targeted metabolites at specific regions of the brain. Seven-day male Sprague-Dawley rats underwent surgical ligation of left common carotid artery, followed by systemic hypoxia with 8% oxygen for 2.5 hours. Subsequently, pups were returned to 21% oxygen at either 38 (normothermia) or 30 (hypothermia) °C for 3 hours. Brain metabolic states were rapidly fixed by *in-situ* freezing. Neurobehavioral outcome was assessed using objective scale of posturing during the reoxygenation period. Non-targeted profiling of 107 metabolites showed that hypothermia causes not only decreases but also increases in metabolites. More specifically, hypothermia diminishes the carbon biomass related to acetyl-moieties such as pyruvate and acetyl CoA; conversely, it increases deacetylated metabolites such as carnitine and choline. Q-IMS revealed that hypothermia diminishes ACh contents specifically in hippocampus and amygdala. In the same anatomical regions, there was an inverse increase in carnitine. These findings imply that therapeutic hypothermia achieves neuroprotective effects by changing cellular acetylation status with coordinated suppression of acetyl-CoA which resides at metabolic crossroads of glycolysis, amino acid catabolism and ketolysis.

P115

Effects of cigarette smoking on retinal circulation in patients with type 2 diabetes

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Background: Although cigarette smoking has been identified as a major risk factor for diabetic retinopathy in patients with type 2 diabetes, the relationship of cigarette smoking and retinal circulation remain to elucidate in detail. The current study examined the effect of chronic smoking on the retinal microcirculation in patients with type 2 diabetes with early stages of retinopathy.
Methods: Using a laser Doppler velocimetry system, we obtained the retinal blood flow (RBF) values by simultaneously measuring the retinal vessel diameter and blood velocity in 75 eyes (75 patients, mean age ± standard deviation, 59.5 ± 9.8 years). The retinal vessel parameter was compared among a current-smoker group ($n = 21$), past-smoker group ($n = 22$) and never-smoked group ($n = 32$) in no or mild nonproliferative diabetic retinopathy of type 2 diabetes.
Results: There were significant decreases in RBF ($p = 0.03$) with decreased blood velocity ($p = 0.009$) but no difference in vessel diameter ($p = 0.75$) in a current-smoker group compared with never-smoked group.
Conclusion: Our results indicates that the blood velocity and RBF in the retinal arterioles decrease in type 2 diabetes patients with chronic smoking, suggesting chronic smoking may be associated with decreased RBF, probably via lower blood velocity in the retinal arterioles in early-phase diabetic retinopathy.

MICROVASCULAR CELL SIGNALING PATHWAYS

P116

Which inward rectifier K^+ channels contribute to resting tone and K^+-induced dilation of skeletal muscle resistance arteries in mice?

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Inward rectifier K^+ channels (KIR) importantly contribute to the regulation of skeletal muscle blood flow. However, the KIR isoforms expressed in murine skeletal muscle feed arteries have not been established. We hypothesized that KIR2.1 are dominantly expressed, contribute to myogenic tone and mediate K^+-induced dilation in superior epigastric arteries (SEAs). SEAs were isolated from abdominal muscles of 3 months old male C57BL/6 mice and either processed for qRT-PCR, or cannulated and studied by pressure myography (37C, 80 cm H_2O). SEAs expressed transcripts for KIR2.1,

4.2 and 4.1 (1:0.3:0.1 relative abundance, resp.), but not KIR2.2. Exposure of cannulated SEAs to the KIR2 inhibitor, ML133 (10 μM), caused a small biphasic constriction (10 $\pm$ 2%, $n = 6$, p $p < 0.05$) indicating that KIR2.1 contribute to resting tone. Elevation of $[K^+]_o$ from 5 to 15 mM produced 53 $\pm$ 15% dilation. In the presence of ML133 (10 μM), the peak of K^+-induced dilation was non-significantly blunted to 33 $\pm$ 9% dilation ($p > 0.05$), but the duration of K^+-induced dilation was reduced to 33 $\pm$ 11% of control (from 254 $\pm$ 52 s to 95 $\pm$ 28 s; $p < 0.05$). In the presence of ML133 (10 μM), application of the general KIR blocker, Ba^{2+} (100 μM) produced 46 $\pm$ 11% constriction. Alone, Ba $^{2+}$ (100 μM) constricted SEAs 17 $\pm$ 4% and abolished K^+-induced dilation ($n = 5$, $p < 0.05$). These findings indicate that either ML133 is not a very efficacious blocker of KIR2.1 in murine SEAs, or that additional KIR channels, such as KIR4.1 or 4.2, which are not blocked by ML133, contribute to resting tone and K^+-induced vasodilation. Supported by R01 HL086483.

P117
The alpha1-adrenergic agonists phenylephrine and noradrenaline enhance the inhibition of myogenic tone by endothelium-dependent vasodilators in rat cremaster resistance arteries

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Activation of vascular smooth muscle (VSM) alpha1-adrenoceptors is hypothesized to regulate endothelium-mediated vasorelaxation via intercellular communication. In the present study, we have investigated this hypothesis by examining the impact of phenylephrine (PE), noradrenaline (NE) and the TP receptor agonist U46619 on endothelium-dependent and independent dilation of cannulated, myogenically active rat cremaster arteries. Endothelium-dependent vasodilators (0.3 μM acetylcholine (ACh) and 3 μM SKA-31) or direct VSM relaxants (10 μM sodium nitroprusside (SNP), 3 μM pinacidil) reversibly inhibited myogenic tone by 50–80%. Bath addition of 25 nM PE further decreased intraluminal diameter, but did not alter the vasorelaxant responses to ACh, SKA-31 or SNP. Increased concentrations of PE (250 nM to 1 μM), along with 1 μM NE, also decreased intraluminal diameter and significantly enhanced the vasodilatory responses to ACh, SKA-31 and pinacidil, but not to SNP. In contrast, exposure to U46619 (10 or 250 nM) did not augment arterial relaxation by either endothelium-dependent or independent agents, even though the extent of U46619-evoked constriction paralleled that of PE. The Kv channel blocker 4-AP (1 mM) also enhanced the inhibitory effects of SKA-31 and pinacidil, suggesting a role for VSM membrane potential. In myogenic arteries, 1 μM PE also caused direct VSM depolarization. In summary, alpha1-adrenoceptor activation in VSM can augment endothelium-mediated inhibition of myogenic tone, which is not mimicked by TP receptor stimulation. Physiologically, these data suggest that alpha1-adrenergic signaling may evoke an endothelium-mediated, negative feedback process to limit VSM contraction in resistance arteries under conditions of elevated sympathetic nerve activity.

P118
N-cadherin, a novel mechano-sensor in small cerebral arteries

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N-cadherin is the major cell-cell adhesion molecule in vascular smooth muscle cells (VSMC). We tested the hypothesis that N-cadherin acts as a novel mechano-sensory mechanism in VSMC, playing a role in the arteriolar myogenic response and being sensitive to changes in vascular tone. In intact and pressurized rat superior cerebellar artery, immunofluorescence revealed the distribution of N-cadherin-adherens junctions (AJ) as linearly aligned punctate sites localized to the borders between adjacent VSMC. When lumen pressure was acutely raised from 50 to 90 mmHg, both the density and average size of these N-cadherin sites increased significantly. Moreover, vasodilation induced by acetylcholine was accompanied by a significant decrease in density and size of N-cadherin sites, while vasoconstriction with phenylephrine induced a significant increase in density of N-cadherin sites at 50 mmHg. Atomic force microscopy (AFM) was employed to further examine the potential of N-cadherin adhesion sites as active mechano-transduction sites in isolated VSMC. AFM probes tipped with an N-cadherin-coated microbead (5 μm) formed strong adhesion with the VSMC surface. During contact between the VSMC and the N-cadherin coated microbead, we observed a progressive clustering of N-cadherin-GFP at the adhesion site. Pulling vertically on the N-cadherin coated bead (~1 nN) induced localized force generation from the VSMC that mechanically opposed the pulling. These observations provide compelling evidence that N-cadherin AJ sites are mechanically sensitive to both externally imposed force and to force generated intracellularly by the contractile apparatus. The data, therefore, support a strong role for these sites in VSMC mechano-trsanduction. (NIH P01HL095486 GAM).

P119

G-protein mediated signaling pathways in myogenic responsiveness of mouse mesenteric artery

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Myogenic responsiveness (MR) is the ability of a small artery to constrict to an increase in intraluminal pressure and dilate to a decrease in pressure. The mechanisms linking an increase in pressure to VSMC contraction are suggested to involve mechanical activation of AT1-receptors. Our aim was to explore the role of alternative G protein-coupled receptor (GPCR) pathways. MR of pressurized mouse mesenteric arteries (MA; 100-200 μm) was measured as the slope of the active diameter curve. The PLC inhibitors U73122 (0.5 μM), ET-18-OCH3 (10 μM), and the PKC inhibitor BIM-X (1 μM) impaired MR. Inhibitors of PLA2 (AACOCF3, 5 u), DAG lipase (RHC80267, 20 μM), PI3-kinase (wortmannin, 0.03 μM), CYP4A (HET0016, 10 μM), and TRPC channels (SKF96365, 10 μM) had no effects. Gq/11 and G12 mRNA and protein were expressed in MA. The Galpha/q inhibitor YM-254890 (0.1 μM) and the AT1-R blocker valsartan (0.3 μM) inhibited MR. The GPCR antagonists prazosin (1 μM), losartan (0.1 μM), BQ-123 (1 μM), and SQ29548 (1 μM) had no effects. The P2Y-R antagonists suramin (100 μM) and PPADS (10 μM) inhibited MR, but the ATP diphosphatase apyrase (20 U/mL) did not. MR was similar in P2Y2-/- vs. age-matched WT mice. Preliminary data suggest a reduction of MR in P2Y6-/- mice vs. WT, and that the Rho-kinase (ROCK) inhibitor Y27632 (3 μM) inhibits MR. Thus, Gq/11 and possibly G12 pathways mediate pressure activation in mouse MA through PLC, PKC, and ROCK. MR may be initiated by mechanical activation of P2Y6-R and AT1-R in VSMCs.

P120

Extracellular histones activate local and propagating endothelial calcium signals

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Histone exposure to endothelial cells causes Ca^{2+} overload and cell death, but the specific cellular ion channels involved have not been identified. We studied the spatial and temporal characteristics of endothelial Ca^{2+} signals after exposure of 3rd order mesenteric arteries to purified histones (Roche). We utilized transgenic mice with endothelial cell-specific expression of a Ca^{2+} biosensor (GCaMP5) to perform spinning disc confocal imaging of endothelial Ca^{2+} signals without interference from vascular smooth muscle or other cell types. We found that histones induce two distinct types of endothelial Ca^{2+} signals. First, at low concentrations (1 μg/mL), histones trigger local Ca^{2+} events that are brief and contained within an endothelial microdomain. At higher concentrations (10 μg/mL), histones trigger much larger, longer duration, calcium waves that propagate within and between endothelial cells. In addition, we found that these two Ca^{2+} signals may involve different ion channels. Histones increase Ca^{2+} signals even in the presence of cyclopiazonic acid (CPA), arguing against a role for IP3-receptor mediated Ca^{2+} events. Treatment with 10 nM GSK219, a transient receptor potential cation V4 channel (TRPV4) antagonist, suppresses the localized Ca^{2+} events induced by 1 μg/mL histones, while the propagating Ca^{2+} events seen at 10 μg/mL are not inhibited by GSK219 (10 or 100 nM). The data demonstrate that extracellular histones are robust activators of endothelial Ca^{2+} signals through TRPV4 dependent and TRPV4 independent pathways.

P121

Histone deacetylase SIRT1 regulates autophagy of vascular adventitial fibroblasts through AKT/mTOR signaling pathway

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Objective: Autophagy functionally links to cellular homeostasis in atherosclerosis. The histone deacetylase Sirtuin 1 (SIRT1) participates in regulating cellular autophagy and inflammation by deacetylating multiple transcription factors. However, there is no direct evidence to demonstrate the effect of autophagy on inflammatory response in vascular adventitial fibroblasts (VAFs). We aimed to determine the effect of SIRT1 on autophagy and inflammation in VAFs and related signaling pathway.

Methods and Results: TNF-α (5 ng/mL) could increase the SIRT1 expression of mRNA and protein in VAFs. Activation of SIRT1 by resveratrol increased TNF-α-induced VAF autophagy by increasing the conversion of LC3-I to LC3-II and beclin1 expression. In contrast, inhibition of SIRT1 by sirtinol or knockdown of SIRT1 decreased autophagy of VAFs. On the other hand, TNF-α decreased the Akt phosphorylation in VAFs. Akt inhibitor MK2206 could enhance the effect of TNF-α on VAF autophagy. Furthermore, knockdown of SIRT1 increased Akt phosphorylation in VAFs. In addition, mTOR inhibitor rapamycin increased TNF-α-induced autophagy of VAFs. The Ingenuity Pathways

Analysis showed that there is a direct interaction between SIRT1 and IL-1β in atherosclerosis. Knockdown of SIRT1 augmented NLRP3 expression and IL-β release in VAFs. The effects of TNF-α on NLRP3 expression and IL-β release were blocked by rapamycin, while 3-MA enhanced the effects of TNF-α on NLRP3 expression and IL-β release in VAFs.

Conclusion: These results indicate that SIRT1 regulates autophagy of VAFs through Akt/mTOR pathway, and SIRT1 inhibits the inflammatory response of VAFs by upregulating autophagy.

Key Words: SIRT1; Autophagy; Inflammation; Vascular adventitial fibroblasts; Akt/mTOR

MICROVASCULAR FLOW REGULATION/OXYGEN DELIVERY/NETWORKS

P122

The role of glial cells in the regulation of retinal microcirculation in response to modulations in systemic oxygen tension

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Purpose: The purpose of this study was to investigate the role of retinal glial cells on the regulation of retinal blood flow in response to modulations in systemic oxygen tension in cats.

Methods: We measured the vessel diameter (D), blood velocity (V) and blood flow (F) simultaneously in first-order retinal arterioles using a Laser Doppler Velocimetry (LDV) system (CLBF model 100, Canon, TOKYO). The subjects were under general anesthesia during hyperoxia (100% O_2, hyperoxia group) or hypoxia (10% O_2, hypoxia group) for 10 minutes after intravitreal injection of L-2-aminoadipic acid (LAA), known as a gliotoxic compound, or diluted HCl used as the control.

Results: In the hyperoxia group, the decreases in D, V, and F in response to hyperoxia were significantly attenuated in the LAA group compared with the control group (LAA: D-8.5 $\pm$ 1.5%, V-13.8 $\pm$ 1.5%, F-27.8 $\pm$ 3.0% vs. control: D-16.8 $\pm$ 1.3%, V-26.3 $\pm$ 2.0%, F-48.9 $\pm$ 2.4%) ($p < 0.01$). In the hypoxia group, the increases in D, V, and F were significantly reduced in the LAA group compared with control group (LAA: D + 6.8 $\pm$ 0.55%, V + 33.0 $\pm$ 0.73%, F + 48.9 $\pm$ 2.8% and control: D + 14.9 $\pm$ 1.8%, V + 42.5 $\pm$ 2.7%, F + 88.9 $\pm$ 4.7%) ($p < 0.05$).

Conclusions: The current findings suggest that retinal glial cells play important roles in the regulation of retinal blood flow in response to modulations in systemic oxygen tension in cats.

P123

Skin microvascular assessments predict outcome of patients treated with extra-corporeal membrane oxygenation (ECMO)

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This study was undertaken to test the hypothesis that maintained integrity and function of skin papillary nutritive microvasculature on ECMO is a predictor of survival, Skin microcirculation in three female and seven male patients; median age 55 years (range 21–78) treated with ECMO for acute heart failure and eight healthy male controls (21–29 years) were examined at the dorsum of the hand between 1st and 2nd metacarpus, using Laser Doppler Perfusion Measurements (LDPM), Computer Assisted Video Microscopy (CAVM). Five to seven films were examined to quantitate peri-capillary bleedings and haloes, Functional Capillary Density (FCD) and Capillary Flow Velocity (CFV). CFV in individual capillaries was scored in a semi quantitative five-category scale.

Results: Five patients survived and five died on ECMO. There was no correlation between central hemodynamic parameters and standard blood test analyses, and survival. Survivors had no capillary bleedings or haloes, and similar microvascular parameters as controls {FCD (loops/mm^2): 70,8/0,23 (mean with CoV) vs. 65,5/0,20; Mean categorical flow velocity (MCFV): 2,7 vs. 2,8; LDPM of 129,8/0,43 vs. 48/0,2}. All non-survivors had peri-capillary pathology (bleedings: $n = 1$ and dark haloes: $n = 4$), as well as highly significant changes in both FCD and CoV FCD (39,4/0,37 vs. 65,5/0,20). MCFV was 1.2 vs. 2.8 < 0.01. LDPM was lower 9.8/0.38 vs. 48/0.2 (ns).

Conclusion: Maintained microvascular integrity in structure and function of skin nutritive papillary capillaries early after establishing ECMO, is a strong indicator of survival. Microvascular dysregulation seems to be incompatible with life.

P124

Relationship between microvascular blood flow and angiogenic factors in pre-eclampsia

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Microvascular dysfunction occurs in preeclampsia with reduced tissue perfusion precedes the onset of the disease. Angiogenic imbalance may play a role in preeclampsia. We hypothesized that an imbalance in pro- and anti-atherogenic factors affects microvascular function in pre-eclampsia and correlates with reduced tissue blood flow. Strain gauge plethysmography was used to measure maternal tissue blood flow and was correlated with circulating angiogenic factors; soluble fms-like tyrosine kinase 1 (sFlt-1), soluble endoglin (sEng) and placental growth factor (PlGF). Tissue blood flow was significantly reduced in the pre-eclampsia group compared to normal pregnancy ($p < 0.0001$). Serum sFlt-1 and sEng were significantly increased in pre-eclampsia compared to normal pregnancy ($p < 0.0001$). In contrast, serum PlGF was significantly reduced in pre-eclampsia compared to normal pregnancy ($p < 0.0001$). There was a strong inverse correlation between microvascular blood flow and sFlt-1 and sEng in the normal pregnancy and pre-eclampsia groups ($p < 0.0001$). There was a positive correlation between microvascular blood flow and PIGF in normal pregnancy and pre-eclampsia ($p < 0.0001$). Blood flow also showed a strong correlation with the sFlt-1: PIGF ratio in normal pregnancy and pre-eclampsia ($p < 0.001$). The data shows that the anti-angiogenic factors studied correlate inversely with microvascular perfusion during both normal pregnancy and pre-eclampsia. In pre-eclamptic patients, the elevated levels of these anti-angiogenic factors inversely correlate with microvascular blood flow whilst they exist at lower levels in patients with normal pregnancies and are associated with normal blood tissue perfusion.

P125

Thrombomoduline improves maternal and fetal outcomes in an experimental pre-eclampsia rat model

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Introduction: Although its pathogenic mechanism of pre-eclampsia has not yet been clarified, it is believed that potential mechanisms might include severe reduction of uterine-placental blood flow. We investigated where the critical sites of deterioration locate and also the protective effect of Thrombomoduline (TM) on placental blood flow (PBF) and tissue oxygenation in the experimental preeclampsia rat model.

Methods: Rats were randomly divided into 4 groups: a control group (C-group), a pre-eclampsia group administered L-NAME and LPS (P-group), a C-group with TM (CT-group), and a P- group with TM (PT-group). On day 21 of pregnancy, uterus exposed for laser-Doppler measurement of blood flow and oxygenation. All fetuses were removed to counts fetus death rates (FDR) and measure a brain weight and VEGFR-1.

Results: It was evident that not only a higher MAP and a FDR, but VEGFR-1 was observed in P-group. Specific reductions of PBF (C-group: 9.4 ± 1.8; P-group: 7.0 ± 1.6 ml/100 g/min; $p < 0.001$), oxygenation of the rat fetal brain and placenta were observed in Pgroup. TM improved not only an increased value of VEGFR-1, but FDR and weight in P-group.

Conclusions: Severe reductions were observed in P-group UBF and placental oxygenation which were not proportional to MAP. These data imply that regional hypoperfusion occurs in dissociation with systemic MAP. These predicted observations might play an important role in fetal maternal and fetal outcome. It is evident that TM which might cause the inhibition of coagulation and inflammation in pre-eclampsia has the potential to improve not deterioration of UBF but also these outcomes.

P126

Skin trauma induces early deep vascular plexus hyperemia, while superficial papillary nutritive perfusion remains unchanged

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The deep skin thermoregulatory plexus has autonomous nerve regulation. The superficial plexus ensures perfusion to locally regulated superficial papillary capillaries, providing nutrition to epithelial proliferation. We have hypothesized that papillary capillary functio. can be assessed by Computer Assisted Video Microscopy (CAVM) and Diffuse Reflectance Spectroscopy (DRS), but not with Laser Doppler Perfusion Measurements (LDPM) because of its dependence on deep plexus perfusion. This study characterizes early microvascular changes in nutritive (CAVM and DRS) and thermoregulatory compartments (LDPM) induced by a standardized skin trauma model. Four female and eight male, healthy volunteers age 20–27 years, were examined with CAVM, DRS and LDPM at baseline, and 30 minutes after the induction of a 5.0 mm long and 1.0 mm deep skin trauma (Surgicutt) on the volar aspect of the forearm. CAVM data were collected at distances between 0 and 1 mm, 2–3 mm and at 30 mm from the skin incision. LDPM and DRS data were recorded at 2–3 mm and 30 mm distances.

Results: LDPM recordings showed a marked hyperemic trauma response at 2–3 mm {36.0(±15.2)a.u.} compared to baseline {8.8(±1.8)a.u.}, but not at 30 mm distance {7.4 (±2.6)a.u.}. Functional capillary density and capillary flow velocities were unaffected by the trauma at all distances {0–1 mm: 9.2(±0.5); 2–3 mm:9.7(±0.8); 30 mm:9.2(±0.4) capillary crossings/mm gridline} compared to baseline {9.4 (±0.8) capillary crossings/mm gridline}. The microvascular oxygen saturation increased at 2–3 mm {71.2(±4.8)%} but not at 30 mm{49.8(±7.9)%} compared to baseline {45.8 (±7.4)%}.

Conclusion: CAVM assesses the papillary microcirculation, while LDPM better mirror the deep plexus perfusion. DRS probably monitor the blood increase in the superficial plexus as it enters the papillary nutritive capillaries.

P127

Metabolic long-term control of microvessel diameters: Roles of oxygen sensitivity, sensor localization and vasodilator or vasoconstrictor signaling

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Aims: Microvascular responses to metabolic signals guarantee adequate tissue supply via negative feedback regulation of perfusion by increasing vessel diameters in undersupplied regions. With decreasing Po2, increasing vasodilator release from vessels, tissue or erythrocytes, and decreasing vasoconstrictor release have been observed. Here, implications of oxygen sensitivity patterns, sensor localisation and vasodilator/vasoconstrictor involvement were investigated and interpreted within a framework for feedback regulation.

Methods: Diameter, length, haematocrit and flow velocity were measured for all vessels ($n = 576$) of a mesenteric microvascular network *in vivo*. Theoretical simulations of hemodynamics, oxygen distribution and long-term diameter adaptation to shear stress, pressure and metabolic stimuli were applied to this network, with metabolic signals acting locally and after transfer to remote vessels. Assumptions on metabolic signalling were varied. Resulting oxygen distributions and deviations of simulated from measured flow velocities were analysed.

Results: (i) For vasodilators, oxygen sensitivity is not needed for negative feedback due to a "dilution effect" causing increasing signal substance blood concentrations with decreasing perfusion. Singular signalling proportional to vessel length gives most realistic results. Tissue-derived signals result in less realistic distributions. Erythrocyte-derived signals cause maldistribution. (ii) Vasoconstrictors cause unrealistic distributions when released into the blood or evoking conducted effects.

Conclusions: Surprisingly, oxygen sensitivity for high Po2 levels may be sufficient for long-term diameter adaptation. Metabolic sensors are probably located in perivascular tissue sleeves. Vasodilators are suited to evoke diameter adaptation locally and in remote vessels, while vasoconstrictors may evoke adaptation only locally.

P128

Rescue of gap junction function restores blood flow in chicken chorioallantoic membrane vessel networks

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Introduction: Theoretical models revealed the relevance of gap junction (connexin) mediated information transfer along blood vessels to ensure adequate flow distribution in microvascular networks. Here, we studied the effects of vascular gap junction blockage and subsequent functional rescue in the chicken chorioallantoic membrane (CAM) vasculature.

Methods: Connexin expression and localization were studied by Western blot and confocal immunofluorescence microscopy. Using intravital microscopy, changes in network morphology were analyzed. 4 selected regions per CAM were treated for up to 24 h with (1) PBS, (2) the gap junction blocker carbenoxolone (175 μM, CBX), (3) the gap junction enhancer dipyridamole (50 μM, DIP), or (4) the combination of CBX and DIP. For CBX blockage maximal vasodilation (acetylcholine 10 μM, adenosine 100 μM, papaverine 200 μM, sodium nitroprusside 10 μM) allowed examining the relative contribution of changes in vessel structure and vascular tone.

Results: Immunohistochemistry revealed basal connexin expression in arteries not in veins. Vessel density was strongly decreased after 24 hours by CBX ($-61.76 \pm 3.27\%$ of control) but significantly less in the presence of DIP ($-13.03 \pm 32.86\%$). CBX led to diameter decrease of arteries and veins to 0.55/0.54 (relative to PBS control) initially effected to about 50% by an increase in vascular tone (59/46 after 3 h), and later almost completely by structural adaptation(96/88%, 6 hours).

Conclusion: Our findings show that vascular gap junction blockage with carbenoxolone evokes maldistribution of CAM blood flow by vessel rarefaction and diameter decrease. These effects can at least partially be neutralized by the gap junction enhancer dipyridamole.

P129

Assessing the microvascular response to insulin in rat skeletal muscle using intravital video microscopy

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Insulin is reported to regulate microvascular volume via *de novo* recruitment of capillaries which previously had not been perfused (Barrett et al, J Physiol 2014). Since this conclusion was based on indirect measurements of microvascular volume (contrast enhanced ultrasound using microbubbles) that have been heavily criticized (Poole J Physiol 2014) we decided to test this concept using direct observation of microvascular blood flow in response to an insulin stimulus.

Methods: Analysis of video sequences obtained using intravital video microscopy was used to quantify microvascular flow in capillaries of the extensor digitorum longus muscle in the rat. Eight Sprague-Dawley rats (165 ± 4 gm) were fasted overnight and studied at basal conditions (saline infusion) and during a hyperinsulinemic euglycemic clamp (HIEC). Immediately following the baseline measurements a bolus (300 pmol/kg) of insulin was infused over 2 minutes followed by a constant infusion rate (30 pmol/kg/min) for 70 minutes. A 40% glucose solution was infused at variable rates (GIR) to maintain euglycemia.

Results: There was no direct evidence of capillary recruitment (total number of functional capillaries containing RBCs unchanged) nor in the number of capillaries with intermittent or stopped flow (15% of all capillaries). Measured RBC velocity was $49 \pm 12\%$ greater during the steady-state portion of the HIEC compared to baseline ($p < 0.01$) with no change in capillary hematocrit. Calculated RBC, plasma and blood flow increased by $80 \pm 25\%$, $53 \pm 12\%$ and $58 \pm 14\%$ compared to baseline ($p < 0.05$), respectively.

Conclusion: Insulin caused a change in microvascular blood flow without evidence of *de novo* capillary recruitment.

MICROVASCULAR MECHANICS/ MICROVASCULAR MODELING/ HEMODYNAMICS/ RHEOLOGY

P130

Is the time lag of pressure pulses in microcirculation associated to wave propagation? A model study

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Background: The time lag of pressure pulses along arteries has been widely investigated and it is recognized to be the result of wave propagation. However, the time lag of pressure pulses in microcirculation is rarely noticed and it remains unclear whether it is also due to the wave propagation.
Methods: The behavior of pulse waves was simulated by computational one-dimensional (1D) and zero-dimensional (0D) models. The time lag associated with the pulse wave propagation is depicted by a 1D model while the 0D model mimics the time lag independent of pulse wave propagation. Both models were applied to a rat mesenteric microcirculation network, and to the human arterial circulation. In the microcirculation, the time lag of pressure pulses was calculated between the main input arteriole and the precapillary vessel, and in arterial circulation it was obtained between the carotid and the femoral arteries.
Results: The simulated time lag in the arterial circulation is 94 ms in the 1D model, and sharply declines to 5 ms in the 0D model. In contrast, in both 1D and 0D models, the time lag of pulse wave in the microcirculation is identical at 22 ms.
Conclusion: The results indicate that differing from the situation in arteries, the time lag of pressure waves in microcirculation is independent of wave propagation properties.

P131

Adaptive mathematical modeling of pulsatile shear stress-mediated nitric oxide-vascular regulation

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Background: Pulsatile shear stress-mediated Nitric oxide (NO) release plays an important role in vascular regulation. However, mathematical modeling of this process, in particular the quantitative description of the governing effect on vessel diameters needs to be developed.
Method: A set of models was integrated to represent the process of shear stress-mediated NO generation, vessel regulation, and the feedback to shear stress. The NO generating model was constructed by transfer functions concerning shear stress rate-sensitive NO response to highlight the effect of pulsatility. A self-adaptive model was built to simulate the diameter change in vessels and its effect on shear stress. The whole model was applied to study the dilation of an arteriole under pulsatile or non-pulsatile shear stress.
Result: In an arteriole (diameter 20 micrometer) under initial shear stress ranging from 6 to 25 dyn/cm^2, the NO release increases up to 4.3% evoked by pulsatile condition, compared with non-pulsatile case. The result is consistent with experiment findings. The corresponding average variation in diameter dilation due to pulsatile shear stress is 12.2%.
Conclusion: The proposed model is able to describe the pathways from shear stress to vascular regulation, which is in particular useful to understand the mechanism of physiological pulsatility in microvessels.

P132

Diffusion of red blood cells in Poiseuille flow

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In blood flow circulation, mass transport plays an important role. Former researches have been performed to understand the self-diffusion of blood flow. However, the gradient diffusion of red blood cells (RBCs) in small vessels is still not well understood. In this study, we investigated the gradient diffusion of red blood cells (RBCs) concentration using blood flow through a Y-shape microchannel. The effects of flow rates, and hematocrit (Hct) on the gradient diffusion were investigated experimentally. Under the same Hct condition, the gradient dispersion coefficient was increased clearly by increasing the flow rate. The scaling argument shows that the main dispersion mechanism of red blood cells is cell-cell interaction. The gradient diffusion dominated over Brownian diffusion in most of high Hct conditions. These findings provide useful information in understanding mass transportation in small vessels.

P133
Increased arterial myogenic tone in an *in vitro* model of vascular insulin resistance

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Vascular insulin resistance is characterized by a decrease in endothelial nitric oxide synthase (eNOS) activity and comparatively increased (unbalanced) endothelin activity. Endothelium-dependent vasodilation is impaired but little is known regarding other vascular responses. Using an *in vitro* model of vascular insulin resistance, this study examined pressure-induced myogenic tone in the rat cremaster muscle artery. Male Sprague Dawley rats (approx. 200 g) were anesthetized (sodium thiopentone, 100 mg/kg i.p.) and the cremaster muscles removed. Pressure-diameter curves over the range 30–120 mmHg were obtained in isolated, cannulated arteries, in both Ca^{2+}-free and normal [Ca^{2+}] solutions. The vessels were then incubated with vehicle solution or insulin (60 μg/mL; intra-lumenal exposure only) for 2 h and the pressure-diameter relationships established again. In arteries incubated with insulin, active myogenic tone was increased at 50, 70, 100 and 120 mmHg, by 20.2 ± 4.8, 12.1 ± 4.8, 13.7 ± 2.4 and 11.6 ± 4.6% respectively ($p < 0.05$, two-way ANOVA, $n = 4$ for all). Insulin also decreased passive (maximum) diameter at 50 and 70 mmHg, by 7.3 ± 1.8 and 4.9 ± 1.4% respectively. There was no significant change in myogenic tone or passive diameter in vehicle-incubated arteries, at any pressure. These observations suggest exposure to high concentrations of insulin increases pressure-induced myogenic tone in isolated arteries and decreases artery distensibility. Such effects may play a role in decreased vascularity and tissue blood flow observed in hyperinsulinemia and insulin resistance.

P134
Quantification of geometrical differences between microangiopathy capillaroscopy images and controls

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The study aimed to quantify geometrical differences between images classified as clear microangiopathy (CM) and not clear microangiopathy (NCM) using a measure of geometrical complexity. Capillaroscopy images were captured by a light microscope ($\times$100 magnification) with a wide field view, using a green light source, and from the ring or middle finger. 11 images were classified as CM, and 11 were classified as NCM by the lead Clinical Scientist (JA). Contrast enhancement was performed on the images. Prior to calculating the Hausdorff fractal dimension (HFD), the images were converted to a binary image using thresholding. The images were also segmented into proximal and distal halves. An algorithm for computing HFD was applied to the whole images and segmented halves, with HFD measuring the rate of addition of structural details with increasing magnification. The more HFD presents towards 2, the more the microvascular configuration fills a space, therefore, the greater the geometrical complexity. Differences in HFD between groups were compared using the statistical programming language R, and presented as medians [q1:q3].No differences were found in HFD between CM and NCM whole images (CM = 1.75 [1.71:1.75]; NCM = 1.75 [1.71:1.76]). The CM group showed increased HFD ($p = 0.02$) in the distal half compared to the NCM group (CM = 1.65 [1.62:1.67]; NCM = 1.59 [1.55:1.60]), with no differences in the proximal half (CM = 1.74 [1.68:1.78]; NCM = 1.70 [1.65:1.72]). Capillaroscopy images that were indicative of CM showed greater geometrical complexity in the distal segment.

P135
Continuous serelaxin infusion alters circumferential wall stiffness but not myogenic tone of mesenteric resistance arteries in spontaneously hypertensive rats

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Vascular stiffness and dysfunction are strongly associated with cardiovascular disease. The peptide hormone serelaxin (human relaxin-2) has beneficial effects in acute heart failure which are attributed to its vascular actions. Previous studies indicate that 3–5 days serelaxin treatment reduces myogenic tone but has little effect on arterial stiffness. Moreover, there are conflicting reports on the optimum duration of treatment because most studies are in healthy animals. Therefore we investigated the vascular actions of serelaxin in spontaneously hypertensive rats (SHRs). Male Wistar Kyoto (WKY) rats and SHRs (25 weeks old) were treated with serelaxin (13.33 μg/kg/hour) or placebo (20 mM sodium acetate) subcutaneously for 10 days. Mesenteric artery passive mechanical wall properties and myogenic tone were analysed using pressure myography. Inner and outer diameters and

volume compliance were significantly reduced in SHRs, but there was no significant difference in circumferential wall stiffness between the two rat strains. Serelaxin treatment significantly reduced circumferential stiffness in SHRs but not WKYs, and this was associated with outward remodelling. Elastase incubation revealed that this difference in circumferential stiffness was not due to elastin. Myogenic tone was not altered in the mesenteric arteries of SHRs, and 10 days of serelaxin treatment did not reduce myogenic tone in either WKYs or SHRs. In summary, 10 days of serelaxin treatment in SHRs has minimal effects on myogenic tone but reduces circumferential stiffness. We suggest that a longer duration of serelaxin treatment may be necessary to induce vascular remodelling.

P136

A novel parameter reflecting rheology and activity of leukocytes in *ex vivo* microvascular model

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Background and Purpose: Leukocytes play an important role in the whole process of atherosclerosis and cardiovascular events. Activated leukocytes show pseudopod formation and up-regulated adhesion molecules. Aim is to explore clinically feasible parameters of blood rheology and activity of leukocytes using a new micro-channel flow analyzer (MC-FAN) served as a myocardial microvascular model *ex vivo*.
Methods: Blood samples collected from 27 healthy volunteers were anti-coagulated by heparin or EDTA-2Na blocking Ca++ dependent activation of leukocytes and platelets. We measured blood passage time through the microchannnels adhesiveness and deformability of leukocytes in whole blood with heparin or EDTA using MC-FAN.
Results: The blood passage time of whole blood with EDTA was shorter than one of blood with heparin. In microscopic analysis, number of leukocytes adhered on the terrace and/or occluded in microchannels were very few in blood with EDTA. Subtraction of the blood passage time with heparin and one with EDTA was negatively correlated to the number of adhering leukocytes ($p < 0.05$).
Conclusion: Subtraction of the passage time of blood with heparin and one with EDTA may be a novel parameter reflecting leukocyte activity in *ex vivo* microvascular model.

P137

Scanning electron microscopic studies on morphological abnormalities of erythrocytes in alcoholic liver diseases

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Methods: The subjects of this study were five patients with alcoholic liver cirrhosis, two patients with alcoholic fatty liver, and two healthy volunteers. Concentrating on erythrocyte morphology in the presence of alcoholic liver disease, we observed erythrocytes under a scanning electron microscope.
Results: Among the five patients with alcoholic liver cirrhosis, stomatocytes accounted for about 14% and acanthocytes for about 10% of all erythrocytes. In the fatty liver group, acanthocytes were absent, and stomatocytes accounted for 23% in one subject and 56% in the other, of the total. When the two patients with liver cirrhosis were examined over time, one of them was found to have 12% stomatocytes, 21% acanthocytes, and a filtration time of 14.7 second at admission. Following 1 month of abstinence, this patient had 1% stomatocytes, 6% acanthocytes, and a filtration time of 10.4 sec, which were accompanied by improvements in peripheral blood parameters and liver function. In another patient with liver cirrhosis, there were no acanthocytea, 8% stomatocytes, and a filtration time of 5 sec at admission; this patient had 1% stomatocytes and a filtration time of 7.7 sec after 1 month of abstinence.In one patient with fatty liver who was examined over time, percentage of stomatocytes was 56%,and the filtration time was 7.7 sec at admission; these parameters were 1% and 5.1 second, respectively, after 1 month of abstinence.
Conclusions: The present study revealed that stomatocytes and acanthocytes are morphologically abnormal erythrocytes observed in the presence of alcoholic liver disease. These abnormal forms of erythrocytes tended to normalize as peripheral blood parameters and liver function were improved by abstinence, which suggests that erythrocyte morphology is related to the pathophysiology of alcoholic liver disease.

MICROVASCULAR PATHOPHYSIOLOGY-PHARMACOLOGY

P138

Effects of alpha-mangostin on ocular hypoperfusion and blood retinal barrier leakage in type 2 diabetic rats

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Background and Objective: Alpha mangostin (alpha-MG) is the first xanthone isolated from bark and dried sap of Garcinia mangostana which has been demonstrated the anti-inflammation and antioxidant properties. The present study examined effects of alpha-MG supplementation on ocular blood flow (OBF) and blood-retinal barrier (BRB) permeability in a type 2 diabetic rat model.

Materials and Methods: Male Sprague-Dawley rats were divided into four groups: normal control and diabetes with or without alpha-MG supplementation. Type 2 diabetic rats were induced by feeding a high fat diet followed by an injection of low dose streptozotocin. Alpha-mangostin (200 mg/day) was administered by gavage feeding for 8 weeks. The effect of alpha-MG on blood glucose, HbA1c, mean arterial pressure (MAP), OBF and BRB leakage were investigated. Additionally, levels of retinal malondialdehyde (MDA), advance glycation end products (AGEs), receptor of advance glycation end products (RAGE), tumour necrosis factor alpha (TNF-alpha), and vascular endothelial growth factor (VEGF) were evaluated.

Results: The elevated blood glucose and HbA1c were observed in DM2 rats. Moreover, DM2 rats had significantly decreased OBF, but statistically increased MAP and leakage of the BRB. The alpha-MG-treated DM2 rats showed significantly lower levels of retinal MDA, AGEs, RAGE, TNF-alpha, and VEGF than the untreated group. Interestingly, alpha-MG supplementation significantly increased OBF while decreased MAP and leakage of BRB.

Conclusion: Alpha mangostin supplementation could restored OBF and improved the BRB permeability through its antioxidant, anti-inflammatory, and anti-glycation activities.

P139

Effects of acute and chronic paracetamol treatments on the alteration of blood brain barrier integrity in the cortical spreading depression migraine animal model

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Adverse effect of paracetamol (APAP) has gradually been reported in the last decade in several systems, including the circulatory system. Since APAP can directly cross the blood brain barrier (BBB), the effect of chronic treatment of this drug on the BBB integrity can be expected. In order to prove this hypothesis, the effect of acute (1 hour) and chronic (15 and 30 days) APAP treatments on the alteration of cerebral microvessels was studied in a cortical spreading depression (CSD) migraine animal model. Rats were divided into the control, CSD, APAP treatment and APAP treatment with CSD group. APAP (200 mg/kg body weight) was i.p. injected as a single or once-daily dose for acute and chronic APAP treated groups, respectively. CSD was induced by KCl application on the cortical surface. The results demonstrated that the CSD induction caused alterations of BBB integrity, as indicated by increase in angle of tight junction alignment between endothelial cells, decrease of tight junction proteins (ZO-1, occludin, claudin-5) expressions as well as increase of IgG extravasation in the CSD group. Pretreatment with acute APAP treatment attenuated those alterations induced by CSD. However, chronic APAP treatment resulted in an enhancement of the abnormalities of BBB integrity induced by CSD. Interestingly, rats that received chronic APAP treatment alone also induced alterations in the BBB integrity as compared with the control group.Based on these results, it can be suggested that long-term treatment with APAP for patients with CSD-related disorders, particularly migraine headaches, might need to be carefully monitored.

P140

Chronic APAP treatment increases alteration of cultured brain endothelial cell line

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Nowadays, non-beneficial effect of paracetamol (APAP) has been discussed in parallel with its protective effect against several pathological conditions. Our previous studies in migraine animal model revealed that chronic use of APAP induced the abnormality of cerebral microvessels. In order to confirm the effect of chronic APAP treatment on the alteration of the cerebral microvessels, the expression of cell adhesion molecule as well as the alteration of tight junction proteins were studied in cultured mouse endothelial cells (bEnd.3 cell) in the condition with and without APAP treatment. bEnd.3 cells were divided into 2 groups; control and APAP treated groups. In APAP treated group, bEnd.3 cells were incubated with APAP (100 μM) for 3 different time points (24 hours, 2 weeks, 4 weeks) while control group was treated with fresh media as well. Expressions of tight junction proteins (ZO-1, occludin, and claudin-5) as well as cell adhesion molecules (ICAM-1 and VCAM-1) were monitored by western blotting. The results revealed that APAP treatment for 24 hours had no effect on the expression of tight junction proteins and cell adhesion molecules as compared with the control. However, chronic APAP treatment (more than 2 weeks) induced alterations in bEnd.3 cells. A significant lower of tight junction proteins levels as well as an increase in cell adhesion molecules were observed in bEnd.3 cells with long-term APAP treatment. These data confirm that chronic APAP treatment induces the alterations of BBB integrity, in which correlates with the results obtained from *in vivo* study.

P141

Somatostatin elicits dilation of isolated porcine retinal arterioles

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Purpose: Retinal blood flow is impaired in patients with type 2 diabetes mellitus with no and mild diabetic retinopathy (DR). Improvement of the impaired retinal circulation could be a novel therapeutic target for DR. Somatostatin (SST) is one of the most important neuroprotective factors synthesized by the retina. In the early stages of DR, there is a downregulation of SST. Topical treatment with SST eye drops prevents retinal neurodegeneration in diabetic rats. SST replacement seems a rational approach for treating DR. Moreover SST induces vasodilatation via endothelium-derived NO in the cat mesenteric artery. However the direct effect of SST on ocular microvascular reactivity remains unknown. We examined the direct effect and the underlying mechanism of the vasomotor action of SST in porcine retinal arterioles.

Methods: Porcine retinal arterioles (90–110 microns in internal diameter) were isolated, cannulated, and pressurized (55 cmH2O) without flow for this *in vitro* study. Video microscopic techniques were used to record the changes in diameter in the retinal arterioles in response to SST.

Results: The retinal arterioles dilated in a SST concentration-dependent (1 nM–30 μM) manner and abolished after endothelial removal. The nitric oxide (NO) synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME) inhibited SST-induced vasodilation comparable to denudation. Inhibition of soluble guanylyl cyclase was comparable to L-NAME.

Conclusions: SST elicits endothelium-dependent dilation of retinal arterioles mediated by NO release.

P142

Active fraction from Bixa orellana leave extract attenuates increased endothelial permeability induced by bradykinin *in vitro*

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Alteration in endothelial permeability is a hallmark of inflammatory processes. It causes severe disruption in endothelial barrier function which eventually leads to vascular pathologies, including atherosclerosis. Previous studies showed aqueous extract of *Bixa orellana* leave (AEBO) exhibited anti-hyperpermeability against bradykinin. Thus, an active fraction (FAEBO) that separated from AEBO through nitric oxide (NO)-guided fractionation was tested for its anti-hyperpermeability activity. Human umbilical vein endothelial cells cultured at 1 μM bradykinin showed increases in endothelial barrier permeability to FITC-dextran compared to cells cultured at media only. Pre-loading of the cells for 60 min with FAEBO before the permeability assay prevented the bradykinin induced increase in permeability. In addition, FAEBO was found to inhibit NO production with maximal inhibition, 82% at concentration 0.2 mg/mL. Furthermore, it was also found that FAEBO attenuated the production of cyclic guanosine monophos-

phate (cGMP) induced by bradykinin with inhibition 79%. Bradykinin induced activation of protein kinase C (PKC) in endothelial cell leading to reorganization of intercellular junction, however, this abolished by pre-incubation with FAEBO. FAEBO suppressed almost 53% of the PKC activity. Collectively, FAEBO exhibited anti-hyperpermeability properties via suppression of NO-cGMP signaling and PKC activity induced by bradykinin. This activity may partly contributed by the dominant compound of the FAEBO, 2-propanamine,2-methyl which was identified through GC-MS. However, further study is needed to determine the activity of the compound.

P143
Arginase inhibition improves endothelial dysfunction in the systemic microvasculature following TBI

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Traumatic brain injury (TBI) is known to cause secondary cardiovascular insults, but the effects on systemic resistance vessels have not been studied. Endothelial dysfunction is a hallmark of many vascular diseases and has been attributed to a decrease in nitric oxide (NO) production due to endothelial nitric oxide synthase (eNOS) uncoupling. Arginase competes with eNOS for the common substrate L-arginine, limiting L-arginine availability for NO synthesis. We hypothesized that TBI results in systemic endothelial dysfunction, due to decreased bioavailability of the eNOS substrate L-arginine and eNOS uncoupling, 24 hours after injury. We found that endothelial-dependent dilations were diminished following TBI. This was attributable to impairment in the NO, and not the endothelial-derived hyperpolarizing (EDH) component of the dilations. Although there was no difference in mRNA expression levels for arginase-1, arginase-2, or eNOS, we found arginase enzyme activity and arginase-1 protein expression were both increased in mesenteric arteries from TBI rats. Immunohistochemistry demonstrated that arginase-1 was localized in both the endothelium and smooth muscle cells of mesenteric arteries. Furthermore, we found that both arginase inhibition and supplementation with exogenous L-arginine improved endothelial-dependent dilations following TBI. TBI was also associated with increased circulating reactive oxygen species (ROS) in plasma and the vascular endothelium as a result of eNOS uncoupling. These results demonstrate 24 hours after a traumatic brain injury, systemic resistance vessels show signs of endothelial dysfunction, manifested as impaired endothelial-dependent vasodilation, due to increased arginase activity and eNOS uncoupling.

P144
Diabetes and glomerular filtration barrier

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We have visualised and quantified hyperfiltration with a confocal laser microscope at the early stage of diabetes in a rat and demonstrated that there was a leakage of large particles with the size of albumin from the glomeruli. We found this leakage was progressive along the duration of being diabetic from the early stage of diabetes. C-peptide, once regarded as physiologically inactive, restored the leakage to the level of control rats. There might be structural changes in the glomerular filtration barrier due to diabetes and C-peptide might restore those structural changes. In particular, we focused on gap length between the secondary foot processes.The purpose of this study was to examine the gap lengths between the secondary foot processes, the last glomerular filtration barrier as well as the slit diaphragm.We used Wistar rats ($n = 51$). Some of control rats ($n = 10$) and STZ induced diabetic rats ($n = 20$) of 17–18 weeks were administered C-peptide continuously for a day. We measured the gap lengths between the secondary foot processes with zipper-like appearance covering the glomerular capillaries in the scan electron microscopic images.The average gap length between the secondary foot processes was larger for diabetic rats than for control rats ($p < 0.05$) but C-peptide widened the gap lengths in both groups ($p < 0.05$). In conclusion, diabetes widened the gap length between the secondary foot processes in the glomeruli in a rat but a nephroprotective effect of C-peptide which restores particle leakage from the glomeruli remains structurally questioned.

P145
Acute treatment with metformin prevents high glucose-induced endothelial dysfunction

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Aims: Metformin is the first line drug used for type 2 diabetes (T2DM). However the molecular and cellular mechanisms remain elusive, particularly with respect to whether metformin has direct actions that protect vascular function. The current study was designed to determine if a 3-hour acute exposure to metformin protects endothelial function against the development of diabetes-related vascular

disease. We investigated the protective effects of metformin on endothelial-dependent vasodilatation (EDV) in thoracic aortae from T2DM db/db mice and on high glucose (HG, 40 mM) induced changes in endothelial nitric oxide synthase (eNOS) signalling in mouse microvascular endothelial cells (MMECs) in culture.

Methods and Results: Acetylcholine (Ach)-induced EDV was significantly ($p < 0.05$) reduced in aortae from db/+ non-diabetic control mice maintained in HG Krebs' for 3 hours compared to aortae maintained in normal glucose (NG, 11 mM) Krebs'. The reduction of EDV was partially reversed following a 3-hour exposure to 50 micromolar metformin; similarly metformin also improved Ach-induced EDV in aortae from diabetic db/db mice. Immunoblot analysis of MMECs cultured in HG versus NG revealed a significant reduction of phosphorylated (p-eNOS)/eNOS and p-Akt/Akt ratio, but not total eNOS or Akt expression. The 3-hour exposure of MMECs to metformin significantly reversed the HG-induced reduction in phosphorylation of both eNOS and Akt, but had no effect on phosphorylation of AMPK or the expression of SIRT1 protein.

Conclusion: The data indicate that a short 3-hour exposure to metformin can reverse/reduce the impact of HG on endothelial function via mechanisms linked to increased phosphorylation of eNOS and Akt.

P146

Microvascular and metabolic responses in the skin during local and systemic hyperinsulinemia: A microdialysis study

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Introduction: Insulin is known to alter microcirculation by production of endothelial nitric oxide and endothelin-1. It is however unclear whether increased insulin levels affect total blood flow in the skin. The urea clearance microdialysis technique is an indirect method to study blood flow *in vivo*. We aimed to study vasoactive and metabolic responses in the skin after locally delivered insulin and after an oral glucose load.

Method: In 9 healthy subjects (age 23.5 ± 3.0 years, BMI 24.7 ± 5.1 kg/m^2), two microdialysis catheters were inserted intradermally in the forearm and perfused at 0.3 μL/min with a perfusate containing Ringer acetate, 2.5% albumin and 30 mM urea. Interstitial glucose and urea levels were sampled every 15 minutes during a baseline period, followed by a period when 17 IU/mL insulin added to one of the catheters and finally during 4 hours following oral intake of 75 g glucose.

Results: Skin blood flow, indirectly measured by urea clearance, increased around the catheter through which insulin was delivered compared to the control catheter ($p = 0.01$), while interstitial glucose decreased around the insulin catheter compared to control ($p < 0.01$). A subsequent oral glucose intake increased skin blood flow around the control catheter ($p = 0.04$) while no further increase in skin blood flow was observed around the insulin catheter. Interstitial glucose concentrations increased equally in both catheters after oral glucose intake.

Conclusion: Local and systemic hyperinsulinemia increase skin blood flow and decrease skin glucose levels in healthy subjects. Microdialysis is a promising minimally-invasive technique for studying microvascular and metabolic effects of insulin in skin.

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Exposure to early life vitamin D deficiency has lifelong implications for vascular and renal function

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Many women of reproductive age are vitamin D (VitD) deficient. The effects of early life exposure to VitD deficiency on cardiovascular health are poorly understood. We examined the effects of *in utero* and early life VitD deficiency in rats on cardiovascular and renal function in the offspring. Female Sprague-Dawley rats were fed either a VitD-deplete or -replete diet for 6 weeks before, and throughout pregnancy and lactation. Offspring were maintained on the same diet as their mothers until 3 months of age, after which, all rats were fed a VitD replete diet. Arterial pressure was measured in conscious rats, and vascular function assessed using wire and pressure myography. Kidney function was measured by clearance methods. We previously reported that young VitD deficient offspring have elevated mean arterial pressure (MAP), heart rate (HR) and endothelial vasodilator dysfunction. We now show that following long term restoration of VitD levels, MAP, HR and endothelial function are normalised, however there are marked and persistent alterations in the function of the kidneys and their vasculature. Lobar arteries have increased responsiveness to angiotensin II, with the maximum constriction increased 4–9 fold ($p < 0.05$), and enhanced neurovascular constriction. Renal glomerular filtration rate is halved ($p < 0.002$). In conclusion, early life VitD insufficiency causes early disturbances in cardiovascular function which can be resolved with subsequent VitD supplementation, except for the effects on

the kidneys and their vasculature which are permanent. Ensuring VitD sufficiency in women of reproductive age is important for the renal health of their offspring.

P148
Enhanced vascular sensitivity to angiotensin II in the mesenteric artery of late-pregnant relaxin deficient mice

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Pregnancy is associated with reduced peripheral vascular resistance, underpinned by altered vascular function. The peptide hormone relaxin plays a major role in regulating maternal renal vasculature during pregnancy, but little is known about relaxin's actions on the mesenteric artery. Therefore, this study tested the hypothesis that vascular reactivity would be compromised in the mesenteric artery of late pregnant relaxin deficient ($Rln^{-/-}$) mice. The vascular responses of small first order mesenteric arteries were measured in non-pregnant (estrus) and late pregnant (day 17.5) wildtype ($Rln^{+/+}$: $n = 7$) and $Rln^{-/-}$ ($n = 7$) mice using wire myography. In $Rln^{+/+}$ mice, there was a significant reduction in sensitivity to the vasoconstrictor angiotensin II (AII) but not the thromboxane mimetic U46619 or phenylephrine in late pregnant compared to non-pregnant mice. In $Rln^{-/-}$ mice, this normal pregnancy adaptation did not occur, resulting in significantly enhanced sensitivity to AII, which was endothelium-independent. Blocking nitric oxide synthase with the inhibitor L-NAME further enhanced the vascular response to AII in both genotypes, whereas inhibition of prostanoid production with the cyclooxygenase inhibitor indomethacin significantly increased AII-induced contraction in day 17.5 pregnant $Rln^{+/+}$ mice but not $Rln^{-/-}$ mice. In conclusion, sensitivity to AII is enhanced in the mesenteric artery of late pregnant $Rln^{-/-}$ mice, and is associated with a decrease in the contribution of vasodilator prostanoids independent of nitric oxide and the endothelium. These data also demonstrate a direct action of relaxin on the vascular smooth muscle and that relaxin deficiency results in vascular complications of pregnancy.

P149
Development of CAST (Cancer Stromal Targeting) therapy

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Although many monoclonal antibodies (mAbs) have already been approved for the treatment of cancer, they are usually used in combination with anticancer agents (ACAs) because of their limited anti-tumor activity when used alone. Antibody-drug conjugate (ADC), the next generation of therapeutic antibodies, is a promising strategy to enhance the cytotoxic effect of a payload. However, most human solid tumors possess abundant stroma that hinders the distribution of ADCs. Moreover, the process of cell uptake is disturbed by the stromal barrier. Therefore, this barrier limits the effectiveness of ADCs, regardless of the internalization ability. To overcome this drawback, we have exploited a novel type of ADC, namely CAST (Cancer stromal targeting) therapy, composed of stroma targeting antibody and ester-bond linker with neighboring PEGylation. Antibody (IgG) itself has an ability of passive tumor targeting based on the EPR (Enhanced permeability and retention) effect. In addition to that, our ADC can actively accumulate in the tumor stroma from which ACAs are released gradually and distribute throughout the tumor, resulting in the arrest of tumor growth due to induced damage to both tumor cells and tumor vessels. Here we present the concept of CAST therapy and discuss the effect on the tumor microcirculation.

P150
Zipper-interacting protein kinase contributes to myogenic reactivity in the resistance microvasculature and hypercontractility in the SHR-model of essential hypertension

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Smooth muscle contractility is primarily dependant on the phosphorylation state of myosin light chain (LC20). While this process is regulated by intracellular calcium, calcium-independent kinases such as zipper-interacting protein kinase (ZIPK) can also play a role, particularly in disease. ZIPK upregulation is observed in animal models of essential hypertension, and its activity is linked to vascular

hypercontractility. We have applied a potent and specific inhibitor of ZIPK to better understand its contributions to normal and pathological function of resistance microvessels. ZIPK was hypothesized to regulate the myogenic response of the resistance vasculature and contribute to the hypercontractility observed in hypertension. Using a newly-developed inhibitor, HS38, we found that ZIPK contributes to the establishment and maintenance of myogenic tone in rat cerebral and cremaster resistance arteries *ex vivo*. The ZIPK contribution to myogenic tone was enhanced in tissues isolated from the spontaneously hypertensive rat (SHR) model of hypertension, particularly at low pressure. We also administered HS38, subcutaneously (0.5 mg/g body mass) daily beginning at 10 weeks, or 18 weeks, for 6 weeks in SHR and found that treatment with HS38 was able to significantly reduce the hypercontractility that typically develops at 10 wks of age and progressively worsens. Furthermore, we found HS38 could suppress the development of myogenic tone in isolated human cerebral resistance vessels. Overall, ZIPK plays an important role in contributing to the development of the full myogenic response in the resistance vasculature. HS38 is a strong candidate molecule for the treatment of hypertension-associated vascular hypercontractility.

NEURON-GLIA-VASCULATURE

P151
Vascular effects on astrocytes Ca2+ dynamics in cerebral cortex

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Recent *in vivo* evidence conducted in anesthetized or slightly sedated animals questions the direct role of astrocyte intracellular Ca2+ in mediating functional hyperemia as spatial and temporal profile of astrocyte Ca2+ transients are poorly associated with the onset of vascular responses. Our objective was to uncover the spatiotemporal basis of the communication between astrocytes and the vasculature in fully awake, behaving mice. A craniotomy over the barrel cortex with the dura removed was performed. A custom build two-photon microscopy was used to image the vasculature and astrocytes Ca2+ from either C56Bl/6 mice or GLAST-cre-LSL-GCaMP3 mice or TEK-cre-LSL-ArchT3 mice. We found that 5-second whiskers' stimulation induced fast vasodilatory responses in penetrating arterioles while generated a delayed onset of endfoot and cell-wide Ca2+ transients. Interestingly, the onset of these Ca2+ transients was typically observed at the peak of the sensory induced vasodilation and toward the end of the vibrissae stimulation. Thus, we tested if the vasculature was communicating back to the astrocytes and consequently modulating astrocytes Ca2+. We showed that manipulation of the vasculature via pharmacology or using optogenetic tools induced vascular changes that were followed by an alteration in astrocyte Ca2+ signals. Our data redefine the uni-directional communication between astrocytes and the vasculature. We introduce a potential role of the vasculature as the modulator of astrocytes Ca2+ transients and propose that astrocytes act as responders to changes in blood flow.

P152
Evaluation of molecular mechanism of retinal neurovascular coupling using isolated porcine retinal arterioles

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Purpose: Retinal vessels run through retinal nerve tissue, and as such, they are constantly influenced by various transmitters released from the nervous system. This close relationship between the nervous system and blood vessels is called "neurovascular coupling", which is known to be involved with the maintenance of retinal microcirculation and the pathogenesis of diseases concerned with retinal blood flow disorder, including diabetic retinopathy. However, it remains unclear which transmitters released from the retinal nerve tissue influence the blood vessels in the retina. Herein, we examined whether prostaglandin E_2, serine, or glutamate, which are known as vasodilatory transmitters released from the nervous system in the brain, affect the retinal arterioles.
Methods: Porcine retinal arterioles were isolated, cannulated, and pressurized without flow *in vitro*. Diameter changes were recorded using microscopic video techniques.
Results: Prostaglandin E_2 induced dose-dependent vasodilation of the retinal arterioles. The highest concentration (0.3 microM) elicited approximately 80% of the maximal dilation. In contrast, serine and glutamate did not alter the vessel diameter ($p > 0.05$). Furthermore, co-administration of serine and glutamate also did not affect the vessel diameter ($p > 0.05$).
Conclusions: Prostaglandin E_2 is a transmitter released from astrocytes which induces vasodilation of the retinal arterioles in a dose-dependent manner. The current finding suggests that prostaglandin E_2 released from retinal neuronal tissue may be involved with the maintenance of retinal blood flow.

P153
Dysfunction of neurovascular coupling in a mouse model of subarachnoid hemorrhage

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Focal increases in cerebral blood flow (CBF) that are matched to local neuronal activation are a necessity for neuron survival and maintenance of brain function. These neurally-triggered focal CBF increases are typically called neurovascular coupling (NVC) when studied *ex vivo* and functional hyperemia when studied *in vivo*. We have previously reported inversion of the NVC response from a physiological vasodilation to a pathological vasoconstriction in freshly prepared brain slices from subarachnoid hemorrhage (SAH) model rats (Koide et al, 2012). Here, we examined *ex vivo* NVC, *in vivo* functional hyperemia and sensory motor function using a mouse endovascular perforation SAH model. In brain slices, astroctye endfoot Ca^{2+} and adjoining parenchymal arteriolar diameter were measured using two-photon and infrared-differential interference contrast microscopy. Local neuronal activation caused an increase in astrocyte endfoot Ca^{2+} and an inverted NVC (i.e. vasoconstriction rather than vasodilation) in brain slices from SAH animals peaking 24 hours post-SAH (with ~80% of slices exhibiting inversion of NVC). *In vivo* functional hyperemia (whisker stimulation-induced CBF increase) measured by laser Doppler flowmetry was also significantly attenuated in mice 24 hours post-SAH. Consistent with impaired NVC, SAH mice showed a decreased ability to perform a battery of sensory motor tasks. These data demonstrate dysfunction of neurovascular coupling occurs following SAH, which may contribute to the development of neuronal deficits. This work was supported by the American Heart Association (14SDG20150027), the NIH (P01HL095488, P30RR032135, P30GM103498), Totman Research Trust and Peter Martin Brain Aneurysm Endowment.

P154
Spatiotemporal dynamics of cerebral blood flow during focal activation with optogenetic photostimulation to the cortical neurons and astrocytes

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Accumulating evidences suggest that both neuronal and glial signaling to the cerebrovasculature contributes to activity-dependent increases in cerebral blood flow (CBF). However, their action sites within the vascular trees and temporal dynamics of the actions remain largely unknown. To explore a role of those complex mechanisms in the neurovascular coupling, the present study examined the CBF response to specific cellular activation using optogenetic animal models that genetically expressed a light-gated cation channel, channelrhodopsin-2 (ChR2), in either cortical neurons or astrocytes. Photostimulation was focally applied using argon laser (0.2 mW, 0.5 sec) to the parietal cortex through the skull, while a spatiotemporal dynamic change in the CBF was evaluated with laser speckle flowgraphy under awake conditions. Pharmacological manipulations were conducted with topically applying drugs to the cortical surface after a removal of the skull and dura, and the CBF responses to photostimulation were compared between pre- and post-treatment in the same hemisphere. We observed that photostimulation to either ChR2-expressing neurons or astrocytes provokes a rapid and robust increase in CBF; $76 \pm 22\%$ ($N = 7$) and $33 \pm 16\%$ ($N = 6$) relative to the pre-stimulation baseline, respectively. The evoked response area, defined by the threshold at half-maximum response in the image, was larger in the ChR2-astrocyte, compared to the ChR2-neuron mice. The pharmacological manipulations further showed a different mechanism participating in the CBF response to the neuronal and astrocytic activations, which imply that a separate, parallel pathway of the neurogenic and gliogenic control of CBF.

OXIDATIVE STRESS, MITOCHONDRIAL METABOLISM AND REDOX

P155

Suppression of high glucose-induced cell apoptosis in PC12 cells by DSePA through inhibition of ROS-mediated DNA damage and AKT inactivation

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Objectives: To evaluate whether diselenodipropionic acid (DSePA), a derivative of selenocystine, was able to protect from high glucose-induced neurotoxicity in PC12 cells and evaluate the underlying mechanism.

Methods: Several methods of cell biology and molecular biology *in vitro* were employed.

Results: The results suggested that high glucose (100 mM) treatment for 48 h induced mitochondria-mediated apoptosis in PC12 cells, accompanied by poly (ADP-ribose) polymerase (PARP) cleavage, caspase activation, depletion of mitochondrial membrane potential. Moreover, high glucose treatment also resulted in DNA damage and dysregulation of MAPKs and AKT pathways through triggering intracellular reactive oxygen species (ROS) overproduction. p53 RNA interference partially suppressed high glucose-induced cytotoxicity and apoptosis. Addition of four chemical inhibitors of MAPKs and AKT pathways further confirmed that MAPKs and AKT pathways both contributed to high glucose-induced neurotoxicity. However, DSePA pretreatment for 6 h effectively attenuated high glucose-induced cytotoxicity, inhibited the loss of mitochondrial membrane potential through regulation of Bcl-2 family expression, and ultimately reversed high glucose-induced apoptotic cell death in PC12 cells. Attenuation of caspase activation, PARP cleavage, DNA damage, and accumulation of ROS all confirmed its protective effects. Moreover, DSePA markedly alleviated the dysregulation of the MAPK and AKT pathways induced by high glucose.

Conclusions: Our findings revealed that the strategy of using DSePA could be a highly effective way in prevention or therapy of diabetic neuropathy.

P156

Transmembrane electron mediators to extract electron energies of RBC glycolysis for prolonged *in vivo* functional lifespan of an artificial oxygen carrier (Hb-Vesicles)

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Electron-energy-rich coenzymes in cells, NADH and NADPH, are re-energized repeatedly through the Embden-Meyerhof and pentose-phosphate glycolytic pathways, respectively. This study demonstrates extraction of their electron energies in RBCs for *in vivo* extracellular chemical reactions using an electron mediator shuttling across the biomembrane. Hemoglobin-vesicle (Hb-V) is an artificial oxygen carrier encapsulating purified and concentrated Hb solution in liposomes. Because of the absence of a metHb-reducing enzymatic system in HbV, HbO_2 gradually autoxidizes to form metHb. Wistar rats received Hb-V suspension (10 mL/kg body weight) intravenously. At the metHb level of around 50%, methylene blue (MB) was injected. The level of metHb quickly decreased to around 16% in 40 min, remaining for over 5 h. *In vitro* mixing of HbV/MB with RBCs recreated the *in vivo* metHb reduction, but not with plasma. NAD(P)H levels in RBCs decreased after metHb reduction. The addition of glucose facilitated metHb reduction. Liposome-encapsulated NAD(P)H, a model of RBC, reduced metHb in Hb-V in the presence of MB. These results indicate that (i) NAD(P)H in RBCs reacts with MB to convert it to leukomethylene blue (MBH); (ii) MB and MBH shuttle freely between RBC and HbV across the hydrophobic lipid membranes; and (iii) MBH is transferred into HbV and reduces metHb in HbV. Some other electron mediators with appropriate redox potentials (phenothiazines, phenoxazines) appeared to be as effective as MB was. We established an indirect enzymatic metHb reducing system for Hb-V using unlimited endogenous electrons created in RBCs that prolongs the functional lifespan of Hb-V in blood circulation.

P157

Traumatic brain injury increases plasma and microvascular reactive oxygen species

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Excessive production of reactive oxygen species (ROS) are implicated in the pathogenesis of numerous disease states and correlates with injury severity. ROS have not been

measured in microvessels or in circulating plasma after trauma. We measured ROS in vascular endothelium from rat mesenteric resistance arteries 1 day after traumatic brain injury (TBI), using dihydroethidium (DHE). ROS levels were elevated in the endothelium but not the vascular smooth muscle. We also measured the oxidation-reduction potential (ORP; mV) in plasma obtained at the same time point, compared to controls. We used a novel diagnostic platform (Redoxsys, Englewood, CO) to measure oxidative stress. The redox potential or ORP is the sum of the total reduction and oxidation potential, measured using single-use sensors. We found the ORP in plasma collected from rats after TBI was significantly higher than controls (181 ± 13 mV, $n = 6$ vs. 159 ± 3.7 mV, $n = 6$, respectively). In addition, we measured ORP in human subjects after TBI compared to healthy controls (154 ± 2.3 mV vs. 132 ± 1.4 mV, $n = 5$ each, respectively). We also show that as a positive control, hydrogen peroxide increased ORP levels in a concentration dependent manner in human plasma (maximal ORP 239 ± 9.7 mV, $n = 3$). These data demonstrate that TBI increases plasma and microvascular endothelial ROS. We also show feasibility of measuring redox imbalance in plasma in a disease model, providing a useful technique for a wide range of circulatory and metabolic studies of oxidative stress.

P158

3, 4-dihydroxyl-phenyl lactic acid ameliorates cardiac reperfusion injury through restoring NADH dehydrogenase 1 alpha subunit 10

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The present study aimed to detect the role of 3, 4-dihydroxyl-phenyl lactic acid (DLA) during ischemia/reperfusion (I/R) induced myocardial injury with emphasis on the underlying mechanism of DLA antioxidant. Male Sprague-Dawley (SD) rats were subjected to left descending artery occlusion followed by reperfusion. Treatment with DLA ameliorated myocardial structure and function disorder, blunted the impairment of Complex I activity and mitochondrial function after I/R. The results of 2-D fluorescence difference gel electrophoresis revealed that DLA prevented the decrease in NDUFA10 expression, one of the subunits of Complex I. To find the target of DLA, the binding affinity of Sirtuin 1 (SIRT1) to DLA and DLA derivatives with replaced two phenolic hydroxyls was detected using surface plasmon resonance and bilayer interferometry. The results showed that DLA could activate SIRT1 after I/R probably by binding to this protein, depending on phenolic hydroxyl. Moreover, the importance of SIRT1 to DLA effectiveness was confirmed through siRNA transfection *in vitro*. These results demonstrated that DLA was able to prevent I/R induced decrease in NDUFA10 expression, improve Complex I activity and mitochondrial function, eventually attenuate cardiac structure and function injury after I/R, which was possibly related to its ability of binding to and activating SIRT1.

P159

Metabolic profiling of ischemic brains reveals multiple control points by cilostazol treatment

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Although cilostazol, an inhibitor of phosphodiesterase3, is known to prevent the recurrence of cerebral ischemic events, biochemical mechanism remains unknown. To understand the mechanisms whereby cilostazol controls metabolic dynamics after the insults, we used metabolomics to target metabolic pathways responding to cilostazol. Male mice (8–12 week old) underwent the permanent ligation of the right common carotid artery followed by the oral gavage of the cilostazol (30 mg/kg or 100 mg/kg). At 60 min after the occlusion, metabolic processes of the brain were rapidly suspended by the *in situ* freezing to minimize autolytic changes. Metabolites were extracted and measured with high-throughput capillary electrophoresis mass spectrometry. We then conducted cluster analysis to compare and contrast changes in 90 metabolites extracted from contralateral (CL) and ipsilateral (IL) hemispheric brains. We found that cilostazol increased cystathionine, taurine, cysteine, and the oxidized form of glutathione that were related to the sulfur amino acid metabolism in the CL. Contrary to our expectation, there was no increase in the expression of cystathionine beta-synthase, which catalyzes the first committed step of the transsulfuration pathway. Furthermore, cilostazol caused remarkable decrease in NADH in both CL and IL. These results suggest that cilostazol acts not only on the platelet, but also on yet identified receptors involving multiple metabolic remodeling via NADH and sulfur amino acid metabolism. Such actions may lead to beneficial therapeutic stratagem in cerebrovascular disease.

P160

Carnosine attenuates early brain injury through its antioxidative and anti-apoptotic effects in a rat experimental subarachnoid hemorrhage model

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Carnosine (beta-alanyl-L-histidine) has been demonstrated to provide antioxidative and anti-apoptotic roles in the animal of ischemic brain injuries and neurodegenerative diseases. The aim of this study was to examine whether carnosine prevents subarachnoid hemorrhage (SAH)-induced early brain injury (EBI) in rats. We found that intraperitoneal administration of carnosine improved neurobehavioral deficits, attenuated brain edema and blood brain barrier permeability, and decreased reactive oxygen species level at 48 h following SAH in rat models. Carnosine treatment increased tissue copper/zinc superoxide dismutase (CuZn-SOD) and glutathione peroxidase (GSH-Px) enzymatic activities, and reduced post-SAH elevated lactate dehydrogenase (LDH) activity, the concentration of malondialdehyde (MDA), 3-nitrotyrosine (3-NT), 8-hydroxydeoxyguanosine(8-OHDG), interleukin (IL)-1b, IL-6, and tumor necrosis factor-a (TNF-a) in rats. Furthermore, carnosine treatment attenuated SAH-induced microglia activation and cortical neuron apoptosis. These results indicated that administration of carnosine may provide neuroprotection in EBI following SAH in rat models.

P161

Effects of NMDA receptor antagonist memantine on NO production, hydroxyl radical metabolism and ischemic change of hippocampal CA1 during cerebral ischemia and reperfusion in mice

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Methods: (1) C57BL/6 mice [$n = 15$] were used. Memantine 25 umol/ kg was given in 5 mice 30 minutes before ischemia, and others were contol group. Both NO production and hydroxyl radical metabolism were continuously monitored by *in vivo* microdialysis. Microdialysis probes were inserted into the bilateral striatum. The *in vivo* salicylate trapping method was applied for monitoring hydroxyl radical formation via 2,3 dihydroxybenzoic acid (DHBA). A Laser doppler probe was placed on the skull surface. Forebrain cerebral ischemia was produced by occlusion of both common carotid arteries for 10 minutes. Levels of NO metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), in the dialysate were determined using the Griess reaction. (2) Survival rate in hippocampal CA1 neurons: Neurons were analyzed into three phases (severe ischemia, moderate ischemia, survive), and the ratio of the number of surviving neurons was calculated as survival rate 7 days after the start of repercussion.

Results: (i) Blood pressure: There were no significant differences between the groups. (ii) CBF: There were no significant differences between the groups. (iii) NO_3^-; Memantine group (97.2 $\pm$ 10.1%; mean$\pm$SD) showed significantly higher than that of the control group (65.3 $\pm$ 21.0) at ischemia ($p < 0.05$). (iv) 2,3-DHBA; Memantine group (90.7 $\pm$ 2.90) showed significantly lower than that of the control (99.5 $\pm$ 2.66) at ischemia, after repurfusion 20, 80-120 minutes ($p < 0.05$). (v) Survival rate in CA1 area: There were no significant differences between the groups.

Conclusion: These *in vivo* data suggest that memantine effects on NO and hydroxyl radical metabolites in mice, and may have neuroprotective effect against cerebral ischemic injury.

P162

Ferritin derived oxidative stress is a risk for liver damage even within reference range in male

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Aim: Ferritin is known to a potent inducer of oxidative stress. In non-alcoholic steatohepatitis (NASH), accumulated ferritin become the second hit pathogenetic factor. However, its subclinical role within reference range is unclear. This study was made to determine influences of subclinical accumulation of ferritin on on liver damage.

Subjects and Methods: Six hundred seventy two recipients of Anti-aging health check-up in Tokai University Tokyo Hospital were included (male: $n = 331$, mean age 63.0 $\pm$ 11.0, female: $n = 341$, mean age 62.3 $\pm$ 11.0). The correlations between serum level of ferritin and 8-OHdG, AST, ALT, Gamma-GT, Fe, high sensitivity CRP (hs-CRP), BMI, abdominal circumference (AC) were analyzed.

Results: Serum levels of ferritin were tend to increase along to aging, and were markedly higher ($p < 0.01$) in male (mean 184.9) than in female (mean 84.6). Positive correlation was observed between Fe, AST, ALT, Gamma-GT, BMI, AC in both male and female ($p < 0.05$). 8-OHdG and hs-CRP were positively correlated only in male. ($p < 0.05$).

Conclusions: Serum level of ferritin is increased along to BMI and AC, then thought to induces liver damage due to oxidative stress, even it's in normal reference range in male.

P163

Cytathionine βsynthase is required to maintain glutathione hydropersulfide, a novel antioxidant molecule, in the murine lens

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Elevated oxidation of proteins in the nuclear region of lens is an indicator of the development of nuclear cataract. Although glutathione (GSH) is known as the principal antioxidant in the lens, glutathione hydropersulfide (GSSH) has been recently recognized as a more effective reductant which can donate a hydrogen atom to one-electron oxidants more readily than the thiol. However, the spatial distribution and the quantification of GSSH remain to be determined. To examine spatial distributions of GSSH, we took advantage of a unique nature of an atmospheric-pressure MALDI imaging mass spectrometry (IMS) where the ionization of tissue metabolites occurs in the presence of molecular oxygen. Under such a condition, GSSH can be readily oxidized to derive glutathione *S*-sulfonate ($GSSO_3H$) whose ionization efficiency is much higher than its native form, making it possible to detect this trace-amount metabolite. It was found that mass intensities of $GSSO_3H$ are high in both the epithelium and the outer cortex of the lens. Mice with targeted deletion of cystathionine β-synthase (CBS), but not that of cystathionine γ-lyase, displayed a decrease in $GSSO_3H$ signals. By contrast, in the CBS-null, the levels of ophthalmic acid were elevated in these regions. In addition, quantitative analysis of reactive sulfur species using monobromobimane-derivatization methods conformed to these IMS findings. Furthermore, morphometric analyses of lens-fiber-cell organization have shown that hexagonal packing geometry typical in the wild type was disturbed in the CBS-null mice. These results suggest that CBS is required to maintain hydropersulfide oxidation states controlling protein functions necessary for lens transparency.

P164

Ganoderma lucidum polysaccharide peptide ameliorates acute kidney injury by reducing the endoplasmic reticulum and the mitochondrial stress cognitive/emotional deficits

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Objectives: The Ganoderma lucidum was reported to have multiple pharmacological roles on chronic hepatopathy, hypertension, hyperglycemia neoplasia, etc. The objective of this study was to determine whether Ganoderma lucidum polysaccharide peptide (GLPP) could attenuate renal ischemia-reperfusion injury (RIRI) both *in vivo* and *in vitro* via reducing Endoplasmic reticulum (ER) stress and mitochondrial damage.

Methods: Male mice were subjected to right renal ischemia for 30 min and reperfusion for 24 h, or to a sham operation with left nephrectomy. Then the renal function, the renal morphology and the renal cell apoptosis were analyzed. The levels of MPO, MDA and SOD were detected in the kidney tissue. Western blot analysis was performed to identify the expression of ER stress hallmarks such as glucose-regulated protein 78 (GRP78), C/EBP-homologous protein (CHOP), caspase 12; and apoptotic parameters involved in mitochondrial dysfunction such as p-p53, p53, Bcl-2, Bax and cytochrome C. NRK-52E cells were used to explore the protective effect of GLPP against hypoxia-reoxygenation and tunicamycin (TM) induced cell injury.

Results: Kidneys undergone ischemia-reperfusion (IR) showed renal dysfunction and characteristic morphological changes while damage was attenuated by GLPP treatment. The abnormal levels of MPO, MDA and SOD caused by IR were dramatically reversed by GLPP. In addition, with the TUNEL assay, more apoptotic cells were labeled in IR group than sham group, while fewer positive signals were seen in the GLPP treated group. Furthermore, the apoptosis induced by ER stress and mitochondrial dysfunction reduced in GLPP treated group. GLPP alleviated hypoxia-reoxygenation induced cell viability loss and $\Delta\Psi m$ dissipation in NRK-52E renal tubular epithelial cells. Also, GLPP pretreatment dramatically reduced hypoxia-rexoxygenation and TM induced cell injury.

Conclusions: Our study suggests that GLPP has a protective effect on acute kidney injury caused by IR via reducing mitochondrial and ER stress induced apoptosis *in vivo* and *in vitro*.

VASCULAR SMOOTH MUSCLE CELLS/PERICYTES

P165

Comparative investigation of Ca^{2+} signalling and vasomotor responses in the ureteric and cremaster muscle microvessels *in situ*

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Microcirculation is the generic name for the finest level of the circulatory system consisting of arterioles, capillaries, and venules whose diameters range from 5 to 50 microns. Intravital microscopy revealed that the response of arterioles and venules to vasonstrictors and vasodilators can vary depending on the tissue, type of stimuli used and the position of the vessel in the network. However the mechanisms underlying these differences have not been investigated.

We have compared microvasculature of two different metabolically sensitive smooth muscle and cremaster muscle tissues. The purpose of this study was to investigate Ca^{2+} signalling in smooth muscle cells and pericytes, induced by agonists such as phenylephrine (PE), serotonin (5-HT), vasopressin (AVP), endothelin-1 (ET-1) and vasomotor responses at all levels of the arteriolar networks and postcapillary venules on *in situ*.

Ca^{2+} imaging of Fluo-4 loaded ureteric microvessels was performed *in situ* using Nipkow disc based confocal imaging system run by Andor IQ software. Images were acquired at 30-60 fps using ×20 (NA 0.7) or ×60 (water immersion, NA 1.2) objectives.

In mark contrast to ureteric microvascular networks all arteriolar and venular branches in cremaster muscle responded to PE. The role of Ca^{2+} entry and sensitivity to caffeine varied in the ureteric and cremaster muscle microvasculature. The results obtained demonstrate that Ca^{2+} signals and vasomotor responses are distinct between distributing arcade and downstream transverse and precapillary arterioles with regard of type of stimuli and mechanisms of Ca^{2+} mobilization.

P166

Pericytes exhibit asymmetric control at capillary bifurcations in the retinal vasculature

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Pericytes, as part of the neurovascular unit, contribute to functional hyperemia, the process whereby metabolically active neurons in the brain stimulate an increase in local capillary blood flow necessary to maintain the moment-to-moment delivery of nutrients and removal of waste. Similar to vascular smooth muscle cells, pericytes can express the contractile machinery capable of generating focal constrictions within the capillary bed. The goal of the current study was to elucidate the functional role of pericytes localized at capillary bifurcations and test the overall hypothesis that junctional pericytes are capable of controlling the direction of flow within the retinal vasculature. Using immunohistochemistry and fluorescence confocal microscopy of isolated mouse retinas, we observed that only a subpopulation of pericytes localized to first and second order branches of capillaries proximal to feeding arterioles express contractile elements, such as alpha-actin, $Ca_v1.2$ and TRPM4, and only these cells contracted to the thromboxane A2 analog, U46619. Notably, contractile junctional pericytes encircling capillary bifurcations exhibited asymmetric coverage and caused asymmetric constriction of branches. Using a novel transgenic mouse expressing a genetically encoded Ca^{2+} biosensor in contractile pericytes (acta2-GCaMP5-mCherry), we observed that Ca^{2+} dynamics were also asymmetric in junctional pericytes, which exhibited Ca^{2+} events that were pharmacologically distinct from those in non-junctional pericytes. In conclusion, junctional pericytes localized proximal to feeding arterioles preferentially constricted one branch of a capillary bifurcation, suggesting that pericytes may play a role in controlling the directional flow of blood. T32HL-007594, P01HL-095488, R37DK-053832, R01HL-121706, Totman Medical Research Trust, American Heart Assocation, and Fondation Leducq.

P167

A novel role for renal NMDA receptors: Regulation of the renal microcirculation

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Glutamate and GABA receptors have been detected throughout the kidney although the precise role(s) these play in the kidney remain unclear. Previously we reported that glutamate and its substrate, GABA, are involved in the regulation of the renal microcirculation. Specifically, we observed GABA and glutamate-mediated vasoconstriction of afferent arterioles in the renal cortex, whereas in the medulla, glutamate evokes vasodilation and GABA evokes vasoconstriction of vasa recta via their actions at contractile pericytes. It is well established that contractile pericytes regulate vasa recta diameter and thus medullary blood flow (MBF). We

have utilised a rat live kidney slice model to investigate glutamate-evoked vasodilation of vasa recta capillaries [4]. We tested numerous glutamate receptor antagonists (MK-801, GYKI, MSOP, LY341495, UBP302) and found that only the NMDA receptor antagonist, MK-801, significantly attenuated glutamate-evoked vasodilation mediated via pericytes. Accordingly, NMDA evoked a pericyte-mediated vasodilation of vasa recta via pericytes, similar to that seen with glutamate. In addition glycine evoked a pericyte-mediated vasodilation of vasa recta (16.5 ± 1.1%, $n = 6$). Interestingly, MK-801 significantly attenuated glycine-evoked vasodilation of vasa recta via pericytes. In conclusion, we suggest that glutamate-mediated vasodilation of vasa recta occurs via NMDA receptors, presumably expressed on contractile pericytes. Moreover, we provide novel observations regarding the differential roles of glutamate and glycine in the regulation of afferent arteriole diameter.

PERIPHERAL CIRCULATION

P168
Accuracy of small sized vessel flow measurements in 3D cine PC MRI with respiratory navigator gating

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Objective: Phase contrast MRI (PC MRI) is a gold standard to measure blood flow rates of various sized vessels. Three Dimensional cine PC MRI (3D cine PC MRI) enables the reconstruction of 3D flow stream in addition to the cross-sectional flow measurement. However, due to the insufficient image quality and vessel movements with breathing in long acquisition time of 3D cine PC MRI, flow rate in small vessels are often underestimated and inaccurate. The objective was to inquire the size limit of the flow rate measurements using 3D cine PC MRI and the effect of respiratory navigator gating on flow measurements.
Methods: We reconstructed 3D main pulmonary geometry based on multi-slice steady-state free procession images, and superposed them to 3D cine PC MRI with and without respiratory navigator gating in a healthy volunteer. Flow streamlines inside the pulmonary arteries were reconstructed and flow measurements were performed in the peripheral pulmonary artery based on the streamlines.
Results: With respiratory navigator gating, peripheral arteries of comparatively small diameters could be depicted by streamline (fifth branch, 3.3 mm in diameter). The flow volume was well preserved, with a small error of 3.2% ± 2.1%. Without respiratory navigator gating, streamline was disturbed and the segmentable vessel diameters was larger (fourth branch, 5.9 mm in diameter). The flow volume had larger error of 16.5% ± 2.6%.
Conclusion: Streamlines reconstructed with 3D cine PC MRI with respiratory navigator gating enable accurate flow evaluation even in small peripheral vessels.

P169
Skin microvascular function in women with peripartum anaemia

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Introduction: During pregnancy approximately 40% of women develop anaemia, with a compensatory decrease in peripheral vascular resistance. However, the effect of anaemia on skin microvascular function is unknown. This study used laser Doppler flowmetry to determine skin microvascular reactivity in women during the third trimester of pregnancy.
Methods: Anaemia was defined as hemoglobin < 105 g/L and hematocrit < 33%. Skin blood flow was measured in anaemic (4) and non-anaemic (11) women in the third trimester (28–40 weeks) of pregnancy. Participants were rested on a recliner chair, back angled at 30 deg, while skin blood flow (cutaneous vascular conductance) was measured using laser Doppler flowmetry on the volar aspect of the dominant forearm. Iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP) evaluated endothelial-dependent and independent vasodilatation, respectively. Local heating evaluated the initial axon-reflex and secondary nitric oxide-mediated vasodilatation.
Results: There was no significant difference in baseline cutaneous vascular conductance ($p > 0.05$). Anaemic women had significantly lower increases in cutaneous vascular conductance following iontophoresis of ACh (272% ± 269% vs. 882% ± 393%, $p < 0.05$), but responses following iontophoresis of SNP were more similar (431% ± 333% vs. 792% ±445%, $p > 0.05$). Heating had no differential effect ($p > 0.05$).
Conclusions: This study is the first to demonstrate a significantly lower increase in skin blood flow to an endothelial-mediated ACh challenge in anaemic women during the third trimester of pregnancy. This finding is in the setting of a preserved nitric oxide-mediated response to heat and suggests decreased bioavailability of endothelial-derived hyperpolarizing factors in the skin microvasculature.

P170

Inhibition of neuronal nitric oxide synthase attenuates flicker-induced increment of retinal blood flow in cats

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Although the retina expresses neuronal nitric oxide synthase (nNOS), it is unknown if nNOS contributes to regulating the retinal blood flow (RBF) during the rest and the flicker stimulation. We therefore examined the effects of nNOS selective inhibitor Nx-propyl-L-arginine (L-NPA) on the retinal blood flow (RBF) in the first-order retinal arterioles from the cats using laser Doppler velocimetry. After intravitreal injections of Nx-Nitro-L-arginine methyl ester (L-NAME), a pan-NOS inhibitor, or Nx-propyl-L-arginine (L-NPA), a selective nNOS inhibitor, we continuously measured baseline RBFs for 2 hours. We then exposed the feline eye to the flicker stimulation (16-Hz) for 3 minutes. Here we used phosphate-buffered saline (PBS) as a control and thromboxane A2 (TXA2) analogue U46619 as a basal tone-adjusted control. We found that both L-NAME and L-NPA caused decreases in baseline RBFs in a dose-dependent manner. Upon the flicker stimuli, the RBF started to increase gradually. After 3 minutes of 16-Hz flicker stimuli, the RBF increased by 53.5% ± 3.4% compared with baseline. In the L-NAME and LNPA groups, the flicker induced increment of RBF induced was attenuated. In the TXA2 group, the reduction in the flicker-induced increase in RBF was comparable to that in the PBS group. These results suggest that increased RBF in response to flicker stimulation is mediated by nitric oxide (NO) production via nNOS activation.

P171

Microvascular angina and skin microcirculation

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Aim: Myocardial microvascular dysfunction is considered to be a chief mechanism responsible for microvascular angina (MVA) to develop and sustain. Cutaneous microvacular function may reflect the state of visceral microcirculation, therefore we examined LDF forearm microvascular flow in MVA patients.

Materials and Methods: 40 patients with MVA (2 men, 38 women; age 55.6 ± 9.6) and 26 healthy volunteers (10 men, 16 women; age 52.1 ± 9.9) were included in the study. Patients were selected if they had symptoms of angina with evidence of myocardial ischemia and intact coronary arteries. Antianginal medication was discontinued for more 5 drug half-lives before investigation. Skin microvascular function was assessed by LDF at rest on the ulnar part of the right forearm. Amplitude-frequency wavelet analysis of blood flow oscillations was performed. Values of the tissue perfusion level (M) and time-averaged vasomotion amplitude was assessed in arbitrary units (AU) in the corresponding frequency band for endothelial (Ae), neurogenic (An), myogenic (Am), venular (Av) and cardiac (Ac) sections of blood flow modulation.

Results: MVA patients had lower basal skin perfusion than healthy volunteers (4.03 AU vs 4.37 AU; $p < 0.57$), decreased amplitude vasomotion Ae (0.210 AU vs 0.284 AU; $p < 0.008$), An (0.262 AU vs 0.304 AU; $p < 0.088$), Am (0.164 AU vs 0.246 AU; $p < 0.005$), Av (0.114 AU vs 0.074 AU; $p < 0.37$) and Ac (0.125 AU vs 0.173 AU; $p < 0.02$).

Conclusions: Our results indicated that impaired cutaneous microvascular function is present in patients with MVA, which appears increasing in endothelial, neurogenic and myogenic tone of precapillary arterioles components.

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Microcirculatory disorders in patients with arterial hypertension and high and very high cardiovascular risk

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Materials and Methods: The study included 60 hypertensive patients and 15 normotensives (age 46.8 ± 9.7 year) with high and very high cardiovascular risk. Study the following risk factors: smoking, BMI, waist circumference, lipids and glucose levels. To assess the state of the microvasculature laser Doppler flowmetry (LDF) with amplitude-frequency wavelet analysis of blood flow oscillations and arterial occlusion test (AOT) were performed.

Results: Hypertensive patients were more often smokers, they showed an increase BMI, waist circumference. Levels of total cholesterol, LDL-C were not significantly different, the level of HDL-C was significantly lower in the hypertension group and was 1.26 ± 0.3 mmol/l vs. 1.46 ± 0.35 mmol/l in the normotensive group ($p = 0.018$). According LDF basal level perfusion oscillation amplitude did not differ in groups. During the occlusion test a reduction in blood flow growth was found in hypertensive group (309.6 ± 142.4 AU in hypertensive group, 321.3 ± 95.0 AU in normotensive group, $p = 0.8$). A significant correlation between lipid profile and myogenic rhythm amplitude at rest in

hypertensive patients (with a total cholesterol level $r = -0.27$, $p = 0.04$, with the level of LDL-C $r = -0.25$, $p = 0{,}035$) was found. A significant correlation between the amplitude of myogenic rhythm during AOP and smoking ($r = 0.39$, $p = 0.037$) was found.

Conclusion: The most important cardiovascular risk factors influencing the microcirculation both at rest and during functional tests in hypertensive patients are lipid profile levels and smoking status.

P173

Impact of Actovegin on microcirculation in patients suffering chronic obliterating diseases of lower limb arteries

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Background searching of optimised treatment mode of patients suffering chronic lower limb ischemia still remains important and unsolved problem.

Objective: To evaluate an Actovegin efficacy in treatment of patients suffering chronic lower limb ischemia caused with stenoocclusive arterial lesions.

Materials and Methods: There were recruited 80 patients suffering chronic lower limb ischemia corresponding to 2B stage according to Fountain classification. Patients were randomly divided to 2 groups. 1st group included 40 patients which were given Actovegin infusions in dosage 1000 mg per day as a monotherapy 10 days. Another 40 patients formed 2nd group and were administrated dextrans and pentoxifylline 10 days. Microcirculation condition before and after treatment was evaluated by means of LDF with wavelet analyses of vasomotions. Basal bloodflow was registered at constant temperature 32C during 10 minutes, following which heating test was performed; heating to 42C during 30 to 40 minutes.

Results: There were detected following changes in 1st group. Amplitude of myogenic huntings increased 56% significantly, p 0.006. Shunting index of basal perfusion decreased, p 0.1. Maximal level of perfusion as well as amplitude of endothelial huntings increased significantly, p 0.006 and p 0.06, consequently. There was revealed significantly reduction of the attainment time of maximal level of heating hyperemia in 2nd group.

Summary: Actovegin infusions impacted tone forming mechanisms. Myogenic tone of precapilar arterioles and capillaric sphincters decreased. Arteriovenular shunting reduced with growing blood supply of capillaries; actovegin showed marked endotelioprotective action. LDF with wavelet analyses of vasomotions is original nonclinical criteria of drug efficacy.

PERMEABILITY/FLUID & SOLUTE EXCHANGE/ GLYCOCALYX

P174

Prolonged shear stress modifies the composition of the endothelial glycocalyx

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Throughout the vasculature a gel-like layer consisting of proteoglycans, glycosaminoglycans, glycoproteins and adsorbed plasma molecules covers the endothelium. Within the endothelial glycocalyx (EG) heparan sulfates and hyaluronan have been proposed to be the most important functional components. The EG has been demonstrated to regulate vascular permeability, transduce shear stress and bind chemokines and growth factors. Glycocalyx dimension and composition both have been shown to be crucial for endothelial function. We hypothesized that long-term continuous laminar shear stress induces changes in the EG composition. For this purpose, human umbilical vein endothelial cells (HUVECs) were cultured under flow for 7 days. Using wheat germ agglutinin (WGA) and the carbohydrate specific antibodies 10E4 and JM403, we observed an increased density and thickness of carbohydrates on top of the endothelium and a change in heparan sulfate composition within the endothelial glycocalyx. Furthermore, by blocking the JM403 epitope within HS in static conditions, we observed a reduction of THP-1 monocyte adhesion upon TNF-alpha stimulation, confirming the predicted pro-inflammatory role of the JM403 binding epitope. Consequently, the observed reduction in JM403 epitope expression within heparan sulfate suggests that long-term shear stress leads to a glycocalyx with anti-inflammatory properties.

P175

Role of mDia1 and Src in vascular hyperpermeability induced by advanced glycation end products

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The disruption of microvascular barrier in response to advanced glycation end products (AGEs) stimulation contributes to vasculopathy associated with diabetes mellitus. Here, to study the role of mDia1and Src and their association

with RAGE, moesin, VE-cadherin and FAK in AGE-induced vascular hyperpermeability, we verified that AGEs induced RAGE-mDia1 binding, followed by phosphorylation of Src, which resulted in increased monolayer permeability in HUVECs. Transfected with either RAGE mutant plasmid which was designed to destroy the specific RAGE-mDia1 binding site, or mDia1 siRNA abolished Src phosphorylation induced by AGEs. Cells over-expressed Src displayed a higher permeability after AGE treatment, accompanied with more obvious F-actin rearrangement. Activation of Src with pcDNA3/flag-SrcY530F alone duplicated these effects. Inhibition of Src with siRNA, PP2 or pcDNA3/flag-SrcK298M abolished these AGE-induced effects. The pulmonary microvascular endothelial cells isolated from receptor for AGEs (RAGE)-knockout mice decreased the phosphorylation of Src and attenuated the barrier dysfunction after AGE-treatment. The *in vivo* study showed that the exudation of dextran from mesenteric venules was increased in AGE-treated mouse. And this was attenuated in RAGE knockout or PP2-pretreated mice. Up-regulation of Src activity induced the phosphorylation of moesin, as well as the activation and dissociation of VE-cadherin, while down-regulation of Src abolished these effects. FAK was also proved to interact with Src in HUVECs stimulated with AGEs. Our studies demonstrated that mDia1 and Src play critical role in AGE-induced microvascular hyperpermeability by phosphorylating moesin, VE-cadherin, and FAK respectively.

P176

Visualisation of small and large transport pores in cultured endothelium and their modification by different types of flow

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Endothelial permeability to circulating macromolecules and patterns of haemodynamic wall shear stress vary from site to site within the arterial system. We aimed to develop new methods to investigate *in vitro* whether there is a causal link.Chronic shear stresses were imposed on cultured porcine aortic endothelial cells (PAECs) grown in multiwell plates by swirling the plates on an orbital shaker: the orbital motion produces a wave of culture medium that rotates around the well. The shear stresses experienced by the endothelial cells at each location on the bottom of the well and at each time during the orbit were obtained using numerical methods. Spatially-resolved measurements of permeability were made by using molecular recognition between the substrate underneath the cells (biotinylated gelatin) and a tracer initially placed above them (FITC-neutravidin or phycoerythrin-neutravidin; 66 and 300 kDa, respectively); the tracer binds to the substrate once it is transported through the monolayer, and is detected by confocal microscopy. FITC-neutravidin accumulated underneath bicellular and tricellular borders whereas phycoerythrin-neutravidin was mostly underneath tricellular borders. Shear stress metrics varied radially in the well. Permeability maps of static cultures exhibited a uniform permeability across the well. Sheared monolayers showed an increase in permeability at the center compared to the edge of the well. The variation in binding locations of the two different size tracers suggests two pathways exist for macromolecule transport. The shear stress studies suggest that more than one feature of shear stress, or a shear stress metric that was not investigated, affects endothelial permeabilty.

P177

Galectin 8 induce increased microvascular permeability via S-nitrosylation of p120 catenin: Role in breast cancer.

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Galectin-8 (Gal-8) is widely expressed in tumoral tissues, promoting endothelial cell migration and angiogenesis. A key characteristic of the tumoral blood vessel is its increased permeability which ensuing the appropriate delivery of oxygen and nutrients for both expansion of malignant cells and maintenance of the cancer stem cell reservoir. The role of gal-8 in vascular permeability has not been addressed yet. Our laboratory showed that the increase in permeability in response to pro-inflammatory agents is correlated with S-nitrosylation of adherens junctions proteins like b-catenin and P-120 catenin (p120). With this background we test the hypothesis that Gal-8 secreted by breast cancer cells MCF7 (MCF7-CM) acts on the endothelium inducing increased microvascular permeability via nitric oxide and S-nitrosylation of adherens junction proteins such us p120. As a model we use EA.hy926 immortalized endothelial cells treated with Gal-8 and MCF7-CM. Permeability tests were performed. eNOS activation was evaluated through western-blot and S-nitrosation of p120 was measured by biotin-switch assay. Gal-8 and MCF7-CM induce: a) increases in permeability in EA.hy926 cells, b) eNOS phosphorylation and c) S-nitrosation of p120. These effects were inhibited in the presence of L-NMA or lactose independently. These results provide a

new mechanism of action of Gal-8 on the endothelial cells that can help to the progress of the tumor.

P178
Use of a novel bioreactor to investigate effects of haemodynamic stresses on endothelial permeability

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The effect of haemodynamic stresses on endothelial permeability to macromolecules is important to normal physiology and in the pathogenesis of atherosclerosis. We have developed and applied novel methods to evaluate effects on such transport of acute or chronic exposure to flow along and across cultured endothelium. Porcine aortic endothelial cells were isolated and cultured at passage 1-3 within the porous capillaries of a FiberCell bioreactor. At confluence they were exposed to acute (4 h) or chronic steady or pulsatile luminal flow (3-10 days; mean shear 3dyne/cm2), with or without transendothelial flow (4 × 10-7 cm/s). Permeability to rhodamine-labelled albumin was assessed by fluorimetry. Confluence of monolayers was confirmed by confocal and scanning electron microscopy. Permeability (cm/s ×10-6, mean+SEM) was increased by acute pulsatile shear (8.78 + 0.63; $n = 14$; $p = 0.0001$) and decreased by chronic pulsatile shear (2.87 + 0.22; $n = 22$; $p = 0.0001$) compared to static controls (5.09 + 0.25; $n = 13$). A decrease in PECAM-1 expression under chronic pulsatile flow was demonstrated by flow cytometry ($p = 0.0239$; $n = 11$). Steady flow (5.94 + 0.9; $n = 10$, $p = 0.002$) gave higher permeability than pulsatile flow. The introduction of transendothelial flow increased apparent permeability more than could be explained by the addition of convective transport (to 15.74 + 2.51; $n = 9$; $p = 0.0001$). 10U/ml thrombin increased permeability (9.28 + 0.84; $n = 6$; $p = 0.0001$), as did 500uM Nw-nitro-L-arginine methyl ester (7.26 + 0.64; $n = 4$; $p = 0.0001$).The hollow fibre bioreactor allowed endothelial permeability to be measured with or without exposure to luminal flow and transendothelial flow over 30 days, permitting the investigation of effects of mechanical stresses on endothelial permeability. (Funded by the BHF Centre of Research Excellence)

P179
Endothelial focal adhesion kinase mediates microvascular hyperpermeability during ischemia/reperfusion injury

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Ischemia/reperfusion (I/R) injury is a common problem associated with many diseases or surgical procedures. Reperfusion or reoxygenation of ischemic tissues causes local microcirculatory disorders including microvascular hyperpermeability, which is contributed by endothelial barrier dysfunction. Focal adhesion kinase (FAK) is one of the key elements that regulate endothelial barrier property by not only controlling focal adhesion activity, but also mediating physical forces and biochemical signals via integrins. We previously reported the involvement of FAK in VEGF-elicited coronary venular hyperpermeability. In this study, we further explored the potential role of endothelial FAK in I/R induced microvascular leakage. Endothelial-specific inducible FAK knockout mice were generated by crossing FAK-floxed (FAKfl/fl) mice with Tie2-CreERT2 transgenic mice. Albumin permeability was measured using intravital microscopy in mouse mesenteric microvasculature. The results showed increased FAK activity in isolated mesenteric microvessels from the mice subjected to I/R. Physiological analyses demonstrated that I/R injury induced albumin leakage across the mesenteric venules. The response was attenuated in the KO mice or in the mice pretreated with FAK inhibitor PF573228. Western blot analyses revealed that I/R injury increased phosphorylation of endothelial junctional proteins through activation of FAK. The increase in phosphorylation was attenuated in the cells derived from KO mice or in the cells treated with FAK inhibitor. Taken together, the data suggest that FAK plays an important role in mediating microvascular hyperpermeability during I/R injury. Supported by NIH RO1HL-120954 and HL-096640.

P180
The effects on the endothelial glycocalyx layer and the microcirculatory parameters under septic condition in mice

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Objective: The endothelial glycocalyx (GCX) is located on the apical surface of vascular endothelial cells and is composed of a negatively charged network of proteoglycans

and glycoproteins. Clinically, it is reported that the disorder of GCX was induced under some pathophysiological status, such as severe sepsis. In this study, to elucidate a physiological role of GCX under septic condition, we observed the GCX behavior and the microcirculatory parameters *in vivo*, by using the mice dorsal skinfold chamber (DSC) model.
Methods and Results: To examine the *in vivo* and real-time imaging, we used BALB/c mice with DSC. To observe the behavior of GCX and microcirculatory parameters, animals were allocated into two groups: control, LPS-administrated septic group. LPS was i.p. administrated at 2 mg/kg at 0 and 18 hours. To examine the GCX behavior, FITC-WGA lectin was i.v. injected to obtain fluorescent images at 24 hours. As the parameters of the microcirculation, we analyzed the leukocytes-endothelial interaction using rhodamine 6G and the vascular permeability using fluorescence dextran (average molecular weight 40 kDa or 75 kDa). Our results showed that the WGA positive layer was attenuated under the septic condition compared to control, which means the GCX is degraded. We also confirmed similar results by using electron microscopy. The adherent leukocytes counts and vascular permeability were increased under the septic condition.
Conclusion: WGA positive GCX degradation and the microcirculatory impairments were observed under septic condition. These results indicate that GCX plays critical roles to keep the stability of microcirculation.

P181
Endothelial glycocalyx is lost in murine malaria infections and is associated with increased urokinase levels and downstream remodelling of the extracellular matrix

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Malaria causes substantial mortality and morbidity. Part of pathogenesis involves cytoadhesion and accumulation of malaria-infected erythrocytes in organs, also in the brain and when present associated with high mortality. We investigated the endothelial glycocalyx; the initial binding partner for malaria-infected erythrocytes and how this vasculoprotective layer is lost upon parasite infections in mice. We addressed this *ex vivo, in situ* by transmission electron microscopy and in plasma using ELISA and dot blots. Furthermore, we did transcardial perfusion with biotin to label endothelial proteins for quantification by western blotting. By using parasite strains inducing either mild or fatal infections we could determine that despite comparable levels of shed glycocalyx components to the plasma during the course of infection the loss from specific organs depend on severity of infection and pathology. Namely, high levels of glycocalyx loss was found in brain and lung in fatal malaria, while mild malaria showed less but significant loss from the lung compared with uninfected mice. Western blotting showed loss of syndecan-1 and 4 from cerebral endothelium. Treatment of mice with dexamethasone prevented fatal malaria but not shedding of glycocalyx. Furthermore, treatment with antithrombin-3, a glycocalyx bound vasculoprotective enzyme, improved survival. However, plasma levels of urokinase were significantly increased only in mice with fatal malaria. In conclusion, endothelial glycocalyx loss is not associated with malaria severity but aberrant urokinase signalling leading to excessive shedding may be linked to loss of endothelial function and local pathology.

P182
Purinergic receptor P2 × 7 is a mediator of blood-brain barrier breakdown and microvascular hyperpermeability

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Microvascular hyperpermeability occurring due to breakdown of the blood-brain barrier (BBB) leads to brain edema which can manifest as elevated intracranial pressure following traumatic brain injury (TBI). The mechanisms by which BBB breakdown/hyperpermeability occur following TBI have not been clearly elucidated. Extracellular ATP that acts on purinergic $P2X_7$ receptors ($P2X_7R$) has been associated with brain edema following TBI. We hypothesize that $P2X_7R$ is a mediator of TBI-induced BBB breakdown/hyperpermeabiltiy. In this experimental model, mild TBI was induced in mice using a controlled cortical impactor with BBB integrity/permeability studied utilizing Evans blue leakage and by intravital microscopy. The role of $P2X_7R$ in TBI-induced hyperpermeability was studied in $P2X_7R$ knockout mice. Rat brain microvascular endothelial cells (RBMEC) were exposed to BzATP (a potent ATP analog) in presence or absence of A740003 (a selective $P2X_7R$ antagonist). Monolayer permeability was studied using FITC-dextran in Transwell inserts. Tight junction integrity and cytoskeletal assembly were studied utilizing zonula occludens-1 immunofluorescence and rhodamine phalloidin staining of F-actin, respectively. Cell viability was studied using Calcein AM assay. Mild TBI induced a significant increase in Evans

Blue and FITC-dextran extravasation indicating BBB breakdown/hyperpermeability compared to sham ($p < 0.05$). TBI in $P2X_7R$ knockout mice showed decreased Evans blue extravasation vs. TBI in wild type mice ($p < 0.05$). BzATP induced monolayer hyperpermeability ($p < 0.05$), tight junction disruption and F-actin stress fiber formation. A740003 attenuated all these derangements. BzATP had no significant effect on cell viability. These results suggest that $P2X_7R$ plays a significant role in modulating BBB integrity/permeability following TBI via tight junction proteins.

P183

Association microvascular endothelial glycocalyx with structural alterations of vessels in hypertension patients

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Purpose: To investigate the association between condition of blood vessels and endothelial glycocalyx of sublingual mucosa region microvessels in patients with arterial hypertension.

Methods: The study included 88 males with arterial hypertension (mean age 51.7 ± 6.7 years). The condition of blood vessels was estimated using the mean common carotid artery intima-media thickness (IMT) and the flow-mediated dilation of the brachial artery (TC). Vascular stiffness was evaluated using the pulse wave velocity (PWV) and cardio-ankle vascular index (CAVI). The endothelial glycocalyx condition was examinated using sidestream darkfield imaging of the sublingual region according to the size of perfused boundary region (PBR). All tests were performed in the a.m. period.

Results: The average values of the analyzed parameters were: IMT 0.85 ± 0.17 mm; PWV 7.95 ± 1.21 m/s; CAVI 7.6 ± 0.9; TC 9.97 ± 6.68%; PBR 1.89 ± 0.25 MICS. The correlation analysis showed the significant positive correlation between PBR and IMT ($r = 0.26$; $p = 0.024$), between PBR and CAVI ($r = 0.27$; $p = 0.011$). The negative correlation between PBR and TC ($r = -0.32$; $p = 0.007$) was determined. There was no significant association between PWV and PBR ($r = 0.11$; $p = 0.325$).

Conclusion: Structural and functional damages of oral mucosa microvessels glycocalyx were associated with the blood vessels structure pathological changes of IMT, arterial stiffness regardless with the blood pressure level, and brachial artery flow-dependent (NO-mediated) vasodilation.

STEM CELLS

P184

Aged bone marrow-derived stem cells display increased pericyte fate in cultured microvascular networks

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Models that mimic angiogenesis are valuable for the investigation of underlying mechanisms and the pre-clinical development of therapies. Our laboratory recently developed a novel tissue culture model that enables time-lapse investigation of cell-cell interactions at specific locations across intact blood and lymphatic microvascular networks. The objective of this study was to demonstrate the usefulness of the rat mesentery culture model for evaluating the effects of aging on stem cell differentiation into vascular pericytes. DiI-labeled cells were seeded onto adult Wistar rat mesenteric tissues and cultured in alpha-MEM + 1% serum for up to 5 days according to four experimental groups: (i) adult human bone marrow-derived stem cells (hBMSCs), (ii) aged hBMSCs, (iii) adult human adipose-derived stem cells (hASCs), and 4) aged hASCs. After 1 day, cells displayed changes in cell morphology indicative of tissue integration. By day 5, aged hBMSCs were observed in typical pericyte location wrapped along blood capillaries during angiogenesis and the number of vessels covered by DiI -positive hBMSCs was increased in the aged versus adult cell group (Aged hBMSC = 18.26 ± 0.47 vessels/mm vs. Adult hBMSC = 2.81 ± 0.39 vessels/mm; $p < 0.05$). No aged hMSCs were observed along initial lymphatic capillaries and no change in hASC cell fate was observed between the respective adult and aged populations. Our results suggest that age influences the ability of BMSCs to become vascular pericytes and establishes the rat mesentery culture model as a valuable tool for *ex vivo* screening of type-specific differences in stem cell fate across an intact microvascular network.

P185

Complex microenvironments consisting of multiple vessel types maintains hematopoietic stem cells

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Blood vessels play important role in directing and regulating the properties of local microenvironments. In the skeletal system blood vessels play crucial roles in osteogenesis. We

have recently identified and reported a new capillary subtype in the murine skeletal system with distinct morphological, molecular and functional properties. These vessels are found in specific locations, mediate growth of the bone vasculature, generate distinct metabolic and molecular microenvironments, maintain perivascular osteoprogenitors, and couple angiogenesis to osteogenesis. Blood vessels in skeletal system have been shown to provide niches for hematopoietic stem cells. The properties of niche-forming vessels and their changes in the ageing organism remain incompletely understood. Our study reveals that endothelial notch activity leads to the expansion of hematopoietic stem cell niches in bone, which is achieved through increases in CD31 and Endomucin-positive capillaries, perivascular cells, arteriole formation, and production of niche factors. While hypoxia-inducible factor signalling in endothelial cells promotes increase in CD31 and Endomucin-positive capillaries and other niche factors, it fails to induce arteriole formation and does not improve vascular niche function. Our findings provide novel insights into the regulation of vascular niches for hematopoietic stem cells and also illustrates that hematopoietic stem cell niche are a part of complex microenviroments involving multiple vessel subtypes and several cell populations.

TRANSLATIONAL RESEARCH

P186

AngioChip: A biodegradable scaffold with built-in vasculature for tissue vascularization and direct surgical anastomosis

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Tissue vascularization has long been the bottleneck in the field of tissue engineering. Numerous tissue types have been successfully engineered *in vitro*, but clinical translation has been achieved only for thin tissues or those with a low metabolic demand (e.g. skin, cartilage and bladder). Large solid tissues (e.g. myocardium, liver) are highly sensitive to oxygen levels and become vulnerable within hours without oxygen supply. These tissues would greatly benefit from rapid vascularization *in vitro* and direct vascular integration *in vivo*. So far direct and immediate surgical anastomosis of vascularized tissues has only been demonstrated using vascular explants, requiring multiple surgeries to harvest the vascular bed. Using a new 3-D stamping technique, we developed an AngioChip scaffold from a synthetic biodegradable elastomer (poly(octamethylene maleate (anhydride) citrate). The AngioChip contains an built-in 3-D, perfusable, branched micro-channel network coated with endothelial cells, embedded into a lattice matrix, supporting assembly of different types of parenchymal cells. The synthetic built-in vascular walls were thin and flexible, yet strong enough to mechanically support a perfusable vasculature in a contracting tissue and enable direct surgical anastomosis. Incorporation of nano-pores and micro-holes in the vessel walls enhanced vessel permeability, permitted inter-cellular crosstalk, extravasation of monocytes, and sprouting of endothelial cells upon stimulation. Vascularized hepatic tissues and cardiac tissues, engineered using AngioChips, were shown to process clinically relevant drugs delivered through their internal vasculature. AngioChip cardiac tissues were also implanted via direct surgical anastomoses to the femoral vessels of rat hindlimbs, establishing immediate blood perfusion.

P187

Does macular thickness vary in the early stages of diabetic retinopathy in type 2 diabetes?

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Whether fovea thickness, assessed by optical coherence tomography (OCT), is altered in early stages of diabetic retinopathy is currently not clear, partly due to variations in DR grading and small sample sizes. As part of the SUMMIT consortium this study aims to examine the relationship between early stages of DR and thickness in all macular regions. 780 participants with type 2 diabetes (461, 162 and 157 with no, microaneurysms only or mild background DR respectively) were recruited across two centres. DR was graded from two-field retinal photography. Macular thickness measured using OCT. The eye with the worse retinopathy and its corresponding OCT data were utilised in the analysis. If retinopathy was the same in both eyes the data from the right eye was utilised. The relationship between

thicknesses in each macular region with DR status was investigated using regression analysis, adjusting for age, gender and centre. There was no relationship between the thickness of the fovea or inner quadrants with DR. In the outer temporal quadrants there was a positive association between thickness and DR (standardised beta = 0.092, $p = 0.012$). This association remained when further adjusted for glycaemic control and diabetes duration (standardised beta = 0.093, $p = 0.020$). This association was not present in the nasal, superior or inferior outer quadrants. This study suggests that there are small differences in the thickness of the outer temporal region in the early stages of DR. Further research is needed to examine whether alterations in this macular region may be an early marker of DR.

P188
Human, *in vivo*, microvascular actions of glucagon-like peptide-1 and its analogues in health, obesity and well-controlled type 2 diabetes

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Limited human studies, supported by in-vitro studies, suggest that GLP-1 and its analogues act as macrovascular vasodilators and attenuate ischaemia-reperfusion injury. However, whether these actions are direct effects are difficult to determine. Additionally, their microvascular actions are unknown.

Aim: To examine (1) microvascular actions of GLP-1 and its analogues, Exenatide and Liraglutide, in health, obesity and well-controlled type 2 diabetes (T2DM); (ii) whether the microvascular response to GLP-1 is related to GLP-1(9.36) amide.

Methods: 3 participant groups were recruited: lean (BMI median(25th,75thquartiles: 23.0(22.0, 24.0)), obese (BMI: 33.0(31.5, 38.0) and T2DM (BMI: 30.0(26.3, 33.0) ($n = 21$ per group). GLP-1 (300 pm), Liraglutide and Exenatide at 1/10th minimum treatment dose (0.06 mg and 0.5micrograms respectively) were microinjected into the dermis of the forearm. Laser Doppler imaging assessed skin perfusion. To determine whether the actions of GLP-1 are related to GLP-1 (9.36)amide the microinjection protocol was performed under DPP-IV inhibition (Linagliptin, 5 mg) and control/placebo conditions.

Results: Liraglutide and Exenatide increased skin perfusion compared to control (saline (0.9%) microinjection) in all groups (lean, saline stabilised response: median 0.61 (25th,75th quartiles: 0.51, 0.73)V; Exenatide: 0.99(0.86, 1.44)V; Liraglutide: 1.19(1.11, 1.50)V, $p < 0.001$) (Obese, saline: 0.52(0.49, 0.63)V; Exenatide: 1.01(0.82, 1.18)V; Liraglutide: 1.19(1.04, 1.47)V, $p < 0.001$) (T2DM, saline 0.59(0.48, 0.67)V; Exenatide: 1.01(0.65, 1.34)V; Liraglutide: 1.18(0.96, 1.27)V, $p < 0.001$). The responses were not altered by obesity or T2DM ($p > 0.455$). The GLP-1 response was not different to control in all participant groups, and was not altered by DPP-IV inhibition.

Summary: This study suggest that Exenatide and Liraglutide, but not GLP-1 (300pM), increase skin perfusion in health, and that the responses are maintained in obesity and well-controlled T2DM.

P189
Dynamics of angiogenesis and blood flow in mouse long bone

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Angiogenesis and osteogenesis are coupled coherent processes occurring during the development of skeletal system. Distinct capillary subtypes have been identified to play an important role in coupling of angiogenesis and osteogenesis. These blood vessels show presence of specialized structures like loops and bulges, but their involvement in mediating non-sprouting angiogenesis is not known. Here, using a novel intra vital imaging technique, we describe the mechanism of blood vessel growth in the mice long bones. The arrangement of blood vessels describes a peculiar blood flow pattern, which attributes the heterogenic phenotypes in capillaries. Blood flow regulates formation of specialized structures in the vascular front through Notch signalling to couple angiogenesis with osteogenesis during early development. Therefore in the aged mice when the blood flow is severely down, activating Notch signalling in endothelial cells could promote formation of these angiogenic structures in blood vessels to promote neo-osteogenesis and thus restore bone mass.

MYOGENIC TONE

P190
A physiological role for TRPV4 sparklets

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Increases in global calcium within vascular smooth muscle cells (SMCs) are crucial for myogenic tone development and maintenance in resistance arterioles, while calcium changes

in endothelial cells (ECs) modulate SMC contractility. Focal calcium influx through TRPV4 channels on ECs, termed sparklets, has been linked to activation of intermediate-conductance calcium-activated potassium channels (IK_{Ca}), hyperpolarization and vasodilation. We examined how these channels can be activated physiologically by altering intraluminal pressure in isolated, pressurized rat cremaster arterioles. ECs were loaded with Oregon Green 488 BAPTA-1 and imaged using confocal microscopy at 3 Hz. Intraluminal pressure was varied between 5 and 80 mmHg, and the frequency of calcium events determined at each pressure. In separate experiments, myogenic tone-pressure response curves were repeated in the presence of an inhibitor of nitric oxide synthase (L-NAME), and then inhibitors of K_{Ca} channels (TRAM-34, and/or apamin). At low intraluminal pressure, ECs spontaneously generated calcium events at a frequency of ~5 events/min which were partially inhibited in the presence of RN1734, a TRPV4 channel antagonist. Spontaneous events significantly decreased from ~5 to ~2 events/min when intraluminal pressure was raised. At low pressures, inhibition of IK_{Ca}, but not SK_{Ca}, resulted in a significant increase in myogenic tone. Immunohistochemical staining for IK_{Ca} and SK_{Ca} channels occurred in myoendothelial projections and in SMCs. At low pressures EC spontaneous calcium events activate IK_{Ca}, which supress myogenic tone by generating hyperpolarization. These data support a role for pressure-dependent TRPV4-mediated spontaneous EC calcium activity in the modulation of myogenic tone.

SATELLITE SYMPOSIUM

SL1
Diverse functions of endothelial NO synthases system: NO and EDH
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The endothelium plays an important role in modulating vascular tone of underlying vascular smooth muscle (VSMC) by synthesizing and releasing several vasodilating substances, including prostacyclin, nitric oxide (NO) and endothelium-dependent hyperpolarization (EDH) factor. It has been repeatedly demonstrated that NO plays an important role in conduit arteries, while the importance of EDH factor increases as the vessel size decreases, irrespective of species or blood vessels tested. Although several factors seem to be involved in EDH, one of the major candidates of EDH factor is hydrogen peroxide (H_2O_2) derived from endothelial NO synthases (NOSs) system in animals and humans. H_2O_2 is formed through dismutation by superoxide dismutase of superoxide anions derived from endothelial NOSs system upon stimulation by shear stress and various agonists. H_2O_2/EDH factor is substantially involved in the regulatory mechanisms of coronary microcirculation *in vivo*, including autoregulation, ischemia/reperfusion and metabolic dilatation. In microvessels, endothelial NO synthase (eNOS) is functionally inhibited by several mechanisms including caveolin-1, functioning as a generating system of EDH factor. Furthermore, direct VSMC relaxation to H_2O_2 is also enhanced in microvessels. This physiological balance between NO and EDH factor in different-sized blood vessels appears to be important as the genetic deletion of endothelial caveolin-1 with a resultant overproduction of NO rather causes coronary microcirculatory dysfunction, nitro stress, and left ventricular hypertrophy in mice *in vivo*. Thus, the endothelial NO synthases system has diverse functions depending on vessel size, maintaining the physiological balance between NO and EDH factor.

SL2-1
Endothelial dysfunction: Regenerate to be old
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The endothelium mediates relaxations of the underlying vascular smooth muscle cells by releasing endothelium-derived relaxing factors (EDRF), which results in endothelium-dependent vasodilatations. The best characterized EDRF is nitric oxide (NO) formed from l-arginine by the constitutive endothelial NO synthase (eNOS). NO diffuses to the vascular smooth muscle where it stimulates soluble guanylyl cyclase with, under normal conditions, the resulting production of cyclic GMP. The release of NO from the endothelium can be mediated by both pertussis toxin-sensitive Gi- (e.g. Alpha2-adrenergic agonists, serotonin) and insensitive Gq- (adenosine diphosphate, bradykinin) proteins. The ability of the endothelial cell to release relaxing factors can be up-regulated by estrogens, increased flow, exercise, diet (omega3-unsaturated fatty acids, polyphenols) and antioxidants, and down-regulated by oxidative stress and increased presence of oxidized low density lipoproteins (LDL). It is reduced chronically by aging, smoking, environmental pollution and in hypertension and diabetes. Following injury or apoptotic death, the endothelium regenerates. However, in regenerated endothelial cells, there is an early selective loss of the pertussis-toxin sensitive mechanisms of EDRF-release. Functional studies suggest that increased presence of oxidized LDL (due to oxidative stress) plays a key role in this selective loss. Genomic studies demonstrate the emergence of fatty acid binding protein-A (A-FBP) and metalloproteinase-7 (MMP7) in regenerated endothelial cells. Inhibition of A-FABP curtails the occurrence of

endothelial dysfunction. The reduced release of NO resulting from the endothelial dysfunction in regenerated areas sets the stage for the occurrence of vasospasm and thrombosis as well as it permits the inflammatory response leading to atherosclerosis.

SL2-2
Importance of coronary microvascular dysfunction

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It is widely known that coronary vascular resistance is largely determined at the level of coronary microcirculation, including the pre-arterioles and arterioles of the coronary arteries. However, along with the advancement of coronary intervention therapy, much attention has been paid to the epicardial coronary arteries, and indeed much attention should be paid to the importance of coronary microcirculation. Coronary microvascular dysfunction appears to be involved in the pathophysiology of almost all cardiovascular diseases, including ischemic heart disease, hypertensive heart disease, heart failure, primary and secondary cardiomyopathy, myocarditis, and intervention-related myocardial dysfunction. Coronary microvascular dysfunction could be caused by impaired vasodilator functions (endothelium-dependent and –independent responses) and/or enhanced contractile responses of vascular smooth muscle (VSMC). Endothelium-dependent vasodilating responses are achieved by nitric oxide (NO) in conduit arteries and endothelium-dependent hyperpolarization (EDH) factor in resistance vessels, while endothelium-independent responses by adenosine and catecholamines. VSMC hypercontracting responses are caused by several mechanisms, including Rho-kinase pathway that plays a key role in vasospastic disorders. It has been recently demonstrated that plasma levels of serotonin could be a useful biomarker of coronary microvascular dysfunction in patients with microvascular angina. Coronary microvascular dysfunction is also an important therapeutic target of cardiovascular risk factors, such as hypertension, hyperlipidemia and diabetes.

SS1-1
Angiogenesis and lymphangiogenesis in cancer metastasis

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Cancer metastasis employs complex mechanisms involving intimate interactions between malignant cells and host cellular components in the tumor microenvironment. While the intrinsic invasive features of cancer cells are necessary for metastasis, they are not sufficient for distal metastasis. We focus our efforts on understanding the roles of angiogenesis and lymangiogenesis in facilitating cancer metastasis. Several of our recent studies have uncovered functions of several angiogenic factors and cytokines including VEGF, VEGF-B, VEGF-C, FGF-2, PDGF-BB and TNF-a in modulation of angiogenesis and lymphangiogenesis for cancer invasion and metastasis. Further, interactions between these and other factors are crucial for acting on various steps of the metastatic cascade. Based on findings, we have identified new biomarkers for therapeutic intervention and we propose new therapeutic strategies for treatment of metastatic diseases.

Our selected publications on this topic:

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Proc. Natl. Acad. Sci. USA, 2015 in press:

Nature Rev Endocrinol. 2014 Sep;10(9):530-9.

Nature Communications 2014 Sep 17;5:4944.

J Clin Invest. 2014 Mar;124(3):1069-82.

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SS1-2
Organotypic lymphatic vessels: Lacteal and Schlemm's Canal

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In small intestine, lacteal as a lymphatic end is located in the center of each villus, which is a crucial transport duct for digested lipids, and lipid-soluble substances and drugs. However, it is a poorly understood how absorbed molecules are transported through the lacteal. We tried to shed light upon novel ways of investigating the dynamics of absorption and drainage into lacteals in the small intestine villi. Utilizing Prox1-GFP reporter mice and a custom-built intravital confocal microscopy system, we directly visualized the lacteals and the absorption and transport dynamics of fluorescence-tagged fatty acids in the villi at cellular level *in vivo*, which consisted of transepithelial absorption via

enterocytes, diffusive distribution over the lamina propria, and subsequent transport through lacteals. Intriguingly, we found active contraction of lacteals, which seemed to be directly involved in dietary lipid drainage. On the other hand, in the eye, Schlemm's canal (SC) is a unique vascular structure for draining out aqueous humor from the intraocular chamber into systemic circulation. Dysfunction of SC has been appeared to be responsible for glaucoma. Here we reveal that SC, which originates from blood vessels during postnatal period, acquires lymphatic identity by upregulating Prox1, the master regulator of lymphatic development. Specific marker analysis revealed that SC is an intermediate between lymphatic and blood vessels. We also evaluated the regulatory mechanisms that alter the fate of SC during both development and pathologic conditions, and found that Prox1 is an accurate and reliable biosensor of SC integrity and identity.

SS1-3
Roles of prostaglandins in regulation of plasticity of lymphatics and lymph nodes

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Angiogenesis is upregulated by prostaglandin (PG)E_2 in inflammation and cancers, however, little is known about its involvement in lymphangiogenesis. When we examined lymphangiogenesis in chronic inflammation, lymphangiogenesis detected by the double immunostaning of VEGFR-3 and LYVE-1 was upregulated during the development of granulation tissues, which were formed around the Matrigel plugs with inductions of cyclooxygenase (COX)-2 and mPGES-1. Administration of a COX-2 inhibitor, celecoxib significantly reduced lymphatic vessel formation in granulation tissues, whereas topical PGE_2 administration enhanced lymphangiogenesis. Lymphangiogenesis was suppressed in mice lacking either PGE receptor 3 (EP3) or EP4 in comparison with wild type counter parts (WT), suggesting that these receptors are relevant to lymphangiogenesis in this inflammatory model. Lymphatic system is an important route for cancer dissemination, and lymph node metastasis (LNM) serves as a critical prognostic determinant in cancer patients. A murine model of Lewis lung carcinoma (LLC) cell metastasis revealed that COX-2 is expressed in dendritic cells (DCs) from the early stage in lymph node subcapsular regions, and COX-2 inhibition markedly suppressed mediastinal LNM. Stromal cell-derived factor-1 (SDF-1) was elevated in DCs before LLC cell infiltration to regional lymph nodes, and a COX-2 inhibitor, an SDF-1 antagonist, and a CXCR4 neutralizing antibody all reduced LNM. Moreover, LNM was reduced in EP3 knockout mice, and stimulation of cultured DCs with an EP3 agonist increased SDF-1 production. Accumulation of regulatory T cells and lymph node lymphangiogenesis were also COX-2/EP3-dependent. These results indicate that COX-2-derived PGE2 modulate lymph node plasticity to form a premetastatic niche.

SS1-4
New lymphology combined with cardiovascular physiology, innate immunology, and oncology

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As one of the lymphatic functions, it is well known that the transport and drainage of hydrophilic substances including plasma protein through the lymphatic system play pivotal roles in maintaining the homeostasis of the internal environment between the cells in tissues in collaboration with the exchange of the substances through the blood capillaries and venules. On the other hand, it is also well known that the initial clinical signs of primary diseases such as inflammation, tumors, and circulatory disorders including infarction and thrombosis appear as functional abnormalities of the internal environment in tissues. These abnormalities of the functions are strongly related to immunological defense reactions around the internal environment and abnormal actions of the transport and drainage of the lymphatic system. Taking into consideration the current inspired findings in lymphatic physiology, innate immunology, and oncology, we have proposed a new lymphology combined with new knowledge of the three above-mentioned academic fields from a defense mechanism points of view. In this lecture, I would like to demonstrate comprehensively our latest studies (Ohhashi T & Kawai Y: J Physiol Sci (review) 65:51-66, 2015; Ohhashi T: Ann Vasc Dis (overview) 5:245-248, 2012; Ohhashi T, Kawai Y et al.: Pharmacol Ther (review) 105:165-188, 2005; Ohhashi T et al.: Jpn J Physiol (review) 44:327-345, 1994; Ohhashi T: Biochem Pharmacol (review) 45:1941-1946, 1993) related to the possibility of establishing the new lymphology, hoping you will evaluate the possibility.

SS1-5
Semaphorin3G provides a repulsive guidance cue from arteries to PlexinD1^{+} lymphatic endothelial cells in the mouse embryonic skin

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The vertebrate circulatory system is composed of closely-related blood and lymphatic vessels whose network patterns have an impact on the integrated microvascular function. It has been shown that lymphatic vascular patterning is regulated by blood vessels during development since lymphatic endothelial cells (LECs) migrate adjacent to arteries and eventually form a random network. However, molecular mechanisms of lymphatic vascular patterning have not fully been understood. The class 3 Semaphorins (Sema3s) represent a family of secreted factors implicated as repulsive regulators of angiogenesis. In this study, we show that an artery-derived ligand Sema3G and endothelial cell receptor PlexinD1 serve as repulsive guidance cues from arteries to LECs. In the mouse embryonic back skin, genetic inactivation of Sema3G or PlexinD1 results in abnormal artery-lymph alignment and reduced lymphatic vascular branching. Conditional ablation in mice demonstrates that PlexinD1 is primarily required in LECs. *In vitro* analysis show that Sema3G does not bind directly to PlexinD1 but to Neuropilin-2 (Nrp2). Co-immunoprecipitation analysis identifies the receptor complex of Nrp2 and PlexinD1, and Sema3G indeed induces the cell collapse in a Nrp2/PlexinD1-dependent manner. Our findings shed light on a molecular mechanism by which LECs distribute away from arteries and form a branching network during lymphatic vascular development.

SS2-1
Role of cyclophilin A in cardiovascular system

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Excessive reactive oxygen species (ROS) deteriorates vascular functions and promotes vascular diseases through multiple pathways. Recently, cyclophilin A (CyPA) has been shown to be secreted from vascular smooth muscle cells (VSMC) and to augment the destructive effects of ROS, linking it to the development of many cardiovascular diseases. In the secretion of CyPA, Rho-kinase plays an important role to organize vicious cycle for augmentation of ROS. The important role of Rho-kinase has been established in the pathogenesis of vasospasm, arteriosclerosis, ischemia/reperfusion injury, hypertension, pulmonary hypertension, stroke and heart failure. Thus, it is important to understand Rho-kinase signaling and the role of downstream effectors such as CyPA in the vascular system in order to develop new therapeutic strategies for cardiovascular diseases. Here, we reported that plasma CyPA levels are increased in patients with coronary artery disease (CAD). Plasma CyPA levels were significantly higher in patients with significant coronary stenosis compared to those without it. A positive correlation was noted between plasma CyPA levels and significant coronary stenosis. The average number of stenotic coronary arteries and the need for coronary intervention were significantly increased in the quartiles of higher CyPA levels. Interestingly, plasma levels of CyPA increased according to the number of atherosclerotic risk factors, all of which induce oxidative stress. Furthermore, plasma levels of CyPA significantly reduced after medical treatment of risk factors. We will discuss the roles of VSMC-derived CyPA in promoting vascular diseases, with particular emphasis on the role of CyPA as a novel biomarker for CAD.

SS2-2
Vascular adrenomedullin-RAMP2 system is essential for vascular integrity and organ homeostasis

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Adrenomedullin (AM) has been recognized to be a pleiotropic molecule involved in both the pathogenesis of cardiovascular diseases and circulatory homeostasis. AM has a short half-life in the blood stream and its application in chronic disease has limitations. Therefore, we have focused on its receptor system. The calcitonin-receptor-like receptor (CLR) associates with one of the accessory proteins, called receptor activity-modifying proteins (RAMPs). By interacting with RAMPs, CLR exhibits a high affinity for AM and its family peptides. We generated RAMP knockout mice and found that phenotypes similar to AM-/- were reproduced only in RAMP2-/-, which showed lethal at mid-gestation with abnormalities of vascular development. To directly analyze the vascular function of AM-RAMP2 system, we then generated endothelial cell-specific RAMP2 knockout mice (E-RAMP2-/-). Most E-RAMP2-/- mice died perinatally. In surviving adults, vasculitis occurred spontaneously. With aging, E-RAMP2-/- mice showed severe organ fibrosis with marked oxidative stress and accelerated vascular senescence. Later, liver cirrhosis, and cardiac fibrosis developed. We next used a line of drug-inducible E-RAMP2-/- mice (DI-E-RAMP2-/-) to induce RAMP2-deletion in adults, which enabled us to analyze the initial causes of the aforementioned vascular and organ damage. Early after the induction,

pronounced edema with enhanced vascular leakage occurred. *In vitro* analysis revealed the vascular leakage to be caused by actin disarrangement and detachment of endothelial cells. Our findings show that the AM-RAMP2 system is a key determinant of vascular integrity and homeostasis from prenatal stages through adulthood. AM-RAMP2 system could be an attractive therapeutic target in cardiovascular diseases.

SS2-3
Role of the endogenous and exogenous NO production systems in the pathogenesis of cardiovascular and metabolic diseases

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Nitric oxide (NO) is synthesized by three distinct NO synthases (NOSs). The roles of NO derived from whole NOSs have been examined in pharmacological studies with non-selective NOSs inhibitors. However, due to non-specificity of the NOSs inhibitors, their authentic roles are still poorly understood. To address this important issue, we developed mice in which all three NOS genes are totally disrupted. The triple NOSs$^{-/-}$ mice manifested phenotypes that resemble metabolic syndrome in humans, including visceral obesity, hypertension, hyper-LDL-cholesterolemia, impaired glucose tolerance, and insulin resistance. Long-term feeding of a high-cholesterol diet markedly increased plasma LDL cholesterol levels in the triple NOSs$^{-/-}$ mice, and these levels were similar to those in apoE$^{-/-}$ mice that manifest severe hyperlipidemia. It has recently been discovered that NO is produced from the NO metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), the latter of which is rich in green leafy vegetables. Based on the background, we examined the effect of a low nitrite/nitrate diet on metabolic phenotypes in wild-type mice. Long-term feeding of a low nitrite/nitrate diet resulted in visceral obesity, hypercholesterolemia, impaired glucose tolerance, and insulin resistance in the wild-type mice. These results suggest that long-term dietary nitrite/nitrate deficiency gives rise to metabolic syndrome in mice, identifying a specific dietary ingredient that causes metabolic syndrome even in the absence of excess calorie intake. Our findings demonstrate that not only the endogenous NO production system, but the exogenous NO production system also plays a role in the pathogenesis of cardiovascular and metabolic disorders.

SS2-4
When NO becomes ugly and causes vasospasm

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In coronary arteries hypoxia causes an acute augmentation of vasoconstrictor responses that is dependent on the presence of nitric oxide (NO) and activation of soluble guanylyl cyclase. This hypoxic effect is due to increases in the intracellular level of inosine 5′-triphosphate (ITP) and the biased activity of soluble guanylyl cyclase which results in the synthesis of inosine 3′,5′-cyclic monophosphate (cIMP). The hypoxic augmentation is due to increased activity of Rho kinase (ROCK), indicating that cIMP may mediate the hypoxic effect by sensitizing the myofilaments to Ca2 + via ROCK. Hypoxia is implicated in exaggerated vasoconstriction in the pathogenesis of coronary artery disease, myocardial infarction, hypertension and stroke. Similar endothelium-dependent,NO-dependent and soluble guanylyl cyclase-dependent contractions can be evoked with thymoquinone, which also augments the levels of cIMP. The understanding of the role of this non-canonical cyclic nucleotide may help identifying novel therapeutic targets for certain cardiovascular disorders.

SS3-1
Mechanotransduction and its failure in the metabolic syndrome due to proteolytic receptor cleavage

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Blood and endothelial cells exhibit a response to fluid shear stress that evokes different responses. For example, leukocytes retract pseudopods under fluid shear stress by a mechanism that utilizes the formyl peptide receptor (FPR) receptor as mechanosensor (Am. J. Physiol, Cell, 290:C1633, 2006). The FPR, a GPCR, signals to agonist binding as well as to physiological fluid shear stress. In the spontaneously hypertensive rat (SHR), a genetic model with elevated blood pressure and other cellular dysfunctions analogous to those seen in the metabolic syndrome, the fluid shear response is attenuated with reduced ability to retract pseudopods. The reduced fluid shear response in the SHR leads to many leukocytes in the circulation with pseudopod formation and multiple complications in the microcirculation (Circ. Res., 95:100, 2004). Investigation of the molecular mechanism for this attenuated shear response shows that the SHR has an elevated proteolytic activity in the plasma and in the tissue due to matrix metalloproteinases (J. Vasc. Res., 47:423,

2010). The proteolytic activity causes cleavage of membrane receptors, including the ectodomain of FPRs, and this reduces the receptor signal when exposed to fluid shear. Blockade of the matrix metalloproteinase activity of the SHR prevents cleavage of the FPR and restores its fluid shear stress response. Other cell dysfunctions in the SHR are also caused by the proteolytic receptor cleavage, e.g. insulin resistance by cleavage of the insulin receptor ectodomain. We suggest that proteolytic receptor cleavage is a major contributor to cell dysfunctions in the metabolic syndrome. Supported by HL10881.

SS3-2
Endothelial cell plasma membrane acts as a mechanosensor that detects fluid shear stress

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Vascular endothelial cells (ECs) sense shear stress and transduce blood flow information into functional responses that play important roles in vascular homeostasis and pathophysiology, however, the mechanism by which ECs perceive shear stress remains unknown. Shear stress may activate various membrane-bound molecules by altering the physical properties of the EC membrane, because membrane properties affect the activities of the membrane-bound molecules. To determine how shear stress influences the cell membrane, cultured human pulmonary artery ECs were examined for changes in the membrane lipid order and fluidity by Laurdan two-photon imaging and FRAP measurements. Generalized polarization (GP) values were calculated from the Laurdan images. The GP images showed a heterogeneous distribution of the GP values across the cell membrane, indicating that the EC membrane has a non-uniform lipid order in which both liquid-disordered phases with low GP values and liquid-ordered phases with high GP values coexist. Upon shear stress stimulation, the GP values rapidly decreased. Shear-stress-induced responses in the membrane lipid order are reversible and dependent on the magnitude of the shear stress. A similar response in lipid order occurred in the artificial membranes of giant liposomes. ECs labeled with DiIC18 were subjected to shear stress and examined by FRAP to identify changes in the membrane fluidity. Diffusion coefficients significantly increased the membrane fluidity in response to shear stress. In conclusion, EC plasma membranes respond directly to shear stress by changing their lipid order and fluidity, and these changes in the membrane physical properties are involved in the shear-stress-sensing mechanisms.

SS3-3
A role of hemodynamic stress on the cerebral aneurysm formation: A series of studies using an animal model of experimentally induced cerebral aneurysms

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Although the cerebral aneurysm (CA) is a major cause of life-threatening subarachnoid hemorrhage, the mechanisms of CA formation still remain unclear. We introduce here a series of reports, using an animal model of experimentally induced CAs, suggesting that enhanced hemodynamic stress associated with wall shear stress (WSS) plays a central role in CA development.

Human CA is more induced in the asymmetric-shaped circle of Willis. CAs in our animal model develop at several sites along the circle of Willis, where blood flow is increased in compensation for unilateral common carotid artery ligation and renal hypertension, suggesting that hemodynamic stress is a key requirement for CA formation.

Hemorheological studies in rat showed that WSS was increased at the CA orifice, in which the initial arterial wall degeneration developed. iNOS was induced at the CA orifice in parallel with development of early CA changes, and either iNOS inhibitor or WSS reduction could decrease the incidence of CA formation after CA-inducing surgery. The data suggest that enhanced WSS is closely associated with CA development. In fact, in knockout mice of P2 $\times$ 4 purinoceptor, one of the vascular endothelial shear-sensors, the number of induced CAs after CA-inducing surgery was significantly smaller than in the control wild type mice. Expressions of several enzymes, such as iNOS, were suppressed in P2 $\times$ 4 knockout mice. The evidence suggests that, during CA formation, vascular endothelial cells may sense excessively high levels of the WSS-associated hemodynamic stress, which may initiate induction of several enzymes, including iNOS, contributing to vascular wall degeneration.

SS3-4
Computational fluid dynamics for simulating the blood flow in arteries: Its applications to hemodynamic analyses of the cerebral aneurysm formation

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Computational fluid dynamics (CFD) can provide detailed information in three-dimensional hemodynamic field. In

particular, the combination of CFD and the image-based modelling technique has been widely used as a powerful tool for the hemodynamic study in large arteries. We introduce here a series of CFD studies to identify wall shear stress-associated hemodynamic factors that are responsible for the cerebral aneurysm formation. In our CFD studies, we employed anatomically realistic models of human middle cerebral artery (MCA) aneurysms, which were segmented from the volume data set of 3D-CT angiographic images. The images were obtained from cases registered with the "CFD ABO Study", which is a National Hospital Organization multi-institutional join clinical trial currently in progress. An aneurysm of each case was artificially removed with previously developed and verified tools to reconstruct arterial geometries just before aneurysm formation. Pulsatile blood flow was simulated using a CFD software package, CFX (ANSYS Inc.), based on the incompressible Navier-Stokes equations. At the inlet boundaries, we imposed flow velocity waveforms based on individual flow velocity measurements using Doppler ultrasonography. We computed hemodynamic indices on the wall surface from a velocity field data set obtained during a pulsatile CFD simulation. The spatial average of the indices were also computed around the aneurysm-developed site and the corresponding site, respectively, and they were compared between the two sites. The results have suggested that the temporal disturbances of wall shear stress, especially in transverse direction to mean flow, may be a key requirement for the formation of cerebral aneurysms.

SS4-1

The dynamics of albumin leakage from mesenteric venules and reflux from collateral lymphatic vessel after superior mesenteric vein constriction

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The abnormality of the dynamic balance between the albumin leakage from intestinal venules and reflux from collateral lymphatic vessel is a major contributor to ascites formation. The present study was to examine the dynamics of albumin leaking from mesenteric microvessel and reflux from collateral lymphatic vessel in rats subjected to superior mesenteric vein constriction, and study the underlying mechanism. Microvessels of male Wistar rats' ileocecal portion of ileum, with collateral lymphatic vessels were selected for fluorescent imaging using FITC labeled albumin. After 10 min of basal observation, the constriction of superior mesenteric vein was accomplished by ligating the superior mesenteric vein. Rb1 (5 mg/kg/h) was administrated through jugular vein starting from 10 minutes before the constriction of superior mesentery vein until the end of observation The dynamic process of albumin leakage from mesentery venule and reflux from collateral lymphatic vessel was observed and recorded continuously for 120 minutes. Superior mesenteric vein constriction increased albumin leakage from venules significantly, peaking at 10 minutes after constriction, a time point from then FITC-labeled albumin started returning from the collateral lymphatic vessel. The expression of claudin-5, occludin, jam-1, ZO-1 and VE-cadherin in intestine decreased significantly after 120 min constriction. Rb1 prevented albumin leaking from mesentery venules and inhibited the down-regulations of tight and adherens junction proteins by constriction. These results suggested that Rb1 was able to protect against albumin leakage from the mesentery venule induced by superior mesentery vein constriction, possibly via inhibiting the down-regulations of tight and adherens junction proteins.

SS4-2

Histamine as an endothelium-derived relaxing factor in mesenteric lymphatic vessels of various ages

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In contrast to advancements in biology of blood vasculature, knowledge of the basic principles of lymphatic functions still remains to a large degree rudimentary and require significant research efforts. Recent studies of the physiology of the mesenteric lymphatic vessels (MLVs) suggested presence of an endothelium-derived relaxing factor (EDRF) other than nitric oxide in mesenteric lymphatic vessels (MLVs). In this study we tested hypothesis that histamine may play a functional role as EDRF in MLVs. We measured and analyzed parameters of lymphatic contractility in isolated and pressurized adult rat MLVs under control conditions and after pharmacological blockade of nitric oxide by L-NAME, 100 uM or/and histamine production by alpha-MHD (alpha-methyl-DL-histidine dihydrochloride), 10 uM). We found that only combined pharmacological blockade of nitric oxide by L-NAME and histamine production by alpha-MHD in 10 uM completely eliminates flow-dependent relaxation of MLVs thus confirming novel role of histamine as EDRF in MLVs. Effectiveness of alpha-MHD was

confirmed immunohistochemically. We also used immunohistochemical labeling and western blot analysis of the histamine-producing enzyme, histidine decarboxylase (HDC) localized inside lymphatic endothelial cells. Additionally we blocked HDC protein expression in MLVs by transient transfection with *vivo*-morpholino oligos. Our results suggest that histamine play a role as EDRF in MLVs. Such functional impact of histamine on MLVs varies over lifespan. This study widens our understanding of the complexity in regulatory mechanisms of functional links between lymphatic functions, lymph flow and content, fluid and macromolecules homeostasis, inflammation in the gut and immune response at various ages. NIH AG-030578 & HL-094269.

SS4-3

New aspects on lymphatic-like structures in human choroid and retina: Relevance to fluid clearance and immune privilege in the posterior eye

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Lymphatics subserve many important functions in the human body including maintenance of fluid homeostasis, immune surveillance, and tumor metastasis. Based on immunohistochemical and transmission electron microscopy analyses, we (Koina et al., IOVS 2015) recently showed numerous blind-ended lymphatic-like sacs just external to the choriocapillaris, as well as infrequent pre-collector and collector lymphatic channels, with an apparent central to peripheral topography of lymphatic formation in the human choroid. This system of blind-ended initial lymphatic segments observed just external to the fenestrated vessels of the choriocapillaris, is ideally placed for recirculating extracellular fluid and strategically placed for immune surveillance. Our findings will be presented within the context of the current controversy of the 'glymphatic' system in the brain and the recent suggestion (Dennistin and Keane, IOVS 2015 Letter to Editor) of paravascular pathways in the retina: the ocular 'glymphatic system'. Recent studies have also shown the presence of lymphatic vessels in the dura mater that drain the cerebrospinal fluid into the deep cervical lymph nodes (Louveau et al., 2015; Aspelund et al., 2015). The presence of a system of lymphatic-like channels in the human choroid provides an anatomical basis for antigen presentation in the posterior eye, with a possible route from the eye to the sentinel lymph nodes, similar to that already described for anterior eye lymphatics. Further studies are needed to determine the fluid transport function of these channels, relationships with blood vessels, roles in immune response and possible pathways from the choroid to the draining lymph nodes.

SS4-4

Immunohistochemical re-evaluation of interrelation between microlympho- and microhemovasculature in normal and cirrhotic human liver: Relevance to ascites formation

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Background and Aims: Recently, differentiation of the microlymphatic system from the microhemovascular system can be done clearly by comparing immunohistochemical (IHC) data of D2-40 and CD34 expressing on the microvasculature in diffuse abnormalities of liver architecture in liver cirrhosis (LC). This study used vascular endothelial growth factor receptor (VEGFR)3 specifically expressed in the endothelium (EC) of the lymphatic systems as well as caveolin (CAV)-1, known as a key component of membrane caveolae, to analyze alterations of microlympho- and microhemovasculature in progressive LC with ascites.
Materials and Methods: LC specimens were obtained as surgical wedge biopsy specimens. Analyses were conducted for VEGFR3, CAV-1, and D2-40 staining for lymphatic capillary marker by IHC and IEM.
Results: The sizes and numbers of microlymphatics for D2-40, CAV-1, and VEGFR-3 are related to the advanced state of LC on regenerative nodules to widely dilated lymphatic vessels. In the advanced state of LC, protein levels of VEGFR3, CAV-1, and D2-40 were higher than those of control and the early state of LC. In fibrotic septa, microlymphatic ECs of newly forming vessels were positive for both CAV-1 and VEGFR-3 staining. The CAV-1 localizations are mainly on vesicles and caveolae in arterial capillary and portal venular ECs, and in lymphatic ECs.
Conclusions: Functional interaction between VEGFR3 and caveolin-1 to modulate EC activation is expected to modulate microlymphatic EC activation during lymphangiogenesis in LC. Overexpression of lymph passing through the proliferative microlymphatics connecting with the liver subcapsular space is necessary for ascites formation in LC.

SS5-1
OCT angiography of the retinal circulation
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Background: Fluorescein and ICG angiography have limited ability to identify the location and extent of vascular lesions due to the lack of direct depth information, and blurring of boundaries by leakage of dye molecules. A no-injection method of detecting blood flow with OCT could overcome these limitations.
Methods: High-speed custom swept-source (100 kHz) and commercial spectral (70 kHz) OCT systems were used. A split-spectrum amplitude decorrelation angiography (SSADA) algorithm was developed to improve the signal-to-noise ratio of flow detection without increasing scanning time. High quality angiograms could be obtained in 3-6 mm square areas. Clinical studies were performed in retinal diseases and glaucoma.
Results: In AMD, choroidal neovascularization could be identified by its presence in the outer retinal layer that is normally avascular, and choriocapillaris defects could be mapped. In diabetic retinopathy, retinal capillary nonperfusion and neovascularization could be identified and the areas mapped. In glaucoma, reduced flow index and vessel density could be detected in the optic disc and surrounding retina with high reproducibility and provided excellent diagnostic accuracy in pilot studies.
Conclusions: OCT angiography could replace dye-based angiography due to its less invasive nature and better delineation and quantification of vascular abnormalities. However, scan area is still limited and processing software is still in the early stages of development.
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David Huang - Carl Zeiss Meditec, Inc. (P), Optovue, Inc. (F, I, P):

SS5-2
Measurement of retinal oxygen extraction in humans
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Adequate function of the retina is dependent on proper oxygen supply. In humans, the inner retina is oxygenated via the retinal circulation. We present a method to calculate total retinal oxygen extraction based on measurement of total retinal blood flow using dual-beam bidirectional Doppler optical coherence tomography and measurement of oxygen saturation by spectrophotometry. These measurements were done on 8 healthy subjects while breathing ambient room air and 100% oxygen. Total retinal blood flow was 44.3 $\pm$ 9.0 microll/min during baseline and decreased to 18.7 $\pm$ 4.2 microl/min during 100% oxygen breathing ($p < 0.001$) resulting in a pronounced decrease in retinal oxygen extraction from 2.33 $\pm$ 0.51 microl(O2)/min to 0.88 + /+ 0.14 microl(O2)/min during breathing of 100% oxygen. The method presented in this paper may have significant potential to study oxygen metabolism in hypoxic retinal diseases such as diabetic retinopathy.

SS5-3
Novel evaluation of diabetic eyes by using En Face OCT angiography
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Background: Diabetic retinopathy is a leading cause of blindness in the world, and retinal microangiopathy is the pathological change in diabetic retinopathy. To elucidate the cause of retinal microangiopathy, many researchers have investigated retinal microcirculation by using fluorescein angiography, laser Doppler velocimetry, laser speckle flowgraphy, etc.

Recently, En Face OCT angiography has been developed, and it enables us to evaluate chorioretinal microcirculation without dye injection.
Methods: En Face OCT angiography (AngioVue, Avanti OCT, Optovue) was performed in healthy and diabetic eyes, and the size of foveal avascular zone was evaluated using by Image J software. The eyes with proliferative diabetic retinopathy and history of laser photocoagulation were excluded.
Results: There was a significant enlargement of foveal avascular zone in diabetic patients ($p < 0.01$). Even in eyes without diabetic retinopathy showed significant enlargement of foveal avascular zone compared to healthy eyes ($p < 0.01$).
Conclusions: Our data suggest that the diabetic eyes show the impairment of retinal microcirculation in the macula even before the retinopathy develops. En Face OCT angiography is useful in non-invasive screening for the detection of the early microcirculatory disturbance in diabetic patients.

SS5-4

Mechanisms of the neurovascular coupling in the retina: Role of neuronal nitric oxide synthase and glial cells in regulating retinal blood flow during flicker-induced hyperemia in cats

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Background: To investigate the mechanism of the regulation of retinal circulation in response to flicker stimulation which is related to "neurovascular coupling" in the retina. In the present study, we focused on the roles of nitric oxide (NO) and glial cells in this regulatory mechanism.

Methods: Using laser Doppler velocimetry, we measured the retinal blood flow (RBF) in first-order retinal arterioles in anesthetized cats. After intravitreal injection of N-omega-propyl-L-Arginine (L-NPA) as a selective nNOS inhibitor or L-2-aminoadipic acid (LAA) as gliotoxic compound, we examined the changes in RBF in response to flicker stimulation of 16 Hz for 3 minutes.

Results: In the PBS group, the RBF increased gradually and reached a maximal level after 2 to 3 minutes of flicker stimuli. In the L-NPA groups, the increases in RBF during flicker stimulation were attenuated significantly by one-third of the baseline. In the LAA-treated eyes, the increases in RBF during the flicker stimulation were significantly attenuated by one-third of those in the control eyes. After injection of L-NPA in LAA-treated eyes, flicker-evoked increases in RBF were almost abolished.

Conclusions: The current results suggest that the increases in RBF in response to flicker stimulation were regulated by NO from nNOS in both neurons and glial cells in the retina and another vasodilatory factors in glial cells. We believe that neurovascular coupling in the retina may be a novel target for the management of retinal vascular disorders, especially diabetic retinopathy.

SS6-1

Regulation of hydroxysafflor yellow A (HSYA) in angiogenesis

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Flowers of Carthamus tinctorius L. are traditionally used in China to treat cerebrovascular and cardiovascular diseases. Hydroxysafflor yellow A (HSYA), the main constituent of Carthamus tinctorius L. flowers, is known for its multiple biological activities. The present study determines the effects of HSYA on angiogenesis *in vitro* and in an ischemic limb animal model. We found that HSYA significantly increased the migration and capillary-like tube formation of human umbilical vascular endothelia cells (HUVECs) in a dose-dependent manner. Using western blotting, we demonstrated that HYSA significantly enhanced expression of angiopoietin-1 (Ang-1) and Tie-2, which have emerged as an important aspect of angiogenesis. In mice limb ischemia model, the results revealed that the recovery of perfusion of ischemic hindlimb tissue after femoral artery interruption was significantly increased in HYSA-treated mice compared with vehicle controls. Consistent with these results, capillary density in ischemic gastrocnemius muscles was significantly increased in HSYA-treated mice. We also found that HSYA significantly increased expression of Ang-1 and Tie-2 in ischemic gastrocnemius muscle. Our results suggested that HSYA promote angiogenesis and that up-regulation of Ang-1/Tie-2 signaling pathway may be involved in the increase of angiogenesis by HSYA. Taken together, these findings illustrate that HSYA promotes ischemia-mediated flow perfusion and capillary formation, and the proangiogenic activity may be associated with the activation of Ang-1/Tie-2 signaling.

SS6-2

A metabolite of danshen formulae,IDHP, attenuates beta-adrenergic receptor mediated cardiac fibrosis depending on NOX2/ROS/p38 pathway

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Background and Purpose: Cardiac fibrosis is a common feature of advanced coronary heart disease and is also a hallmark of heart diseases. However, currently available drugs against cardiac fibrosis are still very limited. In the present study, we have assessed the role of Isopropyl 3-(3,4-dihydroxyphenyl)-2-hydroxylpro panoate (IDHP), a new metabolite of Danshen Dripping Pills, in cardiac fibrosis mediated by beta-adrenergic receptor and it's molecular mechanism.

Experimental Approach: Identification of IDHP was determined by mass spectrometry and 1H and 13C NMR spectra. Myocardial collagen was quantitatively assessed with picrosirius red staining. The mRNA expression of collagen was evaluated with real time PCR. The levels of phosphorylated and total p38 mitogen-activated protein kinase (MAPK), NADPH oxidase(NOX) and superoxide dismutase(SOD) were determined by Western blot analysis. Reactive oxygen species (ROS) generation was evaluated by Dihydroethidium(DHE) fluorescent staining. NOX2 was knocked down using specific siRNA.

Key Results: IDHP attenuated beta-AR-mediated cardiac fibrosis *in vivo* and inhibited ISO-induced neonatal rat cardiac fibroblasts (NRCFs) proliferation and collagen I synthesis *in vitro*. Phosphorylation of p38 MAPK, which is an important mediator in the pathogenesis of ISO-induced cardiac fibrosis, was negatively regulated by IDHP. Role of IDHP in negative regulation of phospho-p38 was dependent on the reduction of ROS generation. Moreover, NOX2 was found to be responsible for this process that IDHP negatively regulated beta-AR mediated ROS/p38 Pathway.
Conclusions and Implications: IDHP attenuates cardiac fibrosis induced by isoproterenol through NOX2/ROS/p38 pathway. These novel findings suggest that IDHP is a potential pharmacological candidate for the treatment of cardiac fibrosis.

SS6-3
Regulation of cerebral blood flow by hemodynamic forces: Maintenance of healthy flow

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Because the cerebral vascular network is enclosed in the rigid cranium any increase in pressure and/or flow-volume can increase intracranial pressure endangering the maintenance of appropriate blood flow to brain tissues (CBF). To prevent this, a very effective autoregulation is present, which is an important feature of the cerebral circulation. Previous studies have investigated this issue and logically assumed that autoregulation is somehow coupled to changes in hemodynamic forces.

Pressure sensitive vasomotor response: For many years, autoregulation of CBF has been primarily explained by the pressure-induced myogenic response of cerebral vessels: the inherent property of vascular smooth muscle to dilate to a decrease and to constrict to an increase in intraluminal pressure. There are two critical mechanisms (Ca^{2+}-dependent and –independent, RhoA/Rho kinase pathway, sensitizing actin-myosin proteins to Ca^{2+}) contributing to the myogenic constriction:

Flow sensitive vasomotor response: Recently, it was shown in certain cerebral vessels, such as the middle cerebral artery a mechanism exists, which is sensitive for changes in blood flow. In contrast to peripheral arterial vessels, in the presence of constant pressure increases in flow elicit constrictions in this type of vessels. The constrictions are mediated by 20-HETE (20-hydroxieicosatetraenoic acid, a constrictor metabolite of arachidonic acid synthesized by cytochrome P450 hydroxylases) and reactive oxygen species (ROS).

On the basis of above, it is plausible that in the cerebral vascular network, during increases in systemic blood pressure when both pressure and flow changes (for example during exercise) the flow-constriction is superimposed on the pressure-constriction. These adaptations of vasomotor tone are important during physical activities and exercise, but also protective in hypertension. By exercising, healthy level of blood flow and prevention of stasis – both in arterial and venous vessels – we can be ensured.

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SS6-4
Targeting AMPK: A new strategy for enhancing HDL function

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AMP-activated protein kinase (AMPK) which is a highly conserved serine/threonine kinase has been found to regulate many aspects of lipid and energy metabolism. However, it is not definite that whether AMPK activators hinder the progression of atherosclerosis by improving HDL function. In the present study, we investigated the effects of AMPK activaton on HDL function and its mechanisms of action in apoE-/- mice. Our results revealed that AMPK activaton induced a signifiant increase in paraoxonase 1 (PON1) activity, the expression of ABCA1 and SR-BI in hepatocytes and enhanced RCT to the plasma, liver, and feces. Furthermore AMPK activation markedly reduced serum myeloperoxidase (MPO) activity, aortic intima-media thickness (IMT) and the percentage of plaque area in the aorta. Our findings reveal that AMPK activation alleviated atherosclerosis by improving HDL function. The mechanisms HDL-include accelerating the process of reverse cholesterol transport, improving the anti-inflammatory and anti-oxidant functions.

SS6-5
Innovative therapeutics of activating Qi and nourish blood in treating cancer diseases via regulating immune system

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Traditional Chinese herbal medicine (TCM) has been accumulating huge amount of experiences in treating different kinds of human diseases including cancer and improving health situation in clinical practice. Development of innovative therapeutics with traditional herbal medicine is

very necessary and meaningful. Through both clinical trial and experimental research, the present studies investigated the therapeutical effects of the traditional Chinese medical herbs on human lung cancer. It discovered that several specific Chinese medical herbs have therapeutic effects on human advanced lung cancer and the Lewis lung cancer in animal models. The results showed that the medical herbs could inhibit the growth of lung tumor, decrease the disease symptoms and improve the living quality and health situation of the host with lung cancer. It demonstrated that the potential cellular immunological mechanisms are associated with improving the host immune system function by these medical herbs. Using the system of culturing K562 cells, a p53 deficient erythroleukemia cell line, the studies determined that the specific medical herb, Hex (Astragalus membranaceus), could stimulate human adult globin gene expression, induce terminal differentiation and apoptosis of erythroleukemia cell lines. Combination of Chinese medical herbs and the extraction with western medical drugs and specific monoclonal antibodies has indicated significant synergic therapeutical effects in treating some malignant cancer diseases such as melanoma. It may provide a rationale and potential for developing new strategies for targeting therapeutic intervention in cancer diseases.

SS6-6
Chemoprevention of lung cancer by using chinese herbs: An update review

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Lung cancer, the most prevalent cancer, leads the highest annual mortality worldwide. Although significant advances have been developed in therapeutic approaches, poor prognosis and survival are inevitable due to drug resistance and side effects. Therefore, chemoprevention, which means prevent cancer progression by using a nature-derived agent with low-toxicity and more effectiveness, gained more attention nowadays. Traditional Chinese Medicines (TCMs), the herbs with multiple functions and low-toxicity, play important roles in lung cancer treatment and prevention. Such as honokiol, the major bioactive compound in *Magnolia officinalis*, was demonstrated to inhibit the development of squamous-cell carcinoma by inducing tumor cell apoptosis. Additionally, artemisinin and astragaloside IV, purified from *Artemisia apiacea* and *Radix astragali* individually, could inhibit growth, migration and invasion of lung adenocarcinoma cells via mitochondria pathway. Other than that, the multiple signaling pathways inactivated or stimulated by these herb-derived compounds could ultimately influence the inter cell homeostasis, which could contribute to the tumor inhibition. This review is to highlight the current state and future prospect of traditional chinese medicines in chemoprevention, which may provide useful information for the regimen and pretreatment of lung cancer.

SS7-1
Roles of increased circulating microparticles in diabetes-associated microvascular dysfunction

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Diabetes is commonly associated with microvascular dysfunction. We found that microvessels of diabetic rats had increased basal permeability, increased leukocyte adhesion, and enhanced permeability responses to inflammatory mediators. Our present study is to investigate the mechanisms involved in diabetes-associated microvascular inflammation and multi-organ vascular complications. We recently found that diabetic rats and diabetic patients had markedly increased circulating microparticles (MPs) comparing to normal control. MPs are small membrane-derived vesicles released upon cell activation or apoptosis. We hypothesized that MPs are not simply by-product, but serve as vectors to actively disseminate inflammation to the whole vasculature. We first characterized the increased MPs in diabetic plasma and compared their differences in number, cell origins, and membrane surface antigens with normal rat MPs using flow cytometry analysis. We then investigated the roles of diabetic MPs in disseminating inflammation in the vascular system using individually perfused intact microvessels. Results showed that most of the increased MPs in diabetic plasma (over 100-fold higher than normal control) originated from platelets, leukocytes, and endothelial cells and existed as aggregates. These small vesicles with larger surface area of phosphatidylserine exposure are highly adhesive with procoagulant properties. Perfusion of diabetic plasma or isolated MPs into a normal rat microvessel causes MP adhesion to endothelium followed by adherent MP-mediated leukocyte adhesion and thrombus formation. These results suggest that increased levels of circulating MPs in diabetic rats are more than simply biomarkers of the disease but mediate leukocyte adhesion and actively disseminate local inflammation to remote regions. Supported by HL56237 & HL084338.

SS7-2
Aquaporins in brain disorders

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Discovery of aquaporin water channel proteins has provided insight into the molecular mechanism of membrane water

permeability. In mammalian brain, Aquaporin-4 (AQP4) is the main water channel and is distributed with highest density in the perivascular and subpial astrocyte end-feet. AQP4 is a critical component of an integrated water and potassium homeostasis. Indeed, AQP4 has been implicated in several neurologic conditions, such as brain edema, seizure and even mood disorders. Expression and regulation of AQP4 have been studied to understand the roles of AQP4 in physiological and pathological conditions. Here we discuss about the mechanisms how AQP4 is dynamically regulated at different levels; channel gating, subcellular distribution, phosphorylation, protein-protein interactions and orthogonal array formation. Interestingly, AQP4 has been identified as a target antigen of autoimmune attack in neuromyelitis optica (NMO). AQP4 may be a potential therapeutic target in several neurologic conditions. Further studies from different aspects are required to develop new drugs against AQP4.

SS7-3

Peroxynitrite could be a molecular target for drug discovery to prevent thrombolysis-induced hemorrhagic transformation in post-stroke treatment

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Stroke is the secondary cause of death and leading cause of disability of human diseases worldwide. Tissue-plasminogen activator (t-PA) remains the only FDA-approved therapy for acute ischemic stroke, with a restrictive treatment time window of 4.5 hours. Developing novel adjuvant therapeutic strategy to extend t-PA's therapeutic time window and reduce the risk of HT is critical for improving outcome of stroke treatment. In this study, we aim to explore whether peroxynitrite, a representative reactive nitrogen species, could be a critical molecular target for drug discovery to prevent such complication.

Methods: We first used peroxynitrite decomposition catalyst (PDC) to test whether peroxynitrite is a player for t-PA-mediated HT and then used peroxynitrite as a molecular target for screening the active compounds from herbal medicine for drug discovery. Male Sprague-Dawley (SD) rats were subjected to middle cerebral artery occlusion (MCAO) with t-PA (10 mg/kg) or t-PA plus FeTMPyP (3 mg/kg, a representative PDC) treatment at MCAO 2 or 5 hours and reperfusion for 22 or 19 hours respectively. Hemorrhagic transformation (HT) was assessed with hemoglobin assay. Neurological deficit were evaluated with Modified Neurological Severity Score (mNSS). Peroxynitrite formation was examined by detecting 3-nitrotyrosine (3-NT) formation. The expression and activity of MMP-9/-2 were assessed by western blotting and gelatin zymorgraphy.

Results: t-PA treatment at 2 hours of MCAO cerebral ischemia did not induce hemorrhagic transformation but attenuated neurological deficit whereas treatment at 5 hours of MCAO cerebral ischemia significantly induced HT and worsened the neurological outcome. Such complications were prevented by FeTMPyP co-treatment. Early t-PA treatment inhibited 3-NT and MMP-9/-2 expression whereas delayed treatment induced 3-NT and MMP-9/-2 expression and activity. FeTMPyP co-treatment down-regulated 3-NT expression and inhibited MMP-9/-2 expression and activity in both time points. The results indicate that peroxynitrite could be a critical molecular target for preventing hemorrhagic transformation and improving neurological outcome ischemic rat brains with delayed t-PA treatment via inhibiting peroxynitrite mediated MMPs activation. We then screened herbal source compounds to scavenging peroxynitrite and prevented hemorrhagic transformation and improving neurological outcome. We found that Baicalin, a flavonoid compound isolated from Scutellaria baicalensis, could be a good candidate to scavenge peroxynitrite and reduce hemorrhagic transformation.

Conclusion: Peroxynitrite could be a molecular target for drug discovery from herbal medicine to prevent thrombolysis-induced hemorrhagic transformation in post-stroke treatment.

Keywords: Peroxynitrite, stroke, hemorrhagic transformation

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SS7-4

Salvianolic acid B ameliorates albumin leakage from mesenteric venules induced by lipopolysaccharide in rats

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Lipopolysaccharide (LPS) causes microvascular barrier disruption, leading to albumin leakage from microvessels resulting in a range of disastrous sequels. Salvianolic acid B

(SalB) is a major water-soluble component derived from Salvia miltiorrhiza. Previous studies showed its potential to attenuate microvascular barrier dysfunction, but the underlying mechanism is not fully understood. The present study was intended to investigate the impact of SalB on endothelial cell barrier *in vivo* in rat mesenteric venules as well as *in vitro* in human umbilical vein endothelial cells (HUVECs). Male Wistar rats were challenged by infusion of LPS (2 mg/kg/h) for 90 min. SalB (5 mg/kg/h) was administrated either simultaneously with LPS or 30 min after LPS infusion. Vesicles in venular walls were observed by electron microscopy. The expression of Zonula occluden-1 (ZO-1), VE-cadherin, caveolin-1 and Src in HUVECs was assessed by Western blot and confocal microscopy, binding of SalB to Src was measured using Surface Plasmon Resonance and BioLayer Interferometry. Treatment with SalB inhibited albumin leakage from rat mesenteric venules and inhibited the increase of vesicle number in venular endothelial cells induced by LPS. In addition, SalB inhibited the degradation of ZO-1, the phosphorylation and redistribution of VE-cadherin, the expression and phosphorylation of caveolin-1, and phosphoirylation of Src in HUVECs exposed to LPS. Furthermore, SalB was found able to bind to Src. This study demonstrates that protection of SalB against microvascular barrier disruption is a process involving both para- and trans-endothelial cell pathway, and highly suggests Src as the key enzyme for SalB to work.

SS7-5
Three dimensional modeling of the endothelial vesicular system with electron tomography

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The membraneous vesicular system of endothelial cells has been implicated in solute and fluid transport across the walls of continuous capillaries. The role of this system in transcapillary transport has been controversial due in part by the difficulty of visualizing its three dimensional configuration in thin sections for TEM. We have perfused mouse abdominal muscle capillaries with terbium, a small, intravital, electron-dense tracer. Semi-thick sections were cut and dual axis tomographic tilt series were acquired from plus 60 to minus 60 degrees at one degree increments with a transmission electron microscope. These tomograms were reconstructed and the TIFF stacks were converted into a 3D data set. Tilt series revealed channels of connected vesicles communicating between the luminal and abluminal compartments and discreet (free) vesicles within the cytoplasm. The 3D data sets were thresholded using the intense electron density of the terbium precipitates to surface render vesicular compartments. The models were rotated through any angle to view the most revealing perspective of the vesicular structures. These techniques permit observation of the endothelial vesicular system throughout a depth of 25-30 nm and overcome ambiguities associated with ultrathin sections. The presence of transendothelial channels of fused vesicles and free vesicles within the cytoplasm is consistent with their hypothesized role as large pores in the transport of solutes across the walls of continuous capillaries.

SS8-1
Effects and mechanism of QiShenYiQi pills attenuating rat cardiac injury induced by ischemia/reperfusion

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Ischemia/reperfusion (I/R) caused myocardium injury is accompanying with energy metabolism disorder. According to the Chinese medicine Qi-blood theory, Qi is the driving force of blood, i.e., myocardium circulation can be improved by modulation of myocardium energy. QiShenYiQi Pills (QSYQ), a compound Chinese medicine with potential of tonifying Qi activating blood, is widely used for treatment of Qi deficiency blood stasis-related diseases in clinic. The present study using a myocardium I/R model explored the effect and mechanism of QSYQ with respect to tonifying Qi activating blood. Male Sprague-Dawley rats were subjected to 30 min occlusion of the left anterior descending coronary artery followed by 90 min or 24 h reperfusion, with or without QSYQ or its major ingredient Astragaloside IV or ginsenoside R1 treatment. Myocardial infarction, histology and ultrastructure were assessed. ATP, ADP and AMP content was determined. F-actin in myocardial cells was evaluated, expression of ATP5D was determined. QSYQ, Astragaloside IV and ginsenoside R1 protected against I/R-induced MBF decrease, myocardial infarction. I/R-induced impairment on cardiac function and structure, decrease in the ratio of ADP/ATP and AMP/ATP and reduction of ATP 5D expression were significantly ameliorated by QSYQ. Furthermore, Astragaloside IV and ginsenoside R1 up-regulated the expression of ATP5D and improved cardiac energy and structure after I/R. The results suggest that QSYQ exerts the effect of tonifying Qi activating blood by upregulation of ATP5D thus improving myocardium structure and function. In this process, Astragaloside IV and ginsenoside R1 contribute to the role of tonifying Qi activating blood of QSYQ.

SS8-2
Qi, blood and biomechanopharmacology

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The concept of Xue in Chinese medicine is almost the same as that in the modern medicine. Qi is considered as the vital energy. The relationship between Qi and blood is of great interesting: Qi governs the blood flow as the general, while blood is supplying nourishment to Qi as the mother. In biomedical interpretation, blood circulation is driven by the energy originated from the heart. Blood pressure <BP> and blood shear stress <SS> are manifestation of the energy. When biomechanical factors <BP, SS> are counted, flowing blood is more than a transporter and deliverer of oxygen, nutrition and metabolic products. SS provided by flowing blood on endothelial interface would be considered as a multi-targeted drug since it influences several important endothelial functions, including thrombosis and thrombolysis. Evidences also show that nourishing Qi to activate blood circulation gain beneficial effects for treatment of cardiovascular and cerebral diseases. We proposed the concept of biomechanopharmacology in 2002. The age-old statement of "Diseases are prevented as blood flowing is promoted "was revealed by the joint elevation of Qi <by exercise> and blood <by herbal medicine> for atherosclerosis prevention. Recently, the principle of Biomechanopharmacology has been extended with the concept of "micro/nanoscaled topography-coupled-mechanics action "for functional biointerfaces. Specially, substrate stiffness plays an important role in the pathophysiological process of tumorigenesis. We believe that biomechanopharmacologically tailored regulation of Qi and blood will play active roles in preventive medicine.

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SS8-3
CNS lymphatic drainage blockade exacerbates cerebral vasospasm and cerebral injury following subarachnoid hemorrhage and partially reversed by Ginkgo biloba extract

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CNS lymphatic drainage pathway to extracranial lymph compartments may play an important role in the removal of substances in the brain and cerebrospinal fluid (CSF).The present experiment was carried out to investigate the possible role of cerebral lymphatic drainage pathway in the development of cerebral vasospasm and related cerebral injury and the influence of Ginkgo biloba extract. Wistar rats were used in the experiment and animals were divided into different groups. SAH models were replicated by double cisternal injection of autologus arterial hemolysate. In some animals the main cerebral lymphatic drainage way out being blocked (cerebral lymphatic blockade, CLB). Two different constituents, Ginkgolides and Ginkgo flavone, were given as interventions. It was found that SAH reduced the drainage of Evans blue-labeled albumin (EBA) from the brain to extracranial lymphatics. A kinetic analysis of 125I-HSA, showed that the clearance rate of macromolecules in the CSF was significantly reduced after SAH. Furthermore, SAH reduced the diameters of basilar artery (BA) and increased thickness of BA. Prominent cerebral injury was found after induction of SAH. The spasm of BA and cerebral injury were partially antagonized by Ginkgolides and Ginkgo flavone. It was concluded that cerebral lymphatic drainage pathway exerts intrinsic protective effects against cerebral vasospasm and cerebral injury by removal of macromolecular substances in the brain and subarachnoid spaces. Ginkgolides and Ginkgo flavone may alleviate the exacerbated cerebral vasospasm and cerebral injury following SAH by CLB.

SS8-4
Target identification of curcumin on ischemic blood flow and anticancer activities by network analysis and biological approaches

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We investigated the angiogenic effects of curcumin on an ischemia and lung cancer model. Unilateral femoral arteries of C57BL/6 mice were disconnected on one side of the mouse and LLC cells were xenografted on the opposite side. Angiogenic effects and underlying mechanisms associated with curcumin were investigated. Molecular targets, signaling cascades and binding affinities were detected by Western blot, 2-DE, computer simulations and SPR techniques. Curcumin promoted post-ischemic blood recirculation and suppressed lung cancer progression in inbred C57BL/6 mice via regulation of the HIF1alpha/mTOR/VEGF/VEGFR cascade oppositely. Inflammatory stimulation induced by neutrophil elastase (NE) promoted angiogenesis in lung cancer tissues, but these changes were reversed by curcumin through directly reducing NE secretion and stimulating alpha1-AT and IRS-1 production. Curcumin had opposite effects on blood vessel regeneration under physiological and pathological angiogenesis, which was effected through negative or

positive regulation of the HIF1alpha/mTOR/VEGF/VEGFR cascade. Curcumin had the promise as a new treatment modality for both ischemic conditions and lung cancer simultaneously in the clinic.

SS9-1
Moesin phosphorylation in T558 is involved in angiogenesis induced by advanced glycation end products

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Object: The study aimed to investigate the role of threonine phosphorylation of moesin in the development of diabetic angiogenesis induced by advanced glycation end products (AGEs).

Methods: CCK-8 assay was used to detect cell viability. Scratch wound healing assay and transwell migration assay were used to test cell migration ability. Tube formation assay by using Matrigel was to test the ability of tube formation in HUVEC. By using siRNA, mutant constructs, and confocal microscope, this study observed the effects of moesin on the proliferation, migration, and tubulogenesis of human umbilical vein endothelial cells (HUVECs). AGE-BSA was prepared by incubating bovine serum albumin (BSA) in PBS with D-glucose for 8 weeks in a sterile environment.

Results: The results demonstrated that (i) Down-regulation of moesin expression attenuated HUVEC proliferation, migration, and tubulogenesis and cellular polarization; (ii) AGE-BSA promoted HUVEC proliferation, migration, and tube formation in dose- and time-dependent manners; (iii) The down-regulation of moesin expression inhibited AGE-induced HUVEC proliferation, migration, and tube formation, and cellular polarization; (iv) The inhibiting mutant at moesin Thr 558 phosphorylation also suppressed AGE-induced HUVEC proliferation, migration, and tube formation; (v) The suppressions of either RhoA activity or ROCK activation reduced AGE-induced moesin phosphorylation and HUVEC angiogenesis.

Conclusion: The phosphorylation of moesin Thr 558 mediates endothelial activation, proliferation, migration, and tubulogenesis. AGEs promoted the diabetic pathological angiogenesis by inducing moesin phosphorylation through RhoA-ROCK pathway.

SS9-2
Shear stress and microvessel permeability

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Acute changes of shear stress (SS) have been implicated to play important roles in the regulation of vascular function. However, most of the SS studies were conducted *in vitro* in the absence of blood components, and the underlying mechanisms remain controversial. This study investigates the effects of acute changes in SS on EC [Ca2 +]i, eNOS activation, nitric oxide (NO) production, and microvessel permeability when intact venules were perfused with blood, RBC perfusate, or cell-free fluid. We quantified SS by measuring flow velocity and fluid viscosity using a high-speed camera and a cone-plate viscometer. EC [Ca2 +]i and NO were measured in Fura-2 AM and DAF-2 DA loaded individually perfused rat venules. EC eNOS activation was evaluated by immunofluorescence staining with confocal imaging. ATP released from RBCs was measured by bioluminescence assay, while EC gap formation was illustrated by fluorescent microsphere accumulation. We found that changes of SS increased EC [Ca2 +]i and gap formation only in blood- or RBC-perfused vessels, whereas SS-dependent NO production and eNOS-Ser1177 phosphorylation occurred in both plasma and RBC perfused vessels. RBCs pretreated with a pannexin-1 inhibitor, or from pannexin-1 knockout mice abolished SS-dependent ATP release and SS-induced increases in EC [Ca2 +]i and gap formation. Our results indicate that when blood flow changes, ECs respond not only to fluid generated wall SS, but also to shear-induced, RBC released ATP. SS-induced ATP release from RBCs causes an increase in EC [Ca2 +]i and gap formation, and that SS-induced NO production can be independent of increased EC [Ca2 +] in intact venules.

SS9-3
Catolpol attenuates hemorrhage from rat mesenteric microvessels exposed to lipopolysaccharide

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Lipopolysaccharide (LPS)-induced microvascular hemorrhage plays a key role in disseminated intravascular

coagulation (DIC), and alleviating hemorrhage is considered to be effective to prevent DIC. Catolpol is a main component of radix rehmannia, a Chinese medicine widely used for the treatment of infectious hemorrhage diseases in clinic. However, it is still unknown whether catolpol is effective to attenuate the LPS-induced microvascular hemorrhage and the underlying mechanism. The present study was aimed to investigate the impact of catolpol on LPS-induced hemorrhage in rat mesenteric microvessels. Male Wistar rats were challenged by infusion of LPS (10 mg/kg/h) through left femoral vein for 120 min. Catolpol (10 mg/kg/h) was continually infused starting from 30 min after LPS infusion through the left jugular vein. The hemorrhage of mesenteric capillaries and venules was observed by a microcirculation inverted microscope. Endothelial cell junctions and basal membrane of venular walls were observed by transmission electron microscopy. The expression of tight junction claudin-5 was assessed by Western blot and confocal microscopy. The expression of MMP2 was analyzed by Western blot. Treatment with catolpol attenuated the LPS-induced hemorrhage and its survival rate, alleviated the breakdown of endothelial junctions and the damage of vessel basal membrane, as well as down-regulated the expression of claudin-5 and MMP2. This study demonstrates that catolpol protects against the LPS-induced microvascular wall damage including both the endothelial junctions and basal membrane.

SS9-4
New strategies to reduce microvascular hyperpermeability, edema, and hypotension in the intoxicated or injured host

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Elevated microvascular permeability is a known problem with traumatic injury. Trauma and acute alcohol intoxication (AAI) often are associated, yet little is known about how AAI affects the microcirculation. Recently we demonstrated using rat model that alcohol intoxication increases microvascular leakage in the gut, and in our current studies we have observed that AAI worsens microvascular leakage elicited by experimental hemorrhagic shock and resuscitation. This effect is accompanied by hypotension during the resuscitation phase in alcohol-intoxicated rats. We also have detected cardiac electrical abnormalities associated with AAI. Taken together, our results demonstrate that alcohol intoxication complicates cardiovascular responses to injury. Our ongoing studies are testing the extent to which the bioactive lipid sphingosine-1-phosphate or its analogs can ameliorate these effects. Supported by NIH grants R01HL098215 and R21AA020049.

Ethics are the responsibility of the authors and their administering institutions.