



- Cluster 1
Atrial natriuretic factor
Taurine transporter
Osteopontin
c-fos
- Cluster 2
Caspase 3
Heat shock protein 70
p21
Connective tissue growth factor
- Cluster 3
Laminin alpha 2
VCAM1
Metallothionein 2A
Myeloid cell leukemia sequence 1
- Cluster 4
Heparin-binding EGF-like growth factor
Activating transcription factor 3
Brain-derived neurotrophic factor
Nebulin

PJ-111

Inhibitory Effects of K201 (JTV-519) on Norepinephrine-induced Diastolic Dysfunction in Rats

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Heart failure patients with a high plasma level of norepinephrine (NE) have a poor prognosis. An increase in the diastolic intracellular calcium level (Ca^{2+}) has been shown in LV dysfunction, but the pathogenesis of diastolic heart failure remains unclear. We investigated induction of diastolic dysfunction in rats using NE with Ca^{2+} loading, and examined the effects of K201 (JTV519) and diltiazem in this model. Animals were examined in four groups: Ca^{2+} loading for 45 min; NE for 25 min; Ca^{2+} loading and NE for 25 min after Ca^{2+} loading (Ca^{2+} -NE); and vehicle. The effects of K201 and diltiazem were studied in the Ca^{2+} -NE group. Hemodynamics and diastolic function were examined using a micromanometer-tipped pressure catheter and Doppler echocardiography. There were no significant changes in LVP and LV-EF among the four groups. A significant increase in LVEDP and decreases in E and Ea waves and deceleration time (DCT) were found in the Ca^{2+} -NE group. NE-induced diastolic contracture (NEIDC) with aortic valve opening in diastole was observed in the Ca^{2+} -NE group. K201 significantly decreased LVEDP, reduced the incidence of NEIDC, and improved diastolic function, but diltiazem did not do so. NE may be important in development of diastolic dysfunction, and K201, which has an inhibitory effect on diastolic Ca^{2+} leakage from the SR, may be useful for improvement of diastolic dysfunction.

PJ-112

Aldosterone Activates Apoptosis Signal-Regulating Kinase 1 (ASK-1) through Nongenomical ROS production and Induces Myocyte Apoptosis.

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[**Background**] Aldosterone is an exacerbation factor of heart failure. In addition to its classical genomic actions, aldosterone influences cell processes through nongenomic mechanism. This study examined whether aldosterone nongenomically induces reactive oxygen species (ROS) production, causing myocyte apoptosis. [**Methods and Results**] Neonatal rat cardiac myocytes were treated with aldosterone (100 nmol/L; 5 min - 48 h) in the presence or absence of following drugs: eprelone, a mineralocorticoid receptor antagonist, RU486, a glucocorticoid receptor antagonist, apocynin, a NADPH oxidase ac-

tivation inhibitor, butylated hydroxyanisole (BHA), a mitochondrial ROS scavenger, and tempol, a free radical scavenger. Aldosterone enhanced NADPH oxidase activity after 5 min. Neither transcriptional nor translational inhibitors blocked this rapid activation, suggesting a nongenomic mechanism. Aldosterone augmented intracellular ROS level, measured by DCF fluorescence, after 5 min and that continued after 48 hours. Nuclear staining with DAPI showed that aldosterone increased the myocyte apoptosis (2.3 fold, $p < 0.001$), coincident with the activation of caspase 3 (1.4 fold, $p < 0.05$) compared with the serum deprived control after 48 hours. In addition, aldosterone activated apoptosis signal-regulating kinase 1 (ASK1), determined by Western blotting. Eprelone, apocynin, and tempol, but neither RU486 nor BHA, significantly inhibited aldosterone-induced NADPH oxidase activation, intracellular ROS accumulation, ASK1 activation, and myocyte apoptosis. [**Conclusion**] The present study demonstrates that aldosterone activates ASK-1 through nongenomical NADPH oxidase-dependent ROS production and induces myocyte apoptosis.

PJ-113

Nkx2.5(+) But Not Flk1(+) Cardiac Progenitor Cells are Fated to Pacemaker Cells Expressing I_f Predominantly Encoded by HCN4 Gene

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Back ground: Hyperpolarization-activated non-selective cation channels (I_f) encoded by HCN gene family play the pivotal role for cardiac automaticity, although its developmental change remains unknown. **Methods and Results:** We examined both transcription, expression and function of HCN in Nkx2.5-positive (+) cardiac precursor cells derived from mouse ES cells. Both the transcript of HCN gene family and the extent of Cs^+ (2mM)-induced inhibition of the automaticity of EBs increased along their differentiation. Flk1(+) cells did not express the transcript of either HCN or Nkx2.5, while Nkx2.5(+) cells expressed HCN. The extent of Cs^+ -induced prolongation of the cycle length of spontaneous action potential and the amplitude of I_f in the Nkx2.5(+) cells was predominantly observed in the late stage (day 15-20th) of their differentiation rather than in the early stage (day 8-10th). Although immunoreactivity against HCN1, 2 and 4 were significantly increased in the Nkx2.5/GFP(+) cells along with their differentiation, the half activation of I_f (-94mV) was close to the kinetics of HCN4. Other channels responsible for automaticity (Cav3.1, Cav1.2, Cav1.3 and Nav1.5) predominantly expressed in the late stage except of Cav3.2. **Conclusion:** These results suggested that Nkx2.5(+) cardiac progenitor cell but not Flk1(+) cell has been committed to the fate to pacemaker cells expressing I_f predominantly encoded by HCN4 gene in the late stage of differentiation.

PJ-114

Erythropoietin Receptor Signaling Mitigates Renal Dysfunction-Associated Heart Failure through Direct Cardioprotective Actions Independent of Erythropoiesis

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Cardiovascular complications including heart failure are the most serious problem in patients with chronic kidney disease (CKD). Although beneficial effects of erythropoietin (EPO) have been reported on cardiac function of patients with CKD-associated heart failure, it is difficult to evaluate whether the effects were resulted simply from improvement of anemia. Expression of EPO receptor on the cardiovascular system may suggest additional cardiovascular effects of EPO beyond hematopoiesis. Mice underwent 5/6 nephrectomy to cause CKD. Four weeks later when renal dysfunction was established, mice were assigned to receive saline (control), EPO (1500U/kg/day), and asialo-EPO (AsEPO; 5000U/kg/day) (n=10 each), and they were followed-up for 8 weeks. AsEPO is known to cause no hematocrit increase; this was confirmed in the

present study. Post-treatment survival was 100% in EPO and AsEPO-treated groups while that of control group was 80%. Compared with control group, AcEPO-treated group as well as EPO-treated group showed a significantly lower heart to body weight ratio (saline, 4.97 ± 0.20 ; EPO, 3.63 ± 0.21 ; AsEPO, 4.08 ± 0.13 mg/g), smaller left ventricular (LV) cavity (LVDd: saline 4.05 ± 0.13 ; EPO, 3.39 ± 0.06 ; AsEPO, 3.64 ± 0.08 mm), and better LV systolic function (EF: saline, 37 ± 1 ; EPO, 58 ± 1 ; AsEPO, $54 \pm 2\%$). These findings clearly indicate direct cardioprotective actions of EPO receptor signaling on CKD-associated heart failure, which is independent of hematopoiesis.

PJ-115

Protein phosphatase inhibitor 1 (I-1) augments a protein kinase A-dependent increase in SR Ca²⁺ loading without changing Ca²⁺ spark

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Background- I-1 is a potent and specific inhibitor of protein phosphatase 1 and the reduced I-1 activity has been implicated in the impaired excitation-contraction coupling in heart failure. However, the precise effects of I-1 on the SR Ca²⁺ handling remain undefined. **Method-** We investigated the effects of PKA and I-1 on the SR Ca²⁺ loading and the spontaneous SR Ca²⁺ release by analyzing caffeine-induced Ca²⁺ transient (Caff CaT) and Ca²⁺ sparks in permeabilized rat ventricular myocytes. **Results-** (1) PKA (10 U/ml) increased Caff CaT from 118 ± 8 nM to 185 ± 10 nM (means \pm SE, $p < 0.01$, $n = 5$). The addition of I-1 (1.6 nM) further increased it to 211 ± 11 nM ($p < 0.01$). (2) PKA increased Ca²⁺ spark frequency from 244 ± 19 /pl/s to 398 ± 85 /pl/s ($n = 6$, $p < 0.05$), and the addition of I-1 increased it further (455 ± 67 /pl/s). Either the amplitude or duration of individual Ca²⁺ sparks did not change after the addition of I-1. (3) The abrupt inhibition of SR Ca²⁺ uptake by cyclopiazonic acid (CPA: 10 μ M) decreased Ca²⁺ spark frequency. The addition of I-1 did not accelerate the CPA-dependent decline of Ca²⁺ spark frequency. **Conclusion-** I-1 is expected to augment PKA-dependent phosphorylation of both phospholamban and ryanodine receptors. Our results suggest that I-1 may stimulate SR Ca²⁺ uptake with less significant effects on SR Ca²⁺ release channels, and can be applied to improve SR Ca²⁺ handling in heart failure.

PJ-116

Cardioprotective Effect of Tetrahydrobiopterin via Inhibiting Oxidative Stress in Failing Rat Hearts

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Tetrahydrobiopterin (BH4) is an essential co-factor of endothelial nitric oxide synthase (eNOS), and previous studies suggested that BH4 could be a molecular target for oxidative stress. We evaluated whether chronic supplementation with BH4 inhibited the NAD(P)H oxidase and protects against cardiac dysfunction and cardiovascular remodeling in failing heart of Dahl salt-sensitive hypertensive (DS) rats. BH4 (5mg/kg/day) was administered from the left ventricular hypertrophy stage (11 weeks) to the failing stage (18 weeks) for 7 weeks. Markedly increased LVEDD and reduced %FS in failing DS rats was significantly ameliorated by BH4. Downregulated levels of eNOS, adiponectin, and SOD-1 expression in failing rats were restored by treatment with BH4. Upregulated expression of NAD(P)H oxidase components, including p22phox, p47phox, gp91phox, and LOX-1 in failing stage was significantly reduced by treatment with BH4. Moreover, increased phosphorylation of p65NF- κ B, PKC β II, p44/p42ERK, and p70S6K was significantly inhibited by BH4. BH4 administration resulted in a significant improvement in cardiovascular remodeling, and suppression of TGF- β 1, type I collagen, MCP-1, TNF- α mRNA. These results suggest that cardiovascular remodeling and failing hearts of DS rats were significantly ameliorated by BH4, which may be due to a increased in eNOS/adiponectin and a decreased in oxidative stress/signal transduction in the LV.

Heart failure, clinical(13) (M)

PJ020

March 28 (Fri)

Poster Presentation Room 1 (Marine Messe Fukuoka 1F Arena)

10 : 10—11 : 00

PJ-117

Noninvasive Prediction of Readmission in Patients with Chronic Heart Failure, Combined Use of Plasma BNP and Bioimpedance Derived Cardiac Index

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BACKGROUND: By measuring whole body bioimpedance, noninvasive cardiac systems (NICaS) estimates cardiac index (NI-CI). We previously described NI-CI had high accuracy compared with thermodilution-method in chronic heart failure (CHF). The study's aim is to find the noninvasive predictor for readmission in outpatients with CHF. **METHOD:** Sixty-five CHF patients, just after discharged, were measured NI-CI, serum BNP, ejection fraction (EF) by echocardiography and exercise threshold (ET) by questionnaire. Also, stroke volume index (SVI), Total vascular resistance index (TVRI) were calculated by NICaS data. By ROC analysis, cut-off point and area under curve (AUC) in each index were calculated for patient's readmission for CHF within one year. **Results:** (Cut-off, AUC): BNP (335.1, 0.95) and NI-CI (2.33, 0.91), ET (4, 0.88), SVI (27.2, 0.87), TVRI (2596, 0.859), EF (37, 0.78). Thus, BNP and NI-CI had high AUC value. And stepwise logistic analysis also showed BNP and NI-CI were the only significant predictors. The formula $Z = 0.1057 + 0.06573 \times \text{BNP} - 1.6898 \times \text{NI-CI}$, ($Z > 0$ predicts readmission) had high predictive accuracy (89%), positive and negative predictive value (61%, 92%) respectively. **Conclusion:** Combined index by plasma BNP and NI-CI may be very useful index for estimating CHF prognosis.

PJ-118

Comparison of peak oxygen uptake and brain natriuretic peptide (BNP) to predict prognosis in patients with heart failure

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Peak oxygen uptake (peak VO₂) and brain natriuretic peptide (BNP) predict survival in ambulatory patients with chronic heart failure (CHF). This study was designed to investigate the prognostic value of the peak VO₂ with respect to BNP. **Methods and Results:** We examined 183 outpatients with CHF who were admitted to our hospital between January 2000 and September 2007 and performed cardiopulmonary exercise testing and BNP measurements after discharge. The cardiac events were defined as cardiac death or readmission due to heart failure. The average follow-up period was 1084.9 ± 740 days, and ejection fraction was 44.7 ± 18.3 %. Cardiac events occurred in 55 patients of our study population, and 49 of those patients were readmitted due to worsening heart failure. Peak VO₂ ($p = 0.01$) and BNP ($p \leq 0.001$) were both independent predictors for cardiac events. The 3-year event-free survival rate was 83.7% in patients with peak VO₂ ≥ 21.0 ml/min/kg (6Mets) and 57.2% in those with peak VO₂ ≤ 17 (5Mets). BNP ($p \leq 0.001$) was a significant and better indicator than