TARGET: Do Tirofiban And ReoPro Give Similar Efficacy Trial?

Eric J. Topol et al

There is considerable debate about the choice of intravenous platelet glycoprotein IIb/IIIa inhibitors for percutaneous coronary intervention, after a meta-analysis of 7 trials and 16,771 patients there has shown a 38% reduction in death or non-fatal MI 30 days after the index procedure. At 149 hospitals in 18 countries throughout North America, Europe and Australia, 4810 patients were randomized between Tirofiban and 2251 patients were treated with TPA bolus and 0.125 mcg/kg/min infusion. Patients qualified by history, physical exam, and diagnostic angiography for "intent-to-treat" lesions addressed by percutaneous revascularization, and were not with evolving ST-segment elevation MI or with urea clearance > 25 mg/dl. The primary endpoint is death or non-fatal MI and the trial has 30% power to determine non-inferiority for tirofiban with an expected event rate in the control (abciximab) group of 5.3% based on the EPISTENT trial. The primary endpoint data will be presented along with the key subgroups such as diabetics. Follow-up data for the trial to 1 year will also be performed.

Late Breaking Science: Linking Genes to Function in the Heart and Vasculature

BASIC ABSTRACTS

Exogenous Hematopoietic Stem Cells Can Regenerate Infarcted Myocardium

Donald Orlic, Jan Kajstura, Stefano Cimini, Baoosheng Li, Stacie Anderson, David Bodine, James Pickel, Annarosa Nardozza, Bernardin-Giraud, Pierre Anversa, New York Medical College, Valhalla, NY. NIH/NIH, Bethesda, MD

To determine whether hematopoietic stem cells (HSC) can transform into cardiomyocytes with the potential to repair dead myocardium after infarction. Lin-koHSC were harvested from transgenic mice expressing green fluorescent protein (GFP) and injected in the region bordering the infarct, 3–5 hours after coronary artery occlusion in mice. A band of closely packed cells was identified at 17 days in nearly 50% of the hearts, between the endocardial and epicardial surface of the infarcted ventricle. This band occupied 50% of the damaged portion of the wall. c-ko/GFP positive HSC were found in the infarcted area shortly after coronary ligation and were still detectable at 7 days. c-ko stained HSC were not labeled by markers of myocytes, α-sarcomeric actin and myosin, endothelial cells, factor VIII, and smooth muscle cells, smooth muscle actin. The band of tissue included in the infarcted zone was constituted 75% by GFP, α-sarcomeric actin, myosin and α-actin positive cardiac muscle cells. Other GFP-positive cell populations were endothelial cells and smooth muscle cells, organized in nascent capillary structures. These results demonstrate that HSC can differentiate into cells with partially aligned myofibrils and resembled late fetal-neonatal cells. GFP-positive replicating myocytes, endothelial cells and smooth muscle cells were c-ko negative. Infarcted mice were injected with BrdU, once a day for 4 days, to establish the extent of cell proliferation in the regenerating myocardium. 28% myocytes, 17% endothelial cells and 12% smooth muscle cells were BrdU positive. These three levels of BrdU labeling were statistically different. Additionally, the percentages of cells positive to Ki67 were measured to evaluate the fraction of cycling cells at this stage of repair: 18% myocytes, 10% endothelial cells and 8% smooth muscle cells were in G1, S, G2. In conclusion, HSC, when injected in the heart, rapidly differentiate into myocardium resulting in significant recovery of muscle mass after infarction.

Mutations in the R1α Regulatory Subunit of Protein Kinase A Cause Familial Cardiac Myxomas

Carl J. Vaughan, Minaed Case, Jie He, Cathy Hatcher, Jordan M. Winter, Stanislawa S-C, Bowles KR, Vatta M, Zintz C, Bowles NE, Pediatric Cardiology, BayCom College of Medicine, Houston, TX

Functional Proteomic Analysis of Protein Kinase C ε Signaling Complexes in Preconditioning

Peipei Ping, Jun Zhang, Roberto Boll, Cardiology Division, University of Louisville, KY.

Studies from our laboratory and others have shown that transgenic (Tg) mice expressing low levels of active protein kinase C ε (PKCε) exhibit resistance to ischemic injury, a cardioprotected phenotype analogous to that observed during preconditioning. Although PKCε has been shown to activate multiple downstream targets in preconditioning, the molecular components of this PKCε-signaling complex and the role these signaling mechanisms play in preconditioning are unknown. We used a proteomic approach to characterize PKCε-signaling complexes. PKCε monomeric antibodies were used to immunoprecipitate cardiac tissues from PKCε Tg mice and wild type mice (n=10 each). Combining 2-D electrophoresis, MALDI mass spectrometry, and immunoblotting, so far we have identified 27 known and 12 unknown molecules in PKCε-signaling complexes. These include signaling proteins (RAKKO, Lck, Src, PKCδ, PKCε, PKb g(170/p65), p38 MAPK, p54/p46 JNKs, ERKs, Hsp27/Hsp71, α-crystallin, INOS, eNOS), and structural proteins (cardiac α-actin, troponin T, α-tropomyosin, prohibitin, desmin, Lap2, caveolin-3). Many of these proteins were not previously suspected to be in PKCε-immuno complexes. In PKCε Tg mice, altered expression and post-translational modification were evident in 33 known and 12 characterized molecules. These data show, for the first time, (i) that PKCe forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore-unrecognized functions of PKCe isoforms; and (ii) that cardioprotection is coupled with dynamic modulation of PKCe-associated proteins and recruitment of signaling molecules to PKCε complexes. Functional proteomic analysis of PKCe signaling complexes is a crucial step toward understanding PKCe-dependent signaling architecture and cardioprotection.

Mutations in the Human δ-Sarcoglycan Gene in Familial and Sporadic Dilated Cardiomyopathy, a Disease of the Cytoskeleton and Sarcolemma

Tsubata S, Bowles KR, Vatta M, Zintz C, Bowles NE, Towbin JA. Pediatric Cardiology, BayCom College of Medicine, Houston, TX

Background: Dilated cardiomyopathy (DCM) is a significant cause of morbidity and mortality due to congestive heart failure and rhythm abnormalities. Approximately 30% of cases are familial and the precise genetic cause is currently specified. The ranges of cardiac phenotypes in these disorders are large. Most mutations reported in DCM are as the most common form of DCM, although X-linked disease is also well described. Two genes have been identified for the X-linked forms (dystrophin and tafazzin), whereas three genes have been identified in autosomal dominant DCM (actin, lamin A/C, desmin). We have hypothesized that DCM is a disease of the cytoskeleton and sarcolemma and have focused our studies on candidate genes whose products are found in these structures. Here we report the screening of δ-sarcoglycan, a member of the dystrophin-associated protein complex (DAPC). Methods: Blood was drawn and DNA extracted from one 4-generation family and 50 sporadic cases of DCM after informed consent. Myocardial samples were obtained after transplantation or autopsy. The δ-sarcoglycan gene was screened for mutations using single strand conformation polymorphism (SSCP), denaturation gradient gel electrophoresis, liquid chromatography-mass spectrometry (LC-MS) and DNA sequencing. Structural protein analysis and immunohistochemistry were performed. Results: Mutation analysis of the DCM pedigree identified a single nucleotide change in exon 8 of δ-sarcoglycan causing an amino acid change from a polar (serine) to nonpolar amino acid (alanine) altering the protein secondary structure. In 2 of the 50 sporadic cases, a 3bp deletion in exon 9, which deletes lysine at position 236, occurred. Neither the missense mutation nor deletion mutation was seen in 200 control patients. Immunohistochemistry demonstrated significant reduction of δ-sarcoglycan staining. Conclusions: Mutation of the δ-sarcoglycan gene causes autosomal dominant DCM. As mutations of this gene are also known to cause the Syrian hamster cardiomyopathy as well as human limb girdle muscular dystrophy, it appears that mutations in this gene place patients at risk for a spectrum of clinical features ranging from cardiomyopathy to skeletal myopathy. This is similar to other DAPC members and dystrophin itself, supporting our common pathway hypothesis which suggests that DCM results from disruption of the cytoskeleton/sarcolemma.

Dual Modulation of Cell Survival and Cell Death by β2-Adrenergic Gi and Gs Signaling in Adult Mouse Cardiac Myocytes

Wei-Zhong Zhu, Ming Zheng, Brian Kobikita, Rui-Ping Xiao, Laboratory of Cardiovascular Science, GRC, MA, NIH, Baltimore, N4D 21224;*Howard Hughes Medical Institute, Stanford Univ Med Cent, Stanford, CA 94305.

Cardiac β2-AR activates both Gi and Gi proteins whereas β1-AR couples only to Gi. The goal of this study is to determine whether β1-AR and β2-AR differ in regulating cardiomyocyte survival and apoptosis, if so, to explore underlying mechanisms. To avoid complicated crosstalks between β-AR subtypes, we express β1-AR or β2-AR individually in the null background (β1-AR and β2-AR double knockdown) mouse cardiomyocytes. Using adult mouse myocyte culture and adenovirus gene transfer techniques. Stimulation of β1-AR, but not β2-AR, markedly induces myocyte apoptosis, as indicated by increased TUNEL or Hoechst staining positive cells and DNA fragmentation. Inhibition Gi signaling with pertussis toxin converts β2-AR to β1-AR in terms of its apoptotic effect, suggesting that Gi is essential for β2-AR-mediated cell survival. To explore the downstream signaling events of β2-AR-coupled Gi, we first examined the possible involvement of PI3K-Akt pathway. The strikingly different effects of β2-AR, on Akt activation, also abolish PI3K-Akt pathway. The strikingly different effects of β-AR subtypes on cardiac cell survival and apoptosis may have important pathophysiological and therapeutic implications in chronic heart failure.