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

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A B S T R A C T

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 constriction (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.

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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;
Berberine, an extensively studied isoquinoline alkaloid derived from several medicinal plant species, possesses many pharmacological actions, including anti-cancer (Lin et al., 1999), antimicrobial (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 hyper trophy (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 deendothelialized portion. 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 - LVESD) / LVEDD \times 100\%
Left ventricular ejection fraction (LVEF) was calculated by the cubic method: 
LVEF = 1 \times \frac{(LVEDD)^2 - (LVESD)^2}{(LVEDD)^3}
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 Tunnel 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 sections were then immersed in 1% osmium tetroxide for 2 h, dehydrated in graded ethanol, and then embedded in epoxy resin. After that, the sections 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).
2.6. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

RNA levels were determined by qRT-PCR with the one-step qPCR kit (Toyobo, Osaka, Japan). Amplification and detection using SYBR-green detection of PCR products were performed with an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA). In this study, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified as an endogenous reference. The rat-specific primer pairs used for brain natriuretic peptide (BNP) were: 5′-GAC CAA GGC CCT ACA A-3′ (forward) and 5′-TCT ATC TTC TGC CCA AA-3′ (reverse); the primer pairs for procollagen I were: 5′-ACT CAG CCC TCT GTG CCT-3′ (forward) and 5′-CTC CTT CCA TAC TC-3′ (reverse); primer pairs for procollagen III were: 5′-CAG TGA AGG AAA TAG-3′ (forward) and 5′-TGT CAT AGG GTG CGA TAT-3′ (reverse); and primer pairs for GAPDH were: 5′-TGT CCG GTA TAC TGG CTT CTT-3′ (forward) and 5′-GAG GGA GTT GTC ATA TTT CTC GTG GT-3′ (reverse).

2.7. Western blotting

Total proteins were extracted and the concentrations were determined by the Bradford method (Kruger, 1994). The equal amount of protein was loaded in each lane and subjected to 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Separated protein bands were then transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were incubated overnight at 4 °C with the respective primary antibodies as follows: anti-LC3B, anti-Beclin-1, anti-phospho-mTOR, anti-total-mTOR, anti-phospho-p38 MAPK, anti-total-p38 MAPK, anti-phospho-ERK1/2 MAPK, anti-total-ERK1/2 MAPK, anti-GRP78/BiP, anti-cleaved caspase 3 (all above from Cell Signaling Technology Inc, USA), anti-cleaved caspase 3 (3 all above from Cell Signaling Technology Inc, USA), anti-cleaved caspase 12 (Abcam, Cambridge, UK), and β-actin (Santa Cruz Biotechnology Inc., CA, USA). After washing in TBST 3 times, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody and developed with an enhanced chemiluminescence system using the enhanced chemiluminescence (ECL) kit (Millipore, USA). The signals were quantified by scanning densitometry with the Image J analysis system (NIH, MD, USA).

2.8. Statistical analysis

Data are presented as mean ± S.E.M. Differences between groups were tested by one-way analysis of variance (ANOVA) and Tukey’s procedure for multiple range tests. Statistical significance was set as P < 0.05.

3. Results

3.1. Berberine prevented TAC-induced cardiac hypertrophy

As shown in Fig. 1, TAC induced a marked increase in the ratio of heart weight to body weight (HW/BW), and also induced mRNA expression of BNP (a well-characterized marker in response to hypertrophy) as compared with the sham + vehicle group. Moreover, when compared with the TAC + vehicle group, the TAC + berberine group reversed an increased ratio of HW/BW (P < 0.05, Fig. 1A), and prevented increased mRNA expression of BNP (P < 0.05, Fig. 1B). In addition, there were significantly lower cardiomyocyte cross-sectional areas (with H&E stained) in the TAC + berberine group than in the TAC + vehicle group (Fig. 1C and D) (P < 0.05). Nevertheless, cardiac hypertrophy induced by pressure-overload was not markedly ameliorated by treatment with 3-MA and berberine.

3.2. Berberine prevented TAC-induced cardiac dysfunction

Four weeks after surgery, transthoracic echocardiography was performed as described above, and a few representative short-axis views from each group are shown in Fig. 2A. According to echocardiographic evaluation, EF and FS in the TAC + vehicle group were significantly decreased (23.6% and 17.8%, respectively) as compared with the sham + vehicle group, but these two parameters were significantly improved in the TAC + berberine group as compared with the TAC + vehicle group (P < 0.05, Fig. 2B). In addition, the geometrical parameters of the heart, including LVESD, LVEDD and IVSD, were also significantly improved in the berberine-treated rats (P < 0.05, Fig. 2B). However, there was no significant attenuation of cardiac dysfunction observed in the TAC rats treated with berberine in combination with the autophagy inhibitor 3-MA as compared with rats treated with berberine alone.

3.3. Berberine attenuated TAC-induced myocardial interstitial fibrosis

Sustained pressure overload induced progressive interstitial fibrosis as determined with Masson’s trichrome staining in the TAC + vehicle group (Fig. 3A). However, berberine administration significantly attenuated the extent of myocardial fibrosis (P < 0.05, Fig. 3B). In addition, higher mRNA levels of procollagen I and procollagen III were observed in the TAC + vehicle group compared with the sham + vehicle group, but these cardiac extracellular matrix proteins were significantly attenuated after berberine administration (P < 0.05, Fig. 3C). Notably, the inhibition of myocardial interstitial fibrosis by berberine was abolished by the specific autophagy inhibitor 3-MA.

3.4. Berberine attenuated TAC-mediated apoptosis and ER stress

To further investigate the potential causes that could lead to cardiac dysfunction and myocardial interstitial fibrosis after TAC, cardiac apoptosis and ER stress were analyzed. For this reason, the expression of biomarkers of ER stress, such as GRP78 (glucose-regulated protein 78) and cleaved caspase-12, was used to evaluate the effect of berberine on ER stress. Berberine treatment led to significantly decreased protein expression of GRP78 and cleaved caspase-12, both of which were induced by pressure overload (P < 0.05, Fig. 4A and B). To confirm the effect of berberine on TAC-induced apoptosis, TUNEL staining was used to examine the rate of cardiac apoptosis. Markedly increased apoptotic cardiomyocytes were observed after TAC, but TAC-induced apoptosis was attenuated by berberine treatment (P < 0.05, Fig. 4C and D). Furthermore, the protein expression of cleaved caspase 3 (a critical mediator of apoptosis) was also assessed, and results indicated that berberine administration markedly decreased TAC-induced cleaved caspase 3 (P < 0.05, Fig. 4E). These findings suggested that berberine could attenuate TAC-induced apoptosis, which was partially related to suppression of ER stress. Meanwhile, there was no significant difference between TAC + berberine and TAC + rapamycin in reducing cardiac apoptosis after TAC treatment (P > 0.05, Fig. 4E).

3.5. Berberine increased the level of autophagy in the TAC-treated rat model

To better understand why berberine could attenuate TAC-induced apoptosis and ER stress, a series of experiments were carried out to determine whether berberine could induce autophagy, a protective
degradation mechanism of the heart against pressure overload (Nakai et al., 2007). Transmission electron microscopy assessment showed that berberine treatment attenuated disorganized sarcomere structure and mitochondrial disarray in the hypertrophic heart induced by pressure overload, which was in parallel with increased autophagosomes (Fig. 5A). The electron microscopy results prompted us to further confirm the effect of berberine on autophagy. Therefore, Western blotting was carried out to measure autophagy-associated biomarkers LC3B (microtubule-associated protein 1 light chain 3) and Beclin-1. As shown in Fig. 5B, there was significantly higher protein expression of LC3B-II/LC3B-I and Beclin-1 in the TAC+berberine group than in the TAC+vehicle group (P < 0.05). Consistent with the results of Western blotting assay, immunohistochemical staining assay showed that increased protein expression of LC3B was found in TAC+berberine group as compared with TAC+vehicle group (Fig. 5C). As expected, such an induction of autophagy by berberine...
was significantly suppressed by the autophagy inhibitor 3-MA, further confirming that berberine can induce autophagy in the rats of cardiac hypertrophy. Moreover, there was a significant difference in the protein expression of LC3B-II/LC3B-I between TAC + berberine and TAC + rapamycin (a well-characterized autophagy inducer), suggesting that the level of autophagy induced by berberine is not as high as that induced by rapamycin.

In order to dissect the underlying molecular mechanism and associated signaling pathways of berberine-induced autophagy, mTOR-signaling pathway that negatively regulates autophagy (Ravikumar et al., 2010) was determined by Western blotting. In this study, compared with TAC + vehicle group (Fig. 6A and B), there was a significant inhibition of the mTOR activity in the group of TAC + berberine or TAC + rapamycin (P < 0.05, respectively), indicating that suppression of mTOR contributes to induction of autophagy by berberine. Because extracellular signal-regulated kinase (ERK1/2) and p38 mitogen-activated protein kinase (MAPK) signaling participate in regulation of mTOR activity, there were markedly decreased levels of phosphorylated ERK1/2 and p38 MAPK in TAC + berberine group compared with TAC + vehicle group (P < 0.05) as shown in Fig. 6A and B.

4. Discussion

To the best of our knowledge, this is the first observation that berberine can prevent cardiac hypertrophy and attenuate cardiomyocyte apoptosis in the TAC-treated rat model, acting through enhancement of autophagy, and that the suppression of the mTOR and its upstream p38 and ERK1/2 MAPK signaling pathways may be contributory.

Autophagy, a highly conserved cytoprotective pathway, serves as a cell survival mechanism, such as recycling amino acids, removing impaired proteins and organelles, and maintaining the function of organelles, including ER and mitochondrion (Levine and Klionsky, 2004; Yang and Klionsky, 2010). Thus, induction of autophagy in the heart can provide energy for the myofiber under the stress condition, such as starvation or pressure overload, which is very important to maintain cardiac myofiber homeostasis and normal cardiac function. The potent induction of autophagy by administration with rapamycin could prevent cardiac hypertrophy induced by TAC and thyroid hormone treatment (Ha et al., 2005; Kuzman et al., 2007). Moreover, rapamycin is more likely to attenuate cardiac dysfunction and to
reverse established cardiac hypertrophy induced by pressure overload (McMullen et al., 2004). However, the exact role of autophagy in response to pressure overload-induced hypertrophy remains debatable. According to previous studies, excessive activation of autophagy has been reported to lead to type II programmed cell death, aggravate cardiac hypertrophy, and speed up the process of heart failure (Bursch, 2001). LC3B, a good indicator of autophagosome formation, is widely used for autophagy assay, because the amount of LC3-II is well correlated with number of autophagosomes (Kabeya et al., 2000). This study observed that protein expression levels of L3B-II/LC3B-I induced by berberine were lower than that induced by rapamycin, suggesting that berberine can induce appropriate enhancement of autophagy in the heart after TAC treatment. Therefore, it can be inferred that berberine inhibited cardiac remodeling and dysfunction through properly inducing autophagy in the hypertrophic heart, which probably supports the concept that activation of autophagy may play a beneficial role in preventing cardiac fibrosis.

Fig. 3. Effects of berberine on myocardial fibrosis. (A) Representative Masson’s trichrome staining, magnification, 200 ×, scale bar = 100 μm. (B) The fibrotic area was calculated as a percentage of total left ventricular myocardial area. (C) Procollagen I and procollagen III mRNA expression was analyzed with qRT-PCR. *P < 0.05 versus the sham + vehicle group; †P < 0.05 versus the TAC + vehicle group; ‡P < 0.05 versus the TAC + berberine group. TAC, transverse aortic constriction; 3-MA, 3-methyladenine.
hypertrophy induced by pressure overload (Chen et al., 2013; Sun et al., 2013).

Hong et al. first reported that suprarenal abdominal aorta constriction-induced cardiac hypertrophy and dysfunction can be inhibited by berberine treatment (Hong et al., 2002). After that, further investigations were carried out to elucidate the mechanism by which berberine exerts its cardioprotection, and the results showed that berberine could modulate sympathetic nervous activity and decrease the level of catecholamine in plasma and left ventricular in rats (Hong et al., 2003). However, these two studies did not find out the exact mechanism to explain why berberine can exert its cardioprotective effects against cardiac hypertrophy. By contrast, our results revealed that berberine could prevent cardiac hypertrophy and dysfunction induced by TAC treatment through enhanced autophagy, which may be the result of inhibition of mTOR signaling pathway, consistent with the findings observed in liver cancer cells (Wang et al., 2010). As an AMPK activator, berberine could increase AMPK activity in many models, including ischemia-reperfusion injury rats (Chang et al., 2012), diabetic mice (Lee et al., 2006), and rat skeletal muscles (Ma et al., 2010). AMPK is responsible for sensing of energy and nutrients, and involved in promoting autophagy by directly activating mammalian autophagy-initiating kinase Ulk1 through phosphorylation of Ser 317 and Ser 777 (Kim et al., 2011). Accordingly, berberine might induce autophagy by activating AMPK except for inhibiting mTOR signaling pathway. Interestingly, some studies indicated that berberine inhibited autophagy in cisplatin-induced kidney damage (Domitrovic et al., 2013). The conflicting results derived from others (Domitrovic et al., 2013) and our studies imply that the effects of berberine on autophagy may vary by different animal species or even by the tissue or organ studied. Another cause may be higher dose of berberine (10 mg/kg/day, 4 weeks) used in TAC rats in our study than that used for cisplatin-induced nephrotoxicity in mice (Domitrovic et al., 2013).

ER stress is a spectrum of ER dysfunction, primarily due to various cellular stress, including ischemia/hypoxia, oxidative stress, sustained pressure overload, and increased unfolded protein synthesis (Ron, 2002). Caspase-12 protein, an ER-specific protease, plays a pivotal role in the initiation of ER stress-induced apoptosis in mice (Nakagawa et al., 2000). Current evidence suggests that excessive ER stress from long-term pressure overload could lead to apoptosis, which has been recognized as the major contributor to aggravating cardiac hypertrophy and dysfunction (Okada et al., 2004). In addition, inhibition of ER stress and subsequently suppressing ER stress-induced apoptosis may play a critical role in protecting the heart against hypertrophy and improving cardiac dysfunction (Groenendyk et al., 2010; Guan et al., 2011; Jung et al., 2010; Minamino et al., 2010). Indeed, as a cellular degradation system that is required for cells to survive via counterbalancing ER expansion under conditions of severe ER...
stress, autophagy alleviates ER dysfunction (Bernales et al., 2006; Ogata et al., 2006). Therefore, enhancement of autophagy may partially ameliorate ER stress-mediated apoptosis. In our study, berberine reduced the TAC-induced apoptosis of cardiomyocytes. In addition, suppressing of autophagy by 3-MA enhanced the expression of cleaved caspase-12 and reduced the cardioprotective effect of berberine on apoptosis. Accordingly, inhibition of TAC-induced apoptosis by berberine seems to be, at least in part, due to autophagy enhancement that suppresses ER stress in cardiac hypertrophy.

Autophagy in cardiomyocytes is a complex process that involves numerous signaling molecules, and one of the most important negatively regulated mechanisms is mTOR signaling (Jung et al., 2010). Previous studies have suggested that rapamycin, a specific inhibitor of mTOR, can activate autophagy in heart tissue (Buss et al., 2009). Several studies have shown that the mTOR signaling pathway is regulated by the MAPK pathways in autophagy (Tang et al., 2008; Wu et al., 2011). Therefore, we assessed the phosphorylation of mTOR and its upstream signaling molecules ERK1/2 and p38, which are important components in the MAPK pathways. The results indicated that berberine suppressed the mTOR activity and its upstream p38 and ERK1/2 MAPK signaling pathways after TAC. Apart from the classical mTOR pathway, there are still several other signaling pathways reported to regulate autophagy (Criollo et al., 2010). Therefore, further studies should be carried out to dissect the molecular mechanism about the effect of berberine on autophagy.

In summary, these results suggest that berberine plays an important role in preventing pressure overload-induced cardiac hypertrophy and apoptosis, which are mainly associated with enhancement of autophagy and inhibition of ER stress. Moreover, these results may shed new light on the cardioprotection exerted by berberine and support its therapeutic potential for cardiac hypertrophy and ventricular dysfunction in clinical settings.

Fig. 5. Effects of berberine on autophagy. (A) Representative autophagic ultrastructure in the heart tissue after TAC under transmission electron microscopy. Arrows indicate autophagosomes, bar = 2 μm. (B) LC3B-II/LC3B-I and Beclin-1 protein expression was shown by Western blotting and presented as a histogram. (C) Representative images of immunohistochemistry of LC3B, magnification, 200 ×, bar = 100 μm. *P < 0.05 versus the sham + vehicle group; †P < 0.05 versus the TAC + vehicle group; ‡P < 0.05, §P < 0.05, ‡P < 0.05 versus the TAC + berberine group. TAC, transverse aortic constriction; 3-MA, 3-methyladenine.
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References


Fig. 6. Effects of berberine on autophagy-related signal pathways. (A) Representative Western blotting images for mTOR, ERK1/2, and p38 MAPK in groups of sham+vehicle, TAC+vehicle, TAC+berberine, and TAC+rapamycin. (B) Quantitative analysis of relative protein expression in each group. *P<0.05 versus the sham+vehicle group; †P<0.05 versus the TAC+vehicle group; ‡P>0.05 versus the TAC+berberine group. TAC, transverse aortic constriction; 3-MA, 3-methyladenine.


