Abstracts

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Translation of Basic Insights Into Clinical Practice

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This annual meeting is the third for this premier research conference sponsored by the American Heart Association Council on Basic Cardiovascular Sciences, the world’s leading organization of cardiovascular scientists. The conference placed emphasis on bridging the gap between basic research and clinical practice, focusing on new concepts and mechanistic insights that have the potential to be translated into clinically-applicable therapies. The meeting featured both invited presentations and poster abstract presentations, with participants from more than 20 different countries.

Abstracts for the poster presentations are provided in this special online supplement available at http://circres.ahajournals.org.
P1

Functional Regeneration of the Canine Ventricles Using Adult Human Mesenchymal Stem Cells Committed In Vitro to a Cardiac Lineage

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We have developed a novel approach to rapidly (7 days) commit adult human mesenchymal stem cells (hMSCs) to a cardiac lineage. These cardiogenic cells express the following cardiac proteins: 1) alpha sarcomeric actin, 2) atrial natriuretic polypeptide, 3) cardiotoxin, 4) alpha myosin heavy chain, 5) Troponin T and 6) Cn2.2 (the alpha subunit of the L-type calcium channel). In some cells anti-alpha sarcomeric actin and anti- Troponin T immunostaining showed myocardial structure. L-type calcium currents of size roughly equivalent to those found in cardiac myocytes have been recorded in some of these cells. More than 50% of these cardiogenic cells stain positive for cyclin D1 in the nucleus indicating retention of the capacity to divide and multiply. We used our right ventricular defect model to test whether these cardiogenic cells can promote regeneration of mechanical function. Circulation 2006; 112:i4-14. In this model a full thickness portion of the right ventricle is removed and replaced with an acellular patch made of extracellular matrix from porcine urinary bladder (ECM). Recovery of mechanical function in the implant region is studied 8 weeks later by an optical technique with submillimeter spatial resolution called high density mapping (HDM). We compared cardiogenic cell seeded ECM (n=8) to EGM seeded with hMSCs that were not committed to the cardiac lineage (n=5). Systolic contraction was significantly improved in cardiogenic cell seeded patches (mean ± sem: 8.2 ± 1.0 vs. 3.7 ± 1.2, cardiogenic seeded vs. HMSC seeded scaffolds; p < 0.05), Regional stroke work was also significantly improved (mean ± sem: 6.7 ± 0.8 vs. 4.1 ± 0.5; cardiogenic seeded vs. HMSC seeded scaffolds; p < 0.05). In conclusion, we have developed a method for rapid commitment of hMSCs to a cardiac lineage that when delivered to the canine heart results in a doubling of systolic contraction and a more than 50% increase in regional stroke work compared to normal ECG analysis

P2

Characterization and Correction of the Cardiac Phenotype in a Mouse Model of Pompe Disease Using rAAV2/9-Mediated Gene Delivery

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The aim of this project is to develop a gene delivery approach to treat Pompe Disease, a form of muscular dystrophy caused by mutations in the alpha glucosidase gaa gene. Deficiency in GAA leads to glycogen accumulation resulting in cardio-respiratory failure in early onset patients within the first year of life. Here we present a characterization study of the cardiac phenotype in our mouse model of Pompe Disease (gaa-/-) using ECG, MRI and tissue assessment. By 2 weeks abnormal amounts of glycogen are observed in lysosomes of cardiac cells. At 3 months, ECG analysis detects a shortened PR interval (gaa-/-: 33.41 ±1.35 ms, control: 44.95 ±1.58 ms) mimicking the conduction phenotype observed in human patients. MRI analysis at 3 months shows a decrease in stroke volume SV (gaa-/-: 36.13 ±1.19 ul, control 51.84 ±3.59 ul), decrease in cardiac output CO (gaa-/-: 7.95 ±0.26 ml/min, control 11.40±7.97 ml/min) and a significant increase in myocardial mass (gaa-/-: 181.99 ±11.40 g, control: 140.79 ±5.12 g) by 12 months of age. We are currently performing PV loop procedure to determine EDPVR in these mice. Using this model of cardiac dysfunction, we are performing PV loop analysis. Characterization of the infarct area was determined by histopathology. Results: The Ees, which is the compliance of the left ventricle at end-systole, was significantly compromised with longer ischemic times when compared to normal controls. (Fig. 1) The Emax data which is an indication of compliance throughout the cardiac cycle paralleled these findings. Histological examination showed progressive damage with increased ischemic time as well as contraction bands classicall associated with reperfusion injury. Conclusions: This study is unique in that it follows functional outcome in vivo at 1 week after ischemia reperfusion injury. We have demonstrated that the optimal ischemic time in mice prior to reperfusion is 30 minutes. In hearts subjected to greater than 30 minutes of ischemia, the Ees and Emax resembled permanent LAD ligation. Although ischemia of 15 minutes does render a significant injury, it is not extensive enough to evaluate if cell therapy will have a significant effect from baseline. This murine model may provide a better translational approach to assess whether patients will benefit from cell therapy following PCI after an acute MI.

P3

Genetic and Genomic Approaches to Study Zebrafish Heart Regeneration

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Mammalian hearts cannot regenerate in response to tissue damage. In contrast, zebrafish hearts regenerate even when up to 20% of the ventricle is amputated. The mechanism of zebrafish heart regeneration is not understood. We analyzed two zebrafish fin-regeneration deficient mutants, no blastoma (nbl) and dorsal of blastoma (dor), to determine whether they have heart regeneration defects. nbl encodes heat shock protein 60 (HSP60) and fails to regenerate hearts. DNA synthesis in cardiomyocytes is decreased in nbl mutants. dor encodes a new family member of fibroblast growth factor, FGF20. No obvious heart regeneration defects were detected in dor mutants. This suggests that heart and fin regeneration are controlled by two partially overlapping set of genes. We decided to systematically characterize and compare heart and fin regeneration by transcriptional profiling with DNA microarrays. Distinct gene clusters were identified based on temporal expression patterns throughout a time course of heart and fin regeneration. Comparisons of gene expression profiles between heart and fin regeneration revealed a set of regeneration core genes as well as tissue specific factors. Further characterization focused on heart regeneration. Most proliferating cardiomyocytes are localized to the lateral sides of the wound. Thus, it is likely that initiation signals for post injury cardiomyocyte proliferation are secreted molecules from the wound. Several secreted molecules identified by microarray analysis are expressed around the wound site, suggesting that they play an important role in heart regeneration. With a set of candidate genes identified from our microarray analysis, we now can use reverse genetic and pharmacological approaches to study the functions of these genes.

P4

Murine Model of Ischemia Reperfusion for Myocardial Restoration with Cellular Transplantation

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Background: While encouraging success has been seen with cellular transplantation after permanent LAD ligation in mice, a model of ischemia reperfusion would better represent the clinical scenario of acute MI followed by percutaneous coronary intervention (PCI). Methods: Mice underwent temporary LAD ligation for 15, 30, 45, and 60 minutes followed by 1 week of reperfusion. The animals underwent assessment of their ventricular performance by pressure volume (PV) loop analysis. Characterization of the infarct area was determined by histopathology. Results: The Ees, which is the compliance of the left ventricle at end-systole, was significantly compromised with longer ischemic times when compared to normal controls. (Fig. 1) The Emax data which is an indication of compliance throughout the cardiac cycle paralleled these findings. Histological examination showed progressive damage with increased ischemic time as well as contraction bands classically associated with reperfusion injury. Conclusions: This study is unique in that it follows functional outcome in vivo at 1 week after ischemia reperfusion injury. We have demonstrated that the optimal ischemic time in mice prior to reperfusion is 30 minutes. In hearts subjected to greater than 30 minutes of ischemia, the Ees and Emax resembled permanent LAD ligation. Although ischemia of 15 minutes does render a significant injury, it is not extensive enough to evaluate if cell therapy will have a significant effect from baseline. This murine model may provide a better translational approach to assess whether patients will benefit from cell therapy following PCI after an acute MI.

P5

Congenital Heart Disease Has a High Incidence in Patients with Anophthalmia/Microphthalmia/Coloboma and May Predict the Presence of Other Malformations

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Introduction: Microphthalmia is a rare birth defect, which is on the spectrum of eye anomalies with Coloboma at the milder end and Anophthalmia at the most severe end. Collectively the eye defect is referred to as MAC, with an incidence of about 21000 births. Several genes that play a role in eye development, including PAX6, PA2X, SIX2, OTX2, CHX10, RX, SHH, and SIX3 have been identified. These genes are highly conserved through evolution and have been proven to cause MAC in animal models. Up to 25% of individuals with MAC have been found to have a cardiac defect. Using this information we use: Chromosomal studies, MRI of head and orbit, renal ultrasound, hearing evaluation, and Cardiology evaluation if a murmur is present. Methods: Data was obtained from the Anophthalmia/Microphthalmia Microphthalmia Registry at Albert Einstein Medical Center. Philadelphia which collects data about malformations associated with MAC. Sub-analysis was performed to evaluate if the presence of Congenital Heart Disease (CHD) could predict the presence of other malformations. Using SSPS software, cross-
tabulations with a two-sided Fisher’s Exact Test (FET) were performed to identify statistically significant differences. Results: Of 225 cases (110 males, 48.9%), 24 (10.7%) had CHD, an incidence significantly higher than the general population (≤1%). The most common malformations were VSD (11 pts. 4.9%) and ASO (6 pts. 2.7%). Interestingly, if CHD was present, there was a significantly higher incidence of other malformations (compared with patients with MAC without CHD) (40% vs. 25.7% vs. 2.5%, FET 0.005; 20% vs. 4.5%, FET 0.008, and 20.6% vs. 7%, FET 0.037), Gastrointestinal (25% vs. 5%, FET 0.003), Pulmonary (20.8% vs. 3.5%, FET 0.004), Vertebral (20.8% vs. 5%, FET 0.013), Cleft lip and palate (16.7% vs. 4%, FET 0.028). Conclusions: The incidence of CHD in patients with MAC is high, and it is associated with a significantly higher incidence of other malformations. Our data suggests that all patients require a Cardiology evaluation.

If CHD is present, then conditions associated with it should be evaluated early on, including X-rays of the chest and vertebrae. Further study of the genes involved in MAC may yield information useful to better characterize mendelian phenomeneons.

Tetrahydrobiopterin Reverses Established Heart Failure by Recoupling of Uncoupled Endothelial NOS
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Background: Pressure overload triggers eNOS as a prominent source of myocardial ROS that contribute to diastolic remodelling and cardiac dysfunction. Administration of tetrahydrobiopterin (BH4) can prevent pressure-load induced cardiac remodelling. The aim of this study was to investigate that BH4 can reverse established non-decompensated heart failure by interacting with the uncoupled eNOS, and on this way prevent the evolution to decompensated end-stage heart failure. Methods: Compensated cardiac remodelling was induced in 25 mice by transverse aortic constriction (TAC). After 4 weeks, mice were randomized to receive BH4 (5mg/d, n=10) or placebo (n=10) for the following 5 weeks. In addition, 5 mice underwent sham-surgery, and 5 mice were sacrificed after 4 weeks. Echocardiography was performed at baseline and after 4 and 8 weeks. Cold SD-OFF was performed to evaluate eNOS dimer/monomer. ROS generation was evaluated with dihydroethidium (DHE) confocal staining (score 1: absent; score 4: markedly present). Myocyte dimensions and fibrosis (score 1: absent; score 5: markedly present) were histologically evaluated.

Results: Administration of BH4 significantly reduced cardiac hypertrophy (myocyte dimensions: 20.32±0.6 μm without BH4 vs. 15.07±0.58 μm with BH4, p<0.001; left ventricular wall thickness resp. 1.11±0.03 mm and 0.91±0.04 mm with p<0.001; heart weight resp. 317±18.13 mg and 200.24±13.07mg with p<0.001; calculated LV mass resp. 221.49±87.72 and 164.18±5.99 with p<0.004) and fibrosis (score 4.6±0.6 without BH4 vs. 2.7±0.3 with BH4). BH4 administration prevented the evolution towards cardiac decompensation (fractional shortening: 20.28±4.82% with BH4, p<0.01). Superoxide generation was markedly reduced by BH4 (n=10 slices, score 4: without BH4 vs. score 2 with BH4). NOS uncoupling (increased eNOS uncoupled and fibrosis) was markedly reduced by BH4 (n=10 slices, score 4: without BH4 vs. score 2 with BH4). NOS uncoupling increased amount of eNOS monomers was markedly reduced by BH4. Conclusion: BH4 can reverse established cardiac remodelling by re-coupling uncoupled NO5 and as a consequence less ROS is generated, leading to less hypertrophy and an amelioration of cardiac function.

Withdrawn

Efficient Direct Delivery of Virus-Conjugated Genes in Mice Heart
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Virus conjugated gene delivery to the heart by direct injection holds promise as a therapeutic target for cardiovascular diseases. Aortic catheterization has proven less successful due to small size of the mice. In this study we present an efficient way to deliver lentivirus mediated gene, expressing enhanced green fluorescence protein (EGFP), in mice heart. Hearts were exposed through left thoracotomy under ventricle. Lenti-EGFP [10^9 PFU/ml] was injected directly at 3 different sites in the ventricle, 15μl each, using a 50 μl Hamilton syringe with a 30-G needle. Sham operated mice received 45 μl of PBS. After 7 d, transfection efficiency was determined in vivo by Xenogen fluorescence live imaging system. The fluorescence intensity (FI) of EGFP expressing cells in mice was quantified at different time points noninvasively. Data showed enhanced FI from the EGFP- transfected mice than the sham-operated controls. Transfection efficiency was further confirmed immunohistochemically after 2 w, which showed presence of green fluorescence in EGFP- transfected cardiomyocytes (Figure 1 A, B). Our results thus indicate that, in vivo mediated genes can be delivered successfully using this method; the genes persist for several weeks after delivery, thus allowing sufficient time for long term experimental protocols and transfection efficiency can be repeatedly checked noninvasively. Hence, this method could be used to stably express recombiant genes or siRNAs in cardiomyocytes in vivo and, as such, may represent an important vector system for myocardial gene therapy.

Single Cardiac Stem Cells Exhibit Mesenchymal Features and Require Stem Cell Antibiotic-1 to Proliferate in Adult Myocardium
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Several recent studies suggest the isolation of stem cells in adult heart; however, the cellular and molecular mechanisms underlie these cardiac muscle-derived stem cells are poorly understood in part due to the lack of definite molecular markers to identify the single cell. In this study, we report the prospective isolation of a novel hierarchy of cardiac stem cells from adult heart based on the distinct proliferative potential in single stem cell to form clonal floating colonies, using mixed culture of single-cell populations derived from the hearts of GFP transgenic and wild-type mice. A small number of individually identified single stem cells clonally proliferated to form spheres, that expressed typical mesenchymal markers, enriched side population cells for more than 200-fold, and were homogeneous with respect to CD45 (-), CD34 (-), and c-kit (-) but heterogeneous for stem cell antigen (Sca-1) expression. Cardiac stem cells formed replatable colonies that could proliferate in vitro over three months retaining high telomerase activity. To determine the essential role of Sca-1 in the adult heart, we targeted Sca-1 transcripts by overexpression of long Sca-1 double stranded RNA, having polyanion II transcriptional pausing to knockdown (KD) Sca-1. The degradation of Sca-1 protein and expression was confirmed in both bone marrow and the heart, with no overt developmental abnormalities at birth. However, cardiac stem cells isolated from Sca-1 KD mice exhibited similar number but smaller size of spheres formation with retarded stem cells self-renewal potential compared with wild-type littermates. These observations were confirmed by the loss of both telomerase activity and BrdU incorporation and relative p53 up-regulation in Sca-1 KD mice. In addition, cardiophoreses isolated from Sca-1 KD mice failed to respond to growth factors-induced Akt and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation. Although the cardiac differentiation potential was comparable in stem cells between KD and control mice, Sca-1 KD mice showed a significant cardiac remodeling after acute myocardial infarction. Thus, beyond the role as a cell surface marker for cardiac stem cells, Sca-1 may be a crucial regulator for stem cells self-renewal in the adult heart.

Identification of Cardioprotective and Cardiogeneative Proteins by Screening 3000 Secreted Proteins
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Background: Ischemic heart diseases are characterized by loss of functioning cardiomyocytes, which are critical for heart function. Current pharmaceutical agents do not address the fundamental issue of this cell loss. We sought to identify cardioprotective and cardiogeneative proteins from FivePrime’s comprehensive secreted protein library, which contains over 3000 secreted proteins, to develop protein therapeutics to promote cell survival and regeneration. Methods: The 3000 plus secreted proteins were produced in a mammalian protein expression system, and individual protein was applied to cell-based assays. The assays include a protection/survival assay in which we detect survival pathway pAKT (phospho-Akt), pERK, and pSTAT3 expression in rat neonatal cardiomyocytes, and a regeneration assay in which we observe adult mouse cardiac stem cell proliferation by cell cluster formation. Results: Twenty-seven proteins showed survival pathways activation, as confirmed by direct cell survival assays. Thirty-one proteins showed promotion of cardiac stem cell proliferation (Figure). Sixteen proteins showed dual effects. We are in the process of testing the top proteins in ischemic rodent models to determine if these proteins can preserve or improve cardiac function in vivo. Conclusion: The described screen is a powerful tool for comprehensive identification of the best cardioprotective and cardiregenerative proteins to develop protein therapeutics for treatment of ischemic heart diseases. Figure: Identification of secreted proteins that promote in vitro cardiac stem cells proliferation. Basal Medium: control. FPT032, FPT033 and FPT034: different proteins.
Cardiac Differentiation in Xenopus Requires the Cyclin-Dependent Kinase Inhibitor p27Xic1

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Cyclin-Dependent Kinase Inhibitors (CDKis) play a critical role in negatively regulating the proliferation of cardiomyocytes, although their role in cardiac differentiation remains largely undetermined. We have shown that the most prominent CDKI, p27Xic1, is expressed in the developing Xenopus heart and has gone on to investigate its role in cardiac differentiation. Microinjection of a translation blocking p27Xic1 morpholino into Xenopus embryos dose-dependently decreased the expression of markers of cardiac differentiation, cardiac Troponin I (Tc) and Nkx2.10, as determined by in situ hybridization. Following injection of 20ng of p27Xic1 morpholino, 44% of embryos (7/163) demonstrated a significant decrease or very significant decrease in the area expressing xNkx2.10 and xTc, compared to only 8% (13/154) of embryos injected with a specific control morpholino. Furthermore, this phenotype could be rescued in 80% (72/90) of embryos by co-injection of 30pg of p27Xic1 RNA construct, but not with 50pg of Gsp1 RNA construct (p27Xic1). These results indicate that p27Xic1 is specifically required for cardiac differentiation and we have further investigated whether this was dependent on its ability to arrest the cell cycle. Upon incubation of Xenopus embryos with Hydroxyurea and Aphidicolin, cell cycle arrest was induced as confirmed by a significant decrease in the number of BrdU positive cells (24–1–3–3); however, both Nk2.10 and Tc were expressed normally suggesting that cardiac differentiation can occur even when cell cycle is arrested. Furthermore, we have shown that full-length p27Xic1 (1–210), deletion mutant N-terminal (1–98), C-terminal (97–210) and (35–96)-p27Xic1 constructs can all arrest the cell cycle (significant decrease in the number of phH3 positive cells); however, only the full length (78%: 25/32) and the N-terminal (82%: 32/39) construct but NOT the C-terminal (51%: 18/35) or (35–96)-p27Xic1 (40%: 16/40) constructs could rescue the p27Xic1 morpholino phenotype (55%: 35/64). Hence, our data strongly suggests that the N-terminus, but not the C-terminus of p27Xic1 is important in Xenopus cardiac differentiation and this is distinct from its ability to arrest the cell cycle.

Cardiac Differentiation Capacity of Human Bone Marrow-Derived Stem Cells

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Introduction: Human bone marrow derived stem cells are believed to be a source of stem cells that are capable of regenerating an injured myocardium. Several clinical studies have demonstrated gross improvement of cardiac function following autologous bone marrow derived stem cell transplantation. There is however little evidence of acquisition of cardiac fate by these stem cells. In this study, we investigated the cardiac differentiation potential of human mononuclear cells and side population (SP) cells following Granulocyte-colony stimulation factor (G-CSF) mobilization. Methods: Athymic nude rats lacking T-cell mediated immunity were utilized to minimize rejection of the human cells. The rats underwent anterior wall cryo-injury by using a liquid nitrogen treated probe. C-CSF mobilized human cells were collected by apheresis and mononuclear cells were isolated by Ficoll gradient centrifugation. SP cells were isolated by flow cytometry based on their ability to efflux the hoescht33342 dye. Both human mononuclear and SP cells underwent DAPI staining to facilitate subsequent detection. The DAPI labeled human cells were delivered in the myocardial injury border zone directly following anterior wall injury (3–4 x 10^5 mononuclear cells or 1–1.4 x 10^5 SP cells). At different time periods, the animals were sacrificed and the DAPI labeled cells were isolated by flow cytometry following enzymatic digestion of the hearts. The SP cells underwent RNA isolation followed by RT-PCR analysis for markers of early cardiac differentiation. The mononuclear cells were fixed on glass slides and underwent immunohistochemical analysis by utilizing a cardiac specific antibody against α-actinin. Results: RT-PCR analysis of the SP cells retrieved from the myocardium indicates that adult human SP cells activate the early cardiac specific genes GATA-4 and NKX 2.5. Moreover, immunohistochemical analysis of the labeled mononuclear cells after recovery from the injured myocardium demonstrated positive expression of the cardiac specific protein α-actinin. Conclusion: Adult human bone marrow derived stem cells activate cardiac genetic programs and express cardiac specific genes following the delivery into the injured myocardium.

In Vitro Cardiogenic Potential of Human Myocardial and Marrow Stromal Cells

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Effort has been made to identify the most appropriate cell for cardiac repair. So far, the bone marrow is the predominant source for the collection of immature progenitors capable of regenerating the damaged myocardium. Importantly, the heart possesses a resident population of stem/progenitor cells that may be employed for the reconstitution of lost myocardium. The present study was designed to compare the growth properties of human cardiac and bone marrow primitive cells in vitro. For this purpose, myocardial fragments (H) and bone marrow specimens (BM) were obtained from 10 patients affected by severe aortic stenosis and a parallel analysis of the in vitro growth characteristics of cells from these organs performed. A similar exponential growth was observed in both cell cultures at multiple passages. The surface phenotype was studied by FAC analysis and immunocytochemistry. The mesenchymal anticytokeratin, CD44, CD73, CD100 and CD105, were detected in cultured H and BM cells. When receptors for SCF, HGF, VEGF and EGF were evaluated, cells positive for c-kit, c-met, Flk1 and CD44, CD73, CD90 and CD105, were detected in cultured H and BM cells. When receptors for SCF, HGF, VEGF and EGF were evaluated, cells positive for c-kit, c-met, Flk1 and c-erb-B2 were found in both cultures. Only 1% of H and BM cells expressed c-erb-B2 but 80% of H cells had already acquired the cardiac phenotype. Interestingly, the expression of c-erb-B2 on a subset of cells of both sources was observed. We are currently testing the biological outcome of agonistic and antagonistic binding of c-erb-B2 in terms of growth, survival and differentiation. Although the expression of GATA-4 and MEF2D was 4-fold higher in H cells, 4% of BM cells were positive for these cardiac transcription factors. Differentiation into myocytes, smooth muscle cells (SMCs) and endothelial cells (ECs) was observed in both cultures in the absence of serum or following the addition of dexamethasone. H cells generated a large proportion of cardiomyocytes than vascular cells whereas BM cells acquired predominantly the SMC and EC lineages. Importantly, co-culture of female H cells with male BM cells enhanced the differentiating ability of both cell types. Cardiomyocytes, ECs and ECs carrying XXY chromosomes were detected by FISH. In summary, resident cardiac progenitors and BM cells possess cardiogenic potential and may be implemented clinically for the treatment of the diseased failing heart.

In Vivo Labeling of Stem Cell Populations

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Introduction: Cell labeling strategies are important in defining the viability and fate of stem cells. While numerous cell labeling strategies are available for animal research, safety concerns limit their use in clinical studies. Feridex is an iron oxide based, FDA approved, MRI contrast agent. Protonate sulfate, an FDA approved heparin analogue, can be used with feridex (Fe-pro complex) to increase its labeling efficiency. We investigated the use of Fe-pro complex in labeling human stem cells. Methods: Mouse skeletal myoblasts C2C12 cells were utilized for in vitro studies evaluating the efficiency of cellular proliferation on loss of Fe-pro complex. We found that Fe-pro efficiently labeled C2C12 myoblasts and had no adverse effects on cellular viability, proliferation or differentiation capacity. To further examine the efficiency of labeling human stem cell populations we collected G-CSF mobilized mononuclear cells and labeled them overnight with Fe-pro complex. In vivo studies were conducted to determine the Fe-pro labeling efficiency of human stem cells in a rat myocardial cryo-injury model. Athymic nude rats lacking T-cell mediated immunity were utilized to minimize rejection. Fe-pro labeled human mononuclear cells were delivered to the border zone directly following anterior wall injury. Each rat received 3–4 x 10^5 Fe-pro labeled human mononuclear cells. Following myocardial injury, the hearts were evaluated using Prussian blue staining. Results: Compared to the intravenous FDA approved dose of feridex (380 µg/ml for a 70 kg patient), we effectively labeled both human mononuclear cells and C2C12 cells at 50 µg/ml of feridex. In addition, Fe-pro complex was efficiently identified at any of the protonate sulfate concentrations tested. Studies in C2C12 studies indicate that iron staining can be detected in 30% of cells after 9 doublings. Human Fe-Pro labeled mononuclear cells were detected in the myocardial injury border zone after two weeks of myocardial injury by prussian blue staining. Observation: Feridex-Protonate sulfite (Fe-pro) complex can be detected by Prussian blue staining of C2C12 cells for up to 9 cellular doublings and can be used to identify human mononuclear cells in vivo following delivery in the myocardial injury border region.

PRISM/PRDM6, a Transcriptional Repressor that Promotes the Proliferative Gene Program in Smooth Muscle Cells

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Smooth muscle cells (SMCs) display remarkable phenotypic diversity and plasticity and can readily switch between proliferative and differentiated states in response to extracellular cues. In an effort to identify novel transcriptional regulators of smooth muscle phenotypes, we compared the gene expression profiles of arterial and venous SMCs by microarray-based transcriptional profiling. Among genes that were differentially expressed in these two SMC types, we discovered an expressed sequence tag encoding a previously uncharacterized zinc finger protein belonging to the PRDM (PRDI-BF1 and RIZ homology domain) family of chromatin-remodeling proteins and named it PRISM (PR domain in smooth muscle). PRISM interacts with a variety of chromatin remodelling enzymes including the class I histone deacetylases, CBX1, EMT2, and p300, thereby identifying PRISM as a novel SMC-restricted epigenetic regulator. Overexpression of PRISM in cultured primary SMCs induces genes associated with the proliferative smooth muscle phenotype while repressing regulators of differentiation, including myocardin and GATA-4. Conversely, small interfering RNA-mediated knockdown of PRISM slows cell growth and induces myocardin, GATA-4, and markers of SMC differentiation. High percentage chimeras from targeted ES cells are currently being bred and we will allow for an in vivo analysis of PRISM function. Taken together, our data suggests that PRISM acts as a novel epigenetic regulator of SMC phenotypic plasticity by suppressing differentiation and maintaining the proliferative potential of vascular SMCs.
An Essential Role of Angiotensin II Type 1 Receptor in the Recipient Arterial Wall, Not in Bone Marrow, in the Pathogenesis of Atherosclerosis

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Background Angiotensin II type 1 receptor (AT1R) is expressed on multiple cell types in atherosclerotic lesions such as bone marrow (BM)-derived cells (leucocytes, progenitor cells) and non-BM intrinsic arterial wall cells (endothelial cells, smooth muscle cells and fibroblasts), which are concerned with cardiovascular diseases. Although pivotal roles of AT1R in atherogenesis have been shown in animals and humans treated with AT1R blocker as well as in ApoAtiR-KO mice, relative roles of AT1R in BM versus AT1R in non-BM-derived intrinsic host cells in the pathogenesis of atherosclerosis have not been addressed. Here, we investigated the role of AT1R in BM versus non-BM intrinsic arterial cells in atherogenesis.

Methods and Results A bone marrow transplantation technique (BMT) was used to create ApoA-KO mice with selective AT1R deficiency in BM cells (BM-AT1R-/- group) and those with selective AT1R deficiency in non-BM host cells (host AT1R-/- group). As control, BM-ApoE-/- and BM-ApoE-/- AT1R-/- groups, in which BM were transplanted from ApoE-/- and ApoA-PoE-/- AT1R-/- mice into ApoE-/- and ApoE-/- AT1R-/- mice, respectively, were generated. Angiotensin II (Ang II) was chronically infused from 10 to 14 weeks of age. En face oil red 0 staining and histopathological analysis of the aorta revealed that Ang II-induced increases in atherosclerotic plaque size were evident in control BM-ApoE-/- (1 ± 0.05 ± 5.2%) and BM-ApoE-/- mice (1 ± 0.3 ± 3%). In contrast, Ang II-induced atherosclerosis was blunted in host AT1R-/- mice (2 ± 2%, P<0.05 vs control) as well as in BM-ApoE-/- AT1R-/- mice (2 ± 2%, P=0.01 vs control). Ang II induction raised arterial pressure in BM-AT1R-/- and BM-ApoE-/- mice, whereas no significant increase in arterial pressure was noted in host AT1R-/- and BM-ApoE-/- AT1R-/- mice. There was no difference in serum lipid levels among groups with or without Ang II induction. Conclusion These results demonstrate an essential role of AT1R in host arterial wall in the pathogenesis of Ang II-induced atherosclerosis. The present study provides novel insights into Ang II-induced vascular pathology and suggests that AT1R-mediated signals in intrinsic endothelial and smooth muscle cells may play a principal role in Ang II-induced atherosclerosis.

VEGF Secretion by Skeletal Muscle-Derived Stem Cells Induces Angiogenesis, Prevents Remodeling, and Improves Function in Ischemic Hearts

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Cardiac cell therapy can improve the performance of ischemia-damaged hearts after myocardial infarction (MI); however, the mechanisms underlying this beneficial effect are not fully understood. We have previously shown that skeletal muscle-derived stem cells (MDSCs) injected into hearts after MI prevent adverse left ventricular (LV) remodeling and improve LV function more effectively than do committed skeletal myoblasts. We largely attributed this difference to the greater ability of MDSCs to induce infarct neovascularization, because very few donor-derived cells differentiated or fused to form new cardiomyocytes and blood vessels. In addition, we observed the donor myocytes in the MDSC-injected hearts to express vascular endothelial growth factor (VEGF) for up to 12 weeks. These results led us to hypothesize that the milieu within ischemic myocardium stimulates transplanted MDSCs to secrete angiogenic factors that induce neovascularization and ultimately improve cardiac function. To test this hypothesis, we subjected MDSCs to hypoxia and cyclic stretch, conditions likely found in ischemic myocardium. We observed a 6-fold and a 2-fold increase in VEGF secretion from MDSCs after 24 hours of hypoxia and cyclic stretch, respectively, when compared to normal culture conditions. To determine the therapeutic impact of cell-secreted VEGF, we used a gain- or loss-of-function approach based on MDSCs engineered to overexpress VEGF or soluble Flt1, a VEGF-specific antagonist. Intramyocardial transplantation of MDSCs overexpressing VEGF or control MDSCs significantly induced angiogenesis, prevented adverse cardiac remodeling, and improved function after MI compared to the saline-injected hearts (P<0.05). In contrast, no such beneficial effects occurred in hearts transplanted with MDSCs expressing soluble Flt1, which displayed no significant improvement in infarct vascularity and LV contractility when compared to control saline-injected hearts. These findings indicate that VEGF secretion by transplanted MDSCs is an important mechanism by which the cells induce neangiogenesis and functional recovery after MI. It is also tempting to speculate that such a mechanism could underlie the greater ability of MDSCs to induce infarct neovascularization, because very few donor-derived cells differentiated or fused to form new cardiomyocytes and blood vessels.

Modulation of the β-Adrenergic Stimulated Intracytoplasmic pDE5a Inhibition Is Blunted and Redox-Sensitive in Myocytes from Chronically Hypertrophied or Failing Heart

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Background We previously reported that inhibition of the cGMP-specific phospho-diesterase pDE5a suppresses β-adrenergic stimulated contractions in normal intact hearts and isolated myocytes. This effect was not observed, however, in a canine model of cardiac failure. Whether this loss of efficacy is intrinsic to the myocyte and/or β-receptor complex is unknown. Methods Adult murine cardiomyocytes were isolated from C57BL/6 mice subjected to 3 weeks of chronic transaortic constriction (TAC), and P3B3/N mice overexpressing activated Gαq. Cells were suspended in Tyrode's solution (1mM Ca2+) and field stimulated (0.5Hz, 25°C). Sarcromere shortening (SS) was assessed by real-time image analysis, Ca2+ transients by indo-1 fluorescence. Results. Myocytes from both TAC and Gαq hearts had normally normal unloaded shortening under rest conditions (3.55±0.03% for TAC, 3.31±0.2% for Gαq, S: 3.59±0.4% and 3.48±0.4% for controls, p=NS); however they exhibited a markedly reduced response to 10mM isoprotrol (ISOP) compared to genotype-specific controls: for example after ISOP infusion, the increase in SS was lower (p<0.01) in TAC in comparison of controls (48.2±2.5% vs 155.1±3.1%). The contraction of TAC and Gαq hearts from isoprotrol (ISOP) and Ca2+ transients in cells. Co-inhibition of the pDE5a inhibitors sildenafil (SL, 1mM, 10μM) blunted FSK inotropy in controls (-26.8% and -15.2% for C57B6L and P3B3/N, respectively, both P<0.01 vs FSK alone) without altering the Ca2+ transients. However, SL had less effect in TAC myocytes (-8.3%, P = 0.03 vs FSK and no impact in Gαq hearts. Pre-inoculation of TAC or Gαq cells with 4M Glutaredoxin (4M GSH), restored SL suppression of FSK responses to -22.6% and -10.3% for TAC and Gαq cells, respectively.
respectively (p<0.05 vs GSH-untreated cells). Conclusions: PDE5A modulation of β-adrenergic contractions is suppressed in myocytes from chronic hypertrophied or failing hearts. The effect is downstream of the β-receptor but involves cAMP-mediated inotropy. Oxidative stress contributes to this behavior, perhaps by diminishing cGMP synthesis via nitric oxide-soluble guanylate cyclase. Rescue by antioxidants may be a useful strategy to enhance effects of PDE5 inhibitors to blunt cardiac adrenergic stress.

PDE5A Inhibition Suppresses Maladaptive but Not Physiological Cardiac Hypertrophy
Thomas J Mulhern, IV, Hazim El-Haddad, Mobusher Mahmud, Milena Gebska, Michael Bridges, Oyadha Berda, Kathlyn Gabrielson, Anna R Hemoes, David A Kass, Hunter C Champion; Johns Hopkins Univ Sch of Med, Baltimore, MD
We have recently shown that PDE5A inhibition has potent antihypertrophic activity in models of in vitro cardiomyocyte hypertrophy and in vivo cardiac dysfunction. Therefore, we were interested in determining whether PDE5A inhibition would affect cardiac hypertrophy in vivo. Here we report on the effects of PDE5A inhibition in a mouse model of cardiac hypertrophy. By echocardiography, LV, and biochemical analysis. Afterload induced hypertrophy (TAC) and physiologic hypertrophy demonstrated an expected increase in cardiac mass (241± 9.7 mg (TAC) vs 152.0± 19.5 mg/swimming; P<0.05). TAC was associated with a reduced cardiac index, depressed systolic, diastolic function when weighed out vs this hypertrophy demonstrated improved cardiac function in all parameters. Sidellin marked improved LV function and reduced heart size in TAC (136± 6.0 mg, P<0.05 compared to TAC) mice but did not alter function or heart size in swimming mice (153.9± 21.3 mg). Swimming mice, while exhibiting significant hypertrophy, did not show increased superoxide activity/staining (by DHE fluorescence) or hydrogen peroxide staining. No differences were observed in the amount of increased NO activity when compared to TAC mice and reduced baseline PDE5A activity when compared to TAC. In summary, physiologic hypertrophy is a beneficial adaptive hypertrophy while TAC is associated with maladaptive hypertrophy and results in the progression to heart failure. Sidellin selectively blocks maladaptive hypertrophy without an effect on physiologic hypertrophy by swimming. Interestingly, both TAC-induced and exercise-induced hypertrophy were both associated with an increase in P38/akt signaling. Sidellin treatment reduced the expression of p-akt in association with reduced hypertrophy, but did not alter the expression of p-akt in exercise-induced hypertrophy. These findings may reflect differences in special localization of Akt and or PDE5A signaling in the heart.

Discovery of Cardiac Marker Expression in Human Embryonic Stem Cells by Gene Expression Profiling
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Background: Despite the huge potential of human embryonic stem (hES) cells, cardiomyocyte availability has been limited. Identifying early cardiac markers on undifferentiated hES cells can be used for improving the efficiency of cardiomyocyte derivation protocols, culture enrichment and isolation of adequate amounts of cardiomyocytes to generate new heart muscle.

We aimed to test the hypothesis that hES cells might express cardiac-specific marker(s) that could be used for cardiomyocyte isolation and drug screening. To this end, hES cells were cultured under different conditions at day 4, the day of POI, and the day of P0. RT-PCR analysis was performed for 381 genes. The expression of 10 genes was confirmed by quantitative real-time PCR. Three of the genes were further analyzed by immunofluorescence and Western blotting. The results indicate that the expression of markers of the cardiac lineage is under the control of the culture conditions and the day of PS. Therefore, the culture conditions can be adjusted in order to isolate cardiomyocytes expressing markers of the cardiac lineage.

Apo E Genotypes Modulate the Plasma Lipid Response to Atorvastatin in Indian Patients with Coronary Artery Disease
Madhu Khullar, Taranjit S Rai, Anil Grover, Rajesh Vijayverghia; Post Graduate Inst of Med Education and Res, Chandigarh, India
Considerable variability exists in the plasma lipid and lipoprotein response to statin treatment due, in part, to genetic factors. The gene for apolipoprotein E (ApoE) is polymorphic and the different genotypes modulate plasma lipids to various extents. The objective of the present study was to evaluate the effect of the apoE genotypes on the lipid response to atorvastatin treatment in patients with coronary artery disease (CAD), followed-up in cardiology clinic at Post Graduate Institute of Medical education and Research, India. Subjects and methods. 100 patients who were clinically confirmed with CAD and treated according to NCEP-ATPIII guidelines were included in the study. Plasma lipids were measured before and after 16 weeks of treatment with 10 mg /day of atorvastatin. Polymorphisms of ApoE were determined by restriction fragment length polymorphism (RFLP). RESULTS. ApoE genotype distributions were 4.28% with epsilon 2/2, 3.5% with epsilon 2/3, 17.41% with epsilon3/3 and 30.0% with epsilon 2/4, 21.4% with epsilon 3/3 and 23.57% with epsilon 4/4 respectively. Apo E allele frequencies for epsilon 2,3 & 4 were 0.21, 0.296 and 0.492 respectively in these patients. ApoE genotype did not have any effect on baseline lipid levels after adjustment for age, gender and body mass index (BMI) (P>0.05). The reduction in total cholesterol level was significantly higher in patients carrying epsilon 4 allele than in those with epsilon 2 or 3 alleles following treatment (-28.4% vs. -13.1%, P< 0.05). Compared with patients carrying epsilon 3 or epsilon 4 allele, those with epsilon 2 allele showed a significantly higher percentage reduction of LDL-C level after treatment (-24.0% vs. -9.7%, P<0.05). CONCLUSION. The plasma lipid level decrease was significantly more prominent in patients carrying epsilon 4 allele, and those with epsilon 2 allele showed a significantly higher percentage reduction in LDL-C level after treatment. Apo E gene polymorphism appears to influence the response to atorvastatin in Indian patients with CAD.

Transcriptional Profiling of Nicotinic Acetylcholine Receptors-Mediated Endothelial Cell Migration
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Gating cation channels. We have previously reported that nicotine, like VEGF and FGF has
angiogenic activity. The effect can be abolished by nAChR antagonists. In this study we examined the contribution of genes encoding different nAChR isoforms, as well as downstream genes, in nicotine-induced EC migration. Methods: Total RNA and protein from HMVECs was subjected to RT-PCR and Western blotting analysis. EC migration and cell proliferation, key events in angiogenesis, were assessed by standard wound and MIT assays. Thioredoxin activity was measured by thioredoxin assay. HMVEC gene expression profiling was performed using cDNA microarrays (Stanford, 43,000 elements) after exposure to vehicle or angiogenic factor for 2, 6, and 24 hours. Results: We detected mRNA transcripts for multiple nAChR subunits. Subunit expression was confirmed by Western blotting. To determine the role of different isoforms as functional nAChR receptors, we used siRNA technology to suppress the expression of each receptor subunit. Knockdown of nAChR α4, α7 subunits fully abolished NIC-induced migration, whereas scrambled RNA and nAChR β subunits (β2, β3, β4) had no effect. Furthermore, we found that nicotine, VEGF and FGF induce HMVECs migration. This activity is accompanied by increased thioredoxin activity. In parallel, message for TXNIP, a key inhibitor of thioredoxin activity, was downregulated by all three angiogenic agents. Heat maps of transcriptional profiles of stimulated EC revealed a large array of genes downstream of nAChR activation that are shared with VEGF or FGF treated cells. A subset of these genes have not previously described to be involved in angiogenesis. Knockdown of several of these commonly regulated genes abolished EC migration. Conclusion: Nicotine promotes angiogenesis via stimulation of nAChR-dependent endothelial cell migration. Furthermore, activation of the EC nAChR induces a transcriptional profile similar to those induced by other angiogenic cytokines. Analysis of transcriptional overlap among angiogenic cytokines reveals genes not previously considered to be involved in angiogenesis.

P29 Genetic Ablation of Bak Protects Against Myocardial Ischemia-Reperfusion Injury

John W Elrod, Weimin Fu, Mark R Duranso, Richard N Kitios, David J Lefer; Albert Einstein College of Medicine, Bronx, NY

Background: Bak is a multidomain, proapoptotic Bcl-2 protein, which together with Bax, controls access of upstream death signals to the mitochondria. Bak is highly expressed in cardiac myocytes. We investigated the effects of genetic ablation of bak in an in vitro murine model of myocardial ischemia-reperfusion (I-R). Methods: bak-/- mice (n = 7) mice and wild-type (WT) littermates (n = 6) were subjected to 30 min left coronary artery occlusion and 72 h reperfusion. Left ventricular (LV) dimensions and function were assessed utilizing high-resolution echocardiography at baseline and post myocardial infarction. Area-at-risk (AAR) and infarct size (IS) were calculated as percentage of LV mass. AAR and LV were subjected to RT-PCR and Western blotting analysis. EC migration and cell proliferation, key events in angiogenesis were assessed by standard wound and MIT assays. Results: bak-/- mice displayed a 53% reduction (p < 0.01, vs. WT) in myocardial infarct size per area-at-risk (AAR/LV) (27.25 ± 4.36%) as compared to wild-type littermates, which displayed an AAR/LV of (58.08 ± 2.56%). In correlation, INFLV was also reduced in bak-/- mice by 50% (p < 0.001) compared to wild-type littermates. AAR/LV was similar in both groups. Echocardiography at baseline revealed no significant differences in LV dimensions or function between WT and bak-/- mice. Following myocardial infarction bak-/- mice displayed significantly decreased LV dilation (LV end-diastolic dimension and LV end-systolic dimension) (p < 0.01 vs. WT mice). Conclusions: This is the first evidence that genetic deletion of bak decreases myocardial infarct size and lessens left ventricular dilatation following I-R injury. Thus, despite the redundancy between Bak and Bax in many systems, Bak plays a critical function in the pathogenesis of myocardial I-R injury.

P30 Myocardial Infarct Size

Bcl-2 Plays an Essential Role in Attenuating Ischemia-Reperfusion Injury in the Naïve Myocardium and Modulates Mitochondrial Permeability Transition

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A role for Bcl-2 has been implicated in myocardial preconditioning, and over-expression of this molecule protects against myocardial ischemia/reperfusion (I/R) injury. However, the participation of Bcl-2 in injury to the naive heart, and its subcellular targets under such conditions, remains elusive. Using a mouse line with ablation of the Bcl-2 gene, we found that myocardial infarct size (IS) was exacerbated (53.5% vs. 53% in WT mice) (p = 0.04, n = 5), demonstrating that Bcl-2 limits IS in the naive myocardium. Furthermore, protection afforded by the NO donor diethylenetriamine/nitric oxide (DETA/NONOate, 0.1 mg/kg x 4 x.i.v., given 24 h prior to 30 min coronary artery occlusion (G4O), was attenuated in the Bcl-2 null mice (a reduction in IS of 34% vs. 53% in WT), indicating a role of Bcl-2 in DETA/NONa cardioprotection. Interestingly, a separate model of cardioprotection (engendered by activation of PKCε) exhibited increased levels of Bcl-2 protein as compared to wild type controls while mice expressing dominant negative PKCε were refractory to preconditioning and did not show an increase in Bcl-2 protein. Isolated cardiomyocytes from Bcl-2 null mice exhibited increased matrix swelling in response to Ca2+ as compared to WT, indicating an increased susceptibility to mitochondrial permeability transition (MPT). Moreover, provision of either recombinant Bcl-2 or PKCε was sufficient to attenuate MPT, suggesting Bcl-2 and PKCε can directly modulate the MPT pore. In addition, reactive oxygen species-induced cytochrome-c release from proteolysis containing the core MPT pore-forming protein, voltage dependent anion channel (VDAC) was prevented by Bcl-2 or PKCε. Finally, treatment with the specific VDAC specific inhibitor Rob68~3400 (0.25mg/kg i.v.) at onset of reperfusion abolished the increase in IS in Bcl-2 null mice. These data suggest that Bcl-2 is pivotal in limiting IS in the naive myocardium by modulating VDAC-dependent MPT, and implicate signaling by the known protective kinases, PKCε, in this process.

P31 Hexokinase I and II Protect Against Oxidant-Induced Cell Death by Increasing Glucose Phosphorylation and Inhibition of Mitochondrial Permeability Transition

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Alterations in glucose metabolism have been demonstrated in heart failure and cardiac hypertrophy. The first step in glucose metabolism is carried out by the hexokinase (HK) family of enzymes. Overexpression of HK I and HK II in tissue cultures protects against oxidant-induced cell death. The protective effects of these enzymes are thought to be due to either an increase in glucose phosphorylation or closure of the mitochondrial permeability transition pore (mPTP) as a result of decreased glucose phosphorylation and inhibition of the mitochondrial permeability transition (MPT). This activity is accompanied by increased thioredoxin activity. In parallel, message for TXNIP, a key inhibitor of thioredoxin activity, was downregulated by all three angiogenic agents. Heat maps of transcriptional profiles of stimulated EC revealed a large array of genes downstream of nAChR activation that are shared with VEGF or FGF treated cells. A subset of these genes have not previously described to be involved in angiogenesis. Knockdown of several of these commonly regulated genes abolished EC migration. Conclusion: Nicotine promotes angiogenesis via stimulation of nAChR-dependent endothelial cell migration. Furthermore, activation of the EC nAChR induces a transcriptional profile similar to those induced by other angiogenic cytokines. Analysis of transcriptional overlap among angiogenic cytokines reveals genes not previously considered to be involved in angiogenesis.

P32Transient Exposure to Extracellular Hydrogen Peroxide Is Associated with a Persistent Increase in Intracellular Calcium and Superoxide Release from the Mitochondria in Ventricular Myocytes Without Apoptosis

Helena M Viola, Peter G Arthur, Livia C Hool; The Univ of Western Australia, Crawley Perth, Australia

Hydrogen peroxide (H2O2) can act as a signaling molecule to mediate cardiovascular pathology. In this study we sought to understand the effects of a transient H2O2 exposure on myocyte function at a concentration insufficient to cause apoptosis or necrosis. Myocytes were exposed to 3.0 μM H2O2 for 5 min followed by 10 μM catalase for 30 min to catalyse H2O2 to water and H2O. Cellular superoxide was measured using dihydroethidium (DHE). Transient exposure to H2O2 caused a 66.1% increase in DHE signal (n = 45, P < 0.05) compared to controls exposed to catalase only (n = 8) without activation of caspase 3 or evidence of necrosis. The increase in DHE signal was attenuated when myocytes were pre-treated with the mitochondrial inhibitor myxothiazol (7.0 μM, n = 14) and when calcium uptake by the mitochondria was inhibited with 5 μM Ruthenium Red (n = 5). We investigated the L-type Ca2+ channel (ICaL) as a source of calcium influx. The increase in superoxide could be attenuated when ICaL function was inhibited with 2 μM nisoldipine (n = 9). Consistent with this, basal channel activity was significantly increased from 5.4 pA/PF (n = 7) to 8.9 pA/PF (n = 25) after H2O2. The response of the channel to β-adrenergic receptor stimulation was used as a functional reporter for changes in cellular production of reactive oxygen species since a decrease in cellular H2O2 is associated with an increase in the sensitivity of the channel to the β-adrenergic receptor agonist isoprenaline (Iso). After exposure to H2O2, the IC50 for activation of the channel by Iso was significantly increased from 5.8 to 27.8 μM. More importantly, this effect and the increase in basal current density persisted for several hours after exposure to H2O2. In addition, intracellular calcium was permanently elevated by a twofold increase from a resting calcium of 68 nM (n = 7, P < 0.05). We propose that extracellular H2O2 is associated with an increase in superoxide production from the mitochondria via calcium influx from the L-type Ca2+ channel. The effect persists because a possible mechanism which exists between increased basal channel activity, elevated intracellular calcium and superoxide production by the mitochondria. This may represent a mechanism for cardiovascular pathology that involves elevated calcium and reactive oxygen species.
metabolism. PPARα and PPARγ are important transcriptional regulators of fatty acid oxidation in the heart. However, the function of PPARγ in the heart remains obscure. To investigate the tissue specific role of PPARγ in the heart, we generated a cardiomyocyte-restricted PPARγ knockout mouse line (CR-PPARγ−/−). CR-PPARγ−/− mice revealed no gross phenotype after birth until reached two months of age. Adult CR-PPARγ−/− mice showed cardiac hypertrophy, progressive heart failure and premature death. Further analyses revealed that expression of Sox2, (encoding manganese superoxide dismutase; MsSOD), a mitochondrial antioxidant enzyme was decreased by about 40% both in transcript and protein levels in cardiac samples from cardiomyocyte-restricted PPARγ knockout hearts prior to major pathological changes compared with those of controls. We further identified that Sox2 is a PPARγ-target gene in the heart. Depressed MsSOD in the CR-PPARγ−/− hearts exhibited myocardial superoxide accumulation, leading to severe oxidative stress. Taken together, this study shows that PPARγ is critical to myocardial redox homeostasis. These findings provide a framework for new therapeutic approaches to heart disease.

## Impaired Mitochondrial Ultrastructure and Metabolic Function in Cardiac and Skeletal Muscle in TauTKO

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**Background:** Tau, a sulfur-containing ϕ-α-amino acid, is the most abundant amino acid in cardiac and skeletal muscle. Tau has previously been shown to play a role in ion movement, calcium handling and tissue protection in muscles, though molecular mechanisms are unclear. Recently, it has been demonstrated that tauire could critically affect mitochondrial function, whereas physiological significance has not been elucidated.

**Methods and Results:** To address the physiological role of tauire in tissues, we generated tauire transmigrator-(Tail) knockout mice (TailKO).KO mice showed a deficiency in tissue tauire content in the hearts tauire muscles (heart; not detectable (TailKO) v.s. 6.7±2.7 (wild) μg/mg wet weight, p<0.001, skeletal muscle; 0.70±0.33 (TailKO) v.s. 16.9±5.1 (wild) μg/mg wet weight, p<0.001) as well as the other tissues. Histological analyses assessed by hematoxylin-eosin stain showed wall thickness and dilatation, increased fibrocellular, and skeletal muscle atrophy in TailKO mice compared with wild-type littermates.

Despite cardiac fibrosis was not observed in TailKO hearts as assessed by Masson’s trichrome stain, TailKO hearts exhibited increased expressions of cardiac failure marker BNP and β-MHC, but not a-skeletal actin, as assessed by northern blot analyses. Transmission electron microscopy analyses showed abnormal mitochondrial ultrastructure in TailKO hearts. Enzymatic histological staining revealed that succinate dehydrogenase (SDH) activity, but not cytochrome c oxidase (COX), was remarkably decreased in the hearts of TailKO compared with wild-type littermates (SDH; 43.16±(p<0.05), COX; 79.30±(not significant), respectively), indicating the impairment of the capacity of oxidative phosphorylation in cardiac and skeletal muscle by tauire depletion.

Interestingly, mitochondrial abnormal ultrastructure and impaired SDH activity were also observed in the skeletal muscles in TauTKO.

**Interestingly, mitochondrial abnormal ultrastructure and impaired SDH activity were also observed in the skeletal muscles in TauTKO.**

**Conclusion:** These results indicate that tauire depletion causes mitochondrial dysfunction in cardiac and skeletal muscle, suggesting that modulation of mitochondrial function may underlie tissue protective roles of tauire.

## Can “Elderly” Hearts Be Rendered Resistant to Ischemia? D-myos-Inositol Trisphosphate, but Not Ischemic Preconditioning, Limits Infarct Size in Cardiac Myocytes

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An α1A-Adrenergic-ERK Signaling Pathway Mediates Survival Signaling in Cardiac Myocytes

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Qiu Ye, Shilpa M Manda, Rani Chirumamilla, Teresa Gallardo, Sean Goetsch, Daniel J Garry, Jing Liu, Sharon Tsang, Tak M Wong; The Univ of Hong Kong, Hong Kong, China

The cardioprotection of ischemic preconditioning appears to be reduced with age in the male, suggesting that testosterone may be responsible. There is evidence of a link between testosterone and heat shock protein 70 (HSP70), which mediates the delayed protection of preconditioning. We therefore hypothesize that testosterone is needed for full activation of HSP70, which mediates the cardioprotection of IP. Male Sprague-Dawley rats (7–6 weeks) underwent sham operation or gonadectomy without (G) or with testosterone replacement (200 μg/d) daily for 8 weeks prior to IP preconditioning. The preconditioning protocol was as follows: 10 min at 5% O2 and 95% N2, with 5 min of reoxygenation. Treatment groups were compared with sham operated male rats (Sham). In addition, we further evaluated α1A activity in knockout mice under basal conditions and during ischemia.

**Role of Testosterone in Heat Shock Protein 70 Activation and Delayed Cardioprotection of Preconditioning in Male Rats**

Jing Liu, Sharon Tsang, Taki M Wong; The Univ of Hong Kong, Hong Kong, China

The cardioprotection of ischemic preconditioning appears to be reduced with age in the male, suggesting that testosterone may be responsible. There is evidence of a link between testosterone and heat shock protein 70 (HSP70), which mediates the delayed protection of preconditioning. We therefore hypothesize that testosterone is needed for full activation of HSP70, which mediates the cardioprotection of IP. Male Sprague-Dawley rats (7–6 weeks) underwent sham operation or gonadectomy without (G) or with testosterone replacement (200 μg/d) daily for 8 weeks prior to IP preconditioning. The preconditioning protocol was as follows: 10 min at 5% O2 and 95% N2, with 5 min of reoxygenation. Treatment groups were compared with sham operated male rats (Sham). In addition, we further evaluated α1A activity in knockout mice under basal conditions and during ischemia.
**Mechanisms of Myocardial Protection by Atorvastatin: S-5’-Nucleotide Is Upstream to eNOS Activation**

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**Background:** Previous studies have shown that statins protect against ischemia-reperfusion injury by activating phosphodiesterase 3-kinase (PI3K), leading to phosphorylation of Akt that activates endothelial nitric oxide synthase (eNOS) by phosphorylation. PI3K also activates ecyo-5’-nucleotide (ESN). Blocking ESN abrogates the infarct size (IS)-limiting effects of statins in the dog. It was suggested that ESN and eNOS are activated in parallel. We have shown in the rat that inducible NOS (iNOS) and cyclooxygenase-2 (COX2) activation are mandatory steps for mediating the IS-limiting effect of atorvastatin (ATV) and are downstream to eNOS. 

**Purpose:** To assess whether ESN activation is downstream or upstream to eNOS.

**Methods:** Wild-type (WT), eNOS -/- and iNOS -/- mice were pretreated with oral ATV (10 mg/kg/d) or water alone (ATV-). For 3 days. Mice were subjected to 30 min coronary artery occlusion and 4h reperfusion (IS protocol), or hearts were harvested, without being subjected to ischemia. For measurement of myocardial ESN (ng/mg protein)/ml, calcium-dependent (cNOS) and independent (ciNOS) NOS activity (X1000 cpm); and adenosine (μg/ml) and 6-keto-PGF1α (the stable metabolite of PG2; pg/ml) levels. 

**Results:** Body weight, LV size and area at risk (AR) were comparable among groups. ATV decreased IS only in the WT mice. cNOS activity was increased in the ATV - WT and iNOS -/- mice. ATV increased cNOS activity and myocardial 6-keto-PGF1α, only in the WT mice. ATV increased ESN activity and tissue adenosine levels in all groups. ESN activity was significantly higher in the eNOS -/- than in the WT mice (p < 0.017) or iNOS -/- group (p < 0.025). 

**Conclusions:** ATV activates ESN in the mouse. ESN upregulation by ATV does not limit IS in eNOS -/- and iNOS -/- mice, suggesting that ESN activation is upstream to eNOS, iNOS and PG2 production.

<table>
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<tr>
<th>WT</th>
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<td>2.91±0.08</td>
<td>2.90±0.13*</td>
<td>2.93±0.09</td>
<td>2.90±0.10</td>
</tr>
<tr>
<td>6-keto-PGF1α</td>
<td>15.7±0.1</td>
<td>15.6±0.1</td>
<td>16.5±0.1</td>
<td>15.3±0.1</td>
</tr>
<tr>
<td>ESN</td>
<td>37.6±6.4</td>
<td>47.66±1.3</td>
<td>45.8±3.8</td>
<td>57.5±4.7</td>
</tr>
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</table>

Adenosine 2.8±0.4 | 5.7±0.5* | 7.0±1.0 | 10.0±0.6 | 3.1±0.5 | 4.9±0.2* |

*p < 0.05 (ATV vs. ATV-).

**P40**

**Pioglitazone Reduces Myocardial Infarct Size and Increases Myocardial PGI2 Levels by Upregulating cPLA2**

Yochai Birbaum, Yumei Ye, Yu Lin, Shaul Atar, Ming-Hue Huang, Jose R Perez-Polo, Barry F Uretsky; Univ of Texas Med Branch, Galveston, TX

**Background:** Several studies have shown that thiazolidinediones (TZD) reduce myocardial infarct size (IS). However, the underlying mechanism is still unclear. It has been demonstrated that the IS-limiting effect of statins involves increased expression and activity of phosphorylated endothelial nitric oxide synthase (eNOS-), inducible NOS (iNOS), phosphatase A2 (pA2), and cyclooxygenase-2 (COX2). Blocking COX2 abrogated the protective effect of atorvastatin. We investigated whether pioglitazone (PIO) shares the same mechanisms, protecting the heart by increasing myocardial PGI2 production. Methods: Sprague-Dawley rats received 3 days of oral treatment with: 1) PIO 10 mg/kg/d or 2) water (control). Rats underwent 30 min ischemia and 4h reperfusion (IS protocol). Arteries were harvested in each group, without being subjected to ischemia (immunoblotting and ELISA for 6-keto-PGF1α, the stable metabolite of PG2). 

**Results:** Body weight, left ventricular weight and the area at risk (AR) were similar between groups. PIO reduced IS (Table). PIO did not affect myocardial expression of P-eNOS, iNOS, and COX2. However, PIO increased expression of cPLA2, and the myocardial content of 6-keto-PGF1α. The protective effect of PIO was abrogated by SC51825 (22.7±1.7%), but not by SC560 (9.5±0.4%; p > 0.004). 

**Conclusions:** IS limitation with PIO is associated with increased expression of myocardial cPLA2, leading to increased PGI2 content. However, it is independent of upregulation of P-eNOS, iNOS and COX2.

<table>
<thead>
<tr>
<th>Control</th>
<th>PIO</th>
<th>p value</th>
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<tr>
<td>IS (% of AR)</td>
<td>29.7±2.1</td>
<td>29.7±2.1</td>
</tr>
<tr>
<td>P-eNOS (%)</td>
<td>100±11</td>
<td>100±11</td>
</tr>
<tr>
<td>iNOS (%)</td>
<td>100±12</td>
<td>98±12</td>
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**P41**

**MuRF3 Maintains the Integrity of the Heart Following Acute Myocardial Infarction**

Jens Feilitz, Eva van Rooij, Jeffrey Spencer, John M Shelton, Univ of Texas Southwestern Med Ctr, Dallas, TX; Leon J de Windt, Hubrecht Lab and Interuniv Cardiology Inst, Utrecht, The Netherlands; James A Richardson, Rhonda Bassel-Duby, Eric N Olson; Univ of Texas Southwestern Med Ctr, Dallas, TX

The muscle-related RING-finger protein 3 (MuRF3), a member of the TRIM family, is restricted to heart and skeletal muscle and is implicated as an E3 ubiquitin ligase within the ubiquitin proteasome pathway. MuRF3 is localized to the sarcoplasm and its expression in muscle cells is induced during muscle differentiation. Yeast-two-hybrid analysis and biochemical characterization revealed gamma-filamin and HLF-2 as MuRF3 interaction partners and targets for proteasome dependent degradation through the MuRF3 coiled-coil domain. To determine the role of the coiled-coil domain for MuRF3 function in vivo, we generated a germline deletion of the coiled-coil domain of MuRF3 (referred to as MuRF3CCc) in mice. Mice homozygous for this mutation are viable and display an increase in gamma-filamin and HLF-2 protein compared to wild type (WT) mice, indicating that both proteins are degraded in vivo by MuRF3. At baseline, echocardiographic analysis of MuRF3CCc hearts showed a mild reduction of systolic left ventricular (LV) function compared to controls. When subjected to acute myocardial infarction (MI) by ligation of the left anterior descending artery, MuRF3CCc mice developed a severe phenotype five days after induction of MI. Strikingly, these mice exhibit a doubling of mortality compared to control mice due to ventricular rupture in the border zone of the MI. This rupture is preceded by LV dilation with a severe decrease in cardiac function and diastolic and systolic posterior wall thickness. Histological analysis showed myocardial necrosis and defective scar formation in the peri-infarct zone and excessive TUNEL positive cells in MuRF3CCc mice compared to WT mice following MI. Cardiac stress markers (ANP, BNP, CTGF) were also upregulated to a greater extent in MuRF3CCc mice. Upon oxidative stress, MuRF3 was co-localized with mitochondria and stress fibers in myocytes. Cells overexpressing MuRF3 showed a reduction of cell death in response to oxidative stress and MuRF3 expression was increased in response to oxidative stress. We conclude that MuRF3 plays a protective role in the heart by maintaining integrity and function of the myocardium. This cardioprotective role is partially mediated through MuRF3 mediated turnover of gamma-filamin and HLF-2.
tations of control and obese rats were similar, while glycosylated hemoglobin concentrations were only mildly elevated in obese rats (0.52 ± 0.06 vs. 0.37 ± 0.10%, p < 0.05). Hearts from obese rats were perfused on the isolated working rat heart perfusion system and subjected to left anterior descending coronary artery occlusion (CAO) and reperfusion. Reperpufusion function, arrhythmias and infarct size was assessed using TTC staining. CAO resulted in an increased infarct size (22.3 ± 2.3% vs. 8 ± 1.6%, p < 0.05) and an increased occurrence of reperfusion ventricular fibrillation (9% vs. 86%, p < 0.05) and a decreased reperfusion aortic output recovery (27.7 ± 3.4% vs. 4.2 ± 4.2%, p < 0.05) in obese compared to control rat hearts. Coronary effluent nitroprusside release before and subsequent to CAO was similar in both groups. The myocardial glucose uptake was however reduced in obese rat hearts when compared to controls (0.56 ± 0.70 mmol/min vs. 0.30 ± 0.10 mmol/min, p < 0.05). Concluding these data indicate that obesity without clinically detectable diabetes mellitus increases myocardial infarct size and incidence of reperfusion arrhythmias and decreases reperfusion function after CAO. This reduced ischemic tolerance was not associated with increased myocardial catecholamine release but with alterations in myocardial glucose metabolism. The effects of the addition of insulin to the perfusion solution is currently under investigation.

**Surgical Ischemia Induces Phosphorylation and Translocation of Heat Shock Protein 27 and β-X-Crystallin in Human Myocardium**

Richard T Clements, Jun Feng, Neel Kodia, Basel Ramli, Shigeki Shiono, Munir Bloodthorn, Shu Xu, Cesario Bianchi, Frank W Sellke; Beth Israel Deaconess Med Ctr, Boston, MA

The small heat shock proteins HSP27 and β-X-cryrb (cryAB) are highly expressed in cardiac and skeletal muscle. Evidence exists that cell cultures indicate that HSP27 and cryAB are dramatically regulated via phosphorylation in response to ischemic insults. The phosphorylation of these proteins is implicated in the regulation of diverse cellular processes including myocyte redox regulation, protein chaperone function, signal transduction scaffolding, and recently, myocyte contractile function. It is unknown if HSP27 and cryAB play a role in the response to ischemia in human myocardium in vivo. Patients undergoing open heart surgery (CABG, valve replacement) are subjected to ischemic insults via cardiopulmonary bypass (CP/CPB). Cardiopulmonary solutions used to arrest the heart during surgery are subjected to ischemic insults including hypoxia and myocardial contractile defects. We performed the following experiments to determine any changes in the phosphorylation and localization of HSP27 and cryAB in human myocardium following CP/CPB. Right atrial appendage and chest wall skeletal muscle samples were collected from patients immediately before and after CP/CPB. In whole tissue lysates, CP/CPB induced a robust increase in the atrial phosphorylation of HSP27 at ser82 and cryAB at ser59. There were no changes in the expression level of either protein. Furthermore, phosphorylation of HSP27 and cryAB following CP/CPB with translocation from a Triton X-100 soluble to an insoluble pool. There was no detectable increase in phosphorylation of either protein in skeletal muscle, suggesting an ischemia specific response. In addition, confocal microscopy of atrial tissue revealed movement of HSP27 and cryAB from a diffuse/membrane localization pre-CP/CPB to a striated sarcomeric pattern after CP/CPB. Double labeling with an atrial specific myosin light chain -2a antibody revealed prominent staining of total ser82 at ser82 and ser59 phosphorylated HSP27 along I-bands of cardiomyocytes after CP/CPB. These results demonstrate that two members of the sHSP family, HSP-27 and cryAB, are phosphorylated and translocate to cardiac myofibrils following surgical ischemia associated with CP/CPB.

**Activation of PKN Causes Cardiac Hypertrophy and Resistance to Ischemia-Reperfusion**

Hiromitsu Takagi, Katsuya Kajimoto, Peiyong Zhai, Junichi Sadohama; UMDNJ New Jersey Med Sch, Newark, NJ

PKN is a serine/threonine kinase, whose catalytic domain is highly homologous to that of protein kinase C. PKN causes ANF gene expression in cardiac myocytes. However, in vivo function of PKN in the heart is poorly understood. In yeast, Pck1, whose amino-terminal regulatory region is highly homologus to that of PKN, is activated by cell wall stress including hypotonic stress (HS). It has been shown that ischemia reperfusion (IR) induces hypotonic cell swelling in mammalian myocytes providing evidence for a protective effect of PKN activation to IR. In the present study, therefore, we examined whether PKN is activated by hypotonic stress in cardiac myocytes in vitro, and by IR in the heart in vivo. We constructed transgenic mice with cardiac specific overexpression of constitutive active (CA) PKN (Tg-CAPKN) and examined whether activation of PKN has a protective effect in the heart. In neonatal rat ventricular myocytes, PKN was activated by HS (37% osmolality) within 15 min. The Activation of PKN by HS reached a peak within 60 min, which was accompanied by Thr774 phosphorylation. Phosphorylation of PKN was also observed in the heart subjected to IR in vivo. In Tg-CAPKN mice, left ventricular (LV) weight to body weight ratio was heavier and LV wall thickness was thicker than in non-transgenic (NTG) mice, indicating that PKN stimulates cardiac hypertrophy. Cardiac function assessed by echocardiography was comparable in both Tg-CAPKN and NTG mice. In NTG hearts, 45min ischemia followed by 24 hr reperfusion caused myocardial infarction (MI), whereas the size of MI was dramatically smaller in Tg-CAPKN heart (NTG vs. Tg-CAPKN, 37.2 ± 6.0 vs. 11.4 ± 6.9%, p < 0.01). The number of TUNEL positive nuclei in the I/R hearts was also significantly smaller in Tg-CAPKN mice than in NTG mice (0.38 ± 0.23% vs. 0.30 ± 0.15%, p < 0.05). These results suggest that PKN is activated by HS in cardiac myocytes and IR in the mouse heart. Furthermore, activation of PKN causes well compensated LV hypertrophy and also has protective effects in the ischemia-reperfusion heart in vivo.

**Reduction of Vagally Induced Atrial Fibrillation and Expression Levels of Connexins by n-3 Polysaturated Fatty Acids in Dogs**

Pascal Daleau, Jean-François Sarrazin, John Kingma, Genevieve Comeau, Dominique Fourrier, Frank Molin; Quebec Heart Inst, Quebec, Canada

Atrial fibrillation (AF) is a common arrhythmia with limited successful treatment. Dietary fish oils are a major source of omega-3 polysaturated fatty acids (n-3 PUFAs) and may lower mortality by reducing sudden cardiac death and in all likelihood ventricular tachyarrhythmias, the exact mechanisms of which remain unclear. Changes in spatial organization of gap junctions or cellular levels of cardiac connexins (Cx) have been linked to arrhythmogenesis. The aims of this open-label, uncontrolled study in dogs were to assess whether n-3 PUFAs prevent AF and to evaluate their impact on atrial tissue expression levels of Cx40 and Cx43. Forty-five dogs were studied using the vagally-induced AF model. Eight dogs were treated with 1.2p PO of n-3 PUFAs daily for 14 days. Of 17 control dogs we were able to induce AF in 8; in the latter we were re-evaluated for AF indubitably following IV treatment with a fish oil emulsion (1.4g in saline). Episodes of AF induced by extra stimuli or burst induction were considered if tissue biopsies were obtained from n-3 PUFAs levels and a western blot analysis of Cx40 and Cx43 protein levels. Total n-3 PUFAs atrial tissue levels were increased significantly in the oral treatment group (5.78 ± 0.71% vs 2.48 ± 0.42% in controls, p < 0.001). No difference was observed for hemodynamic or electrocardiographic parameters between groups. The number of sustained AF in the oral treatment group was reduced by 79% with the extra stimulus technique (10.5 vs. 49.8%, p < 0.001) and by 42% with burst induction (22.5 vs. 38.8%, p = 0.034). Cx40 and Cx43 protein levels were lower in oral treatment dogs (average decrease of 60% vs. p = 0.019 and 42% vs. p = 0.038, respectively); protection against AF appeared to be specifically related to reduced Cx40 expression levels (p < 0.001). In dogs that were given IV n-3 PUFAs, AF inducibility by the extra stimulus technique was reduced by 61.9% (p < 0.0001). In conclusion, oral treatment with fish oils increased plasma and atrial n-3 PUFAs levels and reduced vulnerability to induction of AF in this dog model. Modulation of cardiac Cx by n-3 PUFAs probably contributes to the antiarrhythmic efficacy of oils.
Detrimental Role of Complement Factor C3 After Myocardial Infarction

Stefan Frantz, Helga Wagner, Georg Erli, Johann Bauersachs; Med Universitaetsklinik, Wuerzburg, Germany

Background: Complement contributes to tissue injury in ischemic organs, with or without reperfusion. Activation of complement is an early event in ischemia/reperfusion injury and complement factors like C3 are increased in the myocardium. However, the expression and function of complement in heart failure has not been defined yet. Methods and results: The complement factor C3 is activated in the failing mouse heart up to 6 weeks after large myocardial infarction as determined by immunohistochemistry. After coronary artery ligation mortality was not different between wild-type (WT) and C3 knockout (KO) mice. However, C3 KO mice exhibited significantly reduced ventricular dilation over 3 weeks after myocardial infarction compared to WT controls (end systolic areas by transthoracic echocardiography, WT vs. KO: 19.6 ± 1.2 mm² vs. 13.9 ± 1.2 mm², p = 0.001). C3 levels were absent in KO animals. Furthermore, apoptosis content was significantly lower in the KO mice after myocardial infarction and may account for improved left ventricular remodelling. Conclusion: The complement factor C3 is activated after myocardial infarction. Absence of C3 reduces left ventricular dilation after myocardial infarction. C3 might therefore be an attractive target to treat heart failure.

Apoptosis Inhibition Improves Left Ventricular Function After Prolonged Cold Cardioplegic Arrest

Uwe M Fischer, Werner O Monzon-Posadas, Jürgen H Fischer, Univ of Cologne, Cologne, Germany; Wilhelm Bloch, German Sports Univ, Cologne, Germany; Uwe Meinhof, Thorsten Wahlers; Univ of Cologne, Cologne, Germany

Objectives: Cardioplegic arrest (CA) is associated with myocardial apoptosis induction. Data suggest that apoptosis inhibition during regional myocardial ischemia reduces infarct size and improves regional contractility. We sought to investigate whether inhibition of the apoptosis-signal-pathway would also improve left ventricular (LV) function following prolonged cold CA.

Methods: Ten adult rats were anesthetised and mechanically ventilated. Hearts were arrested by administration of ice-cold crystalloid cardioplegia (Brettschneider solution, Custodiol®; 10 mL/kg) with and without supplementation of a cell permeable non-selective caspase-inhibitor (z-VAD-fmk, 10 µM, n=5 each). Hearts were stored for 4h in cardioplegia (4°C). Subsequently, hearts were reperfused with Krebs-Henseleit-Solution (37°C) on a Langendorf-System. A balloon connected to a pressure transducer was inserted in the LV and inflated to a diastolic pressure of 10 mmHg. LV pressure and heart rate were recorded continuously for 60 min. Five additional rats without ischemic storage served as controls. Results: After 4h cold CA, hearts with apoptosis-inhibition had higher left ventricular pressure throughout the 60min reperfusion as compared to those without apoptosis-inhibition. Apoptosis-inhibition even resulted in significantly higher LV pressures as compared to controls at 30, 40 and 50 min reperfusion (58.6±10, 59.2±8.1 and 58.6±8.6 mmHg vs. 42.7±6.7, 42.8±10.6 and 42.2±8.6 mmHg respectively, p=0.013). Recovery time on reperfusion defined as duration until heart rate and LV pressure reached steady state was significantly shorter with apoptosis inhibition as compared to non-inhibition (13.4±11.6 vs 29.6±8.2 min, p=0.017). Conclusions: Apoptosis inhibition improves LV-function after prolonged cold CA and represents a new promising strategy in myocardial protection for cardiac allografts.

The Stabilization of β-Catenin Inhibits Physiological Hypertrophy in Adult Mouse Hearts

Anthony Baurand, Laura Zelarayan, Sandra Dunger, Russel Betney, Bärbel Pohl, Rainer Dietz, Martin Bergmann; Max-Delbrück-Ctr für Molekulare Med, Berlin, Germany

Constitutive activation of cardiac glycogen synthase kinase-3β (GSK-3β) is known to suppress cardiac hypertrophy in vivo. Among other substrates GSK-3β phosphorylates β-catenin, leading to its degradation. In order to study the role of β-catenin in cardiac hypertrophy, we studied the effect of its stabilization in adult mice at baseline and after angiotensin II (Ang II) stimulation. We employed a cardiac-specific myfibre-inducible Cre transgene to remove β-catenin stabilisation in cardiomyocytes. The exon 3 of β-catenin gene regulates its stability through proteolysis. Recombination of loxp sites flanking exon 3 was confirmed by PCR of genomic heart extracts and the truncated β-catenin product was confirmed by Western blot as well as in the nucleus. Mice with cardiac specific β-catenin stabilization were viable and the echocardiography and histology analysis of the hearts at baseline conditions showed no apparent significant structural modifications. 10 days of Ang II stimulation had significantly lower in the KO mice after Ang II stimulation and may account for improved left ventricular remodelling. Taken together these findings, we could suggest that β-catenin may block the process of physiological heart hypertrophy to increased stress, which lead to an inappropriate adaptation of the heart to enhance its pumping capacity.
 Withdrawn

P57

Characterization of Fibroblast Growth Factor 16 Promoter Activity in Postnatal Cardiac Cells In Vitro and In Vivo
Alina G Sofronescu, Scott Gregoire, Peter A Cattini; Univ of Minnesota, Minneapolis, Canada

The heparin-binding fibroblast growth factor (HGF) family plays important roles in the embryonic growth and development of the heart. FGF-16 expression is reported to be cardiac-specific, with significant induction at birth. This suggests a role for FGF-16 in the postnatal heart and thus its regulation and the mechanisms underlying spatial and temporal control of FGF-16 gene expression are of interest. We hypothesize that spatial control of FGF-16 occurs at the transcriptional level through cardiac-specific promoter activity. Genomic sequences (-6 kb) upstream of the ATG start codon of the murine FGF-16 gene were cloned. A combination of RNA blotting and RT-PCR was used to localize a putative promoter region including CCAAT and TATAA sequences. The transcription initiation site was identified 1073 bp upstream of the ATG codon in this region by primer extension, and was designated nucleotide +1. To test FGF-16 promoter function, a series of hybrid lucerase reporter genes were generated with varying lengths of upstream flanking FGF-16 sequences. These genes (-4.7, -2.7, -1.2 and -0.25FGF-16) were used to transiently transfect neonatal rat cardiomyocytes and rat glial (C6) cells. A Renilla lucerase gene was co-transfected as a control for DNA uptake. Hybrid genes -4.7, -2.7 and -1.2FGF-16:Luc were expressed significantly above ‘background’ promoterless genes (-pLED:Luc) in transfected cardiomyocytes; no activity of -0.25FGF-16:Luc was observed. No significant expression above ‘background’ levels was seen for any hybrid FGF-16/luciferase gene in transfected C6 cells. These data suggest that the FGF-16 promoter is located 1073 bp upstream of coding sequences and contained within 1.2 kb of upstream flanking sequences. Furthermore, sequences located between -1.2 and -0.2 kb are required for cardiac-specific expression. To test the FGF-16 promoter region for activity in vivo, transgenic mice containing a hybrid β-galactosidase reporter gene directed by 4.7 kb of upstream FGF-16 sequences (equivalent to -4.7FGF-16:LacZ) were produced by pronuclear injection. Preliminary data from one line (F8-06; 6wks) suggest the FGF-16 sequence contains promoter activity and works preferentially in the postnatal heart in vivo.

Blockade of Vascular Endothelial Growth Factor by Catheter-Based Adenoviral sFlt-1 Gene Transfer Attenuates Stent-Associated Neointima Formation in Nonhuman Primates
Kouta Funakoshi, Kansuke Egashira, Kaku Nakano, Kenji Sunagawa; Kyushu Univ Graduate Sch of Med Science, Fukuoka-shi, Fukuoka-ken, Japan

Background: We have previously reported that blockade of VEGF by sFlt-1 gene transfer limits neointimal lesion formation induced by balloon injury or high cholesterol diet. Inversely, overexpression of VEGF or PlGF is reported to exacerbate neointima formation. However, the role of VEGF in the pathogenesis of stent-associated neointima formation has not been addressed. This point is important, because (1) the pathogenesis of neointima formation differs considerably between injury models and (2) stent procedure now becomes the major clinical revascularization technique. Therefore, we here investigated the role of VEGF in stent-associated neointima formation in non-primate species. Methods and Results: Male cynomolgus monkeys fed high-cholesterol diet underwent stent implantation procedure in iliac arteries. Immediately after stenting, adenosinovirus solution containing sFlt-1 or LacZ (1-3 x 10^9 pfu, 4mL) was introduced into the stent site through a REMEDY channel balloon catheter. As control, untreated animals undergone stent procedure were studied. The stented iliac artery segments were harvested 1, 3 and 7 days after stenting. The sFlt-1 gene transfer reduced neointima formation (% over untreated Tg mice, p<0.001) 1,5 and 7 days after stenting. The sFlt-1 gene transfer reduced neointima formation (% over untreated Tg mice, p<0.001), 2.5 and 7 days after stenting. These genes (-4.7, -2.7, -1.2 and -0.25FGF-16:LacZ) were generated by protocol for luciferase reporter genes. These data suggest that the first indication of myo or NF-κB specifically and separately using an RNA interference strategy attenuates cardiac hypertrophy and that myotrophin could be used as a therapeutic target for treatment of heart failure.

ErbK5 Activation Inhibits Inflammatory Responses via Peroxisome Proliferator-Activated Receptor-δ Stimulation in Skeletal Muscle: Possible Involvement in Aging
Jun-ichi Abe, Chang-Hoon Woo, Michael P Massett, Tetsuro Shishido, Seigo Itoh, Bo Ding, Carolyn McClain, Wenyi Chi, Sreesatya R Vupalianni, Chen Yan; Univ of Rochester, Rochester, NY

Peroxisome proliferator-activated receptors (PPAR) decrease the production of cytokine and TNFα, which are associated with aging-related inflammation and insulin resistance. Recently, the involvement of the induction of heme oxygenase-1 (H-O1) in regulating inflammation has been suggested, but the exact mechanisms for reducing inflammation by H-O1 remains unclear. We found that overexpression of H-O1 and [Ru(OC)(O2)2], a carbon monoxide (CO)-releasing compound, increased both Erk5 kinase and transcriptional activity in C2C12 cells. [Ru(OC)(O2)2] activated PPARδ transcriptional activity via MEK5/ERK5 signaling pathway. The inhibition of NF-κB activity by ERK5 activation was reversed by a dominant negative form of PPARδ suggesting that ERK5/PPARδ activation is required for the anti-inflammatory effects of CO and H-O1. Finally, we found the concomitant reduction of H-O1 and Erk5, and induction of INOS in skeletal muscle from 19 month old mice, which showed insulin resistance compared with 5 month mice. Interestingly, the ability of PPARδ binding to INOS promoter was also significantly decreased in old mice using chromatin immunoprecipitation assay in vivo. Based on these data, we propose a new mechanism by which CO and H-O1 mediate anti-inflammatory effects via activating Erk5/PPARδ, and Erk5 mediates CO and H-O1-induced PPARδ activation via its interaction with PPARδ.

Nuclear Ca2+/-CaM/CaMK Signaling Regulated β-Mysin Heavy Chain Gene Transcription in Pressure Overload-Induced Hypertrophic Heart of Rat
Jian Liu, Qi Zhou, Xinqiao Hosp, Chongqing, China; Peiyong Wang, Third Military Med Univ, Chongqing, China; Yingbin Xiao; Xinqiao Hosp, Chongqing, China

Regulation of β myosin of heavy chain (β MHC) gene expression by nuclear Ca2+/-CaM/CaMK signaling pathway was investigated in pressure overload-induced hypertrophic rat heart. The model of rat cardiac hypertrophy was established by abdominal aortic constriction. Velocity and isovolumic gradient centrifugation was used to fractionate rat cardiac nuclei. Compare with the control group, β MHC mRNA gene expression was higher in the hypertrophic group and in cultured hypertrophic cardiac myocytes, and they were inhibited by pretreatment with calcium chelator BAPTA-AM, CaM inhibitor trifluoperazine in cultured cell (p<0.05). Nuclear runoff transcription assay showed β MHC gene transcription was markedly enhanced in the hypertrophic group, and that was significantly inhibited by treatment of isolated cardiac nuclei with calcium chelator BAPTA-AM, CaM inhibitor trifluoperazine and CaM antagonist calmidazolium chloride, as well as CaMKi inhibitors KN-62 respectively (p<0.05). Western blot analysis revealed that nuclear CaM, CaMK and CaMKIV were significantly increased, and the activities of CaMKII and CaMKIV in nuclei were also increased in the hypertrophic group and (p<0.05). Thus, we conclude that β MHC gene is regulated at the transcriptional level, which might be required for the nuclear translocation of CaM, CaMKII, and CaMKIV and the activity increase of nuclear CaMKII and CaMKIV. The nuclear Ca2+/-CaM/CaMK signaling pathways might be involved in the β MHC gene regulation and the development of overload-induced cardiac hypertrophy.
of the NOS dimer and NOS activity and reduced expression of $\text{O}_2$-stimulated NOS coupling, which was prevented by treatment with BEC. Pulmonary arterial catherization demonstrated significantly higher mean PA pressure at 6 weeks in AAV-arginase mice compared with AAV/pipal mice (19.8 ± 1 mmHg compared to 13.9 ± 0.9 mmHg, P < 0.05). Arginase transfection was associated with a marked increase in ROS signaling, MMP activation, and pulmonary vascular remodeling. These results clarify the role of arginase in mediating pulmonary hypertension in vivo and provide the first evidence that arginase regulates NOS dimerization and activity.

Cardiac-Specific Abrogation of NF-κB in Cardiomyopathy

Maria A Brown, Michael McGuinness, Univ of Cincinnati, Cincinnati, OH; Jeffery Molkentin, Cincinnati, Cincinnati, OH

Several studies have demonstrated that the proinflammatory cytokine tumor necrosis factor-α (TNF-α) has both injurious and cardioprotective effects, due to the actions of multiple TNF-α-responsive downstream signal transduction pathways. Currently, there is little known regarding the complex TNF-α signaling pathways that affect cardiomyopathy. One of the major TNF signaling pathways involves nuclear factor-κB (NF-κB), a pleiotropic transcription factor that activates genes involved in cellular processes including growth, apoptosis, inflammation, angiogenesis and cardiomycocyte function. It has been shown that NF-κB activation is necessary for G-protein coupled and TNF-induced hypertrophy in vitro. Although it has recently been shown that activation of NF-κB is required for hypertrophy in response to pressure overload and TNF overexpression in vivo, the definitive role of NF-κB in non-cytokine induced cardiomyopathies has not been elucidated in vivo. We show that both TNF-α and NF-κB are activated in cardiomyopathy caused by cardiac-specific transgenic overexpression of calcineurin (CnTg). In order to investigate the role of NF-κB in murine cardiomyopathic models, we engineered transgenic mice with cardiac-specific expression of dominant negative IκBα. Although blockade of NF-κB in double transgenic CnTg/IκBα mice was complete, we were unable to delineate significant in vivo physiological effects from this blockade. This result was in direct contrast to previous results with TNF-induced cardiomyopathy in which blockade of NF-κB activity significantly abrogated cardiac hypertrophy, improved cardiac function and significantly improved survival. Blockade of NF-κB in TNF-induced cardiomyopathy significantly increased Akt activity, which is thought to contribute to the dramatically improved cardiac phenotype. Interestingly blockade of NF-κB did not increase activation of Akt in the calcineurin cardiomyopathic model. This suggests the existence of complex signaling networks in which the same signaling mediator(s) may play different roles dependent upon combinatorial effects of particular mediators. The potential role of other signaling mediators in calcineurin-induced cardiomyopathy is currently under investigation.

Cardiac Autophagy Is a Maladaptive Response to Hemodynamic Stress

Hongxin Zhu, Paul Tannous, Janet Johnstone, Yongli Kong, John M Shelton, James A Richardson, Joseph A Hill, Rhonda Bassel-Duby, Eric N Olson, The Univ of Texas Southwestern Med Ctr at Dallas, Dallas, TX

In an effort to discover regulators of cardiac gene expression and growth, we devised a eukaryotic expression screen for cDNAs encoding activators of the atrial natriuretic factor (ANF) promoter, a cardiac-specific marker of hypertrophy and pathological remodeling of the adult heart. This screen revealed a family of activators of the ANF promoter, called calmodulin-binding transcription activators (CAMTAs), which are conserved from plants to humans. We show that CAMTAs are recruited to the ANF promoter, at least in part, by association with the cardiac homeodomain protein Nkx2-5 and function as inducers of cardiac growth. Overexpression of CAMTA2 in isolated neonatal cardiomycocytes by adenoviral-mediated delivery induces hypertrophic growth of cardiomycocytes. Transgenic mice overexpressing CAMTA2 in the heart display cardiac hypertrophy, which progresses to dilated cardiomyopathy. Mice lacking CAMTA2 display diminished hypertrophy in response to pressure overload and neurohumoral signaling. Through gain- and loss-of-function approaches in vivo and in vitro, we show that class II HDACs restrain the activity of CAMTA proteins. Nuclear export of class II HDACs in response to PKC/PKD signaling releases CAMTAs from HDAC-dependent repression of CAMTA activity. Mice lacking CAMTA2 display diminished hypertrophy in response to pressure overload and neurohumoral signaling. Through gain- and loss-of-function approaches in vivo and in vitro, we show that class II HDACs restrain the activity of CAMTA proteins. Nuclear export of class II HDACs in response to PKC/PKD signaling releases CAMTA from HDAC-dependent repression with consequent expression of genes involved in cardiac growth. These findings uncover a role for mammalian CAMTA proteins as signal-responsive transcriptional activators of cardiac growth and targets for the repression of class II HDACs.

Cardiac Autophagy Is a Maladaptive Response to Hemodynamic Stress

P65

Regulation of Cardiac Growth by CAMTA, a Transcriptional Activator Family That Opposes Class II Histone Deacetylases

Kunhua Song, Johannes Backs, John McNally, Xiaolong Qi, Robert T Gerard, James A Richardson, Joseph A Hill, Rhonda Bassel-Duby, Eric N Olson, The Univ of Texas Southwestern Med Ctr at Dallas, Dallas, TX

In an effort to discover regulators of cardiac gene expression and growth, we devised a eukaryotic expression screen for cDNAs encoding activators of the atrial natriuretic factor (ANF) promoter, a cardiac-specific marker of hypertrophy and pathological remodeling of the adult heart. This screen revealed a family of activators of the ANF promoter, called calmodulin-binding transcription activators (CAMTAs), which are conserved from plants to humans. We show that CAMTAs are recruited to the ANF promoter, at least in part, by association with the cardiac homeodomain protein Nkx2-5 and function as inducers of cardiac growth. Overexpression of CAMTA2 in isolated neonatal cardiomycocytes by adenoviral-mediated delivery induces hypertrophic growth of cardiomycocytes. Transgenic mice overexpressing CAMTA2 in the heart display cardiac hypertrophy, which progresses to dilated cardiomyopathy. Mice lacking CAMTA2 display diminished hypertrophy in response to pressure overload and neurohumoral signaling. Through gain- and loss-of-function approaches in vivo and in vitro, we show that class II HDACs restrain the activity of CAMTA proteins. Nuclear export of class II HDACs in response to PKC/PKD signaling releases CAMTA from HDAC-dependent repression with consequent expression of genes involved in cardiac growth. These findings uncover a role for mammalian CAMTA proteins as signal-responsive transcriptional activators of cardiac growth and targets for the repression of class II HDACs.

P66

Polymorphism

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Case (n = 298)</th>
<th>Controls (n = 520)</th>
<th>P value</th>
<th>Odds Ratio (C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE DD or ACE 8 GG</td>
<td>141</td>
<td>172</td>
<td>&lt;0.0001</td>
<td>1.817 (1.357–2.342)</td>
</tr>
<tr>
<td>+ AGT 235 TT</td>
<td>34</td>
<td>24</td>
<td>&lt;0.0001</td>
<td>2.662 (1.546–4.583)</td>
</tr>
<tr>
<td>ACE DD or ACE 8 GG + MTHFR 1298 AA</td>
<td>75</td>
<td>72</td>
<td>&lt;0.0001</td>
<td>2.093 (1.458–3.000)</td>
</tr>
</tbody>
</table>

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Associated Polymorphisms and the Risk of Coronary Artery Disease

R Palma dos Reis, Ana Isabel Freitas, Sónia Freitas, Ana Célia Sousa, Paula Faría, Susana Gomes, Bruno Silva, António Brehm, António Almada Cardoso, Maria Isabel I Mendonça, Central Hosp of Funchal, Funchal, Portugal

Introduction: Various studies compare coronary artery disease (CAD) patients with controls in order to determine which polymorphisms are associated with a higher risk of disease. The results have often been contradictory. Moreover, the studies have evaluated isolated polymorphisms and not associations of polymorphisms, which is the way they occur in nature. Objective: our purpose was to evaluate the risk of CAD in patients with associated polymorphisms in the same gene or in different genes. Methods: we evaluated in 286 CAD patients and 520 healthy individuals the risk associated with ACE DD, ACE 8 GG, AGT 235 TT, MTHFR 677 TT and 1298 AA polymorphisms. We then evaluated the risk of associated polymorphisms in the same gene. Finally, for the isolate polymorphisms which were significant, we evaluated the risk of polymorphism associations at different functional levels Results: isolated polymorphisms presented with a higher frequency in the cases and are linked in a significant way to the CAD group (ACE DD genotype [P < 0.0001], ACE 8 GG [P < 0.015]; MTHFR 1298 AA [P < 0.011]). The association of polymorphisms in the same gene did not have an additive or synergistic effect, nor did it increase the risk for CAD. The polymorphic associations in different genes (ACE DD or ACE 8 GG + AGT 235 TT, ACE DD or ACE 8 GG + MTHFR 1298 AA) increased the risk for CAD compared with isolated polymorphisms (OR 2.9 vs. 1.8). Conclusions: the association of mutated polymorphisms in the same gene did not increase the risk of the isolated polymorphism. The association of mutated polymorphisms in genes belonging to different enzymatic systems was always linked to an increased risk, when compared with the isolated polymorphisms. This study points to the possibility of understanding the CAD genetic risk from a global point of view and not by regarding each isolate polymorphism.

ASSOCIATED POLYMORPHISM

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CARDIAC AUTOPHAGY AS A MALADAPTIVE RESPONSE TO HEMODYNAMIC STRESS

Hongxin Zhu, Paul Tannous, Janet Johnstone, Yongli Kong, John M Shelton, James A Richardson, Beth Levine, Beverly A Downes, Joseph A Hill, UT Southwestern, Dallas, TX

Background: Autophagy is a highly conserved mechanism of protein and organelle degradation linked to several neurodegenerative diseases. Nothing is known about the possible role of autophagy in heart failure. Methods and Results: Short-term nutrient deprivation, an established trigger of autophagy, induced dramatic increases in the ratio of LC3-II/LC3-I in ventricular lysates, indicative of increased autophagic activity. To determine the specific contribution of cardiomyocyte autophagy, we generated alphaMHC-GFP-LC3 transgenic mice. Short-term starvation induced a robust increase in the punctation pattern of P62-LC3 in myocytes, demonstrating cardiomyocyte autophagy and confirming the validity of our “autophagy reporter” mice. To test for autophagy in failing heart, we induced pressure overload by severe constriction of the thoracic aorta (sTAB). Fluorescence microscopy demonstrated autophagosome-localized GFP-LC3 in left ventricular myocytes 24h after banding which peaked at 48h and subsequently declined (though remaining elevated relative to control,
The mitogen-activated protein kinase (MAPKs) function as central mediators of numerous biologic processes such as control of cellular growth, differentiation, and survival. The extracellular signal-regulated kinases (ERKs) constitute one major branch of the MAPK signaling cascade that is activated in cardiomyocytes through G protein coupled receptors, tyrosine kinase receptors, integrines, stretch, and other diverse stress stimuli. ERK1/2 are directly activated by MEK1, an upstream MAPKK that specifically phosphorylates the TxY motif in the activation loop of ERK1/2. Overexpression of activated MEK1 in the heart by transgenesis induced specific activation of only ERK1/2, which was associated with the early development and near normal maintenance of cardiac hypertrophy of Dcx−/− mice. BMDO J. 2000;19:6341–6350). These results, in conjunction with other published accounts, have solidified the hypothesis that MEK1-ERK1/2 signaling is sufficient to mediate/initiate the cardiac hypertrophic response in vivo. However, the necessity or requirement of ERK1/2 in mediating cardiac hypertrophy in vivo has not been elucidated. Here, we report that ERK1/2, as well as erk1/2−/− gene-targeted mice, as well as mice expressing a bi-transgenic, tet-inducible MKP3 (Usp6p) cDNA in the heart to specifically inactivate ERK1/2 at any time. Loss of erk1 or heterozygosity of erk2 did not alter the cardiac hypertrophic response following pressure overload or neuroendocrine agonist stimulation, despite having a significant reduction in total ERK activity in the heart. Moreover, tet-inducible expression of MKP3 in the heart, which completely inactivated all cardiac ERK activity (but not other MAPKs), did not alter the heart to hyper trophy following pressure overload stimulation. Thus, our results suggest that ERK1/2 are sufficient to induce the cardiac hypertrophic response, but they are not required to mediate it.

The Cardiovascular Transcription Factor Kruppel-like Factor 5 Inhibits Apoptosis Through a Mechanism Involving Interaction with Poly(ADP-ribose) Polymerase-1 and Further Regulation by Acetylation

The low molecular weight GTPases of the Ras super-family are molecular switches cycling between a GTP-bound active state and GDP-bound inactive state. Ras GTPases play critical roles in gene expression, apoptosis, and cytoskeleton regulation in various cell types. Previous in vitro studies in neonatal cardiomyocytes suggested that Ras GTPases are involved in hypertrophy induced by angiotensin II, phenylephrine or leukocyte inhibitor factor. However, very little is known concerning Rho family members functions in vivo, especially Cdc42. Here, we investigated the function of Cdc42 in the heart using a cardiac-specific gene-targeting strategy in which loxP sites flanking the Cdc42 locus were subjected to recombination with a Cre recombinase. This model demonstrates an absence of dystrophin and a decrease in other DAPC components. Mdx mice exhibit mild cardiomyopathy. iSOG deficiency manifests as Limb Muscle Dystrophy (LMD25F), with DCM occurring in patients. A hamster strain that is iSOG deficient (TO2) was identified as a DCM model. We have confirmed chamber dilatation and systolic dysfunction in the TO2 model. This study examined kinase activation in the Mdx and TO2 models. Methods/ Results: Kinase phosphorylation and activation was determined by phospho-specific antibodies. Enhanced ERK activation was observed in the Mdx and TO2 models as compared to C57 and F1B control animals, respectively. ERK activation in these models was accompanied by an increase in Raf Ser259 phosphorylation. This was unexpected in that this Akt mediated phosphorylation event reportedly inhibits the ERK pathway. Additionally, an increase in FAK Tyr927 and Akt Tyr326 phosphorylation was observed in the Mdx model, indicating activation of byyrokinase kinase pathways. Finally, an enhancement of GSK-3 phosphorylation was observed in the Mdx model. This corresponds with the finding of increased Raf Ser259 phosphorylation to suggest that the Akt pathway is activated in the Mdx and TO2 models. Conclusion: This is the first report that correlates enhanced ERK activation and Raf Ser259 phosphorylation in the hearts of Mdx and TO2 animals. DAPC dissociation presumably reverses a negative regulation of the ERK pathway. This corresponds to studies which demonstrate enhanced myocardial ERK activation and cardiomyopathy in a Cav3 knockout mouse model. Raf Ser259 phosphorylation and increased ERK activation might indicate an Akt mediated blockade of the Ras-Raf pathway and the release of a sequestered pool of activated ERK subsequent to the dissociation of DAPC. ERK activation could function as a contributor to the pathology of muscular dystrophy or as a compensatory response.

Role of 14–3–3 Protein in Cardiac Sarcoplasmic Reticulum

The 14–3–3 family of intracellular dimeric phosphoserine-binding proteins regulate signal transduction, cell cycle, apoptotic, and metabolic cascades. Previous work with global 14–3–3 protein inhibitors suggested that these proteins play a critical role in antagonizing apoptotic cell death that occurs in response to provocative stimuli. To determine the specific role of one family member in apoptosis, mice were generated with targeted disruption of the 14–3–3σ gene. Although 14–3–3σ mice did not survive embryonic development, haplosufficient mice appeared normal at birth and were fertile. Baseline cardiac structure and function was normal in 14–3–3σ mice; however, cultured adult cardiomyocytes derived from 14–3–3σ mice were sensitized to the development of apoptosis in response to treatment with hydrogen peroxide or UV irradiation. In addition, 14–3–3σ mice were intolerant of experimental myocardial infarction and most animals died from acute ventricular rupture in the days following surgery. The 14–3–3σ mice that survived myocardial infarction were sensitized to develop pathological ventricular remodeling with increased cardiomyocyte apoptosis in the infarct border zone when compared to wild type littermates. Analysis of the activity of signaling proteins in cardiac lysates revealed that ASK1, JNK and p38 MAPK activation was increased, but ERK1/2 activation was reduced, in 14–3–3σ mice with a general p38 MAPK inhibitor, SB202190, significantly reduced the mortality rate observed after experimental myocardial infarction. These results demonstrate that 14–3–3σ mice plays a critical anti-apoptotic function in cardiomyocytes and therapeutic agents that increase 14–3–3σ activity may be beneficial to patients with MI.

Enhanced Myocardial ERK Activation in Animal Models of Duchenne Muscular Dystrophy and Limb Girdle Muscular Dystrophy 2F

Stephen C Armstrong, Melody A Scott; Cardiovascular Rsch Inst, Sioux Falls, SD

Background: Dystrophin associates with a protein complex (DAPC) which includes: an extracellular protein, α-dystroglycan; the transmembrane glycoproteins, β-dystroglycan and α, γ, δ- sarcoglycans (SG); and caveolin-3 (Cav3). A specific deficiency manifests as Duchenne or Becker muscular dystrophy and dilated cardiomyopathy (DCM). The Mdx mouse model demonstrates an absence of dystrophin and a decrease in other DAPC components. Mdx mice exhibit mild cardiomyopathy. iSOG deficiency manifests as Limb Muscle Dystrophy (LMD25F), with DCM occurring in patients. A hamster strain that is iSOG deficient (TO2) was identified as a DCM model. We have confirmed chamber dilatation and systolic dysfunction in the TO2 model. This study examined kinase activation in the Mdx and TO2 models. Methods/ Results: Kinase phosphorylation and activation was determined by phospho-specific antibodies. Enhanced ERK activation was observed in the Mdx and TO2 models as compared to C57 and F1B control animals, respectively. ERK activation in these models was accompanied by an increase in Raf Ser259 phosphorylation. This was unexpected in that this Akt mediated phosphorylation event reportedly inhibits the ERK pathway. Additionally, an increase in FAK Tyr927 and Akt Tyr326 phosphorylation was observed in the Mdx model, indicating activation of byyrokinase kinase pathways. Finally, an enhancement of GSK-3 phosphorylation was observed in the Mdx model. This corresponds with the finding of increased Raf Ser259 phosphorylation to suggest that the Akt pathway is activated in the Mdx and TO2 models. Conclusion: This is the first report that correlates enhanced ERK activation and Raf Ser259 phosphorylation in the hearts of Mdx and TO2 animals. DAPC dissociation presumably reverses a negative regulation of the ERK pathway. This corresponds to studies which demonstrate enhanced myocardial ERK activation and cardiomyopathy in a Cav3 knockout mouse model. Raf Ser259 phosphorylation and increased ERK activation might indicate an Akt mediated blockade of the Ras-Raf pathway and the release of a sequestered pool of activated ERK subsequent to the dissociation of DAPC. ERK activation could function as a contributor to the pathology of muscular dystrophy or as a compensatory response.

Cardiac-Specific Deletion of the Small Rho GTPase Cdc42 Reveals Its Pivotal Function in Cardiac Hypertrophy

Marjorie S Mailet, Bastiano Sanna, Yi Zheng, Jeffrey D Molkenst; Cincinnati Children’s Resch Foundation, Cincinnati, OH

The low molecular weight GTPases of the Ras super-family are molecular switches cycling between a GTP-bound active state and GDP-bound inactive state. Ras GTPases play critical roles in gene expression, apoptosis and cytoskeleton regulation in various cell types. Previous in vitro studies in neonatal cardiomyocytes suggested that Ras GTPases are involved in hypertrophy induced by angiotensin II, phenylephrine or leukocyte inhibitor factor. However, very little is known concerning Rho family members functions in vivo, especially Cdc42. Here, we investigated the function of Cdc42 in the heart using a cardiac-specific gene-targeting strategy in which loxP sites flanking the Cdc42 locus were subjected to recombination with a Cre recombinase. This model demonstrates an absence of dystrophin and a decrease in other DAPC components. Mdx mice exhibit mild cardiomyopathy. iSOG deficiency manifests as Limb Muscle Dystrophy (LMD25F), with DCM occurring in patients. A hamster strain that is iSOG deficient (TO2) was identified as a DCM model. We have confirmed chamber dilatation and systolic dysfunction in the TO2 model. This study examined kinase activation in the Mdx and TO2 models. Methods/ Results: Kinase phosphorylation and activation was determined by phospho-specific antibodies. Enhanced ERK activation was observed in the Mdx and TO2 models as compared to C57 and F1B control animals, respectively. ERK activation in these models was accompanied by an increase in Raf Ser259 phosphorylation. This was unexpected in that this Akt mediated phosphorylation event reportedly inhibits the ERK pathway. Additionally, an increase in FAK Tyr927 and Akt Tyr326 phosphorylation was observed in the Mdx model, indicating activation of byyrokinase kinase pathways. Finally, an enhancement of GSK-3 phosphorylation was observed in the Mdx model. This corresponds with the finding of increased Raf Ser259 phosphorylation to suggest that the Akt pathway is activated in the Mdx and TO2 models. Conclusion: This is the first report that correlates enhanced ERK activation and Raf Ser259 phosphorylation in the hearts of Mdx and TO2 animals. DAPC dissociation presumably reverses a negative regulation of the ERK pathway. This corresponds to studies which demonstrate enhanced myocardial ERK activation and cardiomyopathy in a Cav3 knockout mouse model. Raf Ser259 phosphorylation and increased ERK activation might indicate an Akt mediated blockade of the Ras-Raf pathway and the release of a sequestered pool of activated ERK subsequent to the dissociation of DAPC. ERK activation could function as a contributor to the pathology of muscular dystrophy or as a compensatory response.

Diaclylglycerol Kinase ζ Prevents Cardiac Remodeling by Mechanical Overload

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Background: Gq protein-coupled receptor (GPCR) signaling pathway including diacylglycerol (DAG) and protein kinase C plays a critical role in the development of cardiac hypertrophy and heart failure. DAG kinase (DGK) phosphorylates DAG and controls cellular DAG levels, and thus is thought to be acting as a regulator of GPCR signaling. We have previously reported that DGK inhibits GPCR agonist-induced cellular DAG accumulation, activation of the downstream signaling cascade and subsequent cardiomyocyte hypertrophy. In this study, we examined
whether DGK modifies cardiac remodeling by mechanical overload. **Methods and Results:** Thoracic transverse aortic constriction (TAC) was created in transgenic mice with cardiac-specific overexpression of DGKeta (DGK-G) and wild-type (WT) mice. Increases in heart weight at 4 weeks after TAC were attenuated in DGK-G mice compared to WT mice. Increases in inter-ventricular septal thickness, dilatation of the left ventricular cavity, and decreases in left ventricular systolic function were observed with echocardiography in WT mice at 4 weeks after TAC surgery. However, these structural and functional changes after TAC were attenuated in DGK-G mice. Up-regulation of atrial natriuretic factor and beta-mimycin heavy chain mRNAs was observed after TAC in WT but not in DGK-G mice. Next, left anterior descending coronary artery (LAD) was ligated in DGK-G and WT mice. Left ventricular chamber dilatation, left ventricular systolic dysfunction, and increases in left ventricular weight and lung weight at 4 weeks after myocardial infarction were attenuated in DGK-G mice compared to WT mice. In the non-infarct area, cardiac fibrosis and up-regulation of profibrotic genes such as transforming growth factor-beta 1, collagen type I, and collagen type III were observed at 4 weeks after myocardial infarction in WT mice. However in DGK-G mice, cardiac fibrosis and profibrotic gene induction were blocked. The survival rate after myocardial infarction was higher in DGK-G mice than in WT mice. **Conclusion:** DGKeta suppresses cardiac structural remodeling after TAC and myocardial infarction. DGKeta may be a potential novel therapeutic target to prevent cardiac remodeling in response to mechanical overload.

**P77**

DnaJb5, an Hsp40 Family Protein, Mediates Anti hypertrophic Effects of Thioredoxin1 in the Heart

Tetsuro Ago, Junichi Sadoshima; UMDNJ-NJMS, Newark, NJ

Thioredoxin1 (Trx1) plays an important role in reducing redox-sensitive proteins, thereby regulating critical cellular functions, such as growth and apoptosis. We have previously shown that cardiac hypertrophy by pressure-overload is suppressed in transgenic mice with cardiac specific overexpression of Trx1 (Tg-Trx1). However, the molecular mechanism by which Trx1 inhibits hypertrophy remains poorly understood. CDNA microarray analyses revealed that expression of DnaJb5, an Hsp40 family protein, is significantly upregulated in Tg-Trx1 hearts. Because another Hsp40 family protein (Mj) was recently shown to inhibit the transcriptional activity of NFAT3 through recruitment of class II HDAC to the nucleus, we hypothesized that Trx1 attenuates NFAT activity through DnaJb5, thereby suppressing cardiac hypertrophy. Protein expression of DnaJb5 was increased in both Trx1-overexpressed cardiomyocytes and Trx1-Tg. Upregulation of Dnajb5 is mediated by HSF-1, a redox-sensitive transcription factor, since both basal expression and activity of HSF-1 were enhanced in Trx1-overexpressed cardiomyocytes and because shRNA-mediated knockdown of HSF-1 attenuated Trx1-induced upregulation of DnaJb5. Immunostaining and western blot analyses indicated that Trx1 and DnaJb5 are colocalized in the nucleus. In vitro pull-down binding assays indicated that DnaJb5 associates with Trx1 through TNP1, a Trx1-binding protein. Both Trx1 and DnaJb5 attenuated phenylpyrene (PE)-induced increases in the transcriptional activity of NFAT as well as hypertrophy without showing additive effects, suggesting that Trx1 and DnaJb5 are on the same pathway leading to inhibition of NFAT and hypertrophy. Furthermore, knocking-down of DnaJb5 attenuated Trx1-induced inhibition of PE-induced cardiac hypertrophy. By making a genetic cross between Tg-Trx1 and transgenic mice harboring the NFAT luciferase reporter, it was found that both NFAT activation and cardiac hypertrophy by PE are significantly suppressed in the presence of Trx1 in vivo. In summary, Trx1 upregulates DnaJb5, colocalizes with the nucleus, and inhibits NFAT. DnaJb5 is a critical downstream effector of Trx1-induced upregulation of DnaJb5, a Hsp40 family protein, is significantly upregulated in Tg-Trx1 hearts. In addition, DnaJb5 expression was increased in cardiomyocytes of Tg-Trx1 hearts and inhibited NFAT activity measured by Amplex Red fluorescence assay kit (P<0.01). Peak LV developed pressure (LVDP) and LV end diastolic pressure (LVEDP) measured using Langendorff-perfusion analysis were significantly increased in WT-iso when compared to sham and hKO-iso groups (P<0.01). But how myotrophin triggers such a signaling cascade for hypertrophic response is unknown. In the present study, using neonatal rat cardiomyocytes, we identified potential sites of the protein in two hairpin-like loops that is responsible for its stimulatory activity by activating NFAT signaling. The increase in reactive oxygen species (ROS) production associated with reduced NO production would create nitric-oxide imbalance, which could contribute to obesity-related cardiac disease. **Methods and Results:** We studied 2–6 month old ob/ob and C57/6 controls. Our results demonstrated that cardiac NOS1 protein abundance (P<0.01) and mRNA expression (P=0.03) were reduced in ob/ob mice compared to WT mice. Decreased cardiac NOS1 protein abundance would reduce NO availability, thereby initiating cardiac hypertrophy, but also initiates cross talk with the ER stress pathway, thereby inhibiting cardiac hypertrophy.

Reduced Neuronal Nitric Oxide Synthesis Expression Concentrations to Increased Oxidative Stress and Nitroso-Redox Imbalance in Murine Model of Obesity

Roberto M Saravia, Khalid M Minhas, Meizi Zhang, Eleanor Pitz, Karl H Schuleri, Koenraad V M Vandekerckhove, Deochun Li, Lili A Barouch, Joshua M Hare; Johns Hopkins Med Inst, Baltimore, MD

Background: Disruption of leptin signaling pathway may contribute to obesity-related cardiac disease, as leptin deficient (ob/ob) mice display cardiac hypertrophy, impaired β-adrenergic signaling, increased cardiac apoptosis and reduced survival. Since leptin maintains a tonic level of neuronal nitric oxide synthase (NOS1) expression in the brain, we hypothesized that leptin deficiency would decrease cardiac expression of NOS1. As NOS1 inhibits xanthine oxidoreductase (XOR), NOS1 downregulation would increase XOR activity. The increase in reactive oxygen species (ROS) production associated with reduced NO production would create nitric-oxide imbalance, which could contribute to obesity-related cardiac disease. **Methods and Results:** We studied 2–6 month old ob/ob and C57/6 controls. Our results demonstrated that cardiac NOS1 protein abundance (P<0.01) and mRNA expression (P=0.03) were reduced in ob/ob mice compared to WT mice. Decreased cardiac NOS1 protein abundance would reduce NO availability, thereby initiating cardiac hypertrophy, but also initiates cross talk with the ER stress pathway, thereby inhibiting cardiac hypertrophy.

Intrinsic Mechanism of Myotrophin-Driven Cardiac Hypertrophy in Neonatal Rat Cardiomyocytes

Biswajit Das, Sudhiranjan Gupta, Subha Sen; Lerner Rech Inst, Cleveland, OH

Myotrophin, an ankyrin repeat protein, is known to play an important role in the initiation of cardiac hypertrophy by stimulating protein synthesis in rat cardiomyocytes that leads to heart failure and is correlated with activation of the NF-κB signaling cascade [JBC, 1998; JCR, 2002; JBC, 2005]. Myotrophin activates NF-κB but how myotrophin stimulates cardiac hypertrophy remains unknown. In the present study, using neonatal rat cardiomyocytes, we examined the localization and trafficking of myotrophin that was added to the external media. Alexa-488 conjugated recombinant myotrophin internalized within 5 min of its introduction. With further time, next to 60 min, myotrophin translocated into the nucleus. NF-κB signaling was upregulated simultaneously. Incorporation of myotrophin into the cell increases protein synthesis (50.39% increase over control), Genistein (a tyrosine kinase inhibitor) pre-treatment significantly inhibited the incorporation of myotrophin into cytoplasm suggesting that internalization of myotrophin is through a tyrosine kinase-mediated pathway. Baisain et al. have shown that myotrophin forms a complex with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and PERK. As GAPDH and PERK are potential targets of myotrophin, we have identified potential sites of the protein in two hairpin-like loops that is responsible for its stimulatory activity by activating NF-κB pathway. By dissect out this structure-function relationship of myotrophin, we have generated 10 myotrophin mutants to define their trafficking, ability to activate NF-κB pathway and subsequent NF-κB activation. Observations obtained from this study will enable us to understand how myotrophin stimulate cardiac hypertrophy.
cardiovascular disease and how myotrophin interacts with components of the NF-κB pathway to initiate cardiac hypertrophy.

**BVCs Symposium Abstracts**

P84 Enhanced Na+/H+ Exchanger Activity Contributes to Myocardial Ischemia/Reperfusion Injury and Cardiac Hypertrophy

Fatima Mraza, Ivanz Baczko, Peter Light, Larry Fiegel; Univ of Alberta, Edmonton, Canada

The Na+/H+ exchanger isoform 1 (NHE1) is a integral membrane protein, found in the myocardium. Recent evidence suggests that NHE1 is an important contributor to myocardial ischemia/reperfusion (IR) injury and a mediator of cardiac hypertrophy. To examine the effect of increased NHE1 expression, we created two transgenic models that express NHE1 in the mouse myocardium. The N-line overexpresses wild type NHE1 and the K-line overexpresses a slightly hyperactive human NHE1. Western blot analysis showed that NHE1 was targeted to the myocardium. NHE activity of adult ventricular cardiomyocytes was elevated in the K-line and N-line (350 ± 12 and 210 ± 2.5 vs. controls [110 ± 3.0]). We examined the sensitivity of field-stimulated N-line cardiomyocytes to IR injury using a metabolic model of inhibition and reoxygenation. The increase in diastolic [Ca2+]i associated with reoxygenation was larger in N-line cardiomyocytes (42 ± 6.8%) vs. controls (22 ± 5.5%, P<0.05). NHE 694 (5 μM) and a NHE1 inhibitor attenuated this difference. To examine the effects of NHE1 expression on heart susceptibility to hypertrophy, we examined cell area and the heart weight to body weight ratio (HW/BW). The cell area of isolated cardiomyocytes was larger in the K-line (111 ± 4.7) vs. controls (100 ± 2.6, P<0.05). The K-line mice also had a greater HW/BW (130.4 ± 4.6%) vs. controls (117 ± 3.8, P<0.05). FcεRI and phospho-ephrin via omeprazole (50mg/kg/day) produced a further increase in the HW/BW in the K-line mice (150.7 ± 10.7). (controls 117.9 ± 7.0, P<0.05). However, angiotensin II (200ng/kg/min) had no effect on the HW/BW in the N-line or K-line mice. These results demonstrate that overexpression of NHE1 directly causes increased diastolic Ca2+ overload during cardiac IR and that enhanced NHE1 activity increases cell area, heart weight to body weight ratio and susceptibility to hypertrophic stimuli.

P85 Cardiac-Specific SUR1 Expression Disrupts Cardiac Sarcolemmal ATP-Sensitive Potassium Channel Activity and Impairs the Cardiac Response to Systolic Overload

Xinti Hu, Xin Xu, Univ of Minnesota, Minneapolis, MN; Thomas Flagg, Washington Univ, St. Louis, MO; Ping Zhang, Univ of Minnesota, Minneapolis, MN; Colin Nichols, Washington Univ, St. Louis, MO; Robert J Bache, Yingjie Chen; Univ of Minnesota, Minneapolis, MN

A recent report described several patients with idiopathic cardiomyopathy who had mutations of ABCG2, the gene that encodes the regulatory subunit SUR2A of the cardiac myocyte sarcolemmal ATP sensitive potassium channels (s-KATP), suggesting that impaired s-K ATP function might be a cause of cardiomyopathy. Consequently, this study examined whether K ATP dysfunction impairs the cardiac response to hemodynamic overload. We recently developed a strain of transgenic mice overexpressing the SUR1 subunit under transcriptional control of the alpha-mysin heavy chain promoter. SUR1-tg mice demonstrate diminished s-K ATP current density, essentially generating a cardiac specific K ATP knockdown model. Transgenic mice develop and grow normally without obvious cardiovascular defects or dysfunction. Although thereby no significant difference in heart weight or left ventricular (LV) function between SUR1-tg mice and wild type littermates (WT) under unstrained conditions, SUR1 over expression resulted in significant increases of myocardial p-mTOR, total-mTOR, p-S6K1, and p-4EBP1, suggesting that expression of SUR1-tg mice after 4 week development more severe LV dysfunction (LV ejection fraction decreased to 49.9 ± 6.8%) vs. controls (22 ± 5.7% in F/F; Vcf 0.6 in F/F, n=3, P<0.05), as compared to 84% in SUR1-tg mice after 4 week development more severe LV dysfunction (LV ejection fraction decreased to 49.9 ± 6.8%) vs. controls (22 ± 5.7% in F/F; Vcf 0.6 in F/F, n=3, P<0.05). The K-line mice also had a greater HW/BW (130.4 ± 4.6%) vs. controls (117 ± 3.8, P<0.05). Administration of omeprazole via osmotic pumps (50mg/kg/day) produced a further increase in the HW/BW in the K-line mice (150.7 ± 10.7). (controls 117.9 ± 7.0, P<0.05). However, angiotensin II (200ng/kg/min) had no effect on the HW/BW in the N-line or K-line mice. These results demonstrate that overexpression of NHE1 directly causes increased diastolic Ca2+ overload during cardiac IR and that enhanced NHE1 activity increases cell area, heart weight to body weight ratio and susceptibility to hypertrophic stimuli.

P83 Myocyte-Specific Excision of the Murine Vinculin Gene Leads to Arrhythmias, Sudden Death, and Heart Failure Due to Dissolution of the Intercalated Disc Structure


Vinculin (Vin) is a ubiquitously expressed actin binding protein that plays a dynamic role in the actin cytoskeleton assembly. Vin is located at intercalated discs (ICDs) as well as in cell-to-matrix adhesions. Metavinculin (MVin) is a muscle-specific splice variant of Vin that is expressed in adult heart. Homozygous Cre-excision of Vin showed no indication of embryonic lethality (n=125). Younger mice (6–10 week-old) with preserved cardiac function nonetheless showed: A) cardiac specific inactivation of the Vin gene. Analysis of this cardiac specific Vin KO model indicates that reduced Vin protein expression at the cell-to-cell junctions causes dissolution of the ICD (cell-to-cell cardiomyopathy) and therefore leads to both electrical as well as contractile dysfunction. This animal model provides new evidence of Vin’s role in mechanical/electrical coupling of cardiac myocytes in the working myocardium.

P86 Overexpression of Calreticulin in Adult Cardiac Myocytes is Not the Cause of Lethality in Mice with Expression of “Activated” α5 Integrin

Iva Neveux, John A McDonald, Maria L Valencik; Univ of Nevada Sch of Med, Reno, NV

We have shown that expression of a constitutively active or “unregulated” α5 integrin subunit, designated α5-I, in perinatal or adult mouse heart resulted in severe conduction defects, rapid onset cardiomyopathy and death. Expression profiling of hearts in which α5-I integrin expression was induced de novo revealed dramatic increases in calreticulin mRNA and protein. Interestingly, the phenotype of mice over expressing calreticulin neonatally resembled that of the α5-I mice. This implicated calreticulin as a proximal mediator of the deleterious effects of α5-I integrin. To answer this question, we used our α5-I integrin specific transgenic mouse model to express high levels of calreticulin in neonatal and adult cardiac myocytes. Induction of increased calreticulin in neonates did lead to cardiac conduction abnormalities as previously reported. However, over expression of calreticulin in the adult heart for up to 2 months did not result in cardiomyopathy. Therefore, while calreticulin expression is increased in α5-I transgenic mice, calreticulin is not the cause of the rapid onset heart failure.
Adverse Effects on Cardiomyocyte Function of Removing the Inhibition of the Sarco(endo)plasmatic Reticulum Ca2+ Transport ATPase by Phospholamban

Virginia Bito, Peter Vangheluwe, William E Louch, Frank Wuytack, Karin R Spido; K U Leuven, Leuven, Belgium

Background. Replacement of the cardiac sarco(endo)plasmatic reticulum Ca2+ ATPase SERCA2a by its SERCA2b splice variant with a higher Ca2+ affinity (skO mice) induces left ventricular hypertrophy (LVH). Although SERCA2a and SERCA2b are both upregulated in the early stages of LVH, SERCA2b mRNA levels declined in 10-month-old skO mice as compared with young skO mice. To investigate whether the severity of LVH phenotypes relates to the observed further reduced cardiac SERCA2a content (33% vs WT), we analyzed cardiomyocyte Ca2+ handling in skO mice. Methods. Control and skO mice were isolated from adult DKO and WT mice. Ca2+ uptake was monitored at 30°C under whole-cell voltage-clamp using X-Fluo-3. Results. In both DKO and WT cardiomyocytes, the amplitude of the [Ca2+]i transient showed a negative frequency dependence. At 8 Hz, the amplitude of the Ca2+ transient was significantly reduced compared to those in skO mice (n = 151; 1:2:3:5 Hz; P < 0.05). The Ca2+ transient was removed in DKO at 1 Hz (n = 101: 8.5 vs 11.1 Hz in WT; P < 0.05), but normalized to WT values at 8 Hz (n = 8.2 in skO, n = 8.3 ± 1.13 in WT). In 1 Hz, diastolic [Ca2+]i was similar in DKO and WT (n = 131: 1:12 in DKO vs. 1:9 ± 1:4 Hz in WT). In both groups LVH was induced. However, increase in Ca2+ in skO was significantly greater when compared to that in WT (Ca2+ i in nM: 196 ± 23 in DKO vs. 312 ± 37 in WT; P < 0.05). The SR Ca2+ content was derived from the Ca2+ transient recorded after application of 10 mM caffeine. In WT, the caffeine-evoked [Ca2+]i transient at 1 Hz was larger compared to WT but in contrast to WT, it did not significantly increase at 8 Hz. Forskolin (10 μM) increased the amplitude of the [Ca2+]i transient and the rate of [Ca2+]i removal in WT, but not in DKO. Conclusions. Increasing the Ca2+ affinity of the SERCA2b/PLB interaction can adequately increase SR Ca2+ uptake even in the context of reduced SERCA2b levels. However, normal SR Ca2+ handling in DKO does not prevent LVH. In addition, the lower case of SR Ca2+ content with frequency and impaired β-adrenergic response is likely responsible for the stress intolerance and high mortality of the DKO mice.

Compensatory Mechanisms Protect the Heart Against Changes in the Ca2+ Affinity of the Sarco(endo)plasmatic Reticulum Ca2+ Transport ATPase

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In skO mice, the cardiac sarco(endo)plasmatic reticulum Ca2+ pump SERCA2a was replaced by SERCA2b, a higher Ca2+ affinity isofrom. Interestingly, the imposed high Ca2+ affinity was countered by a spontaneous increase in phosphorylation (PLB) inhibition, with beneficial effects on cardiac function. The 50% lower cardiac SERCA2 levels in skO may serve the same purpose, i.e., preventing excessive cytosolic Ca2+ uptake at low Ca2+ concentrations [Ca2+], between 0.05–0.4 μM. In fact, the low SERCA2 levels only moderately affected Ca2+ uptake activity in this concentration range. We now (A) compared the compensatory response in heterozygous mice (WT) and (B) crossbred our skO mice with mice overexpressing SERCA2b (M. Ponzacks). We investigated if higher SERCA2 levels would further increase PLB inhibition. (A) In the wild-type (WT) heart, 2% of the SERCA2 content consists of SERCA2b, whereas SERCA2a makes up the remainder. HT mice contain one SERCA2 allele from which only SERCA2b can be formed and one WT allele, of which the transcripts can be spliced to SERCA2a or SERCA2b mRNA. Expression of both alleles would allow a 50/50% SERCA2a/2b distribution in the HT heart. However, cardiac SERCA2a expression predominated (85%) over SERCA2b (15%). In addition, this selective suppression of SERCA2b correlated with an overall reduced SERCA2 content (∼35% vs WT). In contrast to homozygous skO, the lower SERCA2a levels in HT were compensated by a reduced PLB inhibition (2-fold less protein, but increased phosphorylation vs. WT). (B) Overyexpression of SERCA2b in skO mice resulted in a 20% increase in cardiac SERCA2 content, a similar increase in PLB levels and a further reduced PLB phosphorylation vs. skO. Also, the increased SERCA2 levels did not counter hypertrophy, indicating that the hypertrophy in skO is not related to the low SERCA2 content. In conclusion, enforced expression of the higher Ca2+ affinity SERCA2b was spontaneously countered by adjusting SERCA2b expression, PLB expression and PLB phosphorylation. These data support the view that the Ca2+ -uptake activity at lower micromolar Ca2+ concentrations is tightly controlled. Understanding these control mechanisms would open doors for new ways of adjusting the SERCA2 activity in the diseased heart.

Identification of a Progenitor Cell Population from Human Skeletal Muscle that is Superior to Committed Skeletal Myoblasts for Cardiac Cell Therapy

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Skeletal myoblast transplantation is being investigated for human cardiac repair; however, a recent Phase 2 cardiac cell therapy trial based on autologous skeletal myoblasts has ceased to enroll new patients due to lack of efficacy. Here we identify a progenitor cell population from human skeletal muscle biopsies that was superior to committed skeletal myoblasts for cardiac cell therapy. After the human skeletal muscle biopsy was digested, we used fluorescence-activated cell sorting (FACS) to isolate various fractions of mononuclear cells based on expression of the marker CD34 (CD34+CD144−) and two markers CD34+CD144+. We obtained 3 different cell populations: “myogenic” cells (committed skeletal myoblasts) (CD34− /CD34+/CD144− ), “endothelial” cells (CD34+/CD34− /CD144− ), and “myogenic-endothelial” cells (CD34+/CD34+/CD144− ). Each sorted cell population was then expanded in culture. These cells were then injected (105 cells/heart) into the ischemic myocardium of SCD mice after acute myocardial infarction. Two weeks later, echocardiography was performed to assess left ventricular (LV) heart function in n mice group. The hearts injected with myogenic-endothelial cells displayed significant improvement in cardiac contraction, as measured by percent fractional shortening and percent fractional area change, when compared not only with control saline-injected hearts (P<0.005), but also with endothelial cell- or myogenic cell-injected hearts (P<0.005). However, we did not observe improvement in LV cardiac contraction after transplantation of either the myogenic or endothelial cells when compared to control saline-injected hearts. Histologically, the transplanta- tion of myogenic-endothelial cells generated significantly more new skeletal myocytes and induced significantly more neangiogenesis (i.e., blood vessel density) in the infarct zone than did the transplantation of the myogenic and endothelial cell types (P<0.005). In conclusion, these findings suggest that human myogenic-endothelial cells represent a novel cell population that offers superior therapeutic benefit after myocardial infarction when compared to committed skeletal myoblasts or endothelial cells.
Thymus Expands During Correction of Congenital Heart Defects: A New Source of Multilineage Stem Cells
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Introduction: Stem cell transplantation and tissue engineering are attractive future strategies for functional myocardial repair. Multi-lineage stem cells (MSCL) are well qualified for this purpose and have already been isolated from the bone-marrow to far. In the present study, we aimed to isolate MLSC from the thymus gland of infants undergoing cardiac defect repair, where partial thymectomy represent a standard procedure for better cardiac access. Methods: After sternotomy, the thymus gland of 10 neonates/children undergoing cardiac surgery was removed and the tissue was microdissected and cultured. Multi-lineage potential test was conducted for three culture passages using differentiation cultures towards the osteogenic, chondrogenic and adipogenic lineage. Surface antigen expression was investigated in detail by FACS-analysis and results were compared with MLSC from the bone-marrow. Results: While the majority of isolated cells were non-adherent lymphocytes, we observed two types of adherent cells: primary and secondary culture primary. The epithelial cell colonies were refractory to passage. The remaining cells showed almost unlimited proliferation. Throughout the proliferation phase of more than 40 doublings a multi-lineage potential was re-evaluated. Full differentiation potential is maintained during this proliferation phase as confirmed by immunohistochemical staining and primary culture. We described a novel source for isolation of MLSC in neonates and young children undergoing cardiac surgery since the thymus is routinely dissected during pediatric cardiac surgery. Children who might undergo further cardiac surgeries might profit from the use of thymic MLSC for tissue engineering.

protein pre-coating of elastomeric tissue-engineering scaffolds:
Extracellular Matrix Formation and Phenotypic Changes of Culturing Endothelial Progenitor Cells

Background: Optimal cell sources and scaffold chemistry for cardiovascular tissue engineering remain unknown. We investigated seeding endothelial progenitor cell (EPC)-derived endothelial cells on a novel elastomeric scaffold, polyglycolic sebacate (PGS). We hypothesize that EPC and ECM formation could be enhanced by pre-coating PGS scaffolds with ECM proteins. Methods: Characteristic human peripheral blood EPC were seeded onto scaffolds for 3 days followed by 14 days in a laminar fluid flow system. We pre-coated the scaffolds with laminin (LM), fibronectin (FN), fibrin, collagen types I (Coll I), III (Coll III), or elastin (EL) (n=5; control uncoated, unseeded scaffolds). Results: EPC were CD31+/SMA- prior to seeding. Both coated and uncoated scaffolds revealed CD31+ and FN+ cells. Overall, the most enhanced histology was seen in FN pre-coated scaffolds. CONCLUSION: PGS offers the advantages of low stiffness and elasticity, allowing for large deformations. Pre-coating offers the additional advantage of controlling scaffold surface chemistry. Circulating EPC appear to have the potential to provide both interstitial and endothelial functions, and Fibrin pre-coated scaffolds allow a single cell source for construction of autologous heart valves.

AAV SDF-1 Augmented Myoblast Therapy for Cardiac Failure
Bijoy D Thattilaliath, Faris Al-Mousily, Sean Germain, Christina Packac, Stacy Porvanski, Melissa Lewis, Glenn Walter, Barry J Byrne; Univ of Florida, Gainesville, FL

During the past few years the number of patients with cardiac failure has increased following improved therapy and survival of patients following myocardial infarction(MI). Currently there is no approved modality of treatment to repair the resulting damage of ischemic injury. This study was done to evaluate the transplantation of AAV (Adeno-associated virus) SDF-1/Stromal Derived Factor-1 transduced myoblasts to the myocardium versus non-transduced myoblasts in improving cardiac function. SDF-1 is essential for cardiogenesis and vasculogenesis during development. SDF-1 levels are elevated post-ischemic injury and may have a role in the repair and regeneration of the damaged myocardium. We hypothesize that increased levels of SDF-1 with myoblasts will drive cells to the myocardium and promote repair process. AAV SDF-1 transduction into the infracted myocardium. Hydrogel is used in the study groups to improve cell retention and distribution within the graft. Our results confirm the hypothesis that pre-infarcted SDF-1 augmented myoblasts with and without hydrogel showed significant improved cardiac function. The cardiac output improved 8 weeks post-myoblast transplant compared to the media injected controls. The AAV SDF-1 transduced myoblast with and without hydrogel showed significant improvements in cardiac function. Conclusion: AAV transduced myoblasts with SDF1 will be beneficial in improvement of cardiac function compared to using only myoblasts.

Exercise Training Improves Cardiac Function in Diabetic Rats: Noninvasive Assessment Using High-Resolution Magnetic Resonance Imaging
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The diabetic heart is targeted by both coronary and non-coronary pathology that eventually results in cardiac failure. Cardiac function in diabetes can be improved by physical exercise among other interventions. However, the exercise induced benefits on cardiac cycle volume profiles and hemodynamics in diabetes are poorly understood. The importance of this information is underscored by the evidence that diabetic heart disease is associated with both systolic and diastolic left ventricular (LV) dysfunction. We used a non-invasive approach, MRI, to characterize the effects of exercise training on cardiac function in diabetics. Type 2 diabetic animals were randomized into three groups (n=4 per group; sedentary control - SC; sedentary diabetic - SD; and exercised diabetic - ED). At the termination of study, EKG gated cardiac MRI was performed with a 9.4T scanner to resolve LV cardiac cycle events. The systolic hemodynamic indices were derived from the LV volumetric data. The decrease in LV ejection fraction (EF) and diastolic volume in diabetes was prevented by exercise training (end diastolic volumes were 626.70±10.01, 431.94±8.33, and 584.10±7.80 μL in the SC, SD and ED groups, respectively). Exercise training also prevented the increase in LV end systolic volume in diabetes (end systolic volumes were 266.37±4.24, 224.13±2.81, and 269.49±1.73 μL in the SC, SD and ED groups, respectively). Accordingly the LV stroke volume and ejection fraction in diabetes were improved by exercise training of stroke volume was 418.33±8.48, 189.81±8.70 and 385.56±8.33 μL and ejection fraction was 66.74±0.58, 45.80±1.24, 65.65±0.80% in the SC, ED and SD groups, respectively. In diabetes, the LV output was 1.02±5.17 ml, 1.05±0.42 l and 0.99±2.65 l/min in, SC, SD and ED groups respectively, indicating that exercise training was able to prevent the decline in LV output that accompanies a sedentary lifestyle in diabetes. The time derivatives of LV volume in the exercised diabetic group approached the control levels. These results suggest that early intervention in the form of exercise training prevents cardiac dysfunction in diabetes.

Protein Precoating of Elastomeric Tissue-Engineering Scaffolds: Extracellular Matrix Formation and Phenotypic Changes of Culturing Endothelial Progenitor Cells
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Background: Optimal cell sources and scaffold chemistry for cardiovascular tissue engineering remain unknown. We investigated seeding endothelial progenitor cell (EPC)-derived endothelial cells on a novel elastomeric scaffold, polyglycolic sebacate (PGS). We hypothesize that EPC and ECM formation could be enhanced by pre-coating PGS scaffolds with ECM proteins. Methods: Characteristic human peripheral blood EPC were seeded onto scaffolds for 3 days followed by 14 days in a laminar fluid flow system. We pre-coated the scaffolds with laminin (LM), fibronectin (FN), fibrin, collagen types I (Coll I), III (Coll III), or elastin (EL) (n=5; control uncoated, unseeded scaffolds). Results: EPC were CD31+ , FN+ , and α-SMA + prior to seeding. Both coated and uncoated scaffolds revealed CD31+ and FN+ cells. Overall, the most enhanced histology was seen in FN pre-coated scaffolds. α-SMA+ cells were found both on the surface and in the “interstitium” of the scaffold. A range of α-SMA+ cells was observed: uncoated: <EL, <LM, <FN, Coll I< Fibrin, <Coll III. The presence of α-SMA+ cells correlated with enhanced and uniform expression of LM and FN within the surface and “interstitium” of pre-coated scaffolds comparable to native valve. Enhanced FN was observed: uncoated: <Coll III< Coll I< EL< Fibrin< LM. In contrast, Coll III were expressed in the surface. In addition, both CD31+ and α-SMA+ cells produced Coll I. Biochemical assays demonstrated increased DNA content: uncoated: <Coll I< Fibrin< Coll III< FN> Coll I< FN and collagen content (LM< EL< Fibrin< Coll III). Flexure testing demonstrated decreased effective stiffness of the seeded scaffolds, regardless of whether the PGS was pre-coated or uncoated (5.49 ± 0.39 vs. 8.13 ± 0.17 kPa, p<0.05). A range of cellularity was observed: uncoated: <EL< LM< Coll I< Fibrin< Coll III< FN. Overall, the most enhanced histology was seen in FN pre-coated scaffolds. CONCLUSION: PGS offers the advantages of low stiffness and elasticity, allowing for large deformations. Pre-coating offers the additional advantage of controlling scaffold surface chemistry. Circulating EPC appear to have the potential to provide both interstitial and endothelial functions, and can produce ECM on PGS allowing a single cell source for construction of autologous heart valves.

AAV SDF-1 Augmented Myoblast Therapy for Cardiac Failure
Bijoy D Thattilaliath, Faris Al-Mousily, Sean Germain, Christina Packac, Stacy Porvanski, Melissa Lewis, Glenn Walter, Barry J Byrne; Univ of Florida, Gainesville, FL

During the past few years the number of patients with cardiac failure has increased following improved therapy and survival of patients following myocardial infarction(MI). Currently there is no approved modality of treatment to repair the resulting damage of ischemic injury. This study was done to evaluate the transplantation of AAV (Adeno-associated virus) SDF-1/Stromal Derived Factor-1 transduced myoblasts to the myocardium versus non-transduced myoblasts in improving cardiac function. SDF-1 is essential for cardiogenesis and vasculogenesis during development. SDF-1 levels are elevated post-ischemic injury and may have a role in the repair and regeneration of the damaged myocardium. We hypothesize that increased levels of SDF-1 with myoblasts will drive cells to the myocardium and promote repair process. AAV SDF-1 transduction into the infracted myocardium. Hydrogel is used in the study groups to improve cell retention and distribution within the graft. Our results confirm the hypothesis that pre-infarcted SDF-1 augmented myoblasts with and without hydrogel showed significant improved cardiac function. The cardiac output improved 8 weeks post-myoblast transplant compared to the media injected controls. The AAV SDF-1 transduced myoblast with and without hydrogel showed significant improvements in cardiac function. Conclusion: AAV transduced myoblasts with SDF1 will be beneficial in improvement of cardiac function compared to using only myoblasts.
Host-Derived Circulating Cells Generate Hybrid Cardiomyocytes by Cell Fusion in Heterotopic Heart Xenotransplantations

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The possibility to regenerate dead myocardium by cell therapy using either extra-cardiac or cardiac stem cells is the object of an intense investigation. Whereas much work has been done to assess the potential of injected cells to form new myocardium, little is known about the spontaneous recruitment of stem cells through the circulation. Host-derived cardiomyocytes were found in human sex-mismatched heart transplantations, however the relative proportion of this phenomenon was estimated very high by some authors and negligible by others. In heterotopic heart allograft-transplantation, we previously demonstrated that circulating cells engraff the heart transplant but do not significantly contribute to cardiac repair. Possible fusion events, as opposed to transdifferentiation, had been hypothesized but not proved. Here, we took advantage by the use of xenography, in which markers of both donor and recipient cells are available, to address this issue. Xeno-transplantations were performed using GFP+ transgenic rats as hosts, and either Syrian hamsters (n = 8) or transgenic mice expressing the LacZ reporter gene under the control of the cardiac troponin I promoter (n = 6), as heart donors.

In a first trial, MI was induced in 14 w old male spontaneously hypertensive rats (SHR) by ligation of left coronary artery (n = 60). After 24 h, 3x10^6 MN-CBCs (n = 14) or USSCs (n = 15), resp., were directly injected into the MI border zone 24 h after MI in male Sprague-Dawley rats (SD). Medium-injected MI rats served as controls. MI and MI-CTRL (fSD) 17

**Results:** Labeled cells were detected in the border zone adjacent to the infarct area at 6 and 24 h after i.x.-cell injection as well as 1 w after direct injection. Cumulative survival, however, was comparable in cell and medium treated MI SHR rats (57 vs. 61%, resp.) and MI size not significantly different (51:±2 vs. 53:±2%). Moreover, LV end-diastolic pressure and RV systolic pressure, indicating severely impaired heart function, were similar (table). In a second trial, 1x10^6 or 5x10^5 MN-CBCs were injected into C57BL/6 mice, human BMCs (hBMCs) into immunodeficient SCID mice, mBMCs (MNCBCs) into C57BL/6 and SCID mice. Xeno-transplantations were performed using GFP+ transgenic rats as hosts, and either Syrian hamsters (n = 8) or transgenic mice expressing the lacZ reporter gene under the control of the cardiac troponin I promoter (n = 6), as heart donors.

In this study the effect of mononuclear cord blood cells (MN-CBCs) and unrestricted somatic stem cells (USSCs) on survival and heart function after myocardial infarction was analyzed. In a first trial, MI was induced in 14 w old male spontaneously hypertensive rats (SHR) by ligation of left coronary artery (n = 60). After 24 h, 3x10^6 MN-CBCs (n = 23) or medium (n = 23) was injected via a tail vein. Right and left ventricular (RV and LV, resp.) function was assessed using echocardiography and the following parameters were calculated: left ventricular end-diastolic and end-systolic diameter, fractional shortening, ejection fraction, heart rate, and end-diastolic and end-systolic volumes. Mild trauma to the host heart was observed in 2 out of 6 animals. In a second trial, 1x10^6 or 5x10^5 MN-CBCs were injected into C57BL/6 mice, human BMCs (hBMCs) into immunodeficient SCID mice, mBMCs (MNCBCs) into C57BL/6 and SCID mice. Xeno-transplantations were performed using GFP+ transgenic rats as hosts, and either Syrian hamsters (n = 8) or transgenic mice expressing the lacZ reporter gene under the control of the cardiac troponin I promoter (n = 6), as heart donors. All transplants, which were retrieved 15 days after surgery, contained a large quantity of GFP+ inflammatory cells. In 7 of 8 hamster-to-rat heart transplants GFP+ mature cardiomyocytes were found, with percentages ranging from 0.0001% to 0.034%. No more than 15 GFP+ cardiomyocytes were detected in all mouse-to-rat transplants. We also found rare small GFP+ cells expressing markers of cardiac progenitor cells, such as GATA-4 and MEF2C, in all xenographs. All GFP+ cardiomyocytes identified in our study co-expressed GFP (the host’s marker) and either hamster-specific antigens or the LacZ marker of mouse origin (the donor’s markers). Thus, using both an immunological and a genetic approach, we conclusively demonstrate that in our experimental model circulating cells do not significantly contribute to form new myocardium, rather they generate hybrid cardiomyocytes by cell fusion.

**Conclusion:** Other hemodynamic parameters as well as the histological analyses did not provide evidence that in our experimental model circulating cells do not significantly contribute to cardiac repair. Possible fusion events, as opposed to transdifferentiation, had been hypothesized but not proved. Here, we took advantage by the use of xenography, in which markers of both donor and recipient cells are available, to address this issue.

**Objective:** To evaluate the effect of overlapping stents on the integrity of the drug-eluting coating of TAXUS Express® (Boston Scientific Corporation, Natick, MA) stents a short-term study was carried out in healthy swine to assess and compare the outcomes from implantation of stents, either completely overlapping or completely separated. TAXUS Express® (Boston Scientific Corporation, Natick, MA) was chosen for release (8.8%) TAXUS Express stents were deployed into coronary arteries of healthy female domestic swine in a single (32 mm length) or complete overlap (16 mm length) configuration (100%). At termination (n = 4 hours or 48 hours), tissue was digested from the samples, and single (n = 9) and overlapped pairs (n = 9) of stents were visually inspected (inner diameter and outer diameter) at 32X magnification with a light microscope and an area reticle. Coating integrity for overlapping versus single TAXUS Express stents was assessed both qualitatively (stent-to-stent polymer interactions such as protrusions) and quantitatively (total coating displacement area). Results: There were no observations of coating loss, protrusions or any unusual coating features on either single or overlapped pairs of stents deployed in vivo. Overlapped stents did not exhibit any stent-to-stent polymer interactions. Quantitatively, there were no differences in the area measurements of displaced (bare) coating between overlapping and non-overlapping stents. The differences of the two experiments are presented in a completely overlapping configuration. All overapped TAXUS Express stents had intact coatings with low amounts of detectable defects that were well below manufacturing specifications. These results indicate that implanting TAXUS Express stents in an overlapping configuration does not result in the loss of coating integrity.

**Histological Assessment of Myocardial Infarct Size in the Mouse Chronic Infarction Model: Infarct Length Measurement is More Accurate than Infarct Area Measurement**


Efficacy of potential cell- or gene-based treatments for myocardial infarction (MI) is frequently assessed by histological measurement of infarct size (IS) in rodent models. In experiments involving an acute MI setting, measurement of the infarct area in tissue sections of the left ventricle (LV) is a standard approach to determine IS. This approach has also been used in a chronic infarct setting, taking infarct area measurements several weeks post-MI. However, we hypothesized that wall thinning due to cardiac remodeling would invalidate the correlation between a severe infarct and a large infarct scar area measurement, reducing the legitimacy of this approach in a chronic setting. To address this issue, we assessed LV ejection fraction (LVEF) in 23 infarcted mouse hearts and ranked them according to increasing severity. The scar was measured in multiple sections per heart by a blinded investigator using several strategies, the numbers obtained were compared to extract IS as percentage of total LV volume, and the values were compared to the functional measurements for each heart. Our measurement strategies consisted of calculation of the ratio of: (1) summed infarct areas to summed LV myocardial areas (Area Measurement: AM), (2) summed epicardial and endocardial infarct arc lengths to summed epicardial and endocardial LV circumferences (Length Measurement: LM); and (3) summed midline infarct arc lengths to summed midline LV circumferences (Midline Measurement: MLM). Linear correlation analyses showed that IS from all three measurement approaches correlated significantly with LVEF and wall motion score index. However, the IS derived from AM was significantly smaller than those from the other measurements, and the range of values obtained was compressed 0.4-0.64, substantially compromising the accuracy of this approach. We conclude that area-based IS measurements underestimate the extent of the infarct and substantially reduce the sensitivity of this measure compared to measurements based on infarct arc lengths. We further conclude that the estimation of myocardial and infarct midlines provide comparable accuracy to the more cumbersome tracing of epicardial and endocardial lengths and circumferences.

**Improvement of Cardiac Function by Ultrasound-Guided Injection of Bone Marrow Cells into Mouse Myocardium 3 Days Postinfarction**


Considerable interest has focused on intra-myocardial delivery of bone marrow cells (BMCS) and endothelial progenitor cells (EPCs) as a potential treatment for myocardial infarction (MI). However, clinical experiments have been limited to treatment within hours of an MI, in contrast to human trials, in which cell therapy has been performed several days later after patient stabilization and autologous cell harvesting. We compared the therapeutic effects of different putative stem and progenitor cells in a mouse MI model using a novel percutaneous echocardiography injection approach. Cells were delivered at 3 days post-MI. Mice received a bolus of MI, echo-guided injection of cells or saline control (HBSS) into myocardium at the infarct border zone was performed at day 3 post-MI in the following groups (n = 8/group): mouse BMCS (mBMCS) into C57BL/6 mice, human BMCS (hBMCS) into immunodeficient SCID mice, mBMCS into C57BL/6 mice under sterile conditions and into HBSS into SCID mice. High-resolution echocardiography was performed blinded at baseline, 2 days post-MI (before...
injection), and day 28 post-MI. Results: Left ventricular ejection fraction (LVEF) was uniformly reduced from 57.2 ± 4.0% to 38.4 ± 3.7% (p < 0.0001) after MI in all groups. Mice injected with mBMSCs showed the best effect with significant improvement in global LVEF (37.8 ± 2.3 vs 44.4 ± 6.3; p < 0.03) at day 28, and there was a tendency toward less dilation of cardiac dimensions compared with that before injection. The global systolic function of the group injected with mBMSCs was significantly better than that of its corresponding control group (44.4 ± 6.3 vs 31.1 ± 10.4%; p < 0.0005). EPC injection showed a non-significant trend toward improvement of LVEF and less LV dilation, while the LVEF of its corresponding control group decreased, resulting in a significant difference between the EPC group and its controls at day 28 (41.5 ± 4.3 vs 32.1 ± 6.3%; p < 0.0005). Conclusions: Unfractionated mBMSCs attenuated ventricular remodeling and improved LV function when delivered several days post MI. However, different cell types affected LV function to varying extents. The novel approach allowed us to assess the efficacy of cell implantation in the mouse at a clinically relevant time after MI.

Human Umbilical Cord Blood Mononuclear Progenitor Cells Are Attracted to Infarcted Myocardium and Significantly Reduce Myocardial Infarction Size

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There is no consensus on precisely when to inject stem/progenitor cells for treatment of acute myocardial infarction. We therefore determined the attraction of infarcted myocardium from 45 rat hearts for human umbilical cord blood progenitor cells (HUCBC) at 1, 2, 2.5, 3, 6, 12, 24, 48, and 96 hours after LAD occlusion to determine the optimal time to transplant HUCBC after infarction. Our assay is based on migration of fluorescent DAPI labeled HUCBC from wells in an upper chamber of a Boyden apparatus through a semi-permeable membrane into wells in a lower chamber that contained either normal or infarcted myocardium. 100,000 DAPI-labeled HUCBC were placed in separate wells above the membrane that corresponded to normal or infarcted homogenate in the lower wells. The greatest HUCBC migration occurred to infarcted myocardium at 2 hours and at 24 hours after LAD occlusion in comparison with normal controls. 76,331 ± 7,753 HUCBC migrated to infarcted myocardium at 2 hours and 69,911 ± 2,723 at 24 hours after LAD occlusion (both p < 0.001) and significantly exceeded HUCBC migration to normal heart. The HUCBC migration remained greatest at 2 and 24 hours after LAD occlusion when the number of migrated cells was adjusted for infarct size. Injection of 10^6 HUCBC into infarcted non-immunosuppressed rats at 1–2 hours (n=10) or at 24 hours (n=5) after LAD occlusion resulted in infarct sizes one month later of 6.4 ± 0.001% and 6.4 ± 0.02% of the total LV muscle area, respectively, in comparison with infarct sizes of 30.7 ± 0.02% (n=10) in infarcts treated with only isoproterenol (p<0.005). Infarcts treated with only isoproterenol had a myocardial concentration of tumor necrosis factor alpha (TNFα) from 8.9 ± 0.3% to 25.0 ± 1.7% between 2 and 12 hours after LAD occlusion in comparison with controls (all p<0.001). In contrast, the concentrations of these cytokines in infarcts treated with HUCBC did not significantly change from controls. We conclude that: 1) infarcts significantly attract HUCBC, 2) HUCBC can substantially reduce infarct size, and 3) HUCBC can limit expression of cytokines in acute infarcts.

Small Molecules and the Pharmacology of Cardiac Cell Fate

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Developing a pharmacologic agent that could trigger cardiac fate exclusively and promote functional cardiac differentiation is an essential step towards safe and effective embryonic stem cell (ES) cell-based repair and regeneration of the cardiovascular system. We have studied the pharmacology of cardiac fate decisions and differentiation in ES and embryonal carcinoma (EC) cells, using both known drugs and novel synthetic compounds. Our results show that the first step in ES cell differentiation, a physiologic transition from embryonic to somatic cell cycle, is epigenetically regulated and inducible by histone deacetylase inhibitors. To identify specific small molecule inducers of cardiac-mesodermal fate in ES/EC cells, we screened a large (200K) chemical library for drugs that could activate Nkx2.5 and myocardin, two of the earliest signature genes of this lineage. This screen has yielded an extraordinary collection of chemically diverse small molecules capable of activating cardiac genes in ES/EC cells from 0.8% to 51.3 ± 4.6%, monocyte chemoattractant protein (MCP-1) from 10.5 ± 1.1% to 39.2 ± 2.0%, monocyte inflammatory protein (MIP) from 10.6 ± 1.6% to 23.1 ± 0.5% and interferon gamma (INFγ) from 8.9 ± 0.3% to 25.0 ± 1.7% between 2 and 12 hours after LAD occlusion in comparison with controls (all p<0.001). In contrast, the concentrations of these cytokines in infarcts treated with HUCBC did not significantly change from controls. We conclude that: 1) infarcts significantly attract HUCBC, 2) HUCBC can substantially reduce infarct size, and 3) HUCBC can limit expression of cytokines in acute infarcts.

Cell Fusion Was Not Essential for Cardiomyogenesis from Bone Marrow Stem Cells

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Objective: It is well known cell fusion is essential for cardiomyogenesis from bone marrow stem cells (BMSCs) in vitro or in vivo. In this study we investigated other cardiac environmental factors using fetal cardiac milieu instead of cell fusion system and evaluated cardiomyogenesis from BMSCs. Methods: 5.0 x 10^6 BMSCs were prepared from a 3-week-old rat with Sciocey’s Modified Dulbecco Medium including 10% fetal bovine serum. Then supernatant having no cell, made from rat fetal hearts was added into BMSCs culture, which was incubated for seven days without any treatment like 5-azacytidine. Then, culture media was changed twice a week and observed for three weeks. We compared myotubes from BMSCs between bone marrow cells culture alone (BM group, N=8) and those incubated with fetal heart supernatant (BF group, N=8) by microscopy, electron microscopy, immunostaining, and RT-PCR. Results: From day 9 to 21, more myotubes were found in the BF group than in the BM group (19.5% vs 3.5%, p<0.003). These myotubes were long shaped and multinucleated, most of which were beating spontaneously. Under electron microscopy, myofilaments and Z-bands were found. In immunohistochemical study, these myotubes were stained positive by anti Troponin-I and MF20 antibodies specific for cardiomyocyte. In RT-PCR analysis, P20, GATA-4 and Nkx2-5 genes contributing to cardiomyogenesis in embryo, revealed in these myotubes. Conclusions: Cell fusion was not absolutely required for cardiomyogenesis from BMSCs under cardiac environmental factors like fetal cardiac milieu. This study system could enable BMSCs to differentiate into cardiomyocytes more effectively before transplanted into heart.

Long-Term Effect of Gene Therapy for Chronic Ischemic Myocardium Using Platelet-Derived Endothelial Cell Growth Factor in Dogs

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Objective - We previously reported the 2-week benefits of platelet-derived endothelial cell growth factor (PD-ECGF) gene therapy in chronically ischemic myocardium. This study further examined the long-term effects and safety. Methods - Twenty-four beagle dogs were randomly divided into 3 groups and chronic myocardial ischemia was created by stenosing the origin of the LAD using an ameroid constrictor. Two weeks later, a second thoracotomy was performed and a total of 5 mg of pCH17P (pCH17P, n=8) or pCLacz2 plasmid DNA (pCLac2, n=8) was diluted in 2 ml of saline and injected directly into 6 sites supplied by the LAD. Saline group (n=8) received saline alone. All lived dogs were followed up to 6 months. Echocardiography was used to trace the myocardial function (%EF) and myocardial blood volume at different time points as indicated in the results. Results - Myocardial infarction was decreased in the pCH17P group (28.6 ± 1.3% vs 56.3 ± 2.1% in the saline group, p<0.005). Myocardial blood volume and %EF decreased in all 3 groups after ameroid implantation, but recovered 2 weeks in the pCH17P group, and maintained a higher level during the examination period (Figure, * p<0.005, pCH17P vs. pCLac2 and saline). Histologic analysis demonstrated that angionecrosis occurred after PD-ECGF gene treatment. There was a decreased expression of the proapoptotic proteins, active caspase-3 and Bax, and the number of apoptotic myocardial cells was lower in the pCH17P group. Mitochondrial count was higher in pCH17P group. Histologic examination demonstrated that no abnormal histologic changes or neoplasms were found in any organs. Conclusions - We conclude that a therapeutic approach using the PD-ECGF/P gene to treat chronic ischemia is effective and safe.
Inhibiting Calmodulin-Cyclin E Interactions Arrests Cell Cycle Progression of Vascular Smooth Muscle Cells

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BACKGROUND: Understanding the molecular mechanisms underlying vascular smooth muscle cell (VSMC) proliferation may improve the design of therapies aimed vasculo-occlusive diseases. We previously showed that a coordinated elevation in free intracellular Ca2+ concentrations is required for G1-to-S phase cell cycle transitions in VSMC. More recently, our studies suggest that Calmodulin (CaM)-dependent cyclin E/CDC2 kinase activity mediates CaM-sensitivity of this transition, and requires direct binding of CaM to cyclin E. We now show the molecular basis and physiological importance of this interaction. METHODS & RESULTS: A CaM-target database suggested a putative CaM-binding motif in human cyclin E1 (named 'CBS' for CaM-Binding Site). To study the biological function of this putative motif in vivo, we generated and characterized transgenic mouse models harboring a CBS peptide (TC758L6S) that was subject to unlimited hindlimb ischemia. After surgery, Laser Doppler PerfusionImaging demonstrated decreased blood flow recovery in APAKO mice, especially within 2 weeks (ischemic/Non ischemic limb at 1, 2.5, 4; p<0.05 vs wild type mice, Figure). Tissue capillary density at two weeks decreased in APAKO mice, and Aorta Ring Assays revealed mean length of sprouting microvessels was significantly shorter in APAKO mice. (Table) Tube Formation Assay showed endothelial cells isolated from aorta of APAKO mice were tend to form less endothelial network. These results suggested that angiogenesis was impaired in APAKO mice. Conclusion: Aminopeptidase A would be a functional target of ischemia-induced angiogenesis.

CAPILLARY DENSITY AND AORTA RING ASSAY

Wild type APAKO
Capillary Density (Endothelial/Muscle) 1.25±0.12 0.57±0.15
Aorta Ring Assay (µm) 464±80 332±73

P111
Withdrawn

P112
Ischemia-Induced Angiogenesis Is Impaired in Aminopeptidase A Knockout Mice

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Some studies suggested that aminopeptidase A (APA), a membrane-bound metalloproteinase degrading bioactive peptides such as angiotensin II, is associated with angiogenesis of tumor. Although the role of APA on ischemia-induced angiogenesis is unknown. We examined whether ischemia-induced angiogenesis is altered in APA knockout (APAko) mice. Methods and Results: APAko mice and Wild type mice (C57BL/6J) were subject to unilateral hindlimb ischemia. After surgery, Laser Doppler Perfusion Imaging demonstrated decreased blood flow recovery in APAko mice, especially within 2 weeks (ischemic/Non ischemic limb at 1, 2.5, 4; p<0.05 vs wild type mice). Tissue capillary density at two weeks decreased in APAko mice, and Aorta Ring Assays revealed mean length of sprouting microvessels was significantly shorter in APAko mice. (Table) Tube Formation Assay showed endothelial cells isolated from aorta of APAko mice were tend to form less endothelial network. These results suggested that angiogenesis was impaired in APAko mice. Conclusion: Aminopeptidase A would be a functional target of ischemia-induced angiogenesis.

P113
In Vitro Analysis of Signaling by the Selective Corticotropin-Releasing Factor Receptor Agonist Urocortin 2 in Isolated Adult Cardiac Myocytes

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Urocortin 2 (UC2) is one of a family of neuropeptides that activates the corticotropin-releasing factor (CRF) receptor and is currently under development as a novel therapy for acute decompensated heart failure (ADHF). UC2 has been reported to possess cardio-protective properties not observed with existing ADHF therapies and acts by simultaneously increasing cardiac output and decreasing blood pressure. To date, little is known of the mechanisms through which UC2 induces inotropy and lusitropy. Here we show that UC2 signals through the Gi-coupled CRF receptor, receptor antagonist astressin-2B whilst those of dopamine remained unaffected confirming selective activation of the CRF1 receptor. UC2 was >1000-fold more potent than dobutamine at stimulating cyclic AMP accumulation and the phosphorylation of PKA substrates. The efficacy of UC2 were completely blocked by the selective CRF2 receptor antagonist astressin-2B whereas those of dopamine remained unaffected confirming selective activation of the CRF1 receptor. UC2 was >1000-fold more potent than dobutamine at stimulating cyclic AMP accumulation (EC50=1.4nM), while dobutamine was 2.6-fold more efficacious. By recording simultaneous cardiac flux and pressure changes in isolated cardiomyocytes we also demonstrated that UC2 treatment produced a dose-dependent slow increase (~10 minutes to reach maximum) in peak cardiac release, sarcomere shortening, tau and time between peak calcium and peak contraction (EC50=SnSmR), with no significant change in diastolic calcium concentration demonstrating the direct inotropic and lusitropic actions of UC2. These data suggest that the direct inotropic and lusitropic action of UC2 on myocardium occurs through selective activation of a CRF1 receptor-dependent cyclic AMP/PKA pathway.

P114
Treatment of Bone Marrow Mononuclear Cells with Erythropoietin and Platelet-Derived Growth Factor-BB Promotes Cellular Proliferation and Expression of Mesenchymal and Endothelial Cell Surface Markers In Vitro when Compared with Traditional Growth Factors

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Objective: Autologous bone marrow cells are being used in clinical trials for cell-based therapy after myocardial infarction. We developed an in vitro assay to determine if growth factor stimulation would promote specific stem cell growth prior to infusion into a recipient. Methods: Bone marrow mononuclear cells (BM-MNCs) are harvested from 5–6 week old Sprague-Dawley rat's femurs. The contents are flushed with sterile 10% PBS in DSEM-LE. Bone spicules and clots are allowed to settle, the cell suspension is decanted and centrifuged at 1,000 rpm (room temperature). Cells are re-suspended in red blood cell lysis buffer, centrifuged, and washed twice in 20ml of PBS before platting 1×10⁷ cells per well in 24 well plates. Each well is exposed to one of seven of the following growth factor combinations (10 ng/ml): (1) Control (10% PBS + DSEM-LE), 2 platelet-derived growth factor-BB (PDGF-BB), 3 epidermal growth factor (EGF), 4 granulocyte colony stimulating factor (G-CSF), 5 erythropoietin (EPO), 6 EPO + G-CSF, or 7 EPO + PDGF-BB. Media is changed daily for ten days, a sample is removed and the cells counted to calculate total number of cells. This process was repeated in a separate group of BM-MNCs grown to confluence. At passage three, immunohistochemistry is used to detect the presence of CD34, CD44, CD45, CD200, VE-Cad, and Pan-EC. Results: Growth factor stimulation produced a transient increase in the number of cultured BM-MNCs from day one to day five, and decreased thereafter to day 10. At day five EPO treated cells had an increase (P<0.05) in number compared to all other groups. (1) 295,500±27,018 (n=6), (2) 227,250±7,683 (n=5), (3) 185,500±31,815 (n=5), (4) 795,865±74,896 (n=6), (5) 712,333±88,214 (n=3), (6) 913,000±35,355 (n=4). Cells grown in EPO+PDGF-BB demonstrated expression of mesenchymal (CD44+, CD45+, and endothelial (CD34+, VE- Cad+) cells markers. Conclusion: EPO in combination with PDGF-BB stimulates BM-MNC growth 3 fold over no treatment, and express mesenchymal and endothelial cell markers. These data suggest that EPO + PDGF-BB could be explored as growth factor treatment in patients undergoing cell-base therapy after myocardial infarction.

P115
β-Blockers Switch Mitogen-Activated Protein Kinase Signaling in Adult Mouse Cardiac Myocytes

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Beta-adrenergic receptor (AR) antagonists (beta blockers) increase survival in heart failure, but the signaling mechanisms remain uncertain. Among the mitogen activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK) is anti-apoptotic, whereas the MAPK p38 can be pro-apoptotic. Here we tested the hypothesis that beta-blockers switch catecholamine signaling to MAPKs in cardiac myocytes. Myocytes from adult male C57BL/6J mice were cultured overnight in serum-free medium, then stimulated acutely with the natural catecholamines norepinephrine (NE) or epinephrine (EPI) (2 uM). MAPK phosphorylation (activation) was measured by immunoblot. NE or EPI alone markedly decreased ERK phosphorylation (NE 80%, EPI 95%, vehicle 5% of vehicle; n=7). MAPK phosphorylation was measured at 5 min, after 20 min, and after 2 hours. NE or EPI alone caused a decrease in ERK phosphorylation that was not statistically significant. The beta-AR agonist isoproterenol (1 uM) had a similar effect (15±7% of vehicle, NE or EPI alone increased p38 phosphorylation by 6-fold (n=2). In sharp contrast, NE or EPI in the presence of the beta-blocker propranolol (2 uM) caused robust ERK activation (NE 389±70%, EPI 448±127%; n=4; p<0.05). ERK activation was mimicked by the alpha-1-AR agonist phenylephrine (20 uM, 302±56%). NE or EPI in the presence of propranolol had no effect on p38. When myocytes were treated acutely with beta-blockers alone, propranolol had no effect on ERK activity. However, the beta-blockers...
timolol or metoprolol alone activated ERK (timolol EC50 = 23 nM, Emax = 248±2%; n=3, p<0.05, metoprolol EC50 = 27 nM, Emax = 216±4%; n=2). In cultured adult mouse cardiac fibroblasts, in contrast with myocytes, NE or EPI alone activated both ERK (5-fold) and p38 (2-fold). In conclusion, the natural catecholamines NE and EPI alone inhibit anti-apoptotic ERK and activate pro-apoptotic p38 via a beta-AR in adult mouse cardiac myocytes. Beta-blockers switching catecholamine signaling to myocytes to ERK activation via an alpha-1-AR. In addition, certain beta-blockers (timolol and metoprolol but not propranolol) activate ERK directly in the absence of catecholamines. Switching MAPK signaling towards ERK might contribute to the cardioprotective effects of beta-blockers.

Direct Determination of β₁-Adrenergic Receptor Activation Reveals Differential Antagonist Sensitivity of Naturally Occurring Receptor Variants

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Blockade of β₁-adrenergic receptors (βARs) through receptor antagonists (β-blockers) has evolved to one of the most effective and most frequently used therapeutic regimens in cardiovascular medicine. Polymorphisms of these receptors occur frequently, but there is limited and controversial evidence, whether and how these affect the patients response to β-blocker treatment. In this study, we generated a mutant of the β₁-AR in which the cyan- and yellow-emitting variants of the green fluorescent protein (CER and YFP) were inserted at the C-terminus extremity and in the third loop of the receptor respectively. Using fluorescence resonance energy transfer (FRET), the β₁-AR receptor allowed the direct monitoring of conformational changes of the receptors during pharmacological stimulation. After stimulation by its endogenous agonist nor epinephrine, the human β₁-AR was activated (decrease of the FRET ratio of about 4%) at a surprisingly slow speed (about 500 ms) followed by activation of both the CER and YFP variants of the CER. Commonly used β-blockers such as bisoprolol and carvedilol acted as inverse agonists at the human β₁-AR actively inducing a conformational change opposite to that of agonists. The frequently occurring hyperfunctional Arg389 variant of the β₁-AR (associated with a poor prognosis in heart failure patients) displayed similar behaviour to agonists and several antagonists as the Gly389 variant. However, the Arg389 receptor displayed a marked supersensitivity to the β₁-blocker carvedilol compared to the Gly389 variant which we confirmed on the second messenger level (CAMP + 300%). Expression of both receptor variants in cardiac myocytes confirmed the importance of the Arg389-sensitivity to carvedilol for cardiac rate control.

Forkhead Transcription Factors, Foxc1 and Foxc2, Directly Regulate VCAM-1 Gene Expression in Endothelial Cells

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Objective — Although it is known that the induction of vascular cell adhesion molecule 1 (VCAM-1) in endothelial cells promotes leukocyte-endothelium interaction at sites of atherosclerosis initiation leading to the development of atherosclerosis, the precise molecular mechanisms underlying transcriptional regulation of the VCAM-1 gene are incompletely understood. Foxc1 and Foxc2 are forkhead transcription factors that have been shown to play important roles in embryonic blood vessel development and endothelial functions in postnatal life. We investigated their potential regulation of VCAM-1 gene expression. Methods and Results — We found that transcripts of VCAM-1 are significantly upregulated by overexpressing Foxc in endothelial cells. Importantly, consistent with this finding, there are three forkhead-binding elements (FBEs) conserved between mouse and human in the VCAM-1 promoter region, and we confirmed by gel shift and chromatin immunoprecipitation (ChIP) assays that Foxc proteins can bind to these FBEs in vitro and in vivo. Luciferase assays using a series of deletion and mutation constructs for the VCAM-1 promoter further demonstrate that Foxc1 and Foxc2 regulate the promoter activity of VCAM-1 via the FBEs. Most significantly, endothelial cells isolated from adult Foxc2 heterozygous mutant mice show a significant reduction in VCAM-1 transcription compared to wild-type endothelial cells. Conclusions — Our results indicate that Foxc transcription factors directly regulate the VCAM-1 gene and may be critical for endothelial cell function and play a pivotal role in the development of atherosclerosis.

Genetic Polymorphisms of the Renin-Angiotensin System in End-stage Renal Disease and Evolution of Coronary Artery Disease

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Cardiac disease is the leading cause of death in patients having end-stage renal disease (ESRD). Genes encoding for angiotensinogen (AGT), angiotensin-converting enzyme (ACE), are candidates for cardiovascular and renal diseases. The impact of renin -angiotensin system (RAS) gene polymorphism on the prognosis of coronary artery disease in ESRD is still debated. METHODS: Genotyping was performed in 100 ESRD patients and 100 control subjects for the AGT-M235T, AGT -A6G, ACE insertion/deletion (I/D) and the angiotensin II type I receptor gene(ALT/R)(AT1R) A1166C polymorphisms using polymerase chain reaction. Internal medial thickness (IMT) of coronary artery was observed in vivo. Differences were considered significant at p-value of 0.05. RESULTS: Genotype frequencies of AGT-M235T, AGT -A6G, and AT1R A1166C polymorphisms did not differ significantly between ESRD patients and controls. No association of internal medial thickness (IMT) of coronary artery was observed with any genotype in the ESRD patients. A strong association of ACE DD genotype and AGT MM genotype was observed in ESRD patients (p<0.001). The AGT MT genotype was found to be associated with AGT -6G AG genotype only in ESRD population. Thus, polymorphism in genes of the RAS system may influence interindividual differences in the development and course of ESRD, but are not associated with IMT.

Proteomic Identification of Cardiomyocyte Specific Biomarkers of Oxidative Injury

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Numerous lines of evidence indicate a role of oxidative stress in initiation and progression of heart failure. Among the main cell types within the heart, cardiomyocytes (CMCs) undergo hypertrophy or apoptosis, while cardiac fibroblasts (CFs) are responsible for fibrosis during the process of heart failure. In an effort to identify cell type specific biomarkers of oxidative injury, we isolated CMCs and CFs from rat hearts and treated with sublethal doses of H2O2 for detection of secreted protein factors in the conditioned media by ESI-LC-MS/MS based proteomics. Comparison between the profiles of secreted proteins among the two cell types leads to the finding that H2O2 at sublethal doses caused an elevation in the level of Cystatin C protein in CMCs. Not only from CMCs but also from CFs, RT-PCR analyses of Cystatin C mRNA and Western blot analyses of Cystatin C protein confirm that H2O2 induces dose dependent Cystatin C elevation in CMCs but not CFs. To validate the potential of using Cystatin C as a cardiac injury biomarker in association with oxidative stress in vivo, cardiomyopathy was induced in a chronic (Dog) and an acute (Rats) model of rodent ischemia and the condition of liver or kidney injury. Elevated Cystatin C was detected at the protein level in the plasma and heart tissue and at the mRNA level in heart tissue from Dog treated animals. We also tested the level of circulating Cystatin C in a myocardial infarction model induced by left descending coronary artery ligation. An increase in the level of Cystatin C was detected in the heart tissue of animals. These data suggest that Cystatin C can be useful as a biomarker of cardiomyocyte injury associated with oxidative stress in vitro and in vivo.

Molecular Characterization of a Calcium ATPase Gene in an Animal Model of Cardiomyopathy

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Cardiovascular disease, principally heart disease and stroke, is the United States leading killer for both men and women across all racial and ethnic groups. Among the core heart diseases, cardiomyopathy is the most common heart muscle disease usually necessitating a heart transplant. Despite the diverse etiologies of cardiomyopathy, the Ca2⁺ cycling defect is a physiological hallmark of all forms of cardiomyopathy. Therefore, the genes involved in Ca2⁺ cycling have been considered responsible for cardiomyopathy. The calcium ATPase protein involved in regulation of calcium in and out of the intracellular cations stores is thought to be altered in cardiomyopathic patients. The pathophysiological presentation of dilated cardiomyopathy in an avian model of cardiomyopathy (turkey) is similar to the human heart. Therefore, the purpose of this study was to identify and analyze the calcium ATPase gene in furlanoidoinduced cardiomyopathic turkeys. Primers were designed (Prime3 software) from the chicken calcium ATPase mRNA to target the turkey genome. Total RNA was isolated from chicken (control), furlanoido-induced and non-induced turkey heart tissues. A one step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was performed. The expected 195 bp DNA fragment for the calcium ATPase gene was observed in ethidium bromide stained gels with a UVa Violet Gel Documentation system, indicating successful amplification of the calcium ATPase gene from turkey heart.

Increased Mitochondrial Uncoupling Protein 3 Is Associated with the Cardiomyopathy in the Dystrophic Mouse Heart

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Patients with muscular dystrophy have heart failure and abnormal cardiac high-energy phosphate metabolism. Uncoupling protein 5, a muscle-specific form, is involved in the regulation of energy metabolism. Here, we tested the hypothesis that adult dystrophic mdx mouse hearts have cardiac dysfunction and abnormal energy metabolism associated with increased levels of mitochondrial uncoupling protein 3. Non-invasive, in vivo high-resolution cine magnetic resonance (MR) imaging was carried out in control and mdx mice using an 11.7 T MR magnet. High energy phosphate compounds were measured in isolated mouse hearts perfused in the Langendorf mode using 31P MR spectroscopy (MRS). Mitochondrial uncoupling protein 3 levels were measured by Western blotting in control and mdx mouse hearts. Cine MR images showed that mdx mouse hearts had 16% (0.27 ± 0.02 vs. 0.33 ± 0.01 μlms, P < 0.05) lower left ventricular peak filling rates, but the same left ventricular mass, end-diasolic volumes, end-systolic volumes, stroke volumes, ejection fractions and cardiac outputs. 31P MRS showed that mdx mouse hearts had 40% (11.6 ± 1.1 vs. 18.7 ± 1.1 mM, P < 0.01) lower phosphocreatine concentrations, with the same ATP concentrations, as control mouse hearts. mdx mouse hearts had 1.4-fold (P < 0.05) higher mitochondrial uncoupling protein 3 than their controls. We conclude that adult dystrophy-deficient mdx mouse hearts had left ventricular diastolic dysfunction associated with decreased phosphocreatine levels and increased mitochondrial uncoupling protein 3.
study aims to characterize and determine the central regulatory role of mitochondria on tissue redox status and oxygenation. **Methods and Results:** In vivo electron paramagnetic resonance was performed to measure tissue redox status and oxygenation in the ischemic and reperfused myocardium. In vivo fluorometry was performed to measure tissue NADH and reactive oxygen species. High-performance liquid chromatography-electrochemical method was performed to measure tissue reduced and oxidized glutathione ratios. We found that tissue oxygenation level was increased 100% (0.042 to 0.084 mM) during ischemia and decreased 33% (0.042 to 0.028 mM) after reperfusion compared to the non-ischemic myocardium. There was an overshoot of tissue oxygenation after reperfusion. Tissue NADH level was increased to 1.8 fold (1.0 ± 0.2 fold) during ischemia and 1.2 fold (1.0 to 1.2) after reperfusion. There was a brief burst generation of ROS at the beginning of reperfusion. Tissue GSH/GSSG levels showed a 48% (50.0 to 74.0) increase during ischemia and a 29% (50.0 to 35.5) decrease after reperfusion. **Conclusions:** The restrained mitochondria respiration due to hypoxia during ischemia caused an increase in superoxide production persisted to 12 weeks post surgery (39.7 ± 11006 /H11006 increased 70.9 ± 11006 in sham operated controls (n = 13)). At 8 weeks post surgery oxygenation, higher ROS generation, lower NADH content, and lower GSH/GSSG level. The restrained mitochondria respiration due to hypoxia during ischemia caused an increase in superoxide production persisted to 12 weeks post surgery (39.7 ± 11006 /H11006 increased 70.9 ± 11006 in sham operated controls (n = 13)). At 8 weeks post surgery oxygenation, higher ROS generation, lower NADH content, and lower GSH/GSSG level. The restrained mitochondria respiration due to hypoxia during ischemia caused an increase in superoxide production persisted to 12 weeks post surgery (39.7 ± 11006 /H11006 increased 70.9 ± 11006 in sham operated controls (n = 13)). At 8 weeks post surgery oxygenation, higher ROS generation, lower NADH content, and lower GSH/GSSG level. The restrained mitochondria respiration due to hypoxia during ischemia caused an increase in superoxide production persisted to 12 weeks post surgery (39.7 ± 11006 /H11006 increased 70.9 ± 11006 in sham operated controls (n = 13)). At 8 weeks post surgery oxygenation, higher ROS generation, lower NADH content, and lower GSH/GSSG level. The restrained mitochondria respiration due to hypoxia during ischemia caused an increase in superoxide production persisted to 12 weeks post surgery (39.7 ± 11006 /H11006 increased 70.9 ± 11006 in sham operated controls (n = 13)). At 8 weeks post surgery oxygenation, higher ROS generation, lower NADH content, and lower GSH/GSSG level. The restrained mitochondria respiration due to hypoxia during ischemia caused an increase in superoxide production persisted to 12 weeks post surgery (39.7 ± 11006 /H11006 increased 70.9 ± 11006 in sham operated controls (n = 13)). At 8 weeks post surgery oxygenation, higher ROS generation, lower NADH content, and lower GSH/GSSG level. The restrained mitochondria respiration due to hypoxia during ischemia caused an increase in superoxide production persisted to 12 weeks post surgery (39.7 ± 11006 /H11006 increased 70.9 ± 11006 in sham operated controls (n = 13)). At 8 weeks post surgery oxygenation, higher ROS generation, lower NADH content, and lower GSH/GSSG level.
This inhibitory effect can potentially have deleterious effects in the setting of heart failure. Future studies to identify susceptible enzymes will provide important mechanistic information.

**P129**

**Tauroursodeoxycholic Acid Reduces Apoptosis Following Myocardial Infarction in the Rat**

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**Introduction:** Black bear bile extracts have been used in traditional Chinese medicine to treat liver and eye-related illnesses for centuries. Recent analysis of the cellular effects of ursodeoxycholic acid (UDCA) and its taurine conjugated counterpart (TUDCA) have demonstrated antiapoptotic properties through regulation of Bcl-2 family and survival signaling proteins (Bax, Bad, phosphatidylinositol-3-kinase). In this study we assessed the hypothesis that TUDCA administered to Lewis rats prior to a myocardial infarction would exhibit antiapoptotic effects and improve cardiac function. **Methods:** Prior to ligation of the left anterior descending (LAD) coronary artery, TUDCA, n=11, (50mg/kg, 400mg/kg, IV) or PBS, n=15, was given over 40-minutes. Animals were sacrificed at 24 hours after ligation for transferase-mediated deoxyribonuclease biotin nick-end labeling (TUNEL) and immunoassay caspase-3 activity. Additional TUDCA (n=4) or PBS (n=3) treated rats underwent pre-operative, 1-week, and 4-week transthoracic ultrasoundscans to assess shortening fraction (SF) and infarct area. **Results:** TUNEL labeling of the cardiac tissue revealed a significant reduction in apoptotic cells in rats given TUDCA prior to ischemic injury (p<0.05). In support of the reduction in apoptosis, caspase-3 activity in the TUDCA treated animals was lower (p<0.02). For four weeks there was a smaller infarct area in the TUDCA group compared to the PBS group (0.05 vs 0.13 cm$^2$, p<0.11). Furthermore, there was improvement in the SF. **Conclusions:** The results provide evidence for TUDCA as a viable treatment for reducing apoptosis in an experimental model of myocardial infarction. Additional studies will distinguish the functional result of improved cell survival following infarction. Possible clinical applications of this antiapoptotic drug include treatment of acute myocardial infarction.

**P130**

**Sulfaphenazole, a CYP2C9 Inhibitor, Protects Against Ischemia-Reperfusion-Induced Injury in Isolated Rat Heart**

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The cardioprotective effect of Sulfaphenazole, a potent and selective inhibitor of cytochrome p450 isozyme CYP2C9, was investigated using isolated rat heart model of ischemia-reperfusion (I/R) injury. Isolated rat hearts were perfused with Krebs-Henseleit buffer, subjected to 30-min of global ischemia, followed by 45 min of reperfusion. Hearts were perfused with Sulfaphenazole (400 µM) for 15 min before the onset of ischemia and throughout reperfusion. Control hearts subjected to I/R showed significant decrease in contractile function (left ventricular developed pressure, and rate pressure product), increased lactate dehydrogenase and suppressed I/R-induced free radical generation. Sulfaphenazole treatment also showed increased nitric oxide generation. The present study demonstrated that Sulfaphenazole significantly protected hearts against I/R-mediated cardiac dysfunction and injury. The protective effect could be due to the combined effects of CYP2C9 inhibition, antioxidant and as well as enhanced nitric oxide generation.

**P132**

**Effects of Levosimendan plus Noradrenaline on Coronary Blood Flow, Myocardial Infarct Size, and the Extent of the No-Reflow Phenomenon in an Experimental Model of Ischemia-Reperfusion**

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Background: Positive inotropic agents have been used in clinical practice for the treatment of patients with cardiogenic shock. The aim of our study was to examine the effect of high doses of levsimendan plus noradrenaline on coronary blood flow (CBF), myocardial infarct size (MS), and on the extent of the no-reflow phenomenon (NRP) in an experimental model of ischemia-reperfusion. A randomized study was conducted in an experimental model of ischemia-reperfusion. In seventeen piglets, 25–35 kg, after mid-thoracotomy, ischemia was provoked by ligation of the mid portion of the left anterior descending (LAD) coronary artery for 60 minutes, followed by 120 minutes of reperfusion. The animals were randomized 10 minutes before reperfusion into 2 groups: Group A, in which no medical intervention was made, and Group B, in which levsimendan was administered (loading dose 24 µg/kg, followed by continuous infusion 0.4 µg/kg/min with noradrenaline (0.05 µg/kg/min) until the end of reperfusion. CBF was measured by a transit time probe at the site of LAD ligation, and expressed as a percentage of baseline CBF. The extent of NRP was measured with thiocyanate stain, and expressed as a percentage of the myocardial area at risk (AAR). Myocardial infarct size (MS) was measured with triphenyltetrazolium chloride stain (TTC) and expressed as a percentage of the AAR. Results: Eight animals were included in Group A, and 9 in Group B. A trend towards increased CBF was observed in Group B during reperfusion. A trend towards reduction of the extent of NRP was observed in Group B (Group A: 60.9±11.4%, Group B: 50.5±7.6%, p=0.209). The MS did not differ between groups (Group A: 71.1±8.8%, Group B: 80.5±6.0%, p=0.401). Conclusions: In this porcine experimental model of ischemia-reperfusion, high doses of levsimendan plus noradrenaline increased CBF during reperfusion, did not cause further damage to microcirculation, and did not affect MS. According to the results of this study, high doses of levsimendan can be safely used in patients with acute myocardial infarction complicated by refractory cardiogenic shock.

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**Myocardial Apoptosis Induction in Cardiac Surgery Patients Depends on Cardioplegic Solution Type**

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**Objectives:** Experimental studies suggest that myocardial apoptosis induction during cardiopulmonary bypass is not only inhibited by oxygen free radicals but also depends on cardioplegic solution type. We studied apoptosis signal-pathway induction in patients subjected to cardiopulmonary bypass (CPB) using warm blood and cold crystalloid cardioplegia. **Methods:** In twelve patients we collected myocardial biopsies form the right atrial cannulation site before and at termination of CPB. Seven patients had CABG, five patients had aortic valve replacement (AVR). For CABG we used warm blood cardioplegia (Calcisofrome), while AVR hearts were arrested with crystalloid solution (Bretschneider). LV specimens were immuno-cytochemically stained against activated caspase-3 (apoptosis key-enzyme), and 8-isoprostaglandin-F_2alpha (oxidative stress marker). Cardiomyocytes were then quantitatively investigated using TV densitometry (gray units: U). **Results:** Caspase-3 activity significantly increased in CABG (17.8±2.1 to 30.0±1.9 U, p<0.003) while it remained unchanged in AVR (29.3±10.2 to 32.2±12.4 U, p=0.16). 8-Isoprostaglandin-F_2alpha formation remained unchanged in AVR but increased significantly in CABG (by 10.9±4.9 U, p=0.03). While cardiomyocyte apoptosis was significantly higher in AVR (68.8±8 min vs. 38.8±6 min, p<0.03), reperfusion period on CPB was longer in CABG (30.4±5 vs 15.4±5 min, p=0.01). There was no correlation between duration of cardiopulmonary arrest or reperfusion on CPB and caspase-3 activity and 8-isoprostaglandin-F_2alpha formation in either group. **Conclusions:** High potassium warm blood cardioplegia initiates cardiomyocyte apoptosis rather than low sodium, low calcium crystalloid cardioplegia.

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**Protective Actions of Adiponectin on Cardiac Remodeling After Myocardial Infarction**

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Ischemic heart disease is the leading cause of morbidity and mortality in the United States. Obesity-linked diseases are closely associated with the development of ischemic heart disease. However, the link between obesity and the development of ischemic heart disease is poorly understood at a molecular level. Adipose tissue secretes adipokines that directly or indirectly affect obesity-linked disorders. Adiponectin is an anti-atherogenic and anti-diabetic adipokine that is downregulated in patients with obesity-linked diseases including type 2 diabetes and ischemic heart disease. Recently, we demonstrated that adiponectin protects against acute myocardial ischemia-reperfusion injury (Shibata et al. Nature Med. 2005). However, the role of adiponectin in the regulation of chronic cardiac remodeling in response to myocardial infarction has not been investigated. To test the role of adiponectin in regulating cardiac remodeling after chronic ischemia, myocardial infarction was created in adiponectin-deficient (APN-KO) and wild-type (WT) mice by the permanent ligation of left anterior descending (LAD) artery. APN-KO mice showed impaired left ventricular function coupled to increased apoptosis and inflammation, and decreased angiogenesis in the heart after LAD ligation compared with WT mice. These changes were associated with decreased apoptosis and increased inflammation, diminished myocardial apoptosis and promoted angiogenesis in both WT and APN-KO mice that underwent permanent LAD ligation. These observations suggested that adiponectin serves as a functional link between adipose tissue and the heart, and thereby influence the extent of cardiac remodeling following chronic myocardial ischemia. In cultured cardiac myocytes, adiponectin inhibited apoptosis under the conditions of hypoxia, which was reversed by dominant negative AMPK. Adiponectin inhibited LPS-induced TNF-alpha production in cardiac myocytes, which was blocked by inhibition of cyclooxygenase (COX)-2-dependent pathway. These data suggest adiponectin protects the heart from chronic cardiac injury through both AMPK- and COX-2-dependent mechanisms. Adiponectin could represent a molecular target for treatment of obesity-related myocardial disease.
Intracellular Zinc Protects Isolated Rat Hearts from Ischemia/Reperfusion Injury: Involvement of Protein Kinase C

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Background: In addition to its structural role, zinc is an essential component of redox signaling. We have determined that zinc ions play a role of synths in redox switch of Ser/Thr kinase structurally characterized by cysteine-rich sequences in the regulatory domains. Oxidative stress triggers zinc release from protein kinase C (PKC) molecule, the event possibly linked to zinc depletion of cardiac tissue under ischemia/reperfusion (IR). We now examined if replenishment of intracellular zinc at reperfusion improves myocardial recovery after a period of ischemia.

Methods and Results: We analyzed the recovery of left ventricular developed pressure (LVDP), heart rate, and the incidence of arrhythmias in isolated adult rat hearts. Cell viability and apoptosis were analyzed by propidium iodide, annexin V and caspase-3 activation (anti-rabbit caspase-3 antibody) fluorescence quantified by flow cytometry. Results: CRN (50 μM) reduced cardiomyocyte shortening (3.25 ± 0.45% of resting length vs 5.18 ± 0.61% of resting length in controls; n = 14; p < 0.01), intracellular Ca2+ transient amplitude (0.3 ± 0.03 μM Ca2+ vs 0.5 ± 0.04 μM Ca2+ in controls; n = 13; p < 0.001), sarcoplasmic reticulum Ca2+ content (146.0 ± 8.7 μmol/L of cytosol in controls vs 172.8 ± 12.9 μmol/L of cytosol in controls; n = 6; p < 0.01) and L-type Ca2+ channel current (4.6 ± 1.5 pA/pF vs 7.1 ± 1.8 pA/pF in controls; n = 5; p < 0.05). CRN inhibited cardiomyocytes apoptosis induced by Aldo, as analyzed by the percentage of annexin V positive cells (control 5%, 50μM CRN 3%, 10 μM Aldo 76%, and CRN + Aldo 10% vs < 0.001), and caspase-3 activation (17.50%, 15.66%, Aldo 90.91%, and CRN + Aldo 18.83% vs < 0.001). Conclusion: CRN protects cardiomyocytes against apoptosis induced by Aldo. This effect is probably mediated by reducing Ca2+ availability to trigger Ca2+ dependent pathways that induce apoptosis.

Abnormal Activation of the Ubiquitin Proteasome System is a Novel Mechanism that Mediates Doxorubicin-Induced Cardiotoxicity

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Doxorubicin (DOX) is a highly effective antitumor agent known to cause severe cardiotoxicity that culminates in congestive heart failure. A prevailing theory for DOX cardiotoxicity is the DOX-induced generation of reactive oxygen species (ROS), which has been supported by the ability of numerous antioxidants to reduce DOX cardiotoxicity in animal studies. However, clinical trials show very limited effect of antioxidant therapy in humans, suggesting that additional mechanisms might be involved in the pathogenesis of DOX-induced heart failure. Here we show that the Ubiquitin Proteasome System (UPS) is activated by DOX in neonatal rat cardiomyocytes (NRC) and adult mouse cardiomyocytes (AMC) as demonstrated by a GFP reporter that is sensitive to UPS-mediated degradation. DOX induces apoptosis in NRC and AMC as shown by increased cleavage of poly (ADP-ribose) polymerase (PARP), DNA laddering, and TUNEL or Annexin-V positive cells. Importantly, DOX-induced UPS activity leads to the depletion of cardiac survival factor GATA4 as well as its down stream targets Bcl2 and Bclxl. Inhibition of UPS function with specific proteasome inhibitor MG262 or MG132 protects NRC and AMC from DOX-induced apoptosis. Withdrawal of DOX from MG262 treated cultures results in a loss of Bcl2 and Bclxl. Interestingly, several antioxidants are able to block DOX-induced ROS generation but they do not have any effect on UPS activity or GATA4 depletion, suggesting that the UPS-mediated GATA4 depletion is not dependent on ROS. Rather, it is a direct effect of DOX that acts in parallel with ROS leading to DOX cardiotoxicity. Moreover, we have shown that MG262 reduces ROS production possibly due its ability to preserve GATA4 protein levels. Together, these findings suggest that the abnormal activation of the UPS is a novel mechanism that mediates DOX-induced cardiotoxicity. Given the limited effect of antioxidants on reducing DOX cardiotoxicity, a more promising therapeutic modality might be to use DOX and a proteasome inhibitor in combination. This can be easily implemented in clinical settings since the proteasome inhibitor bortezomib has been shown to enhance the antitumor activity of DOX in humans.

Negative Effects of the Atkins Diet on Ischemic Myocardial Metabolism and Function

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Ischemic cardiac muscle utilizes primarily carbohydrates for energy production rather than its normal dependence on lipids. With the increased popularity of the high fat, high protein and low carbohydrate diets (Atkins Diet), the reduced carbohydrate availability could be contraindicated during a myocardial ischemic episode. This study investigated the effects of an Atkins Diet in a canine model and compared the cardiac response to a normal dietary model. Eight animals were used in this study. Six were fed a normal diet (group I) and eight animals on an Atkins Diet (group II). After 6 weeks, anesthetized animals were surgically prepared to measure the myocardial uptake of glucose (Glu), free fatty acids (FFA), lactate (Lac), pyruvate, oxygen and hemodynamic parameters (end systolic elastance (Ees), LV pressure, HR and dp/dt) while increasing the heart rate (50%, 100% and 150%) for 45 minutes. Following 20 min of pacing before β-blockade, a significant increase in the EF (25%) and Lac (125%) uptake, and no change in Glu. Fifteen minutes after β-blockade, FFA uptake was reduced from 3.65 to 1.71 μg/min/100g (LV weight) and was unchanged during pacing. Lac uptake increased 1.15 to 2.27 mg/min/100g following β-blockade and from 1.25 to 5.15 mg/min/100g during pacing. In group II, FFA uptake was elevated and remained during pacing while both Glu and Lac were significantly reduced. Following β-blockade, Lac, Glu and FFA uptakes were markedly reduced following β-blockade and pacing. Cardiac functional parameters dynamically in Group II. EF was significantly elevated during pacing before β-blockade, but after, there was a marked reduction in EES and during pacing, there was little functional activity. In conclusion, increasing cardiac work pacing elevated FFA and/or carbohydrate uptake before β-blockade in Groups I and II. After propranolol (which decreases myocardial FFA uptake with an increased carbohydrate utilization and RQ’s near 1.0), pacing plus the Atkins Diet reduced carbohydrate availability concomitantly with a decreased FFA uptake. The resulting deterioration of myocardial functional activity was predictable and was supported by the present experiments. Therefore, the Atkins Diet with cardiac ischemia or extreme exercise would be contraindicated.
Mast Cell-Deficient Mice Produce Less Circulating IL-6 and Exhibit Less Cardiac Tissue Damage Than Their Littermates Following Myocardial Ischemia Reperfusion Injury

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BACKGROUND Myocardial ischemia reperfusion (IR) injury complicates all forms of coronary artery revascularization. Circulating interleukin-6 (IL-6) has been implicated in cell death following a variety of stimuli. To date macrophages, platelets, neutrophils and the endothelium have been shown to release IL-6 after IR injury. Cardiac mast cells have been implicated in IR; however, their involvement has never been quantified. In this randomized prospective study, we compared cardiac tissue susceptibility and serum IL-6 changes between the mast cell deficient (W/Wv) mice and their normal littermates (+/+). METHODS Thirty male W/Wv mice and +/+ littermates were anesthetized with 2.5% isoflurane. The left coronary artery (LCA) was ligated for 30 minutes or a sham procedure was performed. After 6 hours of reperfusion, the animals were sacrificed. The muscle viability was assessed on fresh whole-mount slices by the nitroblue tetrazolium (NBT) histochemical assay and serum IL-6 concentrations measured with ELISA. RESULTS Cardiac muscle viability was significantly higher in W/Wv than the +/+ mice. Serum IL-6 levels were higher in the +/+ sham mice (16 ± 11 pg/ml, n= 6) than the W/Wv mice (65 ± 11 pg/ml, n= 6), p < 0.001. The IL-6 levels increased significantly after reperfusion only in the +/+ mice (45 ± 14 pg/ml, n= 6, p < 0.001). In addition, a significant increase in PTN mRNA levels (17 ± 11 pg/ml, n= 8, p < 0.783). CONCLUSIONS These results show that the absence of mast cells reduces the myocardial damage associated with IR injury. Furthermore, there is an attenuation is the inflammatory response, as measured by serum IL-6 levels, following this local insult. This finding entertains the prospect of developing prophylactic therapy - targeting selective inhibition of cardiac mast cell activation, in clinical situations involving medical or surgical myocardial revascularization.

Postconditioning the Human Heart: Multiple Balloon Inflations During Cardiac Catheterization May Confer Cardioprotection

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Background: Growing evidence from experimental models suggests that relief of myocardial ischemia in a stuttering manner (i.e., “postconditioning” [Postc] with brief cycles of repertusion/reocclusion) limits infarct size. However, the potential clinical efficacy of Postc has, to date, been poorly explored. Objectives: Our aim was to test the hypothesis that Postc (i.e., clinical surrogate of infarct size) would be attenuated in ST-elevation myocardial infarction (STEMI) patients receiving multiple balloon inflations-deflations during cardiac catheterization and angioplasty. Methods: To obtain initial insight into this question, we conducted an IRB-approved, retrospective chart review of all STEMI patients with single vessel occlusion (LAD and angioplasty. Results: We identify 21 patients receiving 1–3 inflations and 4 patients receiving 4 inflations-deflations, presumably due to more complex coronary lesions, had a length of hospital stay that was modestly (but significantly) prolonged. Nonetheless, peak CK release was considered (0.5 to 4.0 μmol/min/g), while it remained similar in the W/Wv (144.3 ± 1.6) and hypertrophic group, which was significantly attenuated in both control and hypertrophic group by treatment of isolated cardiac nuclei with CA2+-ATPase inhibitor thapsigargin, and ryanodine receptor antagonist ruthenium red (decreased by 20.1%, 115.0 ± 1.3 vs 144.6 ± 1.6) and heparin (reduced by 72.2%, 39.9 ± 1.3 vs 144.6 ± 1.6) on c-fos gene were stronger than the effect of control. Comparison with the control group, the activity of CA2+-ATPase in cardiac nuclei was decreased by 51.9% (4.7 ± 0.5 vs 9.8 ± 0.4 μmol/min/g, p < 0.01); [3H] ryanodine binding assay of ryanodine receptors on nuclear envelope showed Maximal number of binding sites (B max) and dissociation constant (Kd) of ryanodine binding decreased by 57.8% (121.5 ± 11.3 vs 288.0 ± 38.0 fmol/mg protein, p < 0.01); and by 54.4% (2.5 ± 0.8 vs 5.5 ± 1.9 fmol/mg protein, p < 0.05) respectively. [3H] IP3 binding assay of IP3 receptors on nuclear envelope were increased by 1.1 (828.7 ± 77.8 vs 395.6 ± 35.8 fmol/mg protein, p < 0.05) and by 2.15-fold (18.1 ± 2.7 vs 6.3 ± 1.3 fmol/mg protein, p < 0.05) respectively in the hypertrophic group. These results indicated that c-fos gene transcription regulation involved in the change of nuclear calcium handling system, which might at least partly take part in developing overload-induced cardiac hypertrophy.

Gene Expression Changes in Human Trabeculae Treated with the β-Adrenergic Receptor Agonist Isoproterenol

Carmen C Sucharov, Bradley Nelson, Jennifer Morrison, Wayne Minobe, Michael Bristow; Univ of Colorado HSC, Denver, CO

β-adrenergic signaling plays an important role in the natural history of dilated cardiomyopathy (DCM), exerting both compensatory effects on cardiac function and promoting the development and progression of the DCM phenotype. Activation of β-adrenergic receptors (β-AR and β-AR) during periods of cardiac stress initially results in increases in heart rate and contractility, effectively improving cardiac output, but then ultimately harms the failing heart by altering gene expression patterns and cardiac morphology. Of the changes that are observed in failing hearts, increases in β-musky heavy chain (MyHC), skeletal α-actin, and atrial natriuretic peptide (ANP), with coordinate decreases in α-musky heavy chain (αMyHC) and sarcoplasmic reticulum ATPase 2a (SRCA2a), are perhaps the most widely recognized. Gene expression responses in response to β-AR stimulation have been done mostly in animal models, with the exception of one study performed by our group (Lowes et al), in which treatment of heart failure patients with β-blocker therapy results in a partial reversal of the fetal gene program. Here we show that by treating isolated human trabeculae with the β-AR agonist isoproterenol for 48 hours, changes in the fetal gene program are observed, with up-regulation of ANF, BNP and skeletal α-actin. Briefly, individual trabeculae of uniform size (1 to 2 by 6 to 8 mm) were isolated from the free wall of the right ventricle and placed in muscle bath chambers containing Tyrode’s buffer equilibrated with 95% O2/5% CO2 and kept at 37°C. Trabeculae were suspended between plastic mounting clips and treated with 10^-M isoproterenol for 48 hours, and gene expression was measured by RT-PCR. These results suggest that human trabeculae are a valid model system to analyze changes in gene expression in response to hypertrophic agonists in human subjects.
cardiology. However, only few data exist on the impact of RAPA on vascular function. Aim of our study was to investigate the influence of RAPA on vascular function of human internal thoracic arteries (ITA). Methods: Samples of ITA were obtained from 35 patients, undergoing elective coronary artery bypass surgery. Specimen were cut into rings and suspended in organ baths, containing 10 ml Krebs-Henseleit solution for isometric tension recording. After an equilibration period of 2 hours rings were then challenged with 1μm of the contractile agonist noradrenaline (NA). During active tone kept by NA the relaxant compound acetylcholine (ACh) was tested (1μm). Arteries were then incubated with different concentrations of RAPA (10nmol, 1nmol, 0.1μmol, 1 μmol, 10 μmol) for 20 h. In all experiments at least one arterial ring was used as time-matched, “non-treated” control. After that period testing with NA and ACh was repeated on the same rings. Results: The maximal contractile response for 1μmol NA was similar between the different concentrations of RAPA and the controls. Comparing the ACh induced relaxation in relation to the NA (1μmol) induced precontraction before versus after incubation in each group, we found a concentration dependent decrease in relaxation, being significant for 1μmol and 10μmol RAPA compared to the control (in %: control: 46.0 ± 4.6; 1μmol: 30.7 ± 2.8, p < 0.01; 10μmol: 12.9 ± 1.2, p < 0.001). Conclusion: To our knowledge these are the first data investigating the influence of RAPA on vascular reactivity in human tissue in vitro. Our results revealed, that contractility induced by NA revealed no direct effect of RAPA on muscular contractile function. However relaxation of ACh induced by ACh is concentration-dependently decreased by RAPA indicating impaired endothelial function after only short exposure to RAPA.

Lack of microRNA Regulation in Myocardial and Skeletal Muscle in Response to Cardioprotective Arrest and Cardiopulmonary Bypass


Introduction: MicroRNAs(miRNAs) are small, non-coding RNAs that regulate the expression of complementary messenger RNAs (mRNA). The expression of miRNAs in myocytes in response to stress has not been studied. We quantified the abundance of several miRNAs in patients in response to cardioprotective arrest (CPA) and cardiopulmonary bypass (CPB). Methods: Right atrial (RA) and skeletal muscle (SKM) was harvested from similar cardiac surgical patients (N = 6) before and after CPA. RNA was extracted using Ambion’s MELT Total RNA Isolation System. Total RNA was analyzed by Taqman real-time RT-PCR of select mRNA and miRNA targets. Criteria1 and DUSP1 were studied as genes known to be upregulated by CPA as positive controls. GAPDH was used as a negative control. miRNAs were selected based on data in the literature and the potential for stress response and the role in heart failure. Results: Both Criteria1 and DUSP1 were significantly upregulated (p < 0.05) while GAPDH expression was not altered by CPA. There was no significant difference in the regulation of miRNAs tested following CPA/CPB for the 18 targets that were assessed by qRT-PCR. Conclusions: No miRNA targets were identified that were differentially expressed in response to the ischemia reperfusion stress following CPA/CPB in patients.

miRNA gene Detector Delta Ct Delta Ct Std Dev Delta Delta Ct (Biological Reps) Delta Delta Ct Std Dev

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<th>Detector</th>
<th>Delta Ct</th>
<th>Delta Ct Std Dev</th>
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<td>Post RA</td>
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Methods: Samples of ITA were obtained from 35 patients, undergoing elective coronary artery bypass surgery. Specimen were cut into rings and suspended in organ baths, containing 10 ml Krebs-Henseleit solution for isometric tension recording. After an equilibration period of 2 hours rings were then challenged with 1μmol of the contractile agonist noradrenaline (NA). During active tone kept by NA the relaxant compound acetylcholine (ACh) was tested (1μmol). Arteries were then incubated with different concentrations of RAPA (10nmol, 1nmol, 0.1μmol, 1 μmol, 10 μmol) for 20 h. In all experiments at least one arterial ring was used as time-matched, “non-treated” control. After that period testing with NA and ACh was repeated on the same rings. Results: The maximal contractile response for 1μmol NA was similar between the different concentrations of RAPA and the controls. Comparing the ACh induced relaxation in relation to the NA (1μmol) induced precontraction before versus after incubation in each group, we found a concentration dependent decrease in relaxation, being significant for 1μmol and 10μmol RAPA compared to the control (in %: control: 46.0 ± 4.6; 1μmol: 30.7 ± 2.8, p < 0.01; 10μmol: 12.9 ± 1.2, p < 0.001). Conclusion: To our knowledge these are the first data investigating the influence of RAPA on vascular reactivity in human tissue in vitro. Our results revealed, that contractility induced by NA revealed no direct effect of RAPA on muscular contractile function. However relaxation of ACh induced by ACh is concentration-dependently decreased by RAPA indicating impaired endothelial function after only short exposure to RAPA.

Distinct Regulation of Thrombospondin-1 Gene Expression in Loaded Hearts

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The heart responds to pressure overload by concentric hypertrophy, while post-infarction remodeling is associated with thinning of the wall in the infarcted area, followed by eccentric hypertrophy, ventricular chamber dilation and extracellular matrix (ECM) remodeling. Thrombospondin-1 (TSP-1) is a secreted ECM glycoprotein with diverse functions, such as inhibition of angiogenesis and activation of transforming growth factor-β1. However, the role and mechanisms regulating TSP-1 gene expression in the heart are unclear. Here we first studied TSP-1 gene expression during left ventricular (LV) remodeling process after experimental myocardial infarction in rats. TSP-1 mRNA levels were elevated at day 1 and protein levels at 2 and 4 weeks following the myocardial infarction. To study whether also pressure overload affects TSP-1 gene expression, we infused angiotensin II (Ang II) in rats. Subcutaneous infusion of Ang II (30 g/kg/h) by osmotic minipumps for 6, 12 and 72 hours and 2 weeks increased LV TSP-1 mRNA levels up to 2 weeks, the maximal increase being observed at 6 hours (19.4-fold, p < 0.001). Ang II type 1 receptor (AT1) antagonist losartan (400 g/kg/h) completely inhibited the activation of TSP-1 gene expression by Ang II at 6 hours. Finally, angiotensin-vasopressin (AVP) infusion into young (3 months old) and old (20 months old) conscious spontaneously hypertensive rats (SHR) and their age-matched normotensive control Wistar-Kyoto (WKY) rats was used to study whether diastolic hypertensive heart failure influences acute pressure overload induced increases in TSP-1 gene expression. A significant increase in LV TSP-1 mRNA levels were seen after 30 minutes and 4 hours of AVP infusion (0.05 g/kg/min) in young rats (SHR and WKY rats), this increase and greater in SHR. These results indicate that TSP-1 is rapidly up-regulated in response to pressure load well before the development of left ventricular hypertrophy and fibrosis. Moreover, TSP-1 may have a role in the post-infarction remodeling process.

Mao-to, Japanese Herbal Medicine, Prolongs the Survival of Viral Myocarditis in Mice by Reduction of Cardiac TNF-α Expression

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Mao-to, Japanese herbal medicine, has anti-viral and anti-autoimmune effects. Immunomodulatory agents are tried, but the effects were limited. We examined the beneficial effects of Mao-to on the severity of acute myocarditis induced by encephalomyocarditis (EMC) virus in mice with the modification of cardiac tumor necrosis factor-alpha (TNF) gene expression. The C57 mice were randomly divided into 5 groups (n=30 of each). Group N included uninfected control mice. Group A, B, C and D were inoculated with EMC virus. Group A was orally administered saline starting on day 0 to day 9. Group B was orally administered Mao-to (500mg/kg/day) starting on day 0 to day 6. Group C was orally administered with Mao-to at the same dose as group B starting on day 2 to day 9. Group D was orally administered with Mao-to at the same dose as group B starting on day 0 to day 4. The 14 days course of treatment was observed after viral inoculation. Body weight and organ weight containing heart (HW), bilateral lungs, thymus and spleen were examined on days 0, 2, 4, 6 and 14. Cardiac expression of TNF protein and mRNA was examined by immunohistochemistry and qualitative RT-PCR. The survival rates on day 7 were 10% in group A, 18.2% in group B, 23.3% in group C, 50% in group D. Survival rate in group D was significantly higher than that in group A and B. The heart and weight/ body weight ratio in group C on day 6 was significantly (p < 0.05) lower than those in group A, B or D. Myocardial injury and inflammation in group C were significantly (p < 0.05) reduced compared with those in group A and B on day 6. The lung weight/ body weight ratio in group C on day 6 was significantly (p < 0.05) lower than those in group A. Cardiac expression of TNF protein and...
miRNA was significantly (P<0.05) reduced in group C in comparison with in group A, B or D on day 6. Oral administration of Maa-to starting on day 2 is beneficial for improvement of mortality resulting from acute viral myocarditis in mice with reduced expression of cardiac TNF. These findings suggest crucial implication for starting time of herbal medicine in a murine model of viral myocarditis.

MicroRNA Function During Cardiac Hypertrophy

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Diverse forms of cardiac injury evoke a hypertrophic response characterized by an increase in cardiomyocyte cell volume, enhanced protein synthesis, assembly of sarcomeres and activation of a fetal cardiac gene program. Pathological hypertrophy is a major predictor of heart failure and cardiac sudden death. MicroRNAs, which target specific mRNA transcripts for degradation and translational repression, have emerged as key regulators of cell growth, differentiation and death. We have explored the possible involvement of miRNAs in cardiomyocyte hypertrophy and heart failure. Microarray analysis revealed over a dozen miRNAs that were significantly induced or repressed in cardiac tissue from mice that were either exposed to transverse aortic constriction or cardiac-specific over-expression of activated calcineurin, stimuli that induce cardiac hypertrophic remodeling. The changes in expression of these miRNAs were recapitulated in failing human hearts. The potential functions of these hypertrophic miRNAs have been investigated by their cardiac over-expression in vivo and in vitro. Selected cardiac miRNAs have also been inactivated in mice by gene targeting. The functional significance of discreet miRNAs as modulators of the cardiac hypertrophic response will be presented.

Effect of Erythropoietin on Neointima Formation in Rat Carotid Artery Model of Vascular Injury

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Effect of Erythropoietin on Neointima Formation in Rat Carotid Artery Model of Vascular Injury

Maram K Reddy and Vinod Labhasetwar Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198 Various cytotoxic cytokine gene and drug therapies targeting vascular smooth muscle cells (VSMCs) have demonstrated some efficacy in inhibiting VSMCs proliferation and neointima formation following angioplasty and/or stent implantation; however, re-endothelialization of the injured artery is critical to its long-term patency. Previous studies have shown that the bone marrow-derived endothelial progenitor cells (EPCs) are recruited at the vascular injury site and contribute to endothelial recovery. Recombinant human erythropoietin (EPO) has been shown to induce proliferation and mobilization of EPCs and also possesses anti-inflammatory and anti-apoptotic properties. Therefore, we hypothesized that the exogenous administration of EPO would mediate the process of vascular repair by facilitating re-endothelialization and thus could inhibit neointima formation. To test our hypothesis, animals were injected EPO intraperitoneally (5,000 IU/kg) 6 hr prior to vascular injury and then on every alternate day for one week in a rat carotid artery injury model and morphometric analysis of arteries was carried out at three weeks. Although the EPO treated animals demonstrated nearly complete and continuous arterial re-endothelialization (90% endothelial cell coverage vs. < 20% in saline control), the treatment also resulted in excessive neointima formation (Intima/Media ratio 2.1 ± 0.09 vs. 1.6 ± 0.02, n = 5, p < 0.001), resulting in significant reduction in lumen area (0.16 mm² ± 0.01 vs 0.3 mm² ± 0.02, n = 5, p < 0.001). The mechanism of excessive neointima formation in the EPO treated group was found to be due to excessive myoangiogenic response in the injured artery as represented by CD31-positive structures (103 ± 10/mm² vs. 35 ± 5.2/mm² saline control, p < 0.001). Nonetheless, our results explained a common occurrence of vascular access stenosis in patients on EPO treatment during hemodialysis and suggest the cautious use of EPO in patients who are at a risk of vascular injury.

Detection and Monitoring of Brain Recovery After Therapeutic Hypothermia

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Objective: To test the hypothesis that quantitative electroencephalogram (qEEG) can objectively functional electrophysiological recovery of brain after hypothermia in an asphyxial cardiac arrest rodent model. Methods: Twenty-eight rats were subjected to 7-minute (n = 7) and 9-minute (n = 14) asphyxia-cardiac arrest. One half of each group (n = 7) was randomly subjected to hypothermia one hour after CPR (T = 33°C for 12 hours) and the other half (n = 7) was normothermically rewarmed (T = 37°C). Continuous monitoring of blood pressure, ECG and core body temperature and intermittent arterial blood gas (AGB) analysis were undertaken. Neurological recovery after CPR was assessed by serial Neurological Deficit Score (NDS) and qEEG analysis. Information Quantity (IQ), a validated measure of relative EEG entropy, was employed to monitor electrical recovery of the brain. Result: After cardiac arrest, the hypothermia-treated group demonstrated better brain recovery (higher IQ) compared to normothermic controls (P < 0.001). The 72-hour functional recovery by NDS of the hypothermia group was also significantly better compared to the normothermia group (P < 0.01). IQ during the hypothermia maintenance period has the highest correlation within the first 24 hours (Pearson correlation 0.746, 2-tailed significance <0.001) with 72-hour functional recovery by NDS. Conclusion: The qEEG IQ measure was able to detect the effects of hypothermia during the first 24 hours which was corroborated by the functional recovery by NDS at 72 hours. These results demonstrate the potential utility of objective measures such as qEEG to track the response to hypothermia and other potential therapies during the early phase of recovery from cardiac arrest.

Pioglitazone and Atorvastatin Augment Myocardial Production of 15-Epi-Lipoxin A4 in the Rat Heart

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Background: Atorvastatin (AT) increases myocardial expression of phosphorylated Ser-1776 endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS) and Cyclooxygenase-2 (COX2). Although AT increases the expression of COX2 in eNOS-/- mice, there is no increase in COX2 activity as occurs in wild-type (WT) mice. In the rat, COX2 activation occurs by iNOS mediated S-nitrosylation. Aim: To investigate whether the difference in COX2 activity between the AT-treated WT and eNOS-/- is related to S-nitrosylation. Methods: WT, eNOS-/-, and iNOS-/- mice received AT 10 mg/kg/AT (+) or water alone (AT (-)) for 3 days. Hearts were harvested and subjected to ELISA and immunoblotting. S-nitrosylation of COX2 was assessed by the “Biotin Switch” assay. To verify that the immunoprecipitated contained COX2, membranes were stripped and blots with anti-COX2 antibodies. Results: COX2 expression in the AT- groups was very low. AT increased COX2 expression in WT (4,287±104%) and eNOS-/- (4,354±101%), but not iNOS-/- mice (101±3%). However, myocardial 6-keto-PGF1α levels (81.4±0.2 vs. 15.7±0.1 pg/ml; p<0.001) were increased by AT only in WT mice, but not in eNOS-/- (16.5±0.1 vs. 15.3±0.1 pg/ml) or iNOS-/- (15.9±0.2 vs. 15.3±0.1 pg/ml) mice. The “Biotin Switch” assay shows that COX2 was S-nitrosylated only in WT mice. Although eNOS is activated by AT in the iNOS-/- mice, there is no S-nitrosylation and activation of COX2. Conclusions: COX2 is activated by S-nitrosylation only in WT mice. Although iNOS is intact in eNOS-/- mice, it is not activated and therefore, does not S-nitrosylate the AT-induced upregulated COX2.

A Role for Interleukin-Converting Enzyme in Heart Failure

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Apoptosis of cardiomyocytes is increased in heart failure and has been demonstrated to be a crucial factor for the progression of the disease. For the induction of cardiomyocyte apoptosis
hypertrophy and anti-apoptotic signaling. Three independently created BRAP-transgenic (TG) mouse lines in two different genetic backgrounds exhibited grossly enlarged hearts, compared to wildtype littermates (WT). Echocardiography confirmed dilation (left ventricular end-diastolic diameter 4.57 ± 0.17 vs. 3.57 ± 0.04; p < 0.05; BRAP TG vs. WT; p < 0.05; TG vs WT) and significant reduction of fractional shortening (14 ± 3% vs. 40 ± 0.4%; BRAP TG vs. WT; p < 0.05; BRAP TG vs. WT). Stem- and naïve-MHC transgenic Brap1-Tg mice were born alive with 50% of mice dying before the age of 24 weeks. These data indicate that BRAP is differentially expressed in heart failure and controls cardiac size and function via inhibition of the ERK MAPK pathway.

P165 Angiotsin II Receptor Imbalance Associated with Neonatal Cardiac Growth Restriction Is a Prelude to Adult Cardiac Hypertrophy
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The Hypertrophic Heart (HHR) displays spontaneous cardiomyocyte hypertrophy in association with an apparent reduction in myocyte number in adulthood. This suggests the possibility of reduced hyperplasia or increased apoptosis during early cardiac development. The angiotensin AT1 and AT2 receptor subtypes have been implicated in both cellular growth and apoptosis, but the precise mechanisms are unclear. The aim of this study was to determine the relationship between cardiac AngII receptor expression levels and neonatal cardiomyocyte growth and apoptotic responses in the HHR compared with the Normal Heart (NHR) control strain. Cardiac tissues were freshly harvested from male HHR and NHR at several developmental stages (p2 and 4, 6, 8, 12wk). HHR cardiac weight indices were considerably smaller than NHR at day 2 (4.33 ± 0.19 vs 5.01 ± 0.08 mg/g), but ‘caught-up’ to NHR by 4 weeks (5.10 ± 0.15 vs 5.16 ± 0.06 mg/g). By 12 weeks, HHR hearts were 27% larger than NHR. Tissue AT1/R and AT2/R mRNA expression was significantly increased in HHR compared to NHR, and AT1 mRNA expression was significantly higher in HHR neonatal hearts exhibited a 4.6-fold higher AT1/AT2 mRNA expression ratio. Cultured neonatal cardiomyocytes were infected with AT1 and/or AT2 receptor-expressing adenoviruses to achieve a physiological level of receptor expression (150 fmoi receptor/mg total cell protein). In addition, to emulate receptor expression in neonatal HHR hearts, cells were co-infected with AT1 and AT2 receptors at a 4:1 ratio. Apoptosis incidence was studied by morphological analysis after 72 hours exposure to 0.1 μM AngII. When infected with the AT1 receptor alone, a higher proportion of HHR myocytes appeared apoptotic than NHR (27.2 ± 4.1 vs. 12.3 ± 5.6%, P < 0.01). This suggests that the AT1 receptor is upregulated in the HHR hearts to accentuated AT1-mediated apoptosis. Interestingly, the Pax-1/bcl-2 mRNA expression ratio was significantly higher (50%) in HHR neonatal hearts. When cells were co-infected with AT1 and AT2 receptors, evidence of apoptosis in HHR cells virtually disappeared (0.4% ± 0.1%). These findings suggest a novel capacity of AT2 receptors to counteract accentuated AT1 receptor-induced apoptosis in the HHR in early cardiac growth.

P166 Cardiac Myosin Activator CK-1316719 Increases Myocyte Contractility and Myosin ATPase Activation in a Model of Heart Failure
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Improving cardiac contractility by directly activating cardiac myosin may be a preferred treatment for heart failure (HF) over the current isotropic therapies that increase intracellular calcium or act via second messenger activation. Previously we reported that small molecule myosin activators increase cardiac myosin contractility without increasing intracellular calcium or inhibiting phosphodiesterase in non-failing cells. We now report cellular and biochemical responses to the myosin activator CK-1316719 in a rat model of HF. Heart failure was confirmed in vivo by significant decreases in fractional shortening (−M-mode echocardiography) in 12-week post myocardial infarction (Ml) Sprague Dawley rats compared to sham animals. Myocytes were isolated from the left ventricle and septum for cardiac contractility, myosin ATPase, and mRNA analysis. In addition, to emulate receptor expression in neonatal Ml hearts, cells were co-infected with AT1 and AT2 receptors, evidence of apoptosis in Ml hearts virtually disappeared (0.4% ± 0.1%). These findings suggest a novel capacity of AT2 receptors to counteract accentuated AT1 receptor-induced apoptosis in the HHR in early cardiac growth.

P167 Chronic PDE5A Inhibition Reverses Aging-Associated Loss of Akt Activation in the Heart
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With advancing age, the cardiovascular system develops decreased endothelial vasoreactivity, prolonged diastolic relaxation, and a reduction in Akt activation. As both increased NOS abundance and diminished nitric oxide signaling have been reported in the aging CV system, the role of NO in the adaptations of the aging CV system remain controversial. Here we tested...
the hypothesis that phosphodiesterase type 5A (PDE5A), an enzyme that degrades cGMP, contributes to altered ventricular performance with advanced age and is a factor that reduces AKT activation. PDE5A protein abundance, mRNA expression, and enzyme activity were elevated in old (26 month) compared with young adult (6 month) rats (P<0.05). Old and young rats were treated for 2 weeks with chronic sildenafil (60 mg/d by subcutaneous injection) or saline. Sildenafil concentration free sildenafil were 10 nM. After chronic treatment with sildenafil, rats were subjected to pressure-volume loop analysis. Rats chronically treated with sildenafil showed that baseline tau was significantly lower than sham control old rats (n=8, P<0.05) but not significantly different from young control or young treated with sildenafil. Five autopsy animals were analyzed. As with acute administration of sildenafil, chronic treatment resulted in a marked inhibition of the inotropic response to isoproterenol in the old as well as in the young. We have previously observed that under conditions of pathologic cardiac hypertrophy, PDE5A inhibition results in a reduction in AKT signaling. In the present study, we observed an aging-associated reduction in total AKT and activated AKT expression that was partially reversed by chronic treatment with sildenafil. In sum, these data suggest that inhibition of the PDE5A pathway enhances ventricular relaxation in old hearts and restores AKT expression, suggesting that this pathway may be exploited to modulate diastolic function in aged myocardium.

**Adenosine Regulation of the Cardiomyocyte Microtubular Cytoskeleton During Hypertrophy**

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Contractile dysfunction in the hypertrophied heart is in part due to cardiomyocyte cytoskeletal remodeling with accumulation of a dense microtubular network that increases the viscous load on the myofilaments. Adenosine or its stable analogue, 2-chloroadenosine can attenuate hypertrophy and detrimental remodeling in mice subjected to pressure overload in response to transverse aortic constriction (TAC). The mechanism however, by which adenosine attenuates hypertrophy and cardiac dysfunction is unclear. Here, the role of adenosine in cardiac cytoskeletal remodeling during hypertrophy was investigated. Consistent with the ability of adenosine to attenuate hypertrophy, diminished adenosine levels in cd73KO mice were associated with greater hypertrophy and heart failure in response to TAC. Notably, sham cd73 mice already showed increased levels of tubulin compared to WT, and tubulin levels in response to TAC were substantially increased over WT, suggesting that adenosine levels may decrease accumulation of microtubules in cardiomyocytes. In neonatal cardiomyocytes, phenylephrine (PE) caused hypertrophy with increased microtubule accumulation in the cytoskeletal fraction, while CADO treatment reduced hypertrophy and the accumulation of microtubules. To distinguish between anti-hypertrophic effects vs. microtubular effects of CADO, hypertrophied cardiomyocytes containing increased microtubules were treated with CADO. In hypertrophied cells, CADO still diminished microtubules but left sarcomeric actin intact, implying an effect on microtubules independent of an effect on hypertrophy. The CADO-induced loss of microtubules was blocked by DPCPX, implicating the Adenosine A1 receptor in adenosine mediated depletion of microtubules. Furthermore, treatment of cardiomyocytes with PE reduced activation of Lim Kinase (LimK; an enzyme which promotes microtubule destabilization), while CADO restored LimK activation, suggesting a potential mechanism by which adenosine may stabilize microtubules. Together these results imply a novel role for adenosine in preventing the accumulation of microtubules in cardiomyocytes and preserving cardiac function during cardiac hypertrophy.

**Calpain 1 Gene Expression and Calpain-Like Protease Activity Increase with Atrophic Remodeling of the Heart**

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Calpain 1 and 2 are essential regulators of skeletal muscle atrophy. Their role in heart muscle is undefined. Hypothesis: Calpain 1 and 2 regulate cardiomyocyte atrophy. Methods: We used two models of unloading-induced cardiac atrophy and one model of starvation-induced cardiomyocyte atrophy. Failing human hearts of 10 patients (8 men, age: 40.4 ± 5.7) were unloaded with a left ventricular assist device (mean duration was 200 ± 38 days, range: 64–437 days). Myocardial samples were obtained from failing human hearts before and after mechanical unloading. Normal rat hearts were unloaded for 7 days by heterotopic transplantation into the abdomen of a recipient rat. Neonatal rat ventricular cardiomyocytes (NRVM) were starved of amino acids for 18 hours. Using quantitative RT-PCR we measured transcript levels of calpain 1 and calpain 2 at baseline and after treatment. In the unloaded rat hearts we also measured calpain-like protease activity. Results: In patients with left ventricular systolic dysfunction, mechanical unloading increased cardiac transcript levels of calpain 1 but unloading did not significantly change calpain 2 gene expression. The change in calpain 1 transcript levels correlated negatively with the duration of unloading (r=−0.73, p<0.01). Unloading of the heterotopically transplanted rat heart significantly increased calpain 1 transcript levels and calpain-like protease activity. In nutrient deprived NRVM (no amino acids) calpain 1 transcript levels increased. Conclusions: Calpain 1 gene expression and activity increases during cardiomyocyte atrophy suggesting that calpain 1 may regulate reversal of hypertrophy. Future studies will examine the trophic effect of calpain 1 inhibitors in models of atrophy and calpain 1 activators in models of hypertrophy.

**Supramolecular Protein Structure for Extracellular Antithrombin Protection of Vascular Wall**

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Reactive oxygen species damage the blood vessel wall at initial and advanced stages of vascular pathology. Oxidative stress contributes to atherogenic modifications of low-density lipoproteins, development of lipid streaks on the luminal surface, alterations in the glycosylation, accumulation, and uptake of advanced glycation end products. Such adverse modifications damagingly imply the necessity of both intracellular and extracellular protection of vascular wall, which can be realized by gene therapy and exogenous biological antioxidants. Recent our studies in vivo have shown that modified superoxide dismutase (SOD) and catalase (CAT) as well as extracellular superoxide dismutase, an enzyme that employs the specificity of glycosaminoglycan-protein interactions, can be effectively used as antioxidant protectors. Covalent binding of SOD to CAT via the vascular wall glycosaminoglycan chondroitin sulfate (CS) leads to formation of a supramolecular biointerface conjugate (SOD-CAT-CS)-which manifests in vivo the highest anti-inflammatory activity in comparison with SOD and CAT, which were either bound to CS (on the rat model of arterial thrombosis induced by the treatment of vessel with ferrous chloride) that the antithrombotic effects of covalently modified biocatalysts exceed those of native enzymes, free CS and their mixtures. A single-bolus injection of the mixture between SOD and CAT-CS (SOD-CAT-CS) at a significantly lower concentration was compared with that of the SOD-CS-CAT conjugate. This could be explained by different surface distribution of the conjugates in the circulation after their intravenous administration. The biointerface conjugate was effective at doses two orders smaller than those for native SOD and CAT and an order of magnitude smaller than that for CS-modified derivatives, administered either singly or as their mixture. Our results stress the importance of the attachment of a bioantioxidant to the vascular wall and the stable connection of SOD and CAT activities on its surface. Extracellular bioantioxidants are prospective for the development of highly efficient pharmaceutical protectors of the vascular wall against oxidative damage in conjunctive courses of thrombotic, pulmonary, psychiatric or preventive cure.

**Oxidant Stress from Endothelial Nitric Oxide Synthase Uncoupling Plays a Major Role in Pulmonary Hypertension**

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Models of pulmonary hypertension are associated with pulmonary vascular remodeling. Reactive oxygen species (ROS) may importantly contribute to this process, but the sources of ROS remain poorly understood. Endothelial nitric oxide synthase (eNOS) has been shown to be a major producer of ROS in vascular endothelium when deprived of substrate L-arginine and/or its cofactor tetrahydrobiopterin (BH4). Uncoupled eNOS is a monomer while active eNOS forms a dimer. We tested the hypothesis that chronic hypoxia leads to eNOS uncoupling, thereby stimulating ROS generation and contributing to pulmonary vascular remodeling. In C57/B6 mice, 6 weeks of hypoxia induced pulmonary arterial hypertension that was associated with profound pulmonary vascular remodeling in medium (250–400 μm) and small (100–250 μm) arteries. O2 production assessed by luminal chemiluminescence and immunostaining with dithiothreitol increased in the lungs of mice exposed to hypoxia. Nitrrosylation, a marker of end-product of tissue oxidative damage, increased and matrix metalloproteinases (MMP-2 and -9) were activated under conditions of hypoxia. Hypoxia significantly reduced dimeric form of eNOS, accompanied by reduced Ca2+-dependent NO activity, whereas normoxic controls harbored both dimeric and monomeric forms and higher activity. Supplementation of the eNOS cofactor BH4 (1mg/kg food) prevented eNOS uncoupling, and was accompanied by reduced O2 production, nitrosylation formation, and MMP-2 and -9 activation. Moreover, supplementation with BH4 resulted in a significant reduction in mean PA pressure and pulmonary vascular resistance in mice exposed to chronic hypoxia. Supplementation with BH4 (an analog of BH4 that is not a cofactor for NOS) did not reduce these biochemical markers of NO uncoupling and did not alter the pulmonary hemodynamic response to chronic hypoxia. In PA explants from patients undergoing lung transplant, eNOS was also found to be uncoupled. Thus, uncoupled eNOS is associated with pulmonary hypertension in animal models and human pulmonary hypertension and these data support the future investigation of BH4 supplementation as a treatment for pulmonary vascular disorders.

**3'-Azido-3'-Deoxythymidine Inhibits Thymidine Phosphorylation in H9C2 Cells**

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AZT (3'-azido-3'-deoxythymidine, zidovudine) inhibits thymidine phosphorylation in isolated rat liver and heart mitochondria, as shown by previous work from this laboratory. This inhibition may lead to depletion of TTP within the mitochondria and could cause the mitochondrial DNA depletion that observed in tissues experiencing AZT toxicity. In order to further advance this mechanism of toxicity, the H9C2 cell line will be used. H9C2 cells are derived from rat cardiac myoblasts and provide a convenient model system for the effects of toxic insults. We will demonstrate that AZT inhibits thymidine phosphorylation in short term trials conducted with H9C2 cells. The time course of thymidine salvage and phosphorylation was measured by incubating the cells in media supplemented with 1 μM [3H]-thymidine. The H9C2 cells phosphorylated thymidine to a plateau in 20 minutes and then the kinetic studies were performed. The time course of AZT phosphorylation was determined by incubating the H9C2 cells with media containing 1 μM [3H]-AZT. AZT was phosphorylated to AZT-5'-monophosphate (AZTMP), reaching a plateau after 60 minutes of incubation. AZT-5'-triphosphate (AZTTP) was first detectable at 150-200 minutes of incubation. As seen from these two time courses, AZT inhibits thymidine phosphorylation at a linear rate, and the AZT pool consists
Identification of P116 as a New Candidate Gene in Heart Failure by a Genetic Yeast Screen for Secreted Proteins

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Remodeling of the myocardium is of central importance for the development of congestive heart failure. Experiments with conditioned media demonstrated communication between cardiomyocytes and fibroblasts via secreted factors. The aim of our work is the identification and characterization of proteins secreted from the heart. We performed a systematic search for secreted proteins in a murine cardiac cDNA-library using a genetic yeast screen. Out of 1.7 x 10^6 clones putatively secreted from the heart medium after transfection of neonatal rat cardiomyocytes with a P116-expressing plasmid, a genetic screen identified 54 proteins. PI16, a newly identified secreted protein, was strongly and early upregulated in heart failure. Serum from patients with type 2 diabetes showed a significant reduction in glucose level by continuous glucose monitoring (CGM) compared to healthy controls. The mechanism(s) responsible for the enhanced angiogenesis as well as cardioprotective effect of resveratrol, a phytoalexin present in red wine, was investigated using implantable glucose sensors in normal and diabetic rats. Sprague Dawley rats were randomized into three groups: control, resveratrol- and streptozotocin (STZ)-treated rats. After 15 days, the STZ-treated rats showed a significant reduction in glucose level, increased Mn-SOD activity along with the reduction in glucose level for cardioprotection is a promising new therapeutic strategy for treating diabetic myocardium. In conclusion, resveratrol iv) is a potential candidate for the treatment of diabetic myocardium.

Resveratrol: A Fountain of Miracles in Cardiovascular Disease

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Diabetes is the most significant comorbidity of patients with heart failure (HF) which adversely affects outcomes in patients with HF and coronary arteriolar disease. Oxidative stress has been implicated in the pathology and complications of diabetes, which leads to myocardial ischemia-reperfusion injury. Therefore to examine the efficacy of Resveratrol (25mg/kg bw), a compound present in red wine, for 15 days we assessed its effect on striated myoplasm and cytosolic Ca2+ levels in isolated rat hearts. Significant changes in the expression of channel expression.

Calcineurin-Dependent Regulation of Cardiac L-Type Calcium Channel

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Introduction: Evidence from in vivo models of pressure overload suggests that calcineurin, a major mediator of pathological hypertrophy, is capable of regulating its proximal activator, the L-type Ca2+ channel. Based on this, we hypothesized that calcineurin physically and functionally interacts with the L-type Ca2+ channel. Methods and Results: Co-immunoprecipitations from extracts of rat heart and brain using antibodies against calcineurin and α-1,2, the major, pore-forming subunit of the channel, revealed evidence of α-1,2-calcineurin association. GST pull-downs provided evidence for direct interaction between calcineurin and two major regions of α-1,2: the C-terminal domain spanning ser1928, a PKA phosphorylation site. In the presence of PKA, the C-terminal domain spanning ser1928 was phosphorylated in vitro, and recombinant calcineurin antagonized the action of PKA, indicating that α-1,2 is a potential substrate for calcineurin in vitro. To test functional significance, voltage-clamp recordings revealed significant up-regulation of I_{Na} in cultured cardiomyocytes expressing constitutively active calcineurin. Conversely, inhibition of calcineurin with cyclopentorpin (A Ca(3)) and tacrolimus - two structurally distinct calcineurin antagonists - decreased I_{Na}. This inhibition occurred over a time course of several minutes and was partially reversible upon drug washout. Ca3 had no specific effects on I_{Na}. To test for other mechanisms whereby calcineurin might up-regulate Ca2+ channel function, we mapped 2ks of genomic sequence upstream of the α-1,2 gene, identifying several putative NFAT, GATA4, and MEF2 binding sites. Studies performed in cells transiently transfected with an α-1,2 promoter-luciferase reporter suggested that α-1,2 transcription is regulated by calcineurin. Conclusions: These findings are consistent with a model wherein calcineurin regulates the L-type Ca2+ channel at two distinct levels: post-translational modification involving direct binding to the channel, and transcriptional regulation involving NFAT-mediated activation of channel expression.

Coping with Plasma Membrane Calcium Entry to Plasma Membrane Extrusion Revealed by Novel L-Type Calcium Channel Block

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The maintenance of Ca2+ homeostasis is crucial for cardiac myocyte function. In heart failure, as in cardiac development, there is a decrease in the density of L-type calcium channel (Ca2+) currents (I_{Ca,L}). To maintain Ca, homeostasis in plasma membrane Ca2+ influx must be matched by PM efflux. In this study we tested the hypothesis that chronic regulation of PM Ca2+ channels regulates PM efflux. We studied mRNA and Ca2+ handling responses to long-term regulation of influx and efflux induced by over-expression of the small GTPase Rac and the Na-Ca2+ exchanger (NCX). Rac partially inhibited I_{Ca,L}, but not I_{Na}, and increased Ca1,1.2 (n=7, p<0.05), and reduced the transient amplitude. Chronic Rac also slowed Ca2+ transient duration from 1.6 ± 0.2 s (n=4) to 2.5 ± 0.3 s (n=20). To measure SR load and NCX function we perfused the cells with Ca2+ free bath solution for 25 s then rapidly applied 50 mM caffeine. The caffeine-induced transient relaxation phase had a b-exponential decay. The transient phase was eliminated by 10 mM Ni2+, consistent with NCX-mediated efflux. Rem significantly slowed caffeine-induced relaxation (total time 13.2±0.5 s, n=20 vs 8.6±1.0 s, n=14, Rem vs control, p<0.05), suggesting Rem inhibits NCX function. Surprisingly, NCX over-expression also eliminated fast relaxation kinetics (total time 14.7±0.6 s, n=6). To test whether this effect reflected insufficient basal Ca2+ for allosteric NCX activation we increased the bath [Ca2+] from 1.8 to 6mM and measured caffeine-induced relaxation kinetics. 6 mM Ca2+ fully restored the fast kinetic phase in NCX-over-expressed cells and partially restored the fast phase in Rem over expressed cells. 6 mM Ca2+ did not affect the kinetics of control cells. Neither Rem nor Ca2+ channel auxiliary subunits altered heterologously expressed NCX. This data suggests that chronic I_{Na} block by Rem lowers cytosolic Ca2+. In turn, lowered Ca2+ reduces NCX activity via allosteric inactivation of NCX. Elevated Ca2+ influx only partially overcomes this inactivation in Rem over-expressing cells because of tonic I_{Na}. We conclude that Ca, channel-mediated PM entry coordinate controls store loading and Ca2+ efflux.
Increased Sarcoplasmic Reticulum Ca^{2+} Leak Causes Ectopic Contractions in Intact Rat Left Atria

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Objective: Calcium leak from the sarcoplasmic reticulum (SR) of isolated myocytes triggers their depolarization, producing spontaneous mechanical events. Whether SR calcium leak triggers such events in intact heart muscle is less clear. Thus, we investigated whether 2-aminoethoxydiphenyl borate (2-APB), an SR calcium leak inducer, caused asynchronous mechanical contraction in intact, electrically-driven rat left atria. Methods: All experiments were performed on left atria isolated from anesthetized rats. These intact muscles were attached to stimulating electrodes that were placed in 30ml muscle baths containing Krebs-Henseleit superfuse. All preparations were paced initially at 3Hz, and near isometric forces of contraction were measured throughout. Results: Asynchronous mechanical events occurred in paced left atria exposed to 10μM 2-APB. The number of these events increased as pacing rate decreased (i.e., as diastolic interval increased). Atrial mechanical function uncoupled completely from external stimulation with prolonged diastole in the presence of 2-APB. This spontaneous contractile activity (SCA) was reversible as washing 2-APB-treated atria restored normal mechanical function. SCA was sensitive to superfuse ionic composition in that decreasing either superfuse sodium (at constant chloride) or decreasing chloride (at constant sodium) prevented and reversed this phenomenon. DIDS (300μM) and ryanodine (2μM) suppressed atrial mechanical function in the absence or presence of 2-APB, neither suppressed SCA. Conclusions: 2-APB appears to activate a novel SR calcium leak in intact rat left atria which either triggers SCA under physiological salt conditions or depletes SR calcium under conditions which suppress SCA (i.e., low sodium or DIDS) decreased with time when compared to atria exposed to low sodium or DIDS alone, indicating that 2-APB activated SR calcium leak. Importantly, while both nifedipine (20μM) and ryanodine (2μM) suppressed atrial mechanical function in the absence or presence of 2-APB, neither suppressed SCA. Withdrawn

Electrical Remodeling of Ventricular Myocytes from Rats with Volume Overload-Induced Heart Failure

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Recent studies have demonstrated a progressive decrease in myocardial contractility associated with development of marked ventricular dilatation and hypertrophy in response to chronic volume overload in an infrarenal aortocaval fistula model of heart failure. However, possible alteration of myocyte electrophysiology has not been considered in this model. The present study examined the electrical remodeling of isolated ventricular myocytes obtained from hearts subjected to 10 weeks of sustained volume overload relative to myocytes from sham-operated control hearts. Myocyte action potentials and various outward K+ currents were recorded using the whole-cell patch clamp technique. The action potential duration (APD) at both 50% and 90% repolarization were significantly longer in fistula myocytes. Associated with the prolongation of APD, both the transient outward K+ current (Ito) and the delayed rectifier K+ current (IK) were depressed in fistula myocytes. On the other hand, the steady state K+ current (IKs) and L-type Ca2+ current (ICaL) were not significantly changed in fistula myocytes when compared to that of control myocytes. Western blot analyses revealed a significant reduction in the protein expression of Kv4.3 and Kv1.5, while the protein levels of Kv4.2 and Kv2.1 were not altered in fistula myocytes. Our results indicate that decreased K+ channel expression is responsible for the depressed outward K+ currents and prolonged APD in volume overload induced heart failure, which may contribute to cardiac arrhythmias.

Spinal Cord Stimulation Suppresses Bradycardia Responses and Atrial Tachyarrhythmias Induced by Mediastinal Nerve Stimulation in Canines

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Spinal cord stimulation (SCS) with electrical stimuli applied to its dorsal aspect at the cranial thoracic level imparts cardioprotection under conditions of neurally dependent cardiac stress. This study investigated whether neurally induced atrial arrhythmias can be modulated by SCS. In 16 anesthetized canines with intact stellate ganglia and in 5 with bilateral stellatectomy, trains of 5 electrical stimulus were delivered during the atrial refractory period to right- or left-sided mediastinal nerves for up to 20 seconds before and after SCS (20 min). Recordings were obtained from 191 biatrial epicardial sites. Before SCS (11 animals), mediastinal nerve stimulation initiated bradycardia responses alone (12 nerve sites) or followed by tachycardia/fibrillation (50 sites) as well as tachyarrhythmia/fibrillation without a preceding bradycardia (21 sites). Following SCS, the incidence of the bradycardia responses was reduced by 25% (62 to 47 responsive sites) and the magnitude of the cycle length prolongation in the residual bradycardias was reduced. The incidence of tachyarrhythmia induction was reduced by 60% (71 to 29 sites) but once elicited, the residual tachyarrhythmias arose from similar epicardial foci and displayed similar dynamics (cycle length and duration) as in control states. In the absence of SCS, the bradycardia and tachyarrhythmia responses to repeat nerve stimulation were reproducible (5 additional animals). Following bilateral stellactomy, SCS no longer influenced neuronal induction of bradycardia and atrial tachyarrhythmias. These data indicate that SCS obtunds the induction of atrial arrhythmias resulting from excessive activation of intrinsic cardiac neurons, doing so via their sympahtetic neuronal inputs.
infarcted myocardium (1–100 μM) the effect is significant. These results have potential implications for understanding the role of hMSCs and the therapeutic effects of β-blockers in myocardial infarction.

**Mesenchymal Stem Cell Therapy in a Canine Myocardial Infarction Model: Assessment of Regional Persistence and Function Using MRI**

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**Purpose:** To determine the persistence of targeted magnetically-labeled mesenchymal stem cells (MSC) and their effect on regional contractile function in a canine model of myocardial infarction (MI).

**Methods:** A repertused MI was created under X-ray fluoroscopy by closed-chest coronary artery occlusion in 13 dogs. MRI was performed at 72h post-MI to assess MI size and heart function. Using an MRI-compatible injection catheter, autologous MSCs labeled with ferumoxides were injected transendocardially in 7 dogs, targeting to normal (N), peri-infarction (P), and MI. MSC persistence, MI size, and regional cardiac function were studied serially (1, 2, 4 & 8 weeks post-MI) using fast gradient echo MRI, delayed contrast-enhanced (CE) MRI, and myocardial tagging, respectively. A 6-sector per slice model was used to track regional persistence of labeled MSCs, and each sector was classified according to infarct status based on CE-MRI. Systolic strain rate (SSR) was determined from tagged MRI in N, P, and MI regions. 

**Results:** Infarct size was similar between treated and untreated dogs. 

**Conclusions:** Serial MRI of transendocardially implanted ferumoxide-labeled MSCs showed persistence of MSC injections particularly in infarcted myocardium with improvements in regional contractile function demonstrated in noninfarcted myocardium. This study suggests methods that may be useful for tailoring therapy to individual patients, as well as for targeting therapy for improved efficacy.

**Validation of the Wall Motion Score for Assessing Left Ventricular Dysfunction in Mice with Myocardial Infarction**

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**Background:** Wall Motion Score Index (WMSI) is a potentially useful measure of improved cardiac function after cell or gene therapy studies in mouse models of myocardial infarction (MI). While WMSI on a 16-segment model has been clinically validated in post-MI patients, it has not been used in mice because of their small heart size and difficulty interpreting the images. The aim of this study was to determine if WMSI is a practical and accurate method for measuring left ventricular (LV) function in a mouse model of MI using a novel high-resolution echocardiography system. Methods: 46 adult male mice underwent surgery to induce MI by a mid-LAD ligation. Echocardiography was performed under anesthesia one month post MI using a Vevo 660 system (VisualSonics, Toronto) equipped with a 30 MHz mechanical transducer. WMSI was analyzed by a modified 16-segment model on short axis views (4-apical, 6-mid and 6-basal), grading wall motion as 1-normal, 2-hypokinetic, 3-akinetic, 4-dyskinetic and 5-aneurysmal. It was calculated as the ratio of the sum of wall motion score over total segments. We measured LV ejection fraction (LVEF) and volumes (ESV and EDV) by cylindrical-hemisellipsoid model and fractional shortening (FS%) by M-mode. All hearts were harvested after echocardiography. Tissue sections were collected and infarct size (IS) was measured in a blinded manner. Correlations were assessed between WMSI and above-mentioned echocardiographic parameters as well as IS. Results: All recorded images were of good quality and interpretible. The WMSI was 1.64 ± 0.33 and the IS was 34.3 ± 15.3%. Linear correlation analyses showed that WMSI correlated significantly with IS (r = -0.86, p < 0.0005), LVEF (32.76 ± 8.95%) (r = -0.84, p < 0.0005), FS% (23.87 ± 4.42%) (r = -0.39, p = 0.004). Stepwise linear regression analysis revealed that IS was an independent determinant of WMSI with R² = 0.75, p < 0.0005. Conclusions: High-resolution echocardiographic assessment of WMSI in mice is feasible, and correlates strongly with both 2-dimensional measurements of LV systolic function and infarct size. WMSI may be an important tool in assessing regional and global function in mice with experimental MI treated with novel therapies.
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In the Abstracts from the 3rd Annual Symposium of the American Heart Association Council on Basic Cardiovascular Sciences: Translation of Basic Insights Into Clinical Practice (July 31–August 3, 2006, Keystone Conference Center Keystone, CO), which appeared in the September 1, 2006 issue (Circ Res. 2006;99:e18–e50), the abstract “Prenatal Hypoxia Determines Dilated Cardiomyopathy in the Adult Through a VEGF-Dependent Mechanism” (abstract P64) has been withdrawn by the authors. This has been corrected in the current online version of the abstracts.