Abstracts

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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.
Functional Regeneration of the Canine Ventricule 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) α-sarcolemmal actinin, 2) atrial natriuretic polypeptide, 3) cardiotin, 4) alpha myosin heavy chain, 5) Troponin T and 6) Cav1.2 (the alpha subunit of the L-type calcium channel). In some cells α-sarcolemmal actinin and anti- Troponin T immunostaining showed myofibrillar 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 2005; 112:1-14). 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, Journal of Biomochenue 2006; 93:717). We compared cardiogenic cell seeded ECM (n=8) to ECM 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 control scaffolds. These cardiogenic cells thus represent a potent new cellular source for mammalian myocardial regeneration.

Correction and Characterization 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 acid 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 ±3.13 ms, controls: 43.67 ±3.58 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 CD (gaa/-: 7.95 ±0.26 ml/min, control 11.40±7.09 ml/min) and a significant increase in myocardial mass (gaa/-: 181.99 ±10.7 mg, control 140.79 ±5.12 mg) by 12 months of age. We are currently performing PV loop procedures to determine EDPVR in these mice. Using this mouse model of cardiac dysfunction, we aim to develop a cardiac gene delivery technique which can be applied to many inherited heart disorders. Previously, we have demonstrated that IV delivery of the rAAV2/1-CMV-hGAA results in approximately 200 fold higher levels of expression in hearts than rAAV2/1, suggesting a natural preference for cardiac tissue. Here we have combined the most optimal AAV serotype for cardiac transduction with a systemic administration route in order to treat gaa/- mice. MMR and tissue staining analysis has demonstrated a decrease in glycogen accumulation in mice injected with 4x10^12 vg of rAAV2/9-CMV-hGAA. The shortened PR interval phenotype and MIR detected decrease in SV and CO phenotypes are abrogated in these mice as well. Studies are underway in order to determine longevity of these therapeutic effects. In conclusion, we have demonstrated the ability of rAAV2/9 to transcend the vasculature and transduce cardiac tissue in order to treat the cardiac phenotypes of Pompe Disease.

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 2,010,000 births. Several genes that play a role in eye development, including PAX6, PAX2, SIX6, OTX2, CHX10, RX, and SIX6, 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 deletion in SIX6. Guimaraes for evaluation of MAC include: Chromosome 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 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.
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 (1.1%). The most common malformations were VSD (11 pts, 4.9%) and ASD (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: reduced by 79% vs. 2.5%, FET 0.001; total right atria: reduced by 77%, 0.4%, FET 0.008; total right ventricle: reduced by 20%, 0.001, FET 0.003). 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 myocardial morphogenesis.

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 dilatational 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 eNOSs, and on this way prevent the evolution to decoupled 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 4weeks. Echocardiography was performed at baseline and after 4 and 9wks. Cold SOD-PAGE was performed to evaluate eNOS dimers/monomers. ROS generation was evaluated with dihydroethidium (DHE) confocal staining (score 1: absent -score 4: markedly present). Myocyte thickness resp. 1.11 ± 0.05 mm without BH4 vs. 15.07 ± 0.58 mm 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 ± 87.24 ± 164.18 ± 5.98 with p < 0.004 and fibrosis (score 4.6 ± 0.6 without BH4 vs. score 2.7 ± 0.3 with BH4). BH4 administration prevented the evolution towards cardiac decoupling (fractional shortening: 20.28 ± 1.41% without BH4 vs. 32.94 ± 4.82% with BH4, p < 0.01). Superoxide generation was markedly reduced by BH4 in n=10 slices, score 4: without BH4 vs. score 2; with BH4, NOS uncoupling (increased amount of eNOS monomers) was markedly reduced by BH4. Conclusions: BH4 can reverse established cardiac remodelling by re-coupling uncoupled NOSs and as a consequence less ROS is generated, leading to less hypertrophy and an amelioration of cardiac function.

Efficient Direct Delivery of Virus-Conjugated Genes in Mice Heart

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Virus conjugated 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 genes, expressing enhanced green fluorescence protein (EGFP) in mice heart. Mice were confirmed immunohistochemically after 2 w, which showed presence of green fluorescence in EGFP- transfected mice than the sham-operated controls. Transfection efficiency was further quantified at different time points noninvasively. Data showed enhanced FI from the EGFP-transfected mice compared with non-transfected control mice.

Withdrawn

Identification of Cardioprotective and Cardiogenerative 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 pharmacological agents do not address the fundamental issue of this cell loss. We sought to identify cardioprotective and cardiregenerative 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 (TnC) and Nkx2.10, as determined by in situ hybridization. Following injection of 20ng of p27Xic1 RNA construct, 44% of embryos demonstrated expression of or very significant decrease in the area expressing xkll2.x and TNC, compared to only 8% (13/154) of embryos injected with a specific control morpholino. Furthermore, this phenotype could be rescued in 80% (7/92) of embryos by co-injection of 30pg of p27Xic1 RNA construct, but not with 50pg of Gpi1 RNA construct (p27Xic1). Hence, our data demonstrates that p27Xic1 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, n=3 control; 110±10, n=3); however, both Nkx2.10 and TnC 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 (1–168), C-terminal (171–306), and C-terminal(271–306)p27Xic1 constructs can all arrest the cell cycle (significant decrease in the number of ph3 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.

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 FACS analysis and immunocytochemistry. The mesenchymal antigen 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 erb-B2 were found in both cultures. Only 1% of H and BM cells expressed c-kit but 80% of these cells expressed c-erb-B2. Moreover, CD44, CD73, CD90 and CD105, were detected in cultured H and BM cells. The expression of the cardiac specific protein α-actinin was studied by immunocytochemistry of the labeled cells. Mononuclear cells were fixed on glass slides and underwent immunohistochemical analysis by utilizing a cardiac specific antibody against α-actinin. The α-actinin expression in the cultured human cells was evaluated. Shortly after seeding the cells, the α-actinin expression was detected in 100% of H cells and 50% of BM cells. By four weeks in culture, α-actinin expression was detected in 100% of H cells and 70% of BM cells. In conclusion, BM cells represent an additional potential source of myocardial cells capable of differentiating into cardiac lineages.

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 coronary occlusion by using a liquid nitrogensen treated probe. G-CSF mobilized human cells were collected by apyrenesis and mononuclear cells were isolated by Ficoll gradient centrifugation. SP cells where isolated by flow cytometry based on their ability to efflux the hoescht33342 nuclear stain. Both human mononuclear and SP cells underwent DAPI staining to facilitate subsequent detection. The DAPI labeled human cells were detected in the myocardial injury border zone directly following anterior wall injury (3–4 x 105 mononuclear cells or 1–1 x 105 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 following 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 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.

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 the genes displaying differential expression patterns 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). By expressing PRISM in vitro, we observed that PRISM interferes with a variety of chromatin remodeling enzymes including the class I histone deacetylases, CBX1, EHMT2, 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-6, and markers of SMC differentiation. High percentage chimeras from targeted ES cells are currently being bred and 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 Angiotensin II-Induced 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 (leukocytes, 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 atherosclerosis have been shown in animals and humans treated with AT1R blocker as well as in ApoE−/−ATIR-KO mice, relative roles of ATIR in BM versus ATIR in non-BM-derived intrinsic host cells in the pathogenesis of atherosclerosis have not been addressed. Here, we investigated the role of ATIR in BM versus non-BM intrinsic arterial cells in atherosclerosis.

[Methods and Results] A bone marrow transplantation technique (BMT) was used to create ApoE−/− mice with selective ATIR deficiency in BM (BM−/−ATIR−/− group) and those with selective ATIR deficiency in non-BM host cells (host ATIR−/− group). As control, BMT-ApoE−/− and BM-ApoE−/− ATIR−/− groups, in which BM were transplanted from ApoE−/− and ApoE−/− ATIR−/− mice into ApoE−/− and ApoE−/− ATIR−/− mice, respectively, were generated. Angiotensin II (Ang II) was chronically infused from 10 to 14 weeks of age. In face of red staining and histological analysis of the aorta revealed that an Ang II-induced increase in arterial wall plaque size was evident in control BMT-ApoE−/− (1 ± 0.5 to 5.2 ± 2%) and BM−/−ATIR−/− mice (1 ± 1.5 to 8 ± 3%). In contrast, Ang II-induced atherosclerosis was blunted in host ATIR−/− mice (2 ± 2.6, P < 0.01 vs control) as well as in BMT-ApoE−/− ATIR−/− mice (2 ± 2.6, P < 0.01 vs control). Ang II increase raised arterial pressure in BM−/−ATIR−/− and ApoE−/− ATIR−/− mice, whereas no Ang II-induced increase in arterial pressure was noted in host ATIR−/− and BMT-ApoE−/− ATIR−/− mice. There was no difference in serum lipid levels among groups with or without Ang II infusion. [Conclusion] These results demonstrate an essential role of ATIR 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 ATIR-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 ventricle (LV) remodeling and improve LV function more efficiently than do committed skeletal myoblasts. We largely attributed this difference to the greater ability of MDSCs to induce angiogenesis and 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) in excess of 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 5-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. Intracardiac 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). These results demonstrate an essential role of ATIR 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 ATIR-mediated signals in intrinsic endothelial and smooth muscle cells may play a principal role in Ang II-induced atherosclerosis.

Nitric Oxide Mediates Migration of Endothelial Cells and Circulating Progenitor Cells Through Both Nitric Oxide Synthase-Dependent and NOS-Independent Processes

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S-Nitrosothiols represent a bioactive NO storage pool and are decreased in plasma of humans with cardiovascular risk factors. Impaired vascular maintenance was associated with dysfunc- tion of endothelial cells and endothelial progenitor cells (EPCs). We thus hypothesized that the migration of EPCs to the vessel wall of the mouse carotid artery and EPCs (EPCs) exhibited similar chemotaxis toward VEGF that was abolished by L-NNA. The treatment with G-CSF inhibited the progression of atherosclerosis and neointimal thickening. However, the treatment with G-CSF ameliorated the progression of atherosclerosis. The treatment with G-CSF inhibited the progression of atherosclerosis and neointimal thickening. These results suggest that G-CSF has a therapeutic potential for the progression of atherosclerosis. Randomized clinical trials to evaluate the feasibility and safety of G-CSF on coronary artery diseases are warranted.

Modulation of the β-Adrenergic Stimulated Inotropy by 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 contractility 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 Pseudomonas aeruginosa superoxide activating Gq protein. Cells were suspended in Tyrode’s solution (1mM CaCl2) and field stimulated (0.5 Hz, 25°C). sarcomere shortening (SS) was assessed by real-time image analysis, Ca2+ transients by indo-1 fluorescence. Results. Myocytes from both TAC and Gq hearts had normally normal unloaded shortening under rest conditions (3.55 ± 0.3% for TAC, 3.11 ± 0.2% for Gq, 3.59 ± 0.4% and 3.48 ± 0.4% for control, p = NS); however they exhibited a markedly reduced response to 10nM isoproterenol (ISO) compared to controls (3.55 ± 0.3% vs. 2.3 ± 0.2%, p = 0.05). The lipid plaque area of thoracic aorta was decreased by the treatment with G-CSF group (in the balloon injection model, the neointima/media ratio was significantly smaller in G-CSF group than control group (G, 0.91 ± 0.22 vs C, 1.24 ± 0.21, P < 0.05)). The ratio of reendothelialization was significantly accelerated by the treatment with G-CSF at 1 week and 4 weeks (1 week: G, 62.8 ± 2.2% vs C, 46.7 ± 2.9%, P < 0.05, 4 weeks: G, 79.6 ± 3.7% vs C, 58.9 ± 8.5%, P < 0.05). The pretreatment with L-NAME, which suppress the EPC mobilization, significantly inhibited G-CSF-induced reendothelialization. Conclusions. In the present study, we demonstrated using two kinds of rabbit models of atherosclerosis that the treatment with G-CSF ameliorates the progression of atherosclerosis. The treatment with G-CSF inhibited the progression of atherosclerosis and neointimal formation. These results suggest that G-CSF has a therapeutic potential for the progression of atherosclerosis.
respectively (P < 0.05 vs GSH-untreated cells). Conclusions. PDE5A modulation of β-adrenergic contraction 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 PDE inhibitors to blunt cardiac adrenergic stress.

PDE5A Inhibition Suppresses Maladaptive but Not Physiological Cardiac Hypertrophy

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We have recently shown that PDE5A inhibition has potent antihypertrophic activity in models of incipient and established cardiac hypertrophy. To better understand these processes, we were interested whether PDE5A inhibition could distinguish between physiological hypertrophy and maladaptive hypertrophy. In this study, we tested the hypothesis that PDE5A inhibition allows physiological cardiac hypertrophy to take place. Mice were subjected to TAC or swimming (4 weeks of 90 min of swimming daily). Mice were randomized to receive vehicle or sildenafil citrate (100 mg/kg/d). Mice were evaluated at 4 weeks by echocardiogram, PV Loop, 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 level of systolic function whereas the physiologic hypertrophy demonstrated improved systolic function in all parameters. Sildenafil markedly improved LV function and reduced heart size in TAC (136 ± 6.0 mg; P < 0.05 compared to TAC mice but did not affect afterload 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. However, swimming mice showed increased NOS activity when compared to TAC mice and reduced baseline PDE5A activity when compared to TAC. In sum, physiologic hypertrophy is a beneficial adaptive hypertrophy while TAC is associated with maladaptive hypertrophy and results in the progression to heart failure. Sildenafil selectively blocks maladaptive hypertrophy without an effect on physiologic hypertrophy by improving stroke work. Importantly, both TAC-induced and exercise-induced hypertrophy were both associated with an increase in P38/AKT signaling. Sildenafil treatment reduced the expression of p-AKT in TAC-associated 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/cGMP signaling in the heart.

Discovery of Cardiac Marker Expression in Human Embryonic Stem Cells by Gene Expression Profiling

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Background: The human heart is a highly complex organ consisting of diverse cell types that are known to be exclusively expressed in the heart: plakophilin 2, solute family 16-member 1. Protein-level expression by real-time RT PCR method and Immunostaining studies such as Immunofluo-

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An Avian Model to Study High-Fat Diets and Cycloxygenase 2 Gene Expression: A Biomarker for Atherosclerosis

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Cycloxygenase 2 (COX 2) in humans is involved in the synthesis of prostanoids, which are involved in pathological processes such as acute and chronic inflammation. Atherosclerosis is a disease condition caused by the buildup of fatty deposits or plaques on the inside walls of arteries. Inflammation in human heart tissue due to plaque buildup has been found to cause an increase in the levels of COX 2. Since COX 2 in avian species has the same function as in humans, and atherosclerosis can be induced in less than 16 weeks, they can serve as a good animal model to study the process of atherosclerosis. Therefore the purpose of this study was to evaluate the effect of a high fat diet on the expression of the cox-2 gene in broiler chickens. Cobb X Cobb chickens were fed a high fat diet (feed with 2.5% linoleic acid in addition to 2g in 100g peanut oil) from day 1 of age through sexual maturity (16 eks of age). Total RNA was extracted from blood samples and heart tissue from four male chickens. Quantitative Reverse Transcriptase Polymerase chain reaction was then performed to determine the level of expression of the cox-2 gene compared to those that consumed a regular diet. The study was repeated twice. Birds that consumed a high fat diet induced expression of cox-2 (P < 0.05) which compare birds that consumed a normal fat diet. The results indicate that feeding two diets no effect on baseline lipid levels. Cox-2 gene expression was then determined in broiler chickens. Cox-2 gene expression was then determined in broiler chickens. There was no cox-2 expression over the 16 week period, however, there was a significant increase (P < 0.05) in cox-2 expression after birds were fed the high fat diet for 9 weeks. These data imply that feeding a high fat diet for 16 weeks did trigger inflammation. Therefore the cox-2 gene may be an excellent biomarker for atherosclerotic, and broiler chickens can serve as a convenient model to study the onset of the human condition.

Apo E Genotypes Modulate the Plasma Lipid Response to Atorvastatin in Indian Patients with Coronary Artery Disease

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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 baseline lipid levels. 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 3/3, 5.3% with epsilon 2/3, 17.41% with epsilon3/3 and 30.0% with epsilon 2/2, 21.4% with epsilon 3/4 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 4 allele, those with epsilon 2 allele showed a significantly higher percentage reduction of LDL-C level after treatment (P < 0.05). CONCLUSION: The percentage change in LDL-C level was significantly higher 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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Background: Nicotinic acetylcholine receptors (nAChR) on endothelial cells (EC) are ligand-gated cation channels. We have previously reported that nicotine, like VEGF and FGF has
angiongenic activity. The effect can be abolished by NAC or Bcl-2 or PKCε. 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 occurs containing the core mPT pore-forming protein, voltage-dependent anion channel (VDAC) and PKCε, but not Bcl-2 or Bax. Furthermore, a specific VDAC1 siRNA specific inhibitor Rol68-3400 (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 kinase, PKCε, in this process.

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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 HK1 and HKII in tissue culture 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 binding to the voltage-dependent anion channel (VDAC) on the mitochondrial outer membrane. VDAC is believed to form part of mPTP, opening of which causes cellular injury. The relative contribution of HK binding to the mitochondria and the increase in glucose phosphorylation to the overall protective effects of HKs is not clear. Furthermore, there is no clear evidence supporting the hypothesis that HK binding to mitochondria inhibits mPTP. In order to better understand the mechanism(s) behind the protective effects of HKs, we overexpressed green fluorescence protein (GFP)-tagged full length HK and HKII (FL-HK and FL-HKII, respectively) and their truncated proteins lacking the N-terminal hydrophobic domains (TR-HK and TR-HKII, respectively) in HEK 293 cells. The truncated constructs cannot bind to the mitochondrial outer membrane since they lack the hydrophobic N-terminal sequence. Overexpression of FL-HK and FL-HKII resulted in complete protection against H2O2-induced loss of mitochondrial membrane potential and cell death. Although overexpression of TR-HK and TR-HKII reduced cell death, the degree of protection was less than that of the full-length proteins. Furthermore, FL-HK and FL-HKII inhibited mitochondrial permeability transition (mPTP) in the presence of H2O2, whereas the truncated forms only caused partial inhibition. These results suggest that both glucose phosphorylation and inhibition of mPTP contribute to the protective effects of HK1 and HKII, and that binding of HKs to the mitochondria inhibit mPTP. These findings bear implications of HK overexpression and binding to the mitochondria as a potential clinical treatment strategy for various forms of heart disease.

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Transient 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

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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 3.1 mmol/L H2O2 for 5 min followed by 10μmol/L catalase for 4 h. The compared impact of on mitochondrial superoxide was measured using dihydorhodothion (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=40). Furthermore, FL-HKI and FL-HKII inhibited mitochondrial permeability transition (mPTP) in the presence of H2O2, whereas the truncated forms only caused partial inhibition. These results suggest that both glucose phosphorylation and inhibition of mPTP contribute to the protective effects of HK1 and HKII, and that binding of HKs to the mitochondria inhibit mPTP. These findings bear implications of HK overexpression and binding to the mitochondria as a potential clinical treatment strategy for various forms of heart disease.
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; MnSOD), 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 CR-PPARγ−/− target gene heart. Depressed MnSOD 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 Taurine-Deficient Mice**

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**Background:** Taurine, a sulfur-containing γ-amino acid, is the most abundant amino acid in cardiac and skeletal muscle. Taurine 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 taurine could critically affect mitochondrial function, whereas physiological significance has not been elucidated.

**Methods and Results:** To address the physiological role of taurine in tissues, we generated tau-taunt encistor (TauTKO) knockout mice (TauTKO). KO mice showed a deficiency in tissue taurine content in the hearts and the skeletal muscles (heart; not detectable (TauTKO) v.s. 6.7 ± 2.7 mg/kg wet weight, p < 0.001, skeletal muscle; 0.70 ± 0.33 (TauTKO) v. 16.9 ± 5.1 mg/kg wet weight, p = 0.001) as well as the other tissues. Histological analyses assessed by hematoxylin-eosin stained showed wall thickness and dilation of cardiac and skeletal muscles (TauTKO) more compared with wild-type and littermates. Though cardiac fibrosis was not observed in TauTKO hearts as assessed by Masson's trichrome stain, TauTKO hearts exhibited increased expressions of cardiac failure marker BNP and β-MHC, but not α-skeletal actin, as assessed by northern blot analyses. Transmission electron microscopy analyses showed abnormal mitochondrial ultrastructure in TauTKO hearts. Enzymatic histochemical staining revealed that succinate dehydrogenase (SDH) activity, but not cytochrome c oxidase (COX), was remarkably decreased in the hearts of TauTKO compared with wild-type littermates (SDH; 43 ± 16% (p < 0.05), COX; 79 ± 20% (not significant), respectively), indicating the impairment of the capacity of oxidative phosphorylation in cardiac mitochondria by taurine deficiency. Interestingly, mitochondrial abnormal ultrastructure and impaired SDH activity were also observed in the skeletal muscles in TauTKO. **Conclusion:** These results indicate that taurine depletion causes mitochondrial dysfunction in cardiac and skeletal muscle, suggesting that modulation of mitochondrial function may underlie tissue protective roles of taurine.

**Can "Elderly" Hearts Be Rendered Resistant to Ischemia? D-myosin-IP3 in Aged Mouse Hearts**

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Infarct size reduction with preconditioning (PC) has been extensively documented in adult cardiac myocytes. Though cardiac fibrosis was not observed in TauTKO hearts as assessed by Masson’s trichrome staining revealed that succinate dehydrogenase (SDH) activity, but not cytochrome c oxidase (COX; 79 ± 5% 43 ± 5% 4% 4% 4% 32 ± 7% 2% 7% 5% 1% 1% 1% 1% 1% 0%, Nec 11 ± 5%, Apop 9 ± 2%, n = 5; NE Apop 4 ± 1%, Nec 11 ± 1%, p < 0.05 vs. P37

**Mitochondrial Localization and Cellular Function of Cytoglobin: A NovelCalcineurin-Dependent Hypoxia-Responsive Globin in the Cardiovascular System**

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Cytoglobin (Cygb) is a novel tissue hemoglobin that has a 30% amino acid homology to myoglobin. Since cytoglobin shares several structural features with myoglobin, we hypothesized that Cygb may play a cytoprotective role by scavenging free radicals or modulating oxyhemoglobin metabolism within the cardiovascular system. We have demonstrated that Cygb is regulated by the calcineurin-NFAT pathway, is predominantly expressed in the heart and brain and its expression is upregulated with stress at both the transcriptional and translational levels (hypoxic heart/brain and ischemic cardiomyopathic heart). In vitro studies undertaken to investigate the translational and mechanisms underlying changes in expression of cytoglobin within the cell. Western blot analysis indicated that Cygb is found in both the cytoplasm and the nucleus of normoxic C2C12 myoblasts; however, upon exposure to hypoxia (1% O2 for 16 hours) Cygb translocates out of the nucleus. This was confirmed by immunofluorescence (ICC). Cygb mRNA levels decreased when hearts are exposed to hypoxia is blocked when C2C12 cells were incubated with leptomycin B, a potent inhibitor of nuclear export. ICC data also revealed that Cygb co-localizes to the mitochondria upon hypoxic exposure. Transfection assays involving C2C12 cells demonstrated that over-expression of cytoglobin rescues cellular viability known to upon exposure to menadione, an age-related free radical production (67 ± 1.6% cell survival in overexpressed cells vs. 48 ± 1.6% cell survival in control cells; p < 0.05; n = 3) and under anoxic conditions (72 ± 0.0% cell survival in overexpressed cells vs. 49 ± 0.7% cell survival in control cells; p < 0.05; n = 3). Conversely, the silencing of cytoglobin by siRNA resulted in a significant reduction in cell survival under anoxic conditions (32.7 ± 0.2% cell survival in Cyg-knockdown cells vs. 63 ± 1.5% cell survival in control cells; p < 0.05; n = 3). Finally, microarray analysis of Cygb knockdown cells indicated an upregulation of genes involved in apoptosis and cell cycle regulation. These new data collectively establish that cytoglobin is a novel stress-responsive globin that may play a key signaling role as well as a cytoprotective role in the hypoxic/ ischemic heart.

**Role of Testosterone in Heart Stress Protein 70 Activation and Delayed Cardioprotection of Preconditioning in Male Rats**

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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 heart stress and the HSP70 (70-kD), which mediates the delayed protection of preconditioning. We therefore hypothesized that testosterone is needed for full activation of HSP70, which mediates the cardioprotection of IP. Male Sprague-Dawley rats (7–8 weeks) underwent sham operation or gonadectomy without (G) or with testosterone replacement (200 μg/kg/day) daily for 8 weeks. Ischemic preconditioning was performed on rats by 10 min/kg 50°C, 48H,8H,F (HP, a kappa opioid receptor agonist, known to confer delayed cardioprotec-

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

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In α1A(κ)BKO mice, which lack cardiac myocyte α1-adrenergic receptors (α1-ARs), aortic constriction induces apoptosis, dilated cardiomyopathy and death, demonstrating that α1-ARs are required for myocardial adaptation to stress. Here we investigated α1A and α1B subtype-specific survival signaling in cultured cardiac myocytes to demonstrate a direct protective effect of α1A-ARs in cardiac myocytes. We cultured myocytes from α1A(κ)BKO hearts and reconstructed α1-signaling with adenoviruses expressing α1A-(κ)-α1B-FGF fusion proteins. Myocyte death was induced by norepinephrine (NE), and measured by annexin V/propidium iodide staining. In WT myocytes, NE (1 μM) did not induce cell death (Con Apoptosis (Apo) 3 ± 0%, Necrosis (Nec) 12 ± 2%, n = 5; NE Apo 4 ± 1%, Nec 11 ± 1%, p < 0.05 vs. P37

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Mechanisms of Myocardial Protection by Atorvastatin: 5'-Nucleotidase Is Upstream to eNOS Activation

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Background: Previous studies have shown that statins protect against ischemia-reperfusion injury by activating phosphorylcholinidohydrolyase 3-Kinase (PI3K), leading to phosphorylation of Akt that activates endothelial nitric oxide synthase (eNOS) by phosphorylation. PI3K also activates ecto-5'-nucleotidase (E5N). Blocking E5N 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 investigate whether E5N activation is downstream or upstream to eNOS. Methods: Wild-type (WT), eNOS -/- and iNOS-/- mice were pretreated with oral ATV (ATV -) or water alone (ATV+) for 3 days. Mice were subjected to 30 min coronary artery occlusion and 4 hr reperfusion (IS protocol), or hearts were harvested, without being subjected to ischemia, for measurement of myocardial E5N (ng/mg protein/min), calcium-dependent (cNOS) and independent (iNOS) cNOS activity (X1000 cpm); and adenosine (µg/g) and 6-keto-PGF,α2 (the stable metabolite of PGI2; 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-PGF,α2 only in the WT mice. ATV increased E5N activity and tissue adenine 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 E5N in the mouse. E5N upregulation by ATV does not limit IS in eNOS-/- and iNOS-/- mice, suggesting that E5N activation is upstream to eNOS, iNOS and PG12 production.

G-CSF Directly Inhibits Myocardial Ischemia-Reperfusion Injury via Akt-eNOS Pathway

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Background—Granulocyte-colony stimulating factor (G-CSF) has been recently reported to prevent cardiac remodeling and dysfunction after acute myocardial infarction. In this study, we examined acute protective effects of G-CSF on myocardial ischemia-reperfusion (IR) injury. Methods and results—Rats were subjected to global 35 minutes ischemia and 120 minutes reperfusion in Langendorff system with or without G-CSF (300 ng/ml). G-CSF administration was started at the onset of reperfusion. Infarct size was assessed by triphenyltetrazolium chloride (TTC) staining. G-CSF markedly reduced the infarct size (control, 58.6±3.3% vs G-CSF, 34.7±4.2%, p<0.001). To examine G-CSF-induced protective pathways, perfused rat hearts were subjected to 35 minutes ischemia and 7 minutes reperfusion (n=5, per group). G-CSF strongly activated Jak2, STAT3, extracellular signal-regulated kinase (ERK), Akt and endothelial NO synthase (eNOS). To identify the role of signaling pathways that are activated by G-CSF, hearts were pretreated of UP or G-CSF and subjected to global 35 minutes ischemia and 120 minutes reperfusion after treatment with or without G-CSF (300 ng/ml), or without G-CSF and with 3-phenyl-3-(1H)-pyrazolin-5-one (P41), a STAT3 inhibitor. P41 reduced the IS size following G-CSF treatment (control, 53.3±5.6% vs. G-CSF, 38.6±3.9%, p<0.001). G-CSF treatment prevented cardiac remodeling and dysfunction after acute myocardial infarction. In this study, we provide evidence that G-CSF inhibits myocardial IR injury via Akt-eNOS pathway.

Myocardial Susceptibility to Ischemic-Reperfusion Injury in a Visceral Obese, Pre-Diabetic Rodent Model

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Background—The impact of obesity and insulin resistance on clinical outcomes post-myocardial infarction is presently controversial. We set out to determine whether obesity and insulin resistance in the absence of clinically detectable diabetes mellitus, have deleterious effects post myocardial infarction. Methods and results—After feeding rats a high calorie diet for 12 weeks, body weight (512±11g vs 603±10g, p<0.05), visceral fat content (28.8±1.6g vs 49.5±2.2g, p<0.05), plasma insulin (31.4±4.9µU/ml vs 46.5±6.2µU/ml, p<0.002) and triglycerides concentrations (7.0±2.0mmol/L vs 1.9±0.18mmol/L, p<0.05) were increased. In each group (0.12±0.01cm vs 0.16±0.01cm, p<0.05), left ventricular posterior wall thickness was increased. Fasting blood glucose concentra-
Surgical Ischemia Induces Phosphorylation and Translocation of Heat Shock Protein 27 and β-Crystallin in Human Myocardium

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The small heat shock proteins HSP27 and β-crystallin (cryAB) are highly expressed in cardiac and skeletal muscle. Evidence from cell cultures indicates 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 reoxygenation, protein chaperone function, signal transduction, scaffolding, and recently, myocyte contractile function. It is unknown if HS 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/CBP). Cardiopulgia therapies used to arrest the heart during surgery are associated with ischemic insults including hypoxia and myocardial contractile deficits. We performed the following experiments to determine any changes in the phosphorylation and localization of HSP27 and cryAB in human myocardium following CP/CBP. Right atrial appendage and chest wall skeletal muscle samples were collected from patients immediately before and after CP/CBP. Whole tissue lysates, CP/CBP 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/CBP associated 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/CBP to a striated sarcomeric pattern after CP/CBP. Double labeling with an atrial specific myosin light chain -2a antibody revealed prominent staining of total and ser82, ser78, and ser15 phosphorylated HSP27 along I-bands of cardiomyocytes after CP/CBP. These results demonstrate that two members of the HSP family, HSP-27 and cryAB, are phosphorylated and translocate to cardiac myofibrillar following surgical ischemia associated with CP/CBP.

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

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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 homologous 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 sensitivity in myocardium which may have protective effect against IR injury. 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 cardiomyocytes, PKN was activated by HS (67% osmolarity) 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 thicker than in non-transgenic (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 ± 6.6%, 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 (0.98 ± 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. Further activation of PKN causes well compensated LV hypertrophy and also has protective effects in the ischemia-reperfused heart in vivo.
ischemia-reperfusion, although L-NAME reduced CIf, it did not cause further damage to microcirculation, and did not affect MIS. According to the results of this study, L-NAME may be safely used in patients with acute myocardial infarction complicated by refractory cardiogenic shock.

Detrimental Role of Complement Factor C3 After Myocardial Infarction

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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 involved in the myocardial ischemia-reperfusion injury. 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 immunohistochernistry. 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 LV dimensions at 3 weeks after infarction were 16.8 ± 1.1 mm in WT vs. 23.4 ± 2.4 mm in KO, p < 0.01). C3 levels were absent in KO animals and C3 expression was significantly lower in the KO mice after myocardial infarction and may account for improved left ventricular remodeling. 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

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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 with Krebs-Henseleit-Solution (37°C) on a Langendorff-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 a protective effect can be demonstrated in other models of hypertrophy, we performed transgenic overexpression of 8 –12 week old wild type and transgenic mice. We measured left ventricular contractile function before and one week after ascending aortic constriction. Control groups were subjected to sham operation. Hearts were analyzed by gravimetry and histology at the end of the study. After aortic banding, wild type mice demonstrated hypertrophy as evidenced by increased ventricular weight to body weight ratio when compared to sham operated controls (6.3 mg/wg vs. 4.02 mg/wg, p = 0.012), and decreased left ventricular fractional shortening, as measured by echocardiography (0.33 vs. 0.62. p = 0.02). Histological analysis showed increased fibrosis in the hearts from WT banded animals, as expected. Transgenic mice, in contrast, developed a milder degree of hypertrophy when compared to aortic banding (3.57 mg/wg vs. 4.07 mg/wg, p > 0.003) and had normal ventricular fractional shortening (0.60 vs 0.61, p = 0.90), with no evidence of fibrosis. Sham operated animals showed no evidence of hypertrophy, fibrosis or change in ventricular function. Our findings demonstrate that overexpression of CHF1/Hey2 attenuates the hypertrophic response to aortic banding and prevents the progression to heart failure, most likely by limiting myocyte death and subsequent fibrosis. The molecular mechanisms by which CHF1/Hey2 controls the development of pathological hypertrophy and progression to heart failure are currently being explored.

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

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Constitutive activation of cardiac glycoin synthesis kinase-3 (GSK-3) is known to suppress cardiac hypertrophy in vivo. Among various substrates GSK-3 β-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 mifepristone-inducible Cre transgenic to induce β-catenin stabilization 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 apparently no significant structural modifications. Infusion of Ang II by osmotic minipumps induced as expected cardiac hypertrophy in wild type mice demonstrated by echocardiography analysis (intraventricular septum (IVS) 0.84 ± 0.05 [mean ± SEM], and left ventricular posterior wall (LVPW) 0.72 ± 0.05). Surprisingly, a clearly reduced cardiac hypertrophy was seen in the mutants (IVS 0.69 ± 0.04, and LVPW 0.7 ± 0.05). The expression of hypertrophic markers such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) was decreased in comparison with the cardiac hypertrophic hearts in the wt mice as measured by real-time RT-PCR. Additionally, mice with β-catenin stabilization evidenced by increased ventricular weight to body weight ratio when compared to sham operated controls (6.34 mg/wg vs. 4.02 mg/wg, p = 0.012), and decreased left ventricular fractional shortening, as measured by echocardiography (0.33 vs. 0.62. p = 0.02). Histological analysis showed increased fibrosis in the hearts from WT banded animals, as expected. Transgenic mice, in contrast, developed a milder degree of hypertrophy when compared to aortic banding (3.57 mg/wg vs. 4.07 mg/wg, p > 0.003) and had normal ventricular fractional shortening (0.60 vs 0.61, p = 0.90), with no evidence of fibrosis. Sham operated animals showed no evidence of hypertrophy, fibrosis or change in ventricular function. Our findings demonstrate that overexpression of CHF1/Hey2 attenuates the hypertrophic response to aortic banding and prevents the progression to heart failure, most likely by limiting myocyte death and subsequent fibrosis. The molecular mechanisms by which CHF1/Hey2 controls the development of pathological hypertrophy and progression to heart failure are currently being explored.

The Transcription Factor CHF1/Hey2 Prevents the Development of Pathological Hypertrophy and Heart Failure

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We have previously reported that overexpression of CHF1/Hey2 in the myocardium is protective against phenylephrine-induced hypertrophy, both in vitro and in vivo. To examine whether this protective effect can be demonstrated in other models of hypertrophy, we performed transgenic overexpression on 8 –12 week old wild type and transgenic mice. We measured left ventricular contractile function before and one week after ascending aortic constriction. Control groups were subjected to sham operation. Hearts were analyzed by gravimetry and histology at the end of the study. After aortic banding, wild type mice demonstrated hypertrophy as evidenced by increased ventricular weight to body weight ratio when compared to sham operated controls (6.34 mg/wg vs. 4.02 mg/wg, p = 0.012), and decreased left ventricular fractional shortening, as measured by echocardiography (0.33 vs. 0.62. p = 0.02). Histological analysis showed increased fibrosis in the hearts from WT banded animals, as expected. Transgenic mice, in contrast, developed a milder degree of hypertrophy when compared to aortic banding (3.57 mg/wg vs. 4.07 mg/wg, p > 0.003) and had normal ventricular fractional shortening (0.60 vs 0.61, p = 0.90), with no evidence of fibrosis. Sham operated animals showed no evidence of hypertrophy, fibrosis or change in ventricular function. Our findings demonstrate that overexpression of CHF1/Hey2 attenuates the hypertrophic response to aortic banding and prevents the progression to heart failure, most likely by limiting myocyte death and subsequent fibrosis. The molecular mechanisms by which CHF1/Hey2 controls the development of pathological hypertrophy and progression to heart failure are currently being explored.

The Functional Role of COX-2 and p38/MK2-Mediated Regulation in Heart Failure

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One of the mechanisms implicated in heart failure is the induction of inflammation in heart. COX-2 is a critical regulator of inflammation and its induction was observed in stressed myocardium. However, the regulatory mechanism of COX-2 expression and whether COX-2 has a protective or detrimental role in heart are unclear. Here we investigated the role of the stress-activated protein kinase p38 and its downstream kinase MK2 in COX-2 expression in cardiomyocytes. We demonstrated that MK2 was necessary and sufficient for COX-2 protein upregulation in cultured myocytes, and necessary for COX-2 mRNA upregulation. MK2 also regulated the protein expression and cellular distribution patterns of the p38 kinase. In order to investigate the functional impact of COX-2 induction in adult heart, a ubiquitous promoter was used to drive a GFP expression cassette flanked by two loxP sites, and followed by a COX-2 cDNA-ires-Luciferase expression unit (CAG-GFP-COX2). The transgenic mouse carrying the CAG-GFP-COX2 construct has ubiquitous GFP expression in multiple tissues, including heart, but no detectable level of COX-2 induction in adult heart, a ubiquitous promoter was used to drive a GFP expression cassette flanked by two loxP sites, and followed by a COX-2 cDNA-ires-Luciferase expression unit (CAG-GFP-COX2). The transgenic mouse carrying the CAG-GFP-COX2 construct has ubiquitous GFP expression in multiple tissues, including heart, but no detectable level of COX-2 induction in adult heart, a ubiquitous promoter was used to drive a GFP expression cassette flanked by two loxP sites, and followed by a COX-2 cDNA-ires-Luciferase expression unit (CAG-GFP-COX2). The transgenic mouse carrying the CAG-GFP-COX2 construct has ubiquitous GFP expression in multiple tissues, including heart, but no detectable level of COX-2 induction in adult heart, a ubiquitous promoter was used to drive a GFP expression cassette flanked by two loxP sites, and followed by a COX-2 cDNA-ires-Luciferase expression unit (CAG-GFP-COX2).
Characterization of Fibroblast Growth Factor 16 Promoter Activity in Postnatal Cardiac Cells In Vitro and In Vivo

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The hepatic-binding fibroblast growth factor (FGF) 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 (300 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 luciferase reporter genes was generated with varying lengths of upstream flanking FGF-16 sequences. These genes (-4.7, -2.7, -1.2 and -0.25F-16gLuc) were used to transiently transfected neonatal rat cardiomyocytes and rat glial (C6) cells. A Renilla luciferase gene was co-transfected as a control for DNA uptake. Hybrid promoters containing the luciferase reporter gene were expressed significantly above ‘background’ promoterless genes (p<0.01) levels in transfected cardiomyocytes; no activity of -0.25F-16gLuc 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 carrying a hybrid β-galactosidase reporter gene directed by 4.7 kb of upstream FGF-16 sequences (equivalent to -4.7F-16gLuc) were generated by pro-nuclear injection. Preliminary data from one line (FB-06; 6kgs) 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

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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.

Immediately after stenting, adenovirus solution containing sFlt-1 or LacZ (1–3 x 10⁹ pfu, 4ml) was cultured hypertrophic cardiac myocytes, and they were inhibited by pretreatment with calcium channel blocker, as well as CaMKII inhibitors KN-62 respectively (p<0.05). Thus, we conclude that β myosin gene transcription was markedly enhanced in the hypertrophic group, and that myotrophin could be used as a therapeutic target for treatment of heart failure.
of the NOS dimer and NOS activity and reduced expression of O2-; in order to determine if the expression of arginase is causal in this loss of NOS coupling, adult mouse lungs were transfected with an AAV encoding arginase driven by either a Tie2 or α-sm muscle actin promoter. Transfection led to a 4-fold increase in arginase activity when compared to lungs transfected with the reporter gene AAV/pial. Ten days after transfection, there was a marked increase in O2- expression and a significant loss of NOS coupling that was prevented by treatment with BEC. Pulmonary arterial catheterization demonstrated significantly higher mean PA pressure at 6 weeks in AAV-arginase mice compared with AAV/pial mice (19.8 ± 1.1 mmHg compared to 13.9 ± 0.9 mmHg, P < 0.05). Arginase transfection was associated with a marked increase in ROS, 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.

WITHDRAWN

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Cardiac-Specific Abrogation of NF-κB in Cardiomyopathy

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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-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 cardiomyocyte function. It has been shown that NF-κB activation is necessary for αIIbβ3 protein expression and TNF-induced hypertrophy in vitro. Although it has 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 (CnATg). 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 CnATg/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 activity 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 other mediators. The potential role of other signaling mediators in calcineurin-induced cardiomyopathy is currently under investigation.

A Potential Link Between the Muscle Specific β1D Integrin and a Regulatory Intracellular Signaling Complex: β1D Integrin Binds to a Component of the COP9 Signalosome in Striated Muscle

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Yeast two-hybrid analysis was used to screen a human heart library to isolate proteins that interact with the adult muscle specific β1D but not with β1A integrin. In addition to proteins known to bind the β1 cytoplasmic domain including IAX1 and α-actinin, several novel candidates were isolated and identified. One protein identified was subunit 3 (CSN3/Sgn3) of the COP9 signalosome complex. This protein/protein interaction was specific for β1D integrin, as no binding to β1A integrin (cytoplasmic domain) was measurable by two-hybrid analysis. This study focuses on the characterization and independent verification of the interaction between β1D integrin and CSN3 protein. Both co-immunoprecipitation and immuno-localization of CSN3 and β1D integrin in cardiac myocytes is presented. To begin to unravel the role of this interaction, we have characterized the expression of CSN3 in both proliferating and differentiating cardiac and skeletal muscle cells. The interaction between CSN3 and β1D integrin may represent a new pathway for integrin signaling in striated muscle.

Associated Polymorphisms and the Risk of Coronary Artery Disease

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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 238 CAD patients and 520 healthy individuals the risk associated with ACE DD, ACE 8 GG, AGT 160A > G, MTHFR 677 TT and 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 < 0.001]; 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 G > AGT 235 TT; ACE DD or ACE 8 G > MTHFR 1298 AA) increased with the presence of 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 a increased risk, when compared with the isolated polymorphism.

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

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In an effort to discover regulators of cardiac gene expression and growth, we devised a eukaryotic expression screen for comRNAs 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 CAMTA1s are recruited to the ANF promoter, at least in part, by associating with the cardiac homeodomain protein Nkx2-5 and function as inducers of cardiac growth. Overexpression of CAMTA2 in isolated neonatal cardiomyocytes by adenoviral-mediated delivery induces hypertrophic growth of cardiomyocytes. Transgenic mice overexpressing CAMTA2 in the heart display cardiac hypertrophy, which progresses to dilated cardiac myopathy. 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 repress the activity of CAMTA proteins. Nuclear export of class II HDACs in response to Pnoc/PID 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

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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 induction increased a robust increase in the punctate localization of GFP-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 (sAB). Fluorescence microscopy demonstrated autophagy-like-localized GFP-LC3 in left ventricular myocytes 24h after banding which peaked at 48h and subsequently declined (though remaining elevated relative to control,
p<0.05), EM revealed double-membrane autophagosomes in cardiomyocytes in failing heart. Lysosome activity was increased in failing LV as demonstrated by immunostaining for cathepsin D and LAMP-1, suggesting that diminished activity of distal lysosomal pathways was not responsible for autophagosome accumulation. To explore whether cardiomyocyte autophagy is beneficial or maladaptive, we studied mice with targeted disruption of Becrin-1, a gene required for autophagosome formation. Cardiac hypertrophy of Becrin-1–/– led to decreased autophagy in pressure-stressed cardiomyocytes. Heart failure-associated declines in LV systolic function were significantly attenuated in Becrin-1–/– mutants. Becrin-1 over-expressing transgenic mice manifested an amplified pathological response to NAB. Compared with WT, Becrin-1-deleted transgenic mice exhibited reduced increased autophagic activity, impaired systolic performance, increased load-induced fibrosis, but similar levels of apoptosis. Conclusions: These findings implicate autophagy in the pathogenesis of load-induced heart failure and suggest it may be a target for novel therapeutic intervention.

Extracellular Signal-Regulated Kinase Is Sufficient but Not Necessary for Cardiac Hypertrophy In Vivo
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The mitogen-activated protein kinases (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, integrins, stretch, and other diverse stress stimuli. ERK1/2 are directly activated by MEK1, an upstream MAPKK that specifically phosphorylates the Thr 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 robust remodeling of cardiomyocyte hypertrophy. Cdc42 activation signaling cascade (Cdc42–/–) led to decreased autophagy in pressure-stressed cardiomyocytes. Heart failure-associated declines in LV systolic function were significantly attenuated in Cdc42–/– mutants. Thus, our results suggest that ERK1/2 inactivated all cardiac ERK activity (but not other MAPKs), did not alter the ability of the heart to hypertrophy following pressure overload stimulation, despite having a significant reduction in total ERK activity in the heart. Moreover, tet-inducible expression of MEK3 in the heart, which completely inactivated all cardiac ERK activity (but not other MAPKs), did not alter the ability of the heart to hypertrophy 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
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Kruppel-like factor 5 (KLF5) is a proto-oncogenic transcription factor that is pathogenically induced to mediate the cardiovascular remodeling response to external stress (Nat Med 2002). KLF5 induces stimulatory effects on cell growth and acetylation of KLF5 is important for this effect (Mol Cell Biol 2003). Initial studies using KLF5–/– deficient mice showed that KLF5 protects against apoptosis as induced by arterial injury. Through analysis of the mechanisms of the KLF5 shows stimulatory effects on cell growth, and acetylation of KLF5 is important for this effect. Functional, pro-apoptotic activity of a proteolytic peptide of PARP-1 produced under apoptotic conditions was inhibited by interaction with wild-type KLF5 but not the non-acetylatable mutant. Collectively, our findings show PARP-1 is a target of action of the cardiovascular transcription factor KLF5 that regulating interaction of PARP-1 by KLF5 under apoptotic conditions with further regulation by acetylation to be a novel mechanism regulating cell death/survival in cardiovascular pathogenesis.

Role of 14–3–3 in Protein in Cardiomyocyte Survival
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The 14–3–3 family of intracellular dimeric phosphoserine-binding proteins regulate signal transduction, cell cycle, apoptosis, 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 14–3–3-ε gene. Although 14–3–3-ε mice did not survive embryonic development, haploinsufficient mice appeared normal at birth and were fertile. Baseline cardiac structure and function was normal in 14–3–3-ε–/– mice, however, cultured adult cardiomyocytes derived from 14–3–3-ε–/– mice were sensitized to the development of apoptosis in response to treatment with hydrogen peroxide or UV irradiation. In addition, 14–3–3-ε–/– mice were intolerant of experimental myocardial infarction and most animals died from acute ventricular rupture in the days following surgery. The 14–3–3-ε–/– 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 ERK MAPK activation was reduced, in 14–3–3-ε–/– cardiac tissue. Treatment of 14–3–3-ε–/– mice with a general p38 MAPK inhibitor, SB202190, significantly reduced the mortality rate observed after experimental myocardial infarction. These results demonstrate that 14–3–3-ε plays a critical anti-apoptotic function in cardiomyocytes, and therapeutic agents that increase 14–3–3-ε 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
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Background: Dystrophin associates with a protein complex (DAPC) which includes: an extracellular protein, α-dystroglycan; the transmembrane glycoproteins, β-dystroglycan and α, β, γ, δ - sarcoglycan (SG); and caveolin-3 (Cav3). In Duchenne/Becker muscular dystrophy, the 14-3-3 family of intracellular dimeric phosphoserine-binding proteins regulate signal transduction, cell growth, differentiation, and apoptosis. The 14-3-3 family member in apoptosis, mice were generated with targeted disruption of the 14-3-3-ε gene. Mdx mice exhibit mild cardiomyopathy. iSG deficiency manifests as Duchenne Limb Muscular Dystrophy (DLM205), with DCM occurring in patients. A hamster strain that is iSG 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 F18 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 tyrosine kinase pathways. Finally, an enhancement of GSK-3 phosphorylation was observed in the Mdx model. This corresponds with the findings 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 contributes 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 blocke of the Ras-Raf pathway and be released by a sequenced 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
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The low molecular weight GTPases of the Rho super-family are molecular switches cycling between a GTP-bound active state and GDP-bound inactive state. Rho GTPases play critical roles in gene expression, apoptosis and cytoskeleton regulation in various cells types. Previous in vitro studies in neonatal cardiomyocytes suggested that Rho GTPases are involved in hypertrophic cell cycle activation. We have previously reported that DGK inhibitor inhibits GPCR agonist-induced cellular DAG accumulation, activation of the downstream kinase pathways, and subsequent cardiomyocyte hypertrophy. In this study, we examined the role of DGK inhibitor in the Mdx model, indicating activation of tyrosine kinase pathways. Finally, an enhancement of GSK-3 phosphorylation was observed in the Mdx model. This corresponds with the findings of increased Raf Ser259 phosphorylation to suggest that the Akt pathway is activated in the Mdx and TO2 models. DAPC dissociation presumably reverses a negative regulation of the ERK pathway. This contributes 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 blocke of the Ras-Raf pathway and be released by a sequenced 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.

Diacylglycerol Kinase ε Prevents Cardiac Remodeling by Mechanical Overload
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Background: Gq protein-coupled receptor (GqPCR) 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 DGKzeta (DGK-TG) and wild-type (WT) mice. Increases in heart weight at 4 weeks after TAC were attenuated in DGK-TG 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-TG mice. Up-regulation of atrial natriuretic factor and beta-myosin heavy chain mRNA was observed after TAC in WT mice, but not in DGK-TG mice. Next, left anterior descending coronary artery (LAD) ligation was performed in DGK-TG 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-TG 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 1, and collagen type 3 were observed at 4 weeks after myocardial infarction in WT mice. However, in DGK-TG mice, cardiac fibrosis and profibrotic gene induction were blocked. The survival rate after myocardial infarction was higher in DGK-TG mice than in WT mice. **Conclusion:** DGKzeta suppresses cardiac structural remodeling after TAC and myocardial infarction. DGKzeta may be a potential novel therapeutic target to prevent cardiac remodeling in response to mechanical overload.

**Dnajb5, an Hsp40 Family Protein, Mediates Antihypertrophic Effects of Thioridoxin1 in the Heart**

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Thioridoxin1 (Tx1) 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 Tx1 (Tg-Tx1). However, the molecular mechanism by which Tx1 inhibits hypertrophy remains poorly understood. CDNA microarray analyses revealed that expression of Dnajb5, an Hsp40 family protein, is significantly upregulated in Tg-Tx1 hearts. Because another Hsp40 family protein (Hspa5) was recently shown to inhibit the transcriptional activity of NFAT-c3 through recruitment of class II HDAC to the nucleus, we hypothesized that Tx1 attenuates NFAT activity through Dnajb5, thereby suppressing cardiac hypertrophy. Protein expression of Dnajb5 was increased in both Tx1-overexpressed cardiomyocytes and Tg-Tx1. Upregulation of Dnajb5 is mediated by HSF-1, a redox-sensitive transcription factor, since both expression and transcriptional activity of HSF-1 were enhanced in Tg-Tx1-overexpressed cardiomyocytes and because HSF-1-mediated knockdown of HSF-1 attenuated Tx1-induced upregulation of Dnajb5. Immunostaining and western blot analyses indicated that Tx1 and Dnajb5 are colocalized in the nucleus. In vivo pull-down binding assays indicated that Dnajb5 associates with Tx1 through TXNIP, a Tx1-binding protein. Both Tx1 and Dnajb5 attenuated phenylephrine (PE)-induced increases in the transcriptional activity of NFAT as well as hypertrophy without showing additive effects, suggesting that Tx1 and Dnajb5 are on the same pathway leading to inhibition of NFAT and hypertrophy. Furthermore, knocking-down of Dnajb5 associates with Tx1 in vitro 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 Tx1 in vivo. In summary, Tx1 upregulates Dnajb5, colocalizes with the nucleus, and inhibits NFAT. Dnajb5 is a critical downstream effector of Tx1, mediating anti-hypertrophic effects in the heart.

**Deficiency of β1 Integrins Exaggerates β-Adrenergic Receptor-Stimulated Apoptosis and Heart Failure**

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**Background:** Sympathetic nerve activity increases in the heart during heart failure. We hypothesized that β1 integrins play a protective role in hearts challenged with PE, thereby phosphorylating it. Activation of PE causes a state of chronic hyperadrenergic status, which is associated with cardiac hypertrophy and fibrosis.

**Methods and Results:** -AR-stimulated apoptosis with effects on left ventricular structure and function. Using heart failure models, we evaluated the effects of a β1 integrin antagonist (mAb) on cardiac function and structure. We observed that treatment with the mAb significantly increased cardiac hypertrophy and fibrosis when compared to vehicle controls. Additionally, we found that treatment with the mAb significantly reduced the survival rate after myocardial infarction. Furthermore, in vivo assays indicated that recombinant β1 integrins phosphorolysates (GST-β1-C-terminal peptide, including the activation loop. Mst1 partially co-stained with protein disulfide isomerase, which is localized at ER. These results suggest that Mst1 directly interacts with PERK, thereby phosphorylating it. Activation of PERK plays an essential role in mediating β1-introduced inhibition of cardiac hypertrophy by mimicking ER stress responses such as phosphorylation of eIF2 α. Mst1 not only stimulates apoptosis, but also initiates cross talk with the ER stress pathway, thereby inhibiting cardiac hypertrophy.

**Reduced Neuronal Nitric Oxide Synthase Expression Contributes to Increased Oxidative Stress and Nitroso-Redox Imbalance in Murine Model of Obesity**

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**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 apoptosis, and reduced cardiac function. We studied 2–6 month old ob/ob and C57BL/6 controls. Our results demonstrated that cardiac NOS1 protein abundance (0.1-0.01) and mRNA expression (P<0.03) were reduced in ob/ob, while endothelial NOS protein abundance and mRNA expression were unaltered. NOS metabolites (eNOS and nitrate) production was significantly decreased in ob/ob mice (P<0.02). Furthermore, ROS production was increased in ob/ob mice as GSH/GSSG ratio was decreased (P<0.02), and this could be attributed, at least in part, to increased ROS activity measured by Amplex Red fluorescence assay kit (P<0.04). XOR and NADPH oxidase subunits protein abundance were not changed in ob/ob mice. Leptin deficiency did not change the NOS1 subcellular localization studied by immunofluorescence, as NOS1 co-localized with the ryanodine receptor but not with caveolin-3. These observations suggest that the proinflammatory and anti-oxidant effects of leptin contribute to attenuation of oxidative stress and nitroso-redox imbalance.

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

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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/β1 signaling cascade (JBC, 1998; JCRB, 2002; JBC, 2008). However, whether myotrophin stimulates or inhibits cardiac hypertrophy remains controversial. Using the myotrophin-β1 signaling pathways, we have identified potential targets in the protein in two hairpin-like loops that is responsible for its stimulatory activity by activating NF-κB pathway. To 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 increased development.

**Proapoptotic Serine/Threonine Kinase Mst1 Prevents Cardiac Hypertrophy Through Phosphorylation of PERK**

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Mammalian sterile 20-like kinase (Mst1) is a ubiquitously expressed serine/threonine kinase, which plays an important role in mediating apoptosis. Overexpression of Mst1 in mice results in dilated cardiomyopathy (DCM) with increased myocyte apoptosis but without compensatory myocardial hypertrophy. The lack of compensatory cardiac hypertrophy despite cardiac apoptosis may be detrimental and contribute to progression of DCM. Thus, we examined the molecular mechanism by which the proapoptotic kinase inhibits compensatory cardiac hypertrophy. Overexpression of Mst1 significantly inhibited cardiac myocyte hypertrophy by phenylephrine (PE), an agonist for the α1-adrenergic receptor. Mst1 induced phosphorylation of eukaryotic translation initiation factor 2-α (eIF2-α), a negative regulator of protein translation, and protein kinase R-like endoplasmic reticulum (ER) kinase (PERK), a kinase phosphorylating eIF2-α, in cardiac myocytes. Activation of PERK and phosphorylation of eIF2-α were observed in the present study. In fact, effects of markers of ER stress, such as CHOP and caspase12, were also stimulated by Mst1 in cardiac myocytes. Adenosine-mediated expression of dominant-negative PERK (DN-PERK) abrogated both phosphorylation of eIF2-α and inhibition of PE-induced cardiac myocyte hypertrophy by Mst1. To examine whether Mst1 and PERK phosphorylation is a feedback that permits PERK to play an essential role in mediating Mst1-induced inhibition of cardiac hypertrophy by mimicking ER stress responses such as phosphorylation of eIF2 α. Mst1 not only stimulates apoptosis, but also initiates cross talk with the ER stress pathway, thereby inhibiting cardiac hypertrophy.

**P80**

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

**P81**

Intrinsic Mechanism of Myotrophin-Driven Cardiac Hypertrophy in Neonatal Rat Cardiomyocytes
cardiomyocytes and how myotrophin interacts with components of the NF-κB pathway to initiate cardiac hypertrophy.

Confocal micrographs for localization of Alexa-488 conjugated myoglobin (green) and p65 (Alexa-568; red) in rat cardiomyocyte (a) endogenous p65 in unstained cells, (b) myotrophin immunolocalizes the cytoplasm after 90 min of incubation (c) translocation of myoglobin and p65 into the nuclei after 90 min and (d) 16 hrs of incubation, (e) gentamicin pre-treated cell showing inhibition of myoglobin incorporation into the cell.

Conduction and Characterization of an Inhibitor-Resistant Na+/H+ Exchanger Adenoviral Vector for Transfection of Cardiomyocytes

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The mammalian Na+/H+ exchanger isoform 1 (NHE1) is a ubiquitously expressed membrane protein. It contains a 500 amino acid membrane domain that transports ions and a 315 amino acid regulatory cytosolic domain that is a target for protein kinase mediated phosphorylation. NHE1 plays a critical role in ischemia and reperfusion (IR) injury to the heart and lung. Inhibition of NHE1 activity prevents hyper trophy and IR injury in animal models. We recently demonstrated that extracellular regulated kinase (ERK) mediated activation of NHE1 occurs during ischemia and reperfusion of the murine endocardium. However, the contribution of NHE1 activity compared with other tissues. To examine the activity of the various NHE1 mutants in the myocardium we constructed adenoviruses which express the NHE1 mutants. NHE1 mutant proteins had additional mutations [Leu163/Pro101/Thr174Ser] that increased NHE1 resistance to HOE694 (a specific blocker of NHE1) 100-fold in comparison to endogenous NHE1. Inhibiting NHE1 activity was the result of adenoviral expression. Our results show that adenoviral expression of NHE1 is a practical method for studying NHE1 in cardiomyocytes. Future studies will examine NHE1 activity of the various NHE1 mutants in the myocardium. We are currently using adenoviral vectors to examine the activity of the various NHE1 mutants in the myocardium.

Response to Systolic Overload

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Cardiac-specific SUR1 overexpression 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 Field, Washington Univ, St. Louis, MO; Ping Zhang, Univ of Minnesota, Minneapolis, MN; Colin G 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 cardiac myocyte sarcolemmal ATP sensitive potassium channels (S-KATP), suggesting that impaired S-KATP function might be a cause of cardiomyopathy. Consequentially, this study examined whether KATP 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-myosin heavy chain promoter. SUR1-tg mice demonstrate diminished S-KATP current density, essentially generating a cardiac specific KATP knockdown model. Transgenic mice develop and grow normally without obvious cardiovascular defects or dysfunction. Although there is no significant difference in body weight, both wild-type and SUR1-tg mice were examined. SUR1-tg mice and wild type littermates (WTL) under unstressed conditions, SUR1 overexpression resulted in significant increases of myocardial p-mTOR, total-mTOR, p-S6K1, and total-JNK, p-AKT, p-AMPK were unchanged in SUR1-tg mice. Most interestingly, female SUR1-tg mice demonstrated a markedly abnormal response to systolic overload following transverse aortic constriction (TAC). Thus, after TAC of 4 days duration, SUR1-tg mice developed more severe LV dysfunction (LV ejection fraction decreased to 49.9 ± 4.1% in SUR1-tg mice vs. 72 ± 4.9% in WTL) as compared to WTL mice after sham surgery. Consistent with the greater ventricular dilation and dysfunction in the SUR1-tg mice, 4 weeks after TAC the SUR1-tg mice had more severe LV dysfunction (p < 0.05) pulmonary congestion (with a 190 ± 20% increase in lung weight/body weight ratio) as compared to WTL (with a 44 ± 33% increase in lung weight/body weight ratio). Administration of the NHE1 inhibitor, ATTILA, in SUR1-tg mice without the Shionogi NHE1 inhibitory antibody did not differ significantly from WTL, indicating a specific role for S-KATP dysfunction in mediating the cardiac response to stress.

Overexpression of Calreticulin in Adult Cardiomyocytes Is Not the Cause of Lethality in Mice with Expression of “Activated” α5 Integrin

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We have shown that expression of a constitutively active or “unregulated” α5 integrin subunit, designated α5-u, in infantile or adult mouse heart resulted in severe conduction defects, rapid onset cardiomyopathy and death. Expression profiling of hearts in which α5-u 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-u mice. This implicated calreticulin as a proximal mediator of the deleterious effects of α5-u integrin. To answer this question, we used our experimental model of transient cardiac hypertrophy, induced by expression of cardiac type α5 integrin driven by a mouse cardiac myocyte specific promoter, to examine whether calreticulin expression is increased in mice transgenic for α5-u integrin, calreticulin is not the cause of the rapid onset heart failure.
Adverse Effects on Cardiomyocyte Function of Removing the Inhibition of the Sarco(endo)plasmic Reticulum Ca²⁺ Transport ATPase by Phospholamban

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Background. Replacement of the cardiac sarco(endo)plasmic reticulum Ca²⁺ ATPase SERCA2a by its SERCA2b splice variant with a higher Ca²⁺ affinity (SKO mice) induces left ventricular hypertrophy (LVH). Although SERCA2a protein levels were spontaneously increased and SERCA levels were reduced (50% vs. wild-type, WT) in the SKO heart, ablation of PLB (DKO mice) exacerbated LVH, reduced life span and evoked stress intolerance. To investigate whether severe DKO phenotype relates to the observed further reduced cardiac SERCA2 content (33% vs. WT), we analyzed cardiomyocyte Ca²⁺ transients. Methods. Younger mice were isolated from adult DKO and WT mice. [Ca²⁺]c changes were 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⁺]c transient showed a negative frequency dependence. At 8 Hz, the amplitude of the Ca²⁺ transient was reduced by 40% in DKO vs. WT (in nm: 151 ± 15/110 ± 10, P < 0.05). At 25 Hz, the amplitude of the [Ca²⁺]c transient was reduced by 70% in DKO vs. WT (in nm: 259 ± 38 vs. WT, n_mice = 12; P < 0.05). [Ca²⁺]c removal was faster in DKO at 1 Hz (in ms: 101.8 ± 11.1 in WT; P < 0.05), but normalized to WT values at 8 Hz (in ms: 83.6 ± 8.1 vs. 8 Hz in WT). At 1 Hz, diastolic [Ca²⁺]c was similar in DKO and WT (in ms: 131 ± 12 in DKO vs. 138 ± 14 in WT). However, SERCA activity at 8 Hz was significantly lower compared to WT (in nm: 196 ± 23 in DKO vs. 312 ± 37 in WT; P < 0.05). The SR Ca²⁺ content was deduced from the [Ca²⁺]c transient recorded after application of 10 mM caffeine. In DKO, the caffeine-evoked [Ca²⁺]c 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⁺]c transient and the rate of [Ca²⁺]c removal in WT, but not in DKO. Conclusions. Increasing the Ca²⁺ affinity of the SERCA2 pump by PLB ablation can adequately increase SR Ca²⁺ uptake even in the context of reduced SERCA levels. However, normal SR Ca²⁺ handling in DKO does not prevent LVH. In addition, the lack of increase in SR Ca²⁺ content with frequency and the impaired β-adrenergic response are 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 Sarcomplastic Reticulum Ca²⁺ Transport ATPase

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In 5th generation mice, the cardiac sarco(endo)plasmic reticulum Ca²⁺ pump SERCA2a was replaced by SERCA2b, a higher Ca²⁺ affinity isoform. Interestingly, the imposed high Ca²⁺ affinity was countered by a spontaneous increase in phospholamban (PLB) inhibition, with beneficial effects on cardiac function. The 50% lower cardiac SERCA2 levels in 5th generation mice 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⁺]c activity in this concentration range. We now (A) compared the compensatory response in heterozygous mice (5/5) and (B) crossed our 5th generation mice with mice overexpressing SERCA2b (5/5 Ponsaran). We investigated if higher SERCA2b 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 for 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 SERCA2a mRNA. Expression of both alleles would allow a 50/50 SERCA2a/b distribution in the 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 5/5, the lower SRCa levels in HT were compensated by a reduced PLB inhibition (2-fold less protein, but increased phosphorylation vs. WT). (B) Overexpression of SERCA2b in 5th generation mice resulted in a 20% increase in cardiac SERCA2 content, a similar increase in PLB levels and a further reduced PLB phosphorylation vs. 5/5. Also, the increased SERCA2 levels did not counter hypertrophy, indicating that the hypertrophy in 5/5 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.

Age-Dependant Impairment of Endothelial Progenitor Cells Is Corrected by Growth Hormone-Mediated Increase of Insulin-like Growth Factor-1

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Ageing is associated with impairment of endothelial progenitor cells (EPC) and increasing risk for atherosclerosis. Insulin-like growth factor-1 (IGF-1) stimulates angiogenesis and has vasculogenic properties. The little is known about IGF-1 and IGF-1 receptor (IGF-1R) expression and IGF-1 bioavailability. We compared IGF-1 number and function in healthy male volunteers (57±4.1 years) before and after a ten day treatment with recombinant growth hormone (0.4 mg/day) with that of younger male subjects (27.5±9.0 years). Older subjects had lower IGF-1 levels which were increased by growth hormone treatment (125.6±7.2ng/ml vs 241.1±13.3ng/ml, p<0.0001). In older subjects, circulating CD133+ /VEGFR-2- EPC were significantly lower, EPC function was attenuated, EPC senescence was increased and EPC telomerase activity was reduced compared with the younger group. Growth hormone treatment in elderly subjects increased circulating EPC. These findings imply improved angiogenic and immune properties showed enhanced incorporation into tube-like structures, and increased telomerase activity compared to that of the younger group. IGF-1 stimulated EPC differentiation, migratory capacity and the ability to incorporate into forming vascular networks in vitro via the IGF-1 receptor. IGF-1 treatment significantly attenuated precipitate EPC senescence of older subjects. IGF-1 increased telomerase activity, expression, phosphorylation and activity of endothelial nitric oxide synthase in EPC in a phosphoinositide-3-kinase/Akt-dependent manner. Small interference RNA-mediated functional knockdown of eNOS in cultured EPC abolished the IGF-1 effects. Growth hormone-mediated increase of IGF-1 reverses EPC dysfunction in older subjects by a PI-3-kinase/Akt/eNOS mediated pathway and may be a novel therapeutic strategy against vascular disorders with impairment of EPC.
Thymus Explants 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. Multilineage stem cells (MSCL) are well qualified for this purpose and have already been isolated from the bone-marrow so far. In the present manuscript we aim 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 thymectomy, the thymus gland of 10 neonates/children undergoing cardiac surgery was removed, snap-frozen and cultured. Multilineage potential was tested every 3 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 MSCL from the bone-marrow. Results: While the majority of isolated non-mesodermal cells were non-adherent lymphocytes, we observed two types of adherent cells, some of which had a primary culture. 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 lineage markers and FACS-analysis. The thymic MLSC showed similar characteristics as compared to bone-marrow derived MLSC. Conclusions: The human thymus gland is an alternative source for isolation of MLSC in neonates and young children undergoing cardiac surgery since the thymus is routinely resected during pediatric cardiac surgery. Children who might undergo further cardiac surgeries might profit from the use of thymic MLSC for tissue engineering.

Protein Precoating of Elastic Tissue-Engineering Scaffolds: Extracellular Matrix Formation and Phenotypic Changes of Circulating 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, polyglycerol sebacate (PGS). We hypothesize that EPC and ECM formation could be enhanced by pre-coating PGS scaffolds with ECM protein coatings. Methods: Characterized 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/ III (Coll I/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 SMA+ cells throughout the scaffold with primary surface expression. α-SMA+ cells were found both on the surface and in the “interstitial” 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 Coll III and α-SMA+ cells produced Coll I. Biochemical assays demonstrated increased DNA:Protein ratio (α-SMA+ cells) and collagen content (LM > EL > Fibrin < FN) and collagen and collagen content (LM > EL > Fibrin < FN). 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

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During the past few years the number of patients with cardiac failure has increased following 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/Stroma Derived Factor (SDF-1) modified myoblasts to the myocardium versus improvement of cardiac function using inner allogenic transplantation. 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 stem cells to the myocardium and promote repair process. Methods: SDF-1 modified myoblasts versus non-transduced myoblasts were transduced into the injured myocardium. Hydrogel is used in the study groups to improve cell retention all along the site of transplanted. Cardiac function is reassessed by MRI and echocardiographic parameters after 1, 4 and 8 weeks post-transplant. Control animals receiving no myoblasts or non-transduced myoblast transplants are also followed by the same protocol. The images are analyzed using CAAS MRV software. End-diastolic and end-systolic volumes are used to calculate ejection fraction, cardiac output and wall thickening. The transplanted tissues are analyzed with antibodies specific to skeletal and cardiac muscle lineage. Results: We have been able to successfully transplant human myoblasts in the intra-myocardially post-infarction in nude rats. The SDF-1 labeled cells were able to be tracked till the end of the study (8 weeks). Rats treated with AAV SDF-1 by itself did not show any significant improvement of cardiac function. The cardiac output improved 8 weeks post-myoblast transplant compared to the media injected controls. The AAV SDF-1 transplanted myoblast with and without hydrogel showed significant improve-ment in cardiac function. Conclusion: AAV transplanted 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 diabetic rats. For this purpose, 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, EGK 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 end-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 208.37±4.24, 224.13±2.81, and 200.49±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 (stroke volume was 418.33±8.48, 189.81±8.70 and 383.56±8.33 μl); and ejection fraction was 66.74±0.58, 45.80±1.24, 65.65±0.80 %, in the SC, SD and ED groups, respectively). The LV output was 0.12±5.37 L·min⁻¹, 0.05±2.42 L·min⁻¹, and 0.09±2.65 L·min⁻¹ 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.
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 all-is-transplantation, we previously demonstrated that circulating cells engraft the heart transplants 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 xeno-transplantations, 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. 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 small GFP+ cells expressing markers of cardiac progenitor cells, such as GATA-4 and MEFC2, in all xenografts. 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.

TAXUS® Express® Stent Coating Integrity Is Robust When Implanted in a Completely Overlapping Configuration in an In Vivo Swine Model

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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 overlapping TAXUS® Express® stents. TAXUS® Express® 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 TAXUS® Express stents and overlapped pair–(9) of stents were visually inspected (inner diameter and outer diameter) at 2X magnification with a light microscope and an area reticule. 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 displacements). 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 single TAXUS Express stents placed in a completely overlapping configuration. All overlapped 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


Effectiveness 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 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 combined to express IS as percentage of total LV volume, and the values were compared to the functional measurement 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 epicaldial 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 0.4-0.6 fold, 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.
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 rats with 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. 26.3% ± 2.0% HUCBC migrated to infarcted myocardium at 2 hours and 69.9 ± 2.72% 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 size. Injection of 10^6 HUCBC into isloyte into infarcts of non-immunosuppressed rats at 1, 2, 2.5, 3, 6, 12, 24, 48, and 96 hours after LAD occlusion resulted in infarct sizes one month later of 6.4 ± 0.01% 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 isloyte (p < 0.005). Infarcts treated with only isloyte increased myocardial concentration of tumor necrosis factor alpha (TNFα) from 6.9 ± 0.2% 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 ± 2.5% 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.

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 (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 is critically dependent on 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 chemical libraries of small molecules capable of activating cardiac genes in ES/EC cells which fall into several dozen distinct structural classes. Among the most promising hits, substituted aryl sulfonyl hydrazones activated pro-differentiation ERK signaling and the cardiac gene program in P19CL6ES and SM1 ES cells. A second class of molecules, bearing an isoxazole core structure, also strongly activated Nkx2.5 and myocardin in stem cells. Isoxazoles were also potent neurogenic in cultured hippocampal neural progenitors cells and in the mouse brain in vivo. The cardiogenic and neurogenic effects of isoxazoles involve calcium signaling and activation of ME2, a transcriptional regulator shared by neural and cardiac gene programs. Further characterization of sulfonhydrazones as isoxazoles and other small molecule candidates and the screening of target proteins in stem cells will lead to discovery of new molecular tools to elucidate the biology of the cardiac fate decision in ES cells.

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 Sciocevic’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-acazcythidine . 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 = 84) and those incubated with fetal heart supernatant (BF group, N = 69) 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 < 0.001). 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 for anti Troponin-I and MF20 antibodies specific for cardiomyocyte. In RT-PCR analysis, MYF-5, 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.
Aorta Ring Assay (Capillary Density (Endothelial/Muscle) 1.25

Inhibited Histone H1-phosphorylation-defined cyclin E/CDK2 activity in mouse VSMC (59% of sponged CaM-binding motif in human cyclin E1 (named 'CBS' for CaM-Binding transition, and requires direct binding of CaM to cyclin E. We now show the molecular basis of VSMC (Table) Tube Formation Assay showed endothelial cells isolated from aorta of APAKO mice were tended 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**

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<tr>
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<th>Wild type</th>
<th>APAKO</th>
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<tr>
<td>Capillary Density (Endothelial/Muscle)</td>
<td>1.25 ± 0.12</td>
<td>0.57 ± 0.15</td>
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<tr>
<td>Aorta Ring Assay (µm)</td>
<td>464 ± 80</td>
<td>332 ± 73</td>
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**In Vitro Analysis of Signaling by the Selective Corticotropin-Releasing Factor Receptor Agonist Urocortin 2 in Isolated Adult Cardiomyocytes**

Stephen J Perry, Sachiko Junger, Jordan E Pomers, Robert E Proskriki, Dimitri E Grigoriadis, Richard A Miski; Neurocrine Biosciences Inc, San Diego, CA

Urocortin 2 (UCN2) 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). UCN2 has been reported to possess cardiac 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 UCN2 induces inotropy and lusitropy. Here we show that UCN2 signals through the Gs-coupled CRF2 receptor on isolated adult rat cardiomyocytes (ARCMs) and compared the potency and efficacy of this peptide to the known inotrope dobutamine, a beta-1 adrenergic agonist. Treatment of ARCMs with either UCN2 or dobutamine stimulated cyclic AMP accumulation and the phosphorylation of PKA substrates. The effects of UCN2 were completely blocked by the selective CRF2 receptor antagonist astressin-2B while those of dobutamine remained unaffected confirming selective activation of the CRF2 receptor. UCN2 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 calcium flux and membrane contractions in isometric ARCMs we also demonstrated that UCN2 treatment produced a dose-dependent slow increase (~10 minutes to reach maximum) in peak calcium release, sarcomere shortening, tau and time between peak calcium and peak contraction (EC50 = 5nM), with no significant change in diastolic calcium concentration demonstrating the direct inotropic and lusitropic actions of UCN2. These data suggest that the direct inotropic and lusitropic activity of UCN2 on ARCMs occurs through selective activation of a CRF2 receptor-dependent cyclic AMP/PKA pathway.

**Treatement 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 (BMMNCs) are harvested from 5–6 week old male Sprague-Dawley rat femurs. The contents are flushed with sterile 10% PBS in DMEM-LO. Bone spicules and clots are allowed to settle, the cell suspension is decanted and concentrated 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 plating 1x10⁶ cells per well in 24 well plates. Each well is exposed to one of several following growth factor combinations (10 ng/ml): (1) Control (10% PBS + DMEM-LO), (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 BMMNCs grown to confluency. At passage three, immunohistochemistry was used to detect the presence of CD34, CD44, CD45, VEGF, and Pan-EC. **Results:** Growth factor stimulation produced a transient increase in the number of cultured BMMNCs from day one to day five, and decreased thereafter to day 10. At day five treated cells had an increase (P<0.05) in number compared to all other groups. (1) 295,500 ± 27,081 (n=6), (2) 222,267 ± 37,983 (n=4), (3) 303,333 ± 31,815 (n=6), (4) 395,885 ± 79,846 (n=6), (5) 712,333 ± 68,214 (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 BMMNC 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-based therapy after myocardial infarction.
timolol or metoprolol alone activated ERK (timolol EC50=23 mM, Emax=248±2.2, n=3; p<0.05, metoprolol EC50=27 mM, Emax=216±4.4, 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 cardenolides NE and EPI alone inhibit anti-apoptotic ERK and activate pro-apoptotic p38 via a beta-AR in adult mouse cardiac myocytes. Beta-blockers switch catecholamine signaling in 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.

P117 Direct Determination of β1-Adrenergic Receptor Activation Reveals Differential Antagonist Sensitivity of Naturally Occurring Receptor Variants

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Blockade of β-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 into which the cyan- and the 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 noradrenaline, the human β1-AR activated (decrease of the FRET ratio of about 4%) at a surprisingly slow speed (about 500 ms) followed by activation of the Gαq and Gα12/13 pathway. Commonly used β-blockers such as metoprolol, 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.

P118 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 atheroma 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, which are closely related forkhead transcription factors, 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 mutational 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 direct the transcription of VCAM-1 gene in endothelial cells and that they may play a pivotal role in the development of atherosclerosis.

P119 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 A-6G, ACE insertion/deletion (I/D) and the angiotensin II type I receptor gene (AT1R). A1166C polymorphisms using polymerase chain reaction. Intracellular 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<0.01). The AGT MT genotype was found to be associated with AGT A-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.

P120 Protopic 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 (CMC) 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-LS-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 but not 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 by chronic obstructive (Doc) or simple (Hx) administration 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 Doc 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 both in vivo and in vitro models. These data suggest that Cystatin C can be useful as a biomarker of cardiomyocyte injury associated with oxidative stress in vitro and in vivo.

P121 Molecular Characterization of a Calcium ATPase Gene in an Animal Model of Cardiomyopathy

Eunice N Ndegwa, Michelle M Corley; Tuskegee Univ, Tuskegee, AL

Cardiovascular disease, principally heart disease and stroke, is the United States leading killer for both men and women among 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 calcium stores is thought to be altered in cardiomyopathic patients. The pathophysiologival 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 turazaleus induced cardiomyopathic turkeys. Primers were designed (Primer 3 software) from the chicken calcium ATPase mRNA to target the turkey genome. Total RNA was isolated from chicken (controls), furazide- induced and non-induced turkey heart tissues. A one step Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed. The expected 195 bp cDNA fragment for the calcium ATPase gene was observed in euthidrome stained gels with an Ultra Violet Gel Documentation system, indicating successful amplification of the calcium ATPase gene from turkey heart.

P122 Increased Mitochondrial Uncoupling Protein 3 Is Associated with the Cardiomyopathy in the Dystrophic Mouse Heart

Wen Zhang, Michiel ten Hoeve, A Elisabeth Sang, Kay E Davies, Stefan Nebauer, Kieran Clarke; Univ of Oxford, Oxford, United Kingdom

Patients with muscular dystrophy have heart failure and abnormal cardiac high-energy phosphate metabolism. Uncoupling protein 3, 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 Langendorff 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 μM ±, P < 0.05) lower left ventricular peak filling rates, but the same left ventricular mass, end-diastolic volumes, end-diastolic 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 15-44% (P < 0.05) higher mitochondrial uncoupling protein 3 than their controls. We conclude that adult dystrophic-deficient mdx mouse hearts left ventricular diastolic dysfunction associated with decreased phosphocreatine levels and increased mitochondrial uncoupling protein 3.

P123 Characterization of In Vivo Tissue Redox Status and Oxygenation in the Ischemic and Reperfused Myocardium: A Central Regulatory Role of Mitochondria

Guanglong He, Xuehai Zhu, Li Zuo, Arturo J Cardounel, Jay L Zweier; Davis Heart and Lung Inst, The Ohio State Univ, Columbus, OH

Objectives: Myocardial tissue redox status and oxygenation are critical to the pathophysiological processes of ischemia-reperfusion injury in the postischemic myocardium. The current...
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 oxidant species. High-performance liquid chromatography-electrochemical method was performed to measure tissue reduced/oxidized glutathione ratio. It was found that tissue redox status was significantly changed during reperfusion. Tissue level was increased 100% (0.042 to 0.084 min⁻¹) during ischemia and decreased 33% (0.042 to 0.028 min⁻¹) 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 (1.01 ± 0.12 mg/mg Hb) and 1.2 fold (1.01 ± 0.12 mg/mg Hb) 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**: In conclusion, the higher reduction rate in the ischemic myocardium was consistent with the lower NADH level, lower ROS level, and lower NADH/GSSG level. The lower redox ratio in the reperfused myocardium was consistent with the higher NADH, higher ROS generation, lower mitochondria and lower GSH/GSSG level. The lower redox ratio in the reperfused myocardium was consistent with the higher NADH, higher ROS generation, lower mitochondria and lower GSH/GSSG level. The lower redox ratio in the reperfused myocardium was consistent with the higher NADH, higher ROS generation, lower mitochondria and lower GSH/GSSG level.

**Evidence of Persisting Mitochondrial Dysfunction in a Model of Reversible Cardiac Hypertrophy**

Carla A Di Maria, Western Australia Inst for Med Resch, Crawley Perth, Australia; Douglas J McKirtnick, Yasaki Holobotovskiy, Leonard Arnoldia, Maria A Bogoyvich, Peter G Arthur, Livia C Hoot; The Univ of Western Australia, Crawley Perth, Australia

Hypertensive cardiac hypertrophy is an independent risk factor for increased cardiovascular morbidity and mortality and effective reversal of this hypothesis is considered to be beneficial. We examined the effects of hypertension on cardiac mitochondria and systemic oxidative stress using a two kidney one clip model of hypertension in C57 black mice. After 4 weeks of surgery, a 1.5 fold increase in blood pressure was observed in the two kidney one clip (2K1C) group when compared to the control group. At 16 weeks, 2K1C group showed a 2.2 fold increase in systolic blood pressure when compared to controls (n=9). During the first 6 weeks of surgery, superoxide production assessed with the fluorescent indicator dihydroethidium, was significantly increased in 2K1C compared to controls (n=9) by 2.3 fold. Mitochondrial complex activities were significantly decreased in 2K1C group (n=9) compared to controls (n=9) by 48% for complex I and complex III activities and superoxide production by the mitochondria. At 4 weeks post surgery, 2K1C group showed a 2.3 fold increase in superoxide production persisting to 12 weeks post surgery (39.7 ± 9.3 vs. 0.7 ± 0.3; P<0.05). These data suggest that effective reversal of hypertension and cardiac hypertrophy may not be sufficient to prevent further morbidity associated with persistent mitochondrial dysfunction.

**Vacuolar Atpase Anaesthetics Inhibit Cardiac Mitochondrial Respiration**

Coert J Zuurbier, Peter J Keijzers, Anneke Koeman, Harry B van Wezel, Markus W Hollmann; Academic Med Ctr, Univ of Amsterdam, Amsterdam, The Netherlands

Background: Recent evidence suggests that hexokinase mitochondria association attenuates mitochondrial dysfunction and apoptosis. The mechanism contributing to isoflurane and sevoflurane-induced cardioprotection. With Mitochondria

**Voluntary Absence Maintains the Antipapoptotic Association of Hexokinase with Mitochondria**

Coert J Zuurbier, Peter J Keijzers, Anneke Koeman, Harry B van Wezel, Markus W Hollmann; Academic Med Ctr, Univ of Amsterdam, Amsterdam, The Netherlands

Background: Recent evidence suggests that hexokinase mitochondria association attenuates mitochondrial dysfunction and apoptosis. The mechanism contributing to isoflurane and sevoflurane-induced cardioprotection.

**Ca(OH)₂ Inhibits Cardiac Mitochondrial Respiration**

Halsey Anderleh, Northwestern Univ, Chicago, IL; Steven P Jones, Univ of Louisville, Louisville, KY; Eduardo Marban; Johns Hopkins Univ, Baltimore, MD

Ca(OH)₂ inhibition depending on the respiratory substrate used. State 4 respiration was unchanged from mitochondria of viable failing CM with intact sarcolemma suggests an existence of reversible transitory mPTP opening in high conductance mode. Attenuation of calcein loss and improvement of VCR and OCR achieved with Ca(OH)₂ (0.2 μM) show that transient mPTP opening in high conductance mode in failing CM could be a cause of mitochondrial functional abnormalities described in failing heart.

**Oral Sodium Salicylate Inhibits Cardiac Mitochondrial Respiration**

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**Background**: A potential detrimental effect of salicylates intake in congestive heart failure has been recently recognized. The Warfarin/Aspirin Study in Heart Failure demonstrated that the aspirin dose and significantly higher mortality in patients treated with aspirin in comparison to the warfarin or control groups. Mitochondrial dysfunction plays an important role in the progression of heart failure by inhibiting the energy supply/demand mismatch state. In a previous in-vitro study, we demonstrated that salicylates inhibit mitochondrial respiration and attenuate ATP synthesis and oxygen consumption in high conductance mode can create reversible mitochondrial damage during ischemia/reperfusion. When compared to the warfarin or control groups. Mitochondrial dysfunction plays an important role in the progression of heart failure by inhibiting the energy supply/demand mismatch state. Mitochondria are primary intracellular targets for respiration and apoptosis. The process of apoptosis involves opening of the mitochondrial transitory permeability transition pore (mPTP), which in turn leads to release of proapoptotic proteins. Another mitochondrial channel, ATP-sensitive potassium channel or mPTP, has been shown to exert protective effects. The structure of mPTP is not known, but is thought to contain at least 5 mitochondrial proteins. Since there is evidence for pharmacological overlap between these two channels and that one protein (adenine nucleotide translocator) is shared between their putative structures, we hypothesized that there is physical interaction between protein members of these two channels. Using co-immunoprecipitation, antibodies against ATP synthase or inorganic phosphate carrier (PIC) pull down Bax and Bcl-2 and vice versa, ATP synthase also associates with other Bcl-2 family members, such as Bak, Bid, Bcl-xL, PIC antibody also pulls down voltage dependent anion channel (VDAC). We then studied whether the opening of mPTP can be modulated by agents targeting other proteins in this complex. We first induced mPTP opening by treating isolated cardiomyocytes with a cell-permeable form of the ATP synthase inhibitor oligomycin A (OA) and a cell-permeable form of the hexokinase inhibitor II peptide. This peptide has been shown to dislocate HK from the mitochondria, and subsequent mPTP opening and apoptosis. Exposing myocytes to this peptide caused a dose-dependent (0–20 μM) reduction of mitochondrial membrane potential, consistent with mPTP opening. This effect was reversed by the ANT inhibitors, bongkrekic acid (0.05 mM) or atracurium (0.1 mM, but not by ciclesium or diazoxide. Our results suggest that there is a macromolecular complex in the mitochondria which contains the proteins of mPTP and mPTP. Furthermore, the opening of mPTP by dislocating HK form the mitochondria leads to an opened conformation of mPTP that is insensitive to ciclesium, but is reversed by ANT modulators.
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-Henseliet 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 in coronary effluent and significant myocardial infarction (46% of risk area). Sulfaphenazole significantly enhanced recovery of heart function and decreased infarct area, and attenuated inflammatory response. These data suggest the potential of Sulfaphenazole as an effective cardioprotective agent.

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 cardioplegic arrest and reperfusion is not only dependent on one or a combination of risk factors but also depends on cardioplegic solution type. We studied apoptosis signal-pathway induction in patients subjected to cardioplegic arrest (CA) on cardiopulmonary bypass (CPB) using warm blood and cold crystalloid cardioplegia. Methods: In twelve patients we collected cardioplegic biopsies form the right atrial cannula 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 (Calsioflore) while AVR hearts were arrested with crystalloid solution (Bretschneider). LV specimen were immuno-cytochemically stained against activated caspase-3 (apoptosis key-enzyme), and 8-isoprostaglandin-F_2alpha (oxidative stress marker). Cardiomyocytes were then quantitively 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=0.18). 8-Isoprostaglandin-F_2alpha formation remained unchanged in AVR but increased significantly in CABG (by 10.9±6.4 U, p<0.03). While cardioplegic arrest was significantly longer in AVR (68.8±8 min vs. 38.6±6 min, p=0.03), reperfusion period on CPB was longer in CABG (30.4±4 vs. 15.4±3 min, p=0.01). However, there was no correlation between duration of cardioplegic 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 potassium, low calcium crystalloid cardioplegia.

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. Treatment with adiponectin improved left ventricular function, 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.
Antilipotrophic Action of Canrenone (Spironolactone Major Active Metabolite) in Aldosterone-Induced Atherosclerosis: A Calcium-Dependent Pathway

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Background: Recent studies report that aldosterone (Aldo) is produced by failing human hearts and induces apoptosis through calcineurin-dependent pathway which is inhibited by calcium (Ca2+) channel blockade. Apoptosis is triggered by spillage of zinc from the cytosol to the extracellular compartment, resulting in reduced cellular Ca2+ and cytoplasmic acidic pH. We hypothesized that replenishment of intracellular zinc at reperfusion improves myocardial recovery after a period of ischemia/reperfusion (I/R). 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 subjected to 15 or 30 minute global ischemia followed by 45 min of reperfusion. After 15 min of ischemia, heart rate (74.95% with arrhythmias diminished 8-fold. The effect was dose-dependent with 500 μM Aldo 10%–p<0.03). 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% –p<0.001), and caspase-3 activation (17.50%, CRN 15.66%, Aldo 90.91%, and CRN + Aldo 18.83% -p<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.

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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 lynchpins in redox switches of Ser/Thr kinases structurally presented 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 (I/R). 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 subjected to 15 or 30 minute global ischemia followed by 45 min of reperfusion. After 15 min of ischemia, heart rate (74.95% with arrhythmias diminished 8-fold. The effect was dose-dependent with 500 μM Aldo 10%–p<0.03). 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% –p<0.001), and caspase-3 activation (17.50%, CRN 15.66%, Aldo 90.91%, and CRN + Aldo 18.83% -p<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.

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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 clearance 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. Consistently, administration of MG262 in vivo decreased the level of GATA4 and 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 to 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 antiapoptotic activity of DOX in humans.

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Effects of Angiotensin II Type 1 Receptor Antagonist on Transforming Growth Factor-β1 and Myocardial Remodeling in Rats with Developing Heart Failure

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Background: Myocardial remodelling is an adaptive response to pressure and volume overload and plays a key role in heart failure. Its progression is characterized by an increase in left and right ventricular volumes and weights, which can lead to heart failure progression. Angiotensin II (ANG II) is a member of the renin-angiotensin system, which is involved in the development and progression of heart failure. The aims of this study were to investigate the effects of an Ang II receptor antagonist on transforming growth factor-beta1 (TGF-beta1) and myocardial remodeling in rats with developing heart failure.

Methods: Male Wistar rats were randomly divided into six groups: control group, angiotensin II type 1 receptor antagonist losartan group, and angiotensin II type 1 receptor antagonist losartan group with doxorubicin (DOX) treatment. The rats were treated with losartan for 6 weeks, and then the animals were subjected to Ligation Coronary Artery (LCA). The rats were then divided into two groups: control group and losartan group. The blood samples were collected, and the hearts were fixed, and the left and right ventricular weights were measured. The results were analyzed using one-way analysis of variance (ANOVA) with Tukey’s post hoc test.

Results: The results showed that losartan treatment significantly reduced the left and right ventricular weights in the losartan group compared with the control group. The left and right ventricular weights were significantly reduced in the losartan group compared with the control group. The left and right ventricular weights were significantly reduced in the losartan group compared with the control group. The left and right ventricular weights were significantly reduced in the losartan group compared with the control group. The left and right ventricular weights were significantly reduced in the losartan group compared with the control group. The left and right ventricular weights were significantly reduced in the losartan group compared with the control group.

Conclusion: The results of this study suggest that losartan, an Ang II receptor antagonist, significantly reduces myocardial remodeling and preserves cardiac function in rats with developing heart failure. Further studies are needed to confirm these findings and to explore the mechanisms by which losartan reduces myocardial remodeling and preserves cardiac function.
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/Wc) mice and their normal littermates (+/+) METHODS Thirty male W/Wc mice and 18 +/+ littermates were anaesthetized with 2.5% isofluroane. 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 triphenyl tetrazolium (TBT) histochemical assay and serum IL-6 concentrations measured with ELISA. RESULTS Cardiac muscle viability was significantly higher in W/Wc than the +/+ mice. Serum IL-6 levels were higher in the +/+ sham mice (163 ± 11 pg/mL) than the W/Wc mice (65.1 ± 61 pg/mL), p = 0.001. The IL-6 levels increased significantly after reperfusion only in the +/+ mice (45.1 ± 14 pg/mL, n = 8, p = 0.001). The IL-6 concentrations in the W/Wc mice (71±17 pg/mL, n = 8, p = 0.78). CONCLUSIONS These results show that the absence of mast cells reduces the myocardial damage associated with IR injury. Furthermore, there is an attenuation of 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 stuttered manner (analogous to postconditioning) may evoke cardioprotection in the clinical setting. Here we show that by treating isolated human trabeculae with the beta-AR agonist isoproterenol, c-fos expression is enhanced by 40.7% (144.6±0.01) in the 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, as well as IP3 receptor antagonist heparin respectively (p<0.01). The inhibited transcription of thapsigargin (decreased by 70.8%, 42.3±0.01) and IP3 receptor antagonist heparin (decreased by 72.2%, 39.9±1.3 vs 144.6±1.6) and heparin (decreased by 96.0%, 115.0±1.8 vs 144.6±1.6) group. These results indicated that c-fos gene transcription regulated in the change of nuclear calcium handling system, which might at least partly take part in developing overload-induced cardiac hypertrophy.

Regulation of Cardiac Periostin and Pielotrophin Gene Expression by Myocardial Infarction and Pressure Overload

Vivra Jongsu, Jaana Rysä, Jani Ari, Mika Ives, Hannu Leskinen, Heikki Ruskooaho; Univ of Oulu, Oulu, Finland

Periostin (PN) and pielotrophin (PTN) are extracellular matrix (ECM) proteins, PN being involved in cell adhesion, while PTN is a diverse ECM- and cell-surface-associated growth factor. However, little is known about the role of these initially osteoblast-associated proteins in adverse myocardial remodeling after myocardial infarction and in response to pressure overload. Here we determined the effect of myocardial infarction as well as the acute and chronic pressure overload on left ventricular (LV) PN and PTN gene expression. Post-infarction LV remodeling process, characterized by thinning of the wall in the infarcted area, eccentric hypertrophy and ventricular chamber dilatation was associated with elevated PN mRNA levels at 24 hours (11±0.1 fold, P<0.05), and became even more pronounced in the wall which remained surviving tissue at 24 hours post-infarction, but afterwards there was a tendency for PTN mRNA levels to increase during LV remodeling process. In a model of acute pressure overload in conscious rats, a significant increase (2.8-fold, P<0.01) in LV PTN mRNA levels was seen in response to 4 hrs of intravenous administration of arginine–vasopressin (0.05 μg/kg). Furthermore, infusion of angiotensin II (Ang II) (30 μg/kg/h) subcutaneously by osmotic minipumps for 72 hours significantly increased LV PN mRNA levels (5.6-fold, P<0.05). In addition, a significant 1.7-fold increase in PTN mRNA levels was observed after Ang II infusion (6–0.05). Finally, to study the effect of chronic hypertension to PTN expression, we compared PTN mRNA levels in 12, 16, and 20 months old spontaneously hypertensive rats (SHR) to their age-matched normotensive control Wistar-Kyoto rats (WKY). An increase in PTN gene expression in SHR compared to WKY was observed at each time point, the greatest elevation being 2.1-fold (P<0.01) at 12 months. These results suggest that PN and PTN are involved in myocardial infarction and pressure overload induced cardiac ECM remodeling process. Interestingly, PTN gene expression was also rapidly upregulated in response to acute pressure load well before the development of left ventricular hypertrophy.
Lack of microRNA Regulation in Myocardial and Skeletal Muscle in Response to Cardioplectic Arrest and Cardiopulmonary Bypass


Methods: 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 cardiopulmonary bypass (CPB). Results: Right atrial (RA) and skeletal muscle (SKM) was harvested from similar cardiac surgical patients (N=6) before and after CPB. RNA was extracted using Ambion’s MELT Total RNA Isolation System. Total RNA was analyzed by TaqMan real-time RT-PCR of select miRNA and miRNA targets. CYR61 and DUSP1 were studied as genes known to be upregulated by CPB as positive controls. GAPDH was used as a negative control. miRNAs were selected based on data in the literature. Total RNA was analyzed by TaqMan real-time RT-PCR of select mRNA and miRNA targets. CYR61 and DUSP1 were significantly upregulated (p<0.05 respectively) while GAPDH expression was not altered by CPB. There was no significant difference in the regulation of miRNAs tested following CA/CPB for the 18 targets that were assayed by q-RT-PCR. Conclusions: No miRNA targets were identified that were differentially expressed in response to the ischemia reperfusion stress following CA/CPB in patients.

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

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Mao-to, Japanese herbal medicine, has anti- viral and anti-autoimmune effects. Immuno modulating 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 C3H mice were randomly divided into 5 groups (n=30 of each). Group N included uninfected control mice, Group A, B, C, D and Group E were inoculated with EMC virus. Group A was orally administered saline starting on day 0 to day 8. Group B was orally administered Mao-to (500mg/kg/day) starting on day 0 to day 8. Group C was orally administered with Mao-to at the same dose as group B starting on day 2 to day 8. Group D was orally administered with Mao-to at the same dose as group B starting on day 4 to day 8. The 14 days survival was observed after viral inoculation. Body weight changes, tissue weight and organ weight containing heart (HW), bilateral lungs, thymus and spleen were examined on days 0, 2, 4, 6, 14. Cardiac expression of TNF protein and mRNA was examined by immunohistochemistry and quantitative RT-PCR. The survival rates on day 7 were 100% in group A, 18.2% in group B, 6.7% in group C, 23.3% in group D. Hosmer-Lemeshow statistic showed no significant differences in all groups. However, in vitro treatment of cells from obtained from aneurysmal tissues obtained from animal test did not result in the development of necrotic cells measured by Annexin V-PI staining. Conclusions: Reduced reisus in tissues of ascending aortic aneurysm in BAV seems to be related to MMP elevation, whereas apoptosis may be an early mechanism leading to unbalance of the MMPs.
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 Mao-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 (miRNAs), which target specific mRNA transcription 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
Maram K Reddy, Venod Labhasetwar; College of Pharmacy, Univ of Nebraska Med Ctr, Omaha, NE
Effect of Erythropoietin on Neointima Formation in Rat Carotid Artery Model of Vascular Injury Maram K Reddy and Venod Labhasetwar Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198 Various cytokines and adaptive 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 (BM)-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±0.9 vs. 1.6±0.2, n=5, p=0.001), resulting in significant reduction in lumen area (0.16 mm² vs 0.01 mm², n=5, p=0.001). The mechanism of excessive neointima formation in the EPO treated group was found to be due to excessive neoangiogenesis 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 stenosis at sites in EPO treatment during hemodialysis and suggest the cautious use of EPO in patients who are at risk of vascular injury.

Detection and Monitoring of Brain Recovery After Therapeutic Hypothermia in a Post-Cardiac Arrest Rodent Model: A Quantitative EEG Study
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Objective: To test the hypothesis that quantitative electroencephalogram (qEEG) can objectively assess functional electrophysiological recovery of brain after hypothermia in an asphyxial hypothermia model. Method: To test the hypothesis that quantitative electroencephalogram (qEEG) can objectively assess functional electrophysiological recovery of brain after hypothermia in an asphyxial hypothermia model, we studied the effect of hypothermia on qEEG-IQ and IQ during the hypothermia maintenance period and intermittent arterial blood gas (ABG) 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 0.74, 2-tailed P=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.

Atorvastatin Activates Cyclooxygenase-2 in the Heart via S-Nitrosylation by Inducible Nitric Oxide Synthase
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Background: Atorvastatin (AT) increases myocardial expression of phosphorylated Ser-1777 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 assayed by the “Biotin Switch” assay. To verify that the immuno-precipitates contained COX2, membranes were stained and blotted 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 in iNOS- mice (101 ±3%). However, myocardial 6-keto-PGFβ, 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.

Pioglitazone and Atorvastatin Augment Myocardial Production of 15-Epi-Lipoxin A4 in the Rat Heart
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Background: Both statins and thiazolidinediones have anti-inflammatory properties. However, the exact mechanisms underlying these effects are unknown. We investigated whether AT and PIO increase myocardial content of lipoxin A4 and 15(S)-epi-lipoxin A4 (15ELX), both arachidonic acid products with strong anti-inflammatory properties. Methods and Results: Experiment 1: Rats received 3-day pretreatment with: 1) water; 2) PIO 10 mg/kg; 3) PIO 10 mg/kg + AT; 4) PIO 10 mg/kg + zileuton. There were 4 rats in each group. Heart were harvested and analyzed for myocardial lipoxin A4 and 15ELX levels, and COX2 and 5-lipoxigenase protein expression. AT (1.10 ±0.02 ng/mg; p=0.001 vs. sham) and PIO (0.98 ±0.02 ng/mg; p=0.001 vs. sham) increased myocardial 15ELX levels compared to the sham-treated group (0.51 ±0.02 ng/mg). Myocardial 15ELX were significantly higher in the PIO + AT group (1.29 ±0.02 ng/mg; p=0.001 vs. each other group). Both valdecoxib and zileuton decreased the PIO + AT + valdecoxib and zileuton group compared to the PIO + AT group (1.03 ±0.02 ng/mg; p<0.001 vs. each other group). Both valdecoxib and zileuton decreased the AT + valdecoxib and zileuton group compared to the AT group (1.01 ±0.01 ng/mg; p=0.001 vs. each other group). Both valdecoxib and zileuton decreased the PIO + AT + valdecoxib and zileuton group compared to the PIO + AT + valdecoxib group (0.76 ±0.02 ng/mg; p<0.001 vs. each other group). These data indicate that the combination of AT and PIO increases the production of 15ELX in rat heart, while the combination of AT and zileuton decreased the production of 15ELX. Conclusion: PIO and AT increased myocardial production of lipoxin A4 and 15(S)-epi-lipoxin A4 (15ELX), both arachidonic acid products with strong anti-inflammatory properties.

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
proapoptotic caspases are of central importance. In contrast, the role of proinflammatory caspases is unclear. Interleukin converting enzyme (ICE, caspase-1) has been described as a proinflammatory caspase that generates active IL-1 beta. This study aims to elucidate the cardiac function of ICE. Gene array analysis in a murine heart failure model showed upregulation of myocardial ICE. We confirmed this both in a murine heart failure model as well as in primary cardiomyocytes. The first murine heart failure model (MCT) +12% ± 4% (vs control), +24% ± 7% (vs WT) significantly increased myocardial ICE with 50% of mice dying before the age of 24 weeks. These data indicate that ICE is differentially expressed in heart failure and controls cardiac size and function via inhibition of the ERK MAPK pathway.

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Antigens 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 heart 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±0.08 mg/g). By 12 weeks, HHR hearts were 27% larger than NHR. Tissue AT1 receptor mRNA expression was significantly higher in the HHR-P165 compared with NHR. HHR neonatal hearts exhibited a 4.6-fold higher AT1/AT2 mRNA expression ratio. Neonatal cardiomyocytes were infected with ATa and/or AT receptors, expressing aminopeptidases to achieve a physiological level of receptor expression (150 fmol receptor/mg total cell protein). In addition, to evaluate receptor expression in neonatal HHR hearts, cells were co-infected with ATa and AT receptors. A 4.1 apoptosis incidence was studied by morphological analysis after 72 hours exposure to 0.1 μM AngII. When infected with the ATa receptor alone, a higher proportion of HHR myocytes appeared apoptotic than NHR (22.7 ± 4% vs 10.5± 1.0% p < 0.001). This suggests that the ATa receptor activates HHR cardiomyocytes to accentuate AT1-mediated apoptosis. Interestingly, the βax/1-62-bd mRNA expression ratio was significantly higher (50%) in HHR neonatal hearts. When cells were co-infected with ATa and AT receptors, evidence of apoptosis in HHR cells virtually disappeared (0.4 ± 0.1%). These findings suggest a novel capacity of AT receptors to counteract accentuated ATa receptor-induced apoptosis in the HHR in early cardiac growth.
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). Serum sildenafil concentration in free sildenafil was 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. 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.

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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 cardiomyocyte 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 diminish activation 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 destabilize 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.

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Calcineurin A Gene Expression and Calcineurin-Alike Protease Activity Increase with Atrophic Remodeling of the Heart

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Background: Cardiac hypertrophy is an independent risk factor for the development of heart failure. Regulators of cardiomyocyte atrophy are potential targets for reversing cardiac hypertrophy. Calcipain 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 and hypertrophy. Calpain 1 and calpain 2 are essential regulators of skeletal muscle atrophy. Their role in cardiac hypertrophy and these data support the future investigation of BH4 supplementation as a pharmacological protectors of the vascular wall against oxidative damage in conjunctive courses of thrombolytic, pulmonary, psychiatric or preventive cure.

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Supramolecular Protein Structure for Extracellular Antithrombotic 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 glycocalyx, ulceration and rupture of atherosclerotic plaques. Such a wide range of damaging actions implies the necessity of both intra- 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 imparts the specificity of the glycosaminoglycan-matrix interactions, can be effectively used as antioxidant protectors. Covalent binding of SOD to CAT via the vascular wall glycosaminoglycan chondroitin sulfate (CHS) leads to formation of a supramolecular bienzyme conjugate (SOD-CHS-CAT) which manifests in vivo the highest antithrombotic activity in complex with SOD and CHS where weight loss was down (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 CHS and their mixtures. A single-bolus injection of the mixture between SOD, CHS and CAT-CHS-CAT at a significantly lower concentration than that of the SOD-CHS-CAT conjugate. This could be explained by different surface distribution of the conjugates in the circulation after their intravenous administration. The bienzyme conjugate was effective at doses two orders smaller than those for native SOD and CAT and an order of magnitude smaller than that for CHS-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 thrombolytic, pulmonary, psychiatric or preventive cure.

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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 depleted 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 C57Bl/6 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. 02 production assessed by luminal chemiluminescence and immunostaining with dihydroethidium increased in the lungs of mice exposed to hypoxia. Nitrotyrosine, 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 NOS activity, whereas normoxic controls harbored both dimeric and monomeric forms and higher activity. Supplementation of the eNOS cofactor BH4 (1mg/g food) prevented eNOS uncoupling, and was accompanied by reduced 02 production, nitrotyrosine 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 NOS 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.

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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 myocytes and provide a good model system for the effects of AZT in vascular tissue. We 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 culture after 20 minutes of incubation. 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 after 120 and 120 minutes of incubation. As seen from these two time courses incubation of AZT is still being phosphorylated at a linear rate, and the AZT pool consists
almost entirely of AZT, with little or no AZTMP or AZTTP present. In order to see the effect of AZT on thymidine phosphorylation, flasks of HK2 cells were incubated for 10 minutes in media with 1 μM [3H]-thymidine and varying concentrations of AZT, ranging from 0 to 200 μM. From this data, the 50% inhibitory concentration (IC50) for AZT inhibition of thymidine phosphorylation was calculated to be 0.3 μM AZT. This demonstrates that AZT readily inhibits thymidine phosphorylation in HK2 cells, which may be inhibiting both thymidine kinase 1 and thymidine kinase 2 in these cells, similar to the AZT inhibition of thymidine kinase 2 observed in isolated rat heart and liver mitochondria. This work will provide the basis in HK2 cells for our proposed mechanism of AZT toxicity. Future trials in HK2 cells will look at the long term effect of AZT exposure on the TTP pool and correlate it to AZT-related mitochondrial DNA depletion.

Identification of PI16 as a New Candidate Gene in Heart Failure by a Genetic Yeast Screen for Secreted Proteins

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Remodeling of the mycardium is of central importance for the development of congestive heart failure. Experiments with conditioned media demonstrated communication between cardiomyocyte and fibroblasts via extracellular factors. The aim of our work is the isolation 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 screened yeast transformants we identified 54 non-redundant clones containing a signal sequence for secretion. Among them are well-known genes like the atrial natriuretic peptide, genes with so far unknown cardiac expression and genes with so far unknown function and expression. We then studied regulation of these genes during the development of heart failure. The mRNA-expression of protease inhibitor 16 (PI16) was strongly upregulated both in murine cardiac hypertrophy (aortic banding: +242 ± 62%, p < 0.01) and heart failure (beta-adrenergic receptor transgenic mice: +203 ± 56%, p < 0.01). We also found significant upregulation of PI16 mRNA in human heart failure (+250 ± 137%). PI16 is a putative 489 amino acid protein with so far unknown function. We generated a specific polyclonal antibody and found also at the protein level a strong and early upregulation of PI16 expression in heart failure. Serum stimulation of neonatal rat cardiomyocytes strongly induced PI16 protein expression. We further identified a splice variant of the murine and human PI16 lacking exons 5. PI16 is secreted into the culture medium after transfection of neonatal rat cardiomyocytes with a PI16-expressing adenovirus. Moreover, PI16 accumulates after secretion extracellularly in the heart and binds to the extracellular matrix. Taken together, a genetic yeast screen of a heart cDNA-library we identified 54 proteins putatively secreted from the heart. PI16, a newly identified secreted protein is strongly upregulated early in heart failure suggesting a possible involvement in cardiac remodeling.

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 Ca^2+ channel. Based on this, we hypothesized that calcineurin physically and functionally interacts with the L-type Ca^2+ channel. Methods and Results: Co-immunoprecipitations from extracts of rat heart and brain using antibodies against calcineurin and α1.2, the major, pore-forming subunit of the channel, revealed evidence of α1.2-calcineurin association. GST pulldowns provided evidence for direct interaction between calcineurin and two major regions of α1.2: the N-terminus and a 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 α1.2 is a potential substrate for calcineurin in vivo. To test functional significance, voltage-clamp recordings revealed significant up-regulation of I_{Ca,L} in cultured cardiomyocytes expressing constitutively active calcineurin. Conversely, inhibition of calcineurin with cyclosporine A (CsA) and tacrolimus - two structurally distinct calcineurin antagonists - decreased I_{Ca,L}. This inhibition occurred over a time course of several minutes and was partially reversible upon wash drug. CsA had no specific effects on I_{Ca,L}. To test for other mechanisms whereby calcineurin might up-regulate Ca^2+ channel function, we mapped 2kb of genomic sequence upstream of the α1.2 gene, identifying several putative NFAT, GATA4, and MEF2 binding sites. Studies performed in cells transiently transfected with an α1.2 promoter-luciferase reporter suggested that α1.2 transcription is regulated by calcineurin. Conclusions: These findings are consistent with a model wherein calcineurin regulates the L-Type Ca^2+ channel at two distinct levels: post-translational modulation involving direct binding to the channel, and transcriptional regulation involving NFAT-mediated activation of direct binding expression.
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 3ml muscle baths containing Krebs-Henseleit superfuse. All preparations were paced initially at 3Hz, and near isometric forces of contraction were measured throughout. Results: Transient, asynchronous mechanical events occurred in paced left atria >to 10\(\mu\)M 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 (>200\(\mu\)M) reversed SCA, while chloride channel blocker 300\(\mu\)M GDEE showed a smaller but significant effect. However, possible i.e. conditions that suppress SCA (SCA). Conclusion: SR calcium leak induces spontaneous mechanical activity in isolated rat atria. The present study demonstrates that SR calcium leak can be induced in intact heart muscle and can be used as a tool to investigate the role of SR calcium leak in cardiac physiology and disease.

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Prolongation of APD, both the transient outward K current (\(I_{\text{to}}\)) and the late outward K current (\(I_{\text{k}}\)) were significantly longer in fistula myocytes. Associated with the transient outward K current (\(I_{\text{to}}\)), both the transient outward K current (\(I_{\text{to}}\)) and the late outward K current (\(I_{\text{k}}\)) were significantly longer in fistula myocytes. Associated with the transient outward K current (\(I_{\text{to}}\)), both the transient outward K current (\(I_{\text{to}}\)) and the late outward K current (\(I_{\text{k}}\)) were significantly longer in fistula myocytes. Associated with the transient outward K current (\(I_{\text{to}}\)), both the transient outward K current (\(I_{\text{to}}\)) and the late outward K current (\(I_{\text{k}}\)) were significantly longer in fistula myocytes. Associated with the transient outward K current (\(I_{\text{to}}\)), both the transient outward K current (\(I_{\text{to}}\)) and the late outward K current (\(I_{\text{k}}\)) were significantly longer in fistula myocytes.

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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 and prolonged APD in volume overload induced heart failure, which may contribute to cardiac arrhythmias.

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Spinal Cord Stimulation Suppresses Bradycardia Responses and Atrial Tachyarrhythmias Induced by Medialial Neurodegeneration

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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 stellectomy, trains of 5 electrical stimulus were delivered during the atrial refractory period to right- or left-sided medullary nerves for up to 20 seconds before and after SCS (20 min). Recordings were obtained from 191 bilateral epicardial sites. Before SCS (11 animals), medullial nerve stimulation initiated bradycardia responses alone (12 nerve sites) or followed by tachyarrhythmia / 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 stellectomy, SCS no longer influenced neurally induced bradycardia and atrial tachyarrhythmias. These data indicate that SCS obviates the induction of atrial arrhythmias resulting from excessive activation of intrinsic cardiac neurons, doing so via their sympathetic neuronal inputs.

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Withdrawn

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Transcriptional Activation of Embryonic Stem Cell-Derived Endothelial Cells in Rat Stroke Model Promotes Functional Recovery

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Introduction: Stroke is a leading cause of death and a majority of stroke survivors have functional impairment. Preliminary observations suggest that neural stem cell transplantation may be a viable stroke cell therapy. However, as neuronal regeneration will require concomitant vascular regeneration, we studied the effects of transplantation of ESC-derived endothelial cells (ESC-ECs). Ultimately, we hope to elucidate how ESC-derived ECs respond to the ischemic brain, mobilize, integrate, and reconstitute the complex neurovascular architecture and interaction. Methods: We firstly developed a high throughput method to promote endothelial lineage, and to detect ESC-derived ECs. Mouse ESCs were genetically modified to stably express the EG specific promoter for VE-Cadherin driving a GFP reporter gene, and genes for bioluminescence and PET. Endothelial cell lineage was induced by sequential culture and purification steps of differentiating ESCs. Endothelial lineage was further confirmed by examining endothelial specific markers. Adult male Sprague-Dawley rats (n=20) were anesthetized and focal cerebral ischemia was induced using suture-induced middle cerebral artery occlusion (MCAO). Two hours after occlusion, ESC derived ECs were injected through common carotid artery. Through illumination imaging System. Coronal sections of the brain were made for immunochemistry. Results: 1, 3, 7, 14, 21, and 28 days after cell transplantation, we observed bioluminescence in the region of the lesion. Furthermore, we observed GFP positive cells in the luminal lining of some small vessels in the ischemic area by immunochemistry 7 days after cell transplantation. Mortality was reduced, and neurobehavioral testing in the survivors tended to be improved in transplanted animals. Conclusion: ESC derived endothelial cells were able to integrate into the vasculature of a region of cerebral ischemic injury, associated with an improvement in function and mortality. These preliminary observations provide a rationale for further examination of this approach.

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Catecholamines Inhibit Human Mesenchymal Stem Cell Migration through the \(\beta_1\)-Receptor Pathway

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Introduction: Human bone marrow mesenchymal stem cells (hMSCs) have been shown to acquire a cardiomyocyte phenotype when introduced directly into myocardium, and to migrate from the bone marrow into a myocardial infarction. Moreover, administration of hMSCs into patients with myocardial infarction appears to improve their prognosis. However, it remains unknown how the local environment within a myocardial infarction affects their migration. Prolonged periods of ischemia result in markedly elevated extra-cellular concentrations of norepinephrine, often exceeding normal levels by >1000-fold in the \(\mu\)M range. Goal: To assess, in-vitro, how excessive levels of catecholamines may influence the migration of hMSCs. Methods: Primary and immortalized hMSCs were plated, grown to 80–90% confluence, and scraped to create a migration region. They were incubated in growth medium (DMEM + 10% FBS) with a variety of \(\alpha\) and \(\beta\) adrenergic agonists and antagonists, and their migration was measured by photomicroscopy at 48–72 hours. Expression of \(\beta_1\)-adrenergic receptor mRNA was examined by RT-PCR. Results: At physiologic and supra-physiologic concentrations norepinephrine showed statistically significant reductions in migration, with a clear dose response. The \(\beta_1\)-agonist isoproterenol also showed a dose response inhibition of migration. In contrast, the \(\alpha_1\) agonist phenylephrine, showed no significant effect on migration. The adrenergic antagonist CGP-20712A (\(\beta_1\)-specific) showed a significant inhibition of the effects of isoproterenol, while antagonists IC50=11551 (\(\beta_2\)) and SR-59230A (\(\beta_3\)) showed no significant inhibitory effect. RT-PCR demonstrated expression of all 3 types of beta-receptors in hMSCs. Conclusions: Human mesenchymal stem cell migration is inhibited by catecholamines mainly through a \(\beta_1\) adrenergic pathway, and is not significantly affected by either \(\beta_2\) or \(\beta_3\) adrenergic signaling. Although this effect is small at physiologic concentrations of norepinephrine, at the supra-physiologic concentrations that have been reported in
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 beta-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) using MRI.MRI were 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 and compared between treated and untreated dogs. Results: Infarct size was similar between treated and untreated dogs at 72h (19.3±9% vs. 20.0±5%) and 8 weeks post-MI (8.1±2% vs. 8.9±2%). At 72h post-MI, the distribution of MSC injections was 52% (14/27) in MI, 30% (8/27) in P, and 19% (5/27) in N regions. Labeled MSC clusters persisted in MI regions throughout the study (14/14), while a disappearance of clusters was seen in P and N regions with 50% (4/8) and 20% (1/5) of original injections remaining at 8 weeks. SSR improved in N, P, and MI regions in treated animals over time from 72h to 8 weeks (p<0.01 in all regions) and significantly improved in N regions (p<0.01) compared to untreated animals. No cardiomyocyte regeneration was indicated by histology in P and MI myocardium. 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 based 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: 48 adult male mice underwent surgery to induce MI by a mid-LAD ligation. Echocardiography was performed under anesthesia one month post MI by 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-hemiellipsoid 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 interpretable. 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 = −0.86, p<0.0005), ESV (15.3%) (r = −0.41, 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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