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Mitochondrial apoptotic pathway activation in the atria of heart failure patients due to mitral and tricuspid regurgitation

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ABSTRACT

Apoptosis occurs in atrial cardiomyocytes in mitral and tricuspid valve disease. The purpose of this study was to examine the respective roles of the mitochondrial and tumor necrosis factor-α receptor associated death domain (TRADD)-mediated death receptor pathways for apoptosis in the atrial cardiomyocytes of heart failure patients due to severe mitral and moderate-to-severe tricuspid regurgitation. This study comprised eighteen patients (7 patients with persistent atrial fibrillation and 11 in sinus rhythm). Atrial appendage tissues were obtained during surgery. Three purchased normal human left atrial tissues served as normal controls. Moderately-to-severely myolytic cardiomyocytes comprised 59.7 ± 22.1% of the cardiomyocytes in the right atria and 52.4 ± 12.9% of the cardiomyocytes in the left atria of mitral and tricuspid regurgitation patients with atrial fibrillation group and comprised 58.4 ± 24.8% of the cardiomyocytes in the right atria of mitral and tricuspid regurgitation patients with sinus rhythm. In contrast, no myolysis was observed in the normal human adult left atrial tissue samples. Immunohistochemical analysis showed expression of cleaved caspase-9, an effector of the mitochondrial pathways, in the majority of right atrial cardiomyocytes (87.3 ± 10.0%) of mitral and tricuspid regurgitation patients with sinus rhythm, and right atrial cardiomyocytes (90.6 ± 31.4%) and left atrial cardiomyocytes (70.7 ± 22.0%) of mitral and tricuspid regurgitation patients with atrial fibrillation. In contrast, only 5.7% of cardiomyocytes of the normal left atrial tissues showed strongly positive expression of cleaved caspase-9. Of note, none of the atrial cardiomyocytes in right atrial tissue in sinus rhythm and in the fibrillating right and left atria of mitral and tricuspid regurgitation patients, and in the normal human adult left atrial tissue samples showed cleaved caspase-8 expression, which is a downstream effector of TRADD of the death receptor pathway. Immunoblotting of atrial extracts showed that there was enhanced expression of cytosolic cytochrome c, an effector of the mitochondrial pathways, but no expression of membrane TRADD and cytosolic caspase-8 in the right atrial tissue of mitral and tricuspid regurgitation patients with sinus rhythm, and right atrial and left atrial tissues of mitral and tricuspid regurgitation patients with atrial fibrillation. Taken together, this study showed that mitochondrial pathway for apoptosis was activated in the right atria in sinus rhythm and in the left and right atria in atrial fibrillation of heart failure patients due to mitral and tricuspid regurgitation, and this mitochondrial pathway activation may contribute to atrial contractile dysfunction and enlargement in this clinical setting.

1. Introduction

As a large fraction of the population advances into the later decades of life, the incidence of valvular heart disease is expected to increase (Nkomo et al., 2006). Mitral valve disease constitutes the second most prevalent valvular heart disease after aortic valve stenosis (Iung et al., 2002). Left atrial enlargement is frequently observed in patients with mitral regurgitation and left atrial enlargement is a significant predictor of atrial fibrillation (Henry et al., 1976), stroke and mortality (Benjamin et al., 1995). However, the underlying mechanisms of atrial enlargement in patients with mitral and tricuspid regurgitation remain undefined. Atrial myocardial stretch caused by volume overload may play a predominant role in triggering the activation of apoptosis and as a consequence, atrial enlargement (Chang et al., 2011a). Activation of caspases may take place either within death receptor complexes on the cytoplasmic membrane or by a mitochondrion-dependent

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mechanism within the cytosol (Haunstetter and Izumo, 1998). Previous studies showed increased serum and atrial myocardial oxidative stress in patients with severe mitral and tricuspid regurgitation (Chang et al., 2011b; Chen et al., 2009). Intracellular reactive oxygen species can be generated by dysfunction of the mitochondrial electron transport system (Dhalla et al., 2000), a phenomenon leading to the induction of apoptosis (Akao et al., 2001). Dysfunctional mitochondria can contribute to or initiate apoptosis through the release of cytochrome c, which in turn activates caspases 9 and 3 (Haunstetter and Izumo, 1998). Myolysis is defined as disintegration or degeneration of muscle tissue and is an important cause of atrial contractile dysfunction and consequently, atrial enlargement. This study tested the hypothesis that cytochrome c release from mitochondria occurs and mitochondrial pathway activation plays a role in the development of myolysis in atrial cardiomyocytes of heart failure patients due to severe mitral and moderate-to-severe tricuspid regurgitation. Accordingly, this study examined the respective roles of the mitochondrial and tumor necrosis factor-α receptor associated death domain (TRADD)-mediated death receptor pathways for apoptosis in the atrial cardiomyocytes of this patient population. Our group and many other groups have shown that TUNEL positivity of myocytes is not a specific marker for apoptosis and is an unreliable method for identifying apoptosis (Chang et al., 2011a; Kanoh et al., 1999; Kockx et al., 1998; Koda et al., 2003). Therefore, TUNEL reactivity was not specifically assessed in this study.

2. Methods

2.1. Patient population and specimen storage

This study examined eighteen patients with severe mitral regurgitation and moderate-to-severe tricuspid regurgitation who had undergone valve operations for congestive heart failure. Eleven patients had no history and no records of electrocardiograms of atrial fibrillation before surgery (sinus rhythm group). All patients in sinus rhythm group had severe mitral regurgitation, defined by echocardiography according to American society of echocardiography report. In addition, two patients had concomitant severe tricuspid regurgitation, and nine had moderate tricuspid regurgitation. Seven patients had persistent atrial fibrillation before surgery (atrial fibrillation group). All patients had severe mitral regurgitation. Moreover, six patients had concomitant severe tricuspid regurgitation and one had moderate tricuspid regurgitation. Written informed consent was obtained from all study subjects. The study protocol was approved by the Institutional Review Committee for Human Research.

In the mitral and tricuspid regurgitation patients with atrial fibrillation, small pieces of atrial tissue from the right atrial appendage (apical portion) ($n = 7$) and left atrial appendage (apical portion) ($n = 7$) were obtained during surgery; while, in the mitral and tricuspid regurgitation patients with sinus rhythm, owing to ethical considerations by the Institutional Review Committee, only atrial tissue from the right atrial appendage ($n = 11$) was obtained. Some atrial tissues were immediately frozen in liquid nitrogen and stored at −80 °C until later study for immunoblotting. Some atrial tissues were fixed immediately following excision and maintained in 4% buffered formalin overnight at room temperature, then embedded in paraffin and stored until later study for histological analysis.

Three human adult normal left atrial tissue samples (76-year-old female, 70-year-old female, and 24-year-old male) were purchased from BioChain Institute, Inc. (Hayward, CA, USA) for immunohistochemical studies and used as normal controls. In addition, right atrial appendage tissues were obtained from two patients (a 60-year-old male and a 66-year-old female with severe aortic stenosis) without mitral and tricuspid valve disease and adopted as non-mitral–tricuspid control subjects.

2.2. Echocardiography

Transthoracic echocardiographic examinations were performed using a commercially available echo Doppler machine (iE33; Hewlett-Packard; Bothell, WA, USA). Left atrial and ventricular dimensions were determined by conventional M-mode echocardiography. Left atrial volume was obtained by the biplane area–length formula.

2.3. Histological analysis

Tissue sections were deparaffinized in xylene and rehydrated in decreasing concentrations of alcohol. Slides were then stained with hematoxylin and eosin. Atrial cardiomyocytes were analyzed (UTHSCSA, Image tool, Version 3.0) and scored by morphometry as mildly myolytic if <10% of the sarcomere content was absent and as moderately-to-severely myolytic if >10% of the sarcomere was absent (Chang et al., 2011a). The atrial samples were analyzed with at least 100 randomly chosen cells per each sample.

2.4. Immunohistochemistry

Tissue sections (2 μm) were separately immunostained with a rabbit monoclonal anti-cleaved caspase-8 antibody (1:50 dilution; Cell Signaling Technology, Beverly, MA, USA), a rabbit polyclonal anti-X-chromosome-linked inhibitor-of-apoptosis (XIAP) antibody (1:2000 dilution; Imgenex, San Diego, CA, USA), a rabbit polyclonal anti-cleaved caspase-9 antibody (1:1800 dilution; Imgenex, San Diego, CA, USA), and a rabbit monoclonal anti-cleaved caspase-3 antibody (1:200 dilution; Cell Signaling Technology, Beverly, MA, USA). Staining was performed by EnVision + System-HRP labeled polymer anti-rabbit (DakoCytomation, Glostrup, Denmark) in accordance with the manufacturer's instructions. Color development was performed by incubating tissue sections in a 3,3-diaminobenzidine solution. In negative controls, the primary antibodies were omitted and only secondary antibodies were applied. Percentages of positive labeled cells were calculated. The expression of positive immunolabeled atrial cardiomyocytes were further analyzed and scored by morphometry as no expression, weakly positive if less than half of the cell area was positively immunolabeled, and as strongly positive if more than half of the cell area was positively immunolabeled.

2.5. Western blotting

The protein expression levels of membrane, mitochondrial and cytosol extracts of human atrial tissues were examined by Western blot analysis. The membrane fraction was separated by compartmental protein extraction kit (BioChain Institute., Inc Hayward, CA, USA). Mitochondrial and cytosol fractions were separated using a mitochondrial isolation kit (Pierce Biotechnology Inc., Rockford, IL, USA). The HeLa cell lysate (Epitomics, Inc., Burlingame, CA, USA) and Jurkat cell lysate (Cell Signaling Technology, Inc., Boston, MA, USA) served as positive controls for cytochrome c, caspases, XIAP, and TRADD. Aliquots containing 100 μg protein extracts were electrophoresed on 10–15% acrylamide SDS-PAGE gels and immunoblotted onto polyvinylidene difluoride membranes. The membranes were preblocked for 1 h in TBST (10 mM Tris–HCl pH 7.6, 150 mM NaCl, 0.1% Tween-20) containing 5% w/v nonfat dry milk and then incubated at 4 °C over night with various primary antibodies, including mouse anti-cytochrome c (BD Pharmingen, Franklin Lakes, NJ, USA), mouse anti-caspase-3 (Imgenex, San Diego, CA, USA), mouse anti-caspase-8 (Cell Signaling Technology, Inc., Boston, MA, USA), rabbit anti-caspase-9 (Cell Signaling Technology, Inc., Boston, MA, USA), mouse anti-XIAP (R&D Systems, Inc. Minneapolis, MN, USA), rabbit anti-TRADD (Cell Signaling Technology, Inc., Boston, MA, USA), mouse anti-cytochrome c oxidase subunit IV (COX-IV) (Abcam Inc., San Francisco, CA, USA) and mouse anti-α-sarcomeric actin (Sigma Aldrich, Louis, MO, USA). All specific values of proteins evaluated