Novel Mechanisms of Myocardial Ischemia (IHD)

FRS9

March 21 (Sat)

Room 14 (RIHGA Royal Hotel Osaka West Wing 2F KIKU)

8：30—10：10

Keynote Lecture:

Protecting the Ischemic Heart with Pharmacological and Ischemic Postconditioning

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Ischemic preconditioning offers powerful protection against infarction of the ischemic heart but the requirement for pretreatment has made it impractical for treating patients with acute myocardial infarction. However, because preconditioning actually exerts its protection at the time of reperfusion animal studies now reveal that it is not too late to confer this protection to hearts in which ischemia has already begun. Postconditioning with several minutes of brief cycles of ischemia and reperfusion at the termination of a lethal ischemic insult is almost as protective as preconditioning and most studies indicate that the same mechanism is used by both. We found that ischemic postconditioning prevents lethal mitochondrial transition pore formation by keeping the myocardium acidic while it is reoxygenated. That delay seems to allow enough time for the heart to literally precondition itself through its endogenous signal transduction pathways. It is also possible to quickly postcondition the heart using drugs at the time of reperfusion. Activating preconditioning's signal transduction pathway with agents such as adenosine A_{2b} agonists, cyclosporine, erythropoietin, atrial natriuretic peptide, or PKG activators is very protective when these agents are given at reperfusion. Clinical trials with this exciting class of agents are just beginning and already important lessons are being learned on how to more effectively design them. So far, however, the results are very encouraging.

FRS-041

Crucial Role of an Inflammasome Adaptor Molecule ASC (Apoptosis-associated Speck-like Protein Containing a Caspase Recruitment Domain) in Myocardial Ischemia-Reperfusion Injury

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Background: Inflammatory responses play a key role in the pathophysiology of myocardial ischemia-reperfusion (I/R) injury. ASC is an adaptor protein that forms "inflammasome " whose activation leads to caspase-1-dependent interleukin (IL)-1 β generation and subsequent inflammatory responses; however, the role of ASC in myocardial I/R injury remains unclear. **Methods and Results:** Baseline left ventricular (LV) function was unaltered in ASC-deficient ($ASC^{-/-}$) mice. $ASC^{-/-}$ (n=42) and wild-type (WT) (n=42) mice were subjected to 30 min LAD occlusion, followed by reperfusion. $ASC^{-/-}$ mice showed improved LV dysfunction (%FS, $p<0.01$), reduced infarct area /area at risk (IA/AAR, $p<0.01$), and scar formation ($p<0.01$) after myocardial I/R. Immunohistochemistry revealed decreased infiltration of macrophages (Mac3) and neutrophils (Gr-1), but not neovascularization (CD31), in the injured myocardium of the $ASC^{-/-}$ mice. Real-time RT-PCR and ELISA analyses demonstrated that the myocardial mRNA and protein expression of inflammatory cytokines, such as IL-1 β, IL-6, and MCP-1, after I/R were significantly decreased in the $ASC^{-/-}$ mice. Furthermore, we prepared bone marrow-transplanted (BMT) mice and found that IA/AAR was significantly decreased in $BMT^{ASC-/- \text{ to Wild}}$, compared with $BMT^{Wild \text{ to } ASC-/-}$ ($p<0.05$). **Conclusion:** These findings demonstrate that ASC deficiency prevents inflammatory cell infiltration and cytokine expression, thereby resulting in the improvement of LV dysfunction and remodeling after myocardial I/R injury, and suggest that ASC is a novel therapeutic target for myocardial I/R injury.

FRS-042

Elevated Plasma Myeloperoxidase Levels and Coronary Microvascular Dysfunction after Reperfusion in Patients with Acute Myocardial Infarction

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Background: Myeloperoxidase(MPO) is a leukocyte-derived enzyme, and is associated with endothelial dysfunction. This study is aimed at evaluating the association between plasma MPO levels on admission and coronary microvascular dysfunction after reperfusion in patients with acute myocardial infarction(AMI). **Methods:** Plasma MPO levels were measured in patients with ST-segment elevation AMI(n=160) who underwent primary angioplasty within 12 hours of symptom onset. The magnitude of coronary microvascular dysfunction after reperfusion was evaluated by ST-segment resolution (STR) and myocardial blush grade. **Results:** Plasma MPO levels on admission in no STR group (<30%) were significantly higher than in either complete STR group (≥70%) or partial STR group (<70% to 30%)(complete STR group, 59 ± 40ng/ml; partial STR group, 63 ± 49ng/ml; no STR group, 79 ± 49ng/ml; complete STR group vs. no STR group, $P<0.05$). The STR and myocardial blush grade were significantly lower in the high-MPO group (>50ng/ml, median) compared with the low-MPO group(49 ± 29 vs. 62 ± 21%, $P<0.005$; 2.1 ± 0.8 vs. 2.4 ± 0.7, $P<0.05$; respectively). Furthermore, LVEF at 6 months was significantly lower in the high-MPO group compared with the low-MPO group(46 ± 9 vs. 54 ± 9%, $P<0.0001$). Multiple regression analysis showed that elevated plasma MPO level on admission was an independent predictor of incomplete STR(OR, 2.95; 95%CI, 1.43 to 6.11; P=0.0035). **Conclusion:** These findings suggest that elevated plasma MPO levels on admission are independently associated with coronary microvascular dysfunction following reperfusion in AMI patients.

FRS-043

Voglibose Reduces Myocardial Infarct Size via Stimulation of GLP-1 Receptors and PI3 kinase-Akt-eNOS Pathway in Rabbits

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Background: Glucagon-Like Peptide-1(GLP-1) has been reported to protect the heart. We hypothesized that an α-glucosidase inhibitor voglibose might reduce myocardial infarct size via production of GLP-1. Methods: Japanese white rabbits underwent 30 min of coronary occlusion followed by 48h of reperfusion. Rabbits were assigned randomly to 6 groups: a control group, a voglibose group fed diets containing 3.5 mg/kg/day voglibose for 7days, and a voglibose + GLP-1 receptor blocker exendin (9-39) group (3nmol/l), an exendin only (9-39) group, a voglibose + PI3K inhibitor wortmannin group (0.6mg/kg), a wortmannin only group. Myocardial infarct size was measured as a percentage of the risk area. Results: The infarct size was significantly smaller in the voglibose group than in the control group, and this effect was abolished by pretreatment with exendin(9-39) or wortmannin. Ejection fraction in the voglibose group was significantly larger than that in the control group. This effect was completely abolished by exendin(9-39) or wortmannin. Voglibose increased plasma GLP-1 levels. Western Blotting analysis shows that Phospho-Akt and phospho-eNOS were over-expressed in the myocardium of the voglibose group. Conclusion: Voglibose reduces myocardial infarct size and improves LV function via stimulation of GLP-1 receptors and PI3-kinase, Akt and eNOS in rabbits. This finding may provide new insight into therapeutic strategies for di-

abetic patients with coronary artery disease.

FRS-044

ROS-mediated Enhancement of ANT-cyclophilin-D Interaction Underlies Reduced Anti-infarct Tolerance and Lost Response of Cardiomyocytes to Pro-survival Signaling in Hypertrophied Hearts

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Objective We examined the hypothesis that opening of mitochondrial permeability transition pore (mPTP) and consequential cell death during ischemia/reperfusion are facilitated in hypertensive hypertrophied hearts. **Methods and Results** In 12~15-week-old stroke-prone spontaneously hypertensive rats (SHR-SP) and Wistar-Kyoto rats (WKY), myocardial infarction was induced by 20-min ischemia/2-hr reperfusion. Infarct size as % of area at risk (%IS/AR) was significantly larger in SHR-SP than in WKY (69.6 ± 4.5% vs. 54.8 ± 3.7%). Administration of erythropoietin (EPO, 5000 IU/kg) before ischemia limited %IS/AR in WKY (44.2 ± 2.8%) but not in SHR-SP (65.0 ± 5.2%). Phosphorylation levels of Akt, ERK1/2 and GSK-3 β after EPO infusion were similar in SHR-SP and WKY. Baseline levels of adenine nucleotide translocator (ANT), a major subunit of mPTP, and cyclophilin-D (CypD), a trigger of mPTP opening, were comparable in the two groups. However, level of CypD co-immunoprecipitated with ANT and level of carbonylation (a marker for oxidative stress) of mitochondrial proteins upon reperfusion were higher in SHR-SP than in WKY. The inter-group differences were eliminated by infusion of N-(2-mercaptopropionyl)-glycine, a reactive oxygen species (ROS) scavenger. EPO reduced ANT-CypD complex formation upon reperfusion in WKY but not in SHR-SP. **Conclusion** Enhanced interaction of CypD with ANT, leading to mPTP opening, contributes to reduced anti-infarct tolerance and lack of response to EPO-induced protection in hypertrophied hearts of SHR-SP. Augmented ROS is responsible for the enhanced CypD-ANT interaction.

FRS-045

Transient Opening of Mitochondrial Permeability Transition Pore by Reactive Oxygen Species Protects Cardiac Myocytes by Modulating Mitochondrial Ca^{2+}

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Purpose: Reactive oxygen species (ROS) production during ischemia/reperfusion is a critical factor in myocardial injury. However, we have previously reported in Langendorff-perfused hearts that a small amount of hydrogen peroxide (H_2O_2) protected myocardium from ischemia/reperfusion injury through a transient opening of mitochondrial permeability transition pore (mPTP). We further investigated whether ROS would modulate mitochondrial function and mitochondrial Ca^{2+} regulation. **Methods:** The opening of the mPTP (with calcein), mitochondrial membrane potential ($\Delta \Psi_m$; with TMRE), and mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_m$; with rhod2) were measured with a laser scanning confocal microscopy in saponin-permeabilized rat myocytes. **Results:** (1) H_2O_2 (1 μ M) accelerated the calcein leakage from mitochondria, which was canceled by an mPTP inhibitor, cyclosporin A (CsA; 0.1 μ M), whereas H_2O_2 did not depolarize $\Delta \Psi_m$ These results indicate that a low-conductance mode of mPTP was opened by H_2O_2. (2) $[Ca^{2+}]_m$ was decreased by H_2O_2 (H_2O_2; 69 ± 6 %, $p<0.01$ vs. 105 ± 3 % of CTL) in a CsA-sensitive manner (H_2O_2+CsA; 88 ± 4 %, $p<0.01$ vs. H_2O_2). (3) H_2O_2 accelerated mitochondrial Ca^{2+} extrusion, which was attenuated by CsA, indicating the involvement of mPTP opening as a mitochondrial Ca^{2+} efflux pathway. **Conclusion:** We conclude that the transient mPTP opening by H_2O_2 reduces $[Ca^{2+}]_m$ without altering mitochondrial function including $\Delta \Psi_m$, which may prevent mitochondrial Ca2+ overload and irreversible mPTP opening during ischemia/reperfusion.

Updates in Nuclear Cardiology (I)

FRS10

March 21 (Sat)

Room 18 (Hotel NCB 2F MATSU)

8 : 30—10 : 10

Featured Research Session

Keynote Lecture:

Cardiovascular Molecular Imaging

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In the new era, diagnosis and treatment of cardiovascular disease is increasingly being defined by underlying molecular and cellular aberrations rather than by morphological changes and/or clinical signs. In this sense molecular cardiovascular imaging exploits the targeting of expressed cell-surface molecules, intracellular processes, and gene expression. Of particular, nuclear cardiology is considered as a most powerful tool for molecular imaging in clinical setting using suitable radiopharmaceuticals. Cardiac PET has been used for quantitative assessment of myocardial blood flow and coronary flow reserve. This technique nicely identified alteration of coronary flow reserve and endothelial function with various coronary risk factors. In addition, risk factor modifications and various drug therapies indicated a significant improvement in coronary vasomotion. Another molecular target is detection of unstable plaque. While most of imaging techniques have been focused on structural changes of atherosclerosis, nuclear cardiology is used for detection of vascular apoptosis using Tc-99m annexin A5 or macrophage infiltration using FDG. Molecular imaging is also focusing on detection of altered myocardium. We have had tremendous clinical experiences of fatty acid imaging using BMIPP and neuronal function imaging using MIBG. Furthermore, new PET tracers permit quantitative assessment of myocardial energy processes and also beta-receptor function in vivo. Such molecular function beyond perfusion will be helpful for assessing pathophysiology of the diseased myocardium, and optimizing treatment strategy in each patient. Thus, nuclear cardiology has a great potential for providing individualized medicine based on molecular and cellular function in cardiovascular diseases.

FRS-046

"Warranty time" of Normal Stress Myocardial Perfusion SPECT in Patients without Previous Myocardial Infarction

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Background: We previously described prognostic value of stress myocardial perfusion single photon emission computed tomography (SPECT) in patients with known or suspected coronary artery disease. However, "warranty time" of normal stress myocardial perfusion SPECT in a Japanese population is unclear. **Methods:** We identified 1,003 patients who had been judged to have no myocardial perfusion defect (summed stress score =0 calculated by a 5-point scoring. 20-segment model) on rest ^{201}Tl/ stress ^{99m}Tc-tetrofosmin myocardial perfusion SPECT. Averaged followed period was over 2 years. Patients revascularized within 60 days after nuclear testing were censored from the analysis. **Results:** During the follow up period, 2 of nonfatal acute myocardial infarction, 2 of cardiac death, 16 of unstable angina and 7 of admission to hospitals due to heart failure occurred. In patients with normal perfusion SPECT, the event rates at the end of 6, 12, 18, 24 months were <1% per year (0.6%, 0.6%, 0.7% and 0.7%, respectively). After 24 months, the event rate in patients with a normal SPECT increased to 1.1% per year. **Conclusion:** A normal perfusion SPECT portends a benign prognosis (<1% event rate /year) for 24 months. Thus, warranty period for normal myocardial perfusion SPECT is limited within 24 months and repeated scans may be recommended in patients at high risk