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 M-0 to starting on day 2 is beneficial for improvement of mortality resulting from acute viral myocarditis in mice with reduced expression of cardiac TNF. These findings suggest crucial implication for starting time of herbal medicine in a murine model of viral myocarditis.

**MicroRNA Function During Cardiac Hypertrophy**

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Diverse forms of cardiac injury evoke a hypertrophic response characterized by an increase in cardiomyocyte cell volume, enhanced protein synthesis, assembly of sarcomeres and activation of a fetal cardiac gene program. Pathological hypertrophy is a major predictor of heart failure and cardiac sudden death. MicroRNAs, which target specific mRNA transcript or 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, Vinod 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 Vinod Labhasetwar Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198 Various cytokines and growth factors inducted gene and 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 3.5 ± 2.0 vs. 1.6 ± 0.2, n = 5, p < 0.001), resulting in significant reduction in lumen area (0.16 ± 0.01 vs. 0.3 ± 0.02, n = 5, p < 0.001). The mechanism of excessive neointima formation in the EPO treated group was found to be due to excessive neangiogenic response in the injured artery as represented by CD31-positive structures (103 ± 10/mm² versus 35 ± 5.2/mm² saline control, p < 0.0001). Nonetheless, our results explained a common occurrence of vascular access stenosis in patients on EPO treatment during hemodialysis and suggest the cautious use of EPO in patients who are at risk of vascular injury.

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

Yochai Bimbbaum, Yumei Ye, Yu Lin, Sheldon Freeberg, Juan Martinez, Jose R Perez-Polo; Barry F Uretsky; Univ of Texas Med Branch, Galveston, TX

**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-/- groups is related to S-nitrosation. **Methods:** WT, eNOS-/-, and iNOS-/- mice received AT 10 mg/kg/d (AT+) or water alone (AT-) for 3 days. Hearts were harvested and subjected to ELISA and immunoblotting. S-nitrosylation of COX2 was assessed by the “Biotin Switch” assay. To verify that the immuno-precipitates contained COX2, membranes were stripped and blotted with anti-COX2 antibodies. **Results:** COX2 expression in the AT- groups was very low. AT increased COX2 expression in WT (4.27±0.104%) and eNOS-/- (4.35±0.101%) but not iNOS-/- mice (101±3%). However, myocardial 6-keto-PGF1α levels (81.4±0.2 vs. 15.7±0.1 ng/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 ng/ml) or iNOS-/- (15.9±0.2 vs. 15.3±0.1 ng/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-nitrosate the AT-induced upregulated COX2.

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

Yochai Bimbbaum, Yumei Ye, Yu Lin, Sheldon Freeberg, Shawn P Nishi, Juan D Martinez, Ming-He Huang, Barry F Uretsky, Jose R Perez-Polo; Univ of Texas Med Branch, Galveston, TX

**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(E)-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/d; 3) ATV 10 mg/kg/d; or 4) PIO + ATV. Experiment 2: Rats received: 1) water; 2) PIO + ATV; 3) PIO + ATV and valdecoxib, a selective COX2 inhibitor; 4) PIO + ATV and zileuton, a selective 5-lipoxygenase inhibitor; or 5) zileuton alone. There were 4 rats in each group. Heart were harvested and analyzed for myocardial lipoxin A4 and 15ELX levels, and COX2 and 5-lipoxygenase protein expression. AT (1.1±0.02 mg/g; p<0.001 vs. sham) and PIO (0.98±0.02 mg/g; p<0.001 vs. sham) significantly increased myocardial 15ELX levels compared to the sham-treated group (0.51±0.02 mg/g). Myocardial 15ELX were significantly higher in the PIO + ATV group (1.29±0.02 mg/g; p<0.0001 vs. each other group). Both valdecoxib and zileuton abrogated the PIO + ATV increase in 15ELX, whereas zileuton alone had no effect. PIO, ATV and their combination resulted in a small increase in myocardial lipoxin A4 levels, which was not statistically significant. ATV alone, or in combination with PIO markedly augmented COX2 expression. The effect of PIO on COX2 expression was smaller. Myocardial expression of 5-lipoxygenase was not altered by PIO, ATV and their combination. **Conclusions:** Both PIO and ATV increases myocardial levels of 15ELX, an arachidonic acid product with anti-inflammatory properties. This finding may explain the anti-inflammatory properties of both PIO and ATV.
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-1βeta. 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 human failing myocardium via qRT-PCR analysis. ICE expression was significantly increased in NHR (4.3-fold) and increased UVPDqET (176 ± 3.33%). The increased expression in human heart failure is consistent with the findings in the mouse model. Further, the expression of ICE was analyzed in non-failing and failing human myocardium. ICE expression was significantly increased in failing myocardium compared to non-failing myocardium. The increased expression of ICE in failing myocardium suggests a functional role for ICE in cardiomyocyte apoptosis. Our findings indicate a functional role for ICE in protecting from cardiomyocyte apoptosis (3-fold reduction, ICE-KO vs. WT, p<0.05). Importantly, cardiomyocyte hypertrophy is not paralleled by an increased ventricular weight. ICE-TG hearts ultimately displayed left ventricular dilatation and wall thinning. Additionally, these mice exhibit a impaired cardiac function as seen in decreased dp/dt(max)valves (n=9/9; saline vs. 1300 (TG) vs. 1020 mmHg/s) and increased LVEDP-values (176 ± 3.33%). The alterations are not due to increased IL-1βeta formation. Adenoviral expression of ICE in primary cardiomyocytes potently induced both activation of caspase-3 (Immunoblot, +99%; p<0.001) and -9 and cardiomyocyte apoptosis (TUNEL, +40% vs. 2% control, p<0.05). To test the role of endogenous ICE for cardiomyocyte apoptosis we employed ischemia-reperfusion as a strong apoptotic stimulus in ICE-deficient mice. Depletion of endogenous ICE significantly reduced caspase activity, as assessed by fluorescence microscopy and Western blot analysis. These results suggest that ICE is a key mediator of cardiomyocyte apoptosis in response to ischemia-reperfusion-induced apoptosis. These results indicate that ICE is a key mediator of cardiomyocyte apoptosis in response to ischemia-reperfusion-induced apoptosis.

Background: We have shown that atrial natriuretic peptide (ANP) modulates the development of pressure overload-induced cardiac hypertrophy; and ANP, via the cGMP-protein kinase G (PKG) signaling, has direct anti-fibrogenic actions on heart by modulating expression of extracellular matrix (ECM) molecules in cardiac fibroblasts (CFs). Transforming growth factor beta (TGF-β) signaling regulates ECM gene expression and fibrosis in heart under stress conditions. Methods: To test the specific hypothesis that activation of ANP-cGMP-PKG signaling interrupts TGF-β1 signaling by inhibiting TGF-β1-induced phospho-Smad3 (pSmad3) nuclear translocation and myofibroblast (MF) transformation in CFs, quiescent mouse CFs were pre-treated with ANP (1 µM) or 8-but-cGMP (a cGMP analog, 1 mM) for 30 min, and then exposed to TGF-β1 (1 ng/ml) for 30 min (for Western blot and nuclear translocation) or 24 hrs (for MF transformation). pSmad3 protein levels were assessed by Western analysis. CFs were stained with anti-pSmad3 antibody to assess pSmad3 nuclear translocation by confocal microscopy. Numbers of MFs were assessed using α-smooth muscle actin (α-SMA) staining. Separate groups of CFs were pretreated with KT5823 (a PKG inhibitor, 1 µM) 30 min before exposure to ANP or cGMP. Results: ANP and cGMP inhibited TGF-β1-induced nuclear translocation of pSmad3, but did not inhibit TGF-β1-induced phosphorylation of Smad3. Nearly all cells in TGF-β1-treated CFs were α-SMA-positive and contained organized α-SMA filaments, indicating complete transformation of CFs to MFs. Pretreatment with cGMP completely inhibited TGF-β1-induced MF transformation (21% of total cells), but did not change basal (23% of total cells) MF numbers. KT5823 blocked the effects of ANP and cGMP. Conclusion: These results delineate a specific molecular mechanism by which activation of ANP-cGMP-PKG signaling pathway has direct anti-fibrogenic actions on heart by inhibiting TGF-β1-induced pSmad3 nuclear translocation, transformation and ECM expression of CFs. We hypothesize that interaction between the ANP and TGF-β1 signaling pathways at this level may play an important role in the pathogenesis of cardiac fibrosis and remodeling under stress conditions.

Cardiac Myosin Activator CK-1316719 Increases Myocyte Contractility and Myosin ATPase Activation in a Model of Heart Failure

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Improving cardiac contractility by directly activating cardiac myosin may be a preferred treatment for heart failure (HF) over the current inotropic therapies that increase intracellular calcium or act via second messenger activation. Previously we reported that small molecule myosin activators increase cardiac myocyte contractility without increasing intracellular calcium or inhibiting phosphodiesterase in non-failing cells. We now report cellular and biochemical responses to the myosin activator CK-1316719 in a mouse model of HF. Heart failure was confirmed in vivo by significant decreases in fractional shortening (M-mode echocardiography) in 12-week post myocardial infarction (MI) Sprague Dawley rats compared to sham animals. Myocytes were isolated from the left ventricle and septum for cardiac contractility, myofibril ATPase, and mRNA analysis. CK-1316719 (0.4 –5 uM) treatment increased cellular contractility in non-failing cells. We now report cellular and biochemical responses to the myosin activator CK-1316719 in a mouse model of HF. Heart failure was confirmed in vivo by significant decreases in fractional shortening (M-mode echocardiography) in 12-week post myocardial infarction (MI) Sprague Dawley rats compared to sham animals. Myocytes were isolated from the left ventricle and septum for cardiac contractility, myofibril ATPase, and mRNA analysis. CK-1316719 (0.4 –5 uM) treatment increased cellular contractility in non-failing cells.