

# Berberine inhibits the ischemia-reperfusion injury induced inflammatory response and apoptosis of myocardial cells through the phosphoinositide 3-kinase/RAC- $\alpha$ serine/threonine-protein kinase and nuclear factor- $\kappa$ B 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- $\alpha$  serine/threonine-protein kinase (AKT) and nuclear factor (NF)- $\kappa$ B 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- $\kappa$ B p65, inhibitor of NF- $\kappa$ B kinase subunit  $\beta$  (IKK- $\beta$ ), NF- $\kappa$ B inhibitor  $\alpha$  (I $\kappa$ B $\alpha$ ), and NF- $\kappa$ B 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- $\kappa$ B 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.

## 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,

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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)- $\kappa$ B 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- $\kappa$ B signaling pathway.

The NF- $\kappa$ B-mediated phosphoinositide 3-kinase (PI3K)/RAC- $\alpha$  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- $\kappa$ B 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- $\kappa$ B (NF- $\kappa$ BPr; 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<sub>2</sub> 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  $\mu$ g/lane of protein was separated 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- $\kappa$ B p65 (1:1,000; cat. no., ab16502), inhibitor of NF- $\kappa$ B kinase subunit  $\beta$  (1:1,000; IKK- $\beta$ ; cat. no., ab3204), NF- $\kappa$ B inhibitor  $\alpha$  (1:1,000; I $\kappa$ B $\alpha$ ; 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),  $\beta$ -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- $\kappa$ B activity and heart rate assay.** NF- $\kappa$ B 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 cardiometer 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 autoanalyzer (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  $\mu$ l) 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  $\mu$ l of 1M phosphoric acid; the neutralized samples were filtered and analyzed. The samples were kept at 8°C in the autosampler and 50  $\mu$ l 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- $\mu$ m-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 hematoxylin 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  $\pm$  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- $\kappa$ B 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



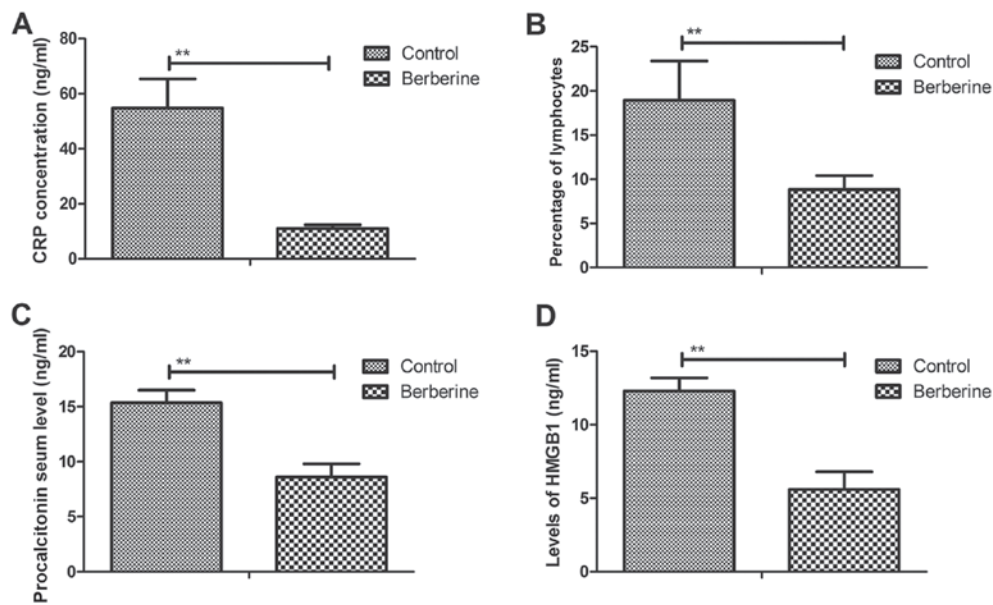


Figure 1. Berberine reduces the expression of inflammatory markers in the serum of a mouse model of ischemia-reperfusion injury. (A) Concentration of CRP. (B) Percentage of lymphocytes. (C) Concentration of procalcitonin. (D) Concentration of HMGB1. \*\* $P < 0.01$  vs. the control group. CRP, C-reactive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

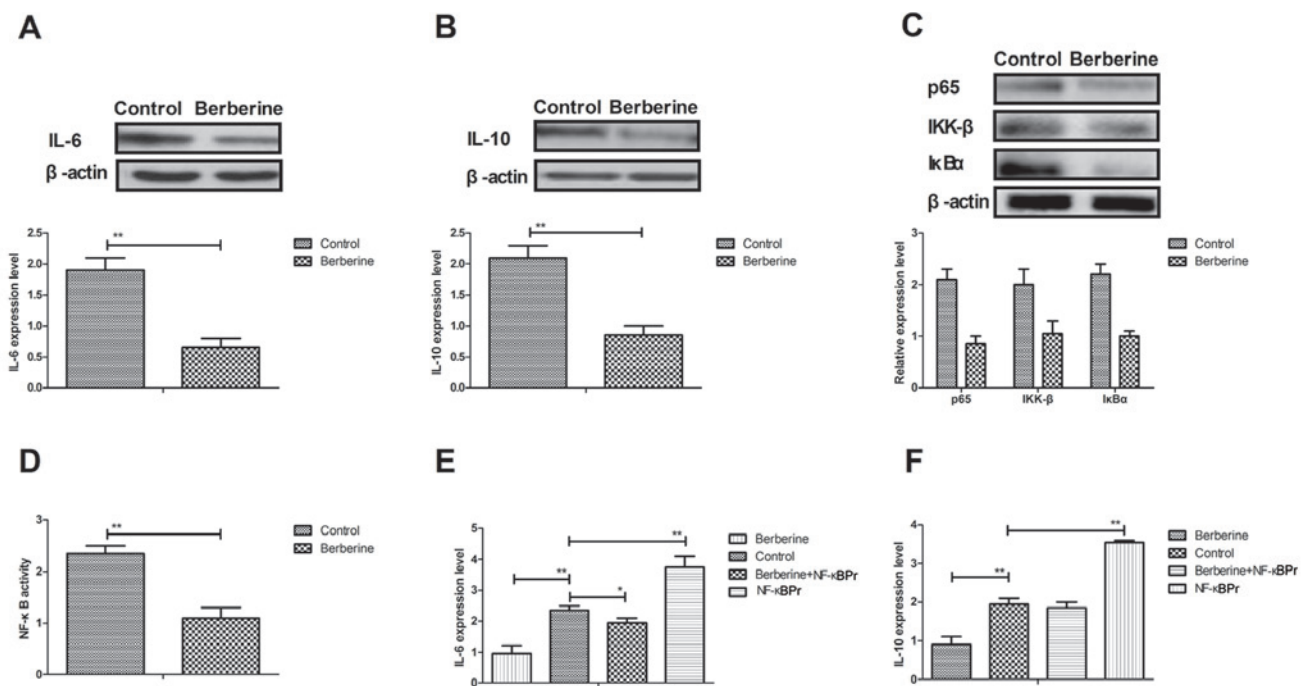


Figure 2. Berberine regulates the expression of inflammatory factors through the NF- $\kappa$ B signaling pathway. Expression of (A) IL-6 and (B) IL-10 in the serum of mice with ischemia-reperfusion injury. (C) Expression of NF- $\kappa$ B p65, IKK- $\beta$  and I $\kappa$ B $\alpha$  in the myocardial cells of the experimental mice. (D) NF- $\kappa$ B 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- $\kappa$ B expression. \* $P < 0.05$ , \*\* $P < 0.01$  vs. the control group. NF, nuclear factor; IL, interleukin; IKK- $\beta$ , inhibitor of NF- $\kappa$ B kinase subunit  $\beta$ ; I $\kappa$ B $\alpha$ , NF- $\kappa$ B inhibitor  $\alpha$ ; NF- $\kappa$ BPr, promoter of NF- $\kappa$ B.

NF- $\kappa$ B p65, IKK- $\beta$  and I $\kappa$ B $\alpha$  were decreased by berberine treatment in the myocardial cells of the experimental mice (Fig. 2C). NF- $\kappa$ B activity was also significantly inhibited by berberine compared to the control (Fig. 2D). The stimulation of NF- $\kappa$ B (NF- $\kappa$ BPr) 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- $\kappa$ B 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

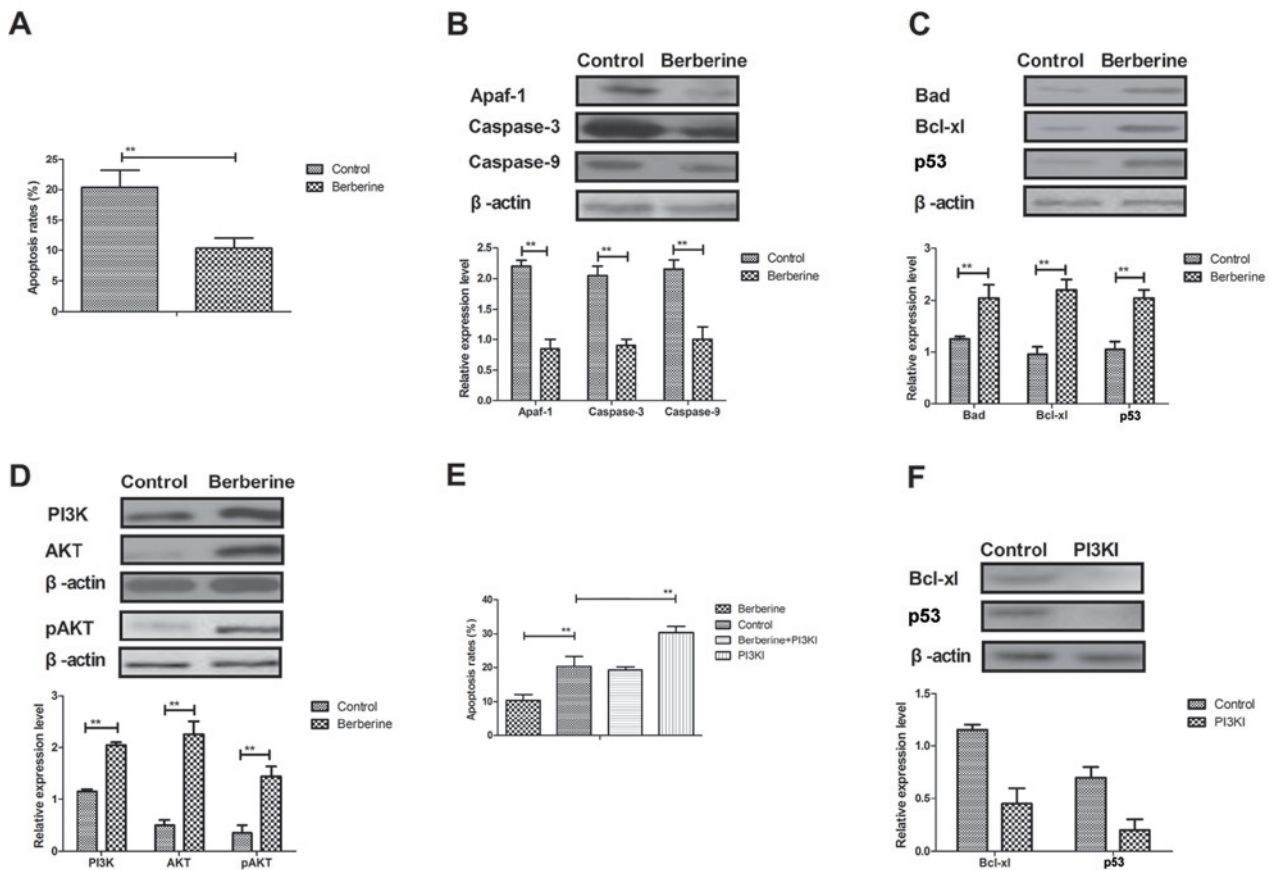


Figure 3. Berberine regulates the apoptosis of myocardial cells through the PI3K/AKT-mediated mitochondrial apoptosis signaling pathway. (A) Apoptosis of myocardial cells in experimental mice after berberine treatment. Expression of (B) Apaf-1, caspase-3 and caspase-9; (C) Bad, Bcl-xl and p53; and (D) 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- $\alpha$  serine/threonine-protein kinase; Apaf-1, Apoptotic protease-activating factor 1; Bad, Bcl-2-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

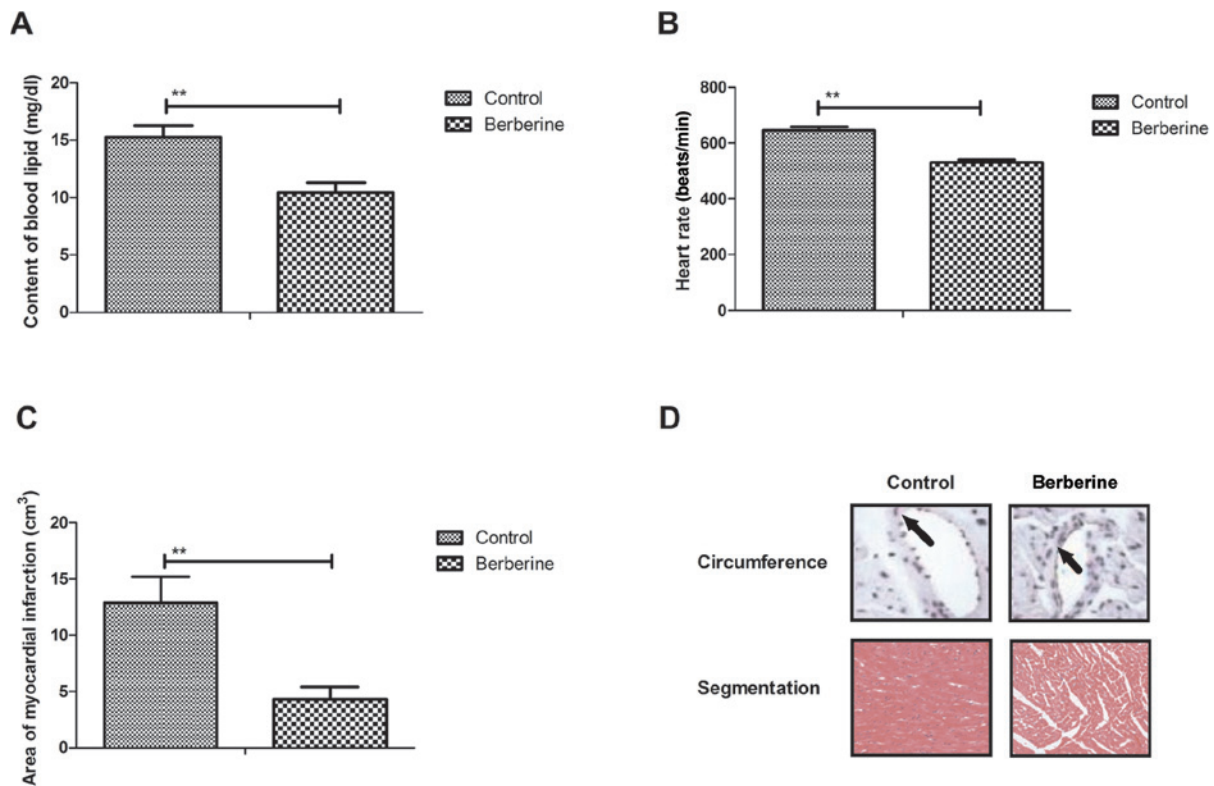


Figure 4. Berberine improves the myocardial function of mice with ischemia-reperfusion injury. (A) Blood lipid levels, (B) heart rate and (C) area of myocardial injury in mice with ischemia-reperfusion injury treated with berberine. (D) Circumference fragmentation and segmentation of the myocardial cells 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- $\kappa$ B and PI3K/AKT signaling pathways, respectively. Notably, although I $\kappa$ B $\alpha$  expression was inhibited by berberine, the expression of NF- $\kappa$ B p65 and I $\kappa$ B $\beta$  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- $\kappa$ B signaling pathway. Additionally, NF- $\kappa$ B has been reported to be associated with myocardial ischemia injury by inhibiting the expression of tumor necrosis factor  $\alpha$ -induced genes associated with ischemia/reperfusion in endothelial cells *in vivo* (49). The present study revealed that expression of NF- $\kappa$ B p65, IKK- $\beta$ , I $\kappa$ B $\alpha$  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- $\kappa$ B 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-x1 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-x1 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- $\kappa$ B 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.

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