Selective Activation of Fibrogenetic Growth Factors Addresses the Pattern of Hypertrophy in Human Aortic Valve Disease

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Background: Myocardial fibrosis develops only in some models of hemodynamic overload suggesting the involvement of non-hemodynamic trophic stimuli in the regulation of connective tissue growth. The aim of the present study was to investigate cardiac expression of fibrogenetic growth factors (GFs) in patients with unremissant, severe myocardial hypertrophy undergoing aortic valve replacement for pure form of aortic stenosis (n=16) or regurgitation (n=14).

Methods: Cardiac platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) formation was evaluated by measuring gene expression in ventricular biopsies collected at cardiac surgery (RT-PCR) and peptide release in coronary circulation. The control group was made of 5 tentatively, excluded from organ donation for non-cardiac reasons and 11 subjects with atypical chest pain undergoing diagnostic cardiac catheterization.

Results: In control subjects and in patients with aortic regurgitation PDGF and bFGF cardiac gene expression was untestable and peptide coronary release was negligible. Conversely, in patients with aortic stenosis myocardial expression of PDGF-A and -B chains and for bFGF were all significantly increased as well as the bFGF release in the coronary circulation (p=0.01 vs control and vs patients with aortic regurgitation for all). In situ hybridization localized growth factor mRNAs in interstitial cells. At multivariate stepwise regression analysis the relative wall thickness was selected as the most predictive independent variable for PDGF (r=0.72, p<0.01) and -8 chains and for bFGF (r=0.62, p<0.01) peptide cardiac release with no significant relationship with indexes of cardiac function or afterload mismatch.

Conclusion: The present results indicate that 1) the gene expression and cardiac formation of fibrogenetic GFs are selectively activated only in aortic stenosis with 2) gene expression mainly sustained by interstitial cells and 3) stepwise regression analysis revealing the significant association between GFs cardiac formation and the pattern of hypertrophy.

1059-24 Patients With Degenerative Aortic Valve Stenoses Have Increased Thermal Heterogeneity in the Stenotic Valve

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Background: Inflammation may be involved in the pathogenesis of aortic valve stenosis (AVS). Aortitis may lead to local heat release. The aim of our study is to investigate whether thermal heterogeneity is present in patients (pts) with and without (AIS) by the first human application of an aortic valve thermography catheter.

Methods: We enrolled 10 pts with AVS undergoing diagnostic catheterization (mean age: 64.6 ± 8.1 yrs) and 8 pts with ASIS (mean age 73.2 ± 11.9 yrs). For the first time we used an 8F aortic valve thermography catheter for the evaluation of mean and maximal pressure gradient. An echocardiography was performed, for the estimation of mean and maximal pressure gradient. Temperature measurement of the AV and the AW was successful and uncomplicated. TD was increased in pts with AVS compared to the subjects without AVS (p<0.05).

Conclusions: The first human application of an aortic valve thermography catheter was successful and uncomplicated. The increased thermal heterogeneity is significantly higher in patients with AVS compared to ASIS.

1059-25 Calcified Rheumatic Heart Valves Are Associated With Osteopontin Bone Matrix Expression


Introduction: Rheumatic Heart Disease is the most common cause of valvular heart disease in developing countries. Despite the high prevalence, the cellular mechanisms are not well-defined. We hypothesized that the mechanism for rheumatic valve calcification is similar to skeletal bone formation and that this process is mediated by synthesis of bone matrix proteins. In this hypothesis, we examined human calcified rheumatic heart valves replaced at surgery and normal human valves removed at autopsy. We examined light microscopic cellular changes and immunoreactivity for bone matrix proteins in paired controls. To test this hypothesis, we examined human calcified rheumatic heart valves replaced at surgery and normal human valves removed at autopsy.

Methods: Hematoxylin and Eosin (H&E), and Masson trichrome (MT) stains were performed. Immunohistochemistry was used to localize osteopontin protein, proliferating cell nuclear antigen (PCNA) and alpha actin (α). Quantification of PCNA was done using a CAS 200 image analyzer (Baccus Labs, Inc; Lombard, IL) and expressed as the percent of nuclear area staining. Osteopontin immunostaining was evaluated from rheumatic heart valves (N=22), and normal valves (N=22). Results: H&E and MT staining confirmed the presence of dense mineralization in the calcified rheumatic heart valves, a finding that was absent in the normal valves. Immunohistochemistry localized osteopontin and alpha actin to areas of calcification in all of the cardiac valves. PCNA expression was positive in 5 of 22 rheumatic cardiac valves. The mean level of PCNA expression in rheumatic valves (N=5) compared to normal controls (N=5) was 1059-26 Interleukin 1 Beta Induces Matrix Metalloproteinase Expression in Human Aortic Valve Myofibroblasts In Vitro and Promotes Cell Proliferation

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Background: Recent studies suggest that calcific aortic stenosis, the most prevalent heart valve disease in the elderly, may be based on a chronic inflammatory process involving invasion and activation of leukocytes. We have shown recently that the pro-inflammatory cytokine tumor necrosis factor alpha is upregulated in stenotic aortic valves, and that it is associated with the remodeling of the extracellular matrix. The role of the pro-inflammatory cytokine interleukin 1 beta (IL-1β) on the pathogenesis of calcific aortic stenosis is unknown.

Methods: Human aortic valve myofibroblasts were isolated from explanted aortic valves and grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin, streptomycin and fungizone. After 24 hours of serum-free culture, subconfluent cells were incubated with IL-1β (10-150 ng/ml) for various durations. Western blotting for MMP-1 and gelatin zymography for MMP-2 and MMP-9 were performed with conditioned media. Cell proliferation with or without IL-1β at various concentrations was assessed by bromodeoxyuridine uptake assay.

Results: Interleukin 1 β (IL-1β) induced expression of matrix metalloproteinase (MMP-1) and -9 in differentiated monocytes in MOMP-1, MOMP-2, and MOMP-9 in conditioned cell culture media as compared to control. Cell proliferation was increased 3.6-fold as compared to control on incubation with IL-1β at a concentration of 0.1 ng/ml (p<0.05).

Conclusion: IL-1β induces matrix metalloproteinase expression in human aortic valve myofibroblasts in vitro and promotes cell proliferation. These results suggest that matrix remodeling in calcifying aortic valve may be actively regulated, involving an inflammatory process. This could be a potential therapeutic target for calcific aortic stenosis.
Inhibition of the c-Jun N-Terminal Kinase Pathway Minimizes Collagen Remodeling in Aortic Regurgitant Hearts

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Background: Fibrosis associated with aortic regurgitation (AR) shows abnormal fibronectin (FN) but normal collagen content. This pathologic fibrosis is important in genesis of heart failure (CHF) in AR. Understanding fibrosis generation may enhance specific therapy to retard CHF. We showed that increased FN expression by cardiac fibroblasts (CF) from rabbits with catheter-induced AR results from activation of the c-Jun N-terminal kinase (JNK) pathway, which also can upregulate collagen-specific metalloproteases (MMPs). To see if JNK activation of MMP is involved in minimizing collagen content in AR, we assessed MMP-2 activity with SP600125, a specific JNK inhibitor. Methods: NL-CF vs AR-CF (3 pairs) from rabbits were grown in triplicate with and without 20uM SP600125. Media were collected for 24 hr intervals over 5 days. Enzymes were separated by gelatin-containing zymograms, stained with coomassie blue, destained, and analyzed by videodensitometry. Band intensities were normalized to total cell protein (AR:NL=2.1:1. p<.03). When CF were grown with SP600125, MMP-2 activity was maximally downregulated by day 2 vs day 0 (NL, 1.0:0.4, p<.001; AR, 1.0:0.5, p<.001, Table). Conclusion: In AR myocardium, fibrotic myocardium featuring abnormal FN but normal collagen content results in part from AR-induced upregulation of MMP-2 by JNK stimulation. Inhibition of this reaction may help mitigate pathologic fibrosis and CHF.

Effect of Candesartan Cilexetil on Left Ventricular Remodeling in Mitral Regurgitation: A Randomized Clinical Trial

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Background: The effect of issue angiotensin blockade in Mitral Regurgitation (MR) due to intrinsic valve disease is disputed due to lack of randomized clinical trials. Of particular importance is the effect of these medications on left ventricular (LV) remodeling, ie., on LV end-diastolic and end-systolic volume index (EDVI, ESVI). Candesartan Cilexetil is an angiotensin receptor blocker with insurmountable receptor attachment allowing prolonged clinical efficacy.

Methods: In 102 patients (age 64±14 years, 36% female) with organic MR of at least moderate degree, in functional Class I or II, with normal renal function (creatinine 1.1±0.2 mg/dL), patients were randomized to treatment with placebo (n=5) or Candesartan Cilexetil 32 mg/daily (n=15) for 1 year. After one year the end-points of EDVI and ESVI were measured.

Results: At baseline, there was no difference between placebo and Candesartan cilexetil groups for age, sex, systolic (141±19 vs. 144±18 mmHg, P=0.69) and diastolic (74±10 vs. 77±14 mmHg, P=0.46) blood pressure; regurgitant volume measured by 2 methods (73±25 vs. 77±14 mL/beat, P=0.97). EDVI (103±21 vs. 104±19 mL/m2) and ESVI (26±9 vs. 25±10 mmHg) were measured.

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