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The Structure-Activity Relationship of Ginsenosides on Hypoxia-Reoxygenation Induced Apoptosis of Cardiomyocytes

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Abstract

Ginsenosides have been studied extensively in recent years due to their therapeutic effects in cardiovascular diseases. While most studies examined the different ginsenosides individually, few studies compare the therapeutic effects among the different types. This study examined how effective protopanaxadiol, protopanaxatriol ginsenosides Rh2, Rg3, Rh1, and Rg2 of the ginsenoside family are in protecting H9c2 cardiomyocytes from damage caused by hypoxia/reoxygenation. In the current study, a model of myocardial ischemia and reperfusion was induced in H9c2 cardiomyocytes by oxygen deprivation *via* a hypoxia chamber followed by reoxygenation. Our data show that structures similar to that of protopanaxadiol, which lacked the hydroxide group at C6, were more effective in lowering apoptosis than structures similar to protopanaxatriol with a hydroxide group at C6. As the compounds increased in size and complexity, the cardioprotective effects diminished. In addition, the S enantiomer proved to be more effective in cardioprotection than the R enantiomer. Furthermore, the immunoblotting analysis demonstrated that ginsenosides activate AMPK but suppress JNK signaling pathways during hypoxia/reoxygenation. Thus, ginsenosides treatment attenuated hypoxia/reoxygenation-induced apoptosis via modulating cardioprotective AMPK and inflammation-related JNK signaling pathways.

Keywords

Ginsenosides; H9c2 cardiomyocytes; apoptosis; hypoxia and reoxygenation

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Conflict of Interest Statement

The authors have declared that no competing interest exists.

Introduction

Panax Ginseng (*Renshen*, Chinese ginseng), a key herb in Chinese medicine, has been used to modulate blood pressure, metabolism, and immune functions [1]. Most ginsenosides consist of a dammarane skeleton (four ring structure composed of 17 carbons) with various moieties attached at the C-3 and C-20 positions. Over 30 ginsenosides have been identified and categorized into two main categories: protopanaxadiol (PPD) and protopanaxatriol (PPT). The difference between the two groups lies in the additional hydroxyl group at C-6 of PPTs [2].

Previous studies have indicated the use of ginsenosides in protecting *in vitro* models from ischemia and reperfusion (I/R) injury by reducing apoptosis and inflammatory responses [3]. *In vitro* and *in vivo* studies have both exhibited potentially positive effects of ginsenosides on heart disease *via* antioxidation, reduced platelet adhesion, vasomotor regulation, and impacting ion channels [4]. Ginsenoside Rb3 has been shown to exhibit cardio protective properties against ischemia reperfusion injury due to its ability to suppress intracellular Ca^{2+} elevation, thus inhibiting apoptosis and caspase activity [5]. A growing number of studies on ginsenosides in recent years have demonstrated the benefits the herbal medication can have on treatment of cardiovascular complications such as ischemia/reperfusion.

Ischemic heart disease is a complicated heart disorder that is a common cause of death in the world, most commonly caused by atherosclerosis or occlusion [3,6]. Ischemia and ischemia coupled with reperfusion has been shown induce programmed cell death [7]. Initial onset of myocardial infarction generates apoptosis, which becomes more severe following reperfusion [6]. Ischemia reperfusion (I/R) generates a complex process, at which time reactive oxygen species (ROS) are generated, cellular calcium overload occurs and the mitochondrial permeability transition (MPT) pore opens, thus leading to cell death or apoptosis [3,8]. Studies the past have shown that certain ginsenosides are capable of inhibiting MPT pores and exhibiting protective effects [9].

Approximately 40 ginsenosides have been identified. Of the 40, 6 were used in this study to evaluate for cardioprotective properties on H9c2 cardiomyocytes that have undergone hypoxia and reoxygenation. The purpose of the study was to compare how effective the 6 types were in lowering apoptosis of H9c2 in response to hypoxia and reoxygenation.

Materials and Methods

Chemical compounds

The 6 groups of ginsenosides used in this study were protopanaxadiol, ginsenoside Rh2, ginsenoside Rg3, protopanaxatriol, ginsenoside Rh1, and ginsenoside Rg2. Protopanaxadiols

and protopanaxatriols had the lowest molecular weights (460.4 and 476.4, respectively) and were also the least polar of the six. Ginsenoside Rh2 structure-wise was similar to protopanaxadiol, with exception to glucopyranosyl attached at the C-3 position. Glucopyranosyl not only increased the molecular weight of Rh2 but also the polarity. Ginsenoside Rh1 and Rh2 are fairly similar in structure, with exception to the glucopyranosyl group being attached at C6 instead of C3. Ginsenoside Rg3 was similar to ginsenoside Rh2, but with an additional glucopyranosyl group attached to the first glucopyranosyl group. Ginsenoside Rg2 was similar in structure to Rh1, but with a rhamnopyranosyl group attached to the glucopyranosyl ligand. The 6 groups each had a chiral center at C-20. The R and S enantiomers were both analyzed for cardioprotective properties. Derivatives of the R and S enantiomers had a different moiety attached at C-20 and were also tested for cardioprotective benefits.

Cell Culture and Hypoxia-Reoxygenation

H9c2 rat cardiomyocytes were cultured in high glucose (4.5 g/L) DMEM supplemented with 10% (v/v) fetal bovine serum and 1% penicillin/streptomycin (v/v). The cells were kept in a CO₂ incubator containing 5% CO₂, maintained at 37°C. Cells were grown in a 60mm plate and underwent treatment once they reached 70–80% confluency. To mimic ischemia, high glucose DMEM medium was changed to none glucose DMEM, obtained from Thermo Fisher. The cells were placed in a hypoxia incubation chamber and normal air was replaced with 90% N₂ and 5% CO₂. The cells were cultured in hypoxia for 24 hours. Following 24 hours hypoxia, the cells were removed from the hypoxia chamber and the medium was

changed from non-glucose DMEM back to high glucose DMEM. The cells underwent reoxygenation for 24 hours.

Drug Treatment

Ginsenosides were diluted to 2 $\mu\text{mol/L}$ in DMSO and added to the cell culture at least an hour prior to treatment. In this experiment, pretreatment of drugs an hour to 20 hours before showed the same result. DMSO concentration did not exceed 1%. Cells cultured in normal medium and kept in a 37°C CO₂ incubator with 95% air and 5% CO₂ was used as a control. The cells undergoing hypoxia/reoxygenation were retreated with the drug when the medium was changed from high glucose DMEM to non-glucose DMEM during hypoxia and also when the medium was changed from non-glucose DMEM during reoxygenation.

Cell apoptosis assay

Cell apoptosis and necrosis was analyzed using the FITC Annexin V Apoptosis Detection Kit I obtained from BD Pharmingen and flow cytometry analysis. Prior to analysis, the cells were collected following treatment and washed in PBS. Afterwards, the cells were centrifuged for 5 minutes at 1200 rpm for 4 minutes. The cells were then suspended in ice-cold PBS. After the cells were centrifuged and suspended another two times, the cells were resuspended in 1X Binding Buffer, as instructed by the manufacturer's protocols. 100 μL of the solution was placed into a 1mL test tube and 5 μL of FITC Annexin V (AV-FITC) and 5 μL of propidium iodide (PI) were added. The test tubes were incubated at room temperature for 15 minutes and then an additional 400 μL of 1X Binding Buffer was added followed by flow cytometry analysis. Necrotic cells were indicated by AV-/PI+ staining. Early stage apoptosis was indicated by AV+/PI- staining and late stage apoptosis was indicated by AV+/PI+ staining. FL3 peak is indicative of PI staining and FL1 is indicative of AV staining.

Western Blot Analysis

Western blot analysis was also used to determine expression of AKT, AMPK, and JNK in H9c2 rat cardiomyocytes pretreated with ginsenosides. The cells were washed with 1X PBS buffer and lysed with RIPA buffer for 5 minutes on ice. After centrifugation for 10 minutes at 8000 g at 4°C, the supernatant was collected for the Western blot assay. Protein concentration was determined based on the BSA standard. 20 μg of protein was loaded on a 10% sulfate polyacrylamide gels (SDS-PAGE). The proteins were transferred onto nitrocellulose membranes at 100V for 2 hours. The membranes were blocked with 5% (w/v) milk powder in TBST. The membranes were then incubated in primary antibodies at 4°C overnight with the appropriate antibodies at a concentration of 1:1000. Afterwards, they were washed thrice with TBST and blocked with secondary antibodies for 1 hour at room temperature. After being washed three times in TBST, the bound antibodies were detected with an enhanced chemiluminescence imaging system.

Results

Effects of ginsenosides on apoptosis of H9c2 cardiomyocytes under hypoxia and reoxygenation (H/R)

H9c2 cells were treated with protopanaxadiol at 2 $\mu\text{mol/L}$. The Flow cytometry results showed that protopanaxadiol was effective at protecting cardiomyocytes that underwent hypoxia/reoxygenation. The X-axis indicates the number of Annexin V-FITC stained cells as FL-1. The Y-axis is indicative of the number of PI stained cells as FL-3. The percentage of apoptotic cells was determined by calculating the ratio of FITC stained positive cells to total cells. (20S)-protopanaxadiol, its derivatives, and its enantiomer all caused a dramatic decrease in apoptotic cells when compared to the H/R control (Figure 1A). H9c2 cells were treated with ginsenoside Rh2 at 2 $\mu\text{mol/L}$. (20S)-Ginsenoside Rh2, (20R)-Ginsenoside Rh2 and their derivatives were tested. The Flow cytometry results demonstrated that ginsenoside Rh2 was effective at protecting cardiomyocytes that underwent hypoxia/reoxygenation. The percentage of apoptotic cells was determined by calculating the ratio of FITC stained positive cells to total cells. (20S)-Ginsenoside Rh2 was slightly more cardioprotective than (20R)-Ginsenoside Rh2. The derivatives of both the S- and R-enantiomers were less effective (Figure 1B). H9c2 cells were treated with ginsenoside Rg3 at 2 $\mu\text{mol/L}$. (20S)-ginsenoside Rg3 and its enantiomer were used to treat H9c2 cardiomyocytes that underwent hypoxia and reoxygenation. The Flow cytometry results showed that ginsenoside Rg3-treated H9c2 cardiomyocytes had slightly less apoptosis than untreated H/R cells. (20S)-Ginsenoside Rg3 treated cells had a slightly lower percentage of apoptotic cells than its enantiomer in cells that underwent hypoxia/reoxygenation (Figure 2A). H9c2 cells were treated with protopanaxatriol at 2 $\mu\text{mol/L}$. The two enantiomers of protopanaxatriol and their derivatives were used to treat cells that underwent hypoxia and reoxygenation. The Flow cytometry results showed that (20S)-protopanaxatriol-treated H9c2 cardiomyocytes had less apoptosis than untreated H/R cells. There was a dramatic difference in the cardioprotective properties of (20S)-protopanaxatriol and (20R)-protopanaxatriol. The S-enantiomer was successful in lowering the apoptosis rate of H/R cells, whereas (20R)-PPT was not. However, the derivatives of the R-enantiomer were more cardioprotective than that of the S-enantiomers (Figure 2B). H9c2 cells were treated with ginsenoside Rh1 at 2 $\mu\text{mol/L}$. The two enantiomers of ginsenoside Rh1 and their derivatives were used to treat cells that underwent hypoxia and reoxygenation. The Flow cytometry results showed that (20S)-Rh1 and (20R)-Rh1, along with their derivatives, all had cardioprotective properties to some degree. The apoptosis rate was determined by calculating the percentage of cells that was positive for the FITC dye. While all of the enantiomers and derivatives of Ginsenoside Rh1 had some cardioprotective effects, there was not a dramatic difference in how well the compounds were at protecting H9c2 cardiomyocytes from undergoing apoptosis (Figure 3A). H9c2 cells were treated with ginsenoside Rg2 at 2 $\mu\text{mol/L}$. The two enantiomers of ginsenoside Rg2 were not effective at protecting cells undergoing H/R. The apoptosis rate was determined by calculating the percentage of cells that was positive for the FITC stain. The apoptosis rate of the untreated H/R group did not differ from the control/untreated cells that underwent H/R (Figure 3B).

Effect of ginsenosides on Akt, AMPK, and JNK phosphorylation

In an attempt to better understand the biochemical mechanisms that control the cardioprotective effects expressed by ginsenosides on H9c2 cardiomyocytes under hypoxic conditions, cardiomyocytes treated with (20S)- and (20R)-protopanaxadiol were collected and subjected to immunoblot analysis using anti-phospho-Akt (Ser⁴⁷³), anti-phospho-AMPK (Thr¹⁷²), or anti-phospho-JNK (Thr¹⁸³/Tyr¹⁸⁵). Basal groups that weren't subjected to hypoxia and reoxygenation had relatively little phosphorylation. Upon treatment with hypoxia and reoxygenation, phosphorylated Akt (Ser⁴⁷³) levels increased dramatically (Figure 4A). In addition to Akt activation, relative phosphorylated AMPK (Thr¹⁷²) levels also increased after hypoxia/reoxygenation (Figure 4B). In comparison to the basal group treated with protopanaxadiol, cardiomyocytes treated with protopanaxadiol in the H/R group expressed dramatically higher levels of phosphorylated AMPK (Figure 4B). Signaling for phosphorylated JNK was also activated after hypoxia/reoxygenation (Figure 4C). Signaling for phosphorylated JNK was dramatically lower in H9c2 cardiomyocytes pretreated with protopanaxadiol (Figure 4C). Western blot analysis was also done to determine the biochemical mechanism of the medication. The study determined what effect ginsenosides had on AMPK, JNK and Akt. AMPK is a highly conserved sensor of adenosine nucleotide levels which is activated at the slightest decrease in ATP production. It has been shown to play a key role in regulating growth and reprogramming metabolism [10]. When ATP production declines, AMPK promotes catabolic pathways for ATP by stimulating the uptake of glucose and enhancing fatty acid oxidation [11]. In addition, AMPK also inhibits anabolic pathways at low ATP production [10]. Under hypoxic conditions, AMPK is activated as a result of reactive oxygen species (ROS) production [11]. The stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK) is categorized as a mitogen-activated protein kinase, which is part a signaling cascade that converge to initiate inflammatory responses [12]. JNK is activated by environmental stress, such as ischemia, reperfusion, and UV radiation, and cytokines. It has been known to be involved in apoptosis, neurodegeneration, cell differentiation, inflammatory conditions, and cytokine production [13,14]. Recent studies have shown that inhibition of JNK can contribute to a reduction in the inflammatory process [15]. Akt has been known to play a key role in cell viability and apoptosis [16]. An increase in cell survival results from inhibition of apoptosis by phosphorylated Akt. Previous studies have shown that Akt reduces apoptosis by serving as an upstream regulator of JNK and c-Jun [17]. There was a relative increase in phospho-AMPK one hour-post reoxygenation. Overall, the H/R groups had higher relative activities of phospho-AMPK than their basal counterparts. (20S)-and (20R)-ginsenosides dramatically increased phospho-AMPK signaling in H/R cardiomyocytes. JNK was phosphorylated as a result of hypoxia/reoxygenation. Phosphorylated JNK signaling was diminished by protopanaxadiol in H/R H9C2 cardiomyocytes (Figure 4C).

Comparison of apoptosis of H9c2 cardiomyocytes under H/R by ginsenosides

The main method used in analyzing the protective benefits of the ginsenosides was flow cytometry. H9c2 cells were exposed to hypoxia for 24 hours followed by 24 hours reoxygenation. Following reoxygenation, the morphological changes of the H9c2 cardiomyocytes were observed through Annexin V and PI staining to determine rate of apoptosis. All of the results showed that the apoptosis rate was higher in cells that underwent

hypoxia/reoxygenation when compared to the basal control H9c2 cardiomyocytes, proving that the H/R model was effective for cell apoptosis measurements. The reduction on apoptosis rates varied among the drugs (Table 1). Overall, compounds that shared a similar chemical backbone structure to protopanaxadiol had a greater effect in decreasing apoptosis rates in cells undergoing hypoxia/reoxygenation than compounds sharing the same structural backbone as protopanaxatriol (Table 1). In both the protopanaxadiol group (PPT, Rh2, Rg3) and the protopanaxatriol group (PPT, Rh1, Rg2), cardioprotective properties were greater in ginsenosides that had smaller molecular weights and were less polar. The S-enantiomer, regardless of the ginsenoside type, was more successful in reducing apoptosis rate.

Discussion

Myocardial ischemia continues to be a common issue in modern times [18,19,20]. Prolonged ischemia causes a drop in ATP levels and an increase in anaerobic metabolism, which results in tissue damage/death [21,22]. Reperfusion of acute or chronic ischemic myocardium is necessary for restoring the flow of blood in order to salvage the myocardium. However, reperfusion has been shown to further increase injury to cardiomyocytes. An influx of reactive oxygen species and pro-inflammatory neutrophils to ischemic tissues further exacerbates tissue damage [23]. Results reported in other studies were confirmed with the current study. The *in vitro* study conducted in the lab showed that hypoxia/reoxygenation (H/R), a model that mimics ischemia/reperfusion (I/R) injury, was capable of causing apoptosis in H9c2.

Ginsenosides in past research have proven to abate damage induced by ischemia reperfusion. Many of the ginsenoside compounds in this experiment have proven to follow this trend. According to the study, S enantiomers, in general, were more favorable in reducing apoptosis than R enantiomers. This suggests that there are more enzyme receptors in cardiomyocytes for the S conformation of ginsenosides than the R conformation. In addition, ginsenosides that share the same chemical backbone as protopanaxadiol had more success than protopanaxatriol in lowering apoptosis rates of H/R H9c2 cardiomyocytes. This trend is validated by previous studies which observed more success with using PPD than PPT [24,25]. A trend seen in both the protopanaxadiol and protopanaxatriol groups is that cardioprotective properties diminish with increasing molecular weight and complexity of the compound. Ginsenoside Rg3 showed less cardioprotective capabilities than other ginsenosides with which it shared a similar chemical backbone with, such as protopanaxadiol and ginsenoside Rh2. Recent studies have shown that ginsenosides metabolites possess greater biological effects than ginsenosides. As a result, ginsenoside Rg3 has less of an effect than its metabolites Ginsenoside Rh2 and PPD in cardioprotection [26,27]. The large molecular weight of many of the ginsenosides may have inhibited its ability to cross the cell membrane and act on the MPT pore.

Polarity is also a factor in determining how effective ginsenosides are in protecting cardiomyocytes from apoptosis. Drugs with high polarity are known to be poorly absorbed when administered [28]. The large number of hydroxide groups on Rg3 and Rg2 may have contributed to their high polarity and lack of effectiveness of protecting H9c2 cardiomyocytes from hypoxia and reoxygenation damage.

Based on the results obtained from flow cytometry analysis, a select number of ginsenosides were chosen for western blot analysis to interpret the biochemical mechanisms employed by ginsenosides. Protopanaxadiol was selected for use in western blot analysis due to its relative success in lowering the apoptosis rate of H/R treated cardiomyocytes. The R and S enantiomers were both selected in order to observe which conformation was more effective at 1 hour post-reoxygenation. The study showed that both ginsenosides were highly effective reducing JNK signaling and activation. JNK has been cited as being involved in apoptosis [29,30]. Protopanaxadiol's ability to decrease JNK levels seen in the H/R control explains how it is able to lower apoptotic levels.

In addition to looking at phosphorylated JNK activity, AMPK and Akt levels were also observed. It is thought that JNK is upregulated by both Akt and AMPK [6,31]. Akt is reportedly required for cell survival [20,32]. It was thought that cardioprotective benefits seen in ginsenosides could be linked to Akt signaling pathway. The study showed that Akt signaling was activated after hypoxia/reoxygenation. However, the Akt signaling was saturated at one hour post-reoxygenation. Hypoxia/reoxygenation did not induce a high level of AMPK activity in H9c2 cells one hour post reoxygenation. However, cardiomyocytes pretreated with ginsenosides had a high increase in AMPK activation, which likely resulted in the deactivation of JNK signaling in the cell. In this study, it can be seen that reduction in apoptosis by ginsenosides stems from the activation of AMPK and deactivation of JNK signals.

Conclusions

This study indicated that hypoxia/reoxygenation lead to increase in apoptotic rates in H9c2 cardiomyocytes. Ginsenosides used in the study played a role in lowering the apoptosis rates of H9c2 cardiomyocytes that underwent hypoxia/reoxygenation via AMPK/JNK signaling pathways. It is thought the S enantiomers of the 6 types of ginsenosides were the more effective of the two enantiomers in targeting apoptosis reduction in H/R cardiomyocytes. In addition, compounds that had a lower molecular weight and were less complex generally had more success in lowering apoptosis. Aside from observing more protective benefits of the S enantiomers, a general trend in how successful the derivatives of the 6 compounds were in lowering apoptosis could not be established. Further studies can look further into the success of ginsenoside derivatives in treating H/R injury as well as finding time-efficient methods for testing all of the compounds.

Acknowledgments

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Highlights

- Ginsenosides as natural products from herb medicine demonstrate the cardioprotective effects on hypoxia and reoxygenation-induced damage.
- There is a structure-activity relationship regarding ginsenosides' inhibition of apoptosis caused by hypoxia and reoxygenation.
- AMPK signaling pathway could be an important mediator for ginsenosides' cardioprotection against hypoxic injury.

B

Figure 1. Effect of protopanaxadiol and ginsenoside Rh2 on Apoptosis Rate of H/R H9c2 cardiomyocytes
(A) Flow cytometry results showed the effect of protopanaxadiol on hypoxia/reoxygenation-induced apoptosis. The percentage of apoptotic cells was determined by calculating the ratio of FITC stained positive cells to total cells. N=4, *p<0.05 vs. Normoxia control; †p<0.05 vs. H/R Vehicle. (B) (20S)-Ginsenoside Rh2, (20R)-Ginsenoside Rh2 and their derivatives were tested. Flow cytometry results showed that ginsenoside Rh2 was effective at protecting cardiomyocytes that underwent hypoxia/reoxygenation. The percentage of apoptotic cells

was determined by calculating the ratio of FITC stained positive cells to total cells. N=4–6,
*p<0.05 vs. Normoxia control; †p<0.05 vs. H/R Vehicle.

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A

B

Figure 2. Effect of Ginsenoside Rg3 and Protopanaxatriol on Apoptosis Rate of H/R H9c2 cardiomyocytes

(A) (20S)-ginsenoside Rg3 and its enantiomer were used to treat H9c2 cardiomyocytes that underwent hypoxia and reoxygenation. Flow cytometry results showed that ginsenoside Rg3-treated H9c2 cardiomyocytes had slightly less apoptosis than untreated H/R cells. N=4, *p<0.05 vs. Normoxia control. (B) The two enantiomers of protopanaxatriol and their derivatives were used to treat cells that underwent hypoxia and reoxygenation. Flow cytometry results showed that (20S)-protopanaxatriol-treated H9c2 cardiomyocytes had less apoptosis than untreated H/R cells. N=4, *p<0.05 vs. Normoxia control.

B

Figure 3. Effect of Ginsenoside Rh1 and Ginsenoside Rg2 on Apoptosis Rate of H/R H9c2 cardiomyocytes

(A) The two enantiomers of ginsenoside Rh1 and their derivatives were used to treat cells that underwent hypoxia and reoxygenation. Flow cytometry results showed that (20S)-Rh1 and (20R)-Rh1, along with their derivatives, all had cardioprotective properties to some degree. N=4, *p<0.05 vs. Normoxia control. (B) The two enantiomers of ginsenoside Rg2 were not effective at protecting cells undergoing H/R. Apoptosis rate was determined by calculating the percentage of cells that was positive for the FITC stain. N=4, *p<0.05 vs. Normoxia control.

A

B

C **Figure 4. The signaling pathways in response to Hypoxia/Reoxygenation**

(A) H9c2 cardiomyocytes were subjected to 24 hours of hypoxia followed by 1 hour of reoxygenation, after which the adherent cells were lysed and collected for western blots. Relative rates of phospho-Akt activity were normalized using the house-keeping protein GAPDH. At one hour post-reoxygenation, phospho-Akt levels were saturated; there was not a dramatic difference in phospho-Akt signaling between H/R control and H/R H9c2 pretreated with ginsenosides. N=3, *p<0.05 vs. Normoxia, respectively. (B) H9c2 cardiomyocytes were subjected to 24 hours of hypoxia followed by 1 hour of reoxygenation, after which the adherent cells were lysed and collected for western blots. Relative rates of phospho-AMPK activity were normalized to total AMPK α . N=3, *p<0.05 vs. Normoxia, respectively; †p<0.05 vs. H/R Vehicle. (C) H9c2 cardiomyocytes were subjected to 24 hours

of hypoxia followed by 1 hour of reoxygenation, after which the adherent cells were lysed and collected for western blots. Relative rates of phospho-JNK activity were normalized using the house-keeping protein GAPDH. N=3, *p<0.05 vs. Normoxia, respectively; †p<0.05 vs. H/R Vehicle.

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Table 1
Cardioprotective Properties of Ginsenosides

Decrease in apoptosis rate was determined by taking the difference between the apoptosis rate H/R control and the apoptosis rate of the H/R groups treated with various types of ginsenosides. The protopanaxadial group overall was more successful in decreasing the apoptosis rate than ginsenosides similar in structure to protopanaxadial. In addition, ginsenosides with smaller molecular weight had better outcomes in reducing apoptosis.