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PRESIDENT'S LECTURE

PL | Effects of memantine and yokukansan on nitric oxide production and hydroxyl radical metabolism during cerebral ischemia and reperfusion in mice

Nobuo Araki

Department of Neurology, Saitama Medical University, Saitama, Japan

The glutamate-mediated neurotoxicity hypothesis of the pathomechanism of cerebral ischemia is well known. The neuroprotective effects of memantine have been demonstrated in ischemia. Yokukansan is a traditional Japanese kampo medicine which was anciently used to reduce the irritability in children. Yokukansan is useful for prevention of abnormal glutamate release, and improvement of glutamate uptake with glutamate transporters. The effects of memantine and yokukansan on NO production and hydroxyl radical metabolism during cerebral ischemia and reperfusion in vivo have never been investigated. C57BL/6 mice were anaesthetized with halothane, and NO production and hydroxyl radical metabolism were monitored continuously using in vivo microdialysis. A microdialysis probe was inserted into the striatum in each hemisphere and perfused with Ringer's solution. The in vivo salicylate trapping method was used to monitor hydroxyl radical formation via 2,3-DHBA and 2,5-DHBA. A laser Doppler probe was placed on the surface of the skull of the right hemisphere. Global forebrain cerebral ischemia was produced by occlusion of both common carotid arteries for 10 minutes. After 2 hours of equilibration, fractions were collected every 10 minutes. Levels of nitrite (NO^{2-}) and nitrate (NO^{3-}) in the dialysates were determined using the Griess reaction. The level of 2,3-DHBA was significantly lower in the memantine group than in the control group after reperfusion. Level of total NO was significantly higher in the yokukansan group than in the control group. The 2,3-DHBA level were significantly lower in the yokukansan group than in the control group. In our experiments, memantine significantly reduced hydroxyl radical metabolites during ischemia and reperfusion, and yokukansan accelerated the production of NO in reperfusion phase and suppress the production of hydroxyl radicals.

SPECIAL LECTURES

SL-1 | Regulation of lymphocytes migration to intestinal microvessels by luminal antigens in health and diseases

Ryota Hokari

Department of Internal Medicine, National Defense Medical College, Saitama, Japan

Recirculation of naïve lymphocytes from blood to lymphoid tissue in physiological condition is generally noted as a key phenomenon in mucosal immune system in the gut. Aberrant leukocyte recruitment to the gut is a key feature of inflammatory bowel diseases. Leukocyte recruitment is regulated by the interaction between adhesion molecules on the endothelium and their specific ligands on leukocytes. The mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1) is specifically expressed on the endothelial cells of gut-associated lymphoid tissue in physiological conditions, and interacts with its specific ligand, $\alpha4\beta7$-integrin, on leukocytes. MAdCAM-1-$\alpha4\beta7$-integrin is the principal module that mediates the gut specific binding of leukocytes to venules. Drugs targeting adhesion molecules have been developed. Environmental factors including food, smoking or hygiene condition are involved in the pathogenesis of inflammatory bowel diseases. In addition, expression of adhesion molecules in the intestine are highly regulated by luminal antigens. Recent advances in this research area and the prospects for clinical application will be presented.

SL-2 | Cerebral microcirculation and glymphatic system

Yoshiaki Itoh

Department of Neurology, Osaka City University Graduate School of Medicine, Osaka, Japan

One of the main roles of microcirculation is to remove toxic waste from the brain. Amyloid beta, cleaved from the cell membrane, was once considered to be discharged across the blood-brain barrier through endothelial cells. Recently, transportation system along the vessels from the parenchyma to the subarachnoid space, named glymphatic system (gliaplus lymphatic) by Nedergaard, is drawing more attention as the potentially main process of clearing up the toxic amyloid. Measurement of perivascular flow is challenging even with multiphoton microscopy since the flow is too slow and the volume is too small. Some papers reported that direction of the flow is from the cortex to the parenchyma along the penetrating artery and in the opposite direction along the vein. Others reported the outgoing flow along the artery, which is reasonable for the pathogenesis of arterial amyloid angiopathy. Even more, bidirectional flow in smooth muscle layer and in perivascular space is suggested.

In our experiments, we observed amyloid transportation from the subarachnoid space to the brain parenchyma in Tie-2 labeled transgenic mouse. Transportation was rapid. At 30 min after the application of amyloid in the subarachnoid space, amyloid was found at 400 micro-meter deep and further accumulation was limited after the period. Amyloid was found both in penetrating artery and vein as well as capillaries. These results suggest that convective flow transporting the amyloid rather than just the diffusive bulk flow through parenchyma. In contrast to the peripheral lymph vessels, cerebral perivascular flow has limited driving force. Arterial pulsation may be involved around the penetrating artery, whereas gradient of hydrostatic pressure may drive convective bulk flow around capillary and veins. The controversy and recently published data will be discussed in the symposium.

SL-3 | Roles of aquaporin-4 (AQP4) in brain lymphatic system and in neurodegenerative diseases

Masato Yasui

Department of Pharmacology, School of Medicine, Keio University, Tokyo, Japan

Aquaporin-4 (AQP4) is the main water channel in mammalian brain, which is distributed with highest density in the perivascular and subpial astrocyte end-feet. AQP4 has been implicated in several neurologic conditions, such as brain edema, seizure and even neurodegenerative diseases. Interestingly, AQP4 has been identified as a target antigen of autoimmune attack in neuromyelitis optica (NMO). NMO is characterized by extensive necrotic lesions preferentially involving the optic nerves and spinal cord. However, previous *in vivo* experimental models injecting human anti-AQP4 antibodies only resulted in mild spinal cord lesions compared to NMO autopsied cases. We generated high affinity anti-AQP4 monoclonal antibodies that recognize the extracellular domains of rodent AQP4. By injecting the monoclonal antibodies against rat AQP4, we have established a severe experimental NMO rat model with highly clinical exacerbation and extensive tissue destructive lesions typically observed in NMO patients. Our data suggest that the pathogenic antibodies could induce immune mediated astrocytopathy with mobilized neutrophils, resulted in early lesion expansion of NMO lesion with vacuolation and other tissue damages. Interestingly, it's been accumulating evidence that AQP4 may be involved in the brain lymphatic pathway and its dysfunction may lead to the neurodegenerative disorders such as Alzheimer's disease and amyotrophic lateral sclerosis (ALS). I will also discuss about this issue with some recent experimental data.

SYMPOSIUM

SY1-1 | Imaging solutes and solvent in the living tissue

Mutsuo Nuriya

Department of Pharmacology, Keio University School of Medicine, Tokyo, Japan

Dynamics of bioactive molecules are among the most critical determinant of their biological activities in the cells and tissues. They are determined by the nature of solutes themselves as well as the dynamics of solvent, water, in the case of living cells. Despite its importance, the dynamics of water inside tissues have been poorly characterized so far. The most critical reason behind it is the lack of appropriate methodology of water imaging; conventional fluorescence tagging cannot be applied to small molecule like water. To overcome the current problems, we are applying the nonlinear Raman imaging technique called CARS (coherent anti-Stokes Raman scattering) imaging. CARS is based on Raman imaging that visualizes particular chemical bond, but has much higher sensitivity than spontaneous Raman scattering. Furthermore, since it is based on the multiphoton phenomena of ultrashort pulsed laser in near infrared (NIR) region, it can realize high resolution 3D imaging in deep positions in the living tissues. In addition, the lasers used in CARS can excite fluorescent molecules, which realize multimodal multiphoton microscopy observation that allows the multifaceted analyses of biological phenomena. Indeed, when acutely prepared murine cortical brain slices were visualized by CARS microscopy system tuned to visualize O-H vibration, it can visualize water in the brain slices. When the extracellular solution is changed from normal H_2O-based solution to D_2O-based one while performing time lapse imaging, the influx of water can be visualized with high temporal and spatial resolution. Furthermore, by taking advantage of multimodal imaging capacity, the dynamics of water in the brain can be visualized together with those of solute molecules as well as cellular markers.

We are currently applying this method to reveal solute and solvent dynamics in the brain in the normal and pathological conditions, to better understand the physiology and pathophysiology of the brain.

SY1-2 | Mechanosensing in vascular endothelial cells

Kimiko Yamamoto[1]; Joji Ando[2]

[1]*System Physiology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan* [2]*Biomedical Engineering, School of Medicine, Dokkyo Medical University, Saitama, Japan*

Vascular endothelial cells (ECs) play critical roles in regulating a variety of vascular functions, including maintenance of the vascular tone, blood coagulation and fibrinolysis, and provision of selective permeability to proteins. It has recently become apparent that ECs show alterations in their morphology, functions and gene expression profile in response to exposure to hemodynamic forces, namely, shear stress and stretch. These responses also play important roles in maintaining normal circulatory system functions and homeostasis, whereas their impairment leads to various vascular diseases, including hypertension, aneurysm and atherosclerosis. The mechanisms underlying the mechanotransduction, however, are not yet clearly understood. Plasma membranes of the ECs have recently been shown to respond differently to shear stress and stretch, by rapidly changing their lipid order, membrane fluidity, and cholesterol content. Artificial lipid-bilayer membranes also show similar changes of the lipid order in response to exposure to shear stress and stretch, indicating that these are physical phenomena rather than biological reactions. Among the membrane lipids, cholesterol has been suggested to play a dominant role in determining the physical properties of the plasma membranes. Shear stress decreased the membrane cholesterol content; whereas, stretch induced by uniaxial stretch and hypotonic swelling increased the membrane cholesterol content. These changes in the physical properties and the membrane cholesterol content were found to be linked to the activation of the growth factor receptors specific to each force. These findings suggest that the plasma membranes of ECs act as mechanosensors, and in response to mechanical forces, they show alterations of their physical properties, with modification of the conformation and functions of the membrane proteins, which then trigger activation of the downstream signaling pathways.

SY1-3 | ^{18}F-FDG-labeled red blood cell PET for blood pool imaging and its application

Yohji Matsusaka[1]; Tadaki Nakahara[1]; Mayumi Kajimura[2]; Masahiro Jinzaki[1]

[1]*Department of Radiology, Keio University School of Medicine;* [2]*Department of Biology, Keio University School of Medicine, Tokyo, Japan*

Purpose: Blood pool scintigraphy has been clinically used to detect active bleeding. Positron emission tomography (PET) has a good image resolution, but there were no appropriate PET agents for blood pool imaging. We found that ^{18}F-fluorodeoxyglucose (^{18}F-FDG) could be a good agent for labeling for red blood cells (RBCs). The aim was to evaluate the optimal conditions of labeling RBCs with ^{18}F-FDG and to demonstrate the usefulness of FDG-labeled RBC PET for detecting active microbleeding and lower legs congestion.

Methods: The optimal labeling procedure was determined by investigating various conditions for labeling RBCs with FDG. Using small-animal PET system, FDG-labeled RBC PET was performed for normal rats. Intraabdominal bleeding models and lower leg congestion models were imaged with FDG-labeled RBC PET. The entire experimental protocols were approved by The Keio University Institutional Animal Care and Use Committee.

Results: The optimal labeling conditions included 60 min of cell fasting and 30 min of labeling time at 37°C. After intravenous injection of the labeled RBCs, blood pool PET imaging was successfully performed in the normal rats. Intraabdominal microbleeding and lower leg congestion were clearly visualized on dynamic PET images.

Conclusions: FDG-labeled RBC PET can be performed for blood pool imaging in rats. This method has a potential of clinical application for detecting intraabdominal microbleeding and lower leg congestion.

SY1-4 | Development of fluorescence probes for hypoxia and/or pH in living samples

Kenjiro Hanaoka

Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

We introduced two fluorescence probes for hypoxia and pH 1) Inadequate supply of oxygen is deeply related to various pathologies such as cancer and vascular diseases. So, fluorescence probes to detect hypoxia are highly required to investigate its biological effects. It is known that the increase of reductive stress is one of the features in hypoxia and azoaromatic compounds are also selectively reduced under hypoxia. Based on these facts, we have developed novel hypoxia-sensitive fluorescence probes, mono azo rhodamine (MAR) and mono azo Si-rhodamine (MASR), based on two different colored fluorophores (*Angew. Chem. Int. Ed.*, 52, 13028–13032, 2013). We further designed and synthesized NIR fluorescence probe, diMe azoSiR640, and successfully achieved real-time imaging of ischemia of their liver and kidney in living mice (*Chem. Commun.*, 54, 6939–6942, 2018). 2) In biological systems, the pH in intracellular organelles or tissues is strictly regulated, and differences of pH are deeply related to key biological events such as protein degradation, intracellular trafficking, renal failure, and cancer. Ratiometric fluorescence imaging is useful for determination of precise pH values, but existing fluorescence probes have substantial limitations, such as inappropriate pK_a for imaging in the physiological pH range, inadequate photobleaching resistance, and insufficiently long excitation and emission wavelengths. We have developed a versatile

scaffold for ratiometric fluorescence pH probes (*J. Am. Chem. Soc.*, 140, 1767–1773, 2018). To demonstrate its usefulness for biological applications, we employed it to develop two probes. i) SiRpH5 has suitable pK_a and water solubility for imaging in acidic intracellular compartments; by using transferrin tagged with SiRpH5, we achieved time-lapse imaging of pH in endocytic compartments during protein trafficking for the first time. ii) Me-pEPPR is a near-infrared (NIR) probe; by using dextrin tagged with Me-pEPPR, we were able to image extracellular pH of renal tubules and tumors *in situ*.

SY2-1 | Neurovascular coupling: focal and global regulation of cerebral microcirculation

Kazuto Masamoto

Brain Science Inspired Life Support Research Center, University of Electro-communications, Tokyo, Japan

A tight coupling between neural activity and vascular responses (i.e. neurovascular coupling) in the brains maintains brain physiology. Recent studies demonstrate that impairment of neurovascular coupling contributes to cognitive decline, which has important implications for prevention and treatment of age-related deterioration of brain functions. However, a causal link between dysfunction of cerebrovascular regulation and neurodegeneration remains incompletely understood. In normal brains, blood flow in the cerebral microcirculation increases and decreases in association with local neural activity. However, whether capillary reacts actively to neural demand is under debate. It is well-known that upstream pial and parenchymal arteries respond and fulfill neural demand. Pial artery forms arterio-arterial anastomosis, which maintains inter-regional balances of blood flow. Parenchymal artery (i.e. perforating or penetrating arteriole) is a terminal artery that has primary responsibility to ensure regional blood flow in the territory regions. Activity-induced dilation of pial arteries is shown to be driven by conducted mechanisms of vasodilation responses (i.e. conduction of membrane hyperpolarization) along the vascular trees in the parenchyma. However, this vasodilation mechanism is not always the case. Optogenetic studies, that stimulate a specific type of the brain cells using light and genetically-targeted light-gated channels, revealed that focal mechanisms of vasodilation (i.e. nitric oxide and cyclooxygenase pathways) also contributes to the responses of the parenchymal arteries, without conduction of the vasodilation. The findings are in good agreement with previous reports that a certain type of the neural activity (e.g. cholinergic stimulation) causes regional vasodilation of the penetrating arterioles, but not the pial arteries. In conclusion, at least two-separate vasodilation mechanisms (i.e. potassium-induced hyperpolarization and NO/COX pathways) are involved in mechanisms of neurovascular coupling. Cellular sources that release those substances are currently under investigated.

SY2-2 | Physiological function of basal forebrain cholinergic fibers projecting to the olfactory bulb and neocortex

Sae Uchida

Department of Autonomic Neuroscience, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

Previous study by our research group has demonstrated in rats that activation of the basal forebrain cholinergic neurons projecting to the neocortex produces vasodilative neural regulation of the neocortex by exciting the nicotinic and muscarinic acetylcholine (ACh) receptors. The olfactory bulb also receives cholinergic basal forebrain input, as does the neocortex. We recently examined the physiological function of the cholinergic projection to the olfactory bulb, comparing that to the neocortex, using urethane-anesthetized rats. Focal chemical stimulation by microinjection of L-glutamate into the horizontal limb of the diagonal band of Broca (HDB) in the basal forebrain which is the main source of cholinergic input to the olfactory bulb increased extracellular ACh release in the ipsilateral olfactory bulb. When the regional cerebral blood flow was measured using laser speckle contrast imaging, the focal chemical stimulation of the HDB did not significantly alter the blood flow in the olfactory bulb, while increases were observed in the neocortex. Our results suggest that the basal forebrain cholinergic projection is less functional regarding blood flow regulation in the olfactory bulb, which is in contrast to that displayed in the neocortex. Odor stimulation is known to increase in regional blood flow in the rodent olfactory bulb, in association with neuronal activities. We investigated the effect of stimulation of nicotinic ACh receptors on the odor-induced olfactory bulb blood flow response. Odor stimulation increased olfactory bulb blood flow, without changes in neocortical blood flow and systemic blood pressure. Intravenous injection of nicotine (30 μg/kg) , a nicotinic receptor agonist, significantly augmented the odor-induced increase response of olfactory bulb blood flow, without changes in the basal blood flow level. The nicotine-induced augmentation was negated by α4β2-preferring nicotinic receptor antagonist. Our results suggest that the activation of α4β2-like nicotinic ACh receptors in the brain potentiates olfactory blood flow response to odor.

SY2-3 | Development of a large-scale simulator for clarification of physical mechanisms of full-scale cerebral circulation

Satoshi Ii[1]; Shigeo Wada[2]

[1]*Graduate School of Systems Design, Tokyo Metropolitan University, Tokyo, Japan*
[2]*Graduate School of Engineering Science, Osaka University, Osaka, Japan*

The cerebral circulation plays an important role to continuously deliver oxygen and glucose to brain tissues. Important features of the cerebral circulation are to maintain a constant cerebral blood flow (CBF) regardless of arterial pressure (cerebral autoregulation) and

control the local and global CBFs as depending on metabolic demands by neural activities[1]. Understanding how the neuro-vascular coupling affects the CBFs is desired to get to the core of the regulation mechanism of the cerebral circulation, that therefore requires to uncover physical aspects of the full-scale cerebral circulation with complex networks derived from anatomical structures (e.g. circle of Willis, vessel anastomosis and capillary bed[2]). The authors tackle this problem by a full-scale cerebral circulatory simulator, which is planned to perform in the ultra-large-scale computer called post-K computer[3]. The simulator consists of two parts, whole-scale circulation and micro-scale circulation. The whole-scale circulation is modeled by a single-phase flow in a cerebrovascular model including up to pial artery/vein, whereas the microcirculation is modeled by a suspension flow including deformable blood cells in branched pipes. Both whole and micro-scale simulations performed on the K computer show the mechanical response through the fluid flow affects the overall flow behavior, suggesting the passive effect by the mechanical balance plays an important role in regulating the cerebral circulation.

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1. P. Blinder, P. S. Tsai, J. P. Kaufhold, P. M. Knutsen, H. Suhl and D. Kleinfeld, The cortical angiome: an interconnected vascular network with noncolumnar patterns of blood flow, *Nat Neurosci* , 16 (2013) 889–897.
2. H. M. Duvenoy, S. Delon, J. L. Vannson, Cortical blood vessels of the human brain, *Brain Res Bull* , 7 (1981) 519–579.
3. http://postk.hgc.jp/home

SY2-4 | Effects of cerebral microcirculation on Aβ and tau pathology in Alzheimer's disease

Satoshi Saito

Department of Neurology, National Cerebral and Cardiovascular Center, Osaka, Japan

Recent investigations have revealed that the cerebral blood flow and the clearance of interstitial fluid from the neuron is much dependent on the state of cerebral microcirculation, suggesting that the maintenance of cerebrovascular integrity is important not only for prevention of cerebrovascular disease but also for amelioration of neurodegenerative diseases including Alzheimer's disease (AD). Pathological hallmarks of AD are senile plaque, cerebral amyloid angiopathy (CAA), and neurofibrillary tangle. The former two changes are based on amyloidosis and the latter is tauopathy. CAA is observed in more than 80% of AD cases. CAA impairs cerebrovascular reactivity and causes both hemorrhagic and ischemic strokes, attributing to cognitive impairment. Most CAA is a consequence of Aβ elimination failure, mainly caused by disturbance of intramural periarterial drainage (IPAD) or glymphatic system. Therefore, facilitation of Aβ clearance would be a potential treatment for dementia. Compared with Aβ, little is known about the relationship between tau and cerebrovascular disease. Recent studies have shown the cellular mechanisms regulating tau release from cytoplasm. However, the clearance mechanisms of extracellular tau protein still remain unknown. Our preliminary investigations showed that crossbreeding of CAA and tauopathy model mice resulted in acceleration of cerebrovascular Aβ pathology and enhanced tau degeneration, suggesting a positive feedforward loop of toxic protein accumulation. The maintenance of cerebrovascular integrity may be effective not only on Aβ but also on tau pathology.

YOUNG INVESTIGATORS AWARD SYMPOSIUM

Y-01 | Protective roles played by heme oxygenase-2 against transhemispheric diaschisis

Shinichi Goto; Akiko Kubo; Takayuki Morikawa; Mayumi Kajimura; Makoto Suematsu

Department of Biochemistry, Keio University School of Medicine, Tokyo, Japan

Objective: Heme oxygenase-2 is a carbon monoxide-generating enzyme, that is expressed in neurons. We have reported the crucial role of this enzyme for regulating hypoxic vasodilation through mechanisms unlocking carbon monoxide-dependent inhibition of H2S-generating cystathionine β-synthase expressed in astrocytes. The objective of the current study is to examine the roles played by heme oxygenase-2 during focal ischemia of the brain.

Methods: Regional differences in metabolites among ipsilateral and contralateral hemispheres were compared between heme oxygenase-2 wild-type and null mice. Local metabolites were measured using quantitative imaging mass spectrometry equipped with an image-processing platform to optimize comparison of local metabolite contents among different animals. Focal ischemia was induced by middle cerebral artery occlusion.

Results: At baseline, the blood flow velocity in precapillary arterioles were significantly elevated in heme oxygenase-2-null mice compared with controls, while metabolic intermediates of central carbon metabolism and glutamate synthesis were elevated in heme oxygenase-2-null mice. These results suggest greater metabolic demands to induce hyperemia in these mice. In response to focal ischemia, heme oxygenase-2-null mice exhibited greater regions of ischemic core that coincide with notable decreases in energy metabolism in the contralateral hemisphere as well as in penumbra.

Conclusion: Heme oxygenase-2 protects against compromised energy metabolism of the ipsilateral hemisphere and also ameliorates transhemispheric diaschisis of the contralateral hemisphere in focal ischemia of the brain.

Y-03 | Effect of bile acid on lymphocyte migration into the small intestine

Naoki Shibuya; Masaaki Higashiyama; Kazuki Horiuchi; Ryota Hokari

Department of Internal Medicine, National Defense Medical College, Saitama, Japan

Aim: Gut immunity is reported to be distinctively affected by each bile acid and the effect on intestinal microcirculation by each bile acid remains to be clarified. We aimed to investigate the effect of several kinds of bile acid on intestinal microcirculation. This study was approved by the ethics committee of animal in our institute.

Methods: (1) Effect of bile acid exposure on intestinal mucosa: Thoracic duct lymphocytes (TDL) were collected from the thoracic duct of donor rat. We intravenously injected CFSE-fluorescence TDL into recipient rats, and migration in intestinal mucosa was observed by a confocal microscope to evaluate the change of TDL migration. In some recipient rats, bile acids were injected into ligated ileum at both ends to evaluate the direct effect on intestinal mucosa. Tauro Colic Acid Natrium (tauro-CANa, 4 mM) or DCA (4 mM) were injected into the intestinal lumen with a 29G needle. MAdCAM-1 neutralizing antibody was administered intravenously in some rats. (2) Effect of bile acid exposure on TDL: TDL were cultured at 4°C for 2 hours with above mentioned bile acids. Expression levels of L-selectin and α4 integrin in the obtained lymphocytes were examined by flow cytometry.

Results: (1) A small number of lymphocytes adhered to intestinal microvessels in control group. TDL adhesion increased in the DCA exposure group. Lymphocyte adhesion was completely blocked by neutralizing antibody of MAdCAM-1. There was no change in TDL adhesion in the Tauro-CANa exposure group. (2) No change of expressions of adhesion molecules such as α4 integrin or L-selectin on TDL was observed with or without addition of bile acids.

Conclusion: DCA increases lymphocyte adhesion to the vascular endothelium in the ileal mucosa, suggesting that the gastrointestinal immunity could be altered by some bile acids via increase in expression of adhesion molecules on microvessels.

Y-05 | Pulmonary endothelial response to cognate anti-HLA antigen and leukocytes in a murine TRALI model

Hideyuki Ochi[1]; Akira Ushiyama[2]; Takehiko Iijima[1]

[1]*Department of Perioperative Medicine, Division of Anesthesiology, Showa University, School of Dentistry, Tokyo, Japan* [2]*National Institute of Public Health, Saitama, Japan*

Background: Transfusion-related acute lung injury (TRALI) is mostly caused by incidental exposure to anti-leukocyte antibody (Ab). Since the severity of TRALI varies widely, Ab does not necessarily induce pulmonary distress. We attempted to confirm the effect of this reaction on the pulmonary microcirculation using intravital microscopy in a murine TRALI model.

Study design and methods: A total of 50 male BALB/c mice were anesthetized and subjected to artificial ventilation. The surface pulmonary microcirculation was observed using an epi-fluorescence microscope through a thoracic window. Monoclonal antibody against H2Kd was administered to the animals (Ab group, n = 25), while saline was to the control group. An arterial blood gas was analyzed. The leukocytes and macro-molecular leakage in the pulmonary circulation were analyzed. The ex vivo wet and dry lung masses were weighed. All experimental protocols were approved by the Committee for Animal Experiments at the National Institute of Public Health.

Results and Conclusion: Leukocytes accumulated in the capillaries (64.6 ± 18.7 leukocytes per designated area in Ab group vs. 20.2 ± 11.0 in control) , and the macro-molecular leakage was confirmed. An arterial blood gas unchanged after antibody administration. The air-containing alveolus area significantly shrank from 2992.6 ± 255.0 μm^2 in the control group to 835.6 ± 624.2 μm^2 in the Ab group. The wet/dry ratio also significantly differed between the groups (6.80 ± 3.88 in the Ab group vs. 4.64 ± 1.16 in the control group). mAb-H2Kd caused the accumulation of leukocytes and hyperpermeability without oxygenation disturbance. These results indicate that disturbances in pulmonary microcirculation can occur without any manifestations of respiratory distress upon exposure to cognate leukocyte antibody.

Y-06 | A case of cerebral air embolism after catheter examination: possible mechanism of microcirculatory disturbance

Hiroko Kimura[1]; Reiko Sawada[1]; Marie Yamamoto[1]; Takato Abe[1]; Kenji Sawa[2]; Yoshiaki Itoh[1]

[1]*Department of Neurology, Osaka City University Graduate School of Medicine, Osaka, Japan* [2]*Department of Respiratory Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan*

Purpose and Methods: Air embolism is a well-known complications of trauma, central venous catheterization, angiography, and surgical procedures. In most cases, air emboli obstructs systemic vein without significant symptoms, whereas paradoxical air embolism through a right-to-left shunt may affect systemic arterial circulation including brain. Brain CT or MRI is reported to be effective in detecting cerebral air embolism although intracranial air may be absorbed very quickly. The detailed mechanism of cerebral blood flow obstruction and reperfusion is still unknown. Here we report a 67 year-old male with cerebral air embolism following the Swan-Ganz's (SG) catheter study. Suspected mechanisms and an animal model of cerebral air embolism supporting the hypothesis will be presented.

Results and Conclusion: A 67 year-old male was admitted to our hospital for unknown cause of hypoxia. He underwent SG catheter angiography which visualized aorta immediately after the right atrium was imaged with contrast enhancement. He was diagnosed as

having patent foramen ovale (PFO) with massive right-to-left shunt. Immediately after the examination, he became drowsy with right hemiparesis. Brain CT scan revealed small air in the superior sagittal sinus, suggesting cerebral air embolism. MRI immediately after CT revealed no hyperintense lesion on diffusion-weighted images and no deficit on MR angiography. One hour later air disappeared on CT. He was treated with heparin for possible thromboembolism. MRI on the next day revealed diffuse high intensity area in the left MCA territory. He had right focal seizure which was successfully treated with an antiepileptic drug. His weakness started to improve on day 2 with complete recovery on day 6. An air bubble plugged at a major trunk of cerebral artery may be quickly dissembled and obstruct microcirculation. Animal model of air embolism might help understand the mechanism.

Y-07 | QiShenYiQi Pills® ameliorates fatigue-induced cardiac hypertrophy and dysfunction via regulation of energy metabolism

Rong Huang[1,2]; Yuan-Chen Cui[1,2]; Xiao-Hong Wei[1,2]; Chun-Shui Pan[1,2]; Quan Li[1,2]; Shu-Ya He[1,2]; Jing-Yu Fan[1,2]; Jing-Yan Han[1,2]

[1]Department of Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University, Beijing, China [2]Tasly Microcirculation Research Center, Peking University Health Science Center, Beijing, China

Aims: Sudden cardiac death is the leading medical cause of death in athletes. This study was to explore the role and mechanism of QiShenYiQi Pills®, a compound Chinese medicine, in protection of fatigue-induced cardiac hypertrophy and dysfunction.

Methods: Male Sprague Dawley rats were used to establish exercise adaption and fatigue model on a motorized rodent treadmill. Echocardiographic analysis and heart function test were performed to assess heart systolic function. Food Intake Weight/Body Weight, Heart Weight/Body Weight hematoxylin and eosin staining, immunofluorescence staining of myocardium sections were assessed to evaluate myocardial structure. ATP synthase expression and activity, ATP levels were assessed using Western blot and ELISA. Expression of proteins related to energy metabolism and IGF-1R signaling was determined using Western blot.

Results: QiShenYiQi Pills® attenuated Food Intake Weight/Body Weight decrease, improved myocardial structure and heart function, restored the expression and distribution of myocardial cx43 after fatigue, concomitant with an increased ATP production and a restoration of metabolism-related proteins expression. QiShenYiQi Pills® upgraded the expression of IGF-1R, P-AMPK/AMPK, PGC-1α, Nrf1, P-PI3K/PI3K, P-Akt/Akt thereby attenuated the dysregulation of IGF-1R signaling after fatigue.

Conclusion: QiShenYiQi Pills® relieved fatigue-induced cardiac hypertrophy and enhanced heart function, which is correlated to its potential to improve energy metabolism by regulating IGF-1R signaling.

Y-08 | QiShenYiQi Pills® inhibits I/R-induced myocardial fibrosis via RP S19 through TGFβ1/Smads signaling pathway

Qian-Ning Zheng[1,2]; Xiao-Hong Wei[1,2]; Chun-Shui Pan[1,2]; Quan Li[1,2]; Yu-Ying Liu[1,2]; Jing-Yu Fan[1,2]; Jing-Yan Han[1,2]

[1]Department of Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University; [2]Tasly Microcirculation Research Center, Peking University Health Science Center

QiShenYiQi Pills (QSYQ) is a compound Chinese medicine widely used in China for the treatment of cardiovascular disease. However, limited data are available regarding the anti-fibrotic role of QSYQ after ischemia-reperfusion (I/R) injury. This study aimed to investigate the effect of QSYQ and its components on I/R-induced myocardial fibrosis, focusing especially on RP S19 and monocyte migration. Male Sprague-Dawley rats were subjected to left coronary anterior descending branch occlusion for 30 min followed by reperfusion with or without administration of QSYQ (0.6, 1.2, or 1.8 g/kg) once daily by gavage for 6 days. Treatment with QSYQ diminished I/R-induced infarct size, retained myocardium structure after I/R, attenuated myocardial fibrosis, and restored heart function and myocardial blood flow after I/R. In addition, the drug significantly inhibited monocyte infiltration and macrophage polarization towards M2, which involved the presence of chemokine RP S19. Moreover, western blots revealed that QSYQ blocked I/R-induced TGFβ1 upregulation and downstream gene expression, such as Smad3,4,6,7, and inhibited expression of MMP2,9. As the components of QSYQ, AsIV, DLA and R1 were assessed as to the effect of each alone or combination between any two on the expression of the proteins concerned. The results showed that AsIV exhibited an effect like QSYQ, while DLA and R1 only partly simulated the effect of QSYQ. The results provide evidence for the potential role of QSYQ in treating myocardial fibrosis following I/R injury. This effect may be associated with QSYQ's inhibition effect on monocyte chemotaxis and TGFβ1/Smads signaling pathway with different component targeting distinct link (s) of the signaling.

FREE COMMUNICATIONS

F-01 | Spreading depolarization evoked during middle cerebral artery occlusion may trigger a development of cerebral infarct in mice

Miyuki Unekawa[1]; Yutaka Tomita[1]; Yoshikane Izawa[1]; Chunhua Tang[1]; Kazuto Masamoto[2]; Iwao Kanno[3]; Norihiro Suzuki[4]; Jin Nakahara[1]

[1]Department of Neurology, Keio University School of Medicine, Tokyo, Japan [2]Brain Science Inspired Life Support Research Center, University of Electro-Communications, Tokyo, Japan [3]Department of Functional Brain Imaging Research, National Institute of Radiological Sciences, Chiba, Japan [4]Department of Neurology, Shonan Keiiku Hospital, Kanagawa, Japan

Background: Spreading depolarization (SD) , sometimes occurs in patients with ischemic or hemorrhagic stroke and trauma, accompanies

hemodynamic response and metabolic dysfunction and may be pathologically harmful during ischemia.

Objectives: To evaluate a correlation of SD with development of cerebral infarction, we analyzed cerebral blood flow (CBF) changes associated with spontaneously occurred SD during ischemia.

Methods: In male C57BL/6J mice (n = 44) , CBF was continuously recorded over the ipsilateral parietal bone with a laser speckle flowgraphy during and after transient (45 min) or permanent occlusion of middle cerebral artery (MCAO) by modified Tamura's method and the spatial response was evaluated with ImagePro software. Cerebral infarction was evaluated by TTC staining after 24 h of MCAO.

Results: Upon MCAO, CBF decreased by -55.7 ± 9.3% in the limited core region and diminished by approximately 20% per 1 mm away from the core region. During occlusion, SD spontaneously or mechanically occurred and concentrically propagated from the core region in 86% of mice. Following SD spontaneously reoccurred and propagated around the ischemic area in 32% of mice. Single propagation of SD wave elicited CBF decrease in the core region, whereas transient decrease/increase followed by slight long-lasting decrease (oligemia) in the normal region. Infarct volume was not significantly correlated with CBF decrease in core region (r = 0.162 in transient and r = 0.167 in permanent occluded mice) or CBF decreased (>40%) area (r = -0.205 in transient and r = -0.084 in permanent occluded mice). Infarction was not observed in mice without SD, and the infarct volume tended to be greater with increasing number of SD during MCAO among transient or permanent occluded mice.

Conclusion: Ischemia-related SD induces a typical change of cerebral circulation as previously reviewed (Hartings, JCBFM, 2017) and might be a trigger of cerebral infarct formation. This study was performed in accordance with the approval (No. 09058) of the Animal Ethics Committee of Keio University (Tokyo, Japan).

F-02 | Effects of memantine on NO production and hydroxyl radical metabolism during cerebral ischemia and reperfusion in mice

Yasuo Ito[1]; Ai Tanaka[2]; Hitoshi Kawasaki[1]; Chika Kitabayashi[1]; Ryoji Nishioka[1]; Makiko Hirayama[1]; Kazushi Takahashi[1]; Toshimasa Yamamoto[1]; Nobuo Araki[1]

[1]*Department of Neurology, Saitama Medical University, Saitama, Japan*
[2]*Department of Neurology, Tottori Medical Center, Tottori, Japan*

Objective: The purpose of this study is to investigate the effects of memantine in brain ischemia, and we measured the nitric oxide (NO) production, hydroxyl radical metabolism in cerebral ischemia and reperfusion.

Methods: Memantine (25 μmol/kg) was administered intraperitoneally to six C57BL/6 mice 30 minutes before ischemia. Seven additional mice received no injection (controls). NO production and hydroxyl radical metabolism were continuously monitored using bilateral striatal microdialysis *in vivo*. Hydroxyl radical formation was monitored using the salicylate trapping method. Forebrain ischemia was produced in all mice by occluding the common carotid artery bilaterally for 10 minutes. Levels of the NO metabolites nitrite (NO_2^-) and nitrate (NO_3^-) were determined using the Griess reaction. Survival rates of hippocampal CA1 neurons were calculated and 8-hydroxy deoxyguanosine (8-OHdG) - immunopositive cells were counted to evaluate the oxidative stress in hippocampal CA1 neurons 72 hours after the start of reperfusion.

Results: The regional cerebral blood flow was significantly higher in the memantine group than in the control group after reperfusion. Furthermore, the level of 2,3-dihydroxybenzoic acid was significantly lower in the memantine group than in controls during ischemia and reperfusion. Levels of NO_2^- and NO_3^- did not differ significantly between the two groups. Although survival rates in the CA1 did not differ significantly, there were fewer 8-OHdG-immunopositive cells in animals that had received memantine than in control animals.

Conclusions: These data suggest that memantine exerts partially neuroprotective effects against cerebral ischemic injury in mice.

F-03 | Ultrasonography monitoring and magnetic resonance angiography in moyamoya disease

Mami Ishikawa[1]; Satoshi Terao[2]; Mutsumi Nagai[3]; Hiroshi Kagami[4]; Makoto Inaba[4]; Heiji Naritaka[5]

[1]*Department of Neurosurgery, Tachikawa Hospital, Tokyo, Japan* [2]*Department of Neurosurgery, Saiseikai Central Hospital, Tokyo, Japan* [3]*Department of Neurosurgery, International university of health and welfare, Tokyo, Japan*
[4]*Department of Neurosurgery, Saiseikai Yokohamashi Tobu Hospital, Kanagawa, Japan* [5]*Department of Neurosurgery, Edogawa Hospital, Tokyo, Japan*

Objective: Ultrasonography (US) provides reliable real-time information during neurosurgery. The latest imaging technique, Superb Microvascular Imaging (SMI) technique, was used for intraoperative US monitoring of two cases with moyamoya disease to examine the pathophysiology. We compared small vessels and their flow between these patients and control patients with SMI technique and contrast agent technique.

Methods: Two patients with moyamoya disease underwent encephalo-duro-arterio-myo-synangiosis (EDAMS), indirect revascularization, and five control patients with unruptured aneurysms underwent clipping operations under US monitoring with the SMI technique, which visualized low-velocity flow with high resolution and high frame rates. We investigated whether the original images of magnetic resonance angiography show vessels in ventricles in other patients with moyamoya disease. All procedures performed were in accordance with the ethical standards of the institutional research committee of Edogawa Hospital and Tachikawa Hospital.

Results: The small cerebral penetrating vessels were clearly visualized by the SMI technique, detailing the characteristics of normal cerebral vessels in patients with unruptured aneurysms. The penetrating vessels were dilated in two moyamoya disease patients.

During neurosurgery, the penetrating flow gathered to the periventricular vessel in infarction type patient, and in the other hemorrhage type patient, flow in the numerous penetrating vessels came from a point of the periventricular vessels. The original images of magnetic resonance angiography show more vessels in ventricles of hemorrhage type patients with moyamoya disease compared to infarction type.

Discussion: The small vessels were dilated, and their enhanced flows were recognized following injection of contrast agent in patients with moyamoya disease. In patients with moyamoya disease, there are three onset pattern, hemorrhage type, infarction type and epilepsy type. Recently, patients visit brain dock and may be diagnosed with moyamoya disease. We cannot judge onset pattern of the patients, but the MR angiography may be useful for the asymptomatic moyamoya disease patients.

F-04 | Ultrasonography using Superb Microvascular Imaging and contrast agent techniques during neurosurgery

Mami Ishikawa[1]; Satoshi Terao[2]; Mutsumi Nagai[3]; Kazuto Masamoto[4]; Ryota Hachiya[4]; Heiji Naritaka[5]

[1]Department of Neurosurgery, Tachikawa Hospital, Tokyo, Japan [2]Department of Neurosurgery, Saiseikai Central Hospital, Tokyo, Japan [3]Department of Neurosurgery, International University of Health and Welfare, Tokyo, Japan [4]Faculty of Informatics and Engineering, University of Electro-Communications, Tokyo, Japan [5]Department of Neurosurgery, Edogawa Hospital, Tokyo, Japan

Objective: Ultrasonography (US) is used during neurosurgical operations as a reliable imaging, providing real-time information. We apply the Superb Microvascular Imaging (SMI) technique for detecting low flow components with the contrast agent technique to neurosurgery. We analyzed the US images and measured the vessel density and vessel flow velocity.

Methods: Seventeen patients diagnosed with a brain tumor (10 meningiomas, 4 glioblastomas, one schwannoma, one hemangioblastoma, one malignant lymphoma) underwent neurosurgery under US monitoring using Aplio US system with SMI and contrast agent techniques. 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. US images of cerebral, cerebellar and tumor microcirculatory flow were obtained following infusion of contrast agent, with fusion image of preoperative MRI. We analyzed a total length of vessel fragments and arrival time of microbubble per unit area using the US images following the contrast agent injection. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee of Edogawa Hospital and Tachikawa Hospital.

Results: Tumor vessels length increased and arrival time of the microbubbles decreased, excluding the malignant lymphoma, compared to those of normal brain. Intraoperative US using SMI and contrast agent techniques produced pioneering images, which distinguished tumor from normal brain using different arrival time of microbubble. The superimposed vessel images also distinguished tumor area from normal brain, using different vessel density in the operative field.

Conclusion: Contrast agent-enhanced US images show microvascular flow of the normal brain and the tumors and provide intraoperative information for neurosurgery.

F-05 | Abstract withdrawn

F-06 | Angioedema and hemorrhage after 4.5 h tPA thrombolysis ameliorated by T541 via regulating mitochondria metabolism

Jing-Yan Han[1,2]; Qing-Fang Chen[1,2]

[1]Department of Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University, Beijing, China, [2]Tasly Microcirculation Research Center, Peking University Health Science Center, Beijing, China

Background: Tissue-type plasminogen activator (tPA) is the only recommended intravenous thrombolytic agent within 3–4.5 hours from stroke onset. How to expand the time window avoiding increasing risk of angioedema and hemorrhage troubled people for decades. T541, a Chinese medicine compound consisted of total Astragalus saponins, total Salvianolic acids and total Panax notoginseng saponins may have potential to alleviate cerebral injury and extend tPA thrombolysis time window.

Methods: Carotid artery thrombosis model was established in male C57BL/6N mice stimulated with 10% $FeCl_3$ for 3 minutes following 10 mg/kg tPA with/without 20 mg/kg T541 at 4.5 hours from femoral vein. Continuous dynamic observation of thrombolysis and cerebral blood flow changes until 24 hours. Then, neurological deficient scores, brain edema and hemorrhage, gap junctions and basement membrane around cerebral microvascular and energy supply in cortex were tested. Rat cerebral microvascular endothelium cultured hypoxia for 4.5 hours and reoxygenation for 3 hours in vitro, testing gap junctions and F-actin.

Results: tPA administered at carotid thrombosis 4.5 hours had worse neurological scores, survival rate, a low efficiency of thrombolysis and cerebral blood flow restoration, high risk of angioedema and hemorrhage in the ischemia hemisphere. Expression decreased and arrangement became discontinuous of junctions (claudin-5, zonula occludens-1, occludin, junctional adhesion molecule-1 and vascular endothelial cadherin), collagen IV and laminin. ATP/ADP, ATP/AMP, ATP5D expression and activities of mitochondria complex I, II, IV declined, whereas an increase of MDP, 8-OHdG and F-actin arranged disordered after 4.5 h tPA treatment. All side effects were reversed by T541 dose-dependently.

Conclusions: Regulating mitochondria metabolism and oxidative stress by T541 may related to protecting endothelial gap junctions and basement membrane of blood-brain-barrier. It provided a new

strategy to reversing angioedema and hemorrhage after 4.5 hours tPA thrombolysis.

F-07 | Analysis of indocyanine green (ICG) angiography during neurosurgical operation for intracerebral hemorrhage and arteriovenous malformation

Satoshi Terao[1]; Mutsumi Nagai[2]; Kazuto Masamoto[3]; Ryota Hachiya[3]; Hiroshi Kagami[4]; Makoto Inaba[4]; Mami Ishikawa[5]

[1]*Department of Neurosurgery, Saiseikai Central Hospital, Tokyo, Japan* [2]*Department of Neurosurgery, International University of Health and Welfare, Tokyo, Japan* [3]*Faculty of Informatics and Engineering, University of Electro-Communications, Tokyo, Japan* [4]*Department of Neurosurgery, Saiseikai Yokohamashi Tobu Hospital, Kanagawa, Japan* [5]*Department of Neurosurgery, Tachikawa and Edogawa Hospital, Tokyo, Japan*

Objective: Indocyanine green (ICG) angiography during neurosurgical operation has been used as an indicator for confirmation of vessel flow. Intraoperative information of blood flow prevents patients postoperative neurological deficits. However, there is no accurate analysis with this image. The flow velocity was measured in patients during neurosurgery following ICG injection and reported by Hachiya et al. at this annual meeting 2017. We measured brain surface velocity during neurosurgical operation for intracerebral hemorrhage (ICH) and arteriovenous malformation (AVM).

Methods: Using an operating microscope (OPMI PENTERO; Carl Zeiss Meditec, Jena, Germany) equipped with fluorescent light source for ICG. ICG (7.5 mg/1.5 ml saline) was rapidly injected as a push bolus, followed by a bolus of 20 ml saline. Images from ICG angiography were recorded under the same conditions (same lens focus, same magnification, and so on) and analyses were performed using the method by the Hachiya et al. All procedures were in accordance with the ethical standards of the institutional research committee of Edogawa Hospital and Saiseikai Central Hospital.

Results: In a patient with ICH, arterial flow tended to recover and venous flow was maintained at the same level after removal of hematoma, compared to those before the removal. In a patient with AVM, cerebral surface arteries drastically dilated and venous flow velocity also drastically increased three to four times after removal of AVM nidus.

Conclusion: We measured vessel velocity of the brain surface in a patient with ICH and AVM. The vessel velocity tend to recover after hematoma removal in a patient with ICH. The arterial and venous flow velocities drastically increased in a patient with AVM after a nidus removal, which means hyperperfusion in the normal brain. Further investigation is important for the safe and precise neurosurgical operation.

F-08 | Microcirculation of cardiac stimuli conduction system: immunohistochemical observation of the microvasculature

Eikichi Okada

Department of Pathology, Toyama City Medical Association Health Control Center, Tokyo, Japan

Objective: To elucidate the features of microcirculation in cardiac conduction system through immunohistochemical analysis of the microvasculatures.

Methods: Anti-CD31-, anti-CD34-, anti-podoplanin-, and anti-von Willebrand factor-immunohistochemistry applied to the autopsied adult hearts without significant cardiac diseases except for mild cardiac hypertrophy due to hypertension.

Results: The density of the blood capillary of the left ventricle is 1690 ± 171/mm^2, the atrium is 690 ± 73/mm^2, the sinus node is 1080 ± 85/mm^2, the AV-node is 1340 ± 148/mm^2, the His' bundle is 1070 ± 94/mm^2, the Purkinje fiber is 0 ± 0/mm^2. The sinus node, the AV node, and the His' bundle have distribution of perimysial and endomysial lymphatic capillaries, while the Purkinje fiber has neither distribution of perimysial nor endomysial lymphatic capillaries. There is no distribution of intrafascicular lymphatic capillaries in all the cardiac conduction systems. The ratio of the blood capillaries to the muscle fibers of the left ventricle is 1. 1. Considering the influence of cardiac hypertrophy due to hypertension, the blood capillary and the muscle fiber ratios of the sinus node, the AV node, and the His' bundle are 1. 1. Same as the left ventricular muscle, the sinus node, the AV node, and the His' bundle are considered to be fostered by the blood circulating in the intrafascicular capillaries. There is no intrafascicular blood capillaries in the His' bundle. It is considered to be fostered by direct diffusion from the intraventricular blood.

F-09 | Unilateral truncal vagotomy facilitates MALT lymphoma formation: relation to substance P and NK-1R on microvessels

Masahiko Nakamura[1]; Shinichi Takahashi[2]; Hidenori Matsui[3]

[1]*School of Pharmacy, Kitasato University, Tokyo, Japan* [2]*School of Medicine, Kyorin University, Tokyo, Japan* [3]*Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan*

Objectives: Vagal nerve plays important role in stomach function. The cholinergic nerves are the nerve most abundantly distributed in the gastric tissue. It has been recently reported that vagal nerve is significantly related to both gastric cancer development and progression, while its relation to the mesenchymal tumor, including MALT lymphoma, is not known. In this study, we investigated the effect of unilateral truncal vagotomy on gastric MALT lymphoma development by using *Helicobacter heilmannii* -infected mouse model. Alteration of substance P and CGRP and

their receptor localization was also investigated by the immunohistochemical method.

Materials and Methods: C57BL/6NCrl mice were infected with *Helicobacter heilmannii*. A total of 38 infected mice underwent unilateral vagotomy under microscopy. The mice were randomized into 4 groups which are the time points of sampling: 2, 3, 4 and 6 months after infection. Both the anterior and posterior sides of the stomachs were removed from each mouse for pathological and immunohistochemical analyses.

Results: The thickness of gastric mucosa was reduced in the vagotomized side compared to the non-vagotomized side. The gastric MALT lymphoma was more prominently found in the vagotomized anterior side of stomach compared with that in the non-vagotomized posterior side of stomach. Substance P-immunoreactive nerves markedly increased in the lymphoma cells and the neurokinin-1 receptor immunoreactive microvessels within the MALT lymphoma in the vagotomized side, compared with the non-vagotomized side. In conclusion, vagotomy enhanced gastric MALT lymphoma development possibly through the substance P-neurokinin-1 receptor pathway. This study was conducted after approval of Kitasato Institute Hospital Ethical Committee, where the principal investigator belonged.

F-11 | Disruption of glycocalyx on cerebral and glomerular blood vessel in a rat model of preeclampsia and protective effects of danaparoid sodium

Hirofumi Hino; Yoshisuke Naitoh; Takanari Yoshikawa

Department of Anesthesiology, St. Marianna University School of Medicine, Kanagawa, Japan

Introduction: It is well recognized that pre-eclampsia (PE) is a major contributor to maternal and fetal mortality. Although the pathophysiology of cerebral edema and proteinuria in PE remains unclear, increased permeability might play a major role in this pathophysiology. We demonstrated that disruption of glycocalyx (GCX), which is involved in the maintenance of vascular permeability, was observed in PE rat model. In the present study, we investigated whether danaparoid sodium (DS), which acts as an anticoagulant and anti-inflammatory agent, possess the protective effect of GCX in the PE rat model.

Methods: After obtaining of local IRBs approval, 27 rats were randomly divided into three groups:Control (C) group, PE and PD-group (PE with DS). To establish experimental PE rats, a modified Sakawi's method using administration of LNAME and LPS was employed in the PE and PD-group. A catheter inserted for the perfusion fixation on the day 21. After the fixation using lanthanum, the both kidney and cerebrum were removed. The image of GCX was archived from each tissue and determined by histogram from the image of electro-microscope.

Results: The disruption of GCX in PE-group was significantly higher than that of C in both tissues (Cerebrum; C: 117 ± 24, PE: 48 ± 6, $P < 0.05$, glomerulus; C: 125 ± 30, P: 72 ± 10 nm, $P < 0.05$, individually). DS significantly improved GCX degradation of PE in both tissues (Cerebrum; 93 ± 27, glomerulus; 110 ± 17, $P < 0.05$).

Conclusion: DS might play a major role in the protective effect of capillary permeability by attenuating GCX damage, because DS possess not only an anti-inflammatory effect and also the similar structure of GCX.

F-12 | Protection of endothelial glycocalyx layer through localization of hydroxyethyl starch in murine models of acute severe hemorrhage

Kohji Uzawa[1]; Shingo Mitsuda[1]; Tomoko Yorozu[1]; Akira Ushiyama[2]

[1]*Department of Anesthesiology, Kyorin University School of Medicine, Tokyo, Japan* [2]*National Institute of Public Health, Saitama, Japan*

Our purpose was to evaluate the effectiveness of hydroxyethyl starch (HES) infusion in protecting the endothelial layer under massive bleeding conditions, using intravital microscopy.

We used 15-week-old male BALB/c mice weighing 25–30 g with non-metallic dorsal skin chambers (DSCs). Experiment 1: We divided 30 mice into six groups including an untreated control group. We drew 1.5 ml blood from the internal jugular vein (0.05 ml/s) of each mouse and infused identical volumes of one of the following fluids (Saline, HES130 kDa, Albumin, Saline+HES130 kDa, and Saline+Albumin) and FITC-WGA Lectin. Observation of the DSC window using intravital microscopy allowed measurement of the thickness of the illuminated parts of the vascular wall as a surrogate for the endothelial surface layer (ESL). Glycocalyx damage was assessed by measuring blood syndecan-1 concentrations, which indicate glycocalyx degradation. Experiment 2: We infused TMR-DEX40 kDa and FITC-HES70 kDa instead of FITC-WGA after blood draw and measured their fluorescence intensity when leaking into the interstitial space, owing to hyperpermeability caused by ESL damage. All experimental protocols were approved by the Committee for Animal Experiments at the National Institute of Public Health (protocol number: 30-006). In the saline group, blood syndecan-1 was significantly increased and the ESL layers became thinner than those in the colloid groups (ALB- and HES-administered groups). Vascular permeability was significantly suppressed in the HES groups compared to that in the saline group at 30, 60 and 90 min following blood draw. HES70 kDa was localized in the intravascular wall in the saline and ALB groups, whereas TMR-DEX40 kDa was uniformly distributed in the blood vessel.

HES administered at the time of massive bleeding protects the ESL from damage. Localization of HES70 kDa to the inner vascular wall of the animal model suggests that hydroxyethyl starch directly protects endothelial glycocalyx layer during massive bleeding.

F-13 | Challenge to clarify the physiological basis of obesity paradox using intravital microscopy

Shingo Mitsuda[1]; Kohji Uzawa[1]; Tomoko Yorozu[1]; Akira Ushiyama[2]

[1]*Department of Anesthesiology, Kyorin University School of Medicine, Tokyo, Japan* [2]*National Institute of Public Health, Saitama, Japan*

Objective: The "obesity paradox" is a medical hypothesis which holds that obese people may be protective and associated with higher survival than lean people. However, little is known about physiological basis that support the mechanism of obesity paradox. Therefore, we aim to elucidate the differences in vascular endothelial glycocalyx (GCX) with intravital microscopy among three groups of mice, that fed different fat content diet.

Methods: Male C57BL/6 mice were divided into three groups; low-fat diet (L, fat = 10%kcal) , medium-fat diet (M, 45%kcal) , and high-fat diet (H, 60%kcal) group. Mice were fed with each diet since 3-week of age, and the chronic cranial window was installed at 8-week of age. At 9-week of age, FITC-labeled wheat germ agglutinin (FITC-WGA) and tetramethyl rhodamine-labeled dextran 70 kDa (TMR-DEX 70) were intravenously injected, and then the brain pial microcirculation within cranial window were observed. We randomly selected three arterioles with a diameter of 20–50 μm per mouse, and captured the images. The mean thickness of GCX (FITC-WGA positive layer) was calculated by offline image analysis and defined as the GCX index (GCXI). All experimental protocols were approved by the Committee for Animal Experiments at the National Institute of Public Health.

Results: The GCXIs of the H and M groups were significantly higher than that of the L group ($P < 0.05$). There was a positive correlation between vessel diameter and GCXI in the H and M group, but not in the L group.

Conclusions: In the H and M group, the GCX layer thickened with an increase in the diameter of the vessels. However, the thickness of GCX remained unchanged in the L groups under similar conditions. Thus, GCX thickness may play a key role in beneficial impact on pathological changes in obesity paradox.