Gypenosides alleviate myocardial ischemia-reperfusion injury via attenuation of oxidative stress and preservation of mitochondrial function in rat heart

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Abstract Gypenosides (GP) are the predominant components of *Gynostemma pentaphyllum*, a Chinese herb medicine that has been widely used for the treatment of chronic inflammation, hyperlipidemia, and cardiovascular disease. GP has been demonstrated to exert protective effects on the liver and brain against ischemia-reperfusion (I/R) injury, yet whether it is beneficial to the heart during myocardial I/R is unclear. In this study, we demonstrate that pre-treatment with GP dose-dependently limits infarct size, alleviates I/R-induced pathological changes in the myocardium, and preserves left ventricular function in a rat model of cardiac I/R injury. In addition, GP pre-treatment reduces oxidative stress and protects the intracellular antioxidant machinery in the myocardium. Further, we show that the cardioprotective effect of GP is associated with the preservation of mitochondrial function in the cardiomyocytes, as indicated by ATP level, enzymatic activities of complex I, II, and IV on the mitochondrial respiration chain, and the activity of citrate synthase in the citric acid cycle for energy generation. Moreover, GP maintains mitochondrial membrane integrity and inhibits the release of cytochrome c from the mitochondria to the cytosol. The cytoprotective effect of GP is further confirmed in vitro in H9c2 cardiomyoblast cell line with oxygen-glucose deprivation and reperfusion (OGD/R), and the results indicate that GP protects cell viability, reduces oxidative stress, and preserves mitochondrial function. In conclusion, our study suggests that GP may be of clinical value in cytoprotection during acute myocardial infarction and reperfusion.

Keywords Gypenosides · Myocardial ischemia-reperfusion · Cardioprotection · Oxidative stress · Mitochondrial function

Introduction

Acute myocardial infarction manifests as ischemic myocardial necrosis localized distal to an abrupt occlusion of an epicardial coronary artery and is a major cause of death in coronary heart disease (White and Chew 2008). At present, myocardial reperfusion is the most effective therapy to reduce ischemic injury and infarct size, and is achieved with the techniques of thrombolysis (Topol 2000), percutaneous coronary intervention (Kushner et al. 2009), and coronary artery bypass grafting (Head et al. 2013a, b). In most circumstances, restoration of blood circulation into the damaged tissue prevents further ischemic cell death and helps in tissue repair and disease management. However, accumulating evidences have indicated that the reperfusion process itself can induce further myocardial cell death, a phenomenon known as myocardial ischemia-reperfusion (I/R) injury (Ambrosio and Tritto 1999). ATP depletion as a result of ischemic hypoxia leads to excessive production of free radicals from the mitochondrial respiratory chain during reperfusion, and such reperfusion-stimulated extensive oxidative damage is believed to be the main trigger for myocardial cell damage and death in the reperfusion injury (Turrens et al. 1991; Yellon and Hausenloy 2007). In addition, calcium accumulation within the mitochondria during ischemia is proposed to activate the mitochondrial permeability transition pore (mPTP) in conjunction with reactive oxygen species (ROS) upon reperfusion, contributing to myocardial cell death and I/R injury (Halestrap et al. 2004). Following success in the reperfusion strategies to reduce myocardial ischemic injury, the focus of research and clinical practice has shifted in the recent years towards

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the development of approaches to prevent or reduce myocardial I/R injury, yet very few of them have successfully passed the proof-of-concept stage (Ibanez et al. 2015). Hence, extensive efforts are currently being invested by scientists and physicians worldwide to search for effective and safe agents that exert myocardial protection against I/R injury.

Gypenosides (GP) are saponins of *Gynostemma pentaphyllum*, a traditional Chinese medicinal herb in the treatment of chronic inflammation, hyperlipidemia, and cardiovascular disease. GP has been demonstrated by the modern scientific methods to possess a variety of pharmaceutical properties such as anti-tumor (Lu et al. 2012), anti-hyperlipidemia (Megalli et al. 2005), and anti-hepatic fibrosis (Chen et al. 2000). GP can also exert cardioprotective effects in rats with induced myocardial infarction by preserving the activity of superoxide dismutase (SOD) and reducing the level of malondialdehyde (MDA) in the myocardium (Xiong et al. 1990). Moreover, GP has been reported to effectively protect the organs against I/R injury presumably via its antioxidant bioactivity (Qi et al. 2000; Zhao et al. 2014). However, whether GP can provide protection against myocardial I/R injury is unclear. Hence, this study aims to evaluate the protective effect of GP on the myocardium in a rat model of myocardial I/R injury, and to investigate the potential mechanism(s) underlying GP-mediated cytoprotection of I/R-injured myocardium. Further, the phenomena observed from the in vivo model are verified in vitro in H9c2 cardiomyoblast cell line with oxygen-glucose deprivation-reperfusion (OGD/R) treatment which mimics myocardial I/R in vivo. The results demonstrate a cardioprotective effect of GP against I/R injury via the reduction of oxidative stress and the preservation of mitochondrial function.

Materials and methods

Animal myocardial I/R model and drug administration

All experimental procedures on animals were conducted in accordance with the national and international guidelines, and approved by the Experimental Animal Ethics Committee of China Medical University. Myocardial I/R injury model was established in 8-week-old male Wistar rats by reversible occlusion in the left anterior descending coronary artery as previously described (Liu et al. 2008; Tu et al. 2013). The rats were randomly divided into seven groups with six animals in each group. At 1 h prior to the surgery, GP (Melonepharma, Dalian, China) was dissolved in distilled water and given to the rats by gavage at a dose of 50, 100, or 200 mg/kg body weight, whereas the rats in the Sham group and I/R group received an equal volume of distilled water. I/R injury was induced by 45-min coronary occlusion followed by 3-h reperfusion. The sham-operated rats underwent the same

surgical procedures without coronary occlusion. Ischemic preconditioning (IPC) was achieved via 3 cycles of 5-min ischemia/5-min reperfusion which was carried out 45 min before I/R induction. Ischemic postconditioning (I-postC), achieved by 3 cycles of 30-s ischemia/30-s reperfusion, was conducted at the end of 45-min coronary occlusion and prior to 3-h reperfusion.

Evaluation of cardiac function

At the end of 3-h reperfusion, left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP), and fractional shortening (FS) were measured with a Phillips IE33 ultrasound system according to the manufacturer's instructions. Thereafter, blood samples were collected to determine the levels of creatine kinase and lactate dehydrogenase (LDH) in the serum using the commercial kits (Jiancheng, Nanjing, China).

Measurement of infarct size

Infarct size of the myocardium was measured based on a previously described method (Zhang et al. 2010). The hearts were excised, and the ventricular tissues were frozen and cut into 2-mm cross sections. The sections were incubated in 1 % 2,3,5-triphenyltetrazolium chloride (TTC, Solarbio, Beijing, China) for 15 min at 37 °C. The red areas, stained by TTC, were ischemic but viable tissues (area-at-risk, AAR), and the pale white areas manifested the infarct myocardium. Areas of total left ventricle (LV), AAR, and infarct size (INF) were measured digitally using Image Pro Plus software (Media Cybernetics), and the infarct size was the average value of the INF/LV ratio of five cross sections.

Histopathological examination

The hearts were fixed in 4 % paraformaldehyde, embedded in paraffin, and sectioned at 5 µm. The sections were stained with hematoxylin and eosin (H&E), followed by dehydration, mounting, and histopathological examination under an Olympus DP73 microscope.

Assessment of oxidative stress

The heart tissue homogenates were centrifuged and the supernatant was collected for the determination of protein concentration with a BCA Assay Kit (Beyotime, Haimen, China). The level of MDA and the activities of SOD and glutathione peroxidase (GSH-Px) in the cardiac protein extract were then determined with the respective assay kit (Jiancheng) according to the manufacturer's instructions.

The level of MDA and the activities of SOD and GSH-Px in H9c2 cell lysates were measured in the same way. To detect

the level of ROS in H9c2 cells, the cells were labeled with 2', 7'-dichlorofluorescein diacetate (DCFH-DA, Beyotime), followed by flow cytometric analysis in a BD Accuri C6 Cell Analyzer (Ann Arbor, MI, USA).

Determination of mitochondrial function

The ATP content in the cardiac total protein extract was measured with an ATP Assay Kit (Jiancheng) according to the manufacturer's instruction. Mitochondria were isolated from the cardiac tissue or the H9c2 cells using a Tissue/Cell Mitochondria Isolation Kit (Beyotime). After centrifugation, the pellet was the mitochondria, and the supernatant, i.e., the cytosolic protein extract, was collected for the determination of the level of cytosolic cytochrome c (Cyt-c) using an enzyme-linked immunosorbent assay (ELISA) kit (USCN, Wuhan, China). The mitochondria were resuspended in PBS and divided into two parts. One part was lysed with the kit to extract mitochondrial protein. The concentration of the mitochondrial protein sample was determined with the BCA kit, and the activities of mitochondrial complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex IV (Cyt-c oxidase), and citrate synthetase were measured with the respective assay kit (Jiancheng) according to the manufacturer's instructions. The relative enzymatic activities of mitochondrial Complexes are expressed as the difference in the absorbance at the corresponding wavelength over a 3-min reaction.

Mitochondrial swelling assay was performed to assess mitochondrial membrane permeability as previously described (Du et al. 2014). Briefly, the mitochondria were suspended in the assay buffer (230 mM mannitol, 70 mM sucrose, 3 mM Hepes, pH 7.4) and adjusted to a final protein concentration of 0.5 mg/ml, and the OD value of the mitochondrial suspension at 540 nm was determined by the UV752 ultraviolet-visible spectrophotometer (YOKE, Shanghai, China).

Cell culture and treatment

H9c2 rat cardiac cell line (ATCC, USA) was cultured in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10 % FBS (Hyclone, Logan, UT, USA) at 37 °C in a humidified atmosphere consisting of 5 % CO₂. The cells were subjected to oxygen-glucose deprivation/reperfusion (OGD/R) to mimic myocardial I/R. Cells were exposed to 0.5 % O₂ (oxygen deprivation) for 4 h in the culture medium deprived of glucose and serum, followed by reoxygenation under a normoxic condition in complete culture medium for 24 h. GP was added to the medium at different concentrations (5, 10, and 20 µM) 24 h before and during OGD/R. Preconditioning (PreC) referred to 10 min/10 min OGD/R that was given 10 min prior to 4-h OGD. Postconditioning (PostC)

was achieved via 3 cycles of 10 min/10 min OGD/R that was performed at the end of 4-h OGD and before 24-h incubation under normal conditions.

Cell viability and death was analyzed at the end of 24-h reperfusion by MTT assay and detection of LDH leakage. After 24-h reperfusion, the culture medium was collected, and the level of LDH in the medium was measured using the LDH Activity Assay Kit (Beyotime). Meanwhile, the cells were incubated with the culture medium containing 0.2 mg/ml 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA) for 4 h at 37 °C. Thereafter, the medium was aspirated, and DMSO was added to dissolve the formazan crystals. Absorbance was measured at a wavelength of 490 nm by the ELX-800 plate reader (BIOTEK, Winooski, VT, USA).

Statistical analysis

All values are expressed as the mean ± standard deviation (SD). The differences between multiple groups were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. $p < 0.05$ was considered to be statistically significant.

Results

Pre-treatment with GP attenuates infarct size and alleviates myocardial injury after cardiac I/R injury

The I/R injury model was established via 45 min of LV ischemia and 3-h reperfusion in rats. The effect of GP pre-treatment on induced myocardial infarction and I/R injury was assessed in comparison with IPC and I-PostC, both of which have been demonstrated to exert beneficial effects on I/R injury in animal models (Ji et al. 2013; Liu et al. 2008). As shown in Fig. 1a, the pale white areas that were not stained by TTC manifested the infarct myocardium. Digital computation of the TTC-stained cardiac tissues indicated that GP pre-treatment of 50, 100, and 200 mg/kg bw significantly reduced the myocardial infarct size in a dose-dependent manner (Fig. 1b). GP of 200 mg/kg, in particular, displayed a higher efficacy in the myocardial protection as compared with IPC and I-PostC.

Cardiographic analysis revealed that I/R caused a significant elevation in LVEDP and a significant decline in LVESP and FS, indicating an impaired cardiac contractility and function (Table 1). Evidently, compared to the I/R hearts, the cardiac function was improved by GP pre-treatment in a dose-dependent manner, and GP pre-treatment at a dose of 200 mg/kg displayed the best cardioprotective effect among all treatment groups.

Histopathological examination showed that I/R resulted in disarrangement of muscle fibers, large areas of necrosis,

Fig. 1 Cardioprotective effect of GP in cardiac I/R. Prior to induction of myocardial ischemia, the rats received 50, 100, or 200 mg/kg GP, or ischemic preconditioning (IPC). A group of rats were subjected to ischemic postconditioning (I-postC) between myocardial ischemia and reperfusion. **a** Representative myocardial cross sections of TTC-stained hearts from the rats with different treatments. White area infarcted tissue; red (TTC-positive) area, viable myocardium. **b** Statistical analysis of the infarct size. Data are expressed as mean $\pm$ SD ($n = 6$ per group). Treatment versus I/R, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **c** Representative H&E-stained histological images ($\times 200$ magnification) of the myocardial sections from different treatment groups. Scales = 100 μ m

interstitial edema, and massive neutrophil infiltration (Fig. 1c). The pathological alternations in I/R were markedly suppressed by GP pre-treatment, IPC, and I-PostC, and

myocardial neutrophil infiltration was minimized by high-dose GP, IPC, and I-PostC. Focal loss of myofibers was observed in the myocardium of the rats receiving 50 and 100 mg/kg GP, whereas the myocardial structure was maximally preserved in the rats receiving 200 mg/kg GP.

Table 1 Post-myocardial I/R cardiographic data of rats with various treatments

	LVESP (mmHg)	LVEDP (mmHg)	FS (%)
Sham	122.7 $\pm$ 8.36	5.22 $\pm$ 1.00	52.62 $\pm$ 5.08
I/R	82.58 $\pm$ 10.47 ^{####}	13.28 $\pm$ 5.07 ^{####}	30.37 $\pm$ 6.04 ^{####}
GP 50 g/kg	87.78 $\pm$ 7.81	9.40 $\pm$ 2.05	39.50 $\pm$ 8.91
GP 100 g/kg	89.38 $\pm$ 9.63	8.80 $\pm$ 2.15	40.98 $\pm$ 8.17
GP 200 g/kg	105.82 $\pm$ 5.74**	6.63 $\pm$ 1.21***	50.42 $\pm$ 10.13**
IPC	97.90 $\pm$ 10.07	8.38 $\pm$ 1.44**	43.98 $\pm$ 7.77*
I-PostC	94.33 $\pm$ 11.08	7.67 $\pm$ 1.10*	47.47 $\pm$ 8.77

LVSEP left ventricular end-systolic pressure, LVEDP left ventricular end-diastolic pressure, FS fractional shortening, I/R ischemia/reperfusion, GP gypenosides, IPC ischemic preconditioning, I-PostC ischemic postconditioning. I/R versus Sham, ^{####} $p < 0.001$; treatment versus I/R, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

GP pre-treatment reduces myocardial necrosis and oxidative stress during I/R injury

The preliminary examination showed a cardioprotective effect of GP against myocardial infarct and I/R injury, and the beneficial effect of the high-dose GP was comparable to IPC and I-postC. We further investigated the potential therapeutic mechanism of GP in the prevention of I/R injury. As shown in Fig. 2a, b, I/R-induced elevation of myocardial necrosis markers, such as serum levels of creatine kinase and LDH, was suppressed by GP pre-treatment in a dose-dependent manner. The high-dose GP exerted a remarkable protective effect against myocardial necrosis, which was comparable to, if not better than, IPC and I-postC.

Fig. 2 GP pre-treatment attenuates I/R-induced elevation of creatine kinase, LDH, and oxidative stress. Rat serum was obtained after I/R, and the activities of **a** creatine kinase and **b** LDH (lactate dehydrogenase) were determined with commercial kits. **c** The level of MDA (malondialdehyde), a marker of lipid peroxidation, in the myocardial protein extract was measured. **d, e** The myocardial protein extract was also subjected to the assessment of enzymatic activities of GSH-Px (glutathione peroxidase) and SOD (superoxide dismutase). Data are expressed as mean $\pm$ SD ($n = 6$ per group). I/R versus Sham, ### $p < 0.001$; treatment versus I/R, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

GP pre-treatment, IPC, and I-postC attenuated I/R-induced oxidative stress in rat hearts. We observed a dose-dependent decrease in the cardiac MDA content, a marker of lipid peroxidation, in the I/R rats that received GP pre-treatment (Fig. 2c). In the meanwhile, the activities of GSH-Px and SOD in the I/R hearts that were significantly suppressed compared to the sham and this effect was attenuated by GP in a dose-dependent manner (Fig. 2d, e). The anti-oxidative effect of the high dose GP was comparable to IPC and I-PostC.

GP preserves mitochondrial function and membrane integrity in cardiac I/R injury

The cardiac mitochondria were isolated, and the enzymatic activities of the complexes in mitochondrial respiration chain were analyzed. Pre-treatment with a high-dose GP prominently maintained the enzymatic activities that were remarkably reduced in I/R, and such protective effect on mitochondrial enzymes was slightly better than IPC and I-PostC (Fig. 3a). Consistently, the high-dose GP protected the cardiocytes from I/R-induced reduction in ATP level, displaying a greater potency than IPC and I-postC (Fig. 2b). Moreover, the high dose of GP maintained the activity of citrate synthase at a high level, the first enzyme in the citric acid cycle for ATP synthesis, in the mitochondria of I/R-injured heart (Fig. 3c).

The collapsed mitochondrial volume and the leakage of Cyt-c into the cytoplasm in the I/R-injured cardiomyocytes indicated a compromised mitochondrial membrane integrity (Fig. 3d, e). Pre-treatment with GP dose-dependently inhibited Cyt-c leakage, and the high-dose GP maintained the mitochondrial volume, suggesting that the mitochondrial membrane permeability was preserved by GP. The effect of the high-dose GP on the preservation of mitochondrial membrane integrity was comparable to, if not better than, IPC and I-PostC.

GP maintains viability of H9c2 cells after OGD/R

The cytoprotective effect of GP against I/R injury was further verified in an in vitro model of OGD/R using H9c2 cardiomyoblast cell line. The cell viability was low after 4-h OGD and 24-h reperfusion, and was progressively higher in a dose-dependent manner after GP (Fig. 4a). The cytoprotective effect GP at a dose of 10 μ M was comparable with PreC and PostC, and 20- μ M GP provided the maximal protection against OGD/R-induced cell injury among all treatment options. Consistently, GP dose-dependently reduced OGD/R-induced cell death in H9c2 cells as assessed by LDH leakage, and the high dose GP exerted a better protective effect against cell death than PreC and PostC (Fig. 4b).

Fig. 3 GP preserves mitochondrial function in rat myocardium after cardiac I/R. **a** The mitochondria were isolated from the cardiomyocytes, and the activities of complex I, II, and IV on the mitochondrial respiratory chain were assessed. **b** ATP level in the myocardium. **c** The mitochondrial protein was extracted, and the activity of citrate synthase was determined. **d** Mitochondrial swelling assay was performed to assess mitochondrial membrane permeability. **e** The content of Cyt-c (cytochrome c) in the cytosol. Data are expressed as mean $\pm$ SD ($n = 6$ per group). I/R versus Sham, ### $p < 0.001$; treatment versus I/R, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

GP inhibits OGD/R-induced ROS production and protects mitochondria in H9c2 cells

Following OGD/R, the oxidative markers, such as MDA level, GSH-Px, and SOC activities, in H9c2 cells were assessed. GP dose-dependently attenuated OGD/R-induced MDA elevation and maintained GSH-Px and SOC activities after OGD/R injury (Fig. 5a–c). Moreover, the intracellular ROS level was analyzed by DCFH-DA staining and the results revealed that GP significantly inhibited OGD/R-induced ROS production in a dose-dependent manner (Fig. 5d); 10- μ M GP showed a comparable anti-oxidative effect to PreC and PostC, while 20- μ M GP showed the best result in combating OGD/R-induced oxidative stress.

The effect of GP on mitochondrial function and integrity was also evaluated. As shown in Fig. 6a, OGD/R resulted in a significant reduction of intracellular ATP compared to control, whereas GP (20 μ M) treatment maintained ATP levels in H9c2 cells after OGD/R, suggesting that GP could protect mitochondrial function in OGD/R-injured cells. On the other hand, the mitochondrial permeability was also preserved by GP dose-dependently as demonstrated by mitochondrial swelling assay (Fig. 6b). Moreover, the high-dose GP

displayed a greater potency in preserving ATP production and mitochondrial permeability in OGD/R-treated cells, as compared with PreC and PostC.

Discussion

I/R injury consists of distinct phases of cellular injury, including ATP depletion and lactate accumulation during ischemia, and production of ROS during reperfusion (Armstrong 2004). In this study, we found that pre-treatment with GP reduced infarct size, ameliorated pathological changes in myocardial morphology, and inhibited necrosis of cardiomyocytes in the rats subjected to cardiac I/R. Moreover, GP attenuated I/R-induced alterations in LVESP, LVEDP and FS. These results indicated that GP pre-treatment exerted structural and functional protection of the left ventricle during cardiac I/R. Intracellularly, the cardioprotective effect of GP was related to its role in the protection of mitochondrial structure and function against I/R injury by reducing ROS production, preserving antioxidant capacity, protecting the enzymatic functions of the mitochondrial respiration chain and citric acid cycle, maintaining mitochondrial membrane integrity, and preventing Cyt-c leakage. Additionally, the cytoprotective effect of GP on

Fig. 4 GP protects H9c2 cells from OGD/R injury. H9c2 cells were subjected to OGD/R with GP treatment at various concentrations, or with preconditioning (PreC) or postconditioning (PostC). **a** Cell viability was assessed by MTT assay. **b** The activity of LDH in the conditioned culture medium was analyzed. Data are expressed as mean $\pm$ SD of three independent experiments. OGD/R versus Control, ^{###} $p < 0.001$; treatment versus OGD/R, ^{**} $p < 0.01$, ^{***} $p < 0.001$

myocytes was verified in vitro in H9c2 cells with OGD/R, and the results indicated that GP protected the cell from OGD/R injury by suppressing oxidative stress, maintaining energy metabolism, and controlling mitochondrial permeability.

Fig. 5 GP reduces OGD/R-induced oxidative stress in H9c2 cells. **a** The level of MDA, and **b**, **c** the activities of GSH-Px and SOD in H9c2 cells after OGD/R were measured. **d** The intracellular ROS were labeled with DCFH-DA, and the labeled cells were subjected to flow cytometric analysis. Data are expressed as mean $\pm$ SD of three independent experiments. OGD/R versus control, ^{##} $p < 0.01$, ^{###} $p < 0.001$; treatment versus OGD/R, ^{*} $p < 0.05$, ^{**} $p < 0.01$, ^{***} $p < 0.001$

Fig. 6 GP protects mitochondrial function in H9c2 cells with OGD/R. **a** Intracellular ATP level. **b** Mitochondrial swelling assay for the determination of mitochondrial membrane permeability. Data are expressed as mean $\pm$ SD of three independent experiments. OGD/R versus Control, ^{###} $p < 0.001$; treatment versus OGD/R, ^{*} $p < 0.05$, ^{**} $p < 0.01$, ^{***} $p < 0.001$

ROS, as a natural byproduct of oxygen metabolism in physiological conditions, play important roles in cell signaling and homeostasis. However, excessive ROS production under environmental stress, such as I/R, may damage cell structure

and induce apoptosis or even necrosis (Turrens et al. 1991). The cardioprotective effect of GP was previously proposed to be correlated with its alleviation of oxidative stress in an experimental model of myocardial infarction (Xiong et al. 1990). With the accumulating attention to the reperfusion injury following ischemia (Ambrosio and Tritto 1999), the effect of GP on I/R injury was evaluated in the experimental models of I/R injury in several organs (Qi et al. 2000; Zhao et al. 2014). A recent study by Zhao et al. suggested that the beneficial effect of GP on hepatic I/R injury was achieved via its anti-oxidative and anti-apoptotic properties (Zhao et al. 2014). In cerebral I/R injury, GP was shown to prevent the loss of DNA and RNA contents in the neurons (Qi et al. 2000), which might be associated with the intracellular oxidative stress and cell death. In the present study, we found that the protective effect of GP against I/R injury was also applicable to the heart. Our results suggested that GP-mediated myocardial protection was related to its interference with oxidative stress, as indicated by the level of MDA, and its enhancement of cellular antioxidant machinery, such as SOD and GSH-Px. Likewise, the enhancement of the activities of SOD and GSH-Px by GP was also observed in oxidative injury in dopaminergic neurons (Wang et al. 2010). Moreover, GP has been demonstrated to boost glutathione synthesis and recycle by upregulating the expression of gamma-glutamylcysteine synthetase and glutathione reductase (Shang et al. 2006). Hence, the possible mechanisms of the antioxidant action of GP include direct binding with the free radicals to stop the chain reaction of ROS generation, protection of the enzymatic activity of the antioxidant system, and transcriptional activation of important antioxidant enzymes.

Mitochondria, the major energy generator, have been lately recognized to contribute to cardiac dysfunction and myocyte injury during cardiac I/R via loss of metabolic capacity and release of toxic products (Lesnefsky et al. 2001). In our study, GP pre-treatment preserved mitochondrial function in a dose-dependent manner after myocardial I/R in vivo, as noted by increased efficiency of complex I, II, and IV on mitochondrial respiratory chain, suggesting that the recovery of electron transmission and proton transport in the mitochondria may account for the elimination of ROS over-production and the restoration of ATP production during reperfusion. Moreover, the mitochondrial swelling assay revealed a striking elevation of mitochondrial volume by high-dose GP after cardiac I/R in vivo or OGD/R in vitro, suggesting a significant role of GP for the inhibition of mPTP opening and the preservation of mitochondrial membrane integrity. Release of Cyt-C from the mitochondria to the cytoplasm as a result of the increased mitochondrial membrane permeability can trigger apoptosis (Gottlieb 2011). Thus, inhibition of Cyt-c release from the mitochondria by GP contributed to the cytoprotective effect in myocardial I/R.

In conclusion, our study shows that pre-treatment with GP limits the extent of myocardial infarction in rats with cardiac I/R. The protection is accompanied by reduction of oxidative stress and preservation of mitochondrial function. Our findings suggest that GP may be of a potential value in myocardial protection.

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