YOUNG INVESTIGATORS AWARDS COMPETITION

410 Young Investigators Awards: Molecular and Cellular Cardiology

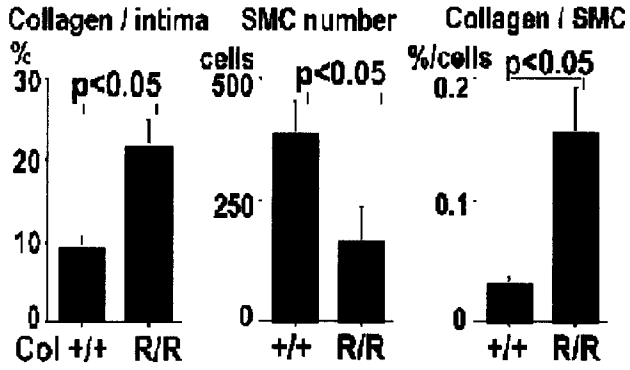
Monday, March 18, 2002, 2:00 p.m.-3:30 p.m.
Georgia World Congress Center, Room 257W

2:00 p.m.

410-1 Genetically Determined Resistance to Collagenase Action Alters Content of Collagen and Smooth Muscle Cells in Atheroma

Yoshihiro Fukumoto, Peter Libby, Elena Rabkin, Nerea Varo, Koichiro Harada, Christopher C. Hill, Patrick R. Lawler, Frederick J. Schoen, Stephen M. Krane, Masanori Aikawa, *Brigham and Women's Hospital, Boston, Massachusetts.*

We have hypothesized that proteolytic activity produced by activated macrophages contributes to instability of atheroma, a common trigger of thrombosis causing acute coronary syndromes such as unstable angina, or myocardial infarction. However, no direct evidence links collagenases with regulation of the collagen content of plaques in vivo. This study tested the hypothesis that collagenolysis critically influences the structure of atheroma in genetically-altered mice. We examined collagen accumulation in atheroma of apolipoprotein E-deficient mice (apoE-/-) expressing collagenase-resistant type I collagen (ColR/R, n=6) or wild-type type I collagen (Col+/+, n=5). Aortic atheroma of both groups contained similar amounts of macrophages expressing matrix metalloproteinase-13 (MMP-13/collagenase-3, the major collagenase in mice), as determined by quantitative color image analysis of immunohistochemically stained sections. Cultured peritoneal macrophages from both groups had similar levels of MMP-13/collagenase-3 mRNA and secreted protein. Atheroma of ColR/R/apoE-/- mice, however, contained significantly more interstitial collagen than those of Col+/+/apoE-/- mice (picrosirius red polarization). Atheroma from ColR/R/apoE-/- contained fewer smooth muscle cells (a major source of collagen in atheroma). These results show that macrophage-derived collagenase action influences a component of plaque structure critical to the clinical complications of atherosclerosis.



2:15 p.m.

410-2 A Novel Inhibitor of _Protein Kinase-C as a Therapeutic for Ischemic Reperfusion Injury in Acute Coronary Syndromes

Felix Lee, Koichi Inagaki, Leon Chen, Lynn Bailey, Fumi Ikeno, Jennifer Lyons, Donna Bouley, Paul Yock, Andrew Carter, Alan Yeung, Daria Mochly-Rosen, *Stanford University, Stanford, California.*

Objective: Although therapies for acute coronary syndromes (ACS) are directed toward timely reperfusion utilizing thrombolytic agents and mechanical interventions, no pharmacological therapies have been developed to protect the myocardium from cellular injury. Ischemic preconditioning (IP) is a natural cardioprotective process whereby short periods of ischemia enhance the tolerance of the heart to subsequent prolonged ischemia. Protein kinase-C (PKC) is thought to play a critical role in the mechanism of IP. We have recently developed isozyme-selective δPKC inhibitor (δV1-1) and previously demonstrated inhibition of δPKC is sufficient to reduce cellular injury in-vitro due to ischemia. Therefore, the ability to modulate δPKC activity in-vivo may allow us to invoke cardioprotection after an ischemic event has already occurred. Methods: Utilizing truncated HIV-1 Tat-protein (TAT) to mediate rapid and efficient transmembrane delivery of peptides, we examined the effect of local delivery of TAT-δV1-1 on protection and functional recovery from ischemic damage. Utilizing a Langendorff model of ischemic injury (20 min) in rat hearts (n=4), delivery of δV1-1 (50 nM) immediately after injury demonstrated improved LVDP (60+/-12% vs 16+/-4%, p<0.00003), reduced LVEDP (56+/-15 vs 107+/-8 mm Hg, p<0.01) and reduced CPK release (2170+/-492U vs 5568+/-446U, p<0.0005) compared to controls (n=4). Utilizing a clinically relevant model of acute porcine (n=4) coronary ischemia (30 minutes) using a balloon catheter inflated in the LAD artery followed by local delivery of δV1-1 (2.5 mM) during the final 10 minutes of ischemia preserved LV EF% 55+/-1% vs 43+/-2%, p<0.0005), reduced hypokinetic areas of the heart (10+/-1% vs 31+/-8%, p<0.02) and reduced infarct size (14%+/-5 vs 34%+/-3, p<0.008) by histomorphometry, versus controls (n=5). Conclusion: Myocardial function preservation and reduced infarct size can be effected by locally delivering a cardioprotective peptide (δV1-1) in a clinically relevant model, after the ischemic event.

2:30 p.m.

410-3 Isolation, Expansion, and Genetic Modification of Bone Marrow Mesenchymal Stem Cells for In-Vivo Repair of Damaged Myocardium

Abeel A. Mangi, Victor J. Dzau, *Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.*

Several groups have reported the use of unfractionated or cell sorted bone marrow derived cells for cardiac repair. The characterization, expansion and conditions for differentiation of these cells need further definition. Here, we describe the isolation, characterization, and propagation of CD34-/c-kit+ subpopulation of mesechymal stem cells derived from adult bone marrow which can differentiate into cardiac myocytes, and be transduced to stably express a reporter gene. These cells can induce a gain of cardiac function when transplanted into the myocardium damaged by ischemic injury. The mononuclear fraction of whole bone marrow from adult Sprague Dawley rats was separated by density centrifugation. Bone marrow stromal cells attached preferentially to uncoated plastic surfaces, and proliferated in mixed culture with hematopoietic cells (HCs) under standard conditions. MSCs were retrovirally transduced with green fluorescent protein (GFP) or Lac Z with over 80% transduction efficiency. MSCs express connexin 43 and c-kit (CD117) but do not express hematopoietic markers CD34, CD45, CD11b; or mature cardiac markers such as troponin, myosin heavy chain or desmin at this stage. MSCs can be separated from HCs by negative immuno-magnetic bead sorting, but cease to proliferate after cell sorting. In separate studies, we have shown that in the presence of 5-azacytidine, MSCs can differentiate into cardiac myocytes as evidenced by the presence of myocyte specific markers by immunostaining and RT-PCR. Lac Z transduced MSCs are injected into the border zone of the ischemic myocardium 60 minutes after ligation of the rat LAD. Two weeks later, the free wall and apex of the left ventricle exhibit extensive blue staining by beta-galactosidase staining, suggesting the presence of Lac-Z expressing cells. Echocardiographic analysis reveals a statistically significant 54% increase in fractional shortening when compared to control, and a 34% increase in ejection fraction. This strategy suggests that bone marrow derived MSCs can be expanded to sufficient scale ex vivo, and genetically engineered to treat damaged myocardium.

2:45 p.m.

410-4 Mesenchymal Stem Cell Injection Induces Cardiac Nerve Sprouting and Tenascin Expression in a Swine Model of Myocardial Infarction

Hui-Nam Pak, Mohammed Qayyum, Angela C. Lai, Dave T. Kim, Akira Hamabe, Yasushi Miyauchi, Michael C. Lill, Kaname Takizawa, Michael C. Fishbein, Behrooz G. Sharifi, Peng-Sheng Chen, Raj Makkar, *Cedars-Sinai Medical Center, Los Angeles, California, UCLA, Los Angeles, California.*

Background - Mesenchymal Stem Cell (MSC) injection into the myocardium is a promising technique to improve cardiac hemodynamic performance. Whether or not MSC injection can result in cardiac nerve sprouting in unclear.

Methods and Results - We created anterior wall myocardial infarction by intraluminal micro-coil embolization of distal left anterior descending coronary artery in 16 swine. One month later, 6 swine were given MSC and fresh bone marrow (BM) into infarcted myocardium (MSC group). Four swine were given fresh bone marrow only (BM group) and 6 swine were given culture media (MI-only group). The swine were sacrificed 95.8±3.5 days after MI. Six normal swine were used as control. Atrial and ventricular tissues were sampled for immunocytochemical studies using antibodies against growth-associated protein 43 (GAP43), tyrosine hydroxylase (TH) and 3 subtypes of tenascin (TnR, C and X). Five fields per slide were counted for nerve density. The results show 1) there were more GAP43-positive nerves in MSC group (117.4±54.9/mm^2) than in BM group (42.7±22.2/mm^2), MI-only group (56.0±35.8/mm^2), or Control (24.3±25.6/mm^2) (p<0.0001). TH staining also showed higher nerve densities in MSC group (26.9±27.2/mm^2) than in MI-only group (15.7±17.3/mm^2, p<0.05) or Control (2.6±1.1/mm^2, p<0.0001). 2) There were more sympathetic (TH positive) nerves in myocardium distant from infarct than in the peri-infarct area (17.0±18.3/mm^2 vs 6.0±4.9/mm^2, p<0.05). 3) Optical intensity and color analyses using Image-Pro plus 4.0 software showed a significantly higher TnR and TnC expression in MSC group or BM group than in MI-only group or Control (p<0.001).

Conclusions - MSC injection into one-month old myocardial infarct results in over expression of cardiac TnR, TnC, and significant cardiac nerve sprouting, resulting in sympathetic hyperinnervation.

3:00 p.m.

410-5 Familial Restrictive Cardiomyopathy Caused by a Missense Mutation in the Desmin Gene: Possible Role of Apoptosis in Disease Pathogenesis

Zeev Perles, Neil E. Bowles, Matteo Vatta, Shinawe Jimenez, Jacqueline Szmuszkovicz, Yassemi Capetanaki, Jeffrey A. Towbin, *Baylor College of Medicine, Houston, Texas, Children's Hospital of Los Angeles, Los Angeles, California.*

Background: RCM is characterized by diastolic ventricular dysfunction with preservation of systolic function and left ventricular end-diastolic dimension and thickness. Amyloid and desmin accumulation are common. Mutations in *transthyretin* have been shown to cause inherited forms of amyloidosis associated with cardiomyopathy, while mutations in the *desmin* gene have been found in patients with RCM or dilated cardiomyopathy.
Methods: The *desmin* and *transthyretin* genes of patients with isolated RCM (2 families and 11 patients with sporadic disease) were screened for mutations by denaturing high performance liquid chromatography and DNA sequencing. Complementary DNA clones