# OE-110

## G-CSF after Transient Myelosuppression Improves Cardiac Function via More Myocardial Regeneration and Vasculogenesis than G-CSF Alone in Ischemia-Reperfusion Hearts

[1]Yu Misao
[1]Masazumi Arai, [1]Xuehai Chen, [1]Chunjuan Lu, [1]Ninguan Wang, [1]Hirohito Onogi, [1]Hiroshi Nagai, [2]Tomoyuki Takahashi, [1]Genzou Takemura, [1]Shinya Minatoguchi, [3]Takako Fujiwara, [1]Hisayoshi Fujiwara
[1]Department of Cardiology, Regeneration Medicine and Bioethics, Gifu University Graduate School of Medicine, Gifu, [2]Department of Gene Therapy and Regenerative Medicine, Gifu University School of Medicine, Gifu, [3]Department of Food Science, Kyoto Women's University, Kyoto

**Background:** It has been reported that G-CSF improved cardiac function after myocardial infarction (MI) via promoted myocardial regeneration in ischemia-reperfusion hearts. We hypothesized that G-CSF after transient myelosuppression with cytoreductives could augment circulating stem cells and myocardial regeneration, and improve the cardiac function, compared with G-CSF alone, in MI hearts. **Methods and Results:** In 30 rabbits, the bone marrow-derived mononuclear cells (BMC) from the hip bone marrow were labeled with 1.1'-dioctadecyl-1to3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI). MI was induced by 30 minute-ischemia and reperfusion of the coronary artery (day 0). Fifteen of the rabbits were treated with 5-fluorouracil and cyclophosphamide, cytoreductives, (day 0 to 2) and G-CSF (day 3 to 7; G+M), while the others with saline and G-CSF (G). FACS revealed that G+M after myelosuppression, had significantly more circulating stem cells than G. On day 28, confocal microscopy showed that G+M had significantly more BMC-derived cardiomyocytes, positive for DiI and troponin I. (G+M:G=8.9 ± 9.1:1.1 ± 1.3%, $p<0.05$) The CD 31 positive-capillaries were increased in G+M, compared with in G. Ejection fraction was significantly improved in G+M (75.6 ± 2.7%), compared with in G. (66.7 ± 3.9%) The infarct area to LV area in G+M (6.6 ± 4.9%) was significantly smaller than in G (13.5 ± 8.1%). **Conclusion:** G+M significantly improved the infarcted cardiac function via augmented myocardial regeneration, compared with G.

# OE-111

## RhoA and Rho-kinase are involved in Interleukin (IL)-18-induced gene expression and apoptosis in cardiac myocytes

Tsuyoshi Sakoda
Toshio Naka, Daizo Kawasaki, Yoshio Fujioka, Takeshi Tsujino, Mitsumasa Ohyanagi
Department of Internal Medicine, Cardiology Division, Hyogo College of Medicine, Nishinomiya

IL-18 is a proinflammatory cytokine with multiple functions. We and others have demonstrated increased levels of circulating IL-18 is a risk factor for acute coronary syndrome and heart failure. A small GTPase, RhoA and Rho-kinase (Rho-K) participate in gene expression. Nuclear factor kappa-B (NF-kB) enhances expression of several cytokines in myocardial ischemia and heart failure. However, the effect of IL-18 on cardiomyocytes is quite unknown. In the present study, we examined the role of RhoA and Rho-K on gene expression and apoptosis induced by IL-18 in neonatal rat cardiomyocytes. The effect of c-fos and NF-kB gene expression by IL-18 was examined by transfection assay with luciferase reporter genes driven by c-fos promoter and multimerized NF-kB sites. IL-18 increased NF-kB and c-fos luciferase activities and these effects were inhibited by dominant negative RhoA N19 and a dominant negative of Rho-K RB/PH(TT). The deletion analysis revealed c-fos serum response element (SRE) accounts for c-fos expression. Furthermore, IL-18 resulted in apoptosis as demonstrated by DNA ladder formation and chromatin condensation. Sinvastatin, a HMG-CoA reductase inhibitor, inhibited not only IL-18-induced NF-kB and c-fos gene expression, but also apoptosis. These results firstly demonstrated that IL-18 induced c-fos SRE and NF-kB gene expression and apoptosis through RhoA and Rho-K. Inhibition of IL-18-induced apoptosis by Sinvaststin might be one of the mechanisms of pleiotropic effect of statins.

# OE-112

## Inhibition of Transforming Growth Factor (TGF)-β Signaling Prevents Left Ventricular Remodeling and Failure after Myocardial Infarction

Masaki Ikeuchi
Hiroyuki Tsutsui, Hidenori Matsusaka, Syouji Matusima, Jing Wen, Toru Kubota, Akira Takeshita
Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kyusyu University, Fukuoka

Myocardial fibrosis plays an important role in the pathogenesis of left ventricular (LV) remodeling and failure after myocardial infarction (MI). Transforming growth factor (TGF)-β has been implicated as a major stimulator of tissue fibrosis. We thus hypothesized that the inhibition of TGF-β signaling might inhibit the development of post-MI LV remodeling and failure. Anterior MI was produced in mice by coronary artery ligation. To block the TGF-β signaling, an extracellular domain of TGF-β receptor (Tβ IIR) plasmid was transfected into the limb skeletal muscles at day 0 and 7 after coronary artery ligation. At 28 days, Tβ IIR significantly inhibited LV dilatation (end diastolic diameter: 6.5 ± 0.1 vs. 5.8 ± 0.1mm, $p<0.01$), and contractile dysfunction (fractional shortening: 13 ± 0 vs. 17 ± 1%, $p<0.01$) in MI mice without affecting infarct size. LV end-diastolic pressure was increased in MI, which was significantly reduced by Tβ IIR (16 ± 2 vs. 10 ± 2mmHg, $p<0.01$). Tβ IIR partially normalized LV $dP/dt_{max}$ and $dP/dt_{min}$. Tβ IIR also inhibited the increase in myocyte cross-sectional area (347.0 ± 24.6 vs. 221.0 ± 19.5 μ $m^2$, $p<0.01$) and collagen volume fraction (14.4 ± 1.9% vs. 8.3 ± 1.2%, $p<0.01$) at the non-infarcted LV. In conclusion, TGF-β plays an important role in post-MI remodeling and failure through promoting myocyte hypertrophy and interstitial fibrosis. The inhibition of TGF-β signaling might be a novel therapeutic strategy to prevent heart failure after MI.

# OE-113

## Cardioprotective effects of MCI-186, a novel free radical scavenger, on acute autoimmune myocarditis

Takaaki Okabe
Chiharu Kishimoto, Miki Hattori, Masaomi Nimata, Toru Kita
Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, Kyoto

<**Introduction**> MCI-186 (3-methyl-1-phenyl-pyrazolin-5-one) is a new free radical scavenger. Free radicals may play a role in the development of myocarditis. <**Hypothesis**> We assessed the hypothesis that MCI-186 protects against autoimmune myocarditis in rats attributing to free radical scavenging actions. <**Methods**> We intraperitoneally administered various dosages of MCI-186 (1mg, 3mg, and 10mg/kg/day, each n=14~17) and saline (control) for 3 weeks to rats with autoimmune myocarditis induce by porcine myosin immunization. <**Results**> MCI-186 administration reduced the severity of myocarditis in a dose-dependent manner; the effect of MCI-186 at a dose of 10mg/kg/day was statistically significant. The formation of hydroxyl radicals in MCI-186-treated heart homogenates was decreased compared with untreated controls. In addition, MCI-186 decreased the myocardial carbonyl contents, and also decreased the myocardial thiobarbituric acid reactive substance protects in rats with myocarditis, reflecting the less oxidative stress state in the treated group. <**Conclusions**> MCI-186 protects against acute autoimmune myocarditis in rats by antioxidant actions due to scavenging hydroxyl free radicals.