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 16,770 patients 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 12/30/99 and 8/25/00. Patients were randomized to either tirofiban or abciximab on a double-blind, double dummy basis. Clopidogrel and aspirin were administered pre-procedurally, along with a 70 U/kg intravenous heparin bolus. The dose of tirofiban was 10 mcg/kg/min infusion for 18–24 hr; for abciximab it was 0.25 mcg/kg bolus followed by 12 mcg/kg/min infusion. Patients qualified by having classic anatomy for “intent-to-stem” lesions addressed by percutaneous revascularization, and were not with evolving ST-elevation MI or with serum creatinine >2.5 mg/dl. The primary endpoint is 30 day or non-fatal MI and the trial has 80% power to determine non-inferiority for tirofiban with an expected event rate in the control (abciximab) group of 5.3% based on the EPIS TENT 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 Campesi, Baosheng Li, Stacie Anderson, David Bodine, James Pickel, Annarosa Papale, Bernardo Nadal-Ginard, Piero Anversa, New York Medical College, Valhalla, NY. NHLBI/NIH, Bethesda, MD.

To determine whether hematopoietic stem cells (HSC) can transform into cardiomyocytes with the potential to repair dead myocardium after infarction, Livi-in-Ki67 HSC 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 densely packed cells was identified at 17 days post-infarction in nearly 50% of HSC in the borders, between the endocardial and epicardial surface of the injured ventricle. This band occupied 50% of the damaged portion of the wall. c-kit/GFP positive HSC were found in the infarcted area shortly after coronary ligation and were still detectable at 7 days. c-kit 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 injured zone was constituted 75% by GFP, α-sarcomeric actin, myosin and α-actinin positive cardiac muscle cells. Other GFP-positive cell populations were endothelial cells and smooth muscle cells, organized in nascent capillary structures and arterioles. Proliferating myocytes were partially aligned myofibrils and resembled late fetal-neonatal cells. GFP-positive replicating myocytes, endothelial cells and smooth muscle cells were c-kit 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 positive to Ki67 were measured to evaluate the fraction of cycling cells in these sick hearts: 15% myocytes, 10% endothelial cells and 8% smooth muscle cells were in G1, G2,M. 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
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Cardiac myxomas arise from primitive pluripotent mesenchymal cells within the subendocardium. In autosomal dominant Carney complex, intracardiac myxomas develop in the setting of hyperplasia, pigmentation, and endocrine overactivity. We previously localized the Carney complex gene defect to human chromosome 17q24. We now demonstrate that mutations in the chromosome 17 PKRAFTα gene encoding the R1α regulatory subunit of cAMP-dependent protein kinase A (PKA) cause inherited cardiac myxomas and Carney complex. We detect heterozygous deletions in the PKRAFTα gene in three unrelated kindreds (J Steroid Biochem 80B in Family YA, J Steroid Biochem 85 in Family YB, J Steroid Biochem 163 in Family YF). These mutations were confirmed by bidirectional sequence analysis and by denaturing acrylamide gel electrophoresis. Each mutant produces a premature stop codon with consequent shift in reading frame and deletion mutation was seen in 200 control patients. Immunohistochemistry demonstrated the most common form of FDCM, 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 FDCM (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 α-sarcoglycan, a member of the dystrophin-associated protein complex (DAPC).

Peipei Ping, Jun Zhang, Roberto Bolli, 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 (PKC-ε) exhibit resistance to ischemic injury, a cardioprotected phenotype analogous to that observed during preconditioning. Although PKC-ε has been shown to activate multiple downstream targets in preconditioing, the molecular components that PKC-ε signaling complex is responsible for those cardioprotective effects are unknown. We used a proteomic approach to characterize PKC-ε signaling complexes. PKC-ε monomeric antibodies were used to immunoprecipitate cardiac tissues from PKC-ε TG mice and wild type mice (n=10 each). Combining 2-DE electrophoresis, MALDI mass spectrometry, and immunoblotting, so far we have identified 27 known and 12 unknown molecules in PKC-ε signaling complexes. These include signaling proteins (RACK-2, Lck, Src, PKCε, PI3 kinase (p170/p85), p38 MAPK, p54/p46 JNKs, ERKs, Hsp27/Hsp71, α-vactinlin, INOS, eNOS), and structural proteins (cardiac α-actin, troponin T, α-tropomyosin, prohibin, desmin, Lap2, caveolin-3). Many of these proteins were not previously suspected to be in PKC-ε, immunocomplexes. In PKC-ε TG mice, altered expression and post-translational modification were evident for 21 known and 11 previously uncharacterized molecules. These data show, for the first time, that PKC-ε forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore-unrecognized functions of PKC-ε isoform; and (ii) that cardioprotection is coupled with dynamic modulation of PKC-ε-associated proteins and recruitment of signaling molecules to PKC-ε complexes. Functional proteomic analysis of PKC-ε signaling complexes is a crucial step toward understanding PKC-ε-dependent signaling architecture and cardioprotection.

Mutations in the Human α-Sarcoglycan Gene in Familial and Sporadic Dilated Cardiomyopathy, a Disease of the Cytoskeleton and Sarcolemma
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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, with the remainder sporadic. Autosomal dominant DCM has been linked to mutations in the α-sarcoglycan gene in at least 21 known and 10 uncharacterized molecules. These data show, for the first time, that PKC-ε forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore-unrecognized functions of PKC-ε isoform; and (ii) that cardioprotection is coupled with dynamic modulation of PKC-ε-associated proteins and recruitment of signaling molecules to PKC-ε complexes. Functional proteomic analysis of PKC-ε signaling complexes is a crucial step toward understanding PKC-ε-dependent signaling architecture and cardioprotection.

Modulation of Cell Survival and Cell Death by β2-Adrenergic Gi and Gs Signaling in Adult Mouse Cardiac Myocytes
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Cardiac β2-AR activates both Gs 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. We show that β2-AR couples more robustly to cardiac myocytes, using adult mouse myocyte culture and adenoviral 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 survival effects. To explore the downstream signaling events of β2-AR-coupled Gi, we first examined the possible involvement of p38 MAPK, since recent studies propose that p38 MAPK underlies Gi-dependent anti-apoptotic effects. We found that although stimulation of either β1-AR subtype increases p38 MAPK activity, this effect is insensitive to PTX, excluding a role of p38 MAPK in β2-AR-mediated cell-survival. In contrast, β2-AR (but not β1-AR) elevates the activity of Akt, a powerful survival signal, this effect is fully abolished by inhibiting Gi with pertussis toxin, scavenging Gβγ with JAK1-κRα, or blocking PI3K with LY294002, indicating that β2-AR activates Akt via Gβγ-Pi3K pathway. Most importantly, inhibition of the Gβγ-Pi3K-Akt pathway converts β2-AR signaling from survival to apoptotic. Thus, β2-AR, unlike β1-AR, activates concurrent apoptotic and survival signals in cardiomyocytes, and the survival effect is mediated by the Gβγ-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.
Functional Proteomic Analysis of Protein Kinase C ε Signaling Complexes in Preconditioning.
Peipei Ping, Jun Zhang and Roberto Bolli

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