YOUNG INVESTIGATOR AWARDS

404

Young Investigators Awards Competition: Physiology, Pharmacology, and Pathology

Monday, March 08, 2004, 9:15 a.m.-10:30 a.m.
Morial Convention Center, Room 257

9:15 a.m.

404-1 In Vivo Magnetic Resonance Evaluation of the Effects of Mouse Embryonic Stem Cells on Cardiac Function

Takayasu Arai, Jorg de Bruin, Theo Kofidis, Ross Venook, Michael V. McConnell, Thomas Quertermous, Robert Robbins, Phillip C. Yang, Stanford University, Stanford, CA

Introduction. Congestive heart failure continues to be a major public health problem despite recent medical advances. Cell transplantation may offer therapeutic potential to salvage the injured myocardium. *In vivo* assessment of the transplanted cells has been described using non-invasive imaging methods. However, systematic longitudinal analysis of the effects of transplanted cells has not been done.
Purpose. This study performs a longitudinal evaluation of cardiac function in infarct mouse model following the transplantation of mouse embryonic stem cells (mESC).
Methods. The mESC were labeled using small paramagnetic iron oxide agents. LAD ligation was done followed by injection of mESC into the infarct area. The mice were divided into 4 groups: sham-operated, LAD-ligated, LAD-ligated with non-labeled mESC, and LAD-ligated with labeled mESC. The images were acquired using ECG-triggered cine sequence. LV ejection fraction (LVEF) and LV mass (LVM) were calculated blindly.
Results. Significant restoration of LVEF and LVM were observed in the mESC treated group at each time point. The mESC treated group demonstrated mean LVEF of 37.9% vs. untreated of 28.6%. A significant increase was also observed in mean LVM in the treated group of 51.4 mg vs. untreated of 34.2 mg. Finally, reliable imaging of the labeled mESC was observed during the 4-week duration.
Conclusions. *In vivo* MR imaging of the labeled mESC enable identification of the cells and assessment of their therapeutic effects on the cardiac function.

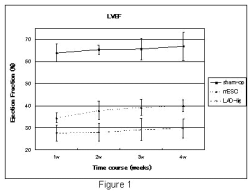

Figure 1

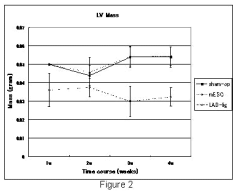

Figure 2

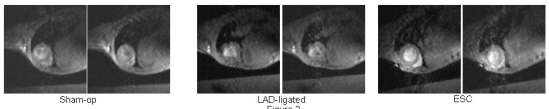

Figure 3

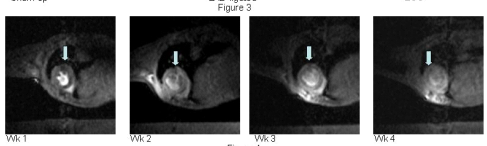

Figure 4

9:30 a.m.

404-2 Aspirin Resistance in Cardiovascular Disease: Underdosing of Overweight Patients

Andrew O. Maree, Ronan Curtin, Michelle Dooley, Peter Crean, Denis Shields, Dermot Cox, Desmond J. Fitzgerald, The Royal College of Surgeons in Ireland, Dublin 2, Ireland, Beaumont Hospital, Dublin 2, Ireland

Background: "Aspirin resistance" may result from failure to inhibit platelet cyclooxygenase and thromboxane (Tx) generation, or platelet activation *via* aspirin-insensitive pathways. We examined the mechanisms involved in a population with stable cardiovascular disease.
Methods: Patients (n=199) on aspirin 75–300mg daily were screened by Platelet Function Analyser (PFA) –100. Serum TxB_2 levels and platelet aggregation to arachidonic acid (AA 1.6mM) were performed in 142 patients. In 50 patients, additional analysis included aggregation to epinephrine (5uM), collagen (0.5ug/ml) and TRAP (5uM) and assay of platelet TxB_2 generated in response to AA. Platelet surface expression of IIb/IIIa, Ib, Ia/IIa and TRAP induced p-selectin were determined.
Results: 29 (15%) patients were aspirin resistant, failing to prolong the PFA-100 closure times (over 193 seconds). PFA-100 closure times and serum TxB_2 were inversely related ($r = -0.23$; $p = 0.002$). Serum TxB_2 in turn correlated strongly with platelet TxB_2 generated in response to AA ($r = 0.69$; $p = 0.0001$) and with platelet aggregation to AA ($R^2 = 0.57$; $p = 0.0007$), epinephrine ($r = 0.3$; $p = 0.04$), and TRAP ($r = 0.4$; $p = 0.002$) but not collagen ($r = 0.16$; $p = 0.24$). Patients with a serum TxB_2 above 4ng/mg (25% of population) generated 14-fold greater levels of platelet TxB_2 (291 +/- 35 vs 21 +/- 12 ng/mg; $p = 0.0001$). In every case platelet aggregation to AA was inhibited by addition of aspirin in vitro. Patient weight correlated with serum TxB_2 levels ($r = 0.17$; $P = 0.05$), particularly in patients on 75mg enteric-coated aspirin (74% of population; $r = 0.29$; $P = 0.002$). Aspirin resistance was also associated with higher platelet GPIIb/IIIa ($p = 0.03$), GPIb ($p = 0.04$) and TRAP induced p-selectin ($p = 0.02$), unrelated to the continued generation of Tx.**Conclusion:** In a stable cardiovascular population, 15% of patients were aspirin resistant. Failure to inhibit platelet Tx was a major contributor, although Tx-independent pathways were also involved. Continued Tx generation was related to weight particularly in patients on a 75mg enteric-coated aspirin preparation. Such preparations may provide an inadequate dose of aspirin in overweight patients.

9:45 a.m.

404-3 Dual Regulation of Endothelial Nitric Oxide Synthase by Rho-Kinase

Yoshiyuki Rikitake, Rosario Scalia, Michael A. Moskowitz, James K. Liao, Brigham & Women's Hospital, Cambridge, MA

Background: Rho-kinase plays an important role in cardiovascular disease. However, the mechanism by which inhibition of Rho-kinase leads to cardiovascular protection is not known. Methods and Results: Human vascular endothelial cells were treated with the Rho-kinase inhibitor, hydroxyfasudil (HF), and Akt activity and eNOS expression/activity were measured. In a concentration-dependent manner, HF stimulates eNOS activity acutely via activating phosphatidylinositol 3-kinase (PI3-K)/Akt pathway and chronically by increasing eNOS expression. Chronic treatment with fasudil inhibits leukocyte-endothelial interaction after ischemia/reperfusion injury and reduced cerebral infarct size and neurological deficits following transient middle cerebral artery occlusion. These effects correlated with decreased Rho-kinase activity and inversely with increased eNOS expression. Indeed, the cardiovascular protective effects of fasudil were blocked by the NOS inhibitor, L-NAME, and were absent in eNOS-deficient mice. In contrast, the rapid or acute cardiovascular protective effect of fasudil in ischemia-reperfusion injury and models of myocardial and cerebral infarction was not associated with changes in eNOS expression but instead, was completely blocked by PI3-K and eNOS inhibitors. Conclusion: These findings indicate that the anti-inflammatory and anti-ischemic effects of Rho-kinase inhibition occur through 2 different mechanisms involving upregulation of eNOS expression and activity. These results suggest that Rho-kinase may be an important therapeutic target for cardiovascular protection.

10:00 a.m.

404-4 Dynamic Changes of Gene Expression in Hypoxia-Induced Right Ventricular Hypertrophy

Saumya Sharma, Heinrich Taegtmeyer, Julia Adrogue, Peter Razeghi, Shiraj Sen, Kholiswa Ngumbela, M. Faadiel Essop, University of Texas Houston Medical School, Houston, TX, University of Capetown Faculty of Medical Sciences, Capetown, South Africa

Background: Hypobaric hypoxia induces right ventricular hypertrophy. The relative contribution of pulmonary hypertension, decreased arterial oxygen, and neuroendocrine stimulation to the transcriptional profile of hypoxia-induced right ventricular hypertrophy is presently unknown. While both ventricles are exposed to hypoxia and neuroendocrine stimulation, only the right ventricle is exposed to increased load.**Methods:** Right and left ventricular tissue was evaluated microscopically to determine myocyte size and fibrosis. We measured the expression of candidate genes by quantitative RT-PCR in the right ventricle of rats exposed to hypobaric hypoxia (11% O_2) and compared the results with the left ventricle to determine the effect of load. **Results:** Hypobaric hypoxia induced right ventricular hypertrophy without fibrosis. In the right but not the left ventricle, ANF transcript levels increased at 7 days and continued to rise at 14 days. Metabolic genes such PPARalpha, PPARalpha-regulated genes, GLUT1 and GLUT4 were differentially regulated in a manner suggesting a substrate switch from fatty acid to glucose oxidation early during hypobaric hypoxia and a switch back to fatty acid oxidation at 14 days. There was also a dramatic switch in myosin isogene expression early in the course of hypobaric hypoxia, whereas later both myosin isoforms were upregulated. SERCA2a expression was downregulated early in the right ventricle, while at 14 days transcript levels were increased. When comparing right and left ventricular gene expression, the transcript levels of all genes, except for myosin isoforms and PDK-4 differed dramatically, suggesting that all these genes are regulated by load.**Conclusion:** Dynamic changes in gene expression occur in hypoxia-induced right ventricular hypertrophy, characterized by an early reactivation of the fetal transcriptional profile followed by a reversion to the "adult" pattern by 14 days. Furthermore, myosin iso-gene and PDK-4 expression is not affected by load suggesting that either hypoxia itself or neuroendocrine stimulation are the primary regulators of these genes