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Original Article

Ginsenoside Rd mitigates myocardial ischemia-reperfusion injury via Nrf2/HO-1 signaling pathway

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Abstract: Ginsenoside Rd (GsRd) reportedly protects the heart against ischemia-reperfusion (I/R) injury. Nrf2/HO-1 signaling plays a key role in attenuating oxidative stress. However, it remains unclear whether GsRd protects against myocardial I/R injury via Nrf2/HO-1 signaling. This study aimed to investigate the role of Nrf2/HO-1 signaling in the cardioprotective effect of GsRd. Rats received 30 min ischemia followed by 2 h reperfusion. Cardiac function, infarct size and serum CK, LDH, cTnI levels were detected. The expression of Nrf2 and HO-1 was detected by western blot. The results suggested that GsRd attenuated myocardial I/R injury as evidenced by improved cardiac function, decreased infarct size and decreased levels of serum CK, LDH and cTnI. In addition, GsRd administration enhanced the expression of Nrf2 and HO-1. In conclusion, the present study shows that GsRd protects against myocardial I/R injury via Nrf2/HO-1 signaling.

Keywords: Ginsenoside Rd, Nrf2/HO-1, myocardial ischemia-reperfusion injury

Introduction

Coronary heart disease exhibits a high morbidity and mortality, and it remains one of the major causes of death all around the world [1]. To give blood supply to the myocardium as soon as possible is the key to alleviate myocardial infarction [2]. However, reperfusion causes a lot of disorders, such as reactive oxygen species (ROS) overproduction, inflammation, apoptosis, calcium overload and mitochondrial dysfunction [3, 4]. To date, much attention has been paid to the overproduction of ROS, and it is indicated that decreasing ROS may alleviated myocardial injury to a great extent [5]. However, there are still few effective therapies to alleviate ischemia-reperfusion injury.

The nuclear factor erythroid-2 related factor 2 (Nrf2), a transcription factor sensitive to the redox state in the cell, plays a key role in fighting against oxidative stress in the cells. Nrf2 activation has been suggested to be protective in many disorders, such as cerebral ischemia, retinal ischemia and myocardial ischemia [6-8]. Under physiological conditions, Nrf2 is located in the cytoplasm and it binds to Kelch-like ECH-associated protein 1 (Keap1). When threatened by oxidative stress and other stimuli, Nrf2 dissociates from Keap1 and then translocates from the cytoplasm to the nucleus [9, 10]. In the nucleus, Nrf2 binds to the antioxidant response element (ARE) sequence, activating the transcription of antioxidative genes, including heme oxygenase-1 (HO-1) and NAD(P)H: quinine oxidoreductase 1 (NQO1) [11].

Ginseng, known as the root of Panax ginseng C.A. Meyer, has been widely used as a valuable medicinal herb for more than 20 centuries in China [12]. There have been more than 40 kinds of ginsenosides isolated from Ginseng, including ginsenoside Rd (GsRd) [13] (**Figure 1**). It has been suggested that GsRd exerts various pharmacological effects, such as removing free radicals [14-16], inhibiting calcium influx [17] and anti-apoptosis [18]. In addition, GsRd promotes neurogenesis in rat brain after transient focal cerebral ischemia and is protective to cerebral ischemia [13, 19]. As for the cardiovas-

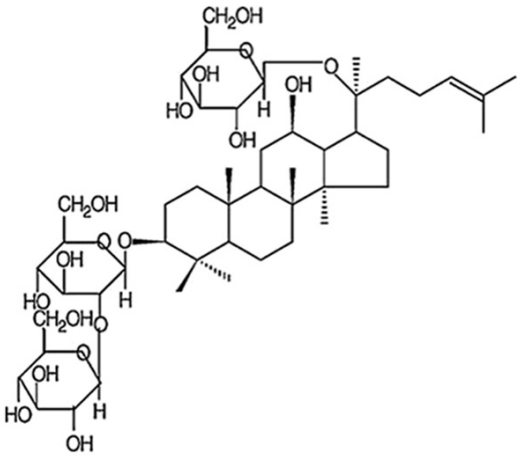

Figure 1. Structure of ginsenoside Rd.

cular system, GsRd has been suggested to be beneficial. GsRd is reported to attenuate myocardial ischemia-reperfusion injury through Akt/GSK-3βsignaling. However, whether GsRd protects the heart from ischemia-reperfusion via Nrf2/HO-1 signaling has not been elucidated. The present study, therefore, aims to testify the hypothesis that GsRd protects against myocardial I/R injury via Nrf2/HO-1 signaling pathway.

Materials and methods

Reagents

GsRd (a purity of 98%) was purchased from Tai-He Biopharmaceutical Co. Ltd (Guangzhou, China). 2,3,5-triphenyltetrazolium chloride (TTC) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against Nrf2, HO-1, NQO1, lamin B and β-actin were purchased from Santa Cruz Biotechnology. CK assay kit, LDH assay kit and cTnI assay kit were purchased from Beyotime (Shanghai, China).

Animals

Male Sprague-Dawley (SD) rats (270-320 g) were purchased from the Experimental Animal Center of the Kunming Medical University. All animals were kept at 22-24°C under a 12 hour/12 hour light-dark cycle. And the rats had free access to food and water. All procedures were performed in adherence with the National Institutes of Health Guidelines for the Use of Laboratory Animals (NIH publication no. 85-23, revised 1996), and were approved by the Kunming Medical University Committee on Animal Care.

Rat myocardial ischemia/reperfusion model

Rats were anesthetized intraperitoneally via the administration of pentobarbital sodium at a dose of 50 mg/kg (Sigma, St. Louis, USA). The n, a left thoracic incision was made to expose the heart. Myocardial ischemia was triggered by occluding the left anterior descending coronary artery with a 6-0 silk slipknot around LAD. After ischemia for 30 min, the slipknot was relaxed allowing for 120 min of reperfusion. The sham group underwent the same procedures except for the coronary slipknot.

Experimental protocol

Rats were randomly assigned into three groups (n = 8 in each group): (1) sham group (2) I/R group: rats were administrated intraperitoneally with vehicle (0.9% NaCl); (3) I/R + GsRd group: GsRd (50 mg/kg, i.p.) was administered 30 min prior to reperfusion. The dose of GsRd was chosen according to previous studies [20].

Assessment of heart function

Rats were anesthetized with the intraperitoneal administration of sodium pentobarbital (50 mg/kg). A catheter was then inserted into the left ventricle through the right common carotid artery for assessing left ventricular ejection fraction (LVEF), left ventricular end-diastolic pressure (LVEDP) and the maximal rate of rise and decline of ventricular pressure (±dp/dt [max]). The data was obtained and analyzed with the AcqKnowledge 4.0 software.

Determination of myocardial infarct size

At the end of reperfusion, the slipknot was retied and Evans blue (2%, 4 ml) was administrated through the aorta into the body. The heart was quickly removed and was conserved in a -20°C refrigerator. The heart was then cut into 2 mm slices, and incubated in TTC solution for 20 min, and then in 4% formaldehyde solution overnight. Evans blue stained area indicates for non-I/R area (blue). TTC stained area indicates for the area at risk (AAR) red. Non-TTC stained area indicates for infarction (white).The size was analyzed with OPTIMAS software. Myocardial infarct area was calculated as follows: infarct area/area at risk% (INF/AAR%).

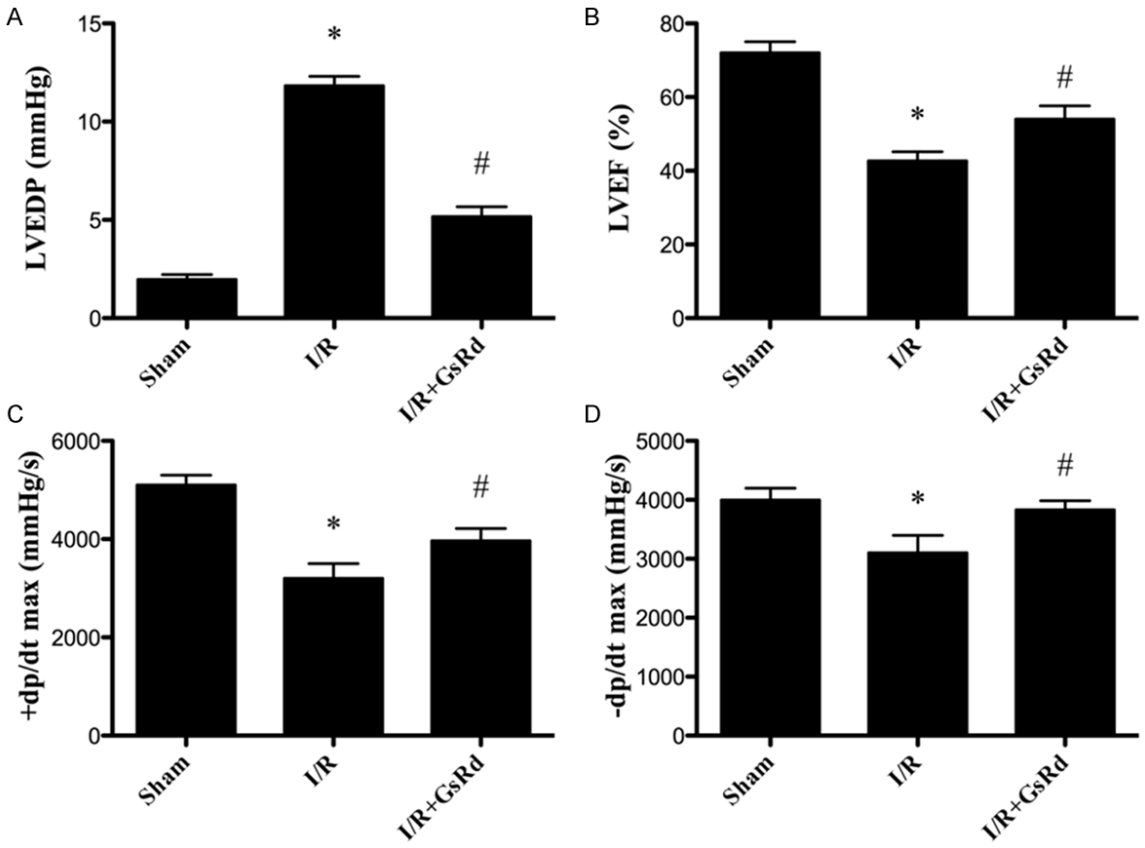

Figure 2. Effect of ginsenoside Rd on cardiac function. A. The effect of ginsenoside Rd on LVEDP. B. The effect of ginsenoside Rd on LVEF. C. The effect of ginsenoside Rd on +dp/dx max. D. The effect of ginsenoside Rd on -dp/dx max. LVEF, left ventricle ejection fraction; LVEDP, left ventricle end-diastolic pressure. Data were expressed as mean ± S.E.M. (n = 8 in each group). $^{*}P < 0.01$ versus the sham group, $^{\#}P < 0.05$ versus I/R group.

Evaluation of CK, LDH and cTnI

At the end of reperfusion, blood was collected from the heart to evaluate the levels CK, LDH and cTnI. The samples were centrifuged and the serum was collected to evaluate the levels of CK, LDH and cTnI according to manufacturer's instruction.

Western blot

Protein extracts were prepared using the heart tissue by homogenization in a RIPA buffer. Protein was measured with the BCA Protein Assay kit (Beyotime, China). And proteins were separated by electrophoresis on SDS-PAGE (10%) and transferred to a nitrocellulose membrane. The membranes were blocked with TBST with 5% nonfat milk for 2 h at room temperature. The membranes were then incubated with the primary antibody rabbit anti-Nrf2, rabbit anti-NQO1 (1:500, Santa Cruz Biotechnology) rabbit anti-HO-1 (1:2000, Santa Cruz Biotechnology). Then, membranes were washed with TBST. The membranes were incubated for 1 h at room temperature with the corresponding secondary antibodies (1:3000, goat anti-rabbit; 1:5000, goat anti-mouse, Santa Cruz Biotechnology). Finally, western blots were detected using ECL Plus Detection kit (Millipore, USA). And the blots were visualized by a Bio-Rad Imaging system.

Statistical analysis

All values are presented as means ± S.E.M. The significance of differences were evaluated by Dunnett's t-test or ANOVAs. A value of $P < 0.05$ was considered statistically significant.

Results

GsRd improved cardiac function

As shown in the **Figure 2**, GsRd significantly increased LVEF and ±dp/dt max and lowered

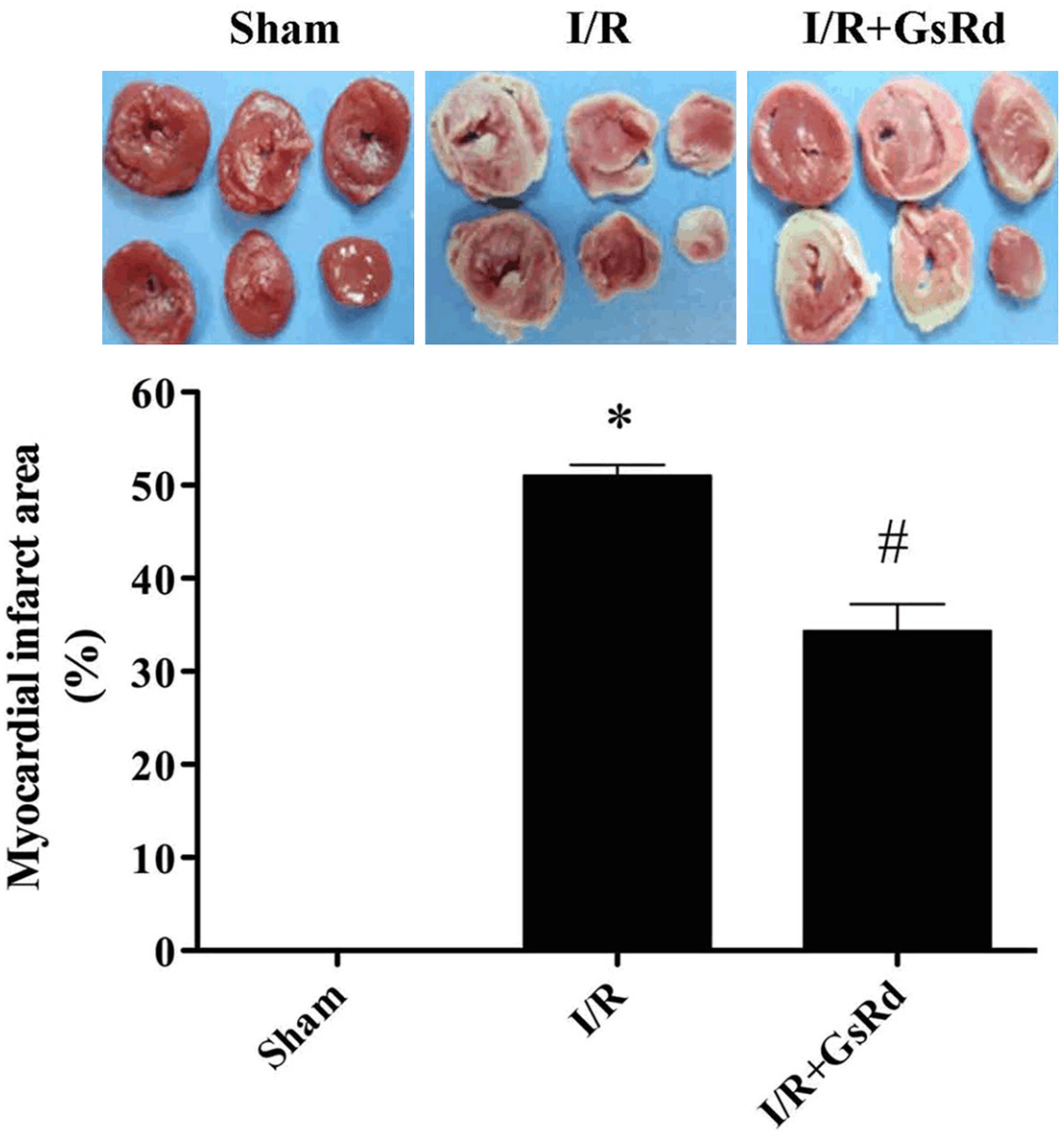

Figure 3. Effect of ginsenoside Rd on myocardial infarction size. Data were expressed as mean ± S.E.M. (n = 8 in each group). $^{*}P < 0.01$ versus the sham group, $^{\#}P < 0.05$ versus I/R group.

LVEDP in I/R + GsRd group compared with those in the I/R group ($P < 0.05$) (**Figure 2**).

GsRd attenuates myocardial infarction

As shown in the **Figure 3**, MI/R resulted in a dramatic infarction. GsRd reduced myocardial infarction area markedly compared with that the I/R group ($P < 0.05$) (**Figure 3**).

Effect of GsRd on the activity of serum CK, LDH and cTnI

In the **Figure 4**, the activity of LDH, CK and cTnI increased dramatically in the I/R group compared with those levels in sham group ($P < 0.05$). However, GsRd treatment significantly decreased CK, LDH and cTnI activity compared with those in the I/R group ($P < 0.05$) (**Figure 4**).

Effect of GsRd on Nrf2/HO-1 signaling

As shown in the **Figures 5**, **6** the expression of Nrf2, HO-1 and NQO1 were detected by Western blot. The nucleus and total Nrf2, HO-1 and NQO1 were dramatically enhanced in I/R group. GsRd treatment significantly increased the expression of nucleus and total Nrf2, HO-1 and NQO1 compared with those in I/R group ($P < 0.05$) (**Figures 5**, **6**).

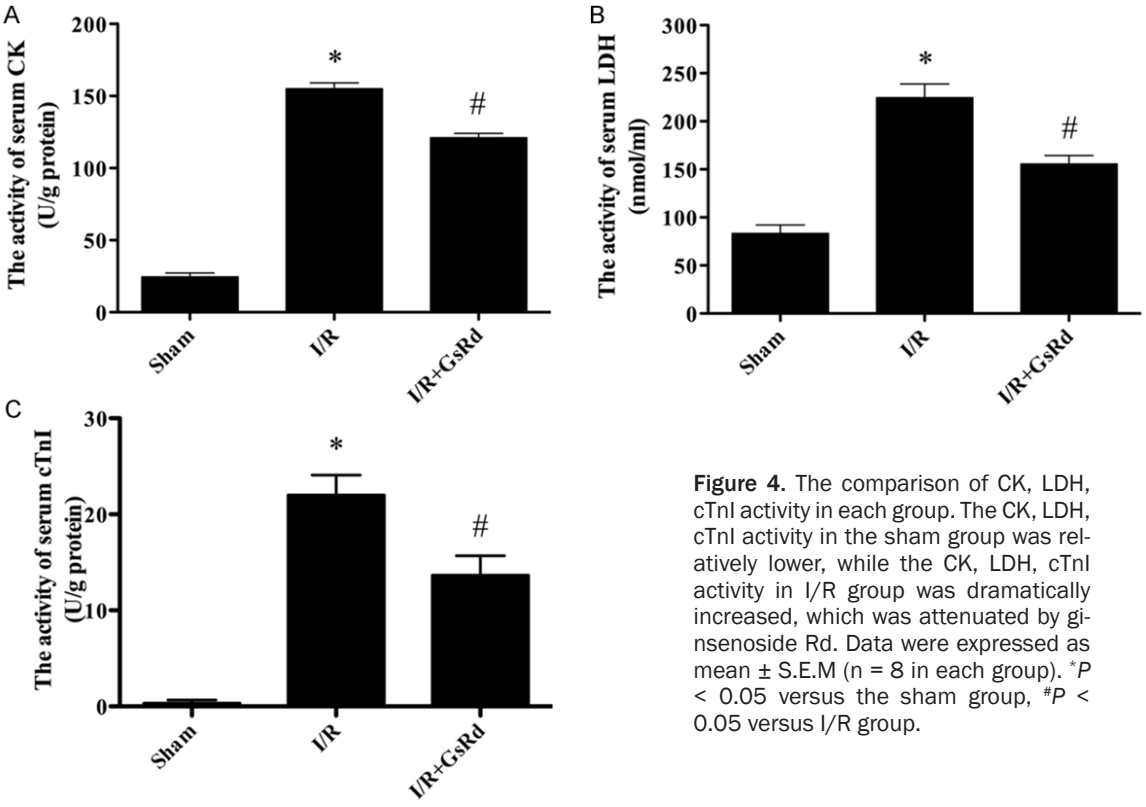

Figure 4. The comparison of CK, LDH, cTnI activity in each group. The CK, LDH, cTnI activity in the sham group was relatively lower, while the CK, LDH, cTnI activity in I/R group was dramatically increased, which was attenuated by ginsenoside Rd. Data were expressed as mean ± S.E.M (n = 8 in each group). $^{*}P < 0.05$ versus the sham group, $^{\#}P < 0.05$ versus I/R group.

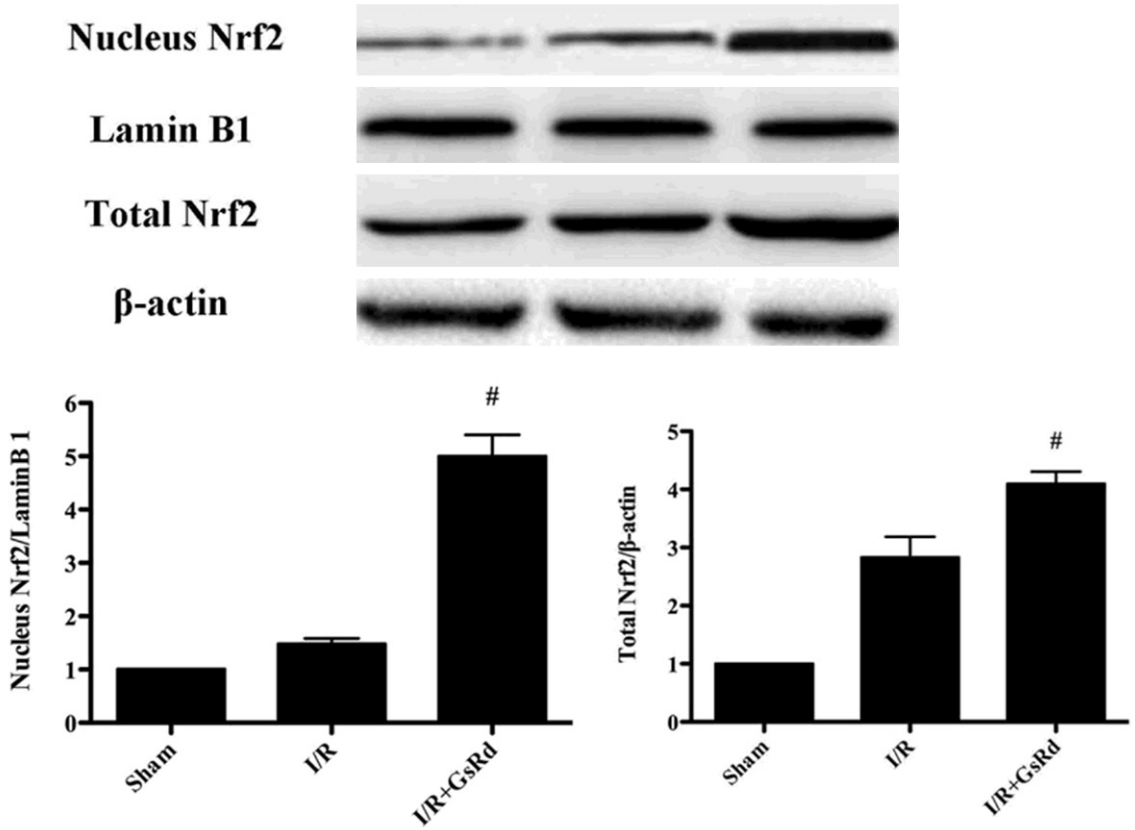

Figure 5. Effects of ginsenoside Rd on Nrf2 expression in rats subjected to myocardial I/R. The representative images of nucleusand total Nrf2 are shown. The results are expressed as the mean ± S.E.M. (n = 8 in each group). #P < 0.05 versus I/R group.

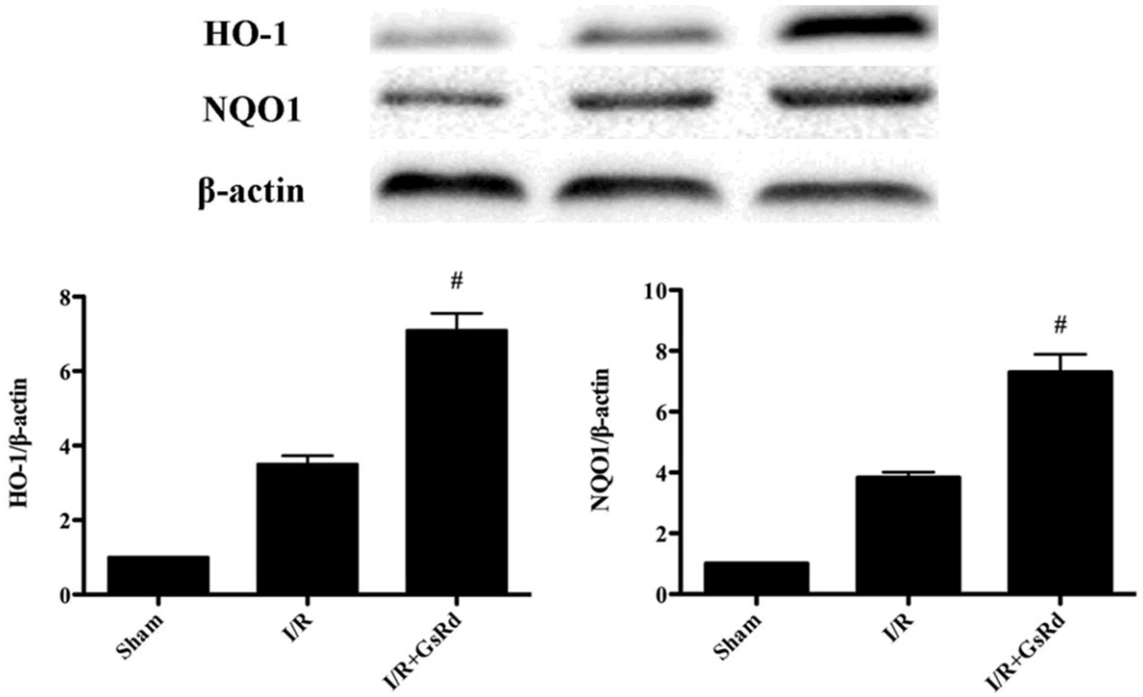

Figure 6. Effects of ginsenoside Rd on HO-1 and NQO1 expression in rats subjected to myocardial I/R. The representative images of HO-1 and NQO1 are shown. The results are expressed as the mean ± S.E.M. (n = 8 in each group). #P < 0.05 versus I/R group.

Discussion

The important observations in our present study are: (1) GsRd attenuates myocardial I/R injury through alleviating cardiac dysfunction. (2) GsRd attenuates myocardial I/R injury by decreasing serum CK, LDH and cTnI activities. (3) The cardioprotective effect of GsRd is associated with the activation of Nrf2/HO-1 signaling.

The ginseng has been used worldwide for a long time, especially in China. Ginsenosides Rd is one of the major ginseng components. In addition, more than 40 ginsenosides have been identified [13]. Various biological effects of ginsenosides have been reported in recent researches. Xie et al. reported that ginsenoside-Rg1 protects against cerebral ischemia/reperfusion injury in rats by downregulating the expression of protease-activated receptor-1 [21]. Zhu et al. reported that ginsenoside Rd ameliorates experimental autoimmune encephalomyelitis in C57BL/6 mice [22]. Wu et al. reported that ginsenoside Rb1 protects rats against sepsis [23]. In addition, previous studies have demonstrated that ginsenosides have a huge protective effect against cardiovascular disorders [24-27]. Wang et al. reported that ginsenoside Rb1 preconditioning protects against myocardial infarction after ischemia and reperfusion via activating phosphatidylinositol-3-kinase (PI-3K) signaling [28]. In the present study, we found that GsRd improved cardiac function, increasing ±dP/dt max and LVEF and decreasing LVEDP, GsRd also decreased myocardial infarct size and lowered the activity of CK, LDH and cTnI, which stands for the severity of myocardial injury.

Ischemia-reperfusion induces the dissociation of Nrf2 from Keap1, leading to the translocation to the nucleus, then binding to the ARE, and activating phase 2 antioxidant and detoxifying genes, such as HO-1 and NQO1 [29]. HO-1 is a rate-limiting enzyme, catalyzing the degra-

dation of heme into biliverdin, carbon monoxide and ferritin [30]. NQO1 is generally regarded as a detoxifying enzyme, since it is able to remove reactive quinones and quinone imines and to diminish toxic hydroquinones [31]. As a result, the up-regulation of HO-1 and NQO1 protects the heart against oxidative stress induced by I/R injury. Moreover, Nrf2/HO-1 signaling pathway have effect on cell survival via various substrates, such as Bcl-2 and Bax [32]. Our results suggest that GsRd attenuates myocardial ischemia reperfusion injury by activating Nrf-2/HO-1 signaling.

In conclusion, our findings suggest that GsRd mitigates myocardial ischemia/reperfusion injury as evidenced by elevated cardiac function and decreased CK, LDH and cTnI activities. The cardioprotective effect of GsRd is tightly related toNrf2/HO-1 signaling pathway.

Acknowledgements

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Disclosure of conflict of interest

None.

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