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High prevalence of pharmacologically active autoantibodies in heart failure, with dual action on serotoninergic 5-HT4 and muscarinic M2 receptors

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Purpose: In adult in congestive heart failure the expression of 5-HT4 receptor is increased 4 folds. Therefore we looked for the presence of anti-5HT4 receptor autoantibodies (AAb) in sera of patients with heart failure.

Methods: Sera from 333 heart failure patients and 150 controls were screened against second extra cellular loop (SEC) of 5-HT4, β1, and 2 adrenoceptors, AT1 and M2 muscarinic receptor, using indirect and inhibition ELISA. The ability of AAbs to bind native corresponding receptors was explored in fluocytometry. AAbs' pharmacological activity was explored using chronothropy on neanatla rat cardiomyocytes and HL-1 cells. Five different truncated peptides from SEC of 5-HT4 were used for eptiope mapping.

Results: We could confirme specific immunoreactivity only for 5-HT4 and M2.We found 21 and 19% of sera positive for 5-HT4 and M2 receptors respectively with 90% of exact identity for both receptors. We found the same result in control population. Peptides derived from SEL of both receptors cross-inhibit sera interactions with 5-HT4 and M2 receptors. They recognised all one and same epitope. IgG fractions patients and controls had negative chronotropic effect. The specificity of the AAbs for 5-HT4 and M2 receptors were verified pharmacologically with ML10375 and atropine specific 5-HT4 antagonist and M2 agonist. Clinical studies revealed that the entire positive control individuals suffered from hypertension, so was the case of heart failure patients positive for the two receptors. There is a high prevalence of pharmacological active cross- reacting AAbs against SEC of 5-HT4 and M2 receptors in hypertensinve heart failure patients. These AAbs may play an important role in on set and pathophysiology of heart heart failure as they are found in hypertesiove patients without yet clinical manifestation of heart disease.

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TGF-beta1 dipendent acquisition of specific adherens junctions in mitral valve prolapse

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Purpose: Mitral valve prolapse (MVP) is the leading cause of mitral incompetence in Western Countries. The pathogenesis of the disease is still unknown, although the dysregulation of TGF-β pathway seems to play a role in familial MVP. TGF-β may induce the expression of stress fibers (α-smooth muscle actin, SMA) in fibroblasts and the acquisition of a myofibroblastic phenotype as well as a switch of the cadherin pattern at junctional level. There are no data on molecular composition of cell-cell junctions in valvular interstitial cells (VICs) from MVP. Aim of this study was to evaluate whether TGF-β pathway is activated in isolated MVP and to study the effect of this activation on differentiation of VICs in myofibroblasts and on the molecular pattern of their junctions.

Methods: Floppy mitral valves were obtained at surgery from patients (12 cases, 10 M and 2 F, mean age 55.5 $\pm$ 12.7 years) who underwent valve repair. Age and sex-matched control samples were obtained from Homograft Tissue Bank (5 cases, mean age 49 $\pm$ 9 years). Valve morphology was assessed on routinely stained histology sections. Antibodies recognizing active form of Smad2 (p-Smad2) in VICs were used. The following antibodies against junctional proteins were applied: N-cadherin, cadherin-11, VE-cadherin, α- and β-catenin, plakoglobin, protein p120. Ki67 was used to assess the proliferation.

Results: MVP leaflets exhibited alterations in architecture with fourfold increase in thickness (2,5 $\pm$ 0,8 vs 0,6 $\pm$ 0,3 mm, $p < 0.0001$) and increased cell density (110,9 $\pm$ 64,6 vs 51,8 $\pm$ 27,5 cells per high power field, $p = 0.04$) compared to the normal valves. A higher density of nuclear staining for p-Smad2 (38% vs 12%, $p < 0.05$) was also observed, indicating an increased activation of TGF-beta pathway.

The VICs in myxomatous leaflets showed a myofibroblast differentiation (expression of α-SMA) and AJs containing high levels of cadherin-11, either exclusively or in colocalization with N-cadherin, unlike VICs from controls that were α-SMA negative and exhibited predominant expression of N-cadherin at AJs level.

Conclusions: Our data support the hypothesis that an increased activation of the TGF-β pathway may contribute to the pathogenesis of sporadic MVP, either with promotion of myofibroblast differentiation of VICs and acquisition of a specific cadherin pattern in AJs, that confer higher resistence of cell-cell contacts in a stress environment. In its turn, myofibroblast contraction may activate latent TGF-beta from extracellular matrix.

Targeting of myofibroblast AJs by specific anti-cadherin peptides may be used to prevent or retard mitral myxoid degeneration.

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Comparison of myocardial structural and molecular changes in rat models of type 1 and type 2 diabetes mellitus

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Cardiovascular complications are one of the main causes of mortality in diabetes mellitus (DM). There is obvious evidence demonstrating that DM leads to structural and functional changes at the level of the myocardium. In this study, we investigated and compared myocardial structural and molecular changes in rat models of type-1 and type-2 DM.

Type-1 DM was induced in Sprague-Dawley rats by single injection of streptozotocin (60mg/kg, i.p.). Control rats receive only vehicle. Experiments were performed 8 wk after the confirmation of diabetes. For type-2 DM, Zucker-diabetic fatty (ZDF) and ZDF-lean rats were used and experiments were performed at the age of 30—32 wk. Heart samples were stained with hematoxylin-eosin and immunohistochemical analysis was performed for nitrotyrosine. TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) was used for detection of DNA-strand breaks. Different gene expression analysis was performed by quantitative real-time polymerase chain reaction.

Histopathological examination showed the characteristics of diabetic cardiomyopathy. DNA-damage in the myocardium of diabetic group was more higher and the nitro-oxidative stress was significantly more pronounced in type-1 compared to type-2 DM. Relative mRNA-expression for alpha-myosin heavy chain (MHC) was decreased whereas beta-MHC and endothelin-1 mRNA-levels were significantly increased in both diabetic compared to corresponding control-groups. Transforming growth factor (TGF)-beta1 mRNA-level was significantly up-regulated only in type-2, whereas c-fos and c-jun only in type-1 DM. Moreover, endothelial NOS, collagen I and III were significantly down-regulated only in type-1 DM. These alterations were significantly more pronounced in type-1 DM.

Our results show that rat model of type-1 DM is associated with significantly higher structural and molecular changes compared with the type-2 DM.

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Incomplete Kawasaki disease as cause of unexpected death in 2 infants: immunophenotype characterization of coronary artery aneurysms

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Introduction: incomplete or atypical Kawasaki Disease (KD) is more common in children younger than one year. In these patients, the diagnosis can be difficult because KD may mimic common childhood illnesses, but the rate of coronary artery aneurysms is much higher if not treated and consequences of missed diagnoses might be serious morbidity or even death.

Purpose: aim of this study is the evaluation of the immunohistochemical profile observed in post-mortem coronary lesions of two infants deceased for KD.

Methods: we describe two patients (a 3-month-old female infant and a 6-month-old male child) with fatal incomplete KD; in the first case a 2D echocardiography showed giant aneurisms in both main coronary arteries; in the second one, diagnosis was done only after post-mortem examination. In both cases a complete autopsy was performed and pathologic specimens were processed for routine histology, histochemical and immunohistochemical stains. The following specific antisera were tested: anti-endothelial antigen CD31, anti-TNF-alpha, anti-Tissue Factor (TF), anti-Thrombopoietin receptor (r-TPO), anti-HLA-DR (Major Histocompatibility Complex, Class II antigen) and anti-monocyte/macrophage antigen CD68.

Results: histopathology showed similar coronary artery lesions in both cases, mainly consisting of aneurysms with diffuse macrophage infiltration and prominent neo-angiogenesis in the arterial wall. Coronary artery aneurysms, and particularly giant aneurysms showed strong immunoreactivity for TNF-alpha, TF and r-TPO, mainly localized in endothelial, smooth muscle cells and macrophages. TF expression was also detected in thrombus. TNF-alfa and HLA-DR expression appeared particularly high in the areas with dense macrophage infiltration. MAcrophage infiltration and HLA-DR immunoreactivity were also shown in the area of the atrioventricular node.

Conclusions : our findings suggest a pathogenetic association of macrophages infiltration, immunological activation (i.e., HLA-DR positivity), tumor necrosis factor and procoagulant factors with coronary artery aneurysms and thrombosis in incomplete KD.

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Time course and distribution of TUNEL nuclear positivity of cardiomyocytes in a chronic rat model of coronary occlusion with and without reperfusion

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Occlusion of a coronary artery either permanent or followed by reperfusion is known to cause irreversible myocellular death mainly by "oncotic" modality. DNA fragmentation by in situ terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) has been frequently detected at light microscopy (LM) in oncotic cells and believed to be an index of a caspase-mediated "apoptotic" type of cellular death, although typical apoptotic ultrastructural features have never been observed. The contribution of this DNA fragmentation- associated oncotic myocellular death to overall irreversible myocardial necrosis after ischemia and ischemia reperfusion is still questionable and its time course unknown.

Purpose: To assess the time course and distribution of TUNEL nuclear positivity of cardiomyocytes within the infarcted and ischemic-reperfused myocardium of rat heart over a 3 day time span.

Methods: Male rats underwent permanent (I, n = 12) or transient (I-R, 30min occlusion followed by reperfusion, n = 12) ligation of the left anterior descending coronary artery and hearts explanted at 1h (groups 1h I, 1h I-R), 1 day (groups 1d I, 1d I-R) and 3 days (groups 3d I and 3d I-R). Sham-operated animals served as controls (C, n = 6). Histology (H&E and Masson trichromic stain) and TUNEL assay were performed on 10 consecutive serial slices at two levels distal to the ligature for LM morphometric assessment of necrosis and nuclear TUNEL analysis.

Results: In groups 1h I and 1h I-R neither cellular necrosis or TUNEL positivity could be detected both in risk and control areas as well as in C hearts. In group 1d I contraction bands in the periphery and coagulation necrotic myocells in the central part of infarction showed 30 $\pm$ 5% and 45 $\pm$ 3% of TUNEL positive nuclei respectively. In group 1d I-R 50 $\pm$ 5% of contraction band necrotic cells had TUNEL positive nuclei. In groups 3d I and 3d I-R most of the still visible nuclei of necrotic myocytes were positive (94 $\pm$ 5 and 95 $\pm$ 4% respectively). TUNEL positivity was never observed in myocytes without LM evidence of cellular necrosis.

Conclusion: The present findings demonstrate that DNA fragmentation: a) invariably coexists with myocellular necrosis prior to complete nuclear demise over a 3 day time span, b) it has a comparable incidence in I and I-R groups, c) it persists at 3 days with an even higher incidence. The possibility that TUNEL positivity could identify a specific caspase-dependent subpopulation of irreversibly damaged cardiomyocytes within the context of ischemic and ischemic-reperfusion necrosis is suggested.