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Differential regulation of mitogen-activated protein kinase signaling pathways in human with different types of mitral valvular disease

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ABSTRACT

Background: Mitogen-activated protein kinases (MAPKs) are considered to play a prominent role in cardiac development, function, and pathogenesis. The different types of mitral valvular disease (MVD), including mitral regurgitation (MR) and mitral stenosis (MS), have different underlying pathophysiologic changes, but the precise intracellular signal transduction mechanisms are not clear. Thus, we investigated the differential regulation of MAPK signaling pathways in humans with different types of MVD.

Methods: Left atrial appendage tissue samples from 32 patients with MVD who were undergoing mitral valve replacement surgery were studied. Serum angiotensin II concentrations were measured using enzyme-linked immunosorbent assay. The expression of MAPK pathway-related genes and proteins was assessed using quantitative polymerase chain reaction, Western blot, and immunohistochemistry.

Results: Echocardiography showed that patients with MS had a greater left atrial pressure overload than those with MR. The relative amounts of angiotensin II, extracellular signal-regulated kinase 1, p38α, c-Jun N-terminal kinase 2, c-Fos, activating transcription factor 2, and c-Jun mRNA were significantly upregulated in those with MS compared with those with MR ($P < 0.05$). The serum angiotensin II concentrations were significantly increased in those with MS compared with those with MR ($P = 0.017$). Substantial changes in the phosphorylated forms of the MAPK proteins were detected. Phosphorylated extracellular signal-regulated kinase 1/2, and phosphorylated p38 were significantly increased in those with MS compared with those with MR ($P < 0.001$), and phosphorylated c-Jun N-terminal kinase in the MR group was significantly greater than that in the MS group ($P < 0.001$). Histologically, more serious myocardial cells losses, myolysis, and interstitial fibrosis were detected in the MS group.

Conclusions: The different types of MVD have different hemodynamic characteristics, and different MAPK pathways were activated in the MR and MS groups, which could lead to diverse left atrial histologic changes.

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1. Introduction

Mitral valvular disease (MVD) is one of the most common cardiac diseases in clinical practice. The different types of MVD, including mitral regurgitation (MR) and mitral stenosis (MS), have different potential hemodynamic mechanisms. For example, pressure and volume overload occur in MS, and relatively pure volume overload occurs with MR [1]. Continued hemodynamic overload would lead to changes of mechanical stress in left atrium, one of the factors to activate histologic changes. However, the precise molecular mechanisms of left atrial histologic changes caused by different hemodynamic overload in different types of MVD are still debatable and not well understood.

The renin-angiotensin system (RAS) is a hormonal system that plays an important role in the regulation of blood pressure and extracellular volume in the body. Activation of the RAS is involved in the pathogenesis of various cardiovascular diseases [2]. In the RAS, angiotensin-converting enzyme converts angiotensin I to angiotensin II (Ang II), the most biologically active peptide of the RAS, and affects several aspects of cardiac function, including contractility, cell metabolism, cellular growth, differentiation, apoptosis, and gene expression [3]. Ang II acts by way of the counter-regulatory Ang II receptors, Ang II type I and II receptors, which induce opposing responses [4]. Ang II type I receptor, activated by Ang II binding, initiates a cascade of phosphorylation processes that activate a family of mitogen-activated protein kinases (MAPKs) [5]. Intracellular MAPK signaling pathways probably play an important role in the pathogenesis of cardiovascular diseases [6], stimulate cardiomyocyte hypertrophy, apoptosis, fibroblast proliferation, accumulation of collagen, and fibrosis, contributing further to structural remodeling [7–9]. The major groups of MAPKs found in cardiac tissue include the extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-Jun NH2-terminal kinases/stress-activated protein kinases, p38 MAPKs, and ERK5 [8]. ERK1/2 are activated to varying extents by growth factors and mitogenic stimuli and play pivotal roles in cell growth and differentiation [10]. However, c-Jun NH2-terminal kinases/stress-activated protein kinases and p38 MAPK are activated in response to inflammatory cytokines, environment stresses (e.g., heat or osmotic shock, hypoxia, ionizing radiation, oxidant stress, and DNA damage), DNA and protein synthesis inhibition, and less often growth factors [10]. ERK1/2 stimulation initiates phosphorylation events, leading to activation of transcription factors such as Elk-1 and c-Fos. c-Jun N-terminal kinase (JNK) phosphorylates and activates transcription factors, such as c-Jun, activating transcription factor 2 (ATF-2), and Elk-1. In contrast, p38 MAPK phosphorylates ATF-2 and CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) [8,11].

Recently, activation of the local RAS and MAPK pathways in atrial tissue was found to be associated with atrial structural remodeling [12,13], including myocyte hypertrophy, apoptosis, myolysis, and interstitial fibrosis [7,14,15]. Therefore, we speculated that Ang II and MAPK activation might be involved in MVDs—promoting left atrial histologic changes. However, to date, little has been known about the differential regulation of MAPK signaling pathways in the different types of MVD. Therefore, the present study was designed to determine (1) the characteristics of left atrial hemodynamic overload in different types of MVD, (2) the changes in the left atrial histologic structure during the development of MR and MS, and (3) whether different MAPK signaling pathways are activated by hemodynamic overload, leading to left atrial histologic changes in different types of MVD.

2. Methods

2.1. Patients

The left atrial appendage was obtained from 32 consecutive patients with MVD (16 with MR and 16 with MS) scheduled for mitral valve replacement surgery. All patients were in New York Heart Association class II-III. The patients were excluded from the study according to previously described criteria [16]. Patients treated with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, who had other valvular moderate to severe stenosis or regurgitation, who had previously undergone cardiac surgery, or who had any other inappropriate medical conditions were excluded.

Before surgery, all patients underwent echocardiography to determine the left atrial dimension, right atrial dimension, left ventricular dimension, right ventricular dimension, left ventricular ejection fraction, and left ventricular fractional shortening. The peak pressure gradient, mean pressure gradient, pressure half time, velocity time integral (VTI), left atrial area (LAA), and left atrial volume (LAV) were used to evaluate the hydrodynamic characteristics of the left atrium.

All patients provided written informed consent. The local ethical committee (reference no. 2011-54) approved the present study, which conformed to the principles outlined in the Declaration of Helsinki.

2.2. Specimen preparation

Samples of venous blood were drawn by direct venepuncture from the radial vein before surgery. The 5-mL samples were transferred into vacutainers without any anticoagulant and immediately centrifuged at 5000 rpm for 15 min at 4°C. The supernatant plasma was stored at −20°C before analysis.

Samples of the left atrial appendage were obtained from each patient during mitral valve replacement surgery before extracorporeal circulation. One part was rapidly frozen in liquid nitrogen and stored at −196°C for further analysis, and the other underwent fixation in 10% neutral buffer formalin and was processed for paraffin histologic examination.

2.3. Serum Ang II concentration measurement

Serum Ang II concentrations were determined using a commercially available enzyme-linked immunosorbent assay kit for human Ang II (Cusabio, Wuhan, China). Absorbance at 450 nm was recorded, and the concentration was calculated from a standard curve generated for each experiment.