Original Article
Protective effect of ginsenoside Rg1 on isoproterenol-induced acute myocardial ischemia in rats

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Abstract: The present study was to investigate the effects of Rg1 against myocardial ischemia by isoproterenol (ISO). ST-segment elevation was measured after the last administration of Rg1. Serum levels of creatine kinase (CK), lactate dehydrogenase (LDH), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) were measured. The hearts were excised for determining heart weight index, microscopic examination, superoxide dismutase (SOD) and malondialdehyde (MDA) measurements. PI3K/PTEN/Akt signaling were determined by western blot. Rg1 decreased the ST elevation induced by acute myocardial ischemia, decreased serum levels of CK-MB, LDH, TNF-α and IL-6. Rg1 also increased SOD activity and decreased MDA content in myocardial tissue. In Rg1 group, the protein levels of PTEN and Bax-2 were significantly decreased, while the protein levels of PI3K, Akt and Bcl-2 were significantly increased in a dose-dependent manner compared to the model group, respectively. Together, these data demonstrate that Rg1 might protect myocardial ischemia via regulating PI3K/PTEN/Akt signaling.

Keywords: Ginsenoside Rg1, myocardial ischemia, PI3K/PTEN/Akt signaling

Introduction

Ischemic heart disease (IHD) is the leading cause of morbidity in the Western world, and according to the World Health Organization, it will be the leading cause of death in the world [1, 2]. Despite advances in basic research and clinical improvements, there have been no fundamental breakthroughs in drug treatment.

Isoproterenol (ISO), β-adrenergic agonist, is known to produce cardiac ischemia due to free radical production by autooxidation [3]. ISO-induced cardiac ischemia results in increased cardiac enzymes and oxidative stress, abnormal electrocardiograph and cardiac functions [4]. PI3K mediates signal transduction through phosphorylation of phosphatidylinositol at position 3 of the inositol ring to produce PtdIns (3,4,5) P3, while lipid phosphatase and tensin homolog on chromosome ten (PTEN) antagonizes PI3K activity by dephosphorylating PtdIns (3,4,5) P3 [5]. Therefore, PTEN serves as a potent negative regulator of PI3K activity in many cellular systems [6]. In cultured myocytes, PTEN activity negatively affected cardiac hypertrophy and myocyte survival [7], and genetic inactivation of *Pten* in muscle tissue resulted in spontaneous cardiac hypertrophy and contractile dysfunction in mice [8]. PTEN activity is diminished after ischemia/reperfusion preconditioning induction and restored when preconditioning is decayed, while partial deletion of Pten reduces the threshold of protection induced ischemia preconditioning [9]. Given the importance of PTEN in the regulation of cell growth and survival, it is somewhat surprising that the role of PTEN in ischemia/reperfusion injury has not yet been fully characterized in intact hearts.

Ginseng, known as the root of Panax ginseng C.A. Meyer, has been widely used as a valuable medicinal herb in China. There have been more than 40 kinds of ginsenosides isolated from Ginseng, including Ginsenoside Rg1. It has been suggested that Ginsenoside Rg1 exerts various pharmacological effects, such as removing free radicals, inhibiting calcium influx and anti-apoptosis [10]. In addition, Ginsenoside Rg1 promotes neurogenesis in rat brain after transient focal cerebral ischemia and is protec-

tive to cerebral ischemia. As for the cardiovascular system, Ginsenoside Rg1 has been suggested to be beneficial. However, the mechanism of Ginsenoside Rg1 protects the heart from ischemia-reperfusion has not been elucidated. The present study was designed to evaluate the effect of Rg1 pretreatment on the ISO-induced myocardial damage in a rat model.

Materials and methods

Materials

50 male healthy Sprague-Dawley rats weighing from 200-220 g were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). Animal experiment was carried out in accordance with the Guide for the Care and Use of Laboratory Animals (US National Research Council, 1996, Jan 12). The animals were maintained in a temperature controlled room at 40-50% humidity, a 12 h light/dark cycle and free access to water. Rg1 with purity of over 98% were purchased from National Institutes for Food and Drug Control (Beijing, China). ISO was purchased from Shanghai Hefeng Pharmaceutical Co. Ltd. (Shanghai, China). Sodium pentobarbital was purchased from Merck (Germany). Propranolol was purchased from Xi'an Li Jun Pharmaceutical Co. ltd. (Xi'an, China). Creatine kinase (CK), lactate dehydrogenase (LDH), total superoxide dismutase (T-SOD), malondialdehyde (MDA), tumor necrosis factor-α (TNF-α), and the interleukin-6 (IL-6) test kits were all purchased from Nanjing Jian Cheng Biological Engineering research institute (Nanjing City, China).

Experimental protocol

The rats were randomly assigned to five groups of 10 rats each. Two groups were given Rg1 (20, 40 mg/kg), one was given 30 mg/kg propranolol. A control group and an untreated model group were given distilled water. All treatments were oral. Rats were pretreated for 14 days and then intoxicated with ISO (ISO, 85 mg/kg except for the control group) by subcutaneous injection on two consecutive days. Blood (3 ml) was collected from the abdominal aorta for serum enzyme assays. After treatment, hearts were excised, rinsed in ice-cold isotonic saline, blotted with filter paper, and homogenized in 0.05 M ice-cold phosphate buffer (pH 7.4) for biochemical assays.

Determination of ST-segment elevation and heart rate

Electrocardiograms (ECG) recorded ST-segment elevation and heart rate at 20 min after the final injection of ISO or other drugs. ECGs were recorded under pentobarbital sodium anesthesia (22.5 mg/kg) using needle electrodes and a BL-420S Biological Function Experiment System purchased from Chengdu Thaimeng Technology Co. Ltd, (Chengdu, China).

Determination of heart weight index

After rats were sacrificed, the heart tissues were excised (excluding large blood vessels, and connective tissue), and weighed after blotting with filter paper. The heart weight index (HWI) was computed as HWI = heart weight (HW)/bodyweight (BW).

Determination of CK, LDH, TNF-α, IL-6 in the serum

CK and LDH levels were measured by a rate assay using an RT-9600 Semi-automatic Biochemical Analyzer (ShenZhenLeiDu life Science, LLC). TNF-α and IL-6 levels were measured by enzyme linked immunosorbent assay (ELISA, Wuhan Boshide Biological Technology Company, Wuhan, China). All measurements were performed according to the kit manufacturers' instructions.

Determination of SOD and MDA in myocardial tissue

Approximately 0.1 g of myocardial tissue was removed from the apical part of the heart, immersed in ice-cold saline solution (1:9 W/V) and homogenized. The homogenate was centrifuged at 3000 rpm for 15 min, and the supernatant was used for the biochemical assays. SOD and MDA levels were measured spectrophotometrically using diagnostic kits according to the manufacturers' instructions.

Histological examination of myocardium

Immediately after the sacrifice of the rats, the hearts were removed and fixed in 10% formalin solution. The heart tissue was processed for sectioning and staining by standard histological methods. Sections (5 mm, Leica RM 2125, Germany) from the left ventricle were stained with hematoxylin and eosin (H&E) and exam-
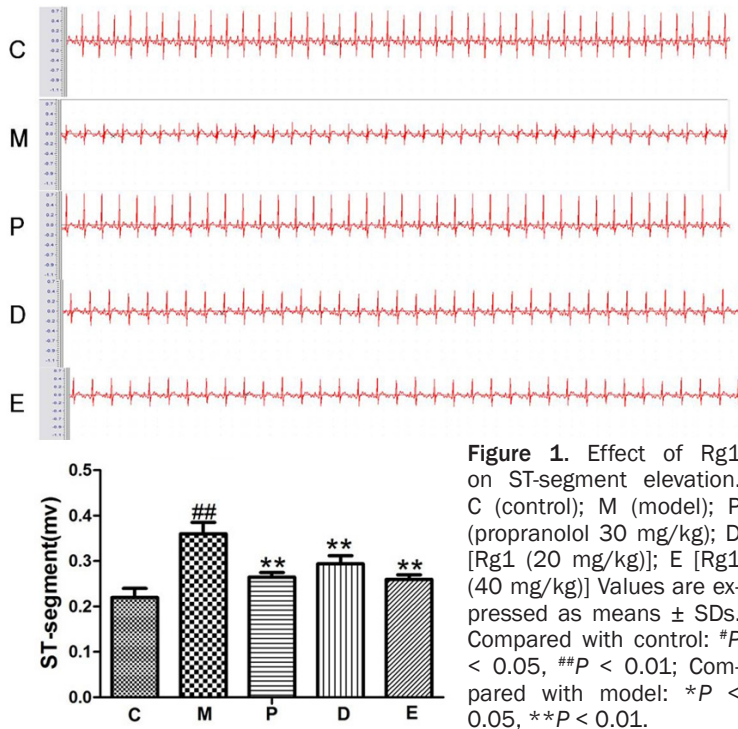
Figure 1. Effect of Rg1 on ST-segment elevation. C (control); M (model); P (propranolol 30 mg/kg); D [Rg1 (20 mg/kg)]; E [Rg1 (40 mg/kg)] Values are expressed as means ± SDs. Compared with control: #P < 0.05, ##P < 0.01; Compared with model: *P < 0.05, **P < 0.01.

Results

Rg1 reduced ST-segment elevation

Five minutes after ISO administration, the ST-segment was elevated in the untreated model group but not in the groups treated with Rg1. These results represented that the myocardial ischemia damage model has been established. Ten minutes after ISO administration, ST-segment elevation was still seen in the untreated group. ST-segment elevation was reduced in the Rg1 groups compared with the untreated model rats. Heart rates tended to stabilize and approximate the rate observed in the propranolol treated group (**Figure 1**).

ined by light microscopy (Nikon, Tokyo, Japan) at 200 × magnification.

Western blotting

The heart tissues were homogenized, washed with PBS, and incubated in lysis buffer in addition to a protease inhibitor cocktail (Sigma, St. Louis, MO) to obtain extracts of proteins. The samples were loaded to 10% SDS-PAGE gels and were electrotransferred to nitrocellulose. The blots were incubated with the appropriate concentration of specific antibody. After washing, the blots were incubated with horseradish peroxidase-conjugated second antibody. The membranes were stripped and reblotted with anti-β-actin antibody (Sigma) to verify the equal loading of protein in each lane. Quantification of protein expression was normalized to β-actin using a densitometer (Imaging System).

Statistical analysis

All values were expressed as the mean ± S.D. and analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test using SPSS version 13.0 software; a P-value of less than 0.05 was considered significant and P < 0.01 was considered to be statistically very significant.

Rg1 decreased CK-MB, LDH, TNF-α and IL-6 serum levels

Significant increases in the myocardial injury marker enzymes, CK-MB and LDH were observed in the untreated model rats compared with the control rats. Pretreatment with Rg1 decreased CK-MB and LDH levels compared with rats in the untreated model group in a dose-dependent manner. Compared with the control group, serum TNF-α and IL-6 levels increased significantly in the untreated model group. Pretreatment with Rg1 decreased serum TNF-α and IL-6 levels compared with the untreated model group rats in a dose-dependent manner (**Figure 2**).

Rg1 decreased heart weight indices

Heart weight indices (HWI) were greater in the untreated model rats than in the control group rats. Pretreatment with Rg1 decreased the HWI compared with the untreated model group rats (**Figure 3**). As the dose increased, the decrease in HWI became larger.

Rg1 showed normal, well preserved of cardiac muscle cell histology

Light microscopy of tissue sections from control rat myocardium showed a normal myofibril-
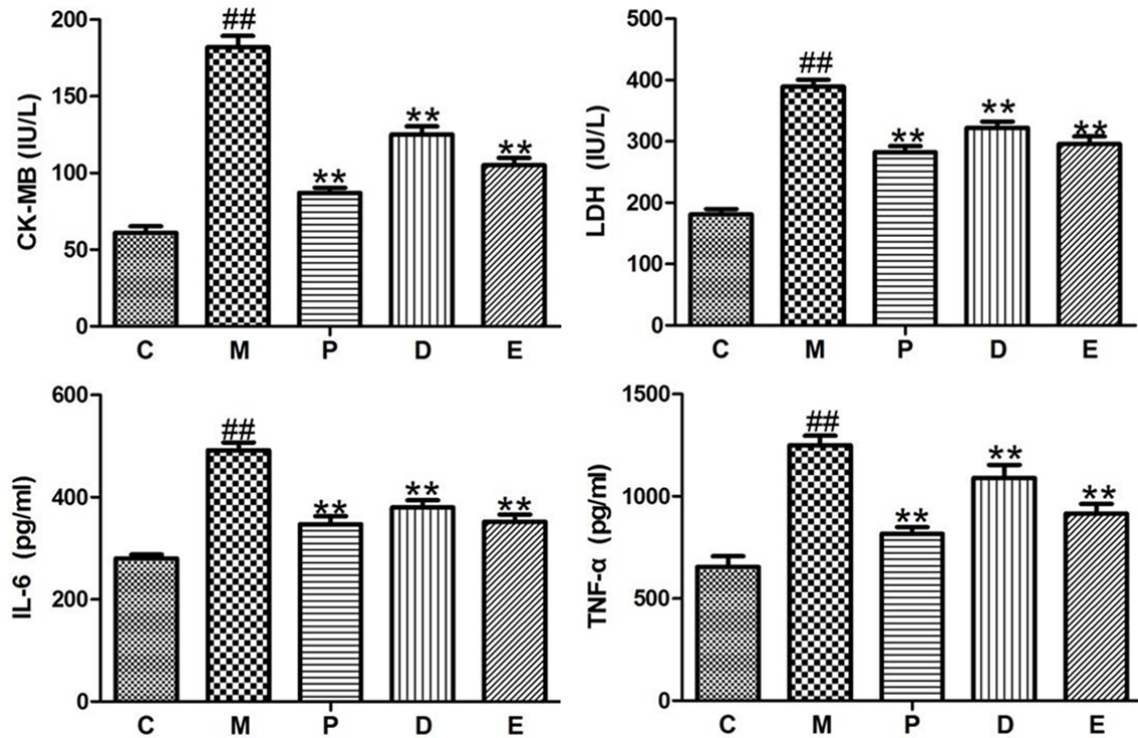
Figure 2. Effect of Rg1 on CK-MB, LDH, TNF-α and IL-6 serum levels. C (control); M (model); P (propranolol 30 mg/kg); D [Rg1 (20 mg/kg)]; E [Rg1 (40 mg/kg)] Values are expressed as means ± SDs. Compared with control: [#]$P <$ 0.05, [##]$P < 0.01$; Compared with model: *$P < 0.05$, **$P < 0.01$.
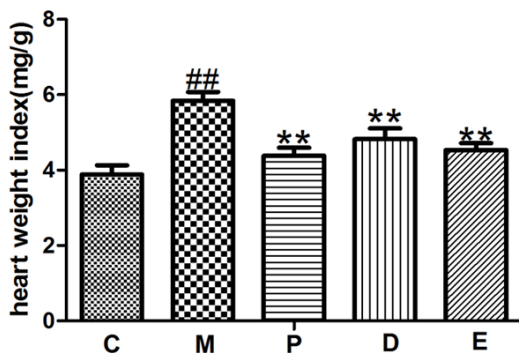

Figure 3. Effect of Rg1 on heart weight indices. C (control); M (model); P (propranolol 30 mg/kg); D [Rg1 (20 mg/kg)]; E [Rg1 (40 mg/kg)] Values are expressed as means ± SDs. Compared with control: [#]$P < 0.05$, [##]$P < 0.01$; Compared with model: *$P < 0.05$, **$P < 0.01$.

lar structure with striations, branched appearance and continuity with adjacent myofibrils. Tissue from the untreated model rats given ISO revealed obvious myocardial cell swelling, degeneration, loss of transverse striations, and large numbers of infiltrating inflammatory cells. Tissues from rats pretreated with Rg1 showed normal, well preserved of cardiac muscle cell histology. Tissue sections from the propranolol group rats revealed approximately normal myofibrillar structure with clear transverse striations, and presence of a few inflammatory cells (**Figure 4**).

Rg1 increased SOD activity and decreased MDA levels

Compared with the control group, SOD levels in the untreated model group decreased significantly. Pretreatment with Rg1 increased SOD activity in a dose-dependent manner compared with the untreated model group rats. Compared with the control group, the MDA levels in the untreated model group increased significantly. Pretreatment with Rg1 decreased MDA levels in a dose-dependent manner compared with the untreated model group rats (**Figure 5**).

Rg1 decreased PTEN, Bax-2 levels, and increased PI3K, Akt and Bcl-2 levels

The expression of proteins of PI3K, PTEN, Akt , Bcl-2 and Bax-2 were changed by ISO in heart.
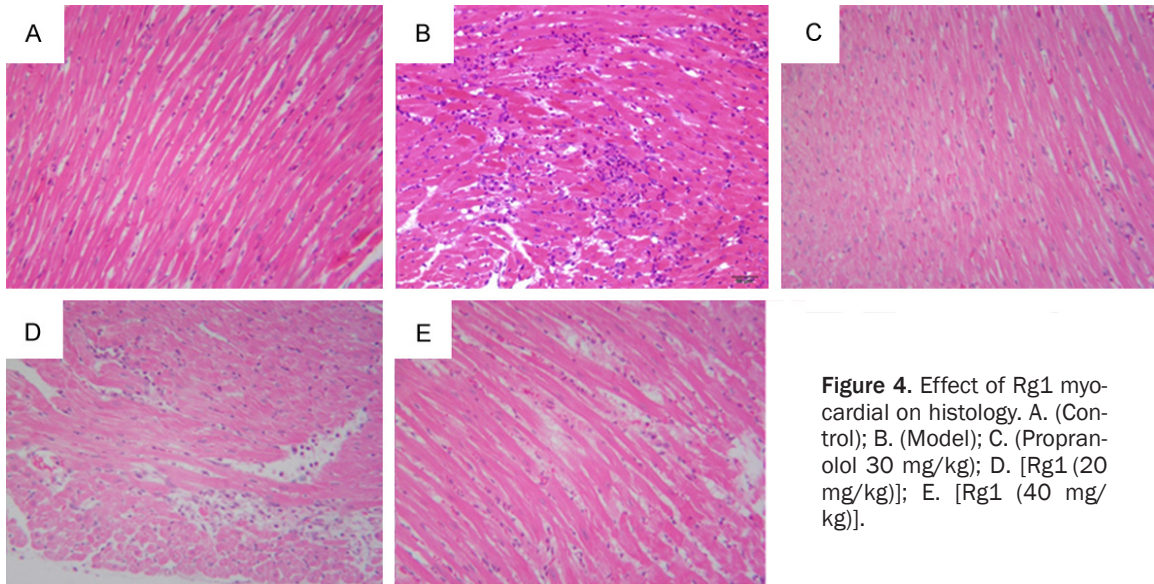
Figure 4. Effect of Rg1 myocardial on histology. A. (Control); B. (Model); C. (Propranolol 30 mg/kg); D. [Rg1 (20 mg/kg)]; E. [Rg1 (40 mg/kg)].
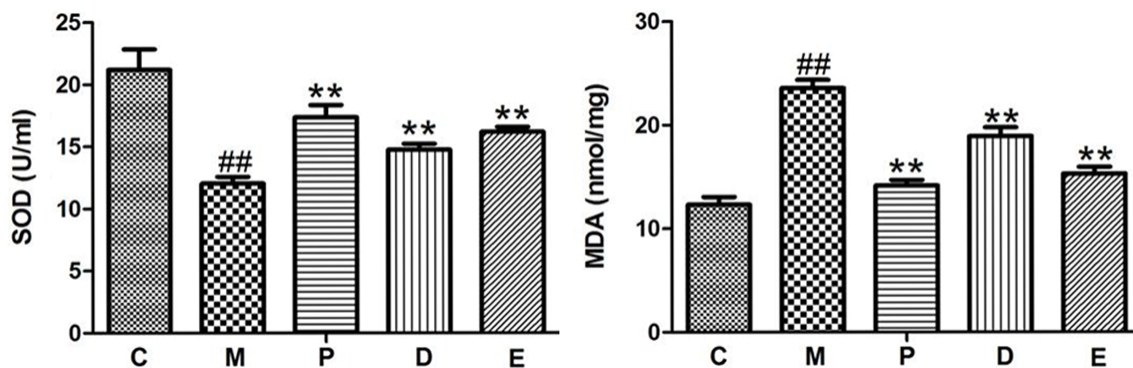

Figure 5. Effect of Rg1 on SOD and MDA levels in the myocardium. C (control); M (model); P (propranolol 30 mg/kg); D [Rg1 (20 mg/kg)]; E [Rg1 (40 mg/kg)] Values are expressed as means ± SDs. Compared with control: #$P < 0.05$, ##$P < 0.01$; Compared with model: *$P < 0.05$, **$P < 0.01$.

As shown in **Figure 6**, compared with the control group, the protein levels of PTEN and Bax-2 in model group were significantly increased. In Rg1, the protein levels of PTEN and Bax-2 were significantly decreased in a dose-dependent manner compared to the model group, respectively. Compared with the control group, the protein levels of PI3K, Akt and Bcl-2 in model group were significantly decreased. In Rg1, the protein levels of PI3K, Akt and Bcl-2 were significantly increased in a dose-dependent manner compared to the model group, respectively.

Discussion

In this study, Rg1 reduced the ST-segment elevation induced by acute myocardial ischemia, alleviated myocardial ischemic injury and decreased HWI. Pretreatment with Rg1 also decreased CK-MB, LDH, TNF-α, and IL-6 levels. Rg1 pretreatment increased SOD activity and decreased MDA levels in the myocardium. These results suggested that Rg1 at the doses used in this study had cardioprotective effects in myocardial ischemia that could be attributed to their anti-oxidative and antiinflammatory properties.

The acute myocardial ischemia induced by ISO was confirmed by loss of integrity of myocardial membranes on histological examination, ST segment elevation and serum elevation of CK-MB and LDH enzymes. Rg1 alleviated myocardial histological injury, reduced heart rate and ST-segment elevation, and decreased
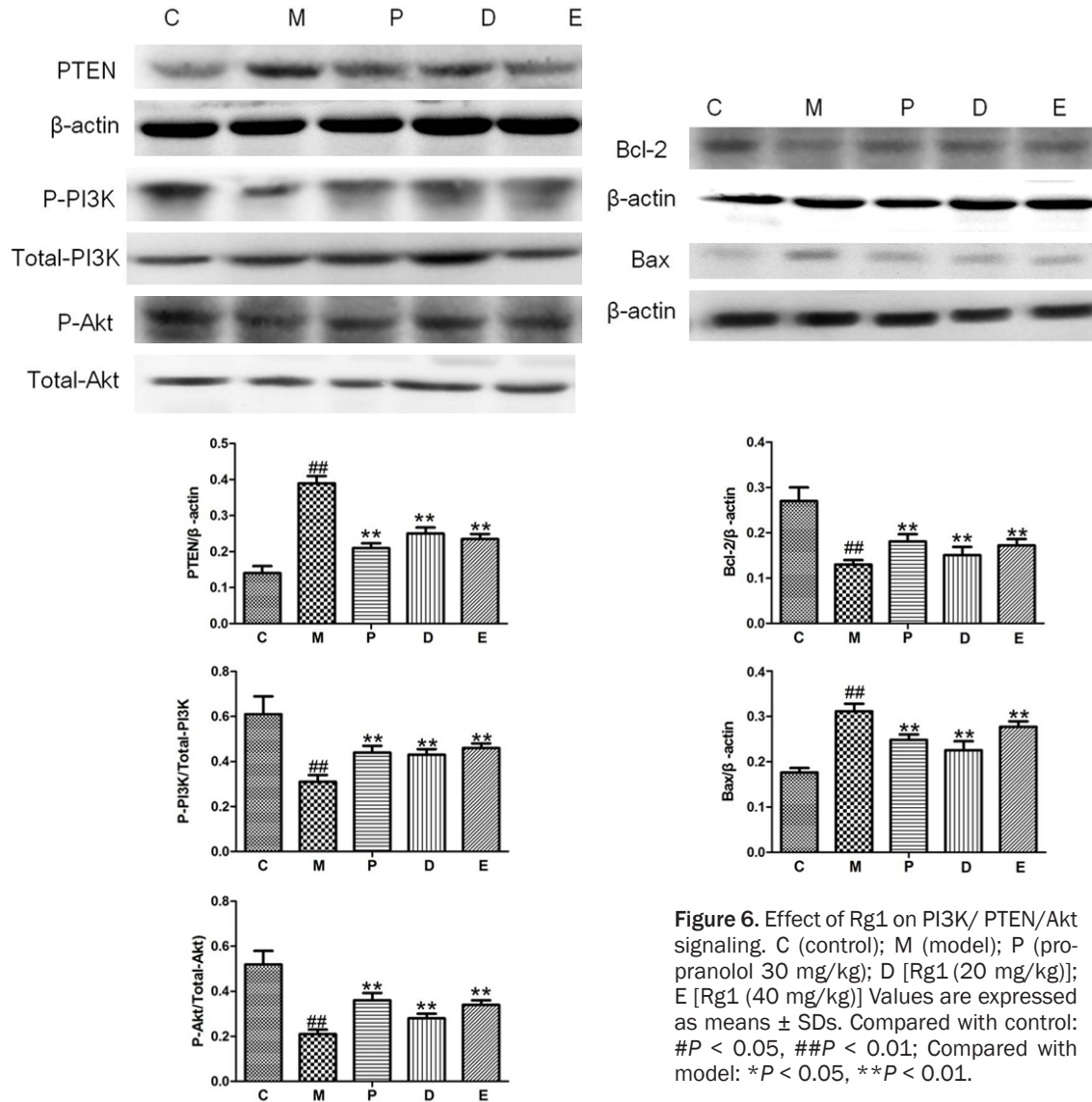
Figure 6. Effect of Rg1 on PI3K/ PTEN/Akt signaling. C (control); M (model); P (propanolol 30 mg/kg); D [Rg1 (20 mg/kg)]; E [Rg1 (40 mg/kg)] Values are expressed as means ± SDs. Compared with control: #P < 0.05, ##P < 0.01; Compared with model: *P < 0.05, **P < 0.01.

CK-MB and LDH enzyme in a dose-dependent fashion.

Inflammation has been recognized as a major driving force in the ischemic process, and increasing evidence has shown that enhanced levels of inflammatory markers are related to ischemia [11, 12]. The proinflammatory cytokines such as TNF-α and IL-6 are small secreted proteins that mediate and regulate inflammation. The inflammatory stresses induced by ISO were reflected by TNF-α and IL-6 elevation, and infiltration of the myocardium by neutrophil granulocytes. Rg1 decreased TNF-α and IL-6 levels in the serum, which suggested that their cardioprotective effects were also associated with anti-inflammatory properties.

The levels of SOD and MDA activity are among the principal pathophysiological parameters in evaluating free radical metabolism. SOD activity reflects the cellular capability of scavenging/quenching free radicals [13]. In this study, SOD activity was significantly decreased in the heart tissue of ISO treated rats as compared to controls. Pretreatment with Rg1 increased SOD activity and decreased MDA level in the myocardium, which suggested that their cardioprotective effects were related to anti-oxidative properties.

PTEN is a well established and important negative regulator for PI3K/AKT and other signaling pathways, yet its in vivo role in cardiac protection remains uncharacterized [14]. In this

report, we investigated the impact of PTEN in intact heart in isoproterenol-induced acute myocardial ischemia. AS shown in **Figure 4**, The expression of proteins of PI3K, PTEN, Akt , Bcl-2 and Bax-2 were changed by ISO in heart. As shown in **Figure 4**, compared with the control group, the protein levels of PTEN and Bax-2 in model group were significantly increased, In Rg1, the protein levels of PTEN and Bax-2 were significantly decreased in a dose-dependent manner compared to the model group, respectively. Compared with the control group, the protein levels of PI3K, Akt and Bcl-2 in model group were significantly decreased. In Rg1, the protein levels of PI3K, Akt and Bcl-2 were significantly increased in a dose-dependent manner compared to the model group, respectively.

In conclusion, this study demonstrated that Rg1 had cardioprotective effects against acute ischemic myocardial injury induced by ISO in rats.

Acknowledgements

Disclosure of conflict of interest

None.

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