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Ginsenoside Rg1 ameliorates oxidative stress and myocardial apoptosis in streptozotocin-induced diabetic rats

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Abstract: We evaluated the cardioprotective effects of ginsenoside Rg1 in a diabetic rat model induced with high-fat diet and intraperitoneal injection of streptozotocin. Ginsenoside Rg1 was injected intraperitoneally for 12 weeks. Myocardial injury indices and oxidative stress markers were determined. Changes in cardiac ultrastructure were evaluated with transmission electron microscopy. Myocardial apoptosis was assessed via terminal deoxynucleotidyl transferase (TDT)-mediated DNA nick-end labeling (TUNEL) and immunohistochemistry. Ginsenoside Rg1 was associated with a significant dose-dependent reduction in serum levels of creatinine kinase MB and cardiac troponin I, and lessened ultrastructural disorders in diabetic myocardium, relative to the untreated diabetic model rats. Also, compared with the untreated diabetic rats, significant reductions in serum and myocardial levels of malondialdehyde were noted in the ginsenoside Rg1-treated groups, and increased levels of the antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) were detected. TUNEL staining indicated reduced myocardial apoptosis in ginsenoside Rg1-treated rats, which may be associated with reduced levels of caspase-3 (CASP3) and increased levels of B-cell lymphoma-extra-large (Bcl-xL) in the diabetic myocardium. Ginsenoside Rg1 treatment of diabetic rats was associated with reduced oxidative stress and attenuated myocardial apoptosis, suggesting that ginsenoside Rg1 may be of potential preventative and therapeutic value for cardiovascular injury in diabetic patients.

Key words: Ginsenoside Rg1, Diabetic cardiomyopathy, Oxidative stress, Apoptosis, Caspase-3 (CASP3)
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1 Introduction

Diabetes mellitus (DM) is a worldwide health problem, in both the developed and developing countries (Beulens *et al*., 2010). In China, the prevalence of DM in adults older than 20 years is about 9.7%, while approximately 15.5% of the entire population is prediabetic (Yang *et al*., 2010). Cardiovascular complications are the most important causes of morbidity and mortality in DM, and DM patients are at higher risk of developing coronary heart disease and congestive heart failure (Asrih and Steffens, 2013). In fact, results of the Framingham Study indicated that the risk of a congestive heart failure event in diabetic males and females was 2- and 5-fold higher, respectively, compared with those without diabetes (Kannel and McGee, 1979).

With improved understanding of cardiovascular injury in DM patients, it is now recognized that DM not only induces endothelial dysfunction and subsequent atherosclerosis, but also directly leads to myocardial damage, perhaps via persistent hyperglycemia or an inflammation-related pathophysiologic mechanism (Aneja *et al*., 2008; Liu *et al*., 2012). The specific myocardial damage known as diabetic cardiomyopathy exists independent of factors such as coronary heart disease or hypertension (Voulgari *et al*., 2010; Falcão-Pires and Leite-Moreira, 2012). Many

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potential mechanisms underlying the pathogenesis of diabetic cardiomyopathy that could eventually lead to cardiac diastolic and systolic dysfunction have been suggested, including inflammation, cardiac fibrosis, oxidative stress, and myocardial apoptosis (Aneja *et al*., 2008; Voulgari *et al*., 2010; Falcão-Pires and Leite-Moreira, 2012).

The development of novel treatment strategies for diabetic cardiomyopathy is undoubtedly important for improving the prognosis of DM patients. An active ingredient in an extract from the dried roots of *Panax notoginseng*, known for its cardioprotective effects, is ginsenoside Rg1 (Lü *et al*., 2009). Previous studies that have evaluated the benefits of ginsenoside Rg1 for the cardiovascular system primarily focused on its anti-remodeling (Deng *et al*., 2010; Li *et al*., 2013; Zhang *et al*., 2013) and anti-ischemic effects (Xia *et al*., 2011; Yin *et al*., 2011). However, to the best of our knowledge previous studies have not evaluated whether ginsenoside Rg1 can prevent myocardial injury related to DM. In the current study, we observed the effects of ginsenoside Rg1 on oxidative stress and myocardial apoptosis in streptozotocin (STZ)-induced DM rats.

2 Materials and methods

2.1 Animal experimental protocols

This investigation conformed to the Guide for the Care and Management of Laboratory Animals published by the Universities Federation for Animal Welfare. The Animal Care and Use Committee of Jilin University approved the study protocols.

Sixty adult male Wistar rats (Animal Center of Jilin University, Changchun, China) weighing (200± 20) g were used for the current study. Rats were randomized into either a high-fat diet group (n=50) or a control group (n=10) and housed at (23±2) °C. The rats of the high-fat diet group were fed a high-fat diet (20% (w/w) lard stearin, 10% (w/w) sucrose, and 0.1% (w/w) bile salt were added to the normal diet) for 4 weeks and then received an intraperitoneal (IP) injection of STZ (40 mg/kg, dissolved in citrate buffer, pH 4.5; Sigma-Aldrich, USA). Rats of the control group were fed standard rat chow and received an IP injection of the same volume of citrate buffer. One week after the administration of STZ, blood samples were obtained from the tail vein after 12 h of fasting. The levels of fasting blood glucose (FBG) were measured in spectrophotometry-based assays using commercially available kits (Invitrogen, USA). Those rats with FBG >7.8 mmol/L were considered diabetic; and 40 rats in the high-fat diet group fulfilled this DM criterion.

The STZ-induced DM rats were subsequently apportioned randomly and equally to a DM control group (administered saline, 1 ml/d IP) and 3 groups administered ginsenoside Rg1 (98% purity, obtained from College of Pharmacy, Jilin University, China) at low (10 mg/(kg·d) IP), medium (15 mg/(kg·d) IP), and high (20 mg/(kg·d) IP) doses, respectively, hereafter referred to as the Rg1-low, Rg1-medium, and Rg1-high groups. The assigned treatments were administered for 12 weeks. The FBG of the rats in the non-diabetic control group was normal, and these rats served as non-diabetic controls in the following experiment.

2.2 Preparation of myocardial tissue and blood samples

Twelve weeks after the Rg1 treatment, rats were anesthetized with 10% chloral hydrate (0.30 g/kg IP) for further study. Rats were euthanized, and the chests were opened to expose the heart. Blood samples were collected from the right ventricles for measurements of blood biochemical parameters and markers of oxidative stress. The left ventricle was removed and sectioned into three slices along a plane parallel to the atrioventricular ring. One part was frozen in liquid nitrogen for measurements of myocardial markers of oxidative stress. The middle section was fixed in 4% (0.04 g/ml) glutaraldehyde for electron microscopy. The remaining portion of the heart sample was fixed in 10% (v/v) formalin and then paraffin-embedded for immunohistochemistry (IHC) assay.

2.3 Measurements of blood glucose, lipids, and cardiac enzymes

Serum samples from rats in each group were collected and sent to the Department of Clinical Biochemistry of the First Hospital Affiliated to Jilin University for further analyses. FBG, total cholesterol (TC), triglyceride (TG), and cardiac enzymes including creatine kinase MB (CK-MB) and cardiac troponin I (cTnI) were measured with automatic analyzer (Hitachi 7060 Automatic Biochemical Analyzer,

Tokyo, Japan) by professional analysts who were blinded to the treatment groups.

2.4 Electron microscopic analyses

The hearts of the rats were removed after perfusion with a modified Karnovsky solution containing 2.5% (0.025 g/ml) glutaraldehyde and 2% (v/v) formalin in 0.1 mol/L sodium phosphate buffer at pH 7.4. The tissue was rinsed in phosphate buffer solution for 15 min at 48 °C. Post-fixation occurred in 1% osmium tetroxide in phosphate buffer solution (PBS; 0.1 mol/L) at 48 °C for 2 h. The tissues were dehydrated in a graded alcohol series (70%–100%) and embedded in Spurr resin (Canemco-Marivac, QC, Canada). Thick sections were obtained using glass knives in an ultramicrotome (Reichert-Jung, Wien, Austria) and the ultrathin sections (90 nm thickness) were mounted on copper grids (200-mesh grid). The grids were counterstained with 4% (0.04 g/ml) uranyl acetate and 0.4% (0.004 g/ml) lead citrate solutions (Watanabe and Yamada, 1983) and examined in a Hitachi H-7100 (Tokyo, Japan) transmission electron microscope at 80 kV at the College of Basic Medical Sciences, Jilin University, China.

2.5 Detection of myocardial and serum markers of oxidative stress

After treatment, the myocardial tissues from each group were weighed and harvested with saline to prepare the tissue homogenate, and centrifuged at (1000–3000)*g* for 10 min. The supernatants were discarded and the cell boluses were sonicated in cold PBS.

The sera from the blood samples obtained from rats in each group were diluted to 1:50 (v/v) with saline. After centrifugation (800*g*, 5 min), the supernatants of tissue and the diluted sera were immediately evaluated for levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH) using commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China) and a spectrophotometer (Ningbo Biocotek Scientific Instrument Co., Ltd., China), in accordance with the manufacturer's protocols. The protein content of cell homogenates was determined via bicinchoninic acid assay in accordance with the instructions provided in the kit (Jiancheng Institute of Biotechnology, Nanjing, China).

2.6 TUNEL and IHC assay

Heart tissue samples were taken from the left ventricles after 12 weeks of treatment, sliced transversely and cut into 5-μm thick sections. Some sections were used for apoptotic assessment with a terminal deoxynucleotidyl transferase (TDT)-mediated dUTP nick-end labeling (TUNEL) assay, and sections were used for IHC staining with caspase-3 (CASP3) and B-cell lymphoma-extra-large (Bcl-xL) antibodies.

Assessment of apoptosis was conducted using a commercially available Dead End Colorimetric TUNEL System (Promega, Madison, WI, USA). Briefly, sections were deparaffinized, digested with proteinase K (20 mg/ml) at room temperature for 15 min, and soaked in PBS for 5 min. Each section was covered with a TDT solution and incubated for 1 h at 37 °C in a humidified chamber. The sections were immersed in a stop buffer to terminate the enzymatic reaction, and then gently rinsed with PBS. Streptavidin-horseradish peroxidase solution was then applied to each section, and incubated at room temperature for 30 min in the dark. Slides were washed in PBS and exposed to 3,3'-diaminobenzidine (DAB; Golden Bridge Biotechnology, Peking, China) for 5–7 min. The slides were then rinsed in water and counterstained with hematoxylin. The number of TUNEL-positive cells was counted in ten randomly selected fields for each individual rat under ocular micrometers (Olympus Optical, Tokyo, Japan) by an investigator without knowledge of the treatment groups.

The tissue expression of CASP3 and Bcl-xL proteins was assessed immunohistochemically using antibody (Santa Cruz, CA, USA). After deparaffinization, endogenous peroxidase activity was quenched with 30% (v/v) methanol and 0.3% (0.003 g/ml) hydrogen PBS. The slides were then boiled in a citrate buffer with microwaves. After blocking nonspecific binding with 5% (0.05 g/ml) bovine serum albumin, the slides were incubated with primary antibodies overnight at 4 °C. The following day, the sections were thoroughly washed in PBS and incubated for 30 min with a peroxidase-conjugated polymer that carries antibodies to goat (1:200, v/v) immunoglobulin. After rinsing with PBS, the sections were exposed to DAB for 7 min. The slides were rinsed in water and counterstained with hematoxylin. The sections were

examined using light microscopy (Olympus BX51, Hamburg, Germany) and analyzed with a computer-assisted color image analysis system (Image-ProPlus 7.0, Media Cybernetics, MD, USA). The positive areas were assessed in at least 10 randomly selected tissue sections from each group studied.

2.7 Statistical analysis

All data are presented as the mean±standard deviation (SD). Statistical analyses were performed with SPSS 16.0 software. Comparisons of parameters were performed by one-way analysis of variance (ANOVA), and then the Newman-Keuls test for unpaired data. Comparisons of parameters between two groups were made with the unpaired Student's *t*-test. *P*-values of <0.05 were regarded as statistically significant.

3 Results

3.1 Effects of Rg1 treatment on blood glucose, lipids, and cardiac enzymes in DM rats

Compared with the rats of the non-diabetic control group, the rats in the DM control group had significantly higher mean serum levels of FBG, TC, TG, CK-MB, and cTnI, and significantly lower mean body weight (Table 1). This indicated that metabolic disorder and cardiac injury had been established in the DM model.

After 12 weeks of Rg1 injections in DM rats, the differences in serum FBG, TC, or TG between the Rg1-treated DM rats and those of the non-treated DM control group were not significant, although rats in the Rg1-high group did show a trend toward lower levels. Notably, serum markers of cardiac injury (i.e. CK-MB and cTnI) were both significantly lower in rats of the Rg1-high group, compared with the rats from DM control group (P<0.05, all). This suggests that Rg1 treatment potentially has a protective effect on diabetic cardiac injury. Moreover, the rats in the Rg1-high group had significantly higher body weight compared with the non-treated DM rats.

3.2 Effects of Rg1 treatment on diabetic myocardium ultrastructure

We investigated the effects of Rg1 treatment on myocardium (left ventricles) via transmission electron microscopy (Fig. 1). In the rats of the non-diabetic control group, myocardial cells were arranged normally with clear structure, less collagen content in the extracellular matrix compared with the DM control (model) rats, normal capillary endothelial cells, and normal mitochondrial structure. In the DM model group, observation of the ultrastructure revealed disorderly arrangements of myocardial cells with ridges and less glycogen than normal; sparse, distorted, and broken myofilament fibers; fewer numbers and swollen mitochondria; vacuolar degeneration; interstitial collagen hyperplasia, swollen capillary endothelial cells, and thickened capillary basement membrane. In rats of the Rg1-high group, there were less interstitial collagen deposition, thinner capillary basement membrane, and more of mitochondria of good structure compared with the untreated DM model rats.

3.3 Effects of Rg1 treatment on serum and myocardial markers of oxidative stress in DM rats

The potential effects of Rg1 on oxidative stress (as reflected by MDA) and antioxidants (SOD, GSH, and CAT), in both serum and myocardium, were evaluated (Figs. 2–5). Compared with rats in the non-diabetic control group, those in the non-treated DM control group had higher levels of MDA and lower levels of the antioxidants. After 12 weeks of Rg1 administration, the serum and myocardial levels of MDH were significantly lower in all three of the Rg1-treated groups compared with the non-treated DM rats. This suggests that Rg1 is associated with reduced oxidative stress in DM rats (Fig. 2). Moreover, we also found that treatment with Rg1 was associated

Table 1 Effects of Rg1 treatment on blood glucose, lipids, and cardiac enzymes in DM rats

Group	FBG (mmol/L)	TC (mmol/L)	TG (mmol/L)	cTnI (μg/L)	CK-MB (U/L)	Body weight (g)
Control	5.42±0.80	0.397±0.066	1.062±0.143	0.007±0.005	0.460±0.082	398.40±20.16
DM	23.44±9.83**	6.533±1.456**	4.594±1.242*	0.320±0.260**	2.300±0.340**	254.60±10.12**
Rg1-low	25.20±9.22	6.352±1.458	4.565±2.016	0.120±0.220	1.660±0.097	260.30±12.20
Rg1-medium	24.40±6.38	6.098±1.852	4.099±1.440	0.080±0.090	1.590±0.091	269.20±10.12
Rg1-high	20.88±10.26	5.520±0.464	3.802±0.201	0.012±0.086$^{\#}$	1.550±0.910$^{\#}$	289.40±17.43$^{\#}$

* P<0.05, ** P<0.01 compared with the control group; $^{\#}$ P<0.05 compared with the DM group. Data are expressed as mean±SD (n=10)

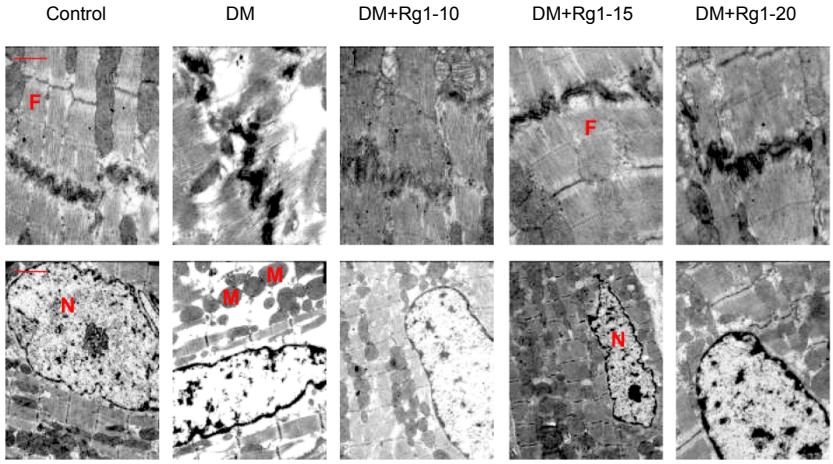

Fig. 1 Effects of ginsenoside Rg1 treatment on the ultrastructure of diabetic myocardium

F: myofilament fibers; N: nucleus; M: mitochondrial. Myocardial cells from the non-diabetic control group were arranged normally with clear structure, less collagen content in the extracellular matrix compared with DM rats, normal capillary endothelial cells, and normal mitochondrial structure. However, observation of the ultrastructure in rats of the DM control group revealed disorderly arrangements of myocardial cells with ridges and less glycogen than normal; sparse, distorted, and broken myofilament fibers; fewer numbers and swollen mitochondria; vacuolar degeneration; interstitial collagen hyperplasia; swollen capillary endothelial cells; and thickened capillary basement membrane. In rats of the Rg1-high group, there was less interstitial collagen deposition, thinner capillary basement membrane, and more of mitochondria of good structure compared with the untreated DM rats. n=10 in each group. Scale bar=0.5 μm in the upper panel; scale bar=1.3 μm in the lower panel

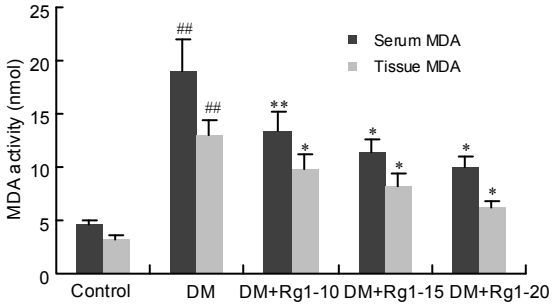

Fig. 2 Effects of ginsenoside Rg1 treatment on serum and myocardial levels of MDA in DM rats

P<0.01 compared with the non-diabetic control group; * P<0.05, ** P<0.01 compared with the DM control group. Data are expressed as mean±SD (n=10)

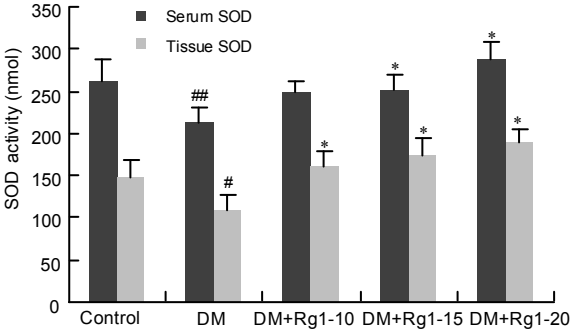

Fig. 3 Effects of ginsenoside Rg1 treatment on serum and myocardial levels of SOD in DM rats

# P<0.05, ## P<0.01 compared with the non-diabetic control group; * P<0.05 compared with the DM control group. Data are expressed as mean±SD (n=10)

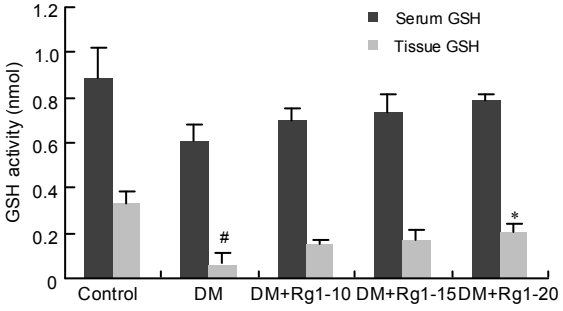

Fig. 4 Effects of ginsenoside Rg1 treatment on serum and myocardial levels of GSH in DM rats

# P<0.05 compared with the non-diabetic control group; * P<0.05 compared with the DM control group. Data are expressed as mean±SD (n=10)

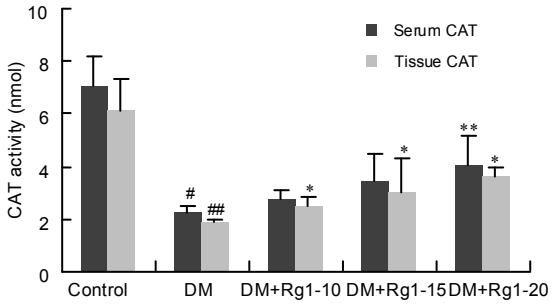

Fig. 5 Effects of ginsenoside Rg1 treatment on serum and myocardial levels of CAT in DM rats

# P<0.05, ## P<0.01 compared with the non-diabetic control group; * P<0.05, ** P<0.01 compared with the DM control group. Data are expressed as mean±SD (n=10)

with dose-dependent higher serum and myocardial levels of the antioxidants SOD, GSH, and CAT (Figs. 3–5). These results suggest that Rg1 treatment attenuates systematic and myocardial oxidative stress in DM rats, and inhibits the reduction of antioxidants.

3.4 Effects of Rg1 treatment on myocardial apoptosis in DM rats

Results of TUNEL analyses show that the percentage of apoptotic myocardial cells was significantly higher in the non-treated DM rats ((62.5±7.59)%) compared with the normal controls ((3.23±1.32)%, $P<0.01$; Fig. 6). The 12-week Rg1 treatment was associated with a dose-dependent attenuation of myocardial apoptosis in DM rats. That is, the percentages of apoptotic myocardial cells in the Rg1-medium ((44.25±6.58)%), and Rg1-high ((30.68±2.88)%) groups were significantly lower than that of the non-treated DM rats ((62.5±7.59)%, $P<0.05$; Fig. 6). These results indicate that cardioprotective effects of Rg1 treatment on DM myocardium may be associated with attenuation of myocardial apoptosis, and the effect appears to be dose-dependent.

3.5 Effects of Rg1 treatment on myocardial levels of CASP3 and Bcl-xL in DM rats

Quantitative IHC analyses revealed that level of the apoptosis-related protein CASP3 in myocardium was higher in rats of the DM control group compared with those of the non-diabetic control group; while level of Bcl-xL (which promotes cell survival) was lower (Fig. 7). Rg1 treatment of DM rats was associated with a dose-dependent inhibition of higher level of CASP3, as well as the restoration of Bcl-xL level. These results suggest that modulation of the expressions of proteins related to apoptosis and cell survival in DM rats myocardium through Rg1 treatment may underlie mechanisms that attenuate apoptosis.

4 Discussion

The putative cardioprotective role of ginsenoside Rg1 has been the focus of several studies (Deng *et al*., 2010; Xia *et al*., 2011; Yin *et al*., 2011; Li *et al*., 2013; Zhang *et al*., 2013), but not with regard to the cardiovascular damage incurred in the pathogenesis of diabetes. The present study was undertaken to investigate the effects of injected treatment of ginsenoside

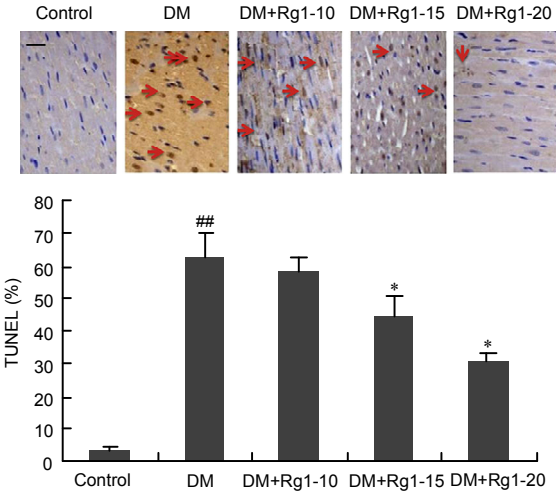

Fig. 6 Effects of ginsenoside Rg1 treatment on myocardial apoptosis in diabetic rats: results of TUNEL analysis
The arrows point to apoptotic cells which are stained in dark brown by TUNEL analysis. The number of TUNEL-positive cells was counted in 10 randomly selected fields for each individual rat under ocular micrometers. ## $P<0.01$ compared with the non-diabetic control group; * $P<0.05$ compared with the DM control group. Scale bar=15 μm (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

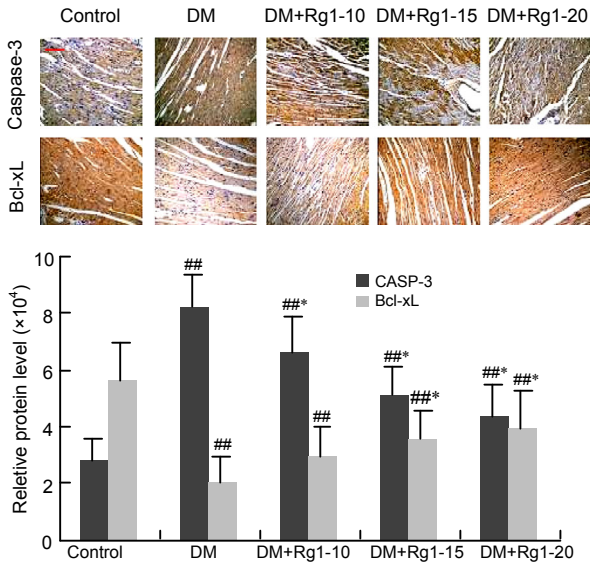

Fig. 7 Effects of ginsenoside Rg1 treatment on myocardial levels of CASP3 and Bcl-xL in diabetic rats: results of IHC analysis
The CASP3 and Bcl-xL proteins are stained in brown. The levels of CASP3 and Bcl-xL proteins were analyzed in 10 randomly selected fields for each individual rat under ocular micrometers. ## $P<0.01$ compared with the non-diabetic control group; * $P<0.05$ compared with the DM control group. Scale bar=10 μm (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

Rg1 in an induced model of diabetes in the rats. We found that ginsenoside Rg1 treatment was associated with protective effects against DM-induced myocardial damage, which may be related to its antioxidant and anti-apoptotic effects.

DM comprises a group of metabolic diseases characterized by high blood glucose (hyperglycemia). Chronic hyperglycemia ultimately leads to damage and failure of multiple systems, including the cardiovascular. Cardiomyopathy in DM is common, and its prevalence could be as high as 75% (Boyer *et al*., 2004), although it is often overlooked because in the early stages it is usually asymptomatic. Early intervention is necessary to delay the development of cardiomyopathy in DM and to reduce mortality.

While Rubler *et al*. (1972) first proposed in 1972 that diabetic cardiomyopathy is an independent type, the understanding of its pathogenesis remains incomplete. In recent years, some researchers have suggested that oxidative stress has an important role in both the pathogenesis and progression of DM complications, including diabetic cardiomyopathy (Brownlee, 2001; Singal *et al*., 2001; Hamblin *et al*., 2007). In the present study we found that diabetic rats not only had higher serum FBG and signs of cardiomyopathy, but also higher levels of oxidative stress markers in serum and myocardial tissue. Our results are thus in accord with previous studies showing that oxidative stress is involved in the pathogenesis of diabetic cardiomyopathy (Giacco and Brownlee, 2010; Khullar *et al*., 2010).

There is still no effective preventative or therapeutic strategy established to treat diabetic cardiomyopathy. General pharmacologic treatment in current clinical practice mainly depends on the control of blood glucose, blood pressure, and lipids. Ginsenosides are natural antioxidants that are considered the source of the efficacy of ginseng in traditional Chinese medicine, used as a muscle relaxant and to improve circulation. A large number of previous studies have shown that ginseng has beneficial effects on the nervous, immune, and cardiovascular systems, largely through attenuation of apoptosis, dilation of vessels, and anti-aging of cells (Lü *et al*., 2009). Ginsenosides specifically have shown potential in the treatment of cardiovascular disorders in rat models (Deng *et al*., 2010; Xia *et al*., 2011; Yin *et al*., 2011; Li *et al*., 2013; Zhang *et al*., 2013), including diabetic rats (Xia *et al*., 2011).

In our study, DM rats treated with the highest dose of ginsenoside Rg1 had lower levels of blood glucose, TG, and TC compared with untreated DM rats. Although these changes were not statistically significant, they suggest the possibility that ginsenoside Rg1 treatment may ameliorate metabolic disorders in DM. More importantly, in ginsenoside Rg1-treated DM rats (at the highest dose) the markers of cardiac injury (CK-MB and cTnI) were significantly lower compared with the untreated DM model group. This indicates that ginsenoside Rg1 may be protective against myocardial injury in DM. The daily dosage levels of ginsenoside Rg1 negatively correlated with MDA levels in serum and myocardial tissue, but positively correlated with antioxidant GSH, SOD, and CAT levels. This further indicates that Rg1 has a strong ability to reduce oxidative damage and ameliorate reductions in antioxidants that are due to DM.

Oxidative stress is caused by excessive formation of reactive oxygen species (ROS) and reactive nitrogen species, as well as by reduced levels of antioxidants necessary for the clearance of ROS (Halliwell, 2007). Under normal physiological conditions, ROS is continuously produced but the antioxidant defense system is sufficient for the prevention of ROS-related injury. If, as in diabetic cardiomyopathy, there is an imbalance between ROS generation and clearance, oxidative stress is amplified and may result in injury and apoptosis in normal tissues (Singal *et al*., 2001). Our study showed that pretreatment with ginsenoside Rg1 was associated with reduced systematic and myocardial oxidative stress in DM rats. Several mechanisms may underlie the anti-oxidative effect of Rg1. For example, it has been suggested that red ginseng extract can elevate the rate-limiting enzyme of GSH-biosynthesis (Park *et al*., 2010). Therefore, as an active component of ginseng, Rg1 may induce the biosynthesis of GSH by up-regulating the rate-limiting enzyme, thereby promoting anti-oxidative activity. Moreover, it has been confirmed that ROS are important inducers of oxidative stress-related injury (Giacco and Brownlee, 2010). Elevated ROS levels were significantly reversed by ginsenoside Rg1 pretreatment, and therefore attenuation of ROS reactions may also be involved in the anti-oxidative effects of Rg1 (Korivi *et al*., 2012). However, the exact molecular mechanisms and pathways

involved in the anti-oxidative effect of Rg1 deserve further study.

Apoptosis due to oxidative stress may be by ways of the mitochondrial, death receptor, or endoplasmic reticulum stress pathways (Aneja *et al*., 2008; Voulgari *et al*., 2010). Throughout the process of apoptosis, proteins of the caspase and Bcl-2 families have crucial roles. Apoptosis is an important mechanism of myocardial cell damage in diabetic disease, and proteins of the caspase and Bcl-2 families are also involved (Li *et al*., 2008; Chen *et al*., 2009; Liu *et al*., 2009; Thandavarayan *et al*., 2009). CASP3 is a member of the caspase family and has been recognized as an important initiator and promoter of apoptosis. Enhanced apoptosis of cardiomyocytes has been noted after myocardial infarction in experimental diabetes; an increase in CASP3 levels after infarction interfered with the remodeling process in the myocardium of rats (Bäcklund *et al*., 2004). Diabetes was found to be associated with enhanced apoptosis and necrosis in both ischemic and non-ischemic human myocardia, an adverse effect that is mediated, at least in part, by CASP3 (Chowdhry *et al*., 2007). Cardiac apoptosis as a major early cellular response in DM is induced by hyperglycemia-derived oxidative stress that activates a mitochondrial cytochrome c-mediated CASP3 pathway (Cai *et al*., 2006). Cleaved CASP3 has also been found to be elevated *in vivo* in STZ- and obesity-induced DM mice (Li *et al*., 2008; Chen *et al*., 2009). Down-regulation of CASP3 can prevent diabetes- and angiotensin II-induced cardiac endoplasmic reticular stress and associated cell death (Xu *et al*., 2009). Our study showed that CASP3 protein levels were higher in STZ-induced diabetic rats than in normal control rats. The CASP3 levels in ginsenoside Rg1-treated DM rats negatively correlated with the Rg1 dose, and were lower than those of the untreated diabetic rats, suggesting that treatment with Rg1 inhibited the expression of CASP3 in a dose-dependent manner.

Bcl-xL is an anti-apoptotic member of the Bcl-2 family (Carrington *et al*., 2009). The up-regulation of Bcl-xL is an important therapeutic mechanism in many cardiovascular diseases such as ischemic heart diseases and congestive heart failure (Ogata and Takahashi, 2003). Estrogen was found to exert a protective effect in cardiomyocytes related to induction of the *Bcl-xL* gene (Morrissy *et al*., 2010). Many signaling pathways, such as p38 and Mitogen-Activated Protein Kinase (MAPK), may prevent apoptosis of cardiomyocytes in an STZ-induced model of DM via up-regulation of Bcl-xL protein expression (Thandavarayan *et al*., 2009). Our study showed that treatment with ginsenoside Rg1 could restore myocardial protein levels of Bcl-xL in a dose-dependent manner, suggesting that regulation of Bcl-2 family proteins may be involved in the anti-apoptotic effect of Rg1 in diabetic myocardium.

Recent studies have suggested many candidate mechanisms and pathways. Wang *et al*. (2013) showed that the protective effect of ginsenoside Rg1 on cerebral ischemia-reperfusion injury correlated with the inhibition of apoptosis of hippocampal neurons, probably by regulating the expression levels of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) pathways. Moreover, it was reported that Rg1 had protective effects against the apoptosis of Aβ25-35-induced endothelial cells, and the ERK signaling pathway may have an important role in this (Yan *et al*., 2013). The involvement of the protein kinase A pathway has also been indicated in the antioxidant and anti-apoptotic effects of Rg1 (Ma *et al*., 2013).

Among the potential mechanisms that could mediate the anti-apoptotic effects of Rg1 is the phosphoinositide-3-kinase (PI3K)/Akt pathway. It has been suggested that the PI3K/Akt pathway is important for insulin signal transduction, cell cycle, cell growth, and survival regulation in DM (Matsui and Davidoff, 2007), and activation of the PI3K/Akt pathway is considered protective against the development of diabetic cardiomyopathy. Indeed, many previous studies have shown that treatment with Rg1 was associated with the activation of the PI3K/Akt pathway in human endothelial cells (Leung *et al*., 2006), hippocampal neuronal cells (Shi *et al*., 2012), and macrophages (Wang *et al*., 2014). However, a recent study indicated that Rg1 may protect chondrocyte from interleukin-1β-induced apoptosis via inhibiting the phosphorylation of Akt (Huang *et al*., 2014). Therefore, it remains to be determined whether interaction with the PI3K/Akt pathway is involved in the anti-apoptotic effect of Rg1 in diabetic myocardium.

5 Limitations

The cardioprotective results of Rg1 in diabetic rats indicated by serum markers of cardiac injury should be further confirmed by evaluation of the parameters appropriate to cardiac hypertrophy and cardiac function. Moreover, the exact molecular mechanisms and pathways underlying the potential anti-apoptotic and anti-oxidative effects of Rg1 in diabetic myocardium should be determined in the future.

6 Conclusions

In summary, the results of our study suggest that ginsenoside Rg1 treatment was associated with reduced systematic and myocardial oxidative stress in DM rats. Moreover, ginsenoside Rg1 could protect diabetic rats from myocardial injury through attenuation of myocardial apoptosis, possibly by inhibiting the expression of CASP3 and restoring Bcl-xL. These results indicate that ginsenoside Rg1 may have potential preventative and therapeutic value for cardiovascular injury in DM patients.

Compliance with ethics guidelines

Hai-tao YU, Juan ZHEN, Bo PANG, Jin-ning GU, and Sui-sheng WU declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：人参皂苷 Rg1 对糖尿病大鼠心肌氧化应激及细胞凋亡的影响

目　的：探讨人参皂苷 Rg1 对高血糖所致心肌损害的保护作用及其机制。

创新点：使用糖尿病大鼠为实验对象，探讨三种浓度的人参皂苷 Rg1 对糖尿病心肌损伤的保护作用及其机制，检测其是否具有浓度依赖性。

方　法：将 60 只 Wistar 大鼠随机分组，其中空白对照组 10 只，另 50 只给予高脂高糖饲养，4 周后腹腔注射 40 mg/kg 链脲佐菌素（STZ）。成功制备糖尿病大鼠模型 40 只，再随机分为糖尿病模型组，糖尿病大鼠+低剂量人参皂苷 Rg1（10 mg/(kg·d)），糖尿病大鼠+中剂量人参皂苷 Rg1（15 mg/(kg·d)），糖尿病大鼠+高剂量人参皂苷 Rg1（20 mg/(kg·d)）。12 周后处死大鼠，取血测定空腹血糖、总胆固醇（TC）、甘油三酯（TG）、心肌酶及氧化应激水平，留取心肌组织使用透射电镜观察心肌细胞超微结构改变，应用 TUNEL 法检测心肌细胞凋亡，免疫组化检测细胞凋亡相关蛋白半胱氨酸天冬氨酸蛋白酶 3（CASP3）和 Bcl-xL 的表达。

结　论：人参皂苷 Rg1 对糖尿病大鼠糖脂代谢无明显影响，人参皂苷 Rg1 可降低糖尿病大鼠血清肌钙蛋白（cTnI）和肌酸激酶同工酶（CK-MB）水平，改善心肌细胞超微结构，减少心肌细胞凋亡，降低大鼠血清和心肌组织中丙二醛（MDA）含量，提高超氧化物歧化酶（SOD）、过氧化氢酶（CAT）和谷胱甘肽过氧化物酶（GSH）水平，降低凋亡蛋白 CASP3 的表达，同时提高 Bcl-xL 蛋白表达。总之，人参皂苷 Rg1 能显著保护糖尿病大鼠心肌损伤，其机制可能与其抗氧化及抗细胞凋亡作用有关。

关键词：人参皂苷 Rg1；糖尿病心肌病；氧化应激；细胞凋亡；半胱氨酸天冬氨酸蛋白酶 3（CASP3）