Abstracts

SL-01

Mechanisms of blood vessel leakiness in inflammation and cancer

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Blood vessel leakiness is a well-documented feature of inflammation and cancer. Plasma leakage under these conditions results from alterations in endothelial barrier function. The cellular basis of this alteration is attributed to the formation of gaps between endothelial cells. When initially described in 1961, endothelial gaps were reported to occur in postcapillary venules after exposure to histamine, serotonin, or bradykinin. Subsequent studies of leaky blood vessels in inflammation revealed that the gaps are small, having a mean diameter of only 0.3 µm (range 0.2 - 1.6 µm). Blood vessel leakage in tumors, which facilitates angiogenesis and delivery of cancer diagnostics and therapeutics, results from larger gaps (mean 1.7 µm, range 0.3 - 4.7 µm), and other endothelial defects. Additional cellular processes, including transcytosis, transcellular holes, and vesiculovacuolar organelles (VVOs), have also been implicated, but evidence for endothelial gaps as routes of plasma leakage remains solid. Although many cytokines, including VEGF and TNF-alpha, can trigger plasma leakage, few agents have anti-leakage effects and even fewer act selectively on endothelial cells. The angiopoietin/Tie family of ligands and receptors are exceptions. Angiopoietin-1 (Ang1, Angpt-1), which activates Tie2 receptor signaling in endothelial cells, suppresses gap formation and reduces leakage induced a wide range of mediators. These actions are strongly influenced by angiopoietin-2 (Ang2, Angpt-2). Unlike Ang1, Ang2 has context-dependent effects on Tie2 signaling. Ang2 promotes Tie2 activation and decreases leakage in some conditions, but paradoxically, competes with Ang1, inhibits Tie2, and increases leakage under other conditions. In studies done to reconcile this paradox, we found that Ang2 acts as a Tie2 agonist in normal mice, where it promotes Tie2 activation and vascular stability. By contrast, in inflammation, Ang2 acts as a Tie2 antagonist, increases gap formation and leakiness, and also increases Ang2 expression. These actions of Ang2 are governed by Tie1 receptors, which are abundant in endothelial cells under normal conditions. Tie1 is an orphan receptor that does not bind angiopoietins but undergoes rapid inactivation by ectodomain shedding in inflammation, and acts as a switch for the agonist/antagonist actions of Ang2. High levels of soluble Tie1 ectodomain and Ang2 are found in blood of critically ill patients and are correlated with poor outcome. In tumors, VEGF destabilizes the vasculature and increases leakiness; Ang2 amplifies these actions. Inhibition of VEGF and Ang2 normalizes tumor vessels and reduces leakage. Together, the evidence shows that in health, Ang1 is dominant, maintains Tie2 activation, suppresses endothelial gap formation, and preserves vascular stability. Ang2 has similar but weaker actions under these conditions. However, in inflammation, when Tie1 is inactivated by ectodomain shedding, Ang2 acts as a Tie2 antagonist that dominates the actions of Ang1. Inactivation of Tie2 promotes endothelial gap formation and vascular leakiness. Elevated Ang2 and soluble Tie1 in blood are biomarkers of endothelial injury. In cancer, VEGF and Ang2 promote vascular instability and leakage. Although angiopoietin/Tie receptor signaling is more complex in tumors than in inflammation, angiopoietins and Tie receptors are promising diagnostic and therapeutic targets in both conditions.

SL-02

Recent Advances in Research on Retinal Microcirculation

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Because the retina is transparent, it is possible to visualize the details of microcirculation in the living eyes non-invasively. Numbers of studies have been performed to investigate the retinal microcirculation not only in experimental animals but in human subjects. Recent advances in the retinal microcirculation research will be discussed.

1. Acridine orange leukocyte fluorography

We have developed the method to visualize flowing leukocytes in the retinal microcirculation of rats, mice, and monkeys with the use of acridine orange and the scanning laser ophthalmoscope. This method allows the evaluation of leukocyte-endothelial interactions such as rolling and adhesion *in vivo*. We have studied the leukocyte dynamics in various experimental models and demonstrated the significance of leukocytes in the retinal microcirculatory disturbances, The studies on experimental diabetic rats revealed that transient leukocyte entrapment in the retinal capillaries occurs in the very early phase of diabetes. The leukocyte entrapment resulted in the occlusion of retinal capillaries in the downstream that caused a relative retinal ischemia. The leukocytes further accumulate in the retinal capillary bed during the course of diabetes progression. These results suggested that diabetic retinopathy is caused by inflammatory reactions which leukocytes play a major role in the pathogenesis.

2. Ultra-wide field fundus angiography

Optos® system has been developed to provide ultra-wide field imaging of ocular fundus without dilation of pupil. The photograph that covers 80% area of the retina can be obtained in 0.25 seconds. Ultra-wide field fundus angiography using fluorescein sodium or indocyanine green has demonstrated the importance of peripheral pathology in numbers of retinal diseases. Diabetic retinopathy usually presents with the retinal lesions predominantly in the posterior pole. However, our study showed that about 10% of patients had diabetic retinal lesions only in the periphery. Recent studies suggested that patients with predominantly peripheral lesions showed faster progression of diabetic retinopathy.

3. Optical coherence tomography (OCT) angiography

OCT is a non-invasive imaging technology based on low-coherence interferometry. It gives high-resolution images to visualize individual layers of the retina. In a recently developed extension of OCT, OCT angiography (OCTA) detects blood flow down to the capillary level by measuring change of the signals. OCTA provides the images of retinal vessels at the capillary level without the use of angiographic dye. It is also possible to analyze retinal vascular lesions in the different layers of the retina. We have applied this technique to study diabetic retinopathy and retinal vein occlusion. We were able to demonstrate the disturbance of the foveal microcirculation of diabetic patients even before the onset of diabetic retinopathy. The studies on retinal vein occlusion suggested that OCTA is very useful for diagnosis and planning the treatment of the disease and that it might replace a conventional dye-based fundus angiography in the near future.

SL-03

Fluid and particle transport via the lymphatic system

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The lymphatic system provides pathways by which fluid, particles and immune cells are drained from the peripheral tissues. These elements are taken up into the initial lymphatics from the interstitial space (lymph formation). Then, the lymph is driven through the lymphatic system by passive and active pump effect (lymph transport). Almost all lymph formed in the initial lymphatics passes through at least one lymph node before entering into the venous system. Failure of lymph circulation causes tissue high-protein edema and local immune deficiency. Thus, from a physiological point of view, lymph formation, transport and nodes' filtering effect must be fundamental issues for lymph circulation.

Periodic tissue deformation serves to enhance lymph flow. Local skin massage dramatically elevated uptake of fluid, protein, particles and cells from the interstitial space to the initial lymphatics. Introduction of local skin massage caused frequency-dependent increase in lymph flow rates, which were elevated linearly with the log of frequency between 0.03 and 3.0Hz. These observations strongly suggest an essential role of periodic tissue deformation in lymph formation.

To examine characteristics of lymph transport, pressure and flow rate of lymph at a certain point of the lymphatic system was determined simultaneously by using graphical analysis. This analysis was performed to equilibrate two relations, such as lymph outflow pressure-flow rate relationship (lymph formation curve) and lymph infusion pressure-flow rate relationship (lymph transport curve). The analysis showed that pressure in the popliteal prenodal lympatics were lower than that in the lumbar lymphatic trunks in rabbits, suggesting that lymphatic pump effect is critical for creating an ascending pressure gradient in the lymphatic system.

Regarding particle uptake, both dispersed particles (extracellular transport pathway) and phagocytosed particles (intracellular transport pathway) were observed in lymph of the rabbit prenodal lymphatics drained from the particles injection site. Initially, the extracellular transport pathway dominated. After subcutaneous injection of latex microspheres (0.5 - 10.0mm in diameter), the decreasing order of amount of dispersed particles taken up in the lymphatic system was as follows: 0.5mm > 1.1mm > 2.0mm-particle. Neither particle with 5.6 nor 10.0mm in diameter appeared in the prenodal lymph. A size barrier may limit particle uptake at the initial lymphatic walls.

After injection of latex microspheres (0.5 - 1.9mm in diameter) into the rabbit popliteal prenodal lymphatics, the decreasing order of amount of particles passed through the lymph node was as follows: 0.5mm > 1.1mm > 1.7mm-particle. No particle with 1.9mm in diameter passed through the lymph node. From these results, size-dependent accumulating mechanisms were confirmed in the lymph nodes.

In this lecture, I am planning to introduce classical but fundamental research for lymph circulation. A lot of studies for lymph formation and transport have been carried out since at least the 1930s. Further development of study for lymphdynamics is large degree expected. Because this development might contribute to treat lymph edema, detect sentinel lymph node, construct drug delivery system, prevent cancer metastasis and improve our knowledge of microcirculation.

SY1-01

Crosstalk between lymphoid cells and vascular systems in intestinal inflammation

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Inflammatory bowel disease (IBD) is associated with angiogenesis and lymphangiogenesis from the existing vascular network. Significant elevation of lymphatic and blood vessel (BV) density was observed in mouse colitis model. The LV density has been reported to increase in the intestinal mucosa of IBD patients. Lymphatic failure and obstruction, recognized as a histopathological features of IBD, are inconsistent with the increase in the lymphatics and lymphangiogenic factors in the intestinal mucosa of IBD patients. The failure to collect accumulating filtered fluid including immune cells and some types of antigens aggravates lymphocytic lymphangitis in IBD. Therefore, improving the lymphatic function may be important for managing IBD. Recent studies show that interaction between platelets and podoplanin on lymphatic endothelial cells (LECs) suppresses lymphangiogenesis. We aimed to investigate the role of platelets in the inflammatory process of colitis, which is likely to be through modulation of lymphangiogenesis. Lymphangiogenesis in colonic mucosal specimens from patients with IBD was investigated by studying mRNA expression of lymphangiogenic factors and histologically by examining lymphatic vessel (LV) densities. Involvement of lymphangiogenesis in intestinal inflammation was studied by administering VEGF-receptor 3 (VEGF-R3) inhibitors to the mouse model of colitis and evaluating platelet migration to LVs. The inhibitory effect of platelets on lymphangiogenesis was investigated in vivo by administering antiplatelet antibody to the colitis mouse model and in vitro by coculturing platelets with lymphatic endothelial cells. Although mRNA expressions of lymphangiogenic factors were significantly increased in the inflamed mucosa of patients with IBD compared with those with quiescent mucosa, there was no difference in LV density between them. In the colitis model, VEGF-R3 inhibition resulted in aggravated colitis with decrease in lymphatic density. Administration of an antiplatelet antibody increased LV densities and significantly ameliorated colitis. Coculture with platelets inhibited proliferation of LECs in vitro. Our data suggest that despite elevated lymphangiogenic factors during colonic inflammation, platelet migration to LVs resulted in suppressed lymphangiogenesis, leading to aggravation of colitis by blocking the clearance of inflammatory cells. Modulating the interaction between lymphoid cells and vascular systems could be a new therapeutic means for treating IBD.

SY1-02

Lipid mediators promote liver repair and regeneration following acute liver injury

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Insufficient liver repair following hepatic ischemia/reperfusion (I/R) determines the outcome of the patients underwent liver surgery. Recent accumulated evidence suggests that lipid mediators including prostanoids and leukotrienes play a role not only in induction of inflammation but resolution of inflammation and tissue regeneration. Recruitment of macrophages through lipid mediators and its receptors is essential for tissue repair and regeneration. We investigated whether lipid mediators are responsible for liver repair from hepatic I/R injury with enhanced recruitment of macrophages. Leukotriene B4 (LTB4) derived from 5-lipooxygenase (5-LOX) acts mainly through LTB4 receptor, BLT1. BLT1 signaling facilitates liver repair through enhanced EGF expression in recruited VEGFR1-expressing macrophage into the injured liver. We confirmed a critical role for VEGFR1 in liver repair and sinusoidal reconstruction during hepatic I/R through EGF production from bone marrow-derived macrophages. Another major and important lipid mediator is PGE2, a metabolite of arachidonic acid produced via cyclooxygenase (COX). The final step of PGE2 generation is catalyzed by a specific PGE synthases (PGESs). Hepatic I/R was associated with increased levels of PGE2 together with inducible mPGES1 in the livers. As compared with WT mice, mPGES1 deficient mice exhibited promotion of liver repair, increasing growth factors, decreasing cytokines and pro-inflammatory macrophage accumulation into the injured regions. Furthermore, mice lacking EP4, one of the PGE2 receptor subtypes, revealed stimulation of liver repair with down-regulated expression of genes related to pro-inflammatory macrophages. Taken together, regulation of several lipid mediators appears to be a therapeutic option for the resolution of inflammation and tissue regeneration during acute liver injury.

SY1-03

Significance of VEGF and VASH2 in Gastric and Hepatic MALT Lymphoma induced by *Helicobacter heilmannii* Infection: Relation to Angiogenesis and Lymphangiogenesis

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During our recent study, the gastric, hepatic and pulmonary MALT lymphoma was formed by the long term infection of Helicobacter heilmannii. We have also 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 and promotor of progress of malignancy.
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. 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 approximately 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 and lung. VASH2 immunoreactivity was found in the lymphoma cells surrounding the irregular microvascular network in the MALT lymphoma. The localization of VEGFA was also observed in the mesenchymal cells. 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 within the gastric and hepatic MALT lymphoma and suggested to have some function in the tumor progression.

SY1-04

Cell-cell interactions for retinal vascular development

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To meet tissue requirements for oxygen and nutrients, blood vessels are properly distributed with an appropriate amount and patterning customized to the function of each organ. In this process, diverse interactions between endothelial cells and other cell types contribute to the establishment of such tissue-specific vascular patternings. Our research is mainly focusing on vascular development of the retina, a part of the central nervous system, now widely utilized as a good model to study the mechanism of angiogenesis. In retinal vascular development, a highly motile population of endothelial cells which positions the leading edge of the vasculature, namely “tip cells”, leads outgrowth of blood vessels. Here we shed light on the mechanisms for the cell-cell communication regulating the direction and movement of tip cells, in particular neuronal and glial (astrocytic and microglial) regulation of endothelial behavior.

SY2-05

Attenuating effect of cilostazol on indomethacin induced small intestinal injury

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Background: Non-steroidal anti-inflammatory drugs (NSAIDs), including indomethacin (IND), are known to frequently cause small intestinal lesions. Neutrophil migration, one of major factors predisposing to intestinal lesions, consists of several steps, including interaction with P-selectin expressed on platelets. Cilostazol, a specific phosphodiesterase (PDE)-3 inhibitor, suppresses the expression of P-selectin from platelets and reduces interaction between platelets and leukocytes, leading to inflammatory amelioration in several disease models. The aim of this study was 1) to clarify that platelet-leukocyte interaction is involved in NSAID-induced small intestinal lesions and 2) to demonstrate the therapeutic effectiveness of cilostazol for these lesions through blocking recruitment of leukocytes by modulating platelet-leukocyte interaction. Methods: 1) IND (2.5 mg/kg) was peritoneally injected into mice daily for 4 days. Anti-PSGL-1 antibody (2 mg/kg) or cilostazol (100 mg/kg) was peritoneally injected one hour before the injection of IND. On day 5, mice were sacrificed for evaluation of small intestinal lesions. 2) Changes in the migration of neutrophils and platelets induced by IND were evaluated in intestinal vessels by an intravital microscopy. Results: 1) IND induced small intestinal lesions with increases in leukocyte migration and MPO activity. Anti-PSGL-1 antibody and cilostazol ameliorated intestinal lesions and histological damage, along with suppression of MPO activity. 2) Intravital microscopy revealed that administration of IND increased migration of platelet-bearing neutrophils. Cilostazol treatment ameliorated neutrophil migration by blocking interaction between platelets and neutrophils. Conclusion: Our results suggest that enhanced neutrophil migration by interaction with platelets is critically involved in the pathogenesis of IND-induced small intestinal lesions and suggest a potential application of cilostazol for prevention of NSAID-induced small intestinal lesions.

SY2-06

Roles of innate immune system in mice small intestinal ischemia-reperfusion injury

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Toll-like receptors (TLRs) recognize microbial components and trigger the signaling cascade that activates innate and adaptive immunity. Recent studies have shown that the activation of TLR-dependent signaling pathways plays important roles in the pathogenesis of ischemia-reperfusion (I/R) injuries in many organs. All TLRs, except TLR3, use a common adaptor protein, MyD88, to transduce activation signals. This Myd88 signaling pathway induce the assembly of the NLRP3 inflammasome, leading to the caspase-1-dependent processing of pro-IL-1β and pro-IL-18, allowing for the secretion of the mature active forms of these cytokines. We investigated the role of innate immune systems in I/R injury of the small intestine. MyD88 and cyclooxygenase-2 (COX-2) knockout and wild-type mice were subjected to intestinal I/R injury. I/R-induced small intestinal injury was characterized by infiltration of inflammatory cells, disruption of the mucosal epithelium, destruction of villi, and increases in myeloperoxidase activity and mRNA levels of TNF-α and the IL-8 homolog KC. MyD88 deficiency worsened the severity of I/R injury, as assessed using the histological grading system, measuring luminal contents of hemoglobin (a marker of intestinal bleeding), and counting apoptotic epithelial cells, while it inhibited the increase in mRNA expression of TNF-α and KC. I/R significantly enhanced COX-2 expression and increased PGE2 concentration in the small intestine of wild-type mice, which were markedly inhibited by MyD88 deficiency. COX-2 knockout mice were also highly susceptible to intestinal I/R injury. Exogenous PGE2 reduced the severity of injury in both MyD88 and COX-2 knockout mice to the level of wild-type mice. I/R also significantly increased NLRP3 and IL-1β mRNA expression and increased the protein levels of both pro- and mature- IL-1β. TLRs and inflammasome mediated inate immune system may play an important role in I/R injury in the small intestine.

SY2-07

The role of heme oxygenase-1 in intestinal ischemia/reperfusion injury in mice

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Aim: Intestinal ischemia/reperfusion (I/R) injury is a complex, multifactorial, pathophysiological process with high morbidity and mortality, leading to serious difficulty in treatment. Although the mechanisms involved in the pathogenesis of intestinal I/R injury have not been fully elucidated, it is generally believed that oxidative stress and subsequent inflammation play an important role. Heme oxygenase (HO) is the rate-limiting enzyme in the catabolism of heme, followed by production of CO, biliverdin, and free iron. In particular, HO-1 (an inducible form of HO) is believed to confer cytoprotection by inhibiting inflammation, oxidation, and apoptosis, and maintaining microcirculation. In this study, we investigated the role of HO-1 on modulation of inflammatory responses in I/R intestinal injury. In addition, the role of BTB and CNC homolog 1 (Bach1), which is a transcriptional repressor of HO-1, and Nuclear factor-erythroid 2-related factor 2 (Nrf2), which has been known to be a transcriptional factor of HO-1, were investigated in this study.
Methods: Intestinal damage was induced by clamping the superior mesenteric artery for 45 min followed by reperfusion in male wild type mice (C57BL/6), Bach1 deficient mice and Nrf2 deficient mice. Subsequently, intestinal damages were evaluated macroscopically, histologically, and biochemically 4h following reperfusion.
Results: Luminal inflammatory markers such as luminal protein and hemoglobin, tissue levels of TNF-alpha and KC, and subsequent PMN accumulation were significantly elevated in I/R-challenged small intestine of WT mice. These changes were significantly attenuated in Bach1 deficient mice, and obviously deteriorated in Nrf2 deficient mice. In addition, the treatment with an HO-1 inhibitor resulted in the reverse of these attenuations in I/R-challenged small intestine of Bach1 deficient mice.
Conclusions: These findings indicate that HO-1 exhibits protective effects against intestinal I/R injury.

Y-01

Impairment of autoregulation of optic nerve head blood flow against elevated intraocular pressure in patients with type 2 diabetes mellitus during vitreous surgery

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Purpose: To examine whether type 2 diabetes mellitus (DM) affect the autoregulation of optic nerve head blood (ONH) flow against elevation of intraocular pressure during vitreous surgery.
Methods: Thirteen eyes of 13 subjects who had DM (from 7 males and 6 female subjects; mean age of 71.7 ± 6.7 years) and 30 eyes of 30 control subjects without DM (from 22 males and 8 female subjects; mean age of 71.1 ± 6.4 years) underwent vitreous surgery for epiretinal membrane or macula hole. Following a standard 25-gauge microincision vitreous surgery, the mean blur rate (MBR) — indexes of ONH blood flow— in the vascular area (vascular MBR) and the MBR in the tissue area (tissue MBR) were measured using laser speckle flowgraphy. Measurements were conducted before and at 5 and 10 minutes after an elevation of approximately 15mmHg in intraocular pressure. Both parameters represent relative values of ONH blood flow (%, compared to baseline).
Results: Ocular perfusion pressure (mmHg) in subjects with DM and without DM was reduced at both 5 and 10minutes after an intraocular pressure elevation ($P < 0.05$, one-way repeated ANOVA with Bonferroni correction). Vascular MBR in subjects with DM (69.5 ± 11.4 and 63.3 ± 14.9) were significantly lower than that in control subjects (77.4 ± 10.4 and 85.6 ± 12.1) at 5 and 10 minutes after intraocular pressure elevation ($P< 0.05$, $P < 0.001$). Tissue MBR in subjects with DM (75.7 ± 8.4 and 73.5 ± 11.9) were also significantly lower than that in control subjects (84.3 ± 12.2 and 91.5 ± 14.9) at 5 and 10 minutes after intraocular pressure elevation ($P< 0.05$, $P < 0.001$).
Conclusion: Our results suggest that DM impair the autoregulation in the vascular and tissue component of ONH blood flow during vitreous surgery.

Y-02

An experimental study on the effect of treadmill exercise using a mouse brain ischemia model

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Aim: The guideline 2009 for stroke treatment, which is published by The Japan Stroke Society, recommends starting rehabilitation as early as possible for preventing disuse atrophy syndrome and improving the ability of daily life. Recently, it is reported that the very early rehabilitation (within 24h) group has no significant difference in comparison with the usual care group. The aim of this study is to research the efficacy of treadmill exercise in the mouse cerebral ischemia models.
Methods and results: Adult male BALB/c mice were used in this study. Animals were anaesthetized with medetomidine, midazolam, and butorphanol. After sufficient anesthesia, the left carotid artery was ligated with 6-0 silk thread. The mice are allowed to walk on the treadmill with speed 5m/min for 10 minutes as a warm-up exercise. After the rest for 10 minutes, the mice are allowed to run on a treadmill with speed 20m/min for 30 minutes. The series of this exercise is performed every day. The result of this experiment was that the density of the intracerebral microvessels was gradually increased in mouse brain ischemia models. Hypoxia induced factor-1a was expressed in the brains of these mice. We thought that hypoxia induced angiogenesis. Microvessels increased with treadmill exercise even in old mice bred for 12 months. The immunohistochemistry was performed using monoclonal antibodies to the stem cell marker, SSEA-1 and SOX2 protein, but these antibodies were not significant.
Conclusion: We discussed the proliferation of the intracerebral microvessels by using the mouse ischemia model. It was found that intracerebral microvessels were increased in proportion to the days of exercise load. From this result, the exercise has an effect on the increase of microvessels even when they are old. The expression of mouse stem cell marker SSEA-1 protein could not be confirmed.

Y-03

2-accetyl-4-tetrahydroxybutyl imidazole ameliorates dextran sulfate sodium-induced colitis via suppression of sphingosine-1-phosphate lyase

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Background: Sphingosine-1-phospate (S1P) receptor 1 (S1P1) is known to be expressed predominantly on naïve T cells and activated DCs, but negligible on gut-homing effectors. Recently S1P agonist ozanimod is reported to be effective on ulcerative colitis and one of the assumed mechanisms is modulation of DC-T cell encounters. 2-accetyl-4-tetrahydroxybutyl imidazole (THI), an inhibitor of sphingosine-1-phosphate lyase (SPL), also has an immunomodulatory activity, whose ameliorating effect on colitis is still unclear. We aimed to clarify the effect of THI on colitis and the migration of naïve lymphocytes in Peyer's patch (PP). Methods: Study1: Male C57BL/6 mice (8 weeks) received 3% dextran sulfate sodium (DSS) dissolved in drinking water for 5 days followed by normal water for 2 days. Some mice were pretreated with THI (50mg/L) in drinking water for 3 days before induction of colitis. We investigated clinical score, histological damage by Cooper's score (grade 0-4) and inflammatory gene expressions. Study2: Male Wistar rats (8 weeks) were used to observe migration of lymphocytes in PP. Lymphocytes previously collected from intestinal lymph of another rat (CD62L+ cells were about 63%) were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) and injected into recipient rats. We observed changes of migration in PP with or without THI by a confocal laser scanning microscope for 3 hours. Results: Study1: THI significantly ameliorated DSS-induced colitis histologically and clinically, and suppressed DSS-induced pathogenic mediators such as TNFα, IFNγ and IL-1β. Study2: Attached lymphocytes on high endothelial venules (HEV) emigrated to stroma in about 30 minutes and some of them migrated to lymphatic capillaries under physiological condition, but THI suppressed lymphocytes migration in stroma significantly and they stayed still around the wall of HEV. Conclusion: Intravital observation proved that THI suppressed lymphocytes migration in stroma, suggesting that suppression of naïve-T lymphocytes migration might be one of the mechanisms of ameliorating colitis by THI. This study suggests that SPL inhibitor may become a novel immunosuppressant for IBD therapy.

Y-04

Development of real-time imaging of lung microcirculation in mice

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＜Introduction＞
The onset of vascular hyperpermeability of the lung capillaries is a critical pathological change during critical care. To improve our understanding of the pathophysiology of lung edema, a vital observation technique is needed. The present study sought to develop a pulmonary window technique for observing the fine structure and microcirculation of lung vessels in mice using intravital microscopy. In addition, we attempted to visualize the vasculature and dynamics of blood cells using fluorescent probes.
＜Materials and Methods＞
Male BALB/c mice (12 weeks old) were used. Mice were anesthetized using sevoflurane and xylazine with butorphanol tartrate for maintenance. The mice were thoracotomized at the level of the 3rd to 5th rib, and the observation window was covered with a transparent film and a circular cover glass (7 mm in diameter); the edge of the cover glass was then sealed. This airtight window enabled the observation of pulmonary blood vessels using epi-fluorescence microscopy and confocal microscopy. Next, FITC-dextran and/or rhodamine 6G was injected into the caudal vein to visualize the plasma flow and leukocytes, respectively. DiI-labeled red blood cells were also used for a blood velocity analysis. The captured videos and photographic images were analyzed using ImageJ software to quantify the dynamics of the red blood cells and the leukocytes.
＜Results and Discussion＞
We were able to observe the pulmonary microcirculation in mice through a pulmonary window for as long as two hours. The blood flow rate, number of leukocytes, endothelial interactions, and vessel density were quantified using captured images. Our model can be applied to studies of the pathophysiology of lung edema.

Y-05

Development and experience of small diameter wire for lymphatic vessels

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[Objective] Lymphatico-venous anastomosis is an effective surgical optiont for treatment of chronic lymphedema. Usually, ICG fluorescent lymphangiography is performed to identify subcutaneously collecting lymph ducts, but it is difficult to identify them in thick parts of subcutaneous tissues such as the thigh. Therefore, we developed a small diameter guide wire (lymphatic vessel guide wire: LGW) that can be inserted into lymphatic ducts and tried to identify lymph vessels in the deep subcutaneous region using it.
[Subject and Method] Fluorescent lymphangiography was performed by subcutaneous injection of indocyanine green on the dorsum of the hind limb of experimental porcines. Collecting lymph ducts were identified around the knee joints, and a small incision was made using a microscope and LGW was inserted. I went up the LGW under X-ray fluoroscopy and identified the collecting lymph duct on the proximal side. After several up and down movements, LGW was withdrawn and valve function impairment associated with LGW operation was confirmed by retrograde contrast from the proximal side. In addition, the lymph ducts in the same area were collected and influence on the inner membrane was evaluated pathologically.
[Results] LGW could be easily inserted into any of the collecting lymph ducts in the experimental porcines, and it went on the lymph ducts without resistance. In retrograde contrast, almost no reflux was observed. Histologically, the findings of intimal damage due to scraping of LGW were recognized.
[Discussion] There are MR lymphangiography and SPCET / CT lymphoscintigraphy as a method to visualize lymph ducts in deep subcutaneous tissue. However, these are not real time evaluation methods. It is suggested that this method is useful in directly identifying lymph ducts in deep subcutaneous tissue during surgery. On the other hand, problems such as requiring auxiliary devices such as X-ray fluoroscopy are pointed out as problems.

Y-06

Lymphatic Vessel Conditions in Lymphedema: Multivariate Analysis on 949 Lymphatic Vessels

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Objective: Supermicrosurgical lymphaticovenular anastomosis (LVA) is becoming a choice of treatment for compression-refractory lymphedema. To maximize therapeutic efficacy of LVA, it is important to know factors associated with lymphatic vessel conditions. This study aimed to clarify factors associated with lymphatic vessel diameter.

Methods: Medical charts of 134 lower extremity lymphedema (LEL) patients who underwent LVA were reviewed. External diameter of lymphatic collectors found in LVA surgery was measured under an operative microscope with a crack scale. Intraoperative lymphatic vessel's findings were assessed according to characteristics of limbs, and preoperative indocyanine green (ICG) lymphography findings. ICG lymphography findings were classified into 3 regions; L-region where Linear pattern was shown, S-region where Splash/Stardust pattern were shown, and D-region where Diffuse pattern was shown. Univariate and multivariate analyses were performed to clarify factors associated with lymphatic vessel's diameter.

Results: In 264 limbs, 949 lymphatic vessels were found at 794 surgical sites. Median (range) of lymphatic vessel diameter was 0.45 mm (0.15-2.00 mm). Multivariate analysis revealed that factors associated with larger lymphatic vessel (0.5 mm or larger) were older age [65 or older; odds ratio (OR) 1.403], radiation history (OR 1.622), incision site in the thigh/leg (compared with the groin; OR 1.607/1.628), and S-region/D-region (compared with L-region; OR 0.529/0.047) on ICG lymphography.

Conclusions: Factors associated with lymphatic vessel's diameter was clarified. ICG lymphography patterns had the lowest ORs to predict larger lymphatic vessels suitable for LVA. A lymphatic supermicrosurgeon can predict lymphatic vessel condition based on patient characteristics, anatomical locations, and ICG lymphography findings before making a skin incision. D-region on ICG lymphography should be avoided for LVA.

F-01

Prevention of glycocalyx degradation on sciatic nerves by anti-coagulant in a septic rat model

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The pathogenic mechanisms underlying critical illness polyneuropathy still remain elusive. Endothelial glycocalyx (GCX) might be involved in the maintenance of the vascular permeability barrier and sepsis causes GCX disruption. Our previous studies have been shown lipopolysaccharide (LPS) reduces EEG amplitude and nerve blood flow, and Danaparoid sodium (DS), an anticoagulant can prevent these phenomena. Therefore, we investigated whether LPS administration causes GCX degradation on sciatic nerves in a septic rat model and whether DS prevents these deteriorations.
[Methods] Eighteen male rats were assigned into the control (C group), LPS (L group: consecutive 3 mg/kg/day LPS administration), or LPS with DS (LD-group: LPS with 300 U/kg/day of DS) groups. A catheter was inserted into the common iliac artery for perfusion fixation and both the sciatic nerves were removed. Electron microscope images of GCX were obtained from 4-20 capillary sites on each sciatic nerve and GCX length was determined. Intergroup differences were assessed by one-way ANOVA.
[Results] The L-group had a lower baseline platelet count than the C-group and a higher interleukin-6 level than the C and LD groups. While GCX degradation in the L group was significantly higher than that in the C group, DS showed a significant protective effect on GCX degradation ($p < 0.001$).
[Discussion] This is the first study to report that GCX degradation in capillary occurs not only in an ordinary organ but also on the sciatic nerve in a septic rat model. Sepsis-induced alterations of this structure may compromise the endothelial permeability, causing interstitial fluid shift and generalized edema. GCX degradation might be involved in nerve degeneration associated with prolonged nerve ischemia resulting from nerve edema. Although DS is known to inhibit the effects of coagulation factor Xa and thrombin during sepsis, it might also act as a precursor of GCX because it consists of a mixture of heparin sulfate, dermatan sulfate and chondroitin sulfate.

F-02

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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Objective: The purpose of this study is to investigate the nitric oxide production, hydroxyl radical metabolism and ischemic change of hippocampal CA1 during cerebral ischemia and reperfusion in mice.
Methods: (1) C57BL/6 mice [n=15] were used. Memantine 25 μmol/ 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), and 2,5-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) 8-OHdG immunopositive cell: To evaluate to oxidative stress in Hippocampal CA1 neurons, the ratio of the number of 8-OHdG immunopositive cell was calculated in 72 hours after the start of reperfusion.
Results: (1) Blood pressure: There were no significant differences between the groups. (2) Cerebral blood flow (CBF): There were no significant differences between the groups. (3) NO_2^-; Memantine group (120.9±5.0 %; mean±SD) showed significantly higher than the control group (88.5±18.0) after repurfusion 60 minutes ($p<0.05$). (4) NO_3^-; Memantine group (97.2±10.1) showed significantly higher than the control group (65.3±21.0) at ischemia. (5) 2,3-DHBA; Memantine group (89.1±4.0) showed significantly lower than the control group (102.6±11.5) at ischemia. (6) 8-OHdG immunopositive cell: Memantine group (8.5±8.6) showed significantly lower than the control group (47.6±30.6) ($p<0.01$). 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.

F-03

Transient neurological symptoms in cerebral amyloid angiopathy patients

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Background : Sporadic cerebral amyloid angiopathy(CAA) is a common age related cerebral small vessel disease, characterized by progressive deposition of amyloid-β(Aβ) in the wall of small to medium sized arteries, arterioles and capillaries of the cerebral cortex and overlying leptomeninges. CAA is not only recognized as a cause of spontaneous intracerebral haemorrhage and cognitive impairment, it is also believed to be the cause of transient neurological episodes, sometimes termed 'amyloid spells'. Methods : The subjects are five patients who were clinically diagnosed with cerebral amyloid angiopathy and confirmed amyloid deposits by amyloid-β positron emission tomography imaging. Retrospectively, the presence or absence of transient neurological symptoms was searched by chart. Results : Two of the 5 patients who were diagnosed clinically as CAA and were PiB positive showed transient ischaemic attack(TIA) -like transient neurologic symptoms.Discussion : Although there are a number of small case reports and series, no large systemic studies have investigated the prevalence or semiology of these phenomena. The underlying mechanisms of CAA transient spells remain unclear but could include sizure-like activity, a direct effect of amyloid or bleeding on local cortical function, or spreading cortical depression.

F-04

Mechanisms for the role of cardiotonic pills® and its major components in I/R-induced myocardial energy metabolism disorder and oxidative stress

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Background： Cardiotonic pills® (CP) is a compound Chinese medicine preparation consisting of Salvia miltiorrhiza (SM), Panax notoginseng (PN) and Borneol. CP is widely used in China for treating angina pectoris and intervention injury and has passed phase III clinical trials for treatment of angina pectoris by the US Food and Drug Administration. 3,4-dihydroxy-phenyl lactic acid (DLA) is the major water soluble ingredient of SM, while notoginsenoside R1 (R1) is the major water soluble ingredient of PN. The present study intended to explore the mechanisms for the beneficial effect of CP and its major components on the I/R-induced myocardial energy metabolism disorder and oxidative stress.
Methods: Male Spragu-Dawley (SD) rats were subjected to left descending artery occlusion for 30 min followed by reperfusion. CP, DLA or R1 was administrated by gavage 2 h prior to cardiac ischemia. Twenty four h after reperfusion, myocardium infarct area, heart perfusion, TUNEL-positive cells in myocardium were evaluated, content of ADP and AMP and expression of mitochondrial respiratory chain complex I and V were assessed.
Results: CP, DLA and R1 all are able to improve rat heart perfusion, and reduce myocardium infarction after I/R. DLA and R1 are able to protect complex I and V, respectively, from decrease in expression, while CP is able to protect both complex I and V from decrease in expression, collectively exhibiting a beneficial effect on both oxidative stress and energy metabolism disorder after I/R.
Conclusion: CP improves I/R-induced rat myocardium injury, a potential attributable to DLA and R1 which contribute to regulation of mitochondrial respiratory chain ameliorating oxidative stress and energy metabolism

F-05

Age-related changes in resting blood flow velocity waveforms from facial cheek skin area in healthy Japanese women examined by Laser Speckle Flowgraphy

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Objective: To investigate age-related changes in facial blood flow velocity waveform (BFVW) parameters based on MBR (Mean Blur Rate) from Laser Speckle Flowgraphy (LSFG) in healthy Japanese women.
Methods: One hundred and eighty-three healthy women (mean 46.7 ± 15.1 years) from 20s to 70s participated in the study as volunteers. Laboratory environmental temperature and humidity were adjusted to 20° C and 50%, respectively. MBR of a whole face of a single subject was monitored by LSFG in the semi-supine posture while simultaneously measuring continuous blood pressure by electrocardiogram (ECG) on his radial artery. Facial BFVW correlation analyses of age-related changes in the following parameters defined by Fujii et al. (2011), 1) Skew, 2) Falling rate, 3) BOT, 4) BOS, 5), FAI, 6) RI, 7) Fluctuation and 8) CVC (Cutaneous Vascular Conductance) from MBRs were performed on an LSFG analyzer. In addition, partial correlations controlled for age between the BFVW parameters in the cheek region and HR, SBP, and DBP were also analyzed .
Results & Discussion: There were significant positive linear correlations of age with Skew, Falling rate, and RI ($p<0.001$), and there were significant inverse linear correlations of age with BOS, BOT and CVC ($p<0.001$), suggesting that aging may reduce facial skin blood flow and reduce the ability to retain blood volume. On the other hand, there were significant positive partial correlations of HR with MBR, BOS, and FAI ($p< 0.05$), and significant negative partial correlations of HR with RI, and Fluctuation ($p<0.05$), suggesting that an increase in blood flow volume may be caused by an increase in the steady component of blood flow due to an increase in HR. Furthermore, there was a significant positive partial correlation between SBP and MBR ($p<0.05$) and between pulse pressure and Fluctuation ($p<0.05$), suggesting that an increase in blood pressure may lead to an increase in blood flow volume and that an increase in pulse pressure leads to an increase in the variable component.
Conclusions: Age-related changes in facial BFVW parameters from LSFG are useful for evaluating skin blood flow, which may be beneficial for human health care.

F-06

Single nephron glomerular filtration rate in a mouse

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Micropuncture technique has been contributing to renal physiology a great deal. To measure single nephron glomerular filtration rate (SNGFR), it was necessary to collect fluid samples at a few sites of a neprhon half a century ago. After administration of radiolabelled probes to a rat, a nephron was punctured to block the glomerular capillary and tubular flows with wax by micropuncture technique. The animal was sacrificed and the neprhon was immediately frozen. SNGFR was figured out after scintillation counting of the target samples. Whole procedure was so difficult and complicated. Recently just before the close of 20th century, multi-photon confocal laser microcopy was introduced into the life science study. It has enabled observation under physiological conditions in various tissues of many small animals. The purpose of this study is to measure in-vivo single nephron glomerular filtration rate in a mouse. After anaesthetisation of a mouse (n=3), the carotid artery was canulated to inject dye solutions. The kidney was exposed and small square surface of the renal cortex was peeled for observation. The intravascular space was visualised with BSA-Alexa 594 using multi-photon confocal laser microscopy. Lucifer yellow, another dye solution for the tubules was injected as an intra-arterial bolus and this process was recorded. In the images of the tubule, the time difference and the length of the urinary flow between 2 points were analysed and the diameters of each point were measured. From these values, SNGFR from 14 trials was calculated assuming the tubule is in a cylinder shape. It was 3.5 ± 0.4 nl/min, which was in the same order of the previous reports. We were able to measure SNGFR without difficulty using multi-photon confocal laser microscopy.

F-07

Lymphatic Supermicrosurgery for Improvement of Lymph Circulation: Reconstructive Supermicrosurgery Based on Pathophysiology of Obstructive Lymphedema on 243 Lymphedema Patients

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Objective: Establishment of supermicrosurgery (microvascular anastomosis of 0.5 mm vessel) has allowed supermicrosurgical lymphaticovenular anastomosis (LVA), which is becoming popular for progressive lymphedema with its effectiveness and minimal invasiveness. This study aimed to investigate efficacy of LVA assisted by indocyanine green (ICG) lymphography.
Methods: ICG lymphography-assisted LVA was performed on 243 secondary extremity lymphedema patients. ICG lymphography findings were classified into linear pattern and dermal backflow (DB) patterns (splash, stardust, and diffuse), and severity stage (DB stage) was determined based on lymphography findings. Intraoperative findings were recorded and analyzed according to ICG lymphography patterns. Lymphedema index was used to evaluate perioperative lymphedematous volume change. Postoperative outcomes were evaluated according to DB stages.
Results: LVA resulted in 2381 anastomoses on 454 limbs. There was no perioperative complications. On regions with Linear pattern, lymphatic vessels could be easily detected via millimeter skin incision, whereas longer skin incision was required for detecting lymphatic vessels on regions with Stardust or Diffuse pattern. Lymphatic vessels were likely to become smaller, as lymphography patterns changed from Linear, Stardust, to Diffuse pattern. Postoperatively, 54% of lymphedema patients could be free from compression, and patients with lower DB stage were more likely to be able to be free from postoperative compression. Significant volume reduction was observed in 89% of the patients, and 93% of the patients could be free from cellulitis.
Conclusions: LVA is the least invasive and effective lymphatic surgery for refractory lymphedema. ICG lymphography guides lymph vessel location and condition, and allows prediction of postoperative outcomes, which facilitates safe and effective LVA. Early diagnosis and early treatment is important to maximize therapeutic effect of LVA.

F-08

Retinal sensitivity in nonperfused areas is correlated with macular edema and microaneurysms formation in eyes with retinal vein occlusion

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PURPOSE: To evaluate a relationship between retinal ischemia and macular edema or microaneurysms using multimodal imaging techniques in eyes with retinal vein occlusion (RVO).
METHODS: Sixty-two eyes of 31 patients (29 eyes with branch RVO, 3 with central RVO, and 30 fellow eyes) were included in this study. In all patients, optical coherence tomography angiography (OCTA) was captured in 3×3 millimeters centered on the fovea, and the retinal sensitivity of the 33 points within the same 3×3 millimeters using microperimetry (MP-3) also was examined. The MP-3 images were transferred to the OCTA system, and superimposed on the OCTA images. The retinal thickness including whole retina (from internal limiting membrane [ILM] to retinal pigment epithelium [RPE]), inner retina (from ILM to inner plexiform layer), and outer retina (from ellipsoid zone to RPE) were also measured using OCTA at all of the 33 points. The location of these points in nonperfused areas (NPAs) was divided into the following 3 groups: A. in the middle of the NPAs, B. at the border between the NPA and perfused area, C. near the foveal avascular zone. Then, we checked whether macular edema or microaneurysms were observed at these points using OCT, OCTA, and fluorescein angiography.
RESULTS: The mean retinal sensitivity at all of the 33 points was positively correlated with the visual acuity. In the NPAs, the retinal sensitivity was significantly decreased compared with that in the perfused area, negatively correlated with a duration after disease onset, and positively correlated with the outer retinal thickness, macular edema, and microaneurysms formation. The retinal sensitivity of the B group was significantly higher than that of the other groups. Also, microaneurysms were significantly more frequently detected in the B group, but macular edema was not so.
CONCLUSIONS: Retinal sensitivity in NPAs was positively correlated with macular edema or microaneurysms as well as the outer retinal thickness. These results suggest that retinal ischemia with a decreased perimetric sensitivity could be a negative predictor for macular edema and microaneurysms.

F-09

Detection of Quiescent Choroidal Neovascularization by Optical Coherence Tomography Angiography

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Purpose: Fluorescein angiography (FA) and indocyanine green angiography (ICGA) have been widely used in the diagnosis of Age-Related Macular Degeneration (AMD). Optical Coherence Tomography Angiography (OCTA) is a new method that enables to image chorioretinal microcirculation without dye injection. The purpose of this study was to determine whether OCTA can detect the quiescent asymptomatic choroidal neovascularization (CNV) in Japanese eyes with AMD (including polypoidal choroidal vasculopathy (PCV)), which have no exudates in OCT B-scan and no signs of CNV in FA and ICGA.
Methods: Retrospective chart review of the patients who were diagnosed or suspected as AMD, and underwent FA, ICGA, OCT and OCTA in Nagoya City University Hospital from January to June 2016.OCTA image was compared with FA an ICGA image (Heidelberg Retina Angiograph 2, HRA2, Heidelberg Engineering, Germany) to detect CNV. Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, California, USA) was used for OCT b-scan. XR RTVue Avanti (Optovue, Inc, Fremont, California, USA) was used for OCTA. Twenty eyes which did not show any exudative changes on FA, ICGA or OCT b-scan were enrolled.
Results: In 4 of 20 eyes (20 %), quiescent asymptomatic CNV was detected by OCTA. Average age was 69.1 (62-75), and average LogMAR visual acuity was -0.06 ± 0.04. Three eyes had PCV and one eye had occult CNV. In PCV cases, the CNVs were located in the choroidal capillary layer, and the occult CNV was found in both outer retina layer and choroidal capillary layer. The average CNV size was 0.21 mm^2 which was relatively small.
Conclusions: Our results demonstrate that spectral-domain OCTA is also useful for the detection of quiescent CNV in AMD patient. Since FA or ICGA could not visualize CNV in our study, OCTA might be the first-line examination for screening patients at risk of AMD.

F-10

A preliminary test to visualize lymphatic flow

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Background
Lymphoscintigraphy (LS) and ICG fluorescence lymphography (ICG-LG) are useful modality to diagnose lymphedema. However, Hospitals that have these are few. Ultrasonography is common modality in daily practice independent for specialties. We report a preliminary study to detect the lymphatic vessels and observe its flow in real time in the lower extremities, using the contrast-enhanced ultrasonography (ce-USG).
Methods
A limb of a healthy volunteer was examined her lymphatic vessels of the lower extremities. First, the ICG-LG was performed to mark the superficial lymphatic vessels. Next, the contrast agent (Sonazoid®, Perflubutane: PFB) was injected to the dorsum and lymphatic vessels were detected by ce-USG. To check the optimal condition of injection, the point of it changed 1, 2 and 4 points and the volume in total changed 0.1, 0.2 and 0.8 ml. The lymphatic flow under the manual lymph drainage (MLD) was investigated. Same method was repeated after enough duration.
Results
There is no remarkable side effect in this study. The lymphatic vessels were visible when the contrast agent injected 0.8 ml and according to the line marked by ICG-LG. They run superficial subctuneous area from dorsum to groin continuously. Most of them were around the great saphenous vein. No perforated lymphatic vessel through the faschia was observed. On the inguinal region, some lymphatic vessels come into the lymphatic node (LN) and the internal side of LN was enhanced clearly. We could detect more lymphatic vessels depend on the injected points. During MLD, they were enhanced clearly according to the rhythm of drainage. For the second time, individual lymphatic flow was weak and narrow.
Conclusions
The study shows that the ce-USG could be useful diagnostic modality to detect clearly normal lymphatic vessels and evaluate its functional flow in real-time fashion in the normal limb.
Sometime the lymphatic flow is unstable, and then making a protocol in this study is important to investigate the flow of lymphedema patients.

F-11

Cerebral and brain tumor microcirculation evaluated by ultrasonography with Superb Microvascular Imaging technique during neurosurgical operation

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BACKGROUND: Ultrasonography has been used as a useful tool for clinical investigation of many organs. Ultrasonography monitoring has also been used during neurosurgical operations as a reliable imaging, providing real-time information. We applied the latest innovative imaging technique, Superb Microvascular Imaging (SMI), to brain tumor surgery for detecting very low-flow components.
Methods: Seventeen patients diagnosed with brain tumor underwent neurosurgical operation with ultrasonography monitoring using a US system (Toshiba, Aplio) with the new SMI technique (imaging frequency: 10-12 MHz, frame rate: 28-31 Hz). Features of the SMI images in the grayscale mode include (1) visualization of low-velocity flow with minimal motion artifact, (2) high resolution of images, and (3) high frame rates.
Results: The tumors, tumor vessels, compressed and shifted vessels, and cistern were clearly visualized on the SMI images in the grayscale mode, detailing the characteristics of normal brain tissue (vertically penetrating, fine, straight vessels), glioblastoma (rounding, dilating, and bending vessels), low-grade glioma (fine and straight vessels), meningioma (many large and branching vessels), and lymphoma (less vascular, low echoic tumor) and demonstrating the tumor-defined border. Contrast-enhanced ultrasonograpy images also showed microvascular flow of the normal brain and the brain tumors.
CONCLUSIONS: Ultrasonography with SMI images and contrast-enhanced ultrasonography in the grayscale mode produce pioneering images to recognize cerebral and tumor vessels and to differentiate tumor from surrounding normal tissue.

F-12

Longitudinal two-photon imaging of microvascular remodeling and microglial response to chronic hypoxia in in vivo mouse cortex.

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We previously reported that chronic exposure to hypoxia induces microvascular remodeling, including capillary dilation and new capillary formation, in *in vivo* mouse cerebral cortex. However, physiological mechanisms of the microvascular remodeling and their impacts on brain functions remain largely unknown. The present study was aimed to determine the dynamic interactions between cerebral microcirculation and microglia, the resident immune cells of the brain, under chronic hypoxia in the mouse cortex *in vivo*. We used transgenic CX3CR1-GFP mice (N = 10) in which the cortical microglia genetically expressed green fluorescence protein (GFP), and dynamic behaviors of the GFP-positive microglia and morphological changes in microvasculature labeled with sulforhodamine 101 (0.1 mL, i.p.) were concurrently visualized with two-photon microscopy through a closed cranial window according to a Tomita-Seylaz method (Tomita et al., JCBFM 2005). After surgery of the cranial window (approximately 3 mm in diameter) made over left temporoparietal region under isoflurane anesthesia, the animals were housed in a hypoxic room (8-9% oxygen and nitrogen in balance) for up to 2 weeks. We observed no detectable differences in the cell size, number density, and number of fine processes of the microglia measured with Matlab-based custom-written software, before and after exposure to chronic hypoxia. In accordance with our previous reports, cerebral microvessels significantly dilate and sprout from the existing capillaries. Longitudinal two-photon microscopy further revealed that the capillary sprouts accompanied with the migrating microglia rapidly extend and form a new capillary connection. The findings indicate that microglia participates in the microvascular remodeling of cerebral cortex responding to chronic hypoxia.

F-13

Flow velocity mapping of human brain microcirculation based on transit times of indocyanine green captured with fluorescent microscopy under neurosurgery

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To develop a novel method for mapping flow velocity in the brain surface microcirculation of the patients under neurosurgery, we tested the previously-reported method with transit-time measurements of fluorescent dye, originally developed for mapping flow velocity in the rodent brain microcirculation (Hoshikawa et al., Microcirculation 2015), to the imaging data of human brain microcirculation. Following bolus injection of indocyanine green (ICG), the brain surface microcirculation in the patient under neurosurgery was imaged using fluorescent microscopy equipped with a conventional CCD camera (640 by 480 pixels, a frame rate of 30 Hz). The imaging data were postprocessed with custom-written Matlab software. To correct respiratory motion artifacts during the image acquisition, spatial alignment was first conducted frame by frame. The images were then manually segmented for individual vessel areas, and a length of the single vessel was measured in parallel with the vessel wall. A transit time of the ICG was determined and compared across several fitting methods, including a linear trend and second-order process with dead time. The flow velocity in the vessel area was then measured by dividing the traveling distance of the ICG with the transit time. In the surface artery (0.2-0.3 mm in diameter), our preliminary results showed a mean velocity of 8 ± 6 mm/sec over a distance of 2 mm along the vessel. However, the obtained velocity was lower than the estimated velocity of 12 mm/sec. This could be due to underestimation of the transit times, which suggest that further optimization is needed to accurately determine the transit times based on the ICG traces in the imaging data of human brain microcirculation.

F-14

Dynamic two-photon microscopic imaging of spatiotemporal fluctuations in the volumes of blood plasma and red blood cells in the capillaries of the anesthetized rat brains.

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The goal of the present study is to characterize the spatiotemporal interplay of blood plasma and blood cell flows among the capillary networks and within the vessels in the brain microcirculation. To visualize both blood plasma and red blood cells (RBCs), we used transgenic rats (N = 3) expressing green fluorescent protein (GFP) on erythrocytes and fluorescently labeled blood plasma with sulforhodamine 101. The GFP-positive RBCs and labeled blood plasma were concurrently imaged with two-photon laser scanning fluorescent microscopy (excitation at 910 nm) at a rate of 30-100 frame per sec in the anesthetized rat brains. The obtained images were then analyzed with custom-written Matlab software. First, we conducted segmentation of the images of the RBCs or blood plasma in each frame using a machine learning method trained with fluorescent intensity data sets of the respective fluorescent RBC and plasma images. Then, number of pixels for RBCs and plasma was counted in each vessel per frame. The ratio of the RBC to plasma areas was also calculated in each vessel segment. We observed that the RBC-plasma ratio was largely fluctuated in capillaries (10-40% of mean) compared to the small arterioles and venules (10-20% of mean). Within the single vessel, some capillaries showed significant negative correlations (R = -0.4 to -0.8) between the areas of the RBCs and plasma passages, but not for all capillaries. The difference could be due to a variation of the focal plane against the cross-section of the vessel. Namely, the vessel parallel to the focal plane was subjected to motion artifacts, whereas the vessel vertical to the focal plane showed stable and reproducible measurements. In conclusion, the present method allows for visualization and characterization of local variations in the plasma and RBC flows within and among the single capillaries of the brains.

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To:The Japanese Society for Microcirculation
Tel : 03-3235-6925 / Fax: 03-6280-7483
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事務局記入欄	受付日	登録日	入金日	完了日	

To:The Japanese Society for Microcirculation
Tel : 03-3235-6925 / Fax : 03-6280-7483
e-mail: jsm_jimukyoku@js-micro.com

日本微小循環学会　変更届

申請日	年　月　日
氏　名	

所属機関変更

機関名					
部門名					
役　職					
住　所	〒				
電　話		内　線		FAX	
E-mail					

自宅住所変更

住　所	〒			
電　話		FAX		
E-mail				

名義変更(※新姓をご記入ください)

氏　名	
フリガナ	

送付先変更　☐所属機関　☐自宅

名簿掲載　☐所属機関　掲載承諾　☐自宅　掲載承諾　☐非承諾(※氏名のみ公開)

個人情報保護のためFAX誤送信にご注意ください
FAX機の種類により、番号の頭に「0」ゼロをつける必要があります。機種をご確認の上、FAXを送信してください。

事務局記入欄	受付日	登　録	確　認	備　考

To:**The Japanese Society for Microcirculation**
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The Japanese Society for Microcirculation

MEMBERSHIP REGISTRATION FORM

	(Family name)	(Middle name)	(Given name)
Name			

	(Month)	(Date)	(Year)
Date of birth	/	/	

Affiliation

Present Position				
Organization				
Office Address				
	Zip		Country	
Phone				
FAX				
E-mail				

Highest degree	
Major field of study (including year of completion)	

Annual membership fee: JPY ¥3,000 per year

Could you please send the membership fee by international bank transfer to the following bank:

Bank name	**Sumitomo Mitsui Banking Corporation, Kojimachi Branch**
Account name	**The Japanese Society for Microcirculation, Makoto Suematsu**
Account number	**2972105**
Swift number	**SMBCJPJT**

JS-Micro Staff Only	acceptance	membership fee			