Abstracts

IL

Interaction of electrical activity with the microcirculation in the visual cortex and the retina

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In the neocortex, neurons with similar response properties are often clustered together in column-like structures, giving rise to what has become known as functional architecture.

Optical imaging of intrinsic signals in the neocortex reaches the striking detail of ~ 50μm, and, it has allowed to characterize the metabolic and haemodynamic responses induced by sensory-evoked neuronal activity. Here, we will review those findings about the spatio-temporal characteristics of neurovascular coupling, and the major finding based on optical imaging of intrinsic signals.

The precise neuronal origin of the hemodynamic responses measured by optical imaging, laser Doppler flowmetry, or fMRI is still disputed, in spite of its importance. Knowing, for example, whether a measured BOLD response reflects neuronal spiking, sub-threshold membrane potential changes, and some non specific vascular activation, is crucial for correctly interpreting the measurement in terms of input, output, and local processing activity in a given cortical volume. It is beyond the scope of this review to cover these important issues. Some of those subjects have been recently reviewed by others (Arthurs OJ & Boniface S, 2002; Attwell D & Iadecola C, 2002; Kim SG, 2003; Lauritzen M & Gold L, 2003; Logothetis NK, 2003; Logothetis NK & Wandell B, 2004; Lauritzen M, 2005, Vanzetta and Grinvald, 2009).

The high complexity of the interactions between neuronal activity and brain microcirculatory responses set a formidable challenge to accurately translating the hemodynamic responses into neuronal ones. A universal neuronal-to-hemodynamic transfer function, valid for all tasks, brain areas and structures might not even exist, especially when different volume scales are to be considered. It is to be hoped that these issues will be elucidated by further research, Recent results from voltage sensitive dye imaging, providing an extraordinary temporal resolution in addition to spatial resolution will be described. The aim is to emphasize the limitations of both optical imaging of intrinsic optical imaging and BOLD f-MRI in exploring real time cortical dynamics.

More recently optical imaging proved to be a useful tool also in functional studies of the retina. Retinal reflectance changes in the absence of photic stimulations or in its presen ce carry information about blood velocity, FA like images without any contrast agent, and qualitative oximerty. The images of these parameters are obtained using the Retinal Function Imager (RFI,). In response to photic stimulation, information is provided about metabolic processes underlying light responses in the retina.

SL1

Roles of gaseous mediators in regulation of neurovascular units

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Brain constitutes a unique microvascular system that responds to metabolic alterations in neuro-glial system. Among small molecular metabolites, gases such as NO, CO and H_2S account for a unique class of mediators that target multiple macromolecules including rate-limiting enzymes. Based on the fact that CO generated by heme oxygenase-2 depends on amounts of O_2 as a substrate, we hypothesize that hypoxia inhibits generation of O_2-derived gases such as CO, and thereby alter the microvascular responses in brain microcirculation. We herein discovered that CO inhibits cystathionine beta-synthase (CBS) (Shintani, et al. Hepatology 2009), the rate-limiting enzyme regulating biosynthesis of thiols and H_2S, a vasodilatory gas. CBS was mined out as a novel CO-responsive enzyme on the basis of metabolome analysis. In the brain slices of newborn wild-type mice, suppression of CO by HO inhibitors causes marked microvascular vasodilation, while application of micromolar CO reverses it. This event did not occur in CBS-KO mice. In both mice, exogenous H_2S causes vasodilation through mechanisms involving K^+ channels. In this system, neural system constitutes a major CO generation, while astrocytes accounts for the H_2S-generating site that might exert vasodilatory effects on pericytes in a paracrine manner. These results suggest that, under hypoxia, neural CO suppression might cause compensatory vasodilation through mechanisms involving the CBS-H_2S system.

SL2

Study of ocular circulation: past, present, and promising future

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Looking back over my over 30-year study of the ocular circulation, first, I focused on the dye-dilution technique with which I showed the retinal blood flow (RBF) alterations during progression of diabetic retinopathy.

Because of the shortcomings of the dye-dilution method, during the second stage of my research period, I focused on the development of the laser Doppler technique with which I measured non-invasively either the relative or even the absolute value of the blood flow especially using the bi-directional method. Since we successfully developed the laser Doppler technique (CLBF model 100, Canon, Tokyo, Japan), with an eye-tracking system, we have been evaluating the RBF in various ocular pathologies.

In the third stage of the retinal blood study, we have developed in vivo and in vitro set-ups. Using cats as an experimental set-up for the in vivo study of the retinal circulation, we showed that flow-induced vasodilation, which is widely used to evaluate vascular endothelial function, occurs in response to the increased RBF during systemic hypoxia. Using a porcine model to study isolated vessels in the retinal circulation in vitro, we also showed that endothelial function is impaired by acute hyperglycemia in the retinal arterioles via increased oxidative stress. In this vitro study, we examined the effects of several drugs on the retinal vascular function and reported for example, that simvastatin and resveratrol cause vasodilation in the retinal arterioles, suggesting that these drugs potentially can improve the RBF in patients with diabetes mellitus.

We believe that translational research, from in vitro, in vivo to clinical set-ups, of the ocular circulation is useful for the development of novel treatments for ocular diseases in the near future. The promising future of the study of the RBF will be discussed based on over 30 years of research experience.

S-1

Keynote lecture: Cellular actions of angiogenesis inhibitors in cancer

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Abnormalities of blood vessels in tumors are widely exploited for diagnosis and treatment of cancer. Inhibitors that block the action of vascular endothelial growth factor (VEGF) or other growth factors that drive angiogenesis take advantage of these abnormalities. Inhibition of VEGF signaling stops sprouting angiogenesis, triggers regression of VEGF-dependent tumor vessels, and makes tumor vessels that survive more normal. These changes in the vasculature have complex effects on tumor cells. Destruction of tumor vessels can lead to intratumoral hypoxia, apoptosis or necrosis of tumor cells, and augmented invasiveness and metastasis in some preclinical tumor models. The mechanism underlying the increased tumor aggressiveness after inhibition of VEGF is not fully understood, but activation of epithelial-mesenchymal transition (EMT) and hepatocyte growth factor (HGF, scatter factor) signaling through its receptor HGFR/c-Met are likely factors. Multi-targeted inhibitors of receptor tyrosine kinases that promote angiogenesis, EMT, invasion, and metastasis have the potential of limiting tumor growth through effects on the tumor vasculature and of concurrently reducing tumor invasiveness and metastasis. Agents currently in development for blocking c-Met signaling together with VEGF-receptor signaling have been found to inhibit angiogenesis and reduce tumor invasiveness and metastasis in preclinical models.

S-2

Roles of prostanoids in enhancement of angiogenesis and lymphangiogenesis in pathological conditions

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Inflammation influences the pathogenesis of cancers by induction of genome damage, proliferation in stromal cells, and generation of inflammatory mediators. Angiogenesis is also a critical step for development and metastasis of cancers. Proinflammatory mediators, such as prostaglandins (PGs) may have cell-autonomous effects on tumor cells in autocrine fashion, however, our results from LLC (Lewis lung carcinoma) implantation models in knockout mice which lack the host receptor signaling clarified that host stromal signaling of a PGE receptor, EP3 has a crucial role in tumor-associated angiogenesis through the induction of proaniogenic growth factors, and exhibited the landscaping effects on tumor cells. An EP3 antagonist inhibited tumor-associated angiogenesis in wild type mice (WT), but not in EP3 knockout mice, suggesting that host EP3 receptor signaling is important in prevention of tumor-associated angiogenesis. Further, bone marrow transplantation experiment revealed that recruitment of bone marrow cells which expressed EP3 is critical for angiogenesis in vivo. Our recent results also suggested that lymphangiogenesis observed during chronic inflammation and tumor growth was regulated by COX-2 and EP signaling. mPGES-1 (microsomal prostaglandin E synthase-1) is a stimulus-inducible enzyme that functions downstream of COX-2 in the PGE_2 biosynthesis pathway. LLC cells implanted subcutaneously into mPGES-1-knockout mice grew more slowly than did those grafted into WT, with concomitant decreases in the density of microvascular networks, the expression of vascular endothelial growth factor, and the activity of matrix metalloproteinase-2. Lung metastasis of intravenously injected LLC cells was also significantly less obvious in mPGES-1-null mice than in wild-type mice. Thus, control of EP signaling and mPGES-1 activity as well as recruitment of EP-expressing cells in the tumor microenvironment is likely to be a novel therapeutic target for cancers.

S-3

Evaluation of shear stress stimulation for gene and protein expression in lymphatic endothelial cells: with special reference to functional regeneration of collecting lymph vessels.

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Recently, we demonstrated that collecting lymph vessels with smooth muscles in the walls are able to regenerate after resection of the vessels in adult murine tissues of hind legs. An activation of VEGF-C-VEGF receptor 3 plays critical roles in the reconnection of the collecting lymph vessels. However, regulation of mechanical forces, i.e., shear stress or pressure on the reconnection of collecting lymph vessels remains unknown. Therefore, we have attempted to evaluate the effects of shear stress stimulation on gene and protein expression in cultured lymphatic endothelial cells isolated from collecting lymph vessels.

Shear stress at 0.5 and 1.0 dyn/cm^2 increased ecNOS immunohistochemical, mRNA, and protein expression in cultured human LECs. The same strength of shear stress also produced a significant release of ATP from the LECs. Exogenous ATP ranging from 10^{-9} to 10^{-6}M produced a significant increase in ecNOS immunohistochemical expression in a dose-dependent manner. The shear stress-mediated increases in ecNOS mRNA expression in human LECs were significantly reduced by 3mM TEA, 10^{-4}M apamin, 10^{-9}M iberiotoxin, 10^{-5}M 2-APB, or 10^{-5}M xestospongin C, but not 10^{-5}M glybenclamide, or 10^{-5}M nifedipine. The shear stress-mediated increases in ecNOS mRNA expression were significantly potentiated by pinacidil or NS1619 in a dose-dependent manner. The immunohistochemical expressions of small- (SK_{Ca}) and big-conductance (BK_{Ca}) Ca^{2+}-activated K^+ channels were confirmed on the surface of human LECs. These findings suggest that shear stress produces a significant release of ATP from LECs, which activates the purinergic P2X/2Y receptor and then facilitates ecNOS mRNA and protein expression through 1,4,5 IP3-mediated release of intracellular Ca^{2+} ions and activation of the Ca^{2+}-activated K^+ channels in LECs. In conclusion, shear stress or intraluminal pressure produced by lymph re-flow through the reconnected lymph vessels may play regulatory roles in gene and protein expression in the endothelial cells or smooth muscles of the reconnected vessels.

S-4

Restoration of angiogenic directions in ischemic retinopathy

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To date, the developmental angiogenesis model in postnatal mouse retinas has greatly contributed to uncovering the general principles involved in sprouting angiogenesis, especially those determining the angiogenic directions. Nonetheless, despite an increasing list of signaling molecules that mediate attraction or retraction of endothelial filopodia, little is known about what signaling defects are responsible for the disoriented vascular outgrowth in pathological settings, including ischemic retinopathies seen in diabetes and premature infants. We addressed these open issues by comparatively analyzing the developmental and pathological angiogenesis in mouse retinas. During development, VEGF upregulates endothelial PlexinD1 expression in growing blood vessels. Neuron-derived Sema3E signals to PlexinD1 and activates RhoJ small GTPase, thereby counteracting VEGF-induced filopodia projections and defining the retinal vascular pathfinding. In a mouse model of ischemic retinopathy, enhanced expression of PlexinD1 in extraretinal vessels prevents VEGF-induced disoriented projections of the endothelial filopodia. By targeting this differentially expressed PlexinD1, intravitreal administration of Sema3E protein selectively suppresses extraretinal vascular outgrowth without affecting the desired regeneration of the retinal vasculature. Our study presents a novel paradigm of vascular regeneration therapy to treat ischemic diseases, not only in retinas but also in other organs.

Y-1

An inducible prostaglandin E synthase, mPGES-1 regulates growth of endometrial tissues and angiogenesis in mouse transplantation model

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Endometriosis is one of the most common gynecological diseases in women of reproductive age. Although cyclooxygenase (COX)-2 inhibitors are effective in the treatment of endometriosis, the adverse cardiovascular effects associated with these inhibitors have limited their use. Microsomal prostaglandin E synthase-1 (mPGES-1) is an inducible enzyme downstream of COX-2 in prostaglandin E_2 biosynthesis. Previously, we developed mPGES-1 knockout mice (mPGES-$1^{-/-}$) and have identified the roles of ectopic lesion- and host-associated mPGES-1 in angiogenesis and the growth of endometrial tissues. When mPGES-$1^{-/-}$ endometrial fragments were implanted into wild type (WT) mice (mPGES-$1^{-/-}$→WT), or WT fragments implanted into mPGES-$1^{-/-}$ mice (WT→mPGES-$1^{-/-}$), the growth of the implants was suppressed at days 14 and 28 after implantation, compared to WT→WT transplantation. A greater degree of suppression was observed in mPGES-$1^{-/-}$ → mPGES-$1^{-/-}$ transplanted mice. After WT→WT transplantation, mPGES-1 expression was localized at the border of the implanted endometrial tissues. Microvessel density, determined by CD31 immunostaining, was markedly suppressed in the endometrial fragments in mPGES-$1^{-/-}$→mPGES-$1^{-/-}$ transplantation mice, with some suppression also observed in the mPGES-$1^{-/-}$→WT and WT→mPGES-$1^{-/-}$ groups. The expression of vascular endothelial growth factor was significantly reduced in the endometrial fragments in mPGES-$1^{-/-}$→mPGES-$1^{-/-}$ transplantation mice at days 14 and 28, in comparison to the WT→WT group. To clarify roles of mPGES-1 in the induction of a proangiogenic factor, VEGF-A, we isolated the endometrial tissues, and determined the mRNA levels of VEGF-A by real time PCR. The expressions were significantly reduced in the mPGES-$1^{-/-}$ endometrial tissues implanted in mPGES-$1^{-/-}$ in comparison with those in WT→WT transplantation. A VEGF-A neutralizing antibody and a VEGFR-2 tyrosine kinase inhibitor suppressed the growth of endometrial tissues and angiogenesis in WT→WT. These results suggested that mPGES-1 enhanced angiogenesis and growth of the endometrial implants, and indicate that mPGES-1 may become a good therapeutic target for endometriosis.

Y-2

Gingival vascular functions are altered in type 2 diabetes mellitus model and/or periodontitis model

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Purpose: Vascular endothelial function has been estimated using plethysmography, which has verified reactive hyperemia of the forearm. The association of vascular reactivity between diabetes and periodontal disease has not been clarified. The alterations in vascular endothelial and smooth muscle functions caused by changes in blood flow as a result of oral gingival reactive hyperemia (GRH) were determined on type 2 diabetes mellitus and/or periodontal disease.
Method: Gingival blood flow was measured by using a laser Doppler flowmetry (LDF) for 31 weeks in Wistar rats (Ws), *porphyromonas gingivalis* (*P. gingivalis*) infection to Ws (Ws+*P.g*; periodontitis animal model), Goto-Kakizaki rats (GKs; diabetes animal model), and *P. gingivalis* infection to GKs (GKs+*P.g*; periodontal and diabetes animal model), respectively. GKs were used as a type 2 diabetes mellitus model. To evaluate the functions of endothelial and smooth muscle, reactive hyperemia was elicited by release of occlusive gingival compression for 1 minute after treatment with acethylcholine and nitroglycerine via gingival mucosa.
Result: GRH in Ws was significantly increased after treatment with acethylcholine or nitroglycerine. However, GRH in GKs was significantly decreased compared to Ws. Also GRH was attenuated by periodontal disease, and this effect was also remarkable in the diabetes model.

We demonstrated that GRH was decreased in diabetes and/or periodontal disease animal models due to increasing oxidative stress in the gingival circulation using by LDF and electron spin resonance (ESR).
Discussion: These results suggest that the disruption of oral vascular functions would be associated with type 2 diabetes. Periodontal disease reduces gingival vascular reactivity and decreases reactive hyperemia, and this effect is accelerated by diabetes. Further, it would be likely that measurements of GRH may be used to estimate vascular endothelial function in the general circulation such as reactive hyperemia of the forearm.

Y-3

Spatiotemporal changes in labile adenine nucleotides upon focal cerebral ischemia revealed by a combined approach of quantitative metabolome analysis and imaging mass spectrometry

Y-4

A pathological role of semaphorin3E in impaired angiogenesis, microcirculatory dysfunction of diabetes

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The axon-guiding molecules known as semaphorins and their specific receptors (plexins) regulate the vascular pattern and play an important role in development of the vascular network during embryogenesis. However, it remains unclear whether these molecules are involved in postnatal angiogenesis. Here we report that semaphorin3E (Sema3E), one of the class 3 semaphorins, and its specific receptor plexinD1 inhibit angiogenesis in adults. Sema3E inhibited cell growth and tube formation by suppressing the vascular endothelial growth factor (VEGF) signaling pathway. Expression of Sema3E and plexinD1 was markedly up-regulated in ischemic limbs of mice, and inhibition of this pathway by introduction of the plexinD1-Fc gene or disruption of Sema3E led to significant improvement of revascularization. We also found that Sema3E-deficient mice showed better blood recovery and a larger vessel area in their ischemic limbs than wild-type mice, suggesting that Sema3E/plexinD1 negatively regulate postnatal angiogenesis. Next, we identified a putative binding element of p53, the tumor suppressor protein, within the promotor region of the Sema3E gene, and found that hypoxia up-regulated Sema3E expression by activating p53 in endothelial cells. Consistent with our in vitro data, up-regulation of Sema3E expression was abolished in the ischemic limbs of p53-deficient mice, suggesting that an increase of p53 promotes Sema3E expression in ischemic tissue and thus inhibits blood flow recovery. It has been reported that angiogenic response to ischemia is attenuated in diabetes. We found that expression of p53 was increased in diabetic mice and this increase was further enhanced by ischemia. Likewise, expression levels of Sema3E were significantly higher in diabetic mice than in control mice. Consequently, blood flow recovery after VEGF treatment was impaired in diabetic mice compared with VEGF-treated control mice. These changes were effectively reversed by additional introduction of the plexinD1-Fc gene. These results indicate that Sema3E negatively regulates postnatal angiogenesis and suggest that inhibition of Sema3E would be a novel strategy for therapeutic angiogenesis, especially when VEGF treatment is ineffective, such as in the diabetic state.

F-1

Beneficial or detrimental effect of hypoxic response in cardiomyocytes

F-2

Cystathionine b-synthase is essential for hypoxia-induced vasodilatation in the brain microcirculation

F-3

Intravital two-photon imaging of penetrating and precapillary arterioles and regional NADH metabolism under hypoxia in murine cerebral parenchyma

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Purpose: Cerebral arterioles dilate when oxygen tension of the surrounding tissue decreases. However, molecular mechanisms of hypoxic-induced vasodilatation in the brain are not fully understood. To understand these mechanisms, it is important to visualize spatio-temporal relationship between regional metabolic changes and microvascular responses in hypoxic brain. We aimed to visualize O_2-dependent regional metabolic changes and microvascular responses *in vivo* in subsurface of cerebral parenchyma and arterioles composing neurovascular unit with surrounding astrocytes and neurons.
Methods: Anesthetized mice were equipped with either a conventional cranial-window with craniotomy or a thinned-skull preparation. Mice were mechanically ventilated to avoid hypercapnia and exposed to systemic isobaric hypoxia which was induced by the inhalation of 10% O_2 for 30 min. Using two-photon laser scanning microscopy, NADH autofluorescence and diameter changes in penetrating and precapillary arterioles within the parenchyma were visualized to examine their temporal alterations.
Results: With the conventional cranial-window preparation, marked vertical displacement of the tissue occurred through edema during hypoxia. With a thinned-skull preparation, however, such hypoxia-induced displacement was diminished, enabling us to examine acute spatio-temporal changes in diameters of penetrating and precapillary arterioles and NADH autofluorescence. Vasodilatation of these microvessels was evoked within 1 min after hypoxia, and sustained during the entire observation period despite the absence of hypercapnia. This event coincided with parenchymal NADH elevation, but the onset and peak dilatory responses of the penetrating arterioles preceded the local metabolic response of the parenchyma.
Conclusion: We visualized rapid vasodilatory responses in penetrating arterioles preceding parenchymal NADH elevation during hypoxia by the thinned-skull preparation combined with two-photon intravital microscopy. This observation suggests the presence of acute hypoxia-sensing mechanisms involving a specific hierarchy of cortical arterioles within the neurovascular unit.

F-4

Astrocytes and pericytes cooperatively maintain capillary-like structure composed of endothelial cells on gel matrix

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Purpose: Gliovascular complex (GVC) is a structural as well as functional unit located at the interface between cerebral blood flow and neural network. Even though its physiological roles are partially revealed, astroglial contribution to the maintenance of cerebral vasculature which is tightly wired with the neuronal network is only incompletely understood. To understand the role of astrocytes and pericytes in maintaining the integrity of the capillaries, GVC model *in vitro* was developed.
Methods: Human brain microvascular endothelial cells (ECs, ACBRI 376) in suspension were plated on gel matrix (Matrigel) and incubated under 5% CO_2 at 37℃. After 6 hours when capillary-like structure (CLS) was formed, human brain astrocytes (ACBRI 371) and /or human cortex pericytes (ACBRI 499) were applied on the CLS. Calcein Green and /or PKH26 Red fluorescent linker kit was used for the cell labeling. In some setting, GFP-expressing human brain microvascular endothelial cells (AGP cAP-0002GFP) were also utilized for the visualization of ECs. The effects of anti-angiogenic factors on astrocytes /pericytes migration and CLS maintenance were evaluated.
Results: After 6 hours, primitive CLS changed its form with the elongated ECs bound to each other more tightly and with the discontinued branches pruned off. Without any supportive cells, CLS became thin and deteriorated at 24 hours, suggesting apoptosis. Pericytes and astrocytes together migrated and adhered to the CLS to construct GVC like structure with pericytes inside and astrocytes outside. Astrocytes alone or together with pericytes to a greater extent suppressed CLS degradation. Fumagillin and suramin, angiogenesis inhibitors, suppressed GVC formation. PDGF receptor inhibitor SU6668 suppressed pericytes/astrocytes migration and accelerated CLS degradation, whereas VEGF receptor inhibitor SU1498 did not suppress pericytes/astrocytes migration and CLS was long maintained with them. Immunohistochemistry revealed aquaporin-4 and agrin expression at the junction of EC and astrocyte.
Conclusion: These results indicate importance of astrocytes as well as pericytes for the maintenance of CLS in cerebral microvasculature.

F-5

Potassium-induced cortical spreading depression suppressed transhemispheric electroencephalogram but did not affect on red blood cell velocity in intraparenchymal capillaries in rats

F-6

Impairment of functional hyperemia is associated with β-amyloid accumulation in small cerebral arteries in somatosensory cortex in APP transgenic mouse

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Purpose: In order to explore the causal relationship between cerebrovascular dysfunction and pathogenesis of Alzheimer's disease, we carried out longitudinal evaluations of CBF response to whisker stimulation and accumulation of β-amyloid (Aβ) in APP transgenic mouse in somatosensory cortex.
Material and Methods: CBF response to whisker stimulation (10 Hz 20 sec) was measured in awake APP transgenic mice with age ranging from 3 to 27 months using LDF every few weeks, and behavior activity was evaluated at the same time using the optical locomotion sensor of a floating ball on which the fixed animal can freely walk (Takuwa et al., 2010). On a separate date, accumulation of amyloid and vessel were imaged using two-photon microscopy with 1024 by 1024 0.5μm-pixels and 4μm z-step. The amyloid and vessels were fluorescently labeled with a 'compound X' (newly-developed by our group) and sulforhodamine-101 (SR101), respectively. The accumulation of vascular amyloid was evaluated by its thickness as a half difference between vessel diameter (SR101) and outer diameter of vascular amyloid (compound X) at several points along a single vessel, and also by its longitudinal gap given by adding all non-amyloid spaces. The accumulation of tissue amyloid was evaluated by added area of all pieces of labeled amyloid in parenchymal tissue.
Results and Discussion: We observed age-dependent decline of evoked CBF over ages from 3 month to 27 month. The decline started about at 17 month. On the other hand, the accumulation of amyloid in the parenchymal tissue and vessel wall of small arteries became detectable about at 14 month. The vascular amyloid extended from small arteries to arterioles from age 14 to 19 month. During this period, the vascular amyloid was increased in thickness (160%) and decreased in gap (80%), and the tissue amyloid was increased to 170%. In conclusion, these preliminary observations suggest that the impairment of neurovascular coupling is tightly linked with the preceding accumulation of Aβ on cerebral small arteries and arterioles.

F-7

Decreased expression of the glycosylated syndecan 4 accompanied with down-regulation of TCRζ in SLE patient T cells

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PURPOSE: Four members of the syndecan (Sdc) families (Sdc1-4) of transmembrane heparan sulfate proteoglycans (HSPGs) are the major source of cell surface HS. HS has been known to play crucial roles in inflammation by binding to the cell surface and matrix proteins, cytokines, and chemokines. Sdc4 (-/-) mice have been reported to present with vascular injury and thrombosis. Previously, we reported down-regulation of TCR/CD3 complex including T-cell receptor ζ (TCRζ) in SLE T cells could lead to aberrant expression of Sdc1 (*J Immunol*, 176: 949, 2006). The aim of this study is to investigate the expression of Sdc families in SLE T cells. METHODS: Peripheral blood T cells were obtained from 11 SLE patients and 8 healthy normal controls. Four members of Sdc family proteins (Sdc1-4) were detected by Western blot (WB) using specific antibody to each Sdc family protein, respectively. Cell surface HS was detected with anti-HS antibody using WB. RESULTS: In WB, there observed no differences in the expression patterns of Sdc1, Sdc2, and Sdc3 between in SLE patient T cells and in healthy normal controls. However, the 35- and 40-kD protein bands detected with anti-Sdc4 antibody were missing in 10 out of 11 SLE patient T cells. TCRζ was also missing in these 10 SLE patients by WB using anti-TCRζ antibody. This Sdc4-related 35-kD protein was absorbed after the treatment of anti-HS antibody. CONCLUSIONS: Decreased expression of the 35-kD glycosylated Sdc4 accompanied by down-regulation of TCRζ could be related to the pathogenesis of SLE, especially with the vascular injury and thrombosis.

F-8

Architecture of blood vessels in the mouse infraorbital nerve

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Purpose: Our study investigates how the effects of a nerve block can be prolonged to maintain therapeutic effects over a long period of time by revealing the dynamics of the microcirculation around peripheral sensory nerves. To establish baseline data for an experiment investigating delayed regeneration after a nerve block of the vascular system in the infraorbital branch of the trigeminal nerve (pure sensory nerve), we examined the normal intraneural vascularisation of the infraorbital nerve from the infraorbital foramen to the peripheral vibrissae to know a normal intraneural vascularisation in a peripheral pure sensory nerve.
Methods: Indian ink was injected into the heart of the mouse for observation of vascular architecture in the infraorbital nerve. Three-dimensional images of blood vessels in the infraorbital nerve were then reconstructed using 3D visualization software.
Result: Optical microscopic observation of the peripheral section of the normal mouse infraorbital nerve (near mouse vibrissae) revealed fascicles of nerve fibers (10-60 μm in diameter) covered with perineurium, with one blood vessel normally present within the fascicle. Optical microscopic observation of the cross-section of normal mouse infraorbital nerve near the infraorbital foramen revealed nerve fiber fascicles (about 150 μm in diameter) covered with epineurium extending from proximal to peripheral areas, increasing in number as they branched. In the nerve fascicle surrounded by the perineurium near the infraorbital foramen, a few blood vessels were distributed. As the nerve fascicle branched and extended, the blood vessels also branched, providing one blood vessel in each nerve fascicle. There were multiple blood vessels in between the epineurium and perineurium. The blood vessels in nerve fascicles consisted of thin branches communicating with blood vessels outside of the perineurium. Nerve fascicles were surrounded by networks of blood vessels communicating with capillaries, arteriolae and venulae. Some blood vessels showed chain-like distribution in nerve fascicles. In the peripheral part of the infraorbital nerve, the blood vessel in the nerve fascicle exited before the nerve entered a vibrissa. There were no blood vessels in the nerve fascicle at the nerve terminal.
Conclusion: The present study demonstrated the significance of the presence of one blood vessel in a nerve fascicle as well as blood vessels surrounding the fascicle.

F-9

Distribution of vascular dendritic cells of bone marrow origin appeared at the non-atherosclerotic normal aorta in bone marrow transplantation chimelic mice

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Purpose: In atherosclerotic lesions or atherosclerosis-predisposed regions, appearance of vascular dendritic cells of bone marrow origin has been reported. However, their distribution has not well known in the non-atherosclerotic normal aorta. Here, using bone marrow transplantation chimeric mice, we examined the appearance and distribution of vascular dendritic cells of bone marrow origin at the non-atherosclerotic normal aorta.
Materials and methods: In this study, we used 6-week old male mice, weighing approximately 10 g. Bone marrow transplantation was performed by using of normal C57BL/6J mice as recipients and EGFP mice (C57BL/6J-Tg: CAG-EGFP transgenic mouse) as donors. Normal C57BL/6J mice were lethally irradiated at a dose of 900 rads per animal by use of an x-ray generator. Then within 4 hours after the irradiation, donor bone marrow cells ($1.5x10^7$ cells/0.3ml) were administered to each recipient mouse via tail vein. After 17, 22, 32, 48, 52, 57, 60, 64, 72 weeks (weighing apporoximately 25-32 g, each n=3), aorta of the chimera mice were perfused with 4% paraformaldehyde, and harvested from the ascending aorta to the abdominal aorta continuously and cut into 16 segments. Specimens were immuno stained with anti-GFP, anti-CD11c antibodies and observed with light and laser scanning confocal microscopes.
Results and Conclusion: No atherosclerotic lesion was seen at the aorta. Vascular dendritic cells of bone marrow origin were mobilized in the non-atherosclerotic aorta in all the mice. In proportion as the mice were older, vascular dendritic cells of bone marrow origin increased and their clusters were seen in almost all the aorta from the aortic orifice to the abdominal aorta. Vascular dendritic cells of bone marrow origin may be some defensive role for atherosclerosis.

F-10

Laser-induced thrombus formation in brain microvasculature of angiotensin II type 2 receptor-knockout mice

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Purpose: Using a laser, we developed a technique to induce thrombus formation in murine brain microvasculature instantaneously. The purpose of this study was to observe the effect of angiotensin II type 2 (AT2) receptor deficient on the process of laser-induced thrombus formation and platelet behavior in the brain microvasculature of mice using intravital fluorescence microscopy.
Methods: C57BL/6J mice (control group, N=7) and AT2 receptor-knockout mice (AT2 knockout group, N=10) were anesthetized with chloral hydrate and inserted a catheter in their cervical vein. Their head was fixed with a head holder, and a cranial window was prepared in the parietal region. Platelets were labeled in vivo by intravenous administration of carboxylfluorescein succinimidylester (CFSE). Laser irradiation (1000 mA, DPSS laser 532 nm, TS-KL/S2; Sankei) was spotted for 4 seconds on pial arteries to induce thrombus formation. Labeled platelets and thrombus were observed continuously with a fluorescence microscope.
Results: After laser irradiation to the pial artery, the complete occlusion rate was not significantly different between in the control group (60%, 12/20 vessels, vessel diameter 26.9±3.3μm) and in the AT2 knockout group (48%, 12/25 vessels, vessel diameter 26.2±3.4μm). Thirty minutes after laser irradiation, the area of platelet thrombus was significantly larger in the AT2 knockout group (555±488 μm^2) than in the control group (358±256 μm^2; P=0.028).
Conclusion: The present study suggests that the AT2 receptor is related to the inhibition of the laser-induced thrombus formation in murine pial arteries.

F-11

The effect of non-newtonian elasticity of vascular walls on aneurism formation in microcirculation

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Purpose: The elasticity of the circulatory vascular wall changes its qualitative nature and is often taken to contribute to aneurism formation even in microcirculation. We have investigated theoretically the relation between non-Newtonian elasticity of an unbroken vascular wall and aneurysm formation in microcirculation, although its primary factor may be some damage to the vascular wall.
Method: By altering the elastic nature of vascular walls devoid of any lesions, we have calculated vascular wall deformations within a network model of circulatory vessels with variable blood flow pressure, one of our assumptions being sinusoidal pressure at the entrance of the thickest circulatory vessel connected to successive bifurcations. We have assumed further that the flow is laminar with Newtonian viscosity, and the deformation of the walls of the vessels is assumed to obey the non-Newtonian polynomial law. Using a nine generation network model with a hierarchical architecture, we have calculated the flow through every vessel and the resultant time-development radius. The calculations had been repeated a countless number of times until reasonable results were obtained.
Results: It turns out that the arteriosclerosis process of the walls in upstream regions would accelerate aneurism formation. The main principle of this phenomenon seems to be similar to that of the familiar high-blood pressure by hyperpiesia enhancement.
Conclusion: A theoretical calculation model of a network vascular system with Non-Newtonian wall elasticity is presented. Using this model we were able to confirm theoretically that the enhancement of aneurism formation in an arteriosclerotic microvascular network can take place, with additional high-blood pressure by hyperpiesia in the peripheral circulation.

F-12

Ribosomal protein S19-prothrombin complex in plasma and its role in coagulum resorption

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Purpose: Ribosomal protein S19 (RP S19) is a component of the small subunit of ribosome. During apoptosis RP S19 is cross-linked by a cellular transglutaminase to become homo-oligomer having monocyte/macrophage selective chemoattractant capacity. The RP S19 oligomer is released extracellularly, recruits monocytes/macrophages and makes them phagocytically clear the apoptotic cells. We have recently reported that RP S19 is present in plasma and is oligomerized by factor XIIIa (plasma transglutaminase) during blood coagulation (Semba et al., 2010 Am. J. Pathol.). Apart from the origin of plasma RP S19, several questions were raised; how RP S19 having only 16 kDa size can stay in the blood stream escaping glomerular filtration, and what the biological role of the RP S19 oligomer in coagulum is. The current experiments were performed to solve these questions.
Experiments: We made a hypothesis that RP S19 would be present in blood stream as a complex with a plasma protein. Using a recombinant RP S19 immobilized on gel beads, we separated a binding protein from plasma and identified to be prothrombin by protein sequencing analysis. We pull down plasma RP S19 with anti-RP S19 antibodies and confirmed co-precipitation of prothrombin. In warfarin-treated patients, the plasma concentration of RP S19 was greatly reduced. Separately, we made another hypothesis that the RP S19 oligomer formed during blood coagulation would recruit monocytes/macrophages to the coagulum and would make the leukocytes clear the coagulum. We took cardiac blood from guinea pig, made the blood clot, and inserted the coagulum into the peritoneal cavity. Various days later we recovered the coagulum and observed macroscopically and microscopically. The coagulum was rapidly covered by macrophages within 24 h concomitant with an intra-coagulum macrophage infiltration and the coagulum was resorbed by day 7. When anti-RP S19 antibodies were premixed into the inserted coagulum, the attachment and penetration by macrophages decreased and the coagulum resorption retarded.
Conclusion: RP S19 is present in the blood stream as a complex with prothrombin. During blood coagulation, plasma RP S19 is oligomerized; the oligomer then recruits monocytes/macrophages and makes the leukocytes phagocytically clear the coagulum.

F-13

Panretinal photocoagulation restores retinal microcirculatory disturbance in diabetic retinopathy - evaluation with retinal function imager®

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Purpose: Retinal Function Imager ® (RFI) (Optical Imaging Ltd., Rehovot, Israel) implements a new, non-invasive diagnostic approach to measuring blood flow velocity, revealing vascular network structure of the retina. Burgansky-Eliash et al reported that decreased retinal blood-flow velocities in diabetic patients by RFI.
The purpose of this study was to evaluate the effect of panretinal photocoagulation (PRP) on the retinal microcirculatory disturbance in patients with diabetic retinopathy (DR).
Methods: Blood flow velocities in the perifoveal retinal vessels were analyzed by RFI in 22 eyes with diabetic retinopathy. Thirteen eyes (59%) had not been treated with PRP, and nine eyes (41%) were treated with PRP more than 1 year ago, and all 9 eyes were stable. And for control, healthy subjects were also analyzed.
Results: The average velocity in the untreated eyes decreased significantly compare to healthy subjects (p=0.02 in arterial velocity, p=0.005 in venous velocity). The average velocity in the retinal arteries in the eyes with PRP was significantly higher than in the untreated eyes (3.75 +/- 0.29 vs. 2.97 +/- 0.16 mm/sec, p=0.02). There was no significant difference in the venous velocity (2.42+/- 0.21 vs. 2.19 +/- 0.14 mm/sec, respectively).
Conclusions: Our data suggested that PRP restores the impaired hemodynamics in the retinal microcirculation in DR and RFI might be a useful modality to evaluate blood flow velocity in the various type of diabetic retinopathy, to assess the efficacy of PRP.

F-14

Relationship between retinal fractal dimensions and retinal circulation in patients with type 2 diabetes mellitus

F-15

Early diabetes and slit membrane

F-16

Effects of ppar gamma agonist on no production, oh- metabolism and ischemic change of hippocampal ca1 during cerebral ischemia and reperfusion in db/db mice

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Methods: (1) male *db/db* mice [n=16] were used. Pioglitazone 20 mg/kg/day was given in 8 mice for 4 days (pioglitazone group), and others were used as contol group. Both NO production and OH^- 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 nitric oxide metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), in the dialysate were determined using the Griess reaction. (2) Hippocampal CA1 neurons were analyzed into three phases (severe ischemia, moderate ischemia, survive), and the ratio of the number of surviving neurons was calculated as survival rate.
Results: (1) Blood Pressure: pioglitazone group (65.8±12.9 mmHg; mean±SD) showed significantly lower than that of the control group (104.7±31.7), 30 minutes before ischemia, ischemia and 10-50 minutes after the start of reperfusion ($p<0.05$). (2) CBF: pioglitazone group (82.4±22.9%) showed significantly higher than that of the control group (59.9±11.0), 50, 90-100 minutes after the start of reperfusion. (3) NO metabolites: 1) NO_2^- ; There were no significant differences between the groups. 2) NO_3^- ; pioglitazone group (121.7±15.9%) showed significantly higher than that of the control group (104.0±10.7), 20-40 minutes after the start of reperfusion. (4) OH- metabolites: 1) 2,3-DHBA; pioglitazone group (98.6±1.45%) showed significantly lower than that of the control group (106.1±4.3), 20 minutes after the start of reperfusion. 2) 2,5-DHBA; There were no significant differences between the groups. (5) Survival rate: There were no significant differences between the groups.
Conclusion: Pioglitazone may protect against cerebral ischemic injury following ischemia and repurfusion.

F-17

Angiotensin type 1 receptor blockers enhances H2O2-induced vasodilatation and improves diabetes mellitus-induced endothelial dysfunction in canine coronary native collateral microcirculation in vivo

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Purpose: We have previously demonstrated that endothelium-derived hydrogen peroxide (H_2O_2) plays an important role in canine coronary microcirculation in vivo. We examined whether angiotensin type 1 receptor blockers (ARB) enhanced H_2O_2-induced vasodilatation in canine coronary collateral microvessels in vivo and if so, whether such beneficial effects of ARB acutely improves coronary collateral vasodilatation in diabetes mellitus (DM).
Methods: Canine subepicardial native collateral small arteries (CSA, >100 μm) and arterioles (CA, <100 μm) were observed by an intravital microscope under cyclooxygenase blockade (ibuprofen, iv). Coronary CSA and CA were visually traced between left anterior descending arteries and left circumflex coronary arteries (LCX) with an injection of indocyanine green. Coronary vascular responses to endothelium-dependent (bradykinin) and -independent (nitroprusside) vasodilators were examined under the following 6 conditions (n=5 each); (i) control, (ii) ARB (olmesartan), (iii) ARB+catalase (an enzyme that dismutates H_2O_2 into water and oxygen), (iv) ARB+L-NMMA (NO synthase inhibitor)+catalase, (v) DM (alloxan) and (vi) DM+ARB. Bradykinin was continuously and retrogradely infused into the diagonal branch of LCX.
Results: ARB significantly increased the bradykinin-induced coronary vasodilatation compared with control in both sized arteries (CSA 10±1 vs. 5±1%, CA 25±4 vs. 20±4%, both $P < 0.01$). ARB+catalase significantly reduced the vasodilatation in CA (10±2%, $P < 0.01$) but not in CSA (8±2%), whereas ARB+L-NMMA+catalase decreased the vasodilatation in both sized arteries (CSA 2±1%, CA 6±1%, both $P < 0.01$). DM significantly decreased the coronary vasodilatation compared with control in both sized arteries (CSA 1±1 vs. 5±1%, CA 7±2 vs. vs. 20±4%, both $P < 0.01$), whereas ARB improved the vasodilatation in both sized arteries (CSA 4±3%, CA 16±3%, both $P < 0.01$). Coronary vasodilatation to nitroprusside was comparable under all conditions.
Conclusions: ARB enhances H_2O_2-induced canine coronary collateral vasodilatation and improves coronary microvascular endothelial function in DM in vivo.

F-18

Dual imaging of spatiotemporal evolution of microvasular red blood cell and plasma flow distribution in anesthetized rat cerebral cortex

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Purpose: To probe the controlling mechanism of red blood cell (RBC) and plasma flow distribution in the brain microcirculation, we performed dual concurrent imaging of fluorescently labeled RBC and plasma in the microvascular networks of rat cerebral cortex.
Methods: Green fluorescent protein (GFP) expressed transgenic Wistar rats (230-480 g, N = 8) were used for the experiments. The animal was anesthetized with isoflurane and a portion of the left skull over the somatosensory area was removed. The opened area was filled with saline. An intravenous bolus injection of Qdot 605 (1 μM in buffered solution, Invitrogen) was performed (1 ml/kg) to label blood plasma. Dual imaging of GFP-expressed RBC (green) and plasma (red-Qdot) were then performed with multi-photon excitation fluorescent microscopy (TCS-SP5MP, Leica Microsystems) at 900-nm excitation (MaiTai HP, Spectra-Physics). An in-plane resolution was 0.89 μm and image size was 512 by 512 pixels (456 by 456 μm). A volume scan was performed from a depth of 0.6 mm to the cortical surface with a step size of 0.01 mm. Three physiological conditions were tested as normal, systemic injection of vasodilator (sodium-nitroprusside), and functional hyperemia (electrical pulse forepaw stimulation, 6 Hz for 3 sec) in each animal.
Results and Discussion: We observed that a width of the cross-sectional intensity was a couple of micro-meters larger in plasma than that of RBC in all sizes of arteries and veins, which indicate a measurable plasma layer (> 0.89 μm at least). In parenchymal capillary regions (< 8 μm in a diameter), separated distribution of RBC and plasma was observed, indicating that florescent signals from RBC and plasma were longitudinally separable along the single capillaries. A ratio of the RBC and plasma occupancy in single capillaries varied vessel-to-vessels. Upon local (functional hyperemia) and global (systemic vasodilator) stimulation, the distribution dynamically changed within and across the networks, which demonstrates a dynamically-variable hematocrit in local microcirculation of the cerebral parenchyma.

F-19

Doppler optical coherence tomography revealed the structure and function of penetrating microvessels of rat cerebral cortex in vivo

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Purpose: Penetrating arterioles and venules are considered to play an important role in maintenance of cerebral cortex. However it is difficult to observe and measure those vessels in vivo because of highly scattering properties of surrounding tissue. Here in this study, they were visualized and the blood flow velocity was measured using Doppler OCT (optical coherence tomography) technique and their functional activity was analyzed in response to the sensory stimulus.

Methods: OCT is a technique to visualize living tissues up to a few mm depth with around 10 μm spatial resolution. To visualize penetrating microvessels in this study, the Doppler effect was used as a marker to find blood vessels embedded within the tissue. On the spectrogram, which was calculated from the OCT signal using short time Fourier transform, penetrating vessels were clearly distinguished from the surrounding tissue, because there was a frequency difference between OCT signals from flowing blood and resting tissue.

Male Wistar rats of 150-200 g were used for cerebral preparation. They were anesthetized with pentobarbital sodium. The cranial window about 3x5 mm^2 was created in the parietal region while the dura mater was kept intact. Systemic arterial pressure was monitored via the femoral artery. The rat hind paw was electrically stimulated to analyze the stimulus-induced changes of blood flow in cerebral microvessels.

Results and Conclusions: The 3-dimensional distribution of blood flow velocity obtained by Doppler OCT technique delineated penetrating microvessels as well as microvessels running deep in the cerebral cortex up to 1.5 mm depth. Penetrating arterioles were discriminated from venules based on the flow direction and pulsatility of blood flow. Electrical stimulus of hindpaw induced significant velocity increase in the penetrating arterioles (about 13% of the control value) and pial arterioles (10%) in the hindpaw area of primary somatosensory (S1) cortex, while the flow velocity did not change significantly in microvessels in the other area of S1 cortex.

F-20

Microscopic imaging mass spectrometry revealed the evidence of deprivation of carbohydrates generated in the host liver by metastatic foci

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Objective: Metastatic foci require carbohydrates from host organ for their progression, although the evidence for such interplay has not yet revealed. This study aimed to examine utilization of carbohydrates generated in the host liver by colon cancer metastases.

Methods: A model of transportal metastasis of human colon cancer was established using superimmunodeficient NOD/scid/γ^{null} (NOG) mice where human-derived colon cancer cell line, HCT116 transfected with the mutant GFP, was injected intrasplenically. Microscopic imaging mass spectrometry (MIMS) combined with capillary electrophoresis mass spectrometry (CE-MS) was carried out to examine differences in energy metabolism between the metastatic foci and surrounding host liver tissues.

Results: The model exhibited hypoglycemia with regenerative responses of the host liver which appeared to results from impaired gluconeogenesis *in vivo* as judges by L-alanine loading test. MIMS provided with evidence for increases of nucleotide phosphates in metastatic foci than hepatocytes that still exhibited comparable amounts of these nucleotides to the controls. In addition, MIMS under loading of excess $^{13}C_3$-alanine led us the heterogeneous distribution of UDP-^{13}C-glucose in tumor-bearing liver and higher level of 13C-nucleotides and ^{13}C-citrate in metastatic foci.

Conclusion: The results indicate that metastatic foci deprive carbohydrates generated in the host liver for their proliferation and energy metabolism.

F-21

Endotoxemia increases the clearance of mPEGylated 5000 MW quantum dots as revealed by in vivo microvascular multiphoton imaging

F-22

Temperature imaging of neural activity in rat brain evoked by whisker stimulation

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Purpose： Local brain temperature is expected to be an important parameter to know the neural activity in a functional neuroimaging. In order to establish infrared (IR) temperature imaging for the study of brain function, it is needed to know the relationship between changes in local brain temperature and cerebral neuronal activity. The purpose of this study was to quantify the regional temperature changes of brain using the IR imaging and to analyze the spatiotemporal transmission of heat following the neural activity.

Methods：The male Wistar rats weighing 150-250g were used for cerebral preparation. These subjects were anesthetized with urethane. A femoral artery was cannulated to monitor the systemic blood pressure. Body temperature was kept at 37-38℃ using a heating pad and a rectal thermometer. A cranial window 1-4 mm posterior and 4-7 mm lateral to bregma was drilled in the skull to expose barrel cortex while the dura mater was kept intact. An IR camera (Merlin MID) imaged 3.8 x 3.1 mm region of the brain surface temperature of rats in vivo with a spatial resolution of 12 μm and a temperature sensitivity of 0.018℃. On each trial image data were collected at 3Hz. Sensory stimulation of vibrissae was given for 10 seconds using an oscillating whisker vibrator (5 mm deflection at 10Hz, 5Hz, and 1Hz).

Results: The brain temperature in the observational region increased significantly with the whisker stimulation. Especially, we found that the temperature increased in the barrel cortex from the acquired images. The peak temperature change was about 40 mK increase at 8 seconds after the start of stimulation. We also observed that the temperature increased from a spot to the periphery during a single whisker stimulation. Furthermore, the peak temperature change decreased, as the stimulus frequency increased.

Conclusion: These results indicate that the IR imaging may be a valuable tool to obtain 2D distribution of temperature reflecting the brain neural activity more quatntitatively than the conventional neuroimaging techniques.

F-23

Analysis of antibody-dynamics in the tumor microcirculation using in vivo imaging-techniques

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Purpose: Abnormal tumor microcirculation, which is formed with leaky vessel and absence of functional lymphatic, causes selective accumulation of antibody in the tumor tissue as macromolecule. Dynamics of antibody in tumor microcirculation has remained unclear despite its commonly use laboratory and clinics. The purpose of this study is to evaluate the features of the antibody-dynamics by using in vivo imaging-techniques.
Method: (1) Extravasation, penetration and retention of fluorescent antibodies (IgM and IgG) in the tumor were examined. (2) The distribution and retention of fluorescent antibodies 〈anti-CD20 for malignant lymphoma (ML) as stroma-poor tumor or anti-EpCAM for pancratic cancer (PC) as stroma-rich tumor〉 were examined in each tumor.
Result: (1) IgG was extravasated from leaky tumor vessel and penetrated into the tumor within 5 minutes, whereas the leakage of anti-fibrin IgM was so restricted that apparent extravascular distribution was not observed during the same period. Although long retention (over 7 days) of IgG was observed, IgM was eliminated by hepatic clearance in a few days. (2) Anti-CD20 mAb distributed over the whole ML tumor area, whereas anti-EpCAM mAb was restricted in the small area of PC tumor. Stromal barrier, which was composed of (a) physical barrier by extra-cellular matrix (b) pharmacological (convection and diffusion) inhibition (c) long distance from vessels to tumor cells, suppressed antibody-distribution in the PC tumor.
Conclusion: In tumor microcirculation antibody-dynamics was influenced both by its own structural feature and stromal barrier of the tumor. In vivo imaging of antibody will provide an aspect in the design of antibody therapy in the oncology.

F-24

Effect of topical nipradilol on retinal microcirculation evaluated by retinal function imager

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Purpose: Retinal Function Imager ® (RFI, Optical Imaging Ltd., Rehovot, Israel) is a novel device offering a noninvasive diagnostic approach to retinal function assessment. The purpose of this study was to evaluate the effect of topical nipradilol, an alpha-beta-blocker as well as a nitric oxide (NO) donor ophthalmic agent for glaucoma, on the retinal microcirculation using RFI in healthy volunteers.
Methods:
Eight healthy volunteers (mean age, 32.9 years) were recruited and microcirculation velocities at perifovea were quantitatively analyzed by RFI. Intraocular pressure (IOP), blood pressure (BP) were also measured. In a single-blind trial, all measurement was done before and after the instillation of nipradilol or saline twice a day for 2 weeks.
Results:
Two weeks instillation of nipradilol decreased IOP from 13.3±2.5mmHg to 11.0±1.8mmHg, ($p = 0.104$). Retinal arterial blood flow velocity significantly increased at 2 weeks after instillation in nipradilol-treated eyes (2.65±0.48mm/sec, 3.02±0.45mm/sec, respectively, $p = 0.015$). There was no significant difference in retinal venous blood flow velocity (2.46±0.84mm/sec, 2.23±0.29mm/sec, respectively, $p = 0.507$). Mean BP decreased from 115±6/71±8mmHg to 108±8/65±6mmHg significantly ($p<0.001$).
Conclusion: Our data showed that topical nipradilol significantly increased arterial blood flow velocity at perifovea in human eyes.
These results suggested that RFI might be useful to evaluate blood flow velocity in the glaucoma patients, to assess the efficacy of topical glaucoma agent.

F-25

Retinal artery response to decreasing ocular perfusion pressure in cats

F-26

Effect of acute increase in systemic blood pressure on retinal microcirculation in cats

F-27

Caffeic acid phenethyl ester elicits dilation of isolated porcine arterioles.

F-28

Fenofibrate elicits dilation of isolated porcine arterioles.

F-29

Half dose photodynamic therapy for chronic central serous chorioretinopathy

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Purpose: Central serous chorioretinopathy (CSC) is characterized by the accumulation of subretinal fluid (SRF) in the macula. Choroidal vascular hyperpermeability is demonstrated by Indocyanine green angiography (ICGA) and leakage from the retinal pigment epithelium is identified by fluorescein angiography (FA). Laser photocoagulation (LP) is a conventional treatment for CSC with extrafoveal focal leakage. However, treatment method has not been established in eyes with subfoveal or indistinct leakage, because LP causes side effect of scotoma in these eyes. Photodynamic therapy (PDT) with verteporfin (6 mg/m^2) is a well-established treatment for age-related macular degeneration. Recently, PDT with half dose verteporfin (3 mg/m^2, 1/2 PDT) has been reported to be effective in CSC. The purpose of this study is to evaluate the short term effect of 1/2 PDT for chronic CSC.

Methods: Forty eyes of 39 patients with chronic CSC of duration of 6 months or longer were enrolled. Chronic cases were chosen because spontaneous resolution of SRF is seen in acute cases. The mean±SD age of the patients was 50±8.8 years (range, 33-70 years) and 30 (76.9%) patients were male. ICGA-guided 1/2PDT was performed, and the mean±SD spot size was 3296±1073 μm. Primary outcome measure was the rate of complete SRF resolution on optical coherence tomography. Secondary outcome measure was the changes in logMAR best-corrected visual acuity (BCVA). SRF and BCVA were evaluated before and at 1 month and 3 months after PDT.

Results: Thirty two (80.0%) eyes at 1 month and 35 (87.5%) eyes at 3 months showed resolution of SRF and no recurrence was seen during the 3-month study period. Mean logMAR BCVA before and 1 month and 3 months after PDT were 0.25, 0.18 and 0.15 respectively. Mean logMAR BCVA at both 1 month and 3 months after PDT showed statistically significant improvements compared with before PDT (both, $p<0.01$). No patients lost vision or suffered any treatment-related complications.

Conclusions: 1/2 PDT for chronic CSC appears to be effective in resolution of SRF and improving visual acuity. PDT could be a standard treatment for chronic CSC, although the dose of verteporfin, timing of the treatment, method to define spot location and size are needed to be discussed.

F-30

Ocular blood flow measured by laser speckle flowgraphy is significantly correlated to aqueous vascular endothelial growth factor in central retinal vein occlusion

F-31

Involvement of autotaxin / lysophospholipase D on intestinal vessels in aggravation of intestinal damage through lymphocytes migration.

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Background: Aberrant leukocyte migration has been implicated in the pathogenesis of inflammatory bowel disease (IBD). Recently, lysophosphatidic acid (LPA) is reported to play a critical role in lymphocyte migration to the second lymph organization. In addition, autotaxin(ATX)/ lysophospholipase D in the vascular endothelium is the main enzyme in the production of LPA. Nevertheless, there have been no studies whether ATX is involved in the aberrant lymphocyte migration to the inflamed mucosa of IBD. In this study we investigated the expression of ATX and its relation to the activity of IBD.
Method: Tissue samples were obtained by colonic and ileal biopsies from patients with Crohn disease(CD) and ulcerative colitis(UC) with informed consent. Lymphocytes infiltration was studied immunohistochemically using anti-CD3 and anti beta7-integrin. Vascular endothelium was assessed by anti-CD34. High-endothelium venule was stained with ATX and anti-mucosal vascular adressin(MAdCAM-1). Degree of expression of mRNA ATX was determined by using quantitative RT-PCR. In murine study, tissue samples were obtained from ileum of SCID mice transferred with CD4 cells of spleen, and colon of BALB/c mouse provided with drinking water containing DSS. The inhibitory effect of bithionol (ATX inhibitor) on aberrant lymphocytes migration to the intestinal mucosa as well as intestinal damages were evaluated.
Result: Enhanced expression of ATX mRNA was observed in the inflamed mucosa from CD and UC patients. ATX expression was co-localized with the MAdCAM-1 positive high-endothelial venules, close to lymphocytes infiltration. The degree of ATX mRNA expression was significantly higher in the actively inflamed mucosa than in the quiescent mucosa in the same patients. In CD4 transferred SCID mice model, degree of expression of ATX mRNA was gradually increased as colitis developed. In DSS mice model, degree of expression of ATX mRNA was significantly higher in colonic mucosa of chronicically developed colitis than that of acute colitis. Administration of bithionol significantly ameliorated both DSS-induced colitis and CD4-induced ileocolitis.
Conclusion: ATX expression on the high endothelium suggests possible involvement of ATX in the aberrant lymphocyte migration to the inflamed mucosa of IBD patients. Blocking of ATX ameliorates intestinal damage. Enhanced expression of ATX in the active mucosa suggests that autotaxin/ lysophospholipase D becomes a new target for IBD treatment.

F-32

Enhancement of lymphangiogenesis and restoration of lymphatic flow in mouse secondary lymphedema model by COX-2

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Purpose: Disturbances in lymph flow can result in the development of secondary lymphedema. Despite genetic analysis of lymphatic malformations and primary lymphedema, the pathophysiology of secondary lymphedema remains poorly understood. Treatments that enhance lymphangiogenesis may offer a therapeutic tool for secondary lymphedema. Because cyclooxygenase (COX)-2-derived prostaglandins (PGs) are known to enhance angiogenesis under pathological conditions, we investigated the role of COX-2 in lymphangiogenesis and the restoration of lymph flow in a mouse tail lymphedema model.
Method: Male C57/BL6 mice were used. The preexisting lymphatic vessels in the tail were removed without damaging the main blood vessels present in the subcutaneous tissues. Measurements of the maximum horizontal diameter of the tails, Quantitative real-time RT-PCR, Microlymphangiography, Immunofluorescent histochemistry, Determination of interstitial pressure in subcutaneous tail tissues, Cell culture of HMVEC were examined.
Result: The diameters of the wounds were markedly increased after surgery, and reached maximum size two weeks after wounding in vehicle-treated mice. Expression of COX-2 in wound granulation tissues was markedly increased one week after surgery compared to unwounded naïve control mice. In vehicle-treated mice, lymphatic flow in the wound granulation tissues was detected three weeks after surgical treatment. By contrast, lymphatic flow was markedly suppressed in mice treated with the COX-2 inhibitor, celecoxib. Three weeks after surgery, LYVE-1 positive lymphatic structures were identified in the granulation tissues formed at wounded lesions on the tails of vehicle-treated mice, whereas formation of LYVE-1 positive lymphatic structures was markedly suppressed with celecoxib treatment. In addition, interstitial tissue pressures in the distal areas of the tail wounds were markedly increased with celecoxib, and VEGF-C expression was reduced by celecoxib one week after surgical treatment.
Conclusion: The present study suggests that lymphangiogenesis, together with recurrence of lymph flow after surgical induction of lymphedema, is upregulated by endogenous PGs derived from COX-2.

F-33

Angiogenesis and lymphangiogenesis in hepatic MALT lymphoma in helicobacter heilmannii-infected mouse

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Background & Aims: We established the low-grade MALT lymphoma model in C57BL/6 mouse infection of *Helicobacter heilmannii* obtained from cynomolgus monkey (Infect. Immun. 75 (3): 1214-1222, 2007). After long term infection, we found the MALT lymphoma formation in the liver and lung in addition to gastric MALT lymphoma. Thus, the present study was undertaken to clarify the mechanism of the formation of the MALT lymphoma in the liver.
Methods: We used an *Helicobacter heilmannii* isolated from the stomach of a cynomolgus monkey and maintained in C57BL/6 mouse stomachs. Mucosal homogenates were used to inoculate C57BL/6 mice which were then examined over 24 months. Macroscopic observations were carried out, and histochemical studies as well as PCR analysis of the bacteria of the Helicobacter species were performed at intervals over the observation period. In addition, the histochemical characteristics of the microvascular network in the hepatic and gastric MALT lymphoma was investigated using monoclonal and polyclonal antibodies against CD31, podoplanin, VEGF-A, VEGF-C, Flt-1, Flk-1, Flt-4, and MadCAM-1.
Results: 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 about 50% of the infected mice. PCR and in situ hybridization analysis showed the existence of *Helicobacter heilmannii* not only in the fundic mucosa but in the liver. Twelve and eighteen months after the infection, about 100% of infected mice had the liver and pulmonary lesion. Some of the MALT lymphoma was defined as high grade group by the PCNA positivity. Within the tumor, irregular microvascular network including capillaries, high endothelial venules and lymphatics was distributed, which was similar to that of the gastric MALT lymphoma.
Conclusion: Long term infection of Helicobacter heilmannii in C57BL/6 mouse induced the low and high grade hepatic MALT lymphoma as well as gastric lymphoma. From the viwepoint of the microvascular network, the hepatic lesion was found to be very similar to the gastric lesion.

F-34

The expression of podoplanin and P-cadherin on mouse choroid plexus

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Purpose: Cerebrospinal fluid (CSF) is supplied from choroid plexus in all ventricles. Choroid plexuses consist of a single layer of choroid plexus epithelial cells (CPE) enclosing a vascular core constructed by choroidal capillary and choroid plexus interstitial space. The CPE, choroid plexus fibroblasts (CPF), and a single layer of choroid plexus vascular endothelial cells (CPVEC) forms a selective barrier between the central nervous system (CNS) and the blood. The interstitial space including CPF, the endothelial basement membrane with pore at interstitial side of CPVEC, and the epithelial basement membrane intermediate between CPE and CPVEC. The CPE adheres CPVEC or the adherence is interrupted by CPF. The cerebral ventricle environment is maintained by epithelial cells lining the ventricles (CSF-brain barrier) and the choroid plexus (blood-CSF barrier). However, few differences in the molecular composition of these barriers have been described except for VE-cadherin and cadherin-10. The purpose of this study is evaluate the novel findings about the expression of lymphatic endothelial marker podoplanin, and about cadherins in the murine choroid plexus.
Materials & Methods: The expression of podoplanin, and P-cadherin, N-cadherin, and VE-cadherin on mouse choroid plexus was immunohistochemically investigated using confocal microscopic study.
Results & Conclusions: Podoplanin was expressed on ependymal cells and at the surface of ventricular side on CPE. N-cadherin was expressed on on ependymal cells but not on CPE. VE-cadherin was expressed at cell-cell junctions of CPVEC as reported previously, and between CPVEC and CPE or CPF at the basal side of CPVEC. P-cadherin was strongly expressed between CPE and vascular core. These distinctive expression pattern may suggest that podoplanin and N-cadherin function as CSF-brain barrier, and that in blood-CSF barrier of the choroid plexus, podoplanin functions between CSF and CPE like CSF-brain barrier, and not only VE-cadherin but also P-cadherin interrupts between CPE and CPVEC.

F-35

Assessing therapeutic responses of anti-tumor agents in pancreatic ductal adenocarcinoma using xenografts and genetically engineered mouse models

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Pancreatic cancer is one of most hard-to-treat cancer with the mortality rates almost identical to the incidence rate. Gemcitabine is the current standard chemotherapeutic agent used as first-line treatment for patients; however, it results in only a marginal survival benefit. Common chemotherapeutics including gemcitabine reported to exhibit dose-dependent cytotoxicity under the nutrient sufficient conditions, whereas these effects were abolished by nutrient deprivation. Thus, we screened arctigenin as a candidate substance from among the natural product, which show preferential cytotoxicity under nutrient-deficient conditions.

To predict clinical therapeutic responses of anti-tumor agent of arctigenin, we evaluate the utility of generally used cancer derived cell xenograft models and the genetically engineered mouse model of pancreatic cancer. Recently, pancreas-specific type II TGF-beta receptor (tgfbr2) knockout mice have been generated, in the context of active Kras (Kras(G12D)) expression, using the Cre-loxP system both driven by the endogenous Ptf1a (pancreatic transcription factor-1a) locus (Ijichi et al., Gene Dev 2006). Homozygous deletion of Tgfbr2 with Kras(G12D) expression developed well-differentiated pancreatic ductal adenocarcinoma with 100% penetrance. The clinical and histological features of these knockout mice recapitulated human PDAC. In this study, histopathological and biomedical imaging analyses were used in conjunction of overall survival to establish timelines for disease onset and tumor progression.

Daily administration of arctigenin significantly suppressed the tumor growth of mouse pancreatic cancer cell line xenografts as a single agent. In mouse model of Tgbr2 knockout with Kras expression, we performed high resonance in vivo MRI to observe tumor formation non-invasively. The MR image and the pathological characteristics of dissected section were well correlated. Daily administration of arctigenin gave no effects on tumor mass formation *in situ*, but the histopathological features were different between non-treated and arctigenin treated group. Further, a modest improvement in overall survival was observed in a few mice, thought neither attained statistical significance.

F-36

Development of a hepatic lobular model for large-scale simulation of metabolic systems

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Hepatocytes occupy a large part of the cellular volume in liver have very extensive and complex metabolic pathways, and play an important role in regulation of vast variety of small molecules in the body. However, due to the striking complexity in metabolic pathways and regulations, it is very difficult to comprehensively understand the dynamics of hepatic metabolism and its underlying mechanism as a whole. We therefore developed a large-scale computational model of metabolism in hepatocytes including approximately 500 defined substances and about 250 dynamic reactions based on the reaction kinetics obtained by literature search.

In the model, the metabolism in the mitochondria and cytoplasm are calculated separately and simultaneously (considering the intracellular compartmentation). The local heterogeneity of enzyme expression pattern between the upstream (portal vein) and downstream (central vein) of sinosidal blood flow, which is called metabolic zonation, is also taken into consideration in the model (considering the intercellular compartmentation).

The simulation model was parameterized by metabolome data of acute ischemia-induced dynamics which covers key intermediates of comprehensive metabolic pathways.

Using the model, we compared a predicted dynamics under hypoxic and hypoglycemic condition from a couplet model in which two connecting hepatocytes having portal vein and central vein metabolic properties with those from the models in which both hepatocytes have a property of portal vein or central vein only. As a result, by coupling cells with different properties, over-all changes in metabolites in response to these stimulations were suppressed and the metabolically important fluxes (e.g. ammonia scavenging, net gluconeogenesis or net glycolysis) were enhanced, suggesting that the robustness and efficiency of the metabolism would be improved at an organ level.

This result may contribute not only to understanding how hepatic metabolism is optimized using heterogeneity, but also to understanding of metabolic failure in the intercellular material exchange disorder such as a case of hepatic cirrhosis. The application possibility of the computational model for other diseases will be discussed.

F-37

Vascular endothelial growth factor receptor-1 tyrosine kinase activity facilitates liver repair and sinusoidal restoration through recruitment of macrophages after acetaminophen hepatotoxicity

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Aims; Vascular endothelial growth factor (VEGF) and its receptors promote liver regeneration. The objective of the present study was to examine the role of VEGF receptor 1 (VEGFR1) signaling in hepatic tissue repair after acetaminophen (APAP)-induced liver injury.
Methods; Male 8 week-old VEGFR1 tyrosine kinase knockout mice (VEGFR1 TK$^{-/-}$) and wild-type (WT) mice were treated with APAP (300 mg/kg, i.p.). We determined levels of ALT, necrotic area of the liver, and hepatic tissue levels of mRNA expression, and examined hepatic microvascular injury using in vivo and electron microscopy. The expression of VEGFRs and the recruitment of macrophages were assessed by immunohistochemistry.
Results; In WT mice, serum levels of alanine aminotransferase (ALT) and the necrotic area peaked between 8 and 24 hrs, and then declined. In VEGFR1 TK$^{-/-}$ mice, ALT levels remained high at 48 hrs and extensive hepatic necrosis and hemorrhage were observed, as well as high mortality. Down-regulation of hepatic mRNA expression of VEGFR1 and VEGFR2 was noted in VEGFR1 TK$^{-/-}$ mice. VEGFR1 TK$^{-/-}$ mice displayed lower expression of proliferating cell nuclear antigen and of growth factors including hepatocyte growth factor and basic fibroblast growth factor than WT. The hepatic microvasculature in VEGFR1 TK$^{-/-}$ was compromised as evidenced by impaired sinusoidal perfusion, suppressed endocytosis in liver sinusoidal endothelial cells (LSECs), and the formation of large gaps in LSECs. In WT mice, immunofluorescence revealed that recruited VEGFR1+ cells in the necrotic area were positive for CD11b. VEGFR1 TK$^{-/-}$ exhibited fewer VEGFR1+ cells.
Conclusions; These results suggest that VEGFR1 signaling facilitates liver recovery from APAP hepatotoxicity by preventing excessive hemorrhage and by reconstituting the sinusoids through recruitment of VEGFR1-expressing macrophages to the injured area and through affecting expression of genes including hepatotrophic and pro-angiogenic growth factors.

F-38

Protective role of HIF-1 in the development of alcohol-induced steatosis in mice

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Purpose: Chronic alcohol intake evokes liver hypoxia, however, its pathological role in the development of alcoholic fatty liver remains unknown. In this study, we aimed to elucidate roles of hypoxia inducible factor (HIF)-1, a master transcription factor of adaptive responses to hypoxia, in the disease.
Methods: Wild-type and HIF-1α-deficient mice were exposed to an ethanol-containing diet for 4 weeks, and genes involved in the hepatic lipid metabolisms were examined quantitatively.
Results: Deletion of HIF-1α gene aggravated lipid accumulation in liver when exposed to ethanol. Quantitative PCR revealed increased expression of SREBP1c and its downstream target, acetyl CoA carboxylase in HIF-1α-deficient liver. These alterations were accompanied by enhanced DEC1 expression, a HIF-1 target transcription repressor. Over expression of DEC1 restored the deteriorated fatty infiltration in the mutant mice. Conversely, co-administration of the HIF hydroxylase inhibitor dimethyloxyalylglycine improved markedly the ethanol-induced fatty liver in mice.
Conclusion: The current results provide direct evidence for protective roles of HIF-1 induction in the development of ethanol-induced fatty liver.

LS1

緑内障と微小眼循環
Glaucoma and ocular blood flow

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緑内障は慢性進行性の多因子性視神経疾患であり、その最大の危険因子は眼圧である。しかし、眼圧下降にもかかわらず進行する例は多数存在し、眼圧以外の重要な危険因子は循環であると考えられている。緑内障性視神経症の首座である視神経乳頭は、特異な微小循環環境を持っている。血圧と眼圧に関連する眼灌流圧低下による緑内障の有病率との関係が疫学調査から報告され、眼圧変動や体位変換による乳頭血流の自動調節能の異常も緑内障の発症や進行に関係するとされる。本セミナーでは緑内障と微小眼循環因子の関係を、解剖、疫学的側面から解説し、臨床の現場での循環動態の把握の困難さと現在可能性のある循環改善治療法についてお話ししたい。

Employment

1989-1990	Resident in Dept. of ophthalmology, University of Tokyo, Japan
1990-1994	Instructor in Dept. of ophthalmology, University of Tokyo, Japan
1994-1998	Fellow in Dept. of Biochemistry and Molecular Biology, University of Tokyo, Japan
1998-2000	Chief instructor in Dept. of ophthalmology, University of Tokyo, Japan
2000-2002	Research fellow and Clinical Instructor in Glaucoma Center, UCSD, USA
2002-2003	Assistant research scientist and Principal Investigator in Glaucoma Center, UCSD, USA
2003-	Assistant Professor in Dept. of ophthalmology, University of Tokyo

LS2

Basic research for the effects of the medicines related to diabetes on the vascular damage by diabetes

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In this study, the mechanisms of vascular damage by high concentrations of glucose and insulin were first investigated with respect to endothelial cells-neutrophils adhesion and migration accompanied with expression of the related endothelial adhesion molecules. High glucose concentrations significantly increased the endothelial cells-neutrophils adhesion by over-expression of the endothelial adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, P-selectin, and E-selectin through activation of an intracellular protein kinase C (PKC) pathway.

The elevated adhesion by high glucose was significantly inhibited by antihypertensive agents, an angiotensin II receptor antagonist (ARB) and an angiotensin-converting enzyme inhibitor (ACEI) with reducing endothelial nitric oxide (NO) production but not intracellular PKC activation. These findings are thought to support the clinical data in which one of the ARB, candesartan significantly protected and improved the diabetic retinopathy compared to the placebo. The high glucose-induced endothelial cells-neutrophils adhesion was also blocked by the medicines often used for the patients with diabetes, such as a sulfonylurea, gliclazide, an aldose reductase inhibitor, epalrestat, hydroxymethylglutaryl-CoA reductase inhibitors, and an anti-platelet agent, cilostazol.

On the other hand, high insulin concentrations significantly enhanced the endothelial cells-neutrophils adhesion by over-expression of only ICAM-1 through a PKC and a tyrosine kinase, especially a mitogen-activated protein kinase (MAPK) pathways. High insulin also significantly enhanced the endothelial cells-neutrophils migration by over-expression of one of the adhesion molecules, platelet endothelial cell adhesion molecule (PECAM)-1 through only an intracellular MAPK pathway.

Pioglitazone, an insulin sensitizer significantly ameliorated the high insulin-induced endothelial cells-neutrophils adhesion by decreasing intracellular PKC and MAPK activity but not endothelial NO production. Similarly, the agent significantly improved the high insulin-induced migration by inhibiting intracellular MAPK activity but not endothelial NO production. These data may confirm that pioglitazone is thought to have not only an anti-hyperglycemia action but also direct anti-atherosclerosis actions. Actually, pioglitazone is reported to significantly reduce the plaque volume in the coronary artery of the patients with diabetes compared to a sulfonylurea, glimepiride.

I hope this lecture will be useful in the near future for the audience, especially treating for the patients with diabetes.

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ES

Anti-VEGF therapy on the ophthalmologic field

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血管内皮増殖因子（VEGF）は眼科領域においても、種々の疾患の病態と密接に関わっていることはよく知られている。その中でも糖尿病網膜症、加齢黄斑変性、未熟児網膜症などに代表される眼内血管新生を主因とする疾患はVEGFが最も深く関わり、重篤な視力障害を残す難治疾患である。近年、病態の主因をなすVEGFを分子ターゲットとした抗VEGF治療は眼科領域にも導入され、その効果が確認されている。その中でも加齢黄斑変性に対しては本邦でもラニビズマブおよびペガプタニブが実用化されており、今まで困難であった視力回復が可能となる症例も認められるようになった。現在、新たな抗VEGF薬も開発中であり、今後、さらにVEGFをターゲットとした治療は眼科領域においても重要な役割を担っていくと考えられる。

略　歴

石橋　達朗（いしばし　たつろう）

1975年　九州大学医学部卒業
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1986年　帰国後、九州大学医学部眼科　講師
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