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ABSTRACT

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Presidential Lecture

Cerebral microcirculation from physiology to pathophysiology

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Apparently core function of the brain is based on neural network, connecting neuronal activities through synapses. Physiologically, however, these neuronal activities require various types of glia and vasculature, that is, neurovascular unit. Although glucose is known as a sole energy source of the brain in physiological condition, neurons take up lactate released from astrocytes more than glucose transported from microcirculation. It is now well known that astrocytic endfeet surround the capillaries and function as an efficient pathway of the energy substrates and various mediators. Interestingly, "true capillaries", which means most terminal capillaries, are not equipped with any vasomotor apparatus such as smooth muscle cells. Enhanced neuronal activity may be transmitted to astrocytes and astrocytic endfeet with pericytes may function to transmit the signal along the capillary to smooth muscles in the proximal arteriole (proximal integration model), the system of which is known as neurovascular coupling.

Ischemic stroke is most often caused by obstruction of major cerebral arteries. Recanalization with thrombolysis and/or mechanical thrombectomy is the most efficient treatment. Microcirculation, however, may play a pivotal role in saving ischemic penumbra where suppression of secondary thrombosis and maintained collateral flow may reduce final size of infarction. Chronic ischemia at the border zone of major arterial territories may induce remodeling of microcirculation, developing meningo-vascular anastomosis. Microcirculatory disturbance is also important in "No reflow phenomenon". Diffuse intravascular coagulopathy and Trousseau syndrome may also affect microcirculation, causing shower emboli.

Recent studies also suggest that stroke is not the only disease involving microcirculatory disturbance. Headache, dementia, and immune-mediated encephalomyelitis may also develop with impaired microcirculation. Aura in the migraine is caused by cortical spreading depression and throbbing pain following the aura is induced by trigemino-vascular inflammation of the dura matter. Alzheimer's disease may initially develop with oligomer formation of amyloid β which may be disposed through perivascular space.

In this presidential lecture, recent advance and future direction of such studies will be presented.

Special Lecture

Development and application of imaging tracers for neuropsychiatric disorders

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In-vivo imaging technologies with small-molecule tracers have allowed the pursuit of key processes involved in the etiology of neuropsychiatric diseases in humans and animal models. Depositions of misfolded protein aggregates composed of amyloid-beta, tau, alpha-synuclein, and TDP-43 are core pathological events in neurodegenerative dementias. We have developed bimodal PET (positron emission tomography) and optical imaging agents for diverse tau pathologies, and high-contrast target detection with these probes has offered the diagnosis and differentiation of dementing illnesses on an individual basis, along with longitudinal PET and two-photon

microscopic assays of progressive tau depositions in model mice. More recently, bimodal tracers for alpha-synuclein fibrils have been generated and applied to animals recapitulating the propagation of alpha-synuclein aggregations and then humans. The pathological protein accumulations in the brain are also known to be mechanistically and reciprocally linked to the activation of inflammatory microglia and astrocytes, which can be captured by probe-assisted imaging of molecular markers for either homeostatic or detrimental glial phenotype. Using such neuroimaging approaches, we have revealed the efficacy of a brain-entering amino acid composite for suppression of neuroinflammation and neuronal loss in a mouse model of neurodegenerative tau pathologies, and this potential therapeutic component is currently being evaluated in a clinical study with the aid of a PET tracer for reactive astrocytes. Moreover, tau depositions have been found in late-life psychiatric disorders exemplified by psychosis and bipolar illnesses. As inflammatory microglial and astrocytic responses have been commonly noted in young-onset and late-life mental diseases, deleterious gliosis and loss of homeostatic neuron–glia coordination could be pivotal pathological substrates of psychological and behavioral manifestations and would be clarified with emerging methods targeting additional glial biomarkers.

Symposium 1: Ischemic Brain/Heart Disease

Recent advances in treating neonatal hypoxic–ischemic encephalopathy

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Hypoxic–ischemic encephalopathy (HIE) occurs in 0.5–1.0 per one thousand newborns and leads to cerebral palsy (CP). Although therapeutic hypothermia (TH) improved mortality of newborns suffering from HIE, there still exist areas for continued development of therapeutic strategy for prevention of CP. Establishment of treatment methods for the sequelae of brain damages will benefit many patients with CP and their families with high care burden. Accumulating evidence suggests that administration of umbilical cord blood-derived stem cells (UCBSCs) have therapeutic potentials for neuroregeneration and improved functional outcome. With the support of Neonatal Encephalopathy Consortium Japan, we successfully completed a multicenter open-label pilot study of autologous UCBSCs treatment for neonatal HIE as a concomitant therapy with TH (ClinicalTrials.gov ID: NCT02256618). Phase II clinical trial is ongoing. We also evaluated potential of umbilical cord-derived mesenchymal stromal cells (UC-MSCs) therapy on a mouse model of neonatal stroke. Intravenous administration of UC-MSCs was safe and improved motor function by modulating the microglial reaction in the peri-infarct cortex. We further elucidated the metabolic changes that occur following the neonatal stroke mouse model and successfully visualized the distribution of glucose, carnitine glutamate, and glutathione by using MALDI-MS imaging. While administration of hematopoietic stem cells improved cerebral blood flow, MSC therapy affected the TCA cycle, glycolysis/gluconeogenesis, and ketone body production by restoring carnitine level in the periinfarct area. Further accumulations of data are anticipated to develop effective treatment strategies.

Symposium 1: Ischemic Brain/Heart Disease

Microcirculation in neurosurgery for stroke

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Strict control of rapidly changing cerebral microcirculation is quite important in the perioperative period of neurosurgery for stroke, and its success or failure affects outcomes. For delayed cerebral vasospasm in subarachnoid hemorrhage, administration of Rho kinase inhibitor and thromboxane synthetase inhibitor is performed in addition to thorough subarachnoid hematoma scavenge during surgery. Recently, the usefulness of systemic administrated phosphoesterase inhibitors (cilostazol) and selective endothelin-A receptor inhibitors (clazosentan) has also been reported.

Cerebral hyperperfusion may occur after carotid endarterectomy or resection of cerebral arteriovenous malformation (AVM), which are chronic revascularization procedures. To avoid the symptomatic transformation, systemic management centered on blood pressure control is required.

On the other hand, after surgery of extracranial/intracranial artery anastomosis for moyamoya disease, not only cerebral hyperperfusion but also cerebral hypoperfusion due to watershed shift or thrombus formation can occur in rare cases. To prevent the additional ischemic neuronal damage, postoperative management with systemic administration of free radical scavenger and minocycline hydrochloride, which suppresses the expression and activity of matrix metalloproteinase (MMP)-9 for protection of blood-brain barrier play an important role.

Symposium 2: Microcirculatory Disturbance and Pathological Protein Accumulation in the Brain

Pathophysiology of Alzheimer's disease—Mechanisms of amyloid-β accumulation and propagation

Akitoshi Takeda; Itsuki Hasegawa; Keisuke Togawa; Marie Tanaka; Hiroto Namba; Yoko Hirayoshi; Shinobu Minatani; Toshikazu Mino; Yoshiaki Itoh

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Alzheimer's disease (AD) and other neurodegenerative diseases are characterized by intracellular and extracellular accumulation of proteins, such as amyloid-β (Aβ) and tau, and degeneration of neurons. Recent studies elucidate the accumulation and propagation of proteins involved in the progression of neurodegenerative diseases. Aβ proteins are cleared by specific transport across the blood–brain barrier (BBB). Several studies have revealed a lymphatic-like system (glymphatic pathway) that drains into the cervical lymphatics in the rodent brain, which might constitute an unappreciated, complementary aspect of brain clearance. In the glymphatic pathway, the convective influx of the cerebrospinal fluid (CSF) is balanced by perivenous efflux of the interstitial fluid, which removes the neuropil of toxic proteinaceous metabolites, including Aβ. Glymphatic function robustly increases during sleep, thereby eliminating Aβ from the extracellular space that accumulates during wakefulness. Influx and clearance rates of CSF tracers were reduced in the AD model animals, and, interestingly, glymphatic dysfunction was observed in aged transgenic animals with insoluble Aβ plaques as well as in young animals with no visible Aβ plaque formation. Prions are infectious protein assemblies that can be transmitted between individuals by serving as templates that convert normal protein molecules to their pathogenic conformation. In the last decade, compelling basic research has linked prion mechanisms to AD. Walker et al. found that the source of Aβ fibrils into which they were injected dictates unique patterns of neuropathology in mice. Subsequent studies have also reported similarities between Aβ propagation and prion strain-like protein behavior. Although mice with Aβ conformation have not been demonstrated to propagate indefinitely in vivo, distinct amyloid structures produce unique patterns of neuropathology upon inoculation. Elucidation of these mechanisms of Aβ clearance and propagation will play an important role in future therapeutic strategies for AD.

Symposium 2: Microcirculatory Disturbance and Pathological Protein Accumulation in the Brain

Relationship between α-synuclein accumulation and glymphatic system

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Recent advances in pathology and molecular biology have led to the understanding of most neurodegenerative diseases as proteinopathies in which specific proteins (amyloid-β (Aβ), α-synuclein, tau protein, TDP-43, etc.) accumulate abnormally at the affected site. Although much research has focused on the mechanisms of production and degradation of the abnormally accumulated proteins, the mechanisms of their efflux out of the brain have recently attracted attention. The mechanism of excretion of waste products from the brain has long been unknown. Recently, the circulation of cerebrospinal fluid, including the perivascular space, has been proposed as a route for excretion of waste products in the brain and named the glymphatic system. It has been shown in animal studies that impairment of the glymphatic system impairs the efflux of α-synuclein from the brain and increases their accumulation in the brain. For example, the decreased expression of aquaporin 4, which plays an important role in the glymphatic system, accelerated pathologic deposition of α-synuclein and facilitated the loss of dopamine neurons. It has also been shown that glymphatic influx of cerebrospinal fluid tracer was reduced in mice overexpressing mutated human α-synuclein, accompanied by perivascular aggregation of α-synuclein and impaired polarization of aquaporin 4 expression in substantia nigra. These findings suggest that dysfunction of the glymphatic system is involved in the pathogenesis of α-synucleinopathies. However, since there is no established method to visualize and evaluate the glymphatic system in humans, it has not been fully investigated whether the function of the glymphatic system is impaired in patients with α-synucleinopathies and the dysfunction of the glymphatic system is involved in the pathogenesis of α-synucleinopathies. Recently, a method has been reported that may allow noninvasive evaluation of the function of the glymphatic system in humans using MRI.

Symposium 2: Microcirculatory Disturbance and Pathological Protein Accumulation in the Brain

Pathobiochemical features and transmission potential of TDP-43 proteinopathy

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TDP-43 (Transactive response DNA-binding protein, 43 kDa) was originally discovered as a TAR-binding protein. TAR (trans activation responsive region) is an RNA regulatory sequence located in the terminal repeat of the HIV-1 gene. In 2006, TDP-43 was shown to be a major component of ubiquitin-positive, tau-negative α-synuclein inclusion bodies found in the autopsied brains of patients with ALS (amyotrophic lateral sclerosis) and some cases of FTLD (frontotemporal lobar degeneration).

TDP-43 proteinopathy is characterized by TDP-43-positive inclusion bodies in the brain. FTLD is classified into Types A–E based on the histopathological features, and this classification partially correlates with the clinical manifestations and genetic abnormalities in an individual. Immunoblotting the surfactant-insoluble fractions of brains with ALS and Types A–C FTLD using phosphorylated TDP-43 antibodies shows differences in the detected C-terminal fragments and protease-resistant bands. This suggests that the structural

conformation of TDP-43 aggregates differs in each disease type. Currently, attempts are being made to resolve the structure of TDP-43 aggregates using cryo-electron microscopy.

Furthermore, in TDP-43 proteinopathy, the TDP-43-positive inclusion bodies are found in major lesions as well as the entire central nervous system, with similar pathobiochemical characteristics. In ALS, they are also found in the intramuscular nerve bundles—the peripheral nerves. This suggests that TDP-43 can propagate throughout the nervous system. In fact, TDP-43-positive inclusion bodies were observed in culture cells and mice after administering them the insoluble fractions derived from autopsied brains.

In ALS, the plasma TDP-43 was elevated when measured using Simoa (Single Molecular Array). This shows that TDP-43 circulates to the peripheral blood and could be used as a biomarker.

Symposium 3: Recent Advance in Microcirculation Study of Gastroenterology

Activation of inflammasome and microcirculatory disturbance in gastrointestinal inflammation

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Background and Aim: The inflammasome is a large, multiprotein complex that consists of a nucleotide-binding oligomerization domain-like receptor (NLR), an apoptosis-associated speck-like protein containing a caspase recruitment domain, and pro-caspase-1. We have investigated the activation mechanism of the inflammasome and microcirculatory disturbance in various models of drug-induced enteritis and colitis.

Methods: Indomethacin (10μg/kg) was orally administered, 5-fluorouracil (5-FU) (500mg/kg) was intraperitoneally injected, or oxazolone (1%) was intrarectally administered to wild-type and NLR family, pyrin domain-containing 3 (NLRP3)$^{-/-}$ mice.

Results: Indomethacin-induced small intestinal damage was macroscopically visible in multiple ulcers characterized by mucosal defects. NLRP3$^{-/-}$ mice showed damage inhibition with mature interleukin (IL)-1β reduction, while exogeneous IL-1β aggravated the damage. NLRP3 was mainly expressed in inflammatory cells, represented by macrophages. Treatment with colchicine, an NLRP3 activation inhibitor, inhibited the indomethacin-induced damage by inhibiting mature IL-1β. Although treatment with IL-1β did not change the severity of the damage, the preventive effects of colchicine were abolished by supplementation with IL-1β. Small intestinal damage induced by 5-FU was microscopic mucositis characterized by the decrease in villus height and neutrophil infiltration in the lamina propria and submucosal layer. NLRP3$^{-/-}$ mice exhibited less mucositis than wild-type mice, and exogeneous IL-1β aggravated the mucositis. Oxazolone-induced colitis was a rapid-onset colitis characterized by the epithelial cell loss and neutrophil infiltration. NLRP3$^{-/-}$ mice exhibited more severe colitis than wild-type mice with the increased expression of IL-4 and IL-13, but this phenotype was rescued by exogenous IL-1β.

Conclusion: NLRP3 inflammasome activation and IL-1β exacerbate non-steroidal anti-inflammatory drug-induced small intestinal damage and cytotoxic anti-cancer agent-induced small intestinal mucositis. In contrast to these two types of experimental damage, NLRP3 inflammasome and IL-1β may have a protective role in the oxazolone-induced experimental colitis.

Symposium 3: Recent Advance in Microcirculation Study of Gastroenterology

The expression of mucosal adhesion molecules is involved in the clinical course and pathogenesis of ulcerative colitis

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Background: Adhesion molecules such as mucosal addressing cell adhesion molecule (MAdCAM)-1 and vascular cell adhesion molecule (VCAM)-1 are highly expressed in vascular endothelium in ulcerative colitis (UC). These molecules are involved in lymphocyte homing to tissues by binding to integrins on the surface of lymphocytes. Recently, antiα4β7 antibody treatment has been effective against refractory UC, suggesting that interaction between α4β7 in lymphocytes and MAdCAM-1 in vascular endothelium play an important role in the pathogenesis of UC. In this study, we investigated the relationship between the expression of adhesion molecules in the colonic mucosa and the clinical course of UC patients.

Methods: Sixty-eight patients with UC in remission visited Kyoto Prefectural University of Medicine and were included in the study. In all patients, biopsies were taken from the rectum during colonoscopy, and various adhesion molecules were quantified by real-time PCR. We compared the expression of mRNA for various adhesion molecules between patients who had subsequent relapse (relapse group) and those who remained in remission (remission group) and examined the correlation between the expression of mRNA and endoscopic severity and histological activity.

Results: Twenty-nine UC patients (42.6%) relapsed, and the expression of VCAM-1, MAdCAM-1, ICAM-1, ICAM-2, E-selectin, and P-selectin was significantly higher in the relapse group. The expression

of these adhesion molecules increased in correlation with the severity of endoscopic classification. The expression of adhesion molecules other than ICAM-2 was significantly higher in histologically active mucosa than in inactive mucosa. In addition, MAdCAM-1 expression tended to be higher in patients receiving maintenance therapy with vedolizumab.

Conclusion: The expression of adhesion molecules in the rectal mucosa of UC patients correlated well with mucosal inflammation and related to subsequent relapse. The expression of MAdCAM-1 was associated with the efficacy of maintenance therapy with vedolizumab and may be an indicator of treatment choice for UC.

Symposium 4: Microcirculatory Disturbance in Ophthalmic Diseases

New approach to the treatment of diabetic retinopathy: Role of neurovascular coupling and therapeutic perspectives

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The retinal blood flow is well-regulated by the interaction with retinal neurons and glia to maintain the retinal tissue demands, referred to as retinal neurovascular coupling (NVC). We found that impairment of the NVC, which can be evaluated by a flicker-induced increase in retinal blood flow, may precede the onset of diabetic retinopathy in diabetic mice (Hanaguri, Nagaoka, 2021, *Sci Rep*). To develop a minimally invasive treatment for diabetic retinopathy, I would like to introduce three new approaches for DR: a nano-particle eye drop, a therapeutic vaccine, and oral medications. We have recently developed a nano-eyedrop of fenofibrate to penetrate to the posterior segment and confirmed restoration of the impaired NVC in diabetic animals. We developed a peptide vaccine that improved the NVC function in diabetic animals. We also confirmed that systemic administration of sodium-glucose cotransporter-2 inhibitors and supplements may improve the NVC in diabetic animals. I would like to introduce new minimally invasive treatment strategies for diabetic retinopathy based on our new findings.

Symposium 4: Microcirculatory Disturbance in Ophthalmic Diseases

Association between diabetic retinopathy and ocular blood flow

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Diabetic retinopathy has become one of the leading causes of blindness in developed countries. The concept that altered retinal and choroidal blood flow may have a role in the development of diabetic retinopathy was emphasized more than 30 years ago. Therefore, measurements of ocular blood flow in diabetic retinopathy are necessary to understand the role played by alterations of the ocular hemodynamics in the progression of retinopathy. Furthermore, autoregulation is a very important function in understanding ocular circulation in detail.

Autoregulation plays an important role in controlling blood flow in tissue to enable a constant supply of oxygen and nutrients to maintain organ function. Autoregulation is achieved by alteration in resistance to blood flow and changes in blood vessel tone and involves dilatation of the vessels in response to decreases in ocular perfusion pressure (OPP). However, autoregulation of the retinal vessels in patients with type 2 diabetes mellitus may be impaired. We have previously reported on impaired autoregulation of blood flow at the retinal and optic nerve head tissue in response to a decrease in OPP during surgery in subjects with systemic disorders such as hypertension and hyperlipidemia using laser speckle flowgraphy (LSFG), which can measure ocular blood flow non-invasively.

There have been several reports of fluctuation of OPP during intraocular surgery, and a change in intraocular pressure during surgery has been suggested to increase the risk of ischemia at the optic nerve head and at the retina, which causes vision loss and visual field defects. Therefore, it would be important to investigate ocular circulation in patients with type 2 diabetes mellitus during surgery. In this symposium, we would like to discuss the “impaired autoregulatory capacity” in the retina and optic nerve tissue in patients with type 2 diabetes mellitus.

Symposium 4: Microcirculatory Disturbance in Ophthalmic Diseases

The outer blood-retinal barrier in central serous chorioretinopathy

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The outer blood-retinal barrier consists of a single layer of retinal pigment epithelial cells (RPE) lined on Bruch's membrane is a barrier between the choroid and the neural retina, which protects the neural retina from blood flow through the choroid that has abundant fenestrated blood vessels and carries out necessary material transportation together. The RPEs are bound by tight junctions and various transporters present on the plasma membrane are responsible for amino acid transport between the choroid and the retina. Furthermore, as multifunctional cells, RPE also phagocytizes and degrades photoreceptor outer segments and regenerates visual substances, which is different from the blood–brain barrier and the inner blood-retinal barrier. Central serous chorioretinopathy (CSC) is a disease associated with dysfunctions of the outer blood-retinal barrier and choroidal vascular abnormality, but the detailed

mechanism remains unclear. The increase of serum cortisol or aldosterone was reported to be associated with CSC but how those corticosteroids act to induce CSC is unknown. Some recent genome-wide association studies reported the association between CSC and the variants in several genes distributed in the RPE that may be involved in the pathogenesis of this disease. In this presentation, I will discuss the role of the outer blood-retinal barrier in normal conditions and in the pathologic state of CSC based on the latest findings of basic, genetic, and clinical studies.

Free Paper

Dextran dynamics revealed by two-photon microscopy

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Background: In Alzheimer's disease, the perivascular flow in the brain, also known as the lymphatic system, may function as a clearing mechanism for large molecules, including amyloid-β (Aβ). We previously reported that Aβ perfused over the cerebral cortex could be transported to the pericapillary parenchyma via penetrating vessels (IJMS 2022). However, the detailed mechanisms of such transport under physiological conditions were not fully elucidated. In this study, we developed a continuous perfusion system with a cranial window and observed the dynamics of cerebrospinal fluid in the perivascular space.
Methods: A cranial window was installed on the brain of a C57BL/6 mouse (CLEA Japan) under isoflurane anesthesia ($n = 9$). Before closing the window, a small amount of 100 μM dextran solution (40 kDa) labeled with FITC was instilled, and images were taken with two-photon microscopy every 30 min up to 150 min. In another experiment, a cranial window with inlet and outlet was placed over the brain surface, and the same dextran solution was continuously injected ($n = 10$). The current study was approved by the Animal Ethics Committee.
Results: The dextran instilled onto the brain surface penetrated into more than 100 μm deep from the beginning of the observation and the increase was not remarkable thereafter. Perivascular space of arteries and veins was equally depicted as a ring-shape. There was no delay in the dynamics of dextran between the periarterial and perivenous regions. Contrary to the superfusion experiment, dextran was gradually delivered into the deep parenchyma down to 150 μm deep through the perivascular space.
Conclusion: Perivascular space flow that gradually convects from the brain surface to the deep parenchyma was observed by continuous perfusion of the brain surface.

PCSK9 inhibitor can improve outcome after brain ischemia

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Objective: The correlation of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) expression after brain ischemia is still unclear. Here we explore the neuroprotective effect via PCSK9 inhibition focused on acute inflammation in ischemic injury.
Methods: Initially, SH-SY5Y cells were used as an in vitro cell culture model. These cells were grown under acidic condition for 24 h and normal pH (7.4) condition. As an inflammatory model, lipopolysaccharide (LPS) was also administrated under normal condition and cells were grown for 24 h. Immunochemistry and Western blotting for PCSK9 were performed to compare this expression in acidic condition with in normal condition. In addition to it, the treatment model with PCSK9 inhibitor (evolocumab) was also compared with them. Subsequently, 2–3 months old C57/B6 male mice ($n = 14$) were subjected to distal middle cerebral artery occlusion (dMCAO). To assess the neurological outcome, and ischemic brain injury in relation to PCSK9 expression and treatment by PCSK9 inhibition, 14 mice were used in our study and divided into 2 groups; treatment by vehicle ($n = 7$) and evolocumab ($n = 7$). Mice were euthanized 7 days after MCA occlusion to evaluate neurological dysfunction and ischemic volume. To evaluate neurological dysfunction, we performed several behavior studies: modified Bederson Score, elevated body swing test, sticky tape test, and corner test.
Results: PCSK9 expression were significantly increased in acidic condition ($p < .01$), and it was suppressed via PCSK9 inhibitor ($p < .01$). Neurological assays at 1, 3, and 7 d post-dMCAO were significantly improved in the dMCAO-treatment group ($p < .001$), compared with dMCAO-vehicle. PCSK9 inhibition also significantly reduced infarct volume ($p < .05$).
Conclusions: PCSK9 inhibitor can improve neurological outcome via suppression of PCSK9 expression in brain ischemia.

Comparison of dye-based fundus angiography and optical coherence tomography angiography in diabetic macular edema

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Purpose: To compare the detection rate of microaneurysms (MAs) in diabetic macular edema (DME) between dye-based fundus angiography and optical coherence tomography angiography (OCTA).

Methods: Retrospective chart review of 15 eyes with DME and 6 × 6 mm AngioVue OCTA (Optovue, Inc.) and fluorescein angiography (FA)/indocyanine green angiography (IA) using HRA2 (Heidelberg), SD-OCT (Macular cube, Cirrus HD-OCT, Carl Zeiss) evaluation at Nagoya City University Hospital. The images of OCTA (6 × 6 mm) were overlayed onto the images of FA early phase or ICGA late phase. The number of MAs detected by FA early phase, IA late phase, OCTA superficial capillary plexus (SCP), and OCTA deep capillary plexus (DCP) were evaluated. The MAs which were detected both by FA and OCTA, or ICGA and OCTA were counted. This study has been approved by the institutional review board.

Results: Fifteen eyes of 11 patients with DME consisted of three males and females females, with a mean age of 65 ± 6 years. The number of MA detected in FA was 57 ± 29 and 22 ± 15 in IA, and the number of MA detected in IA was significantly lower ($p < .01$). The number of MA detected by OCTA (SCP) was 17 ± 8 and by OCTA (DCP) was 29 ± 10, with significantly fewer MA detected by SCP ($p < .01$). The concordance rate of MA detected by FA/IA and OCTA were 16.2% (FA and OCTA (DCP)), 17.9% (FA and OCTA (DCP)), 15.9% (IA and OCTA(SCP)), and 30.0% (IA and OCTA (DCP)). It was significantly higher results between IA and OCTA (DCP) ($p = .016$).

Conclusions: The MAs detected by OCTA and located in DCP, and MAs detected in IA late phase showed significant more agreement. The MAs that can be found in IA late-phase and DCP by OCTA might share the pathological characteristics.

Clinical features and microcirculatory dynamics of cancer-related thrombosis

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Objective: Intravascular coagulation is increased in cancer patients, often complicated by nonbacterial endocarditis (NBTE) and resulting in cerebral embolism. In this study, we examined the clinical aspects of cancer-related thrombosis in our hospital and discussed microcirculatory dynamics.

Methods: Twelve patients who were thought to have had cerebral infarction due to cancer-related thrombosis during a 1-year period at our hospital were included. The mode of onset, blood coagulation markers, tumor markers, and imaging findings were examined in each patient.

Results: Age ranged from 33 to 88 years, 5 males and 7 females. Primary tumor types were choriocarcinoma in one case, duodenal cancer in two cases, anal cancer in one case, lung cancer in five cases, ovarian cancer in one case, and bladder cancer in two cases. Transesophageal echocardiography confirmed the finding of NBTE in one case. Blood coagulation markers showed elevated D-dimer or FDP levels in all patients. Seven patients had infarct foci larger than 1 cm and suspected embolization of large vessels, while small infarcts smaller than 1 cm were bilaterally scattered in the cortex and white matter. Most patients were treated with anticoagulation with intravenous heparin during hospitalization, but a few were forced to switch to warfarin or antiplatelet agents upon discharge. One case of deep vein thrombosis was treated with apixaban and another with subcutaneous injection of calcium heparin. The prognosis was poor, with 4 of the 12 patients dying within 3 months.

Discussion: In cancer-related thrombosis, increased coagulation capacity and thrombus formation occur via exposure to tissue factor, mucin production, PAI-1 production, cytokine activation, and fibrin deposition on tumor cells. In animal models, tumor-derived tissue factor-positive microvesicles and neutrophil extracellular traps have been associated with thrombosis in cancer-bearing mice. Elucidation of the mechanisms of cancer-associated thrombosis is expected to lead to the development of new therapeutic strategies.

Brain infarction and vasculitis in neuropsychiatric systemic lupus erythematosus: A clinico-pathological case

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Objective: Nervous system involvement is poorly understood manifestation of SLE. We show the postmortem histopathology of an SLE patient and discuss about the relationship vasculitis and stroke in neuropsychiatric SLE (NPSLE).

Methods: We report an 84-year-old female with SLE, who had been diagnosed with SLE 40 years ago. She was transferred to our hospital due to appetite loss and general fatigue for 1 month. After admission, her consciousness level was decreased. Fluid attenuated inversion recovery (FLAIR) images and diffusion-weighted images of brain MRI revealed high signal lesions in pons that did not match the vascular territory. Although the patient received immunosuppressive therapy, died on hospital day 67, and suffered from several complications including aspiration pneumonia. Finally, the brain lesions were enlarged to the whole brain stem, bilateral hypothalamus, thalamus, and basal ganglia continuously. This presentation is approved by the Ethics Committee of Moriguchi-Ikuno Memorial Hospital.

Results: An autopsy showed lytic necrosis of the pons, encephalomalacias of the thalamus and basal ganglia, multiple brain infarctions and hemorrhages. Infarcted parenchyma was surrounded by small blood vessels, which showed prominent vasculitis change. Fibrinoid necrosis of the basal ganglia vessels with an inflammatory infiltrate in the perivascular (Virchow-Robin) spaces was revealed. The blood vessels were nearly obstructed by abscess and hemorrhage. Whereas, arteriosclerotic changes of the basilar artery and vertebral arteries were mild. Besides, severe vasculitis was not shown in other organs.

Conclusion: We showed that vasculitis was associated with cerebrovascular disease in the NPSLE patient. Considering from lymphocyte

infiltration to the Virchow-Robin spaces, continuously exposing the cerebral endothelium to autoantibodies might cause complement activation. NPSLE patients, in which various immunosuppressive therapies are ineffective as above case, may benefit from treatment with a complement inhibitor.

Microcirculatory disturbance in a case of eosinophilic granulomatosis with polyangiitis causes multiple cerebral infarction in the bilateral border zone areas

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Introduction: Eosinophilic granulomatosis with polyangiitis (EGPA) is known to cause peripheral neuropathy, whereas reports of central nervous system involvement are quite rare. Here, we report a patient with EGPA who developed multiple cerebral infarctions with subcortical and subarachnoid hemorrhages colocalized at bilateral border zone areas.
Case Report: The patient is a 69-year-old woman with a history of asthma, eosinophilic otitis media, sinusitis, and hemorrhagic colitis. One day, she suddenly noticed left-hand weakness while performing household tasks. Subsequently, she became unsteady on the bicycle and noticed left shoulder pain and purpura a few centimeters in diameter on all extremities. She was referred to our hospital by a local clinic. Laboratory tests showed WBC of 6200/μL, eosinophil 30%. ESR was 36 mm/1 h. Total IgE was 440 IU/mL Head magnetic resonance imaging revealed multiple cerebral infarctions with subcortical and subarachnoid hemorrhaging colocalized at the bilateral border zone areas. She was diagnosed as having cerebral infarction caused by EGPA and treated with prednisolone. Neurological symptoms, including left hemiparesis and unstable gait, gradually improved with normalization of eosinophilia. One year after discharge, she had no recurrence of stroke, including asymptomatic ischemic lesions on MRI, with low-dose prednisolone.
Discussion: The border zone area is characterized by low hydrostatic pressure, and its microcirculation may easily be affected by hypereosinophilia and vasculitis. In the present case, ischemic lesions were accompanied by subcortical and subarachnoid hemorrhage, suggesting that vasculitis can make the vessel wall vulnerable while disturbing the peripheral flow in the same lesion. The initial occurrence of cerebral infarcts and the apparently delayed detection of microhemorrhages and subarachnoid hemorrhages may indicate that disruption of the vessel wall may follow initial disturbance of microcirculation.
Conclusion: Inflammation in the small cerebral arteries in EGPA may induce bilateral border zone infarctions with colocalizing subcortical and subarachnoid hemorrhages.

Applicants' Presentation for Young Investigator Award

Microcirculatory disturbance after recanalization with endovascular thrombectomy

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Background: Recent development of mechanical thrombectomy has dramatically improved the prognosis of cerebral infarction. However, recanalization of major arteries does not always induce complete recovery of brain function. To elucidate the role of microcirculatory disturbance including reperfusion injury, we retrospectively analyzed cases of endovascular thrombectomy conducted in our department in 2022.
Methods: Patients with acute cerebral infarction who were treated with endovascular therapy in Osaka Metropolitan University Hospital were retrospectively analyzed. We evaluated NIHSS, ASPECTS, vascular lesion and recanalization status in addition to basic demographic data.
Results: Case 1 is an 84-year old female, who was found with total aphasia, right hemiparesis (MMT 3), and conjugate eye deviation to the left. NIHSS was 28. MRI revealed high-intensity lesion at the left basal ganglia and a part of the temporal cortex on DWI and left M1 occlusion on MRA. Intravenous thrombectomy followed by three passes of mechanical thrombectomy resulted in recanalization of TICI2b with distal occlusion at M2. Follow-up MRI revealed an enlarged area of cerebral infarction. In the other 6 cases, enlargement of cerebral infarction after successful recanalization was not observed.
Discussion: Microcirculatory disturbance after reperfusion, known as reperfusion injury, may be caused by secondary thrombosis, activation of leukocytes, endothelial damage to capillaries, and free radical generation. Distal embolization by fragmented thrombus during the recanalization procedure, observed in case 1, might be another mechanism.
Conclusion: Recanalization with endovascular therapy can successfully rescue the ischemic brain in most cases. Microcirculatory disturbance may not contribute to the prognosis significantly although distal embolization can induce severe neurological deficit.

Establishment of pericyte-specific optogenetic animal tool

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Purpose: Vascular mural cells are classified into smooth muscle cells located around arteriovenous endothelial cells, and pericytes

located around capillary endothelial cells. Smooth muscle cells relax in response to increased intracellular cAMP concentration, resulting in increase in cerebral blood flow. On the other hand, it remains unclear whether pericytes themselves are involved in the regulation of cerebral blood flow by vasoconstriction/dilation. One reason is that transgenic animals for pericyte-specific stimulation have not been established, while smooth muscle-specific stimulation has been established using cell-specific gene targeting. NG2 and Pdgfrb are known as pericyte markers, but both are also expressed in smooth muscle. In this study, we aimed to establish a pericyte-specific gene expression induction method, called the set-subtraction method.

Methods: A tetracycline-inducible gene expression system was used with photoactivatable adenylate cyclase (PAC), an optogenetics tool. Specifically, PAC was expressed in all mural cells in double transgenic mice crossed between NG2-tTA and tetO-PAC-2A-GFP, then the tetO cassette was removed from smooth muscle cells in triple transgenic mice crossed with Myh11-Cre (smooth muscle-Cre). Perfusion was conducted through the cardiac route using glyoxal fixative (pH = 5, containing 3% glyoxal) for immunohistochemical staining. The procedures were approved by the Animal Research Committee of Keio University (18081–1).

Results: GFP expression was quantified by immunohistochemical staining, and more than 90% of GFP expression in smooth muscle cells was removed in the triple transgenic mice, indicating that the set-subtraction system successfully created mice that express GFP in a pericyte-specific manner. More experiments are needed to investigate the effects of light stimulation of PACs expressed only on pericytes and the accompanying increase in intracellular cAMP concentration on changes in blood flow.

Conclusion: We established a new pericyte-specific optogenetic stimulation system using the set-subtraction method. Further experiments are needed to investigate the role of pericytes in regulating cerebral blood flow.

Subarachnoid hemorrhage induces neuroinflammation and neuronal cell death throughout the entire brain

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Background: Subarachnoid hemorrhage (SAH) caused by rupture of a cerebral aneurysm is a disease with a high mortality rate, and early brain injury (EBI) occurring within 72 hours after SAH has attracted attention as a prognostic factor. The pathogenesis of EBI is not fully understood, and effective treatment has not yet been established. Although neuroinflammation is important prognosis factor of EBI, whether neuroinflammation spreads throughout the cerebrum and the extent of its depth in the cerebral cortex remain unknown.

Methods: In this study, we induced SAH in mice by injecting hematoma into prechiasmatic cistern and created models of mild to severe SAH. Brain sections were prepared 24 hours after SAH creation, and neuroinflammation and neuronal cell death were analyzed in the control, sham, mild SAH, and severe SAH groups by immunofluorescence staining of Iba-1 and NeuN/TUNEL from the anterior to posterior cerebral cortex. In particular, the cortex was divided into superficial (layers I-III) and deep (layers IV-VI) layers, and the hippocampus, which is located deeper than the cortex, was also analyzed. All experimental procedures were approved by the ethics committee of Keio University.

Results: In the cortex and hippocampus, Iba-1-positive active microglia were significantly increased in the SAH group compared to the control and sham groups, and neuroinflammation was more exacerbated in the severe SAH group, causing neuronal death mainly in the superficial cortical layers. In contrast, there was no significant increase in neuronal death in the granular layer of the hippocampus.

Conclusions: Neuroinflammation caused by SAH had spread throughout the cerebrum, causing neuronal cell death. Considering that the cerebral cortex is responsible for long-term memory and movement, suppressing neuroinflammation in all layers of the cerebral cortex may improve the prognosis of patients with SAH.

Establishment of deep learning algorithm for retinal vascular area measurement: An alternative biomarker of brachial-ankle pulse wave velocity

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Purpose: Retinal microcirculation reflects the alteration of systemic circulatory dynamics. The purpose of this study was to develop a novel method to automatically evaluate retinal vascular changes associated with systemic diseases including hypertension and arteriosclerosis.

Methods: The institutional review board for clinical research of Hokkaido University Hospital (012-0106) approved the study protocol. We established a deep learning algorithm that fully automatically identifies retinal blood vessels and measures the total area of retinal vessels by pixels in fundus images. Retinal photographs ($n = 10\,571$) obtained from 5598 individuals during routine health checkups were used to measure the total retinal vascular area. Blood pressure was also measured in each patient. Brachial-ankle pulse wave velocity (baPWV), a key indicator of arterial stiffness, was also measured in 372 individuals. Automatic segmentation of retinal vessels was performed using our deep learning algorithm to measure the total arteriolar area (AA) and the total venular area (VA). The correlation between blood pressure, baPWV and AA, VA were evaluated.

Results: Both AA and VA showed a negative correlation with blood pressure and baPWV. Furthermore, the predicted values of baPWV, which were estimated from regression equations using variables that included AA, showed a better correlation with the measured values of baPWV compared to the predicted values that did not include AA.

Conclusions: The retinal vascular area, which is measured full-automatically from a fundus image, could serve as a convenient index for the evaluation of systemic vascular status including hypertension and arteriosclerosis.

Evaluation of oxidative stress by fluorescence immunostaining of novel therapeutic agents in type 2 diabetic mice

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Purpose: We have previously reported that oral administration of tofogliflozin, supplement with *Trapa bispinosa* Roxb. extract (TBE) and lutein, fenofibrate nano-eyedrops (FenoNano) improved retinal blood flow dysregulation in diabetic mice. We focused on oxidative stress as a cause of retinal blood flow dysregulation due to diabetic mice and investigated the effect of suppression of oxidative stress by therapeutic agents.
Methods: We divided the mice into five groups, untreated type 2 diabetic group (db/db mice), Tofogliflozin group (feed containing tofogliflozin), Supplement group (feed containing TBE and lutein), FenoNano group (1% FenoNano-eyedrops twice daily), and normal blood sugar control group (db/m mice) ($n = 6$ each). And compared the intensity of fluorescence immunostaining with oxidative stress markers Nitrotyrosine (NO2-Y) and 8-OHdG in retinal tissue sections from the ganglion cell layer (GCL) to the outer nuclear layer (ONL) and the retinal vascular area. The Ethical Committees of Nihon University Committee Guidelines for the Care of Laboratory Animals in accordance with the principles of the Association of Research in Vision and Ophthalmology approved the animal experiments in the current study.
Results: NO2-Y and 8-OHdG from the GCL to ONL and retinal vascular areas were increased in the untreated diabetic group compared to the control group, and significantly decreased in both the tofogliflozin, supplement and FenoNano groups. NO2-Y and 8-OHdG in the control and each treatment group were not significantly different in any of the groups.
Conclusions: Retinal blood flow dysregulation observed in type 2 diabetic mice may be ameliorated by suppressing oxidative stress.

Modulation of lymphocytes migration to intestinal microvessels by succinate in mice

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Introduction: The gut microbiota and its metabolic products interact with the host in several ways to influence homeostasis and many diseases. Compositional and metabolic changes in the gut microbiota are a well-established contributing factor in inflammatory bowel disease (IBD), although the mechanisms remain unclear. Succinate, an intermediate of bacterial breakdown of indigestible dietary fiber, accumulates in the intestine of patients suffering from IBD. Succinate has been reported to activate immune cells and enhance inflammation via succinate receptor 1(SUCNR1), leading to the activation of macrophages and antigen-specific T-cell activation. However, the mechanism of how succinate is involved with the pathogenesis of IBD remains unclear. It is known that IBD is associated with increased adherence of lymphocytes to vascular endothelium via adhesion molecules in the intestinal tract. Therefore, we hypothesized that succinate would modulate the adherence of lymphocytes by enhancing adhesion molecules. We aimed to investigate the effect of succinate on intestinal microcirculation.
Methods: Male wild-type mice (C57BL/6) were used for the study. Lymphocytes were obtained from the spleen and stained with a fluorescent dye (CFSE). Stained splenocytes were injected from the jugular vein slowly. Small intestinal microcirculation was observed under confocal laser scanning microscopy (CLSM). Succinate was injected into the lumen or superfused directly onto the mesentery in some mice. This study was conducted after obtaining approval from the ethics committee of the institution to which the researcher belongs.
Results: Succinate-treated mice showed adhesion of splenocytes in intestinal mucosa about 30 minutes after the administration compared with control mice.
Conclusions: Our results suggest that succinate might be involved with intestinal inflammation in IBD by enhancing adherence of leukocytes. Further studies are necessary for the exact mechanisms to be clarified.

Irregular and tortuous vasculature in colonic mucosa of active ulcerative colitis observed by endocytoscopy

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Introduction: Treat to Target (T to T) strategy has been widely spread for the management of ulcerative colitis (UC) and endoscopic or histological mucosal healing (MH) is the treatment target. Although serum or fecal biomarkers are developed, nevertheless, endoscopy is the gold standard for monitoring UC activity. In addition to image enhanced endoscope (IEE), ultra-high magnifying endocytoscope (ECS: x520) has been developed, and combination with narrow-band imaging (NBI; EC-NBI) allows in vivo visualization of intramucosal capillary network in colonic mucosa. Here we report two cases of UC with the observation of the architecture of vasculature in inflammatory colonic mucosa by ESC.
Case 1: A 66-year-old male showed bloody diarrhea and abdominal pain in Jan 202X. Patient visited the nearby clinic and colonoscopy demonstrated diffuse inflammatory and edematous mucosa and was diagnosed as left-sided UC. Mesalazine was administered to the

patient, however, diagnosed with mesalazine-intolerance, and was referred to our hospital. Mesalazine was discontinued, prednisolone (PSL) 1 mg/kg, and intensive granulocytapheresis (GMA) was administered. This combination therapy was ineffective, therefore, tacrolimus (Tac) was administered and remission was achieved. ESC showed narrow and irregular blood vessels with coarse distribution in ulcer scar. In contrast, irregular and tortuous blood vessels were observed in mild active inflammatory mucosa of Mayo endoscopic score (MES) 1 in the rectum. Treatment was changed based on T to T.

Case 2: An 81-year-old female showed bloody stool in 202X-2. Patient was diagnosed as left-sided UC. Mesalazine, PSL, and azathioprine (AZA) were administered and clinical remission was achieved. ESC showed irregular and tortuous blood vessels with dense distribution showing MES1 activity. Biologics were added based on T to T.

Conclusion: Endocytoscope may be a useful tool for evaluating mild inflammatory mucosa to monitor UC and perform T to T.

Pirfenidone prevents experimental esophageal stricture after ulcer healing by inhibiting NLRP3 inflammasome activation

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Background and Aim: Esophageal injury often results in a scar, leading to refractory strictures. The NLRP3 inflammasome activates caspase-1, causing the maturation of interleukin (IL)-1β. Here, we aimed to investigate the preventive effect of pirfenidone (PFD), an antifibrotic drug, on esophageal stricture after healing of an acetic acid-induced ulcer and studied its mechanism by focusing on the activation of the NLRP3 inflammasome.

Methods: Esophageal ulcers were induced in rats via the local application of acetic acid in the serosa. PFD was intraperitoneally administered to the rats 3 days after ulcer induction. The effect of PFD on esophageal stricture after ulcer healing was assessed by esophagography on day 9. The protein levels of mature caspase-1 and IL-1β were assessed by western blotting.

Results: The ulcers fully developed 3 days after induction and were almost scarred by day 9 with severe strictures. PFD promoted ulcer healing and attenuated fibrotic collagen in the submucosa by suppressing the increase in NLRP3, cleaved caspase-1, and mature IL-1β expression, thereby preventing esophageal stricture. Exogenous IL-1β abolished the therapeutic effects of PFD on ulcer healing and stricture formation. Furthermore, NLRP3 inhibitor (Glyburide) and caspase-1 inhibitor (ac-YVAD-cmk) mimicked the effects of PFD on ulcer healing and stricture formation, with suppression of the increase in cleaved caspase-1 and mature IL-1β proteins and expression of fibrosis-related molecules including transforming growth factor (TGF)-β1.

Conclusion: The NLRP3 inflammasome promotes esophageal stricture formation following ulcer healing and PFD exerts potential prophylactic activity against strictures possibly via the inhibition of the NLRP3/IL-1β/TGF-β1 axis.