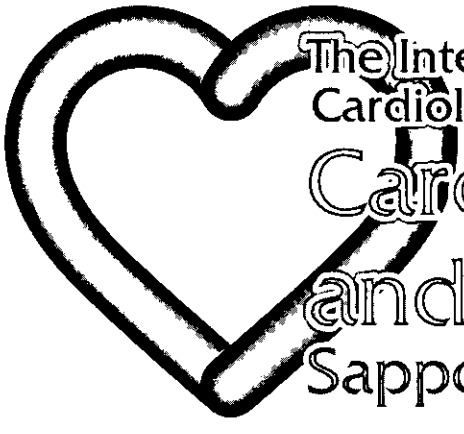
Poster Session



The International Symposium of Cardiology Frontiers:

# Cardiomyopathy and Heart Failure 2003

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# P-I-1-1

## Chronic Myocarditis Induced by CM2 Myosin Autoreactive T cell

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As emphasized anyplace, it has been the topics that chronic persistent myocardial inflammation forced either by viral infection and/or autoimmune response must be developing into dilated cardiomyopathy. So far we have proposed a unique experimental model of autoimmune myocarditis to broaden this understanding. The lethal myocarditis happens inescapably with cardiac myosin immunization using Kohno fragment <30a.a, reported by us in 2000>. Despite monophasic clinical course, the heart is gradually predisposed to dilate the chambers and reduce the contractility through repetitive immunization. In the process, myosin autoreactive T cell is playing a crucial role in processing the disease. Recently we succeeded to identify autoreactive and myocarditogenic T cells against another fragment of cardiac myosin, CM2 <17a.a; reported by Wegmann KW et al in 1994>. In transfer experiments using the CM2 autoreactive T cell, the myocarditis became more serious in acute stage, and caused longer inflammation in chronic stage in comparison with naive autoimmune myocarditis with CM2. The transferred myocarditis was persisting active inflammations until 6 months later. Besides T cell balance between Th1 and Th2 was never leaning to Th2 priority during the experimental period, and T cell repertoire did not restrict into a few lines. In mRNA level, trend of inflammatory cytokines was exactly corresponding with histology of this chronic myocarditis. Especially, NO release was likely sustained to the end.

# P-I-1-2

## Proarrhythmic Autoantibodies Produced Against Muscarinic 2 Acetylcholine Receptors in Patients With Dilated Cardiomyopathy

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Objectives: To study the clinical significance of muscarinic 2 acetylcholine (M2) receptor autoantibodies in patients with dilated cardiomyopathy (DCM).
Background: Atrial fibrillation (Af) is one of the aggravating factors in patients with DCM, and autoimmune mechanism is suggested to be involved in development of both arrhythmia and DCM.
Methods: Sera from 104 patients with DCM, age-matched 100 patients with lone Af and 100 healthy control subjects were screened for M2-Abs by enzyme-linked immunosorbent assay (ELISA). Purified IgG by Protein-G or Protein-A was used as a primary antibody of ELISA. Correlations of the M2 receptor autoantibodies to clinical variables were assessed by multivariate analysis. The electrophysiologic effects of M2 receptor autoantibodies were also determined in chick embryos.
Results: In DCM, M2 receptor autoantibodies were detected in 40 % of patients using Protein G and in 36 % of patients using Protein A. These autoantibodies were also found in certain patients with lone Af without cardiac abnormalities (24 %, 24%), which was significantly higher than that in healthy subjects (8%, 8%). Af was more common in autoantibody-positive than autoantibody-negative patients with DCM. Multivariate analysis confirmed that M2 receptor autoantibody was an independent predictor of the presence of Af in such patients. Purified IgG from both Af and DCM patients were able to display negative chronotropic effects and induce supraventricular arrhythmias in chick embryos, which could be eliminated by pre-administration of M2 epitope peptides.
Conclusions: M2 receptor autoantibodies may play a role in mediating the development of Af in patients with DCM.

# P-I-1-3

## Osteopontin Is Essential for Cardiac Fibrosis in Angiotensin II-Induced Hypertrophy

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Background-Angiotensin II (Ang II) induces both cardiac hypertrophy and fibrosis. Osteopontin (OPN) is a phosphoprotein induced by Ang II and is up-regulated in several experimental models of cardiac hypertrophy and fibrosis. In the present study, we examined the hypothesis that OPN is important to the development of cardiac fibrosis by Ang II infusion in mice lacking the OPN gene.
Methods and Results-Male OPN knockout (OPNKO) mice and age-matched wild-type (WT) mice were treated with Ang II, infused at a rate 2000ng·kg-1·min-1 for 4 weeks. Ang II elevated systolic blood pressure to comparable levels in OPNKO and WT mice. Ang II significantly increased left ventricle/ body weight ratio, myocyte area, and atrial natriuretic factor (ANF) mRNA expression in OPNKO and WT. Moreover, Ang II significantly increased collagen I, collagen III, transforming growth factor (TGF)-beta1 mRNA expression and cardiac collagen contents in WT but not in OPNKO.
Conclusions-Chronic depletion of OPN by gene targeting abolished cardiac fibrosis in mice with Ang II-induced hypertension. Thus, OPN has a pivotal role in the development of cardiac fibrosis.

# P-I-1-4

## Tenascin-C Expression in the Myocardium Obtained by Partial Left Ventriculectomy From Patients With Dilated Cardiomyopathy

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Tenascin-C (TNC), an extracellular matrix glycoprotein, is not normally expressed in adult hearts but appears under pathologic conditions such as acute myocardial infarction and acute myocarditis. However, the role of TNC is unknown in slowly progressive myocardial diseases such as idiopathic dilated cardiomyopathy (DCM). In this study the expression of TNC was immunohistochemically examined in the myocardium from 62 patients with DCM (54 men, 8 women; mean age, 50 ± 15 years) obtained by partial left ventriculectomy. The immunoreactivity was semi-quantitatively graded from 0 to 3+ depending on the extent. The degree of interstitial fibrosis was evaluated by the sirius red staining. The positive TNC staining was occasionally but not very often observed in focal fibrotic area associated with a few mononuclear cell infiltration. With polalized microscopy, the sirius red staining of the TNC-positive area revealed collagen III rich fine fibrils that could be newly formed fibrotic lesion rather than mature fibrosis, where recent myocardial cell loss was suspected. The grading scores of TNC staining were 0 in 22, 1+ in 16, 2+ in 18, and 3+ in 6 patients with DCM. TNC expression level was not clearly correlated with the percent fibrosis. Preoperative left ventricular ejection fraction was 20.9 % in the score 0 group and 21.1 % in the score 3 group. Further investigation will be warranted about the relationship between TNC expression and the clinical features. The expression level of TNC was generally low in the myocardium of DCM patients with refractory heart failure and indicative of partial left ventriculectomy. It is suggested that TNC may not play a key role with regard to ventricular remodeling in DCM hearts, although TNC possibly expresses associated with focal myocardial tissue alterations.

# P-I-1-5

## The Pivotal Link Between Hepatitis C Virus Infection and Aortic Stiffness in Patients With End-Stage Renal Disease

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Background: Recent reports have been emphasized a link between infection with micro-organism such as Chlamydia pneumoniae, and an increased risk of cardiovascular diseases. Although the high prevalence of hepatitis C virus (HCV) infection in patients with end stage renal failure (ESRF) has been well-known, the role of HCV in pathogenesis of these atherosclerotic diseases is unclear. Recently, aortic pulse wave velocity (PWV), a marker of arterial stiffness, has been reported to be a major predictor of mortality in ESRF patients. In this study, we evaluate the relationship of aortic PWV and HCV infection in patients with ESRF. Methods: Seventy-six outpatients (age: 64 years, 59 male, duration of dialysis treatment: 3517 days) undergoing chronic dialysis treatment in single center were examined. We measured their blood pressure and aortic PWV by VaSera VS-1000 (FUKUDA DENSHI, Japan), left ventricular mass index (LVMI) by echocardiography, serum HCV-RNA by RT-PCR, and plasma brain natriuretic peptide (BNP) by highly sensitive RIA. Determinants of aortic PWV were analyzed by multiple stepwise regression analysis. Results: Twenty patients (26%) were seropositive for HCV, and twelve patients (16%) had HCV viremia. Aortic PWV was significantly higher in patients with HCV viremia than those without it (11.7 ± 3.0 vs. 9.7 ± 2.2 m/sec, $p < 0.01$). Serum transaminase levels were within normal limits in both groups. Using simple regression analysis, aortic PWV correlated significantly with age ($r2=0.21$, $p < 0.0001$), HbA1c ($r2=0.32$, $p < 0.0001$), mean blood pressure ($r2=0.26$, $p < 0.0001$), and did not correlate with duration of dialysis treatment, total cholesterol, creatinine, calcium and phosphate. Multiple regression analysis indicated that independent determinants of aortic PWV were age ($\beta=0.08$, $p < 0.0001$), HbA1c ($\beta=1.09$, $p < 0.0001$), mean blood pressure ($\beta=0.05$, $p < 0.0001$), and the presence of HCV-RNA ($\beta=2.03$, $p < 0.0001$) (multiple $R=0.88$). Conclusion: These data show for the first time that HCV infection is strongly associated with the progression of atherosclerosis in patients with ESRF.

# P-I-1-6

## SOCS1 and Viral Myocarditis

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Enteroviral infection, including coxsackievirus B3 (CVB3), is a common cause of acute myocarditis that can lead to heart failure, arrhythmias and death, especially among young adults and infants. In addition, enteroviral infection has been implicated in the development of dilated cardiomyopathy, one of the main indications for cardiac transplantation. However, little is known regarding intrinsic signaling mechanisms within the infected cardiac myocyte that contribute to the host defense against viral infection. To determine whether JAK-STAT pathway is altered in CVB3 infected heart, we examined the activation of JAK-STAT pathway in the CVB3 infected mice heart. Both STAT1 and STAT3 were strongly activated at 3 days after CVB3 infection. Importantly, SOCS1, a cytokine-inducible inhibitor of JAK/STAT pathway, was also strongly induced at a similar time as the activation of STATs. We therefore inoculated alpha-MHC-SOCS1 transgenic mice to determine whether expression of SOCS1 and subsequent inhibition of JAK-STAT signaling could have a functionally significant effect in the setting of infection with the cardiotropic CVB3. We found robust viral replication in the heart, acute cardiomyopathy and high mortality in SOCS1 transgenic mice during CVB3 infection. Furthermore, we demonstrated that inhibition of SOCS1 in the cardiac myocyte via adeno-associated virus-mediated expression of a dominant-negative SOCS1 increased the myocyte resistance to the acute cardiac injury caused by CVB3 infection in vivo, indicating that strategies aimed at inhibition of SOCS1 could potentiate the intrinsic antiviral actions of cytokines that stimulate JAK-STAT pathway. Thus, SOCS1 might be a novel therapeutic target for acute cardiomyopathy during CVB3 infection.

# P-I-1-7

## Serial Measurement of Myocardial Contractility During Mechanical Support

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**Purpose:** Evaluation of myocardial contractility during ventricular assist device (VAD) support is important for recognizing the mechanism of myocardial recovery, but such evaluation is difficult because of the changing preload and afterload conditions. We serially investigated a load independent index and compared it with conventional indexes during VAD support in goats with severe heart failure after myocardial infarction (MI).
**Materials and Methods:** Nine adult goats weighing 61.6 ± 5.7 kg were installed pulsatile bi-VADs. Acute MI (AMI) was created by ligation of the left anterior descending coronary artery. Ultrasonic crystals were inserted into both sides of the endocardium for measuring the LV short axis dimension. Fractional shortening (FS), a conventional index, and end-systolic pressure-dimension relationship (ESPDR), a useful load independent index, were measured for 4 weeks after AMI using the short axis dimension and simultaneous LV pressure.
**Results:**FS decreased for five days (from 22.4 ± 13.4% to 15.7 ± 7.9%), but it turned to improve within 1 week. However the slope of ESPDR significantly decreased after AMI (from 27.1 ± 10.8 mm Hg/mm to 12.2 ± 4.5 mm Hg/mm), and reached to a minimum about 2 weeks later. This discrepancy may mean that FS reflected the compensated movement of the non-ischemic myocardium, although the infracted myocardium continued to show deteriorating contractility.
**Conclusion:** Serial analysis using load independent index provides important information for recognizing myocardial recovery during VAD support.

# P-I-1-8

## Circulating Levels of IL18 and Its Site of Production in Patients With Asymptomatic Chronic Heart Failure

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Patients with chronic heart failure have elevated levels of various proinflammatory cytokines. Recently, IL-18 level was identified as a strong predictor of death from cardiovascular causes in patients with ischemic heart disease. We sought to determine whether elevated IL-18 levels are also present in patients with left ventricular dysfunction. METHODS: Twelve asymptomatic patients with ischemic and non-ischemic cardiomyopathy underwent cardiac catherterlization to evaluate hemodynamic state. Blood samples for measurement of plasma cytokine level were performed in the ascending aorta and coronary sinus. Levels of IL-18 were measured with enzyme-linked immunosorbent assay kits. The yield of cytokine from myocardium was calculated with IL-18 concentration and blood flow in coronary sinus, which was measured by thermodilution catheter. RESULTS: IL-18 concentrations in both coronary sinus and aorta tend to correlate with left ventricular ejection fraction (p=0.18, 0.10, respectively). However, no correlation was found between IL-18 spillover from coronary sinus, namely IL-18 production in myocardium, and left ventricular ejection fraction or plasma BNP concentration. CONCLUSIONS: Elevated IL-18 levels are present in patients with LV dysfunction even in the absence of the clinical symptom. These data suggest that IL-18 may be involved in the progression of subclinical LV dysfunction to clinical CHF, and that myocardium is not the main site of production of IL-18 in patients with heart failure.

# P-I-2-1

## A New Experimental Model of Hypertrophic Cardiomyopathy Induced by a K Channel Blocker in Rats

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Background: Idiopathic hypertrophic cardiomyopathy(HCM) exhibits assymmetric septal hypertrophy(ASH), concentric hypertrophy, or dilated phase. However, the mechanisms for these morphological differences are not known.
Aim: To establish a new experimental animal model of HCM which exhibits ASH, diffuse concentric hypertrophy or dilated phase of HCM.
Methods: Wister rats age at 4 weeks were divided into 7 groups. In 6 groups, a K channel blocker with 3,4-diaminopyridine(DAP; 5, 10, 20, 30, 40 or 80mg/day) was administered orally for 8 weeks and histological examination of the heart was performed.
Results: Heart weight/body weight(HW/BW) was increased with 20mg/kg or over, left ventricular wall area(LVWA; an indicater of left ventricular mass) was increased with 10mg/kg or over, ASH(IVS thickness/PW thickness >1.3) was induced at 5 and 10mg/kg and concentric hypertrophy with larger doses, left ventricular cavity(LVC) was reduced with 5-30mg/kg but was increased again with 40mg/kg, myocyte diameter was increased from control 8.4 up to 31um dose-dependently, interstitial fibrosis and edema was induced with 40 mg/kg or over. No significant differences in heart rate and systolic blood pressure were noted among the groups. Myocardial disarray was not observed.
Conclusion: The results indicate that although myocardial disaaray was not induced, ASH, concentric hypertrophy, dilated phase resembling that of HCM can be induced by changing doses of DAP. Probably, blocking of K channels(K ATP, K Ca) resulted in increased Ca influx and resulted in myocyte hypertrophy and finally in left ventricular failure.

# P-I-2-2

## Novel Gene Expression and Drug Screening for Cardiac Remodeling Using by Cardiac Transplantation-Coronary Ligation Rat Model

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**OBJECTIVES** - We developed a novel heterotopic cardiac transplantation-coronary ligation (MI/RE) model capable of inducing MI with the absence of consequent hemodynamic and neurohormonal affects during the development of cardiac failure, and allowing differential quantification of indexes of cardiac remodeling *in vivo* (Nakamura H, et al. JACC 2003). Aim of this study is to screen out candidate genes for cardiac remodeling and examine the effect of ACE inhibitor, perindopril on these genes in both the conventional MI (MI/HF) and MI/RE models.
**Methods** - We performed isogenic heterotopic cardiac transplantation and simultaneous coronary ligation to produce MI in the donor heart, and to evaluate the donor heart in Lewis rats at 1 week after the operation. Total 3.8K genes were analyzed by BD Atlas™ Glass Microarrays for each samples. Variation of each gene expression was evaluated by the relative rate corresponding to the sham operation. Treated rats were administered with oral perindopril (1mg/kg/day) for 14 days through the operation.
**Results** - Although 42 candidate genes were screened out in the MI/HF model, finally 7 genes were focused by the additional MI/RE model (cut off ratio, 2.5). Perindopril up-regulated as many as 44.4% genes in the MI/RE model, whereas it down-regulated 93.3% genes in the MI/HF model. Perindopril did not only surpressed the expression of these novel genes, but also modulated angiotensin related genes in the MI/RE model.
**Conclusion** - MI/RE model of this invention where heart failure can be excluded permits efficient screening test of drug effects and/or genes expression for cardiac remodeling. Renin-angiotensin system may play a pivotal role in modifying the specific gene expression for cardiac remodeling after MI.

# P-I-2-3

## Hydrostatic Pressure-Overload Induces Cardiomyocyte Hypertrophy

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Although numerous experiments using the cell-stretching model have reported that the mechanical stress to the cultured cardiomyocytes by itself could induce cardiomyocyte hypertrophy, little information is available with regard to the effect of the enhanced hydrostatic pressure on cardiomyocyte physiology. In this study, we isolated rat neonatal cardiomyocytes, cultured them in an atmospheric pressure chamber, and applied hydrostatic pressure to the cultured cardiomyocytes for 60 minutes. Quantitative RT-PCR analysis revealed that hydrostatic pressure induced the expression of immediate early response genes including c-fos. Significant increase of c-fos expression was observed at 80mmHg, and the level of expression was linearly increased up to 160mmHg. In order to dissect the signaling pathway involved in this hypertrophic response, various pharmacological agents were used prior to the pressurized cardiomyocytes culture. Nifedipine (calcium channel antagonist) and cyclosporine A (calcineurin inhibitor) significantly suppressed pressure-induced c-fos gene expression, and PD98059 (p42/44 MEK inhibitor) partially suppressed c-fos expression. However, valsartan (AT1 receptor antagonist), genistein (tyrosine kinase inhibitor), wortmannin (PI3 kinase inhibitor), staurosporin (PKC inhibitor) and rapamycin (mTOR inhibitor) did not suppress the hydrostatic pressure-induced c-fos expression. These results suggested that hydrostatic pressure could induce cardiomyocyte hypertrophy, and calcineurin / NFAT signaling was involved in this process.

# P-I-3-1

## Role of Angiotensin II in Altered Expression of Molecules Responsible for Coronary Matrix Remodeling in Insulin-Resistant Diabetic Rats

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In the diabetic heart, extracelluar remodeling based on abnormal collagen deposition may be associated with potential interactions between the matrix metalloproteinase (MMP) system which regulates extracellular matrix turnover, and the fibrinolytic system which is involved in fibrin degradation process. We examined the expression profile of MMP and fibrinolytic system in the heart from OLETF rats, a model of human type 2 diabetes mellitus (DM), at an early insulin-resistant stage (20 weeks old), and compared it with that from the age-matched genetic control, LETO rats. Transforming growth factor (TGF)-$\beta_1$ expression increased in coronary vessels from OLETF rats. Increased coronary expression of plasminogen activator inhibitor type 1(PAI-1), a primary physiological inhibitor of fibrinolysis, was evident in OLETF rats; whereas, both tissue and urokinase plasminogen activators were expressed equally in coronary vessels from LETO and OLETF rats. In contrast, OLETF rats exhibited a significant reduction in coronary expression of MMP-2, which degrades ultrastructure collagen, and membrane-type-1 (MT-1)-MMP, which activates proMMP-2. However, there was no significant group difference in coronary expression of tissue inhibitor of MMP-2 (TIMP-2). No significant difference between diabetic and nondiabetic coronary vessels was seen for expression of another gelatinase (MMP-9), and collagenase-3 (MMP-13). Both intra- and extra-vascular collagen types I and III immunoreactivity and fibrin deposition were seen in diabetic vessels. These alterations were reversed to nondiabetic levels by an angiotensin II type 1 (AT-1) receptor blocker, candesartan, and it prevented the development of perivascular fibrosis. Thus, in addition to upregulation of PAI-1, downregulation of MMP-2 and MT1-MMP may play a crucial role in cardiac matrix remodeling at the insulin-resistant stage of type 2 DM. These molecules appear to be regulated by angiotensin II via stimulation of TGF-$\beta_1$, which would underlie the clinical usefulness of AT-1 blocker in type 2 DM.

# P-I-3-2

## Inhibition of Angiotensin Converting Enzyme (ACE) Attenuates Hypofibrinolysis and Reduces Cardiac Perivascular Fibrosis During the Development of Diabetic Cardiomyopathy in Genetically Obese Mice

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Obesity and insulin resistance are associated with accelerated coronary disease and increase in blood of plasminogen activator inhibitor (PAI)-1, the major physiologic inhibitor of fibrinolysis. Using a mice model of obesity (ob/ob), we determined the role of hypofibrinolysis in blood and tissue in causing cardiac complications. Active PAI-1 antigen in plasma was elevated in obese mice (ob/ob 32.2 ± 5.0 ng/ml vs lean 8.5 ± 2.1, ELISA, $p<0.05$) at 20 weeks. Collagen content of the left ventricle (LV) was increased in obese mice (2.23 ± 0.67 $\mu$ mol/g vs. 1.7 ± 0.84, $p<0.05$, hydroxyproline assay). Morphometric analysis demonstrated perivascular fibrosis in coronary arterioles and small coronary arteries (fibrosis to lumen ratio 2.9 ± 1.7 vs 1.3 ± 0.8, $p<0.05$; fibrosis to wall ratio 1.4 ± 0.5 vs 0.8 ± 0.4, $p<0.05$). Strong immunoreactivity for PAI-1 and tissue factor (TF), an initiator of coagulation, in the vessel wall and type-1 collagen in the perivascular area were noted. mRNA of PAI-1 (15.1 ± 6.5 fold over lean) and TF (1.5 ± 0.6 fold) were increased (RT-PCR). ACE inhibition (temocapril 20mg/kg/day) in obese mice from 10 to 20 weeks attenuated perivascular fibrosis (fibrosis to lumen ratio 1.7 ± 1.0, $p<0.05$; fibrosis to wall ratio 0.7 ± 0.3, $p<0.05$) and arrested the increase in plasma PAI-1 (21.9 ± 5.7) and LV collagen content (1.8 ± 0.42). Treated mice exhibited faint immunoreactivity for PAI-1, TF and type-1 collagen and marked reductions of mRNA of PAI-1 (4.3 ± 2.0 fold over lean) and TF (1.2 ± 0.3 fold). Thus, hypofibrinolysis and hypercoagulation can precede and contribute to coronary perivascular fibrosis and collagen deposition in obesity before the development of overt diabetes, and ACE inhibition can attenuate perivascular fibrosis.

# P-I-3-3

## Inhibition of NRSF in the Heart Leads to Cardiac Dysfunction and Sudden Death

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**Introduction** Recently we found that NRSF, a transcriptional repressor, plays an important role in the expression of multiple fetal cardiac genes. Cardiac specific transgenic (Tg) mice of dominant negative (dn) mutant of NRSF showed cardiac dysfunction and sudden death. In this study, we examined the electrophysiological properties in dnNRSF Tg mice. **Method** We performed ECG monitoring and in-vivo intracardiac electrophysiological study (EPS) in both non-Tg and Tg mice. **Results** ECG monitoring revealed that 1st and 2nd degree AV block and VPCs including short run occurred spontaneously in Tg mice, but not in non-Tg mice. At the time of death in Tg mice, VT/VF followed by asystole was recorded. In in-vivo EPS, prolongation of AV interval was detected in Tg mice, but any other parameters were not significantly different between them. Ventricular tachycardia was induced only in Tg mice. **Conclusion** We successfully showed that the cause of sudden death and high inducibility of ventricular arrhythmias in dnNRSF Tg mice. NRSF may play an important role in maintenance of normal cardiac electrophysiological properties by controlling several gene expressions.

# P-I-3-4

## Defect of VEGF/KDR Signaling System in the Heart of Stroke-Prone Spontaneously Hypertensive Rat With the Development of Malignant Hypertension : Involvement of Rho-Kinase for Akt Downregulation

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Stroke-prone spontaneously hypertensive rat (SHRSP), a genetic animal model of malignant hypertension, demonstrates a remarkable hypertrophy of cardiomyocytes and a decreased capillary density in left ventricular (LV) wall in the malignant hypertensive stage. The present study aimed to investigate age-related changes in protein and mRNA expressions of a variety of molecules that are pertinent as possible mediators of angiogenesis in SHRSP heart with the development of malignant hypertension. Cardiac expressions of angiogenetic molecules were evaluated in SHRSP at 6 weeks of age (SHRSP6: prehypertensive stage), 20 weeks of age (SHRSP20: established hypertensive stage) and 40 weeks of age (SHRSP40: malignant hypertensive stage). The expressions of target molecules were compared with those of age-matched Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) heart. VEGF, a potent angiogenic growth factor, was highly upregulated in SHRSP20, whereas its angiogenic receptor, KDR, was downregulated, as compared to the age-matched WKY. In SHRSP40, both VEGF and KDR were significantly downregulated. Total coronary capillary density was already decreased in SHRSP20. LV systolic function was almost unchanged in SHRSP20 compared to WKY, but LV diastolic function was impaired. Endothelial NO synthase (eNOS) and Akt were downregulated in SHRSP heart in an age-related manner. In contrast, Rho-kinase was upregulated in SHRSP heart in a time-dependent manner. These age-related changes in cardiac expressions of target molecules were less pronounced in SHR. In conclusion, there was a defect of the VEGF/KDR signaling system in SHRSP heart with the development of malignant hypertension. Rho-kinase seemed to downregulate Akt-NO pathway in SHRSP heart. This could result in an impairment of physiologic angiogenesis leading to a decline in the coronary capillary density. Thus, in SHRSP that develops cardiac hypertrophy, this defect may play a pivotal role as molecular pathogenesis for the development of deterioration of cardiac function resulting from myocardial hypoperfusion.

# P-I-3-5

## Identification of a Caveolin-3 Mutation in Familial Hypertrophic Cardiomyopathy

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Recent genetic analysis has revealed 14 and 15 different disease genes for hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), respectively. However, mutations in these disease genes could be identified only in a part of patients, suggesting that there are other disease genes. That the known disease genes for cardiomyopathy overlapped with the disease genes for limb-girdle muscular dystrophy (LGMD) reasoned us to investigate an LGMD gene for mutations in HCM and DCM. A total of 146 HCM patients and 130 DCM patients were analyzed for mutation in caveolin-3 (*CAV3*) gene, and a missense mutation, replacing serine for threonine at codon 63, T63S, was identified in an HCM patient. The mutation was found at the evolutionary conserved residue, not present in healthy controls, and co-segregated with HCM in the multiplex family, suggesting the causative role in HCM. To reveal the functional deficit caused by the T63S mutation in comparison with the LGMD-related mutations involving the same 63th residue, T63P and del63-65, we investigated the distribution of GFP-tagged CAV3 proteins with or without these mutations. Confocal microscopic analysis and Western blot analysis showed that the T63P mutation caused cytoplasmic retention of CAV3 to less extent than the LGMD mutations. These observations strongly suggest that HCM is a clinical spectrum of *CAV3* mutations.

# P-I-3-6

## Role of Caveolin in Cardiac Hypertrophy

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Caveolae are omega-shaped organelles of the cell surface. Caveolin-3, a structural component of cardiac caveolae, is associated with cellular signaling. We investigated the morphological changes of caveolae and caveolin-3 expression in hypertrophied cardiomyocytes and the effect of overexpression of caveolin-3 on hypertrophic responses in cardiomyocytes. Cultured rat neonatal cardiomyocytes were used for the experiments. Phenylephrine (PE) induced cellular hypertrophy associated with an increase of the number of caveolae and an upregulation of caveolin-3. Although PMA increased the number of caveolae and the caveolin-3 expression, the extent of these upregulations was less than that of PE. The PE-induced upregulations of caveolae and caveolin-3 expression were inhibited by BAPTA. Inhibitors of calcineurin and CaMKII attenuated the PE-induced upregulation of caveolin-3. Therefore, the number of caveolae and the expression of caveolin-3 were upregulated in rat hypertrophied cardiomyocytes, possibly via the alterations of intracellular $Ca^{2+}$. Next, we investigated the role of caveolin-3 in hypertrophied cardiomyocytes. We constructed an adenovirus encoding human wild-type caveolin-3 (Ad.Cav-3), dominant negative caveolin-3 (Ad.Cav-3D), or bacterial beta-galactosidase (Ad.LacZ). In non-infected cells, PE and endothelin-1 (ET) increased cell size and [$^3$H] leucine incorporation, indicating myocyte hypertrophy. Ad.Cav-3 prevented the PE- and ET-induced alterations. Ad.Cav-3 also blocked the PE- and ET-induced phosphorylations of ERK, but did not affect JNK and p38 MAP kinase activities. In contrast, Ad.Cav-3D significantly augmented hypertrophic responses to ET, associated with increased ET-induced phosphorylation of ERK1/2. These results suggest that caveolin-3 behaves as a negative regulator of hypertrophic responses, probably through suppression of ERK1/2 activity.

# P-I-3-7

## p300 Are Involved in Rho Kinase-Mediated c-fos Gene Expression in Cardiac *Myocytes*

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A small GTPase, RhoA, participates in cytoskeletal organization and gene expression. We have previously reported that RhoA stimulates c-fos gene expression through c-fos SRE in cardiac myocytes (CM). Rho kinase (Rho-K) has been reported to bind with RhoA and modulate Endothelin-1 (ET-1)-induced cardiac myocyte hypertrophy. Transcriptional coactivator p300 plays an important role for transcription in CM. However nothing is known about the role of p300 in RhoA-mediated c-fos gene expression. c-fos promoter/enhancer linked to the luciferase reporter gene (c-fos luciferase) was activated by dominant active type (DA) RhoA (RhoAval14), DA Rho-K (Rho-K CAT) and p300. RhoA-induced c-fos luciferase expression was inhibited by dominant negative type (DN) Rho-K (Rho-K RB/PH.TT) and DN p300 (p300A). Also Rho-K-induced c-fos luciferase expression was inhibited by p300A. The deletion and mutation analysis revealed that c-fos serum response element (SRE) accounts for c-fos luciferase expression by RhoA, Rho-K and p300. Rho-K fused GFP (Green Fluorescence Protein) (GFP-Rho-K) was located in the cytosol under no stimulation. However GFP-Rho-K was translocated into nucleus by RhoAval14 or ET-1 stimulation. In vitro protein-protein interaction assay, 35S methionin-labeled p300 bound GST fusion Rho-K protein. Furthermore, in mammalian two-hybrid assay, Gal4-p300 (p300 fused to Gal4) and VP16-Rho-K (Rho-K fused to VP16) activated Gal4 luciferase expression, which means the possibility that Rho-K bound with p300. These results indicated that activated Rho-K translocated into nucleus and bound with p300, subsequently c-fos gene expression was upregulated through SRE in CM.

# P-I-3-8

## Overexpression of p300 Promotes Left Ventricular Endothelin-1 Expression and Remodeling Following Myocardial Infarction in Adult Mice *in Vivo*

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A p300 protein serves as a coactivator of hypertrophy-responsive transcriptional factors such as MEF-2 and GATA-4. p300 also possesses histone acetyltransferase activity, which induces acetylation of certain DNA binding transcription factors as well as of histone. In addition, our recent study shows that hypertrophpic stimuli induce acetylation and DNA binding of GATA-4 in cultured neonatal cardiac myocytes and that p300 is involved in these processes. However, the role of p300 in left ventricular (LV) remodeling following myocardial infarction (MI) *in Vivo* is unknown. To solve this problem, transgenic mice overexpressing p300 in the heart (TG) or wild-type mice (WT) were subjected to MI or sham operation at the age of 12 weeks. At this stage, echocardiography revealed that left ventricular size and function did not differ between WT and TG. Five weeks after MI, however, TG revealed more progressive LV remodeling than WT, exemplified by lower LV ejection fraction and by larger LV end-diastolic and systolic dimensions. There were no significant differences in infarct size between WT and TG. Western blots showed that LV expression of p300 but not that of GATA-4 was induced in the TG mice. Compatible with p300-mediated acetylation of GATA-4, electrophoretic mobility shift assays revealed that MI induced binding of cardiac GATA-4 to endothelin-1 GATA element. Overexpression of p300 in the heart enhanced DNA binding of GATA-4 but did not change its expression level. In both WT and TG, LV levels of endothelin-1, a downstream target of p300/GATA-4 pathway, were higher in the MI group than the sham-operated group. However, this increase was further exaggerated in TG compared with WT. These findings demonstrate that cardiac overexpression of p300 promotes LV endothelin-1 expression and remodeling following MI in adult mice *in Vivo*.

# P-I-3-9

## Increase in Protein Phosphatases Activity is Associated With Impaired Contractility Without Hypophosphorylation of $Ca^2$ Regulatory Proteins in Hamster Cardiomyopathy

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Although increase in protein phosphatase (PP) activity and subsequent dephosphorylation of $Ca^{2+}$ regulated protein is proposed as a detrimental mechanism in heart failure, the pathophysiological role of PP has not been fully understood. We characterized changes in PP activity, protein kinase A (PKA) activity and phosphorylation levels of $Ca^{2+}$ regulatory proteins with progression of UMX7.1 hamster cardiomyopathy and aging. Left ventricular (LV) function, PP1 and PP2A activity, PKA activity, phosphorylation of ryanodine receptor at serine 2809 (p-RyR) and phospholamban at serine 16 (p-PLN) were assessed at 6, 10, and 28 weeks (wk) of age in cardiomyopathic hamsters(CMH) and age-matched normal hamsters(NH) (n=6 at each stage). In CMH, LV systolic function showed progressive decrease from 10 to 28 wk with LV dilation and elevated LV end-diastolic pressure. PP1 activity was elevated by 26% ($p<0.05$) at 6 wk, by 80% ($p<0.05$) at 10 wk and by 96% ($p<0.05$) at 28 wk compared with age-matched NH which was accompanied by 1.73-1.8 folds increase in PP1$\beta$ expression. PKA activity was elevated by 41% ($p=0.08$) at 10wk and by 53% ($p<0.05$) at 28 wk compared with age-matched NH. Interestingly, p- RyR and p-PLN in CMH showed less decrease with aging and were significantly higher at 10-28wk than age-matched NH.
In summary, 1) in CMH, increase in PP1 activity was well correlated with increased PKA activity till 10 wk, whereas PP1 activity appeared to be dominant at 28 wk. 2) Although difference of phosphorylation level of RyR and PLN between NH and CMH was relatively smaller than its age-dependent decrease, p-RyR and p-PLN were significantly increased at 10-28wk than age-matched NH. These data suggest that increase in PP1 activity may be associated with increased PKA activity with progression of LV dysfunction and thus decreased phosphorylation of $Ca^{2+}$ regulatory proteins might not be a requisite event.

# P-I-3-10

## Defective Regulation of Inter-Domain Interaction Within Ryanodine Receptor as a Cause of Heart Failure

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Two domains within ryanodine receptor (RyR) in sarcoplasmic reticulum (SR) {N-terminal (N:0-600) and Central (C:2000-2500)}, where 7 out of 11 mutations have been found in patients with ARVD/polymorphic VT (pVT), have been recently shown to interact with each other as a regulatory switch for channel gating. Here, we assessed whether the abnormality in the inter-domain interaction induces the defective FKBP12.6-mediated stabilization of RyR seen in heart failure.
SR vesicles were isolated from dog LV muscles (n=6), then RyR was labeled with the fluorescent conformational probe methylcoumarin acetate (MCA) using the following synthetic peptide as a site-directing carrier. 1)The peptide corresponding to Gly2460-Pro2495 of RyR (that contains a potential mutation site in pVT), designated DPc10 (10-100 $\mu$ M), induced unzipping mode of N- and C-domain interaction. The unzipping mode was confirmed by the quenching of the MCA fluorescence by a large-size fluorescence quencher QSY-BSA. 2)The DPc10 (10-100$\mu$ M) induced SR $Ca^{2+}$ leak, associated with a dissociation of FKBP12.6 from RyR. 3) In the DPc10-introduced isolated myocytes, a peak of intracellular $Ca^{2+}$ transient decreased with a prolonged time from peak to 50% fall, in association with a decrease in cell shortening. 4) In the SR taken from pacing-induced dog failing hearts, the unzipping mode of the domains has already occurred in association with both FKBP12.6 dissociation and $Ca^{2+}$ leak. The specific domain interaction within RyR critically regulates the gating property of RyR and it' s defectiveness may be involved in the common pathogenic mechanism of ARVD/pVT and heart failure.

# P-II-1

## Analysis of Enterovirus Genome in the Myocardium of Murine Myocarditis in Subacute Phase and Patients With End-stage Dilated Cardiomyopathy

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We detected enterovirus genome in the myocardium of patients of dilated cardiomyopathy (DCM), however enteroviruse was not able to be isolated by conventional methods. Some papers suggest mutation of enterovirus genome in DCM hearts. We analyzed enterovirus genome in the myocardium of murine myocarditis in sub-acute phase and patients with end-stage dilated cardiomyopathy to show mutation of enterovirus genome.
We extracted total RNA and made cDNA with poly T and virus specific primers from mouse and human samples. We designed many primers in 5' NTR lesions of enterovirus genome for analyze of 5' NTR of enterovirus. In sub-acute phase of murine coxsackievirus myocarditis model, enterovirus genome was detected by RT-PCR, however enteroviruse was not able to be isolated by conventional methods. In sub-acute phase of murine coxsackievirus myocarditis model, PCR products from 80 to 644 in 5' NTR lesion was positive and PCR from 5' end to 644 was negative. These results suggested deletion of 5' end of enterovirus genome. We made cDNA with poly T from human samples, and we detected PCR products in 5' NTR lesion from these cDNA. So, we made a success in making nearly full-length cDNA from human samples, and PCR from 5' end was also negative. Next, we tried nearly full-length PCR products of enterovirus genome in the myocardium. We detected long PCR products (from 5' NTR to middle and middle to 3' end) from murine myocarditis model with GIBCO BRL Elongase enzyme mix. We started long PCR from human samples.
It is suggested that enterovirus genome deleted 5' end in sub-acute phase of murine coxsackievirus myocarditis model. Further examination including sequencing of PCR products is need for analysis of enterovirus genome in the myocardium of DCM.

# P-II-2

## The Clinical Features of *Takotsubo* Cardiomyopathy

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Background: In recent years, cardiologists have come to recognize a reversible form of heart failure of unknown origin characterized hypokenetic, *takotsubo*-shaped left ventricle on left ventriculography. This study was designed to clarify the clinical features of *takotsubo* cardiomyopathy.
Methods: Fifteen patients with *takotsubo*- like ventricular dysfunction were followed for a period of 1 to 4 years. The clinical course, routine test result, and cardiac catheterization data were assessed in each patient.
Results: The subjects were 12 elderly women and 3 men (mean age; 73.5 years), and 5 patients had been exposed to stress. On admission, cardiac enzymes were not markedly increased, but the serum norepinephrine and plasma brain natriuretic peptide levels were high (1.48 ng/ml and 489.0 pg/ml, respectively). Coronary angiography revealed normal coronary arteries. However, left ventriculography showed apical akinesis, together with basal hyperkinesis (a *takotsubo*-shaped ventricle). The left ventricular ejection fraction, the initial pulmonary artery wedge pressure, and cardiac index were 39.5 %, 8.8 mmHg, and 1.9 l/min/$m^2$, respectively. These abnormalities resolved within 17.4 hospital days without any treatment in 13 cases, and with hemodynamic support for 3 days in 2 cases. New cardiac events did not occur over a 1-4 year follow-up period. One patient died of a non-cardiac event.
Conclusion: Coronary vasospasm, myocarditis, and other previously reported diseases previously described excluded as the cause of *takotsubo* cardiomyopathy in our subjects. This cardiomyopathy had a good prognosis without any specific treatment, but, there may be a possibility of it contributing to cardiac or non-cardiac sudden death.

# P-II-3

## Elevation of C-reactive Protein Associated With Sleep-Disordered in Patients With Congestive Heart Failure

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[Objectives] Sleep-disordered breathing (SDB) and C-reactive protein (CRP) have been reported as risk factors of the prognosis of patients with congestive heart failure (CHF). We evaluated the prevalence of SDB in CHF patients and observed the prognosis of them. Furthermore, we analyzed the relation between CRP and SDB in patients with CHF.
[Methods] We studied one hundred consecutive patients with left ventricular ejection fraction <50% admitted to our hospital because of worsening CHF. Just before the discharge, oxygen saturation during sleep for two nights was monitored with a pulse oximeter and blood sample was taken. We observed their prognosis.
[Results] Forty-one patients had sleep-disordered breathing, defined as 4% oxygen desaturation index more than 5 events/hour. CHF patients with SDB showed significantly higher CRP concentration than those without SDB (CRP. 1.0 and 0.4mg/dl, respectively, p= .03). During follow-up period (mean 17 months, range from 3 to 34 months), 6 died and 23 patients were readmitted because of CHF. Among them, 6 (100%) and 14 (61%) had SDB, respectively (vs. those without SDB, p= .02, p= .03).
[Conclusion] The present study suggested that SDB was the risk factor of prognosis in patients with CHF and it was possible that SDB may be responsible for the elevation of CRP. Accordingly, early recognition and treatment of SDB is very important and further investigations are warranted to confirm the relationship between SDB and CRP in patients with CHF.

# P-II-4

## Prognostic Value of Left Ventricular Flow Propagation Velocity in Patients with Chronic Left Ventricular Systolic Dysfunction

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Left ventricular (LV) flow propagation velocity (FPV) measured using color M-mode Doppler technique has been shown to be a useful nonivasive index of LV diastolic function. However, little information is available about the prognostic implications of this index for congestive heart failure. This study aimed to determine the usefulness of FPV as a prognostic marker in patients with chronic LV systolic dysfunction. In consecutive 98 patients with impaired LV systolic function (61 with previous myocardial infarction, and 37 with idiopathic dilated cardiomyopathy), New York Heart Association (NYHA) functional status was deterimined, and LV end-diastolic (LVDd) and left atrial (LAD) diameters, LV mass, and LV ejection fraction (EF) were measured using echocardiography. The peak early (E) and late (A) diastolic transmitral velocities, E/A, deceleration time of the E wave and isovolemic relaxation time were measured using the conventional Doppler techniques, and RFP was defined as E/A>=2.0. FPV was measured using color M-mode Doppler technique and FPV/E was calculated. Over a mean follow-up of 37 months, 26 had cardiovascular events (death in 4, congestive heart failure in 13, ventricular tachycardia in 6, and cerebral infarction in 3). On univariate analysis, NYHA, LVDd, LAD, LV mass, EF, RFP and FPV were significantly associated with cardiac events, but EF was the single independent predictor of cardiac events on multivariate analysis. After stratifying the patients into two groups according to EF (group A with EF<0.35 and group B with EF>=0.35), univariate analysis revealed that only NYHA was significantly associated with cardiac events in group A and FPV/E was the single significant predictor of cardiac events in group B, respectively. RFP was not significantly associated with cardiac events in the subgroups. To evaluate LV diastolic function using color M-mode Doppler derived FPV/E may contribute to predicting prognosis in patients with systolic dysfunction, especially in those with mildly depressed systolic function.

# P-II-5

## Diagnostic Impact of Myocardial Velocity Profile in the Hypertrophied Left Ventricular Wall in Patients With Cardiac Amyloidosis : Comparison With Hypertension and Hypertrophic Cardiomyopathy

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Background – The myocardial velocity profile (MVP), derived from color-coded tissue Doppler imaging (TDI), can identify transmural heterogeneity based on the physiology and pathology of the myocardium. This study sought to clarify whether the MVP can differentiate cardiac amyloidosis (CA) from other causes of left ventricular hypertrophy.
Methods and Results – We recorded MVP and determined its myocardial velocity gradient (MVG) in the ventricular septum and LV posterior wall using color-coded TDI in 10 patients with CA, 25 patients with hypertensive hypertrophied LV wall (HT), 25 patients with asymmetric septal hypertrophy of hypertrophic cardiomyopathy (HCM), and 20 clinically normal controls. End-diastolic ventricular septal thickness was similar among the CA, HT and HCM groups. Percent systolic thickening of the ventricular septum and LV posterior wall calculated from M-mode LV echocardiogram was lower in the CA group than in the HT, HCM or control group. The peak MVGs during systole and early diastole were lowest in the CA group, followed, in order, by the control group, HT group and HCM group. The systolic and early diastolic MVPs in the ventricular septum and LV posterior wall showed a characteristic serrated pattern in all patients with CA but not in other patient groups.
Conclusion – MVPs in the ventricular septum and LV posterior wall show a distinctive serrated pattern, that may be related to amyloid deposition in the myocardium. Myocardial tissue characterization using color-coded TDI provides a diagnostic information in CA.

# P-II-6

## Long-term Influence of Left Ventricular Diastolic Dysfunction on the Clinical Course of Patients With Hypertrophic Cardiomyopathy

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Background: Left ventricular (LV) filling flow propagation velocity (FPV) has been shown to well reflect diastolic function in patients with hypertrophic cardiomyopathy (HCM). Purpose: To determine how LV diastolic dysfunction modifies the clinical course of patients with HCM. Methods: In 43 HCM patients, an echocardiography was performed to measure LV end-diastolic dimension, wall thicknesses, percent fractional shortening, left atrial dimension (LAD), early diastolic and late diastolic peak transmitral flow velocities (E and A, respectively), deceleration time (DT) of E, E/A, isovolumic relaxation time, FPV and FPV/E. They were then followed up for a mean period of 42 ± 23 months. Cardiac events included hospitalization for atrial fibrillation or flutter (AF event), that for ventricular tachycardia or fibrillation (VT event), that for heart failure (HF event), and that for the other serious cardiac problems such as cardiogenic embolism and sudden death. The total events included any of the above events. Results: The Cox proportional hazards model analysis revealed that FPV/E was the single independent predictor each for the Af event (n=9, $\chi^2$=4.35, p=0.037) and for the HF event (n=11, $\chi^2$=7.16, p=0.047). Both LAD and FPV/E were the independent predictors for the total events (n=21, X2=6.172, p=0.0130 for LAD, X2=3.932, p=0.0474 for FPV/E). The HF event was associated with the AF event in 6 of 11 patients (54%). Conclusion: Diastolic dysfunction indicated by reduced FPV/E can be a strong predictor of cardiac events in patients with HCM. Diastolic dysfunction may be a principal cause of AF that frequently leads to more serious cardiac events such as CHF.

# P-II-7

## Longitudinal Myocardial Function Assessed by Strain Tissue Doppler Imaging in a Case of Isolated Noncompaction of Left Ventricular Myocardium

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A 15-year-old man was referred to our hospital for further examination because of ECG abnormality. He had no family history of sudden death. The 2-demensional echocardiography showed numerous prominent trabeculations and deep intertrabecular recesses in the apex of the left ventricular (LV) wall. LV ejection fraction was 68%. The patient underwent tissue and strain Doppler imaging at the basal, mid, and apical LV walls on the apical 4-chamber view. With the use of tissue Doppler imaging, there were no significant differences in the peak systolic LV wall motion velocities between patient and normal patients (8.3 and 8.7cm/sec, respectively). In contrast, the ratio of apical to basal peak systolic strain was significantly lower in the patient (-0.38 / -1.16 = 0.33) than in the normal control (-0.71 / -1.23 = 0.58). LV myocardium with isolated noncompaction is characterized by an early impairment in systolic myocardial function at a time when LV pump function remains normal. This abnormality precedes the onset of congestive heart failure and can be detected by strain but is not apparent by tissue Doppler imaging.

# P-III-1

## Early and Sustained Efficacy of Percutaneous Catheter Alcohol Ablation for Medically Refractory Patients With Hypertrophic Obstructive Cardiomyopathy

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Percutaneous catheter treatment with alcohol ablation (PTSMA) to responsible septal branch demonstrates outstanding improvement in symptomatic patients with hypertrophic obstructive cardiomyopathy(HOCM). Since we started this treatment in 1998, fifty-three procedures in forty-two patients are performed in our institution, and their results were analyzed. The study patients consist of 16males and 26 females, aged 15-79 year old, who are diagnosed to have HOCM with NYHA class II m-IV symptom on drug treatment and intra-left ventricular pressure gradient (PG) >60mmHg. Super-selective myocardial contrast echo was applied for confirming proper target vessel. Number of treated vessel was 1-3 (average 1.4) and 1.0-3.9ml(average 2.2ml) of ethanol was injected. PG decreased from 90.5 +/- 32.9mmHg to 25.1+/-24.8mmHg on catheter ($p<0.0001$) and NYHA functional class improved to 1.3 +/-0.5 from 2.6+/- 0.5($p<0.0001$). Successful (>50%) and excellent (>80%) reduction of PG was obtained in 92% and 48% of patients respectively. Seven patients developed transient complete heart block, however nobody required permanent pacemaker implantation. Five patients received repeat PTSMA procedure after 6 months time as intended second session for combined outflow and mid-ventricular obstruction, and another 6 patients required repeat PTSMA for recurrence of pressure gradient with residual symptom. In follow-up period at 22 +/- 19 months time patient's symptom was preserved in NYHA class 1.4+/-0.5 except two late deaths (1 pneumonia+AMI, 1 non-cardiac). Thus PTSMA appears safe and excellent treatment in early and late stage for Japanese patients, and should be positively indicated to symptomatic patients with close watch to its long-term result.

# P-III-2

## Successful Catheter Treatment for Hypertrophic Cardiomyopathy With Isolated Mid-Ventricular Obstruction

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In contrast to left ventricular outflow tract obstruction, percutaneous transluminal septal myocardial ablation (PTSMA) has not yet been recognized for hypertrophic cardiomyopathy (HCM) with mid-ventricular obstruction (MVO) as the alternative treatment. Since extensive myocardial hypertrophy involving free wall in addition to inter-ventricular septum contributes formation of obstruction in this type, PTSMA has not been considered effective and safe similar to LVOT type. In view of being less invasive, however, overcoming possible problems and applying the interventional treatment to this type would be beneficial for the patients with significant symptom. We report the results of six procedures of PTSMA in five patients suffering from isolated MVO. Myocardial ablation was performed by injection of alcohol into 1.5 ± 0.5 branches after verification of target vessels by super selective myocardial contrast echocardiogram. Pressure gradient decreased to 24 ± 13 mmHg from 78 ± 21 mmHg ($P<0.01$) and NYHA functional class improved to 1.3 ± 0.5 from 2.8 ± 0.7. Major complication such as rupture of free wall or papillary muscle was not observed. PTSMA was safely feasible and effective for MVO. In conclusion, this nonsurgical treatment would be applicable therapeutic strategy for patients with MVO who do not show satisfactory response to medical therapy, although further experience is required.

# P-III-3

## Protective Effects of Benidipine, a Calcium Antagonist, Against Myocardial Damage Following Viral Infection

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**Background** We have reported that some antioxidants have beneficial effects in experimental viral myocarditis. Benidipine, a second generation calcium antagonist, has been reported to possess lipid antioxidant properties. However, the effects of this drug has not been evaluated in that animal model.
**Objectives** This study was designed to examine the effects of benidipine in a murine model of viral myocarditis due to encephalomyocarditis virus (EMCV) infection.
**Methods** Four-week-old DBA/2 mice were inoculated with EMCV (day 0). Benidipine (3 or 10 mg/kg) or diltiazem (60 mg/kg) was given daily, and control mice received the vehicle only. Survival rates were determined on day 14. Myocardial histopathology and the amout of plasma malondialdehyde (MDA) formed by the thiobarbituric acid method were examined on day 7.
**Results** Treatment with benidipine improved the survival in a dose-dependent manner. The 14-day survival rate was significantly higher in the 10 mg/kg benidipine group (67%, 26/39) than in the control group (42%, 17/41, $p < 0.05$). The 14-day survival rate in the 3 mg/kg benidipine group was 50% (5/10), and that in the diltiazem group was 40% (4/10). The pathological score were decreased in a dose-dependent manner. The cellular infiltration score and the myocardial necrosis score were significnatly lower in the group treated with 10 mg/kg benidipine ($1.0 \pm 0.2$ and $0.8 \pm 0.3$, respectively) versus the control group ($2.0 \pm 0.1$ and $1.8 \pm 0.4$, respectively, $p < 0.05$). Plasma MDA levels were significantly decreased in the group treated with 3 mg/kg benidipine ($11.9 \pm 1.4$ nmol/mL) and 10 mg/kg benidipine ($10.4 \pm 1.3$ nmol/mL), compared with the control group ($39.1 \pm 9.8$ nmol/mL, $p < 0.05$). Diltiazem had no effect in this model.
**Conclusions** These results suggest that treatment with benidipine prevents myocardial injury following viral infection. The cardioprotective effects of benidipine may be due to antioxidant effects.

# P-III-4

## Beneficial Effects of Arotinolol, a $\beta$-blocker, in a Murine Model of Congestive Heart Failure Induced by Viral Myocarditis

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**Background** We have reported that the third generation $\beta$-blocker carvedilol was beneficial in a murine model of viral myocarditis. The beneficial effects of carvedilol may be due to an antiviral effect from an increase in interferon-$\gamma$ (IFN-$\gamma$). However, the effects of the other third generation $\beta$-blockers have not been analyzed in this model.
**Objectives** This study designed to examine the roles of arotinolol, a nonselective $\beta$-blocker with $\alpha$ 1-adrenergic receptor blocking properties, in a murine model of congestive heart failure induced by viral myocarditis due to encephalomyocarditis virus (EMCV) infection.
**Methods** 4-week-old DBA/2 mice were inoculated with EMCV (day 0). Arotinolol (1 or 3 mg/kg) was given daily, and control mice received the vehicle only. Survival rates were determined on day 14. Myocardial histopathology, IFN-$\gamma$ levels in the heart by ELISA assay on day 5.
**Results** The 14-day survival rate increased in a dose-dependent manner, and was significantly higher in the 3 mg/kg arotinolol group (70.0%, 7/10) than in the control group (26.7%, 4/15, $p < 0.05$). The 14-day survival rate in the 1 mg/kg arotinolol group was 60.0% (6/10). The pathological score were decreased in a dose-dependent manner. The cellular infiltration score and the myocardial necrosis score were significnatly lower in the group treated with 3 mg/kg arotinolol ($1.4 \pm 0.2$ and $1.6 \pm 0.2$, respectively) versus the control group ($2.2 \pm 0.2$ and $2.4 \pm 0.2$, respectively, $p < 0.05$). IFN-$\gamma$ levels in the heart were significantly increased in the group treated with 3 mg/kg arotinolol ($5.6 \pm 0.2$ pg/mg of heart), compared with the control group ($3.6 \pm 0.2$ pg/mg of heart).
**Conclusions** These results suggest that arotinolol exerts some of its beneficial effects by increasing the production of IFN-$\gamma$. Arotinolol, like carvedilol, may be effective in patients with viral myocarditis by boosting IFN-$\gamma$ production.

# P-III-5

## Atenolol, Bunazosin, and Carvedilol: Which of Them Should be Prescribed in Heart Failure From Diabetes Mellitus?

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**Background:** The use of alfa1-blocker is recommended for obese hypertensives because of the favorable effect on glucose metabolism. Beta-blockers have been known to improve prognosis of patients with heart failure, but it is still controversial for patients with diabetes mellitus. The aim of this study was to evaluate the effect on cardiac function and fine structure in diabetic cardiomyopathy among alfa1-blocker, bunazosin, beta1-selective-blocker, atenolol, and non-selective alfa-beta-blocker, carvedilol. **Materials and Methods:** The male OLETF rats (n=20) at 36 weeks of age, exhibiting type 2 diabetes mellitus, were divided into 4 groups and treated for 4 weeks; (a) atenolol 20 mg/kg/day. (b) bunazosin 10 mg/kg/day, (c) carvedilol 10 mg/kg/day, and vehicle. After hemodynamic measurements, the heart was examined immunohistochemically using monoclonal antibody against major lipid peroxidation product (4-HNE). **Results:** The OLETF rats showed significantly increased left ventricular (LV) end-diastolic pressure (12 ± 4 vs. Control; 6 ± 2 mmHg), decreased LV peak negative dP/dt (2358 ± 224 vs. 3543 ± 285 mmHg/sec), and hypertrophy of cardiomyocytes (22.1 ± 0.7 vs. 18.0 ± 0.3 $\mu$m, $p<0.05$ respectively). Moreover, TBARS (thiobarbituric acid-reactive substances) in the plasma was increased (7.8 ± 1.8 vs. 3.6 ± 0.2 nM/ml). Atenolol and carvedilol effectively improved the cardiac function, whereas bunazosin and carvedilol preserved the fine structure of LV myocardium. Though atenolol showed no effect on the oxidative stress, both bunazosin and carvedilol decreased 4-HNE in LV. Furthermore, carvedilol was effective to reduce TBARS (4.8 1.2, $p<0.05$ vs. vehicle). **Conclusion:** This study suggests that alfa-beta-blocker which possesses antioxidant effect is suitable in the treatment of heart failure associated with diabetes mellitus.

# P-III-6

## Cardioprotection by Angiotensin II Antagonism in Heart Failure is Mediated Through Stabilization of Ryanodine Receptor

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Background. Although angiotensinII-receptor blockade is considered to be useful for the treatment of human heart failure, little beneficial hemodynamic effect has been shown in some experimental failing hearts. Here, we assessed the effect of an angiotensinII-receptor blocker, valsartan, on sarcoplasmic reticulum (SR) function, defectiveness of which is a major pathogenic mechanism in heart failure.
Methods and Results. SR vesicles were isolated from dog LV muscle (normal, or 4-weeks rapid RV pacing with or without valsartan). In the untreated and valsartan-treated paced dogs, cardiac function showed similar deterioration (compared with pre-pacing). However, both the density of b-receptors and the contractile response to dobutamine were greater in the valsartan-treated paced dogs than in the untreated paced dogs. In untreated paced hearts, the ryanodine receptor (RyR) was PKA-hyperphosphorylated, showed an abnormal $Ca^{2+}$ leak, and had a decreased amount of RyR-bound FKBP12.6. No such phenomena were seen in the valsartan-treated paced hearts. Both the SR $Ca^{2+}$ uptake function and the amount of $Ca^{2+}$-ATPase were decreased in the untreated failing SR, but both were restored in the valsartan-treated SR. Conclusions. During the development of pacing-induced heart failure, valsartan may inhibit b-adrenergic receptor-mediated signaling at presynaptic nerve endings and concurrently restore SR function without improving resting cardiac function (leading to "uncomfortable" hemodynamics, but probably "comfortable" sub-cellular conditions devoid of $Ca^{2+}$ overload).

# P-III-7

## Increased Myocardial Microvessels and Improved Left Ventricular Function by Intracoronary bFGF in Patients With Idiopathic Dilated Cardiomyopathy

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Background: Except cardiac transplantation, there are no fundamental cuarative treatments for patients with idiopathic dilated cardiomyopathy(DCM). Abnormal myocardial microcirculation may be aggavating this serious disease.
Aim: To examine the effects of intracoronary injection of bFGF on left ventricular function and coronary angiogenesis in patients with DCM. Methods: Approved by University Ethical Committee, seven patients diagnosed as DCM by LVG classification underwent LVG, CAG and cardioscope-guided endomyocardial biopsy followed by intracoronary administration of 100ug human recombinant bFGF. Three months later, they underwent re-study.
Results: Three months later, functional capacity by NYHA was improved in 5 patients. Left ventricular function by LVG was improved(EDVI from 121.5 ± 28.6 to 97.7 ± 19.5; EF from 0.46 ± 0.16 to 0.50 ± 0.13). Endocardial color of the same left ventricular wall segment observed by cardioscopy was changed from white or light brown to pink or reddish brown, indicating improved myocardial blood flow in 4 patients. The number of microvessels per unit area of the biopsied specimen was increased.
Conclusion: The results indicate that left ventricular function can be improved by administration of bFGF probably through angiogenesis and resultant blood flow restoration in patients with DCM.

# P-III-8

## Novel Mutations in the Lamin A/C Gene and Familial Dilated Cardiomyopathy

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Background: We reported that some families with dilated cardiomyopathy (DCM) had mutations in the lamin A/C gene. Most mutations in the lamin A/C gene are located in the rod domain of lamin A/C structure and families which have mutations in the lamin A/C gene had variable degrees of atrio-ventriclar (AV) conduction defects. Therefore, we screened families with DCM associated with AV conduction defects. And also we screened families with DCM without AV conduction defects. Methods: We obtained genomic DNA from all probands who were eligible for this study after we obtained informed consent. Finally, 23 families had DCM associated with AV conduction defects and 20 families had DCM without AV conduction defects. Results: We found 4 mutations in the lamin A/C gene in families with DCM and AV conduction defects (Family 1: R190W, Family 2: I210N, Family 3: L215P, Family 4: R377L). All mutations are located in the rod domain of lamin A/C structure and all are missense mutations. None of the 4 sequent variants were found in controls and also each altered residue is highly conserved throughout evolution, indicating that these defects probably have functional consequences. In contrast, no families with DCM had a mutation in the lamin A/C gene. Conclusions: We identified 4 mutations in the lamin A/C gene in 4 families with DCM associated with AV conduction defects but we could not find any mutations in families with DCM. Three of them (I210N, L215P, R377L) are novel missense mutations, and all mutations which were identified in this study are located at the rod domain of lamin A/C structure. This will suggest that the location of mutations in the lamin A/C gene is important for cardiac phenotype, especially AV conduction defects.