1P-056

Experimental Autoimmune Myocarditis (EAM) Model in Nonhuman Primates

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The ultimate treatment for cardiomyopathies of all etiologies is cardiac transplantation. Recently, autoimmune myocarditis has been reported as one of the etiologies of dilated cardiomyopathy. For development of new treatment and/or early diagnostic methods, elucidation of the causes and mechanisms of the disease is vital. In this study, we aimed to establish a primate model of experimental autoimmune myocarditis (EAM) using five cynomolgus monkeys. For this, monkeys were given an intradermal injection of plain myosin mixed with IFAon the medial aspect of the thighto stimulate an immune reaction. One monkey, to serve as a control, was injected with OVA. Cardio-specific examinations, including echocardiography and other evaluations were performed pre-injection and at several weeks after the injection. We confirmed the symptoms of myocarditis and progress of the medical condition after second injection. Lastly, we conducted clinicopathological examinations of all the monkeys, which revealed that the immunized monkeys showed notable increases in cardiac hormone levels, prolonged QTc interval, ventricular dilation. Pathologically, there was cardiac fibrosis and cardiomyocyte deficiency. These findings mimicked human myocarditis and cardiomyopathy. In conclusion, we established a nonhuman primate model of EAM. Several pathological changes were observed, as with human EAM, suggesting that this model of EAM in nonhuman primates could be a useful model for the human disease. (COI: Properly Declared)

1P-057

Physiological role of TRPC6 upregulation in hyperglycemia-exposed mice hearts

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<Purpose> Receptor-activated Ca^{2+}-permeable cation channels (RACCs) have been attracted attention as novel pharmacological targets of heart failure. Transient receptor potential canonical (TRPC) 3/6 are molecular entities of RACCs and reportedly upregulated in pathologically remodeling heart. We have recently reported that increased TRPC3 positively regulates reactive oxygen species (ROS) production with NADPH oxidase 2 (Nox2). In contrast, a relation between TRPC6 and heart failure is unclear. In this study, we analyzed the role of TRPC6 upregulation in hyperglycemia-induced heart failure, focusing on crosstalk with TRPC3 and Nox2.

<Methods/Results> TRPC6 was upregulated in streptozotocin (STZ)-treated mice hearts and neonatal rat cardiomyocytes (NRCMs) treated with high glucose while Nox2 was downregulated. After STZ treatment, only TRPC6-deficient mice showed decreased cardiac function and increased oxidative stress. TRPC6-silenced NRCMs treated with high glucose showed high levels of ROS. In TRPC3/TRPC6/Nox2-expressing cells, TRPC6 inhibited increase of Nox2 protein expression by TRPC3 in its channel activity-independent manner.

<Conclusions> Upregulation of TRPC6 in hearts exposed to hyperglycemia inhibited formation of TRPC3-Nox2 complex and suppressed Nox2-dependent ROS production, suggesting that TRPC6 upregulation contributes to adaptation for hyperglycemic stress in the heart. (COI: No)

1P-058

IL-6 may have protective roles in Lmna-related cardiomyopathy

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Purpose: Mutations in *LMNA* which encodes A-type lamins can cause several human diseases including fatal cardiomyopathy with conduction defects. Interleukin-6 (IL-6) is a multi-functional cytokine and known to promote fibrosis in heart. In addition, the IL-6 receptor antibody (MR16-1) was reported to suppress inflammation after myocardial infarction, and improved cardiac remodeling in mice. In this study, we investigated the roles of IL-6 in Lmna-related cardiomyopathy and the therapeutic effects of MR16-1. **Methods**: We used Lmna p.H222P knock-in mice (H222P) and C57BL/6J mice as control (WT). We performed qRT-PCR to check gene expression, ELISA to measure IL-6 levels in serum and heart, and histological analyses of Masson's trichrome staining. **Results**: mRNA of IL-6 was significantly increased in H222P heart, but its protein levels was not different from WT. After MR16-1 treatment, mRNA of IL-6 in H222P heart was decreased, whereas mRNA of IL-6 receptorα, ANP, collagenIα1, and TGFβ2 was increased. IL-6 protein levels in heart were maintained constantly, and there was no notable histological changes. **Conclusions**: These results may suggest protective roles of IL-6 in Lmna-related cardiomyopathy, although short time treatment could not show prominent histological improvement. (COI: No)

1P-059

Sonic hedgehog signaling regulates the mammalian cardiac regenerative response

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Certain organisms, including zebrafish, are capable of complete cardiac regeneration in response to injury. This response has also been observed in newborn mice, although in this case, the regenerative capacity is lost at approximately one week of age. The mechanisms regulating this short temporal window of cardiac regeneration in mice are not well understood.

Here, we show that sonic hedgehog (Shh) signaling modulates the neonatal mouse regenerative response. In particular, we demonstrate that following apical resection of the heart on postnatal day 1, mice activate Shh ligand expression and downstream signaling. This response is largely absent when surgery is performed on non-regenerative, postnatal day 7 pups. Furthermore, an enhanced cardiac regeneration response was detected in ptch heterozygous mice which have a genetically-based constitutive increase in Shh signaling. We further show that Shh ligand is produced in the myocardium by non-myocytes and appears to regulate cardiomyocyte proliferation, as well as the recruitment of monocytes/macrophages to the regenerating area. Finally, we demonstrate that a small molecule activator of Shh signaling promotes heart regeneration, whereas an inhibitor of Shh signaling impairs the regenerative response. Together, these results implicate Shh signaling as a regulator of mammalian heart regeneration and suggest that modulating this pathway may lead to new potential therapies for cardiovascular diseases. (COI: No)

1P-060

Analysis of Diabetic Cardiomyopathy with type 2 Diabetes Mellitus in Nonhuman Primate

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Cardiovascular diseases and the subsequent cardiomyopathies have diverse etiologies and mechanisms and require variable treatment strategies. Diabetes mellitus is one of the many causes of cardiomyopathy. The mechanism of diabetic cardiomyopathy (DCM) is unknown. The present study aimed to determine the symptoms and clinicopathological signs of cardiovascular disease in cynomolgus monkeys with spontaneous type 2 DM by blood test. And cardiac biomarkers (atrial and brain natriuretic peptides, ANP and BNP), electrocardiography (ECG), echocardiography and chest X-ray were evaluated for evidence of severe heart failure. We performed also histopathological and immunohistochemical analyses for the phyisiological evidence of DCM. In echocardiography, that aimed, indicated depression of cardiac function, and cardiac biomarkers were clearly increased. Cardiomyocytes showed steatosis and fibrosis with excessive deposition of amyloid polypeptide (amylin). These results were concordant with the most recent research on DCM and indicated the novel pathological mechanisms of DCM. This nonhuman primate model faithfully mimicked the pathophysiology of human DCM. This model will be useful for development of new therapies and diagnostic procedures for DCM. (COI: Properly Declared)

1P-061

Role of Cardiac Hormones in a Nonhuman Primate Model of Cardiac Disease

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Nonhuman primates are commonly used as experimental animals because of their biological resemblance to humans. In patients with cardiac diseases, levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) tend to increase due to cardiac damage. Therefore, the levels of ANP and BNP are used as indicators in the diagnosis of human heart failure. However, there are no reports of reference values for ANP and BNP with heart disease in nonhuman primates. In this study, we recorded the age, sex, and weight of 162 cynomolgus monkeys. We then performed evaluations to assess ANP, BNP, electrocardiography and echocardiography, and accordingly divided the monkeys into two groups: healthy monkeys and those with spontaneous cardiac disease (i.e. those who showed symptoms of valvular disease and heart failure due to dilated cardiomyopathy). Statistical analysis was performed using IBM SPSS to compare the relationship between ANP and BNP and the factors of age, sex and weight. There were no significant relationships between factors such as age, sex, and weight and ANP and BNP. On the other hand, both ANP and BNP were significantly different between the two groups. Similar to human beings, ANP and BNP levels tend to increase with cardiac disease in monkeys. Based on these results, we conclude that ANP and BNP are important leading indicators of cardiac disease in nonhuman primates, and that this nonhuman primate cardiac disease model is useful for research in cardiology. (COI: Properly Declared)