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Berberine improves pressure overload-induced cardiac hypertrophy and dysfunction through enhanced autophagy

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ABSTRACT

Cardiac hypertrophy is a maladaptive change in response to pressure overload, and is also an important risk for developing heart failure. Berberine is known to have cardioprotective effects in patients with hypertension and in animal models of cardiac hypertrophy. In the current study, we observed that transverse aortic contraction (TAC) surgery induced a marked increase in heart size, the ratio of heart weight to body weight, cardiomyocyte apoptosis, myocardial fibrosis, and hypertrophic marker brain natriuretic peptide, all of which were effectively suppressed by berberine administration. In addition, berberine enhanced autophagy in hypertrophic hearts, which was accompanied by a decrease in heart size, cardiac apoptosis, and the attenuation of cardiac dysfunction. Furthermore, use of autophagy inhibitor 3-methyladenine (3-MA) blocked berberine-induced autophagy level, and abrogated the protection of berberine against heart hypertrophy, cardiac dysfunction, and apoptosis. Berberine ameliorated TAC-induced endoplasmic reticulum stress, which was also abolished by 3-MA. Moreover, berberine significantly inhibited the upstream signaling of autophagy, such as the mammalian target of rapamycin (mTOR), extracellular signal-regulated kinase (ERK1/2), and p38 mitogen-activated protein kinase (MAPK) phosphorylation. We conclude that berberine could attenuate left ventricular remodeling and cardiomyocyte apoptosis through an autophagy-dependent mechanism in a rat model of cardiac hypertrophy, which is, at least in part, associated with enhanced autophagy through inhibition of mTOR, p38 and ERK1/2 MAPK signaling pathways.

1. Introduction

Physiological left ventricular hypertrophy with normal or appropriately enhanced contractile function is an adaptive remodeling process. However, further development of pathological cardiac hypertrophy may irreversibly result in cardiac dysfunction or heart failure in response to chronic pressure overload, and consequently worse prognosis (Frey and Olson, 2003; Vakili et al., 2001). Despite significant advances in understanding of the mechanisms underlying this process, current treatment for cardiac hypertrophy remains rudimentary. Recently, autophagy, a dynamic process involving the bulk degradation of cytoplasmic organelles and proteins, has been known to be responsible to the pathogenesis of cardiac hypertrophy (Gottlieb and Mentzer, 2010; Nakai et al., 2007).

Autophagy is the major intracellular degradation system, characterized by the sequestration of cytosolic proteins and organelles in autophagosomes, fusion of autophagosomes with lysosomes, and degradation by lysosomal acid hydrolases and proteases (Levine and Klionsky, 2004). The constitutive level of autophagy in the heart is a homeostatic and protective mechanism (Terman and Brunk, 2005). Autophagy also serves as a dynamic recycling system for cellular renovation and homeostasis, contributing to the quality control of proteins and organelles within cardiomyocytes under stress conditions (Gottlieb and Mentzer, 2010; Nakai et al., 2007). The major properties of cardiac hypertrophy induced by pressure overload are increased size of cardiomyocytes, elevated protein synthesis, strengthened organization of the sarcomere, and accumulation of misfolded proteins, all of which can be cleared by the autophagic process (Kim et al., 2007; Nakai et al., 2007;

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Sadoshima and Izumo, 1997). In addition to degrading impaired proteins, autophagy is also important to remove the dysfunctional organelles, in particular mitochondrion and endoplasmic reticulum (ER) (Kim et al., 2007). Recent studies have suggested that ER stress-meditated apoptosis is caused by sustained pressure overload, resulting in loss of hypertrophic cardiomyocyte and heart failure (Okada et al., 2004). Therefore, activation of autophagy provides an adaptive mechanism to retard these changes during pressure overload-induced cardiac hypertrophy.

Berberine, an extensively studied isoquinoline alkaloid derived from several medicinal plant species, possesses many pharmacological actions, including anti-cancer (Lin et al., 1999), anti-microbial (Yi et al., 2007), and cholesterol-lowering effects (Kong et al., 2004). Recently, there is fast-growing interest in exploring the effects of berberine on cardiovascular diseases, such as arrhythmia (Wang et al., 2012), hypertension (Liu et al., 1999), and hypertrophy (Hong et al., 2002). Although several studies have proposed that berberine induces autophagy in human hepatoma cells and lung cancer cells (Hou et al., 2011; Peng et al., 2008; Wang et al., 2010), little is known about the relationship between the cardioprotection of berberine and autophagy enhancement in cardiac hypertrophy. In this study, we sought to determine whether enhanced autophagy could contribute to the cardioprotection of berberine in a rat model of transverse aortic constriction (TAC)-induced cardiac hypertrophy and dysfunction, and to further delineate the underlying molecular mechanisms and signaling pathways by which berberine would exert its effects.

2. Materials and methods

2.1. Experimental animals

Sprague-Dawley male rats, weighing 200–220 g, were bred in the animal facility of Nanjing Medical University, according to the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised in 1996). The rats were housed in individual cages under controlled conditions with constant temperature and humidity, and were exposed to a 12-h day/night cycle and had free access to food and water. The protocols adopted in the present study were approved by the Ethics Committee for the Use of Experimental Animals, Nanjing Medical University, China.

2.2. TAC-induced cardiac hypertrophy

Pressure overload-induced cardiac hypertrophy was achieved by TAC as described elsewhere (Zhang et al., 2013). Briefly, rats were anesthetized by 10% chloral hydrate (0.03 ml/kg, intraperitoneal injection). During the surgery period, rats were ventilated using a rodent ventilator with positive pressure (ALC-V8S, Alcott Biotech Co., Shanghai, China). A 4-0 silk suture was placed under the transverse aorta between the innominate artery and left common carotid artery, and tied against a 22-gauge needle, and then the needle was promptly removed to create a defined constriction. Rats subjected to a sham operation served as controls. There were five experimental groups: sham+vehicle ($n=18$); TAC+vehicle ($n=18$); TAC+berberine ($n=18$); TAC+berberine+3-methyladenine (3-MA) ($n=18$); and TAC+rapamycin ($n=8$). All above chemicals (including berberine) were given alone or in combination immediately after the completion of TAC surgery as designed. Berberine (Sigma-Aldrich, St. Louis, MO, USA) was administered at a dose of 10 mg/kg/day (Hong et al., 2002) for 4 weeks via oral gavage administration. The autophagy inhibitor, 3-MA (Sigma-Aldrich CO.), was given at a dosage of 100 mg/kg on alternate days (Lu et al., 2009) for 4 weeks by intraperitoneal injection. Rapamycin (Sigma-Aldrich), an inhibitor of mammalian target of rapamycin (also known as mTOR) that was used as a positive control to induce autophagy, was dissolved in dimethyl sulfoxide (25 mg/ml) and then diluted with phosphate-buffered saline before intraperitoneal injection (1 mg/kg/day) (Xie et al., 2013). At the end of the observation periods and after echocardiography, rats were sacrificed, and the hearts were explanted for further analyses.

2.3. Echocardiographic assessment

Cardiac function was evaluated by echocardiography with a Vevo 770 cardiac system (VisualSonics Inc., Toronto, Canada). M-mode tracings were used to digitally measure intraventricular septal thickness diastole (IVSD), left ventricular end-systolic dimension (LVESD), and left ventricular end-diastolic dimension (LVEDD). Left ventricular fractional shortening (FS) was calculated as: $FS=(LVEDD\times LVESD)/LVEDD\times 100\%$. Left ventricular ejection fraction (LVEF) was calculated by the cubic method: $LVEF=[(LVEDD)^3-(LVESD)^3]/(LVEDD)^3\times 100\%$. All measures were averaged with five consecutive cardiac cycles and performed by observers blinded to the identity of the tracings.

2.4. Histopathological analyses

Heart tissues for histopathological analyses were fixed in 10% formalin and embedded in paraffin. Cardiomyocyte cross-sectional areas were evaluated by hematoxylin and eosin (H&E) staining. The degree of fibrosis was determined by Masson's trichrome staining. Immunohistochemical staining with antibodies for rabbit monoclonal anti-LC3B (1:3200; Cell Signaling Technology Inc., Beverly, MA, USA) was performed to determine the relative content of LC3B in the left ventricle. Photomicrographs of stained sections were digitalized and analyzed by an automated image analysis system (Image-Pro Plus 6.0 software, Media Cybernetics, Silver Spring, MD, USA).

Apoptosis was tested by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) staining using an in situ cell death detection kit-AP (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's instructions. In brief, paraffin-embedded heart tissue sections (thickness, 4 μm each) were deparaffinized and permeabilized with 0.1 mol/L sodium citrate, pH 6.0, at 65 °C for 2 h. Sections were then exposed to a TUNEL reaction mixture containing terminal deoxynucleotidyl transferase and nucleotides. After 1 h of incubation at 37 °C in a humidified atmosphere, TUNEL-positive nuclei were detected by light microscopy. As a positive control, the cross sections of fixed and permeabilized heart tissues from sham+vehicle group were treated with DNase I (3000 U/ml, Roche Applied Science, Indianapolis, IN, USA) to make the DNA stand broken prior to the Tunel procedure (Communal et al., 1998).

2.5. Transmission electron microscopy

Cardiac tissue was quickly cut into 1 mm cubes, and fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) overnight at 4 °C. After fixation, the selections were then immersed in 1% osmium tetroxide for 2 h, dehydrated in graded ethanol, and then embedded in epoxy resin. After that, the selections were incised into ultrathin sections (60–70 nm) with an ultramicrotome and post-stained with uranyl acetate and lead citrate, and then examined under a JEM-1010 transmission electron microscope (JEOL, Peabody, MA, USA).