Berberine inhibits the ischemia-reperfusion injury induced inflammatory response and apoptosis of myocardial cells through the phosphoinositide 3-kinase/RAC-α serine/threonine-protein kinase and nuclear factor-κB signaling pathways

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Abstract. Myocardial ischemia-reperfusion injury is one of the most common cardiovascular diseases, and can lead to serious damage and dysfunction of the myocardial tissue. Previous studies have demonstrated that berberine exhibits ameliorative effects on cardiovascular disease. The present study further investigated the efficacy and potential mechanism underlying the effects of berberine on ischemia-reperfusion injury in a mouse model. Inflammatory markers were measured in the serum and levels of inflammatory proteins in myocardial cells were investigated after treatment with berberine. In addition, the apoptosis of myocardial cells was investigated after berberine treatment. Apoptosis-associated gene expression levels and apoptotic signaling pathways were analyzed in myocardial cells after treatment with berberine. The phosphoinositide 3-kinase (PI3K)/RAC-α serine/threonine-protein kinase (AKT) and nuclear factor (NF)-κB signaling pathways were also analyzed in myocardial cells after treatment with berberine. Histological analysis was used to analyze the potential benefits of berberine in ischemia-reperfusion injury. The present study identified that inflammatory responses and inflammatory factors were decreased in the myocardial cells of the mouse model of ischemia-reperfusion injury. Mechanism analysis demonstrated that berberine inhibited apoptotic protease-activating factor 1, caspase-3 and caspase-9 expression in myocardial cells. The expression of Bcl2-associated agonist of cell death, Bcl-2-like protein 1 and cellular tumor antigen p53 was upregulated. Expression of NF-κB p65, inhibitor of NF-κB kinase subunit β (IKK-β), NF-κB inhibitor α (IκBα), and NF-κB activity, were inhibited in myocardial cells in the mouse model of ischemia-reperfusion injury. In conclusion, the results of the present study indicate that berberine inhibits inflammatory responses through the NF-κB signaling pathway and suppresses the apoptosis of myocardial cells via the PI3K/AKT signaling pathway in a mouse model of ischemia-reperfusion injury. These results suggest that berberine is a potential drug for the treatment of patients with ischemia-reperfusion injury.

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Introduction

Cardiovascular disease is a generic term including cardiovascular and cerebrovascular diseases caused by hyperlipidemia, atherosclerosis and hypertension (1,2). Ischemic heart disease caused by ischemia-reperfusion injury is a major public health concern and is the leading cause of mortality for patients with nonfatal acute myocardial infarction, ischemic heart failure, cardiac failure, angina pectoris and other coronary heart diseases (3,4). Epidemiologic studies have indicated that the risk of ischemia-reperfusion injury is linked to the association between age, sex and genetic polymorphism, and ischemic heart disease (5,6). Although previous studies have demonstrated that early reperfusion of the ischemic myocardium is one of the most therapeutic approaches in restoring cardiac function for the remission of ischemia-reperfusion injury (7), reperfusion can lead to further ischemia-reperfusion injury, and myocarditis and myocardial infarction (8). At present, numerous theories and signaling mechanisms to explain this effect have been proposed and explored in myocardial cells and in animal models of ischemia-reperfusion injury (9-11).

Inflammation is one of the most common characteristics of cardiovascular diseases, including myocardial infarction, anoxia-reoxygenation injury of heart, ischemia-reperfusion injury and ischemic heart disease (12). Previous studies have suggested that preventing inflammation serves an efficient role in protection against myocardial ischemia-reperfusion injury,

and highlights novel perspective viewpoints for the diagnosis and treatment of cardiac disease (13,14). Wang *et al* (15) indicated that berberine can be regarded as having antiarrhythmic effects in a type II diabetic myocardial infarction model via depression of inward rectifier potassium channel 2. A previous study indicated that inhibition of apoptosis and inflammation contributes to the rehabilitation of myocardial ischemia/reperfusion injury (16). In addition, Jiang *et al* (17) demonstrated that berberine can attenuate lipopolysaccharide-induced inflammation in rat mesangial cells via regulation of the nuclear factor (NF)-κB signaling pathway. Furthermore, berberine exhibits anti-inflammatory effects in patients with acute coronary syndrome who have suffered percutaneous coronary intervention (18). Therefore, the present study hypothesized that berberine could regulate inflammation in the progression of ischemia-reperfusion injury through the NF-κB signaling pathway.

The NF-κB-mediated phosphoinositide 3-kinase (PI3K)/RAC-α serine/threonine-protein kinase (AKT) signaling pathway is associated with myocardial fibrosis in rats (19,20). Previous studies have suggested that the apoptosis of myocardial cells serves a crucial role in cardiac dysfunction following acute myocardial infarction in the development of coronary heart disease (21,22). Numerous strategies aimed at preventing or mitigating the extent of apoptosis have attempted to protect the heart against the coronary heart disease-induced apoptosis of myocardial cells (23-25). Berberine has been identified to attenuate ischemia-reperfusion injury-induced myocardial cell apoptosis by reducing the stimulation of the 5'-AMP-activated protein kinase (AMPK) and PI3K/AKT signaling pathways in a diabetic rat model (26). In addition, berberine has been reported to inhibit the apoptosis of human umbilical vein endothelial cells induced by *Staphylococcus aureus* in preclinical research (27). Furthermore, berberine inhibits ischemia-induced apoptosis through activation of the PI3K/AKT signaling pathway (28). The present study hypothesized that berberine could regulate the apoptosis of myocardial cells via the PI3K/AKT signaling pathway in a mouse model of ischemia-reperfusion injury.

The present study investigated the cardioprotective effects of berberine in a mouse model of ischemia-reperfusion injury. This demonstrated that the NF-κB and PI3K/AKT signaling pathways were involved in the anti-inflammatory and antiapoptotic effects of berberine on myocardial cells in the progression of ischemia-reperfusion injury. These results suggest that berberine is a potential therapeutic agent for the treatment of ischemia-reperfusion injury.

Materials and methods

Animal study. A total of 100 male C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA, USA) and housed under pathogen-free conditions. Mice were maintained in an environment with a 12 h light/dark cycle and free access to food and water. The mouse model of ischemia-reperfusion injury was generated according to a protocol described in a previous study (29). The mice were randomly divided into two groups (n=40 per group). In the first group the animals received 10 mg/kg berberine orally once per day, whilst the second group received the same volume of PBS. The treatment continued for 30 days. On day 30, the myocardial function of the mice was then analyzed to evaluate the efficacy of berberine.

The current study was approved by the Tab of Animal Experimental Ethical Inspection of the General Hospital of the Chinese People's Armed Police Forces (Beijing, China) and performed in accordance with their recommendations. All surgery and experiments were performed under anesthetic to minimize pain.

Cell culture. Myocardial cells were isolated from experimental mice on day 30, in accordance with a previous study (30), and cultured in minimum essential medium with 10% fetal bovine serum (both Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Myocardial cells were treated by a promoter of NF-κB (NF-κBPr; 1:800; NEMO; cat no., AF2684; R&D Systems, Inc., Minneapolis, MN, USA) or PI3K inhibitor (PI3KI; 1:500; cat. no., HY-17645; MedChemExpress, Monmouth Junction, NJ, USA) and cultured at 37˚C with 5% CO_2 in a humidified atmosphere. Then, the myocardial cells were treated with 10 mg/ml berberine (cat. no., PHR1502; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 24 h to analyze its effect *in vitro*.

ELISA. In the protein detection assay, mouse C-reactive protein (CRP; cat. no., MCRP00), procalcitonin (cat. no., DY8350-05) and high mobility group box 1 protein (HMGB1; cat. no., AF1690) (all R&D Systems, Inc.). ELISA kits were used to determine inflammatory responses. The procedures were conducted according to the manufacturer's protocol. The final results were recorded at 450 nm using a microplate reader.

Western blot analysis. Myocardial cells isolated from berberine treated mice were homogenized in a radioimmunoprecipitation assay buffer containing protease inhibitors (Gibco; Thermo Fisher Scientific, Inc.). Then, the myocardial cells were centrifuged at 2,000 x g at 4˚C for 10 min and 2 µg/lane of protein was separeated by a 12% SDS-PAGE gel and transferred to polyvinylidene fluoride membranes (Thermo Fisher Scientific, Inc). After blocking with 5% skimmed milk for 12 h at 4˚C, the polyvinylidene fluoride membranes were incubated with goat anti-mouse primary antibodies directed against the following proteins: Interleukin (IL)-6 (1:2,000; cat. no., ab6672), IL-10 (1:2,000; cat. no., ab34843), NF-κB p65 (1:1,000; cat. no., ab16502), inhibitor of NF-κB kinase subunit β (1:1,000; IKK-β; cat. no., ab3204), NF-κB inhibitor α (1:1,000; IκBα; cat. no., ab309300), apoptotic protease-activating factor 1 (1:2,000; Apaf-1; cat. no., ab32372), caspase-3 (1:2,000; cat. no., ab2172), caspase-9 (1:2,000; cat. no., ab32539), PI3K (1:2,000; cat. no., ab189403), AKT (1:2,000; cat. no., ab8805), Bcl2-associated agonist of cell death (Bad; 1:2,000; cat. no., ab32445), Bcl-2-like protein 1 (Bcl-xl; 1:2,000; cat. no., ab32370), β-actin (1:2,000; cat. no., ab8226) and cellular tumor antigen p53 (p53; 1:2,000; cat. no., ab61241; all Abcam, Cambridge, UK) for 2 h at 37˚C. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (cat. no., HAF010; Bio-Rad Laboratories, Inc., Hercules, CA, USA) for 1 h at 37˚C at a 1:5,000 dilution. The results

were visualized using an enhanced chemiluminescence detection system (cat. no., D5905-50TAB; Sigma-Aldrich; Merck KGaA). Protein expression was analyzed using BandScan software (version, 5.0; Glyko, Inc.; BioMarin Pharmaceutical, Inc., San Rafael, CA, USA).

Flow cytometric analysis of the apoptosis of myocardial cells. Apoptosis rates of the myocardial cells were evaluated using an Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) apoptosis detection kit (BD Biosciences, San Jose, CA, USA). Myocardial cells were collected and suspended with Annexin V-FITC and PI according to the manufacturer protocol. Fluorescence was measured with a fluorescence-activated cell sorting flow cytometer using FCS Express™ IVD software (version 4; De Novo Software, Los Angeles, CA, USA).

NF-κB activity and heart rate assay. NF-κB activity in the myocardial cells was analyzed according to a previously described method (31). The heart rates of the experimental mice treated with berberine or PBS were measured using a cardiotachometer on day 0 and 30 after treatment. The results were recorded to analyze the effects of berberine on myocardial function.

Total lipid content and percentage of lymphocytes analysis. Blood samples were collected from experimental animals on day 30. An autohumalyzer (Dimension® RxL Max®; Siemens Healthineers, Erlangen, Germany) was used to assess total cholesterol according to the manufacturer's protocol.

The total lipid content from total cholesterol was determined by high-performance liquid chromatography as reported previously (32). In brief, the supernatant (200 μl) of the blood samples were transferred into a fresh 1.5 ml amber microcentrifuge tube and prepared with 12 mM potassium ferricyanide solution in 3.3 M sodium hydroxide to start the thiochrome reaction. The thiochrome reaction quenched by 25 μl of 1M phosphoric acid; the neutralized samples were filtered and analyzed. The samples were kept at 8˚C in the autosampler and 50 μl was injected into an Agilent Eclipse Plus C18 (4.6x150 mm, 5 mm; Agilent Technologies, Inc., Santa Clara, CA, USA) column protected by a SecurityGuard C18 (4x3 mm) guard column (Phenomenex, Inc., Torrance, CA, USA) at 40˚C. A total of 150 mM potassium phosphate dibasic (solvent A; pH 7.0) and methanol (solvent B) served as the mobile phase at a flow rate of 1.5 ml/min and an 8 min gradient as follows: 0 min (85% solvent A), 1 min (80% solvent A), 3 min (80% solvent A), 6 min (50% solvent A), 7 min (85% solvent A), 8 min (85% solvent A). The samples were analyzed using Agilent 1200 HPLC System equipped with a fluorescence detector and operated by ChemStation Rev. B.02.01 SR1 (both Agilent Technologies, Inc.).

In addition, the percentage of lymphocytes was analyzed by flow cytometric analysis using conjugated antibodies directed against CD8-FITC (cat. no. ANT-282; Prospec-Tany TechnoGene, Ltd., East Brunswick, NJ, USA), CD4-PE (cat. no. A07751; Beckman Coulter, Inc., Brea, CA, USA) and CD3-PC5 (cat. no. A07749; Beckman Coulter, Inc.) (all 1:1,000) at 4˚C for 12 h according to a previous study (33). Briefly, peripheral blood mononuclear cells were aseptically separated by density gradient centrifugation (speed, 8,000 x g) at 4˚C for 10 min. Cells were blocked with 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) at 37˚C for 2 h and then incubated with the aforementioned antibodies and washed three times with PBS. The percentage of lymphocytes analyses was performed using a FACSCalibur™ flow cytometer (BD Biosciences) equipped with 488 nm argon laser. A minimum of 10,000 events was acquired and analyzed using BD CellQuest Software (version 3.3; BD Biosciences).

Immunohistochemistry. Paraffin-embedded 4-μm-thick sections of myocardial tissue were prepared and epitope retrieval was performed as described previously (34). The paraffin-embedded sections were treated with hydrogen peroxide (3%) for 10-15 min, and were subsequently blocked using 5% skimmed milk powder for 10-15 min at 37˚C. Finally, the sections were stained with hemotoxylin and eosin at 4˚C for 12 h. All sections were washed three times with PBS. The area of myocardial injury, circumference fragmentation and segmentation of myocardial cells were determined in six random fields through a fluorescence microscope (BZ-9000; Keyence Corporation, Osaka, Japan).

Statistical analysis. All data are presented as the mean ± standard error of the mean from three independent experiments. Unpaired data was analyzed using a Student's t-test. Data between multiple groups were compared using one-way analysis of variance followed by a post hoc Tukey's range test. P<0.05 was considered to indicate a statistically significant difference using SPSS software (version 19.0; IBM Corp., Armonk, NY, USA).

Results

Berberine decreases inflammatory responses in a mouse model of ischemia-reperfusion injury. The effects of berberine on inflammatory responses in a mouse model of ischemia-reperfusion injury were investigated. As shown in Fig. 1A, CRP was upregulated in ischemia-reperfusion injury mice and significantly downregulated by berberine. In addition, berberine significantly decreased the percentage of lymphocytes in the serum of ischemia-reperfusion injury mice compared with the control group (Fig. 1B). Plasma levels of procalcitonin were significantly decreased in mice treated with berberine compared to the control group (Fig. 1C). Furthermore, the expression of HMGB1 was significantly downregulated by berberine in the serum of mice with ischemia-reperfusion injury compared with the control group (Fig. 1D). Collectively, these results suggest that berberine markedly decreases inflammatory responses in the mouse model of ischemia-reperfusion injury.

Berberine regulates the expression of inflammatory factors through the NF-κB signaling pathway. The molecular mechanisms underlying ischemia-reperfusion injury-induced inflammatory responses were investigated in the myocardial cells of the mouse model of ischemia-reperfusion injury. The results revealed that berberine significantly downregulated the expression of IL-6 and IL-10 in the serum of mice with ischemia-reperfusion injury (Fig. 2A and B). The levels of

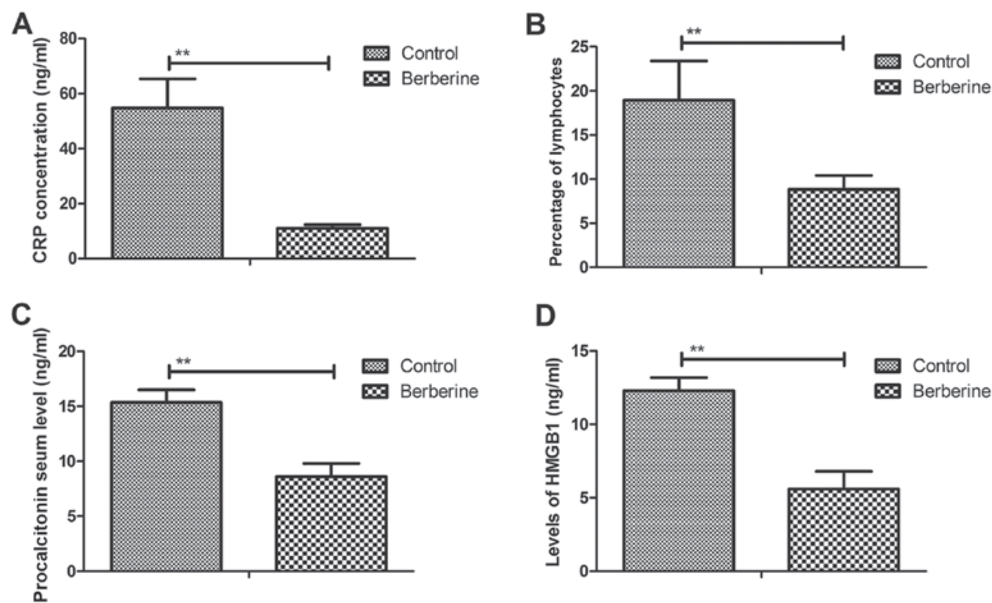

Figure 1. Berberine reduces the expression of inflammatory markers in the serum of a mouse model of ischemia-reperfusion injury. (A) Concentration of CRP. (B) Percentage of lymphocytes. (C) Concentration of procalcitonin. (D) Concentration of HMGB1. **P<0.01 vs. the control group. CRP, C-reactive protein; HMGB1, High mobility group box 1 protein.

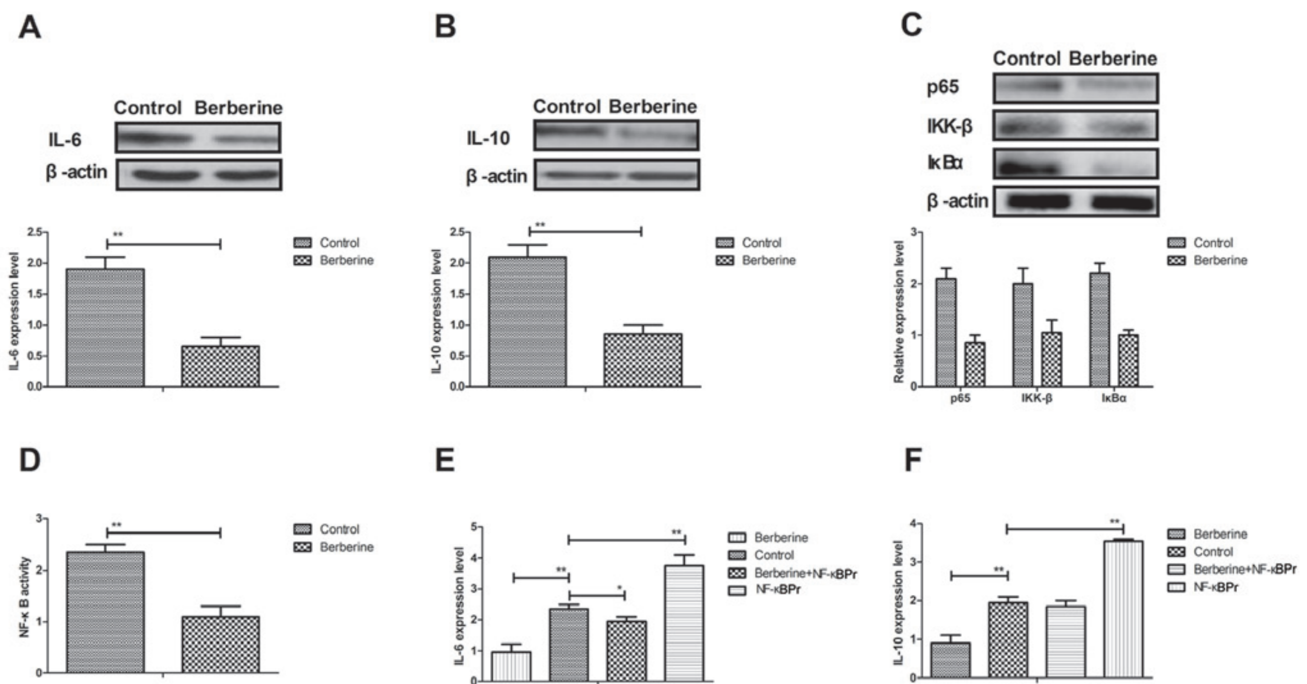

Figure 2. Berberine regulates the expression of inflammatory factors through the NF-κB signaling pathway. Expression of (A) IL-6 and (B) IL-10 in the serum of mice with ischemia-reperfusion injury. (C) Expression of NF-κB p65, IKK-β and IκBα in the myocardial cells of the experimental mice. (D) NF-κB activity in the myocardial cells of the experimental mice. Expression of (E) IL-6 and (F) IL-10 in the myocardial cells after inhibition of NF-κB expression. *P<0.05, **P<0.01 vs. the control group. NF, nuclear factor; IL, interleukin; IKK-β, inhibitor of NF-κB kinase subunit β; IκBα, NF-κB inhibitor α; NF-κBPr, promoter of NF-κB.

NF-κB p65, IKK-β and IκBα were decreased by berberine treatment in the myocardial cells of the experimental mice (Fig. 2C). NF-κB activity was also significantly inhibited by berberine compared to the control (Fig. 2D). The stimulation of NF-κB (NF-κBPr) activity significantly blocked berberine-suppressed IL-6 and IL-10 expression in the myocardial cells (Fig. 2E and F). This data indicates that berberine regulates the expression of inflammatory factors through the NF-κB signaling pathway.

Berberine inhibits the apoptosis of myocardial cells through the PI3K/AKT-mediated mitochondrial apoptosis signaling pathway. The apoptosis rate of myocardial cells in ischemia-reperfusion injury mice and the mechanism of

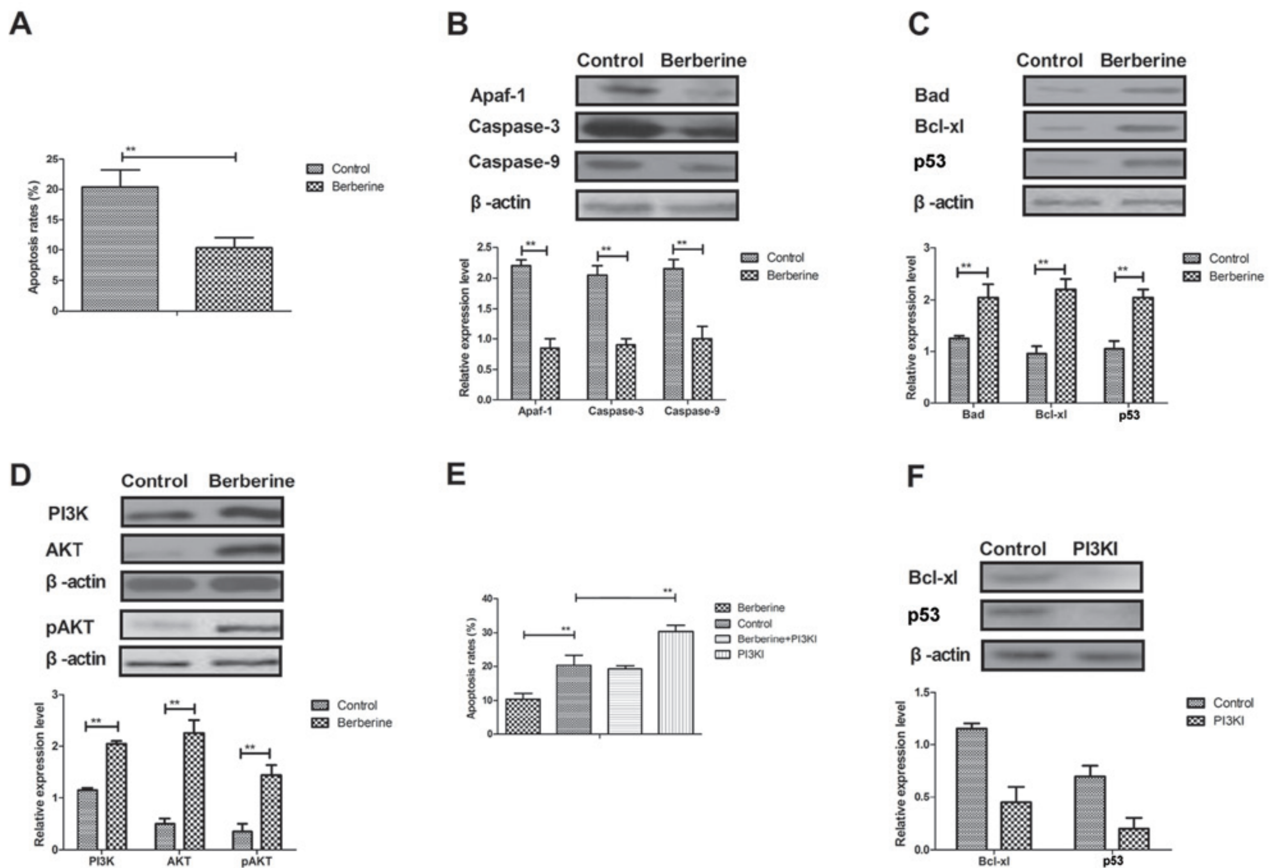

Figure 3. Berberine regulates the apoptosis of myocardial cells through the PI3K/AKT-mediated mitochondrial apoptosis signaling pathway. (A) Apoptosis of myocardial cells in experimental mice after berberine treatment. Expression of (B) Apaf-1, caspase-3 and caspase-9; (C) Bad, Bcl-xl and p53; and (D) PI3K and AKT in the myocardial cells. (E) Effect of PI3K inhibition on the antiapoptotic effect of berberine on myocardial cells. (F) Effect of PI3K inhibition on Bcl-xl and p53 expression in myocardial cells *in vitro*. **P<0.01 vs. the control group. PI3K, phosphoinositide 3-kinase; AKT, RAC-α serine/threonine-protein kinase; Apaf-1, Apoptotic protease-activating factor 1; Bad, Bcl2-associated agonist of cell death; Bcl-xl, Bcl-2-like protein 1; p53, cellular tumor antigen p53.

the antiapoptotic effects of berberine were investigated in the present study. Berberine significantly inhibited the apoptosis of myocardial cells induced by ischemia-reperfusion injury compared with the control group (Fig. 3A). Berberine also significantly inhibited the expression of Apaf-1 and cleaved caspase-3 and caspase-9 in myocardial cells compared with the control group (Fig. 3B). In addition, the expression of Bad, Bcl-xl and p53 was upregulated by berberine in the myocardial cells of the mouse model of ischemia-reperfusion injury (Fig. 3C). In addition, the expression of PI3K and AKT was significantly increased by berberine in myocardial cells (Fig. 3D). Furthermore, the inhibition of PI3K significantly inhibited the antiapoptotic effect of berberine on myocardial cells (Fig. 3E). Treatment with the PI3K inhibitor (PI3KI) decreased Bcl-xl and p53 levels in the myocardial cells *in vitro* (Fig. 3F). These results suggest that berberine inhibits the apoptosis of myocardial cells through the PI3K/AKT-mediated mitochondrial apoptosis signaling pathway.

Berberine improves blood lipid levels, blood pressure and myocardial function of mice with ischemia-reperfusion injury. The benefits of berberine on the myocardial function of experimental mice with ischemia-reperfusion injury were analyzed. The results revealed that the blood lipid content was significantly decreased by berberine in the mouse model of ischemia-reperfusion injury (Fig. 4A). In addition, heart rate was significantly decreased by berberine in mice with ischemia-reperfusion injury (Fig. 4B). Furthermore, the area of myocardial infarction was significantly decreased in the myocardial tissue of mice treated with berberine (Fig. 4C). Additionally, as determined by histological analysis, the circumference fragmentation and segmentation of myocardial cells were markedly improved by treatment with berberine (Fig. 4D). Taken together, these results suggest that berberine markedly improves myocardial function in mice with ischemia-reperfusion injury.

Discussion

Cardiovascular diseases present as systemic vascular lesions in clinical patients, and are the frequent causes of mortality in the adult population of economically developed countries (35,36). Ischemia-reperfusion injury is one of the most common coronary heart diseases and may further develop into coronary atherosclerosis heart disease, coronary arterial atherosclerosis, or vascular cavity stenosis or occlusion (37,38). Previous reports have indicated that inflammation and apoptosis contribute to the initiation and development of ischemia-reperfusion injury (39,40). Recently, berberine

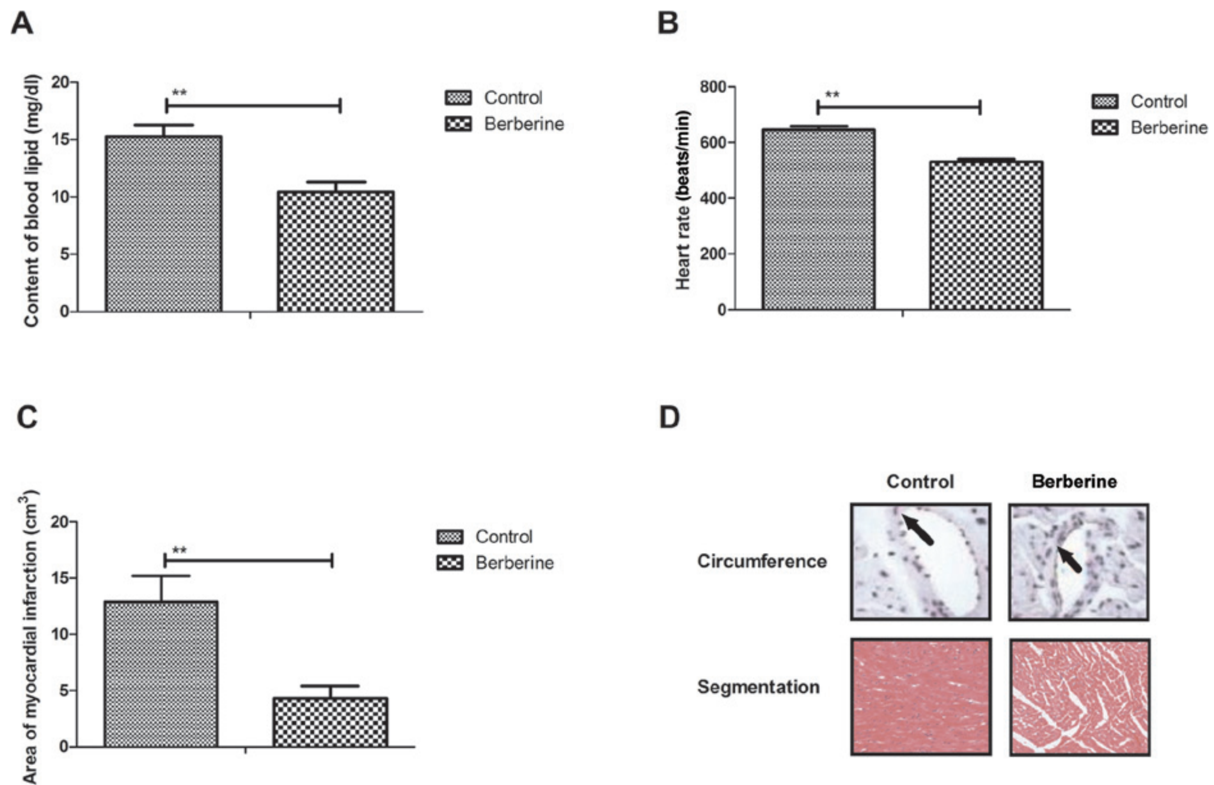

Figure 4. Berberine improves the myocardial function of mice with ischemia-reperfusion injury. (A) Blood lipid levels, (B) heart rate and (C) area of myocardial injury in mice with ischemia-reperfusion injury treated with berberine. (D) Circumference fragmentation and segmentation of the myocardial cells from mice with ischemia-reperfusion injury treated with berberine. Magnification, x40. **P<0.01 vs. the control group.

has been demonstrated to have a protective function against myocardial ischemia-reperfusion injury (41). The present study investigated the therapeutic effects of berberine on ischemia-reperfusion injury, as well as analyzing the molecular mechanism of berberine-mediated regulation of inflammation and apoptosis in the myocardial cells of a mouse model of ischemia-reperfusion injury. The findings of the current study suggest that berberine regulates the expression of inflammatory factors and apoptosis in myocardial cells through the NF-κB and PI3K/AKT signaling pathways, respectively. Notably, although IκBα expression was inhibited by berberine, the expression of NF-κB p65 and Ikkβ were also inhibited by berberine in myocardial cells, which may contribute to the anti-inflammatory effects observed. These investigations suggest that berberine is a potential therapeutic agent for the improvement of myocardial function in ischemia-reperfusion injury.

Ischemia-reperfusion injury of the heart is a major public health concern, and coronary heart disease caused by myocardial ischemia-reperfusion injury is the greatest cause of mortality and disability among cardiovascular events, including myocardial infarction, cardiopulmonary bypass surgery and heart transplantation (42,43). Ischemia-reperfusion injury of the heart frequently results in irreversible fatal injury (44). Therefore, understanding the molecular mechanism of anoxia-reoxygenation is essential for the prevention and treatment of cardiovascular disease. Vinten-Johansen *et al* (45) demonstrated that inflammation and proinflammatory mediators are involved in myocardial ischemia-reperfusion injury, post ischemic injury and gradually restoring blood flow. In addition, an earlier study identified that berberine could attenuate vascular remodeling and inflammatory responses in a rat model of metabolic syndrome (46). Furthermore, Adil *et al* (47) investigated the ameliorative effects of berberine against gentamicin-induced nephrotoxicity in rat models and the results indicated that berberine attenuated oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. Researchers have also suggested that the PI3K/AKT signaling pathway is involved in the cardioprotection of preconditioning during myocardial ischemia and reperfusion (48). The present study demonstrated that berberine reduced the inflammation caused by ischemia-reperfusion injury through inhibition of the NF-κB signaling pathway. Additionally, NF-κB has been reported to be associated with myocardial ischemia injury by inhibiting the expression of tumor necrosis factor α-induced genes associated with ischemia/reperfusion in endothelial cells *in vivo* (49). The present study revealed that expression of NF-κB p65, IKK-β, IκBα and p53 were decreased by berberine treatment, which contributed to the decrease in inflammatory responses in the mouse model of ischemia-reperfusion injury. These findings suggest that berberine regulates the NF-κB and PI3K/AKT signaling pathways in myocardial cells.

Recently, berberine has been reported to prevent nigrostriatal dopaminergic neuronal loss, and suppress the apoptosis of hippocampus cells and human umbilical vein endothelial cells (27,50). Chen *et al* (26) revealed that berberine can reduce ischemia/reperfusion-induced myocardial apoptosis through activation of AMPK and

PI3K-AKT signaling in diabetic rats. In addition, pretreatment with berberine can protect myocardial cells against lipopolysaccharide-induced myocardial dysfunction via the inhibition of apoptosis in mice (51). Furthermore, the cardioprotective roles of berberine against myocardial ischemia/reperfusion injury have been identified through its attenuation of mitochondrial dysfunction and apoptosis in myocardial cells (52). The present study identified that berberine inhibited the expression of Apaf-1, caspase-3 and caspase-9, and promoted the expression of Bad, Bcl-xl and p53 in myocardial cells through regulation of the PI3K/AKT signaling pathway. Although levels of the proapoptotic protein Bad were upregulated, the levels of the antiapoptotic proteins Bcl-xl and p53 were high enough to inhibit the apoptosis induced by Bad in myocardial cells. These results suggest that berberine inhibits the mitochondrial apoptosis signaling pathway in the myocardial cells of the mouse model of ischemia-reperfusion injury.

In conclusion, the present study highlights the efficacy of berberine against ischemia-reperfusion injury. Inflammatory markers were downregulated by berberine in mice with ischemia-reperfusion injury. Blood lipid levels, heart rate, and circumference fragmentation and segmentation of myocardial cells were markedly improved by berberine. Notably, the findings of the present study also indicated that berberine inhibits inflammation via the NF-κB signaling pathway, as well as the PI3K/AKT signaling pathway, by which berberine inhibited the mitochondrial apoptosis signaling pathway in myocardial cells. These results suggest that berberine is a potential therapeutic agent for the treatment of ischemia-reperfusion injury.

References

1. Kaligis RW, Adiarto S, Nugroho J, Pradnyana BA, Lefi A and Rifqi S: Carotid Intima-media thickness in indonesian subjects with cardiovascular disease risk factors who were not receiving lipid-lowering agents. Int J Angiol 25: 174-180, 2016.
2. Lines SW and Carter AM: Complement and cardiovascular disease-the missing link in haemodialysis patients. Nephron 134: 104, 2016.
3. de Luna AB, Zareba W, Fiol M, Nikus K, Birnbaum Y, Baranowski R, Goldwasser D, Kligfield P, Piotrowicz R, Breithardt G and Wellens H: Negative T wave in ischemic heart disease: A consensus article. Ann Noninvasive Electrocardiol 19: 426-441, 2014.
4. Virtanen JK, Mursu J, Tuomainen TP and Voutilainen S: Dietary fatty acids and risk of coronary heart disease in men: The Kuopio ischemic heart disease risk factor study. Arterioscler Thromb Vasc Biol 34: 2679-2687, 2014.
5. Cai G, Zhang B, Weng W, Shi G and Huang Z: The associations between the MCP-1-2518 A/G polymorphism and ischemic heart disease and ischemic stroke: A meta-analysis of 28 research studies involving 21,524 individuals. Mol Biol Rep 42: 997-1012, 2015.
6. Krause N, Brand RJ, Arah OA and Kauhanen J: Occupational physical activity and 20-year incidence of acute myocardial infarction: Results from the Kuopio ischemic heart disease risk factor study. Scand J Work Environ Health 41: 124-139, 2015.
7. Lu YZ, Wang J, Song J, Zhang CY, Ji JQ, Li BH, Tian XX and Song XL: Effect of ischemic postconditioning on the expression of myocardium matrix metalloproteinase-2 induced by ischemia/reperfusion in rats. Zhongguo Ying Yong Sheng Li Xue Za Zhi 30: 81-84, 2014 (In Chinese).
8. Ge G, Zhang Q, Ma J, Qiao Z, Huang J, Cheng W and Wang H: Protective effect of Salvia miltiorrhiza aqueous extract on myocardium oxidative injury in ischemic-reperfusion rats. Gene 546: 97-103, 2014.
9. Qu D, Han J, Ren H, Yang W, Zhang X, Zheng Q and Wang D: Cardioprotective effects of astragalin against myocardial ischemia/reperfusion injury in isolated rat heart. Oxid Med Cell Longev 2016: 8194690, 2016.
10. Wang TH, Liu HT, She XJ, Ma Q and Song N: Study of the protective effect of L-arginine on relative ischemic/reperfusion injury in myocardium of rat. Zhongguo Ying Yong Sheng Li Xue Za Zhi 21: 266-269, 2005 (In Chinese).
11. Mastrocola R, Collino M, Penna C, Nigro D, Chiazza F, Fracasso V, Tullio F, Alloatti G, Pagliaro P and Aragno M: Maladaptive modulations of NLRP3 inflammasome and cardioprotective pathways are involved in diet-induced exacerbation of myocardial ischemia/reperfusion injury in mice. Oxid Med Cell Longev 2016: 3480637, 2016.
12. Yang M, Chen J, Zhao J and Meng M: Etanercept attenuates myocardial ischemia/reperfusion injury by decreasing inflammation and oxidative stress. PLoS One 9: e108024, 2014.
13. Liu ZZ, Kong JB, Li FZ, Ma LL, Liu SQ and Wang LX: Ischemic postconditioning decreases matrix metalloproteinase-2 expression during ischemia-reperfusion of myocardium in a rabbit model: A preliminary report. Exp Clin Cardiol 18: e99-e101, 2013.
14. Chen L, Wang Y, Pan Y, Zhang L, Shen C, Qin G, Ashraf M, Weintraub N, Ma G and Tang Y: Cardiac progenitor-derived exosomes protect ischemic myocardium from acute ischemia/reperfusion injury. Biochem Biophys Res Commun 431: 566-571, 2013.
15. Wang LH, Yu CH, Fu Y, Li Q and Sun YQ: Berberine elicits anti-arrhythmic effects via IK1/Kir2.1 in the rat type 2 diabetic myocardial infarction model. Phytother Res 25: 33-37, 2011.
16. Loubele ST, Spek CA, Leenders P, van Oerle R, Aberson HL, Hamulyák K, Ferrell G, Esmon CT, Spronk HM and Ten Cate H: Activated protein C protects against myocardial ischemia/reperfusion injury via inhibition of apoptosis and inflammation. Arterioscler Thromb Vasc Biol 29: 1087-1092, 2009.
17. Jiang Q, Liu P, Wu X, Liu W, Shen X, Lan T, Xu S, Peng J, Xie X and Huang H: Berberine attenuates lipopolysaccharide-induced extracelluar matrix accumulation and inflammation in rat mesangial cells: Involvement of NF-κB signaling pathway. Mol Cell Endocrinol 331: 34-40, 2011.
18. Meng S, Wang LS, Huang ZQ, Zhou Q, Sun YG, Cao JT, Li YG and Wang CQ: Berberine ameliorates inflammation in patients with acute coronary syndrome following percutaneous coronary intervention. Clin Exp Pharmacol Physiol 39: 406-411, 2012.
19. Fan H, Ma L, Fan B, Wu J, Yang Z and Wang L: Role of PDGFR-β/PI3K/AKT signaling pathway in PDGF-BB induced myocardial fibrosis in rats. Am J Transl Res 6: 714-723, 2014.
20. Lovell MJ, Yasin M, Lee KL, Cheung KK, Shintani Y, Collino M, Sivarajah A, Leung KY, Takahashi K, Kapoor A, *et al*: Bone marrow mononuclear cells reduce myocardial reperfusion injury by activating the PI3K/Akt survival pathway. Atherosclerosis 213: 67-76, 2010.
21. Hashemian M, Poustchi H, Mohammadi-Nasrabadi F and Hekmatdoost A: Systematic review of zinc biochemical indicators and risk of coronary heart disease. ARYA Atheroscler 11: 357-365, 2015.
22. Farvid MS, Ding M, Pan A, Sun Q, Chiuve SE, Steffen LM, Willett WC and Hu FB: Response to letters regarding article, 'Dietary linoleic acid and risk of coronary heart disease: A systematic review and meta-analysis of prospective cohort studies'. Circulation 132: e23-e24, 2015.
23. Liu LL, Lin LR, Lu CX, Fu JG, Chao PL, Jin HW, Zhang ZY and Yang TC: Expression of inflammatory and apoptosis factors following coronary stent implantation in coronary heart disease patients. Int Immunopharmacol 11: 1850-1854, 2011.
24. Salmina AB, Shul'man VA, Nikulina SY, Trufanova LV, Fursov AA, But'yanov PA, Kuskaev AP, Bol'shakova EV and Kotlovskii MY: Apoptosis of leukocytes as a marker of neutrophil-endotheliocyte interaction in coronary heart disease. Bull Exp Biol Med 144: 39-41, 2007 (In English, Russian).
25. Geng YJ: Molecular mechanisms for cardiovascular stem cell apoptosis and growth in the hearts with atherosclerotic coronary disease and ischemic heart failure. Ann N Y Acad Sci 1010: 687-697, 2003.
26. Chen K, Li G, Geng F, Zhang Z, Li J, Yang M, Dong L and Gao F: Berberine reduces ischemia/reperfusion-induced myocardial apoptosis via activating AMPK and PI3K-Akt signaling in diabetic rats. Apoptosis 19: 946-957, 2014.

27. Xiong CY, Fu YH, Hu HB, Bi AF and Pei DC: Berberine inhibited apoptosis of human umbilical vein endothelial cells induced by *Staphylococcus aureus*: An experimental research. Zhongguo Zhong Xi Yi Jie He Za Zhi 34: 710-713, 2014 (In Chinese).
28. Kim M, Shin MS, Lee JM, Cho HS, Kim CJ, Kim YJ, Choi HR and Jeon JW: Inhibitory effects of isoquinoline alkaloid berberine on ischemia-induced apoptosis via activation of phosphoinositide 3-kinase/protein kinase B signaling pathway. Int Neurourol J 18: 115-125, 2014.
29. Mallavia B, Liu F, Sheppard D and Looney MR: Inhibiting integrin αβ5 reduces ischemia-reperfusion injury in an orthotopic lung transplant model in mice. Am J Transplant 16: 1306-1311, 2016.
30. Zhao P, Gao J, Jiang J, Peng X, Wu W, Zheng L and Yao L: Myocardial cells and mitochondrial autophagy in sepsis mice induced by lipopolysaccharide. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 32: 177-181, 2016 (In Chinese).
31. Fan YH, Zhao LY, Zheng QS, Dong H, Wang HC and Yang XD: Arginine vasopressin increases iNOS-NO system activity in cardiac fibroblasts through NF-kappaB activation and its relation with myocardial fibrosis. Life Sci 81: 327-335, 2007.
32. Komprda T, Zelenka J, Fajmonova E, Bakaj P and Pechová P: Cholesterol content in meat of some poultry and fish species as influenced by live weight and total lipid content. J Agric Food Chem 51: 7692-7697, 2003.
33. Shah W, Yan X, Jing L, Zhou Y, Chen H and Wang Y: A reversed CD4/CD8 ratio of tumor-infiltrating lymphocytes and a high percentage of CD4(+)FOXP3(+) regulatory T cells are significantly associated with clinical outcome in squamous cell carcinoma of the cervix. Cell Mol Immunol 8: 59-66, 2011.
34. Vidal-Laliena M, Romero X, March S, Requena V, Petriz J and Engel P: Characterization of antibodies submitted to the B cell section of the 8th Human Leukocyte Differentiation Antigens Workshop by flow cytometry and immunohistochemistry. Cell Immunol 236: 6-16, 2005.
35. Seaman CD, George KM, Ragni M and Folsom AR: Association of von Willebrand factor deficiency with prevalent cardiovascular disease and asymptomatic carotid atherosclerosis: The Atherosclerosis risk in communities study. Thromb Res 144: 236-238, 2016.
36. Morgia G, Russo GI, Tubaro A, Bortolus R, Randone D, Gabriele P, Trippa F, Zattoni F, Porena M, Mirone V, *et al*: Prevalence of cardiovascular disease and osteoporosis during androgen deprivation therapy prescription discordant to EAU guidelines: Results from a Multi-center cross-sectional analysis from the CHOsIng treatment for prostate canCEr (CHOICE) study. Urology 96: 165-170, 2016.
37. Heikkila K, Koskinen OA, Agarwal A, Tikkinen KA, Mäki M and Kaukinen K: Associations of coeliac disease with coronary heart disease and cerebrovascular disease: A systematic review and meta-analysis. Nutr Metab Cardiovasc Dis 25: 816-831, 2015.
38. Tully PJ, Turnbull DA, Beltrame J, Horowitz J, Cosh S, Baumeister H and Wittert GA: Panic disorder and incident coronary heart disease: A systematic review and meta-regression in 1131612 persons and 58111 cardiac events. Psychol Med 45: 2909-2920, 2015.
39. Nakano Y, Matoba T, Tokutome M, Funamoto D, Katsuki S, Ikeda G, Nagaoka K, Ishikita A, Nakano K, Koga J, *et al*: Nanoparticle-mediated delivery of irbesartan induces cardioprotection from myocardial ischemia-reperfusion injury by antagonizing monocyte-mediated inflammation. Sci Rep 6: 29601, 2016.
40. Schubert C, Raparelli V, Westphal C, Dworatzek E, Petrov G, Kararigas G and Regitz-Zagrosek V: Reduction of apoptosis and preservation of mitochondrial integrity under ischemia/reperfusion injury is mediated by estrogen receptor beta. Biol Sex Differ 7: 53, 2016.
41. Yu L, Li F, Zhao G, Yang Y, Jin Z, Zhai M, Yu W, Zhao L, Chen W, Duan W and Yu S: Protective effect of berberine against myocardial ischemia reperfusion injury: Role of Notch1/Hes1-PTEN/Akt signaling. Apoptosis 20: 796-810, 2015.
42. Weinreuter M, Kreusser MM, Beckendorf J, Schreiter FC, Leuschner F, Lehmann LH, Hofmann KP, Rostosky JS, Diemert N, Xu C, *et al*: CaM Kinase II mediates maladaptive post-infarct remodeling and pro-inflammatory chemoattractant signaling but not acute myocardial ischemia/reperfusion injury. EMBO Mol Med 6: 1231-1245, 2014.
43. Ren-an Q, Juan L, Chuyuan L, Wenjuan F, Chunyan H, Xuemei Y, Lin H and Hong N: Study of the protective mechanisms of Compound Danshen Tablet (Fufang Danshen Pian) against myocardial ischemia/reperfusion injury via the Akt-eNOS signaling pathway in rats. J Ethnopharmacol 156: 190-198, 2014.
44. Yndestad A, Sandanger Ø, Jong WMC, Aukrust P and Zuurbier CJ: Response to letter from Toldo et al. on 'NLRP3 inflammasome activation during myocardial ischemia reperfusion is cardioprotective'. Biochem Biophys Res Commun 474: 328-329, 2016.
45. Vinten-Johansen J, Jiang R, Reeves JG, Mykytenko J, Deneve J and Jobe LJ: Inflammation, proinflammatory mediators and myocardial ischemia-reperfusion Injury. Hematol Oncol Clin North Am 21: 123-145, 2007.
46. Li XX, Li CB, Xiao J, Gao HQ, Wang HW, Zhang XY, Zhang C and Ji XP: Berberine attenuates vascular remodeling and inflammation in a rat model of metabolic syndrome. Biol Pharm Bull 38: 862-868, 2015.
47. Adil M, Kandhare AD, Dalvi G, Ghosh P, Venkata S, Raygude KS and Bodhankar SL: Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. Ren Fail 38: 996-1006, 2016.
48. Hu X, Xu C, Zhou X, Cui B, Lu Z and Jiang H: PI3K/Akt signaling pathway involved in cardioprotection of preconditioning with high mobility group box 1 protein during myocardial ischemia and reperfusion. Int J Cardiol 150: 222-223, 2011.
49. Kim HJ, Tsoy I, Park JM, Chung JI, Shin SC and Chang KC: Anthocyanins from soybean seed coat inhibit the expression of TNF-alpha-induced genes associated with ischemia/reperfusion in endothelial cell by NF-kappaB-dependent pathway and reduce rat myocardial damages incurred by ischemia and reperfusion in vivo. FEBS Lett 580: 1391-1397, 2006.
50. Kim M, Cho KH, Shin MS, Lee JM, Cho HS, Kim CJ, Shin DH and Yang HJ: Berberine prevents nigrostriatal dopaminergic neuronal loss and suppresses hippocampal apoptosis in mice with Parkinson's disease. Int J Mol Med 33: 870-878, 2014.
51. Wang YY, Li HM, Wang HD, Peng XM, Wang YP, Lu DX, Qi RB, Hu CF and Jiang JW: Pretreatment with berberine and yohimbine protects against LPS-induced myocardial dysfunction via inhibition of cardiac I-[kappa]B[alpha] phosphorylation and apoptosis in mice. Shock 35: 322-328, 2011.
52. Wang Y, Liu J, Ma A and Chen Y: Cardioprotective effect of berberine against myocardial ischemia/reperfusion injury via attenuating mitochondrial dysfunction and apoptosis. Int J Clin Exp Med 8: 14513-14519, 2015.

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