**POSTER SESSION**

**1087 Matrix Metalloproteinases, Fibrosis, and Diastolic Function in Heart Failure**

Monday, March 08, 2004, Noon-2:00 p.m.
Morial Convention Center, Hall G
Presentation Hour: 1:00 p.m.-2:00 p.m.

**1087-105** Plasma Metalloproteinase Levels Are Correlated With Natriuretic Peptide and Endothelin-1 Levels in Patients With Heart Failure

Raymond T. Yen, Andrew T. Yan, Himai R. Gurusinghe, Peter P. Liu, Francis G. Spinale, Robert S. McKelvie, McMaster University, Hamilton, ON, Canada, University of Toronto, ON, Canada

**Background:** Several neurohormones can activate matrix metalloproteinases (MMPs) which regulate extracellular matrix turnover and left ventricular (LV) geometry in the failing myocardium. However, the relationships between the various neurohormonal and MMP systems in heart failure (HF) have not been elucidated.

**Methods:** Patients with symptomatic HF and LV ejection fraction <0.40 were randomized in the RESOLVD trial to various combination regimens of candesartan, enalapril, and metoprolol. In this study, plasma MMP-2, MMP-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), angiotensin II, aldosterone, and endothelin-1 were measured by ELISA in 184 patients at baseline. Epinephrine and norepinephrine were measured by HPLC.

**Results:** The relationships between plasma MMP and neurohormone levels are tabulated below.

<table>
<thead>
<tr>
<th>Kendall’s tau correlation</th>
<th>MMP-2</th>
<th>MMP-9</th>
<th>TIMP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP</td>
<td>0.26</td>
<td>0.06</td>
<td>0.21</td>
</tr>
<tr>
<td>BNP</td>
<td>0.21</td>
<td>0.03</td>
<td>0.14</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>-0.084</td>
<td>0.067</td>
<td>0.079</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>0.005</td>
<td>0.096</td>
<td>-0.061</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.067</td>
<td>0.025</td>
<td>0.06</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>0.041</td>
<td>-0.006</td>
<td>0.047</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>0.11</td>
<td>0.010</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Conclusion:** Increased plasma MMP-2 and TIMP-1 levels were related to elevated levels of ANP, BNP and endothelin-1. In contrast, the renin-angiotensin-aldosterone and the sympathetic nervous systems, both of which have been historically associated with promoting fibrosis, did not correlate with MMP levels. Therefore, MMP and TIMP levels appear to be differentially regulated by certain bioactive signals, rather than a non-specific global induction in HF. These new findings suggest that these proteolytic pathways can be modulated through specific pharmacological targets.

**1087-106** Pioglitazone Modulates Collagen Synthesis in Fibroblasts in Response to Angiotensin II

Kui Chen, Dayuan Li, Xing Jiang Zhang, Jawahar L. Mehta, University of Arkansas for Medical Sciences, Little Rock, AR, Central Arkansas Veterans Healthcare System, Little Rock, AR

**Background and Objectives:** Angiotensin II (Ang II) and free radicals are potent mediators of ischemic cardiac dysfunction. Fibroblast growth and activity (collagen formation) characterize this process in response to Ang II and free radicals. Recent studies suggest that PPAR-γ ligands have the potential to modulate the process of acute ischemic injury and its long-term consequences. This study examined the modulation of fibroblast activation in response to Ang II by the PPAR-γ ligand pioglitazone.

**Methods and Results:** Cultured rat cardiac fibroblasts were treated with Ang II (10^{-8} to 10^{-6} M) before exposure to Ang II (10^{-7} M). Treatment with Ang II resulted in increased collagen-1 and reduced MMP-1 protein expression in cardiac fibroblasts (both P<0.01). Ang II treatment also reduced protein kinase B (PKB/Akt) expression. Pretreatment of cells with pioglitazone completely blocked the decreased PKB/Akt expression in Ang II-treated cells, and simultaneously inhibited the effects of Ang II on collagen and MMP expression (all P<0.01 vs. Ang II alone).

**Conclusion:** This study shows that Ang II enhances collagen and reduces MMP-1 protein expression in fibroblasts. Importantly, pioglitazone blocks these effects of Ang II, most likely by PKB/Akt activity in fibroblasts.

**1087-107** Synergistic Effects of the Addition of Candesartan to Enalapril on Myocardial Fibrosis and Left Ventricular Diastolic Function in Dogs With Heart Failure

Koaru Funahibi, Katsuyoshi Onishi, Koaru Dohi, Takafumi Koji, Masaki Tanabe, Tetsuya Kitamura, Masaaki Ito, Takeshi Nakano, Miyake University School of Medicine, Tsu, Japan

**Backgrounds:** Angiotensin II (Ang II) type 1 receptor blocker, candesartan reduced relevant cardiovascular events when added to ACE inhibitors in patients with congestive heart failure (CHF). Candesartan prevents myocardial fibrosis due to the effective inhibition of Ang II in animals with non-ACE pathway, while ACE inhibitor acts on myocardial fibrosis partly through the bradykinin-mediated mechanism as well as the inhibition of Ang II. Therefore, we hypothesized that the addition of candesartan to enalapril synergistically prevented myocardial fibrosis and improved left ventricular (LV) diastolic function to the greater extent than each drug alone in CHF.

**Methods:** Twenty-eight dogs were assigned to the following treatment protocols on the 8th day of a 4-week rapid pacing: (A) rapid ventricular pacing (240 bpm, n=7), (B) enalapril (1.9 mg/kg/day) and pacing (n=7), (C) candesartan (1.5 mg/kg/day) and pacing (n=7), (D) combined enalapril (0.95 mg/kg/day) and candesartan (0.75 mg/kg/day) and pacing (n=7).

**Results:** Although there was no difference in myocardial Ang II levels among all groups, concomitant use of enalapril and candesartan significantly decreased LV end-diastolic pressure and LV stiffness estimated by LV pressure-volume plane to the greater extent than each drug alone. Furthermore, it suppressed the expression of the collagen-type 1 and 3 mRNA (p<0.05) and decreased the levels of LV collagen volume fraction evaluated with picrosirius red stain (A 6.8±1, B 6.3±1, C 3.6±1, D 2.5±1, %, respectively, p<0.05 vs. A, tp<0.05 vs. B, tp<0.05 vs. C). Conclusion: Concomitant use of enalapril and candesartan synergistically prevented LV fibrosis and improved LV diastolic function during the development of CHF. Thus, the addition of candesartan to ACE inhibitor may become an important strategy for the prevention of cardiac remodeling.

**1087-108** Transforming Growth Factor Beta and Factor XII Are Constituents of the Cardiacoid Heart Endocardial Layer: A Study by Confocal Laser Microscopy

Bernhard Maisch, Dorgit Diepho, Reinhard Funck, Andreas Wilke, Rudi Arnold, Philipps-University, Marburg, Germany

The detailed composition of the cardiacoid right ventricular endocardial layer and the pathogenesis are still unresolved. One common explanation is that fibrosis occurs as a consequence to microlesions induced by high levels of circulating serotonin and bradykinin.

**Methods:** The right ventricular endomycardial biopsies of 7 patients (5 female, mean age 43.9 ± 11.4 y) with established cardiacoid syndrome and right ventricular disease
Enhanced Instability of Membranous Type 1 Matrix Metalloproteinase in Response to Mechanical Loading in Left Ventricular Myocardium

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Background: The extracellular matrix (ECM) is essential for maintaining the structural integrity of the heart. Among matrix metalloproteinases (MMPs), membranous type 1 MMP (MT1-MMP) is recognized to be a key enzyme by degrading ECM proteins directly as well as by activating the other MMPs, including MMP-2. However, it remains to be elucidated if mechanical loading and unloading influence the process of MT1-MMP activity. We therefore investigated whether mechanical load is responsible for MT1-MMP activity and consequently affects the induction of MMP-2 activity in ventricular myocardium.

Methods and Results: Unloading (UL, n=5) and loading (LO, n=5) of the left ventricle in dogs were achieved by constriction of the inferior vena cava and by rapid ventricular pacing (180 beats/min) for 10 days, respectively. Although the expression of the active form (37-40kDa) of MT1-MMP in UL and LO did not differ from control (n=6), the inactive form of MT1-MMP (43-43kDa) significantly (p<0.01) increased by 158% in LO, and significantly (p<0.05) decreased by 55% in UL, assessed by Western blotting. In addition, tissue inhibitor of MMP (TIMP)-2 significantly (p<0.01) decreased by 56% with the reduction of zymographic MMP-2 activity in LO.

Conclusion: In summary, mechanical loading initiates the autocalcification of active form MT1-MMP into inactive form concomitant with TIMP-2 reduction, resulting in decreasing MMP-2 activity. These in vivo findings suggest the ECM degradation pathway mediated by MT1-MMP down-regulates in response to mechanical loading to counteract against the overload. Thus, we speculate the important compensatory biological system of MMPs/TIMPs for maintaining the adequate ECM circumstance in the development of heart failure.

Assessment of Ventricular Untwisting Recoil Velocity by Doppler Tissue Echocardiography: An Index of Relaxation Time Constant

Yuichi Notomi, Takahiro Shiota, Zoran B. Popovic, Hirotsugu Yamada, Maureen G. Martin-Miklowitz, Dimitri G. Deseran, Don W. Wallack, Neil L. Greenberg, Mario J. Garcia, James D. Thomas, Cleveland Clinic Foundation, Cleveland, OH

Background: The rate of recoil of left ventricular (LV) torsion that occurs during isovolumetric relaxation, when determined by tagged magnetic resonance imaging (MRI), is a strong predictor of the relaxation time constant (τ). Doppler tissue echocardiography (DTE) can detect myocardial velocity directly with higher spatio-temporal resolution than MRI. We assessed the relationship between τ and LV untwisting velocity (LV/U) measured by DTE.

Methods and Results: We obtained micrometer-recorded LV pressures and DTE data in 4 anesthetized closed-chest dogs at baseline, during right and left ventricular pacing, and during dobutamine and esmolol infusion (total 21 stages). τ was calculated by the equation: LVP = p(t) - p(t)<sup>0</sup> - P<sub>b</sub> - LV/U (rad/s) was defined as the difference between rotational velocities at the basal and apical short-axis levels of the LV. The rotational velocity was calculated from the septal and lateral regional velocity obtained from the LV short-axis and corrected by the instantaneous LV radius. The LV/U showed a strong nonlinear relation with τ (r = 0.80, p < 0.001, see figure). LV/U showed moderate correlation with minimum dP/dt (r = 0.41, p = 0.05 by repeated-measures linear regression).

Conclusions: Ventricular untwisting recoil rate, as determined by DTE using our novel method, may facilitate measurement of LV pressure decay as an index of relaxation. This novel method may provide an estimation of ventricular relaxation in individual patients, allowing serial noninvasive evaluations.

Improved Calcium Homeostasis and Antiarrhythmic Effects of a Novel Chimeric Molecule That Inhibits Both Type III Phosphodiesterase and L-Type Calcium Channel in Failing Hearts

Raza Mahaji, Katyatoun Derakhshan, Gregory Hamilton, Marc-Antoine Gillis, Daniel Bednarik, Peter Suzdak, Stanley Nattel, Artesian Therapeutics, Gaithersburg, MD, Montreal Heart Institute, Montreal, PQ, Canada

Background: Congestive heart failure (CHF) is becoming an increasing contributor to mortality rate as a function of the aging population. Because of their ability to improve cardiac performance and symptoms of decompensation, phosphodiesterase inhibitors (PDEIs) have been used in the treatment of patients with severe CHF. However, several trials have shown that PDEIs result in a >25% increase in mortality rate, apparently due to arrhythmias. We have developed a novel dual-pharmacophore compound (ATI21107) designed to simultaneously inhibit the cardiac phosphodiesterase PDE3 and the L-type calcium channel (LCC). We hypothesized that ATI21107 would preserve the beneficial effects of a pure PDEI, while minimizing deleterious responses through antagonism of LCC.

Methods: Hemodynamic, echocardiographic, and electrocardiogram data were measured in anesthetized dogs with tachycardia-induced (220-240 bpm x 5 weeks) CHF. Effects of graded intravenous doses of ATI21107, and a pure PDEI, milrinone (Mi), on hemodynamic and arrhythmic indices were compared to those of vehicle control.

Results: Both ATI21107 and Mi resulted in a comparable decline in mean arterial pressure and left ventricular end-diastolic pressure, while heart rate increased <5% in both groups at all doses. However, Mi infusion resulted in non-sustained ventricular tachycardia (frequency of 173±12 bpm, meansSEM) at all doses, while none were observed with ATI21107. ATI21107 produced a dose-dependent increase in the rate of ventricular relaxation (<p<<sub>b</sub><sub>LV</sub>), with a maximal increase of 120±7% of control, 87% of the maximal effect of Mi (138±11%). ATI21107 also produced a dose-dependent increase in <p<sub>b</sub><sub>LV</sub> ejection fraction, and fractional area shortening, with the maximal increase in contractility at the highest dose being only 55–70% of the maximal effect of Mi.

Conclusions: ATI21107 improves hemodynamic indices in dogs with CHF, and has selective antiarrhythmic effects. This novel pattern of hemodynamic action, coupled with a reduced arrhythmogenic profile, may make ATI21107 a promising agent for CHF therapy, particularly in patients with a significant component of diastolic dysfunction.

Poster Session 1088

Heart Failure: Treatment

Monday, March 08, 2004, Noon-2:00 p.m.
Morial Convention Center, Hall G
Presentation Hour: 1:00 p.m.-2:00 p.m.

Partial Fatty Acid Inhibition by Trimetazidine Improves Left Ventricular Function in Patients With Heart Failure of Different Etiologies

Gabriele Fragasso, Attil Pallossi, Carmen Silipigni, PierMarco Piatti, Lucilla Monti, Emanuela Setola, Giorgio Bassanelli, Chiara Montano, Alberto Margonato, Istituto Scientifico/Universita' San Raffaele, Milano, Italy

We assessed whether the addition of the partial fatty acid inhibitor trimetazidine (TMZ) to standard conventional therapy (CT) in patients (pts) with heart failure, can effectively improve symptoms, resting left ventricular function and exercise tolerance. Forty-one pts (37 males, age 64±4 yrs) were randomly allocated in an open label fashion to either CT (21 pts) or conventional therapy plus TMZ (20 mg tid) (20 pts). All pts underwent 2D-echocardiography and exercise testing before and at follow up (11±3 months). NYHA functional class, ejection fraction (EF), exercise time (ETsec) and metabolic units during exercise (METS-3.5 ml O2kcal min<sup>-1</sup>) were evaluated. Physicians performing functional tests were blind to patients' treatment arm. At baseline NYHA class, EF (37±8 vs 38±6), exercise time (411±129 vs 397±143) and METS (7.2±3.1 vs 7.8±2.5) were not different in the TMZ and CT groups, respectively. At follow-up, in the TMZ group 11 pts improved NYHA class and none worsened, while in CT NYHA class improved only in 2 pts and worsened in other 2. EF significantly increased in TMZ pts compared to CT (43±3 vs 35±8, p=0.008). Finally, although not significantly, exercise time (414±166 vs 399±137, p=0.25) and METS (8.5±2.7 vs 7.7±1.7, p=0.26) tended to be higher in TMZ pts. In conclusion, long term TMZ, by shifting the energy substrate preference away from fatty acid metabolism and toward glucose metabolism, improves left ventricular function and symptoms in pts with heart failure, regardless of its etiology. The observed TMZ benefit contrasts with the natural history of the disease, as confirmed by the mild but consistent decrease of EF in pts when on CT only. Whether these benefits would translate into improved survival should be ascertained by a multicenter trial.