Increased Bioavailable Berberine Protects Against Myocardial Ischemia Reperfusion Injury Through Attenuation of NFκB and JNK Signaling Pathways

Yang Yu,1,* PhD, Ming Zhang,1,* PhD, Yali Hu,1 MSc, Yali Zhao,1 MSc, Fei Teng,1 MSc, Xiaoyan Lv,1 PhD, Ji Li,1 PhD, Ying Zhang,2 MD, Grant M. Hatch,1 PhD and Li Chen,1 PhD

Summary

Activation of Janus kinase (JNK) is involved in the pathogenesis of cardiac ischemia reperfusion injury. We previously demonstrated that oral treatment of rats with high doses of berberine (BBR) improved cardiac function in ischemia reperfusion injury. It is unknown if BBR modulates JNK activation. We developed a new formula, solid dispersion of BBR with sodium caprate (HGSD), which increases its bioavailability and membrane permeability. The present study examined if HGSD-mediated inhibition of JNK protects the heart from ischemia reperfusion injury.

The cardioprotective effect of HGSD was examined in rat hearts subjected to global 45 minutes ischemia followed by 30 minutes reperfusion. Hemodynamic parameters and troponin levels in the perfusate, and TNF-α, IL-6, JNK, and NFκB levels in the heart were determined. To further explore the cardioprotective mechanism of HGSD, H9c2 cells subjected to hypoxia/reoxygenation were incubated with serum containing HGSD in the absence or presence of an activator or inhibitor of JNK.

Pretreatment of rats with HGSD for 7 days significantly improved recovery of heart function in animals subjected to ischemia reperfusion injury compared to untreated controls. In addition, HGSD pretreatment inhibited cardiac production of TNF-α and IL-6, and attenuated ischemia reperfusion induced cardiac JNK activation and nuclear translocation of NFκB compared to untreated controls. In H9c2 cells subjected to hypoxia/reoxygenation, the presence of JNK activator diminished the release of TNF-α and IL-6 and the nuclear translocation of NFκB.

HGSD treatment protects the heart from ischemia reperfusion injury through attenuation of NFκB and JNK signaling pathways.

Key words: Solid dispersion of berberine with sodium caprate (HGSD), Ischemia reperfusion heart injury, H9c2, Hypoxia/reoxygenation

Myocardial infarction is one of the leading causes of death worldwide. Although restoration of blood flow is the only way to save the myocardium from necrosis, reperfusion subsequent to coronary re-canalization further aggravates cardiac dysfunction, leading to myocardial cell death.1-3) Thus, the development of effective and low toxic inhibitors of myocardial ischemia reperfusion injury has a great clinical significance.

Janus kinase (JNK) is a mitogen activated protein kinase (MAPK) that modulates cellular proliferation, differentiation and apoptosis.4) JNK is activated by cell stress including heat shock and ultraviolet irradiation. In addition, JNK is activated in cardiac ischemia reperfusion injury, but not during ischemia.5) JNK activation is correlated with nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) activation and its translocation into the nucleus.6) As part of the inflammatory response, NFκB...
activation induces the release of tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6). It was previously demonstrated that cell necrosis and apoptosis induced by cardiac ischemia reperfusion injury were attenuated in JNK knockout mice compared to wild type.7 Thus, a drug targeting the activation of JNK might serve as an effective strategy for the treatment of ischemia reperfusion heart injury.

Berberine (BBR) is an isoquinoline alkaloid originally isolated from the Chinese herb Coptischinensis (Huang lian). The beneficial effects of BBR treatment on metabolic disease are well documented.8 Although BBR exhibits multiple therapeutic activities, its poor oral bioavailability has limited its clinical application. A new formula, solid dispersion of BBR with sodium caprate (HGSD), was recently developed, which markedly improves bioavailability of BBR.9 We previously demonstrated that BBR exhibits beneficial effects on endothelial function, ischemia reperfusion heart injury and the abnormalities associated with diabetic myocardium.10,11 Other studies have shown that BBR attenuates ischemia induced cardiac dysfunction by reducing the release of TNF-α and IL-6.12 However, the potential protective effect of HGSD on ischemia reperfusion heart injury is unknown. In addition, whether the protective effects of HGSD on ischemia reperfusion heart injury are mediated through inhibition of JNK activity and blockade of nuclear translocation of NFκB is unknown.

In the present study, we evaluated the protective effects of HGSD in a global ischemia reperfusion heart injury model and in H9c2 cells subjected to hypoxia/reoxygenation. Our results suggest that treatment with HGSD protects the heart from ischemia reperfusion injury, and the mechanism is through a reduced inflammatory injury mediated by decreased activation of JNK and NFκB.

**Methods**

**Materials:** JNK activator (Anisomycin), JNK inhibitor (SP600125) and other reagents were obtained from Sigma-Aldrich (Shanghai, China). Antibodies for P-JNK, JNK, NFκB and secondary antibody were purchased from Cell Signaling Technology Inc. (Shanghai, China). GAPDH antibody was obtained from Epitomics Inc. (Hangzhou, Zhejiang, China). BBR was purchased from Northeast Pharmaceutical Factory (Beijing, China). HGSD pretreatment were then perfused for 105 minutes without ischemia (Control, Con), or subjected to global ischemia for 45 minutes following reperfusion for 30 minutes with HGSD solution (Model, M). Hearts from the rats with HGSD pretreatment (12.5, 25, 50 mg/kg/day) were perfused for 30 minutes, and then subjected to global ischemia for 45 minutes following reperfusion for 30 minutes with HGSD treatment, (HGS). Left ventricular pressure (LVP), left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), maximum rate of ventricular pressure rise and fall (± dp/dtmax), and heart rate (HR) were recorded. Left ventricular developed pressure (LVDP) was calculated as LVSP-LVEDP and the recovery of LVDP was calculated as (LVSP-LVEDP)/LVEDPbaseline × 100%. Cardiac output was calculated as LVDP × HR. Subsequent to perfusion, the hearts were immediately frozen by immersion in liquid nitrogen.

**Determination of cardiac troponin I (cTnI) levels:** Hearts were perfused as above, and 1 mL of perfusate was collected prior to ischemia and at 2, 5, 20, and 30 minutes of reperfusion. The level of cTnI in the perfusate was determined by ELISA. Perfuse protein concentration was measured using the bicinchoninic acid (BCA) assay (Thermo, Beijing, China) with bovine serum albumin as standard. Forty mL of perfusate was used for the ELISA.

**Determination of TNF-α and IL-6 levels:** Hearts were perfused as above, and total protein was isolated from heart tissue using the RIPA buffer as per the manufacturer’s protocol (Beyotime, Nanjing, Jiangsu, China). Protein concentration was measured using the bicinchoninic acid (BCA) assay with bovine serum albumin as standard. Eighty mL of protein was used for the ELISA. The levels of TNF-α and IL-6 were determined by ELISA.

**Western blot analysis:** Total protein, cytoplasmic protein, and nuclear protein were extracted from heart tissue or from H9c2 cells using the RIPA buffer according to the manufacturer’s protocol. Protein concentration was measured as described above. Protein (80-120 μg) was separated in a 12% SDS polyacrylamide gel and transferred onto polyvinylidenefluoride (PVDF) membranes (Bio-Rad, Shanghai, China). Membranes were blocked with 5% (w/v) skim milk or 5% BSA for 2 hours at room temperature, and then incubated with rabbit polyclonal antibodies (P-JNK, 1:500; JNK, 1:1000; NFκB, 1:1000; GAPDH, 1:3000) with light shaking overnight at 4°C. The membranes were washed 3 times with 15 mL of 10 mM Tris-HCl, 150 mM NaCl and 0.1% (v/v) Tween-20, and then incubated with secondary antibody (1:2000) at room temperature for 2 hours. Protein was visualized with enhanced chemiluminescence, and images were generated
using a GENE Imaging system. The images were quantified using Image Analysis Software (Quantity One). The P-JNK results are expressed as phosphorylated protein relative to total protein. GAPDH was used as the loading control.

Preparation of Huang-Gui Solid Dispersion (HGSD) containing serum: Wistar male rats (180-200 g) were treated with HGSD (1,000 mg/kg) orally twice daily for 3 days. Blood was then obtained from the main ventral artery under aseptic conditions, and centrifuged at 3,500 rpm for 15 minutes (Legend 17R, Thermo, Beijing, China). Sera were isolated and referred to as serum containing HGSD. All sera were filtered through a 0.22 μm filter membrane and inactivated at 56°C for 30 minutes, and then stored at -20°C for subsequent studies. The concentration of BBR in the serum was determined by HPLC as previously described.13,14

Hypoxia reoxygenation of H9c2 cardiac cells: H9c2 rat cardiac myoblastic cells were obtained from Cell Resource Center (IBMS, Beijing). Cells were maintained in high glucose Dulbecco’s Modified Eagle’s medium (DMEM) with 10% fetal calf serum (FCS), 2% L-glutamine, 10% sodium bicarbonate, 10% sodium pyruvate, 5% HEPES, 1% penicillin/streptomycin, and 1% gentamycin in an incubator (37°C, 5% CO2) with 10% sodium bicarbonate, 10% sodium pyruvate, 1% penicillin/streptomycin, and 1% gentamycin in an incubator (37°C, 5% CO2) in 48-well plates. Cells were incubated for 24 hours in DMEM supplemented with 1% FCS to differentiate the cells into cardiomyocytes. To study the effects of HGSD during hypoxia/reoxygenation, differentiated H9c2 cells were incubated for 4 hours with 10% or 20% serum containing HGSD. The medium was then replaced by an isotonic solution devoid of nutrients: Tyrode’s solution in mM (NaCl 130, KCl 5, Hepes 10, MgCl2 1, CaCl2 1.8). Cells were then placed in an air tight chamber for up to 8 hours at 37°C. Hypoxia was induced by flushing a stream of 95% N2 and 5% CO2 into the chamber, until the oxygen saturation reached a value between 0.5 to 1%. At the end of the hypoxic incubation period, reperfusion was mimicked by replacement of the Tyrode’s solution with DMEM, and reoxygenation was performed by flushing a stream of 95% O2 and 5% CO2 into the chamber. Cells were then incubated under normoxic conditions for 12 hours. Subsequent to incubation, the cells were then lysed, and the lysates were used for immunoblotting. In some experiments, the cells were incubated as above in the absence or presence of the JNK inhibitor SP600125 (10 μM) or the JNK activator anisomycin (0.01 μg/mL).

Cell viability analysis: Cell viability was determined by MTT assay in 96-well cell culture plates. H9c2 cells were treated in the presence or absence with hypoxia/reoxygenation, and the culture medium was removed from the wells after treatment, and 200 μL of MTT reagent at a concentration of 1 mg/mL in PBS was added to each well. After 3 hours incubation at 37°C, MTT reagent in PBS was removed, and then the blue-colored formazan product was solubilized in 150 μL of DMSO for 20 minutes. The absorbance of converted dye was measured at a wavelength of 570 nm.

Statistical analysis: All data are expressed as the mean plus or minus standard error of the mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA). The level of significance was defined as P < 0.05. All statistical analyses were carried out using SPSS 20.0.

Results

Pretreatment of rats with HGSD attenuates cardiac dysfunction induced by ischemia reperfusion injury: To investigate whether HGSD treatment improves the recovery of left ventricular function in ischemia reperfusion cardiac injury, rats were pretreated in the absence or presence of HGSD (12.5, 25, 50 mg/kg/day) for 7 days, and the hearts were removed and perfused under normoxic conditions (Con) or subjected to global ischemia for 45 minutes followed reperfusion for up to 30 minutes (I/R). The recovery of left ventricular developed pressure (LVDP), left ventricular end diastolic pressure (LVEDP), and left ventricular developed pressure (LVDP) × heart rate (LVDP*HR) were significantly different before the ischemia among all the groups, including the control group, model group, and HGSD treatment group, which might indicate the HGSD treatment had no effect on the hemodynamic parameters under normoxic conditions. Furthermore, after 45 minutes of ischemia, systolic and diastolic functions were significantly depressed, with declines in LVDP and cardiac output in the model group. After 30 minutes of reperfusion, LVDP and cardiac output had slightly recovered, but remained significantly lower than at the beginning of the perfusion. LVEDP was increased significantly after 10 minutes of ischemia reperfusion. Pretreatment of rats with HGSD (12.5, 25, 50 mg/kg) significantly improved cardiac dysfunction caused by ischemia in a dose-dependent manner. After 30 minutes of reperfusion, HGSD (25 and 50 mg/kg) pretreatment significantly enhanced the recovery of cardiac LVDP 2-fold (P < 0.05), cardiac output 2.5-fold (P < 0.05), and decreased cardiac LVEDP 1.8-fold (P < 0.05) compared to the hearts of untreated animals. These data indicate that HGSD treatment of rats protects against cardiac dysfunction induced by ischemia reperfusion heart injury.

Pretreatment with HGSD decreases cardiac release of cTnl and production of cardiac TNF-α and IL-6 induced by ischemia reperfusion injury: Cardiac troponin I (cTnl) is a marker of cardiac muscle tissue injury. Rats were pretreated in the absence or presence of HGSD 25 mg/kg/day for 7 days, and the hearts were removed and perfused under normoxic conditions (Con) or subjected to global ischemia for 45 minutes followed by reperfusion for up to 30 minutes (I/R). Also, the release of cTnl into the perfusate was determined at 2, 5, 20, and 30 minutes of reperfusion. As shown in Figure 2A, the level of cTnl released into the perfusate remained unaltered under normoxic (Con) conditions. In contrast, ischemia followed by reperfusion (I/R) resulted in a significant increase in cTnl released into the perfusate by 2 minutes compared to Con. Pretreatment of rats with HGSD significantly attenuated the release of cTnl into the perfusate after ischemia reperfusion. Thus, HGSD pretreatment attenuates myocardial damage induced by ischemia reperfusion injury.
Figure 1. Pretreatment of rats with HGSD attenuated cardiac dysfunction induced by ischemia reperfusion injury. Rats were pretreated in the absence or presence of HGSD (12.5, 25, 50 mg/kg/day) for 7 days, and the hearts were removed and perfused under normoxic conditions (Con) or subjected to global ischemia for 45 minutes followed by reperfusion for 30 minutes (I/R). Recovery of left ventricular developed pressure (LVDP) (A), left ventricular end diastolic pressure (LVEDP), (B) and LVDP*HR (C) were determined as described in the Materials and Methods. Data are presented as the mean ± SEM, n = 6. *P < 0.05, **P < 0.01 compared to Con, *P < 0.05 compared to I/R.
Increased production of cytokines plays a key role in cardiac ischemia reperfusion injury. Rats were pretreated in the absence or presence of HGSD 25 mg/kg/day for 7 days, and the hearts were removed and perfused under normoxic conditions (Con) or subjected to global ischemia for 45 minutes followed by reperfusion for up to 30 minutes (I/R). Cardiac TNF-α and IL-6 were subsequently determined. I/R hearts exhibited a significant increase in TNF-α and IL-6 levels compared to Con (Figures 2B, 2C). Pretreatment of rats with HGSD resulted in significant 35% (P < 0.05) and 36% (P < 0.05) reductions in TNF-α and IL-6 levels, respectively, compared to I/R. Thus, HGSD pretreatment attenuates production of cardiac cytokines induced by ischemia reperfusion injury.

**Pretreatment of rats with HGSD reduces cardiac JNK activation and nuclear translocation of NFκB in response to ischemia reperfusion injury:** To explore the mechanism by which HGSD attenuates production of TNF-α and IL-6 in ischemia reperfusion injury in the heart, rats were pretreated with the absence or presence of HGSD 25 mg/kg/day for 7 days, and the hearts were removed and perfused under normoxic conditions (Con) or subjected to global ischemia for 45 minutes followed by reperfusion for up to 30 minutes (I/R). The expressions of JNK, P-JNK and NFκB were determined. Total levels of JNK were unaltered under all conditions (Figure 3A). In contrast, the ratio of P-JNK/JNK was significantly increased in I/R hearts compared to Con. In rats pretreated with HGSD, the ratio of P-JNK/JNK was significantly reduced compared to I/R. Total levels of NFκB were unaltered under all conditions (Figure 3B). In contrast, cytosolic NFκB was reduced 4-fold, and nuclear NFκB in-
Figure 3. Pretreatment of rats with HGSD reduced cardiac JNK activation and nuclear translocation of NFκB in response to ischemia reperfusion injury. Rats were pretreated in the absence or presence of HGSD 25 mg/kg/day for 7 days, and the hearts were removed and perfused under normoxic conditions (Con) or subjected to global ischemia for 45 minutes followed by reperfusion for up to 30 minutes (I/R). The expressions of JNK and P-JNK (A) and cytoplasmic and nuclear NFκB (B) were determined as described in the Materials and Methods. Representative blots are depicted. A: Left panel, representative blots of P-JNK, JNK and GAPDH. Right panel, relative ratio of P-JNK/JNK. B: Top left panel, representative blots of cytosolic, nuclear and total P65-NFκB and GAPDH. Lower left panel, relative ratio of total P65-NFκB/GAPDH. Top right panel, relative ratio of cytosolic P65-NFκB/GAPDH. Lower right panel, relative ratio of nuclear P65-NFκB/GAPDH. Data are presented as the mean ± SEM. n = 6. *P < 0.05 compared to Con, †P < 0.05 compared to I/R.

creased 5-fold in I/R hearts compared to Con. In rats pretreated with HGSD, the translocation of NFκB to the nucleus was significantly reduced compared to I/R. Thus, HGSD pretreatment reduces cardiac JNK activation and nuclear translocation of NFκB in response to ischemia reperfusion injury.

Serum from HGSD pretreated rats protects H9c2 cells from hypoxia/reoxygenation injury, and JNK activation stimulated loss in H9c2 subjected to hypoxia/reoxygenation injury: We further explored the potential mechanism of the protective effect of HGSD on ischemia reperfusion heart injury in differentiated H9c2 cardiac myocytes. Serum containing HGSD was prepared by pretreating rats orally twice daily with 1,000 mg/kg HGSD for 3 days. The serum concentration of BBR from HGSD pretreated rats was determined by HPLC to be 27.9 ± 2.7 μM (n = 10). We initially examined the viability of H9c2 cells subjected for up to 8 hours to hypoxia followed by 12 hours of reoxygenation and incubation in the absence (H/R) or presence of 10% (10% HGSD) or 20% (20% HGSD) concentrations of serum prepared from HGSD pretreated rats. Control (Con) cells were incubated under normoxic conditions, and received 20% serum from untreated rats. The 10% and 20% HGSD serum contained 2.79 μM and 5.59 μM BBR, respectively. Cell viability was unaltered by 4 or 6 hours of hypoxia/reoxygenation (Figure 4A, B). In contrast, cell viability was significantly reduced by 8 hours of hypoxia/reoxygenation compared to
Serum from HGSD pretreated rats protected H9c2 cells from hypoxia/reoxygenation injury, and JNK activation stimulated loss in H9c2 subjected to hypoxia/reoxygenation injury. H9c2 cells were incubated with 20% serum (Con) or incubated in the absence (H/R) or presence of 10% (10% HGSD) or 20% (20% HGSD) concentrations of serum prepared from HGSD pretreated rats, and cell viability was determined in cells subjected to 4 hours (A), 6 hours (B), or 8 hours (C) of hypoxia followed by 12 hours of reoxygenation.

Figure 4. Serum from HGSD pretreated rats protected H9c2 cells from hypoxia/reoxygenation injury, and JNK activation stimulated loss in H9c2 subjected to hypoxia/reoxygenation injury. H9c2 cells were incubated with 20% serum (Con) or incubated in the absence (H/R) or presence of 10% (10% HGSD) or 20% (20% HGSD) concentrations of serum prepared from HGSD pretreated rats, and cell viability was determined in cells subjected to 4 hours (A), 6 hours (B), or 8 hours (C) of hypoxia followed by 12 hours of reoxygenation.

Con (Figure 4C). Incubation of cells with 20% serum from HGSD pretreated rats attenuated the loss in cell viability mediated by 8 hours of hypoxia/reoxygenation. Thus, serum from HGSD pretreated rats protects H9c2 cells from hypoxia/reoxygenation injury.

Serum from HGSD pretreated rats attenuates the production of TNF-α and IL-6 in H9c2 cells subjected to hypoxia/reoxygenation through downregulation of JNK activity and reduced nuclear localization of NFκB: H9c2 cells were incubated for 12 hours in 20% serum prepared from untreated rats or rats pretreated with HGSD, and incubated plus or minus the JNK inhibitor SP6000125 or the JNK activator anisomycin for 4 hours, and then subjected to hypoxia/reoxygenation, and cell availability was determined. As shown in Figure 5A, incubation of cells with anisomycin reduced cell viability, and attenuated the protective effect of HGSD on cell viability. In contrast, incubation of cells with SP6000125 attenuated the loss in cell viability mediated by hypoxia/reoxygenation. Incubation of cells with both SP6000125 and HGSD serum resulted in a further increase in cell viability, but this did not differ from cells incubated with SP6000125 alone. Thus, JNK activation stimulates loss in H9c2 cell viability mediated by hypoxia/reoxygenation injury.

The production of TNF-α and IL-6 is an important factor involved in cardiomyocyte damage mediated by hypoxia/reoxygenation. We examined if an HGSD mediated reduction in activation of JNK contributed to the attenuation of production of TNF-α and IL-6 in H9c2 cells. H9c2 cells were subjected to hypoxia for 8 hours followed by 12 hours of reoxygenation incubation in the absence or presence of 20% HGSD serum prepared from HGSD pretreated rats. The productions of TNF-α and IL-6 were also determined. Control cells (Con) were incubated under normoxic conditions, and received 20% serum from untreated rats. In H9c2 cells subjected to hypoxia/reoxygenation, TNF-α and IL-6 levels were increased 1.9-fold and 2.7-fold, respectively, compared to Con (Figure 5 B, C). HGSD significantly decreased the productions of TNF-α and IL-6 in H9c2 cells subjected to hypoxia/reoxygenation. The presence of the JNK activator anisomycin reversed the effect of HGSD on the production of TNF-α and IL-6.

The above data indicate that the inhibitory effect of HGSD on the production of TNF-α and IL-6 might be mediated through reduced activation of JNK. We thus ex-
Figure 5. Serum from HGSD pretreated rats attenuated the production of TNF-α and IL-6 in H9c2 cells subjected to hypoxia/reoxygenation through JNK. H9c2 cell viability was determined in H9c2 cells incubated in the absence or presence of 20% serum prepared from HGSD pretreated rats in the absence or presence of the JNK activator anisomycin or the JNK inhibitor SP600125, and then subjected to 8 hours of hypoxia followed by 12 hours of reoxygenation. Data are presented as the mean ± SEM. n = 6. *P < 0.05 compared to 1, †P < 0.05 compared to 7, ‡P < 0.05 compared to 8. B and C: Expressions of TNF-α and IL-6. *P < 0.05 compared to Con, †P < 0.05 compared to H/R, ‡P < 0.05 compared to HGSD.
examined the ratio of P-JNK/JNK. The ratio of P-JNK/JNK was increased 37.8% in H9c2 cells subjected to hypoxia for 8 hours followed by 12 hours of reoxygenation compared to Con (Figure 6A). HGSD significantly decreased the ratio of P-JNK/JNK in H9c2 cells subjected to hypoxia/reoxygenation. The presence of the JNK activator anisomycin reversed the effect of HGSD on P-JNK/JNK. Thus, serum from HGSD pretreated rats attenuated the production of TNF-α and IL-6 in H9c2 cells subjected to hypoxia/reoxygenation through down regulation of JNK activity.

We next examined the expression of NFκB in these cells. Nuclear NFκB was significantly increased by 130% and cytosolic NFκB decreased by 65% in H9c2 cells subjected to hypoxia for 8 hours followed by 12 hours of reoxygenation compared to Con (Figure 6B). HGSD significantly reduced the amount of nuclear NFκB and increased the amount of cytosolic NFκB in H9c2 cells subjected to
hypoaxia/reoxygenation. The presence of the JNK activator anisomycin reversed the effect of HGSD on the translocation of NFκB between nuclear and cytosolic fractions. Thus, serum from HGSD pretreated rats reduced nuclear localization of NFκB in H9c2 cells subjected to hypoaxia/reoxygenation.

Discussion

Myocardial infarction disturbs the balance between oxygen supply and demand, resulting in myocardial dysfunction or injury. Restoration of blood flow is a key strategy that diminishes injury induced by ischemia. However, restoration of blood flow triggers an inflammatory cascade, which results in further damage to the ischemic myocardium. This is known as reperfusion injury. Berberine (BBR) is an alkaloid derivative from the plant Berberis vulgaris (European barberry), which has been extensively studied for its multiple pharmacological activities. Our group and others have reported that BBR could attenuate heart injuries induced by ischemia reperfusion. However, the protective mechanism of BBR on anti-inflammatory injury remains unclear. Moreover, the bioavailability of BBR is poor, and extensive efforts to improve it have been made over the past 10 years. Solid Dispersion of BBR with sodium caprate (HGSD) was developed by our laboratory as a new formula for increasing the bioavailability of BBR. It was unknown whether HGSD could protect the heart from ischemia reperfusion injury.

In the present study, we evaluated the protective effect of HGSD pretreatment on ischemia reperfusion mediated heart injury in the rat heart. HGSD pretreatment improved the recovery of left ventricular developed pressure and cardiac output, and decreased cTnI release in rat hearts subjected to 45 minutes of ischemia followed by 30 minutes of reperfusion compared to control. The protective effect of HGSD on ischemia reperfusion heart injury was consistent with previous studies reported for BBR treatment alone. For example, in an acute ischemia reperfusion heart injury model, BBR pretreatment attenuated the infarct size and occurrence of arrhythmias through regulation of AMPK activity. The cardioprotective mechanism of BBR may be related to suppression of autophagy activation. Another study reported that treatment of rats with Coptisine, which contains 98% BBR, protected hearts from myocardial ischemia reperfusion injury. The mechanism was proposed to be mediated through suppression of myocardial apoptosis and inflammation mediated by inhibition of the Rho/ROCK pathway. In view of the re-injury caused by an inflammatory response in the reperfusion period, we further wanted to examine whether BBR could protect myocardial ischemia reperfusion by anti-inflammatory reaction, and explored the potential mechanism.

The increased production of cytokines is a major complication in the inflammatory cascade during ischemia reperfusion heart injury. Ischemia reperfusion injury induces the release of many cytokines and chemokines, including tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6), which have been implicated with most pathophysiological responses in cardiovascular disease. Cardiomyocyte damage or heart injury are induced when extracellular and endogenous TNF-α and IL-6 accumulate to excess levels in the heart. The productions of TNF-α and IL-6 are promoted through increased NFκB. Release of TNF-α and IL-6 further activate upstream of NFκB to translocate to the nucleus, and promote production of these cytokines. JNK is an inflammation triggered MAP kinase that can be activated through various extracellular stimuli, such as ischemic stress and lipopolysaccharide. It is a key upstream regulator of NFκB as well. It has been reported that some drugs inhibit JNK-mediated NFκB activation to protect the heart from ischemia reperfusion injury. However, it was unknown if JNK mediated NFκB activation is a target of BBR in the protection of ischemia reperfusion heart injury. In our study, pretreatment of rats with HGSD in vivo decreased the production of TNF-α and IL-6, and inhibited the activation of JNK and nuclear translocation of NFκB in hearts subjected to ischemia reperfusion injury.

Moreover, in the present study, we attempted to explore the pharmacological effects of HGSD in vivo. The serum pharmacology method of Huang et al was used. Sera were isolated from animals administered with drug, and this was referred to as serum containing drug. This method could reflect the true pharmacological action of a drug (especially multi-component formulas) in the body. Therefore, serum pharmacology in cell experiments was used to mimic the condition in vivo. However, since the BBR was the main pharmacological component in the HGSD, the concentration of BBR in HGSD pretreated serum was first evaluated. The concentration of BBR in HGSD pretreated serum was 2.79 μM in 10% serum and 5.59 μM in 20% serum, and this dose was also in the range of other BBR pharmacological studies. The in vivo pharmacological action of HGSD was confirmed at the cellular level, which also might indicate the critical role of BBR in this new formula. Furthermore, treatment of H9c2 cells in vitro with serum from HGSD pretreated rats inhibited the activation of JNK, nuclear translocation of NFκB, and attenuated the production of TNF-α and IL-6 in cells subjected to hypoaxia/reoxygenation. The presence of the JNK activator anisomycin reversed the effects of HGSD on the productions of TNF-α and IL-6, and nuclear translocation of NFκB. These data indicate that HGSD exerts its protective role, in part, through inhibition of the JNK-mediated NFκB pathway, suggesting that HGSD may serve as a potential therapeutic for myocardial ischemia/reperfusion injury.

In summary, our study demonstrates that HGSD, as a new formula of BBR, protects the heart from ischemia reperfusion injury, and the mechanism is through the anti-inflammatory effect of BBR. The attenuations of JNK mediated NFκB activation and cytokine production may contribute to the protective effect of BBR on ischemia reperfusion heart injury. Since several other signaling pathways regulate upstream of NFκB, such as the ERK, P38MAPK, and PI3K/Akt-related signaling pathways, future studies may identify novel molecules involved in the regulation of NFκB activation through HGSD.
Disclosures

Conflicts of interest: The authors have declared no conflict of interest.

References