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Ginsenoside Rg1 Protects Rat Cardiomyocyte From Hypoxia/Reoxygenation Oxidative Injury Via Antioxidant and Intracellular Calcium Homeostasis

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ABSTRACT

Ginsenoside Rg1 is a major active ingredient of *Panax notoginseng radix* which has demonstrated a number of pharmacological actions including a cardioprotective effect in vivo. This study investigated the protective effect and mechanism of ginsenoside Rg1 in cardiomyocytes hypoxia/reoxygenation (H/R) model. Pretreatment with ginsenoside Rg1 (60–120 μM) reduced lactate dehydrogenase release and increased cell viability in a dose-dependent manner. Fluorescence analysis demonstrated ginsenoside Rg1 reduced intracellular ROS and suppressed the intracellular [Ca^{2+}] level. Cell lysate detected an increase of T-SOD, CAT, and GSH levels. The myocardial protection of ginsenoside Rg1 during H/R is partially due to its antioxidative effect and intracellular calcium homeostasis. J. Cell. Biochem. 108: 117–124, 2009.

KEY WORDS: GINSENOSIDE Rg1; HYPOXIA/REOXYGENATION; ANTIOXIDANT; REACTIVE OXYGEN SPECIES; Ca^{2+} OVERLOAD

Notoginseng, the root of *Panax notoginseng (Burk.) F.H. Chen*, is a precious traditional Chinese medicine employed in the treatment of trauma and bleeding due to internal and external injury. It has been demonstrated as having a number of pharmacological effects on the cardiovascular system, liver, kidneys, hematological system, diabetic angiopathy, immune function, anti-inflammatory function, lipid metabolism, antitumor activity, and central nervous system [Ng, 2006]. Saponins are the major active constituents. More than 50 saponin have been isolated from notoginseng [Wang et al., 2006]. Some of these are the same as ginsenosides isolated from Panax ginseng.

In China, a preparation of *Panax notoginseng* saponins (PNS), named Xuesaitong, when used in the treatment of patients with cerebral ischemia, cerebral hemorrhage, angina pectoris, and hyperviscosity syndrome, produced a marked therapeutic effect [Lin, 2006]. The mechanisms of pharmacological and therapeutic effects were shown through antioxidant and influencing calcium ion [Wu and Chen, 1988; Li et al., 1990; Ma and Xiao, 1998; Han et al., 2000; Liu et al., 2002; Zhu et al., 2003; Yuan et al., 2005; Shi et al., 2007].

Ginsenoside Rg1 (Fig. 1), one of the richest components in PNS, was shown antioxidative protection [Liao et al., 2002; Chen et al., 2003; Cheng et al., 2005]. Therefore, the present study was designed to investigate its effect and mechanism on hypoxia–reoxygenation (H/R) damage in cadiomyocytes.

METHODS

CHEMICALS AND REAGENTS

Ginsenoside Rg1 (purity >99%), was purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological products (Beijing, China). The Cell Titer 96 Aqueous One Solution Cell Proliferation Assay Kit was purchased from Promega;

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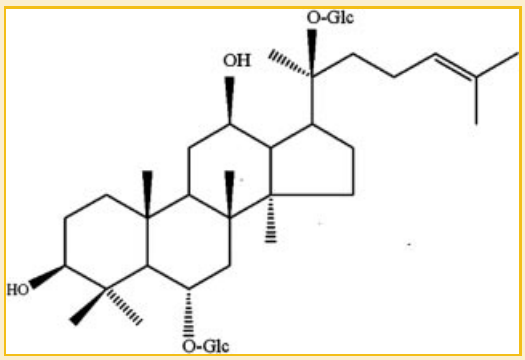

Fig. 1. Structures of ginsenoside Rg1.

glutathione (GSH), catalase (CAT), DCFH-DA, were purchased from Fluka, Germany; NAC, Verapamil, Collagenase, and propidium iodide (PI) were purchased from Sigma–Aldrich; Fluo-4-AM was purchased from Molecular Probes and Dojindo, Japan; DMEM, F_{12}, DMEM glucose-free and other medium components were supplied by Gibco.

ANIMALS

The investigation was performed with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

PRIMARY CULTURE OF CARDIOMYOCYTE

Cardiomyocytes were isolated from hearts of neonatal Sprague–Dawley rats of both sexes that were up to 3 days old and were isolated and cultured as described previously [Simpson and Savion, 1982]. Cells were preplated for 90 min to allow fibroblasts to attach and yield a purer cardiomyocyte culture. After enriched culture for 2 days in a selective medium, the cardiomyocytes were maintained in a low-serum medium (DMEM/F12 1:1 with 0.1% insulin–transferrin–selenium G supplement, 0.5% horse serum, and 0.3% penicillin–streptomycin 100 IU/ml) so as to inhibit the growth of nonmyocyte. After 4-day culture, cardiomyocyte were used in subsequent experiments. The yield of cardiomyocyte was more than 90% as determined by Mayer's hem alum-eosin Y staining.

HYPOXIA/REOXYGENATION TREATMENT PROTOCOL

Pretreated with ginsenoside Rg1, the cultured cardiomyocytes were washed with Hank's solution (5 mM HEPES, 137 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1.5 mM $CaCl_2$, pH 7.2). The cells were incubated in a glucose-free DMEM base medium and then subjected to hypoxia to mimic the in vivo condition of myocardial ischemia. The cells were placed in an incubator at 37°C. N_2 (95%) and 5% CO_2 were flushed into the incubator to bring the oxygen content down to 1% monitored by an oxygen probe. After 3 h of hypoxia, the cells were subjected to reoxygenation by changing the medium into a DMEM base medium with 5.5 mM glucose (pH 7.4) followed by incubation under normoxia for 1 h.

LDH ASSAY

Lactate dehydrogenase (LDH) was released from injured cells and was used as an index of cell injury. LDH activities in the hypoxia and the reoxygenation media and in the cells lysate (by freeze–thawing in distilled water) were determined. The total of LDH activities in the hypoxic and reoxygenation media and the cell lysate obtained from the same well would give the total LDH activity. The percentage of LDH released during hypoxia or reoxygenation in each of these media was standardized against the total LDH activity. The percentages of LDH released during hypoxia and reoxygenation would be combined to give the percentage of LDH released during H/R [Liu et al., 2004].

DETERMINATION OF CELL VIABILITY

After 1 h reoxygenation, cells were washed once in Hank's solution, and the medium was switched to 100 μl of Hank's solution in each of the 96-well plate. Cell viability was assessed by MTS method [Chen et al., 2002]. The Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit was purchased from Promega, WI. The cellular level of 3-(4,5-dimethylthiazol-2-yl-5)-(MTS), an indicator of mitochondrial function of living cells, was linearly proportional to the number of viable cardiomyocytes. Briefly, MTS and PMS were

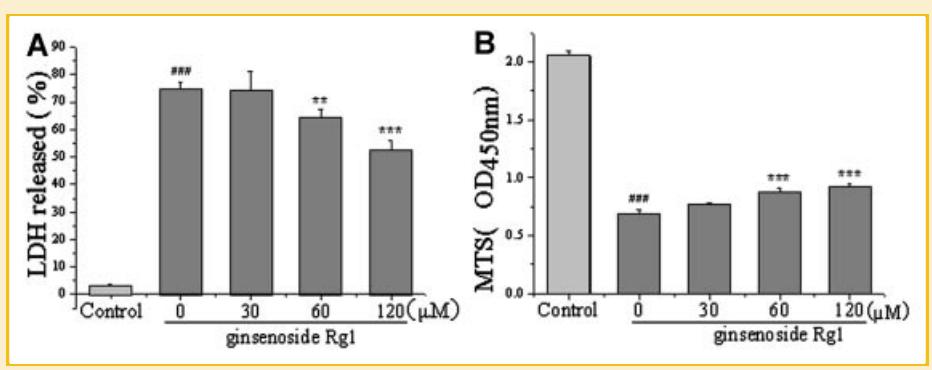

Fig. 2. Pretreatment with ginsenoside Rg1 reduces H/R damage in cardiomyocytes. Cardiomyocytes were pretreated for 48 h with ginsenoside Rg1. The cells were subjected to H/R as described in Methods Section. The percentage of LDH released (A) and cell viability (B) during H/R were determined and expressed as the mean ± s.e.m. ($n = 6$). $^{###}P < 0.001$ as compared with control without H/R. $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with the drug-free control.

mixed at the ratio of 20:1 immediately before being added to the samples. Twenty microliters of MTS/PMS solution was added to each of the 96-well plate and incubated at 37°C in a humidified 5% CO_2 atmosphere for 3 h. The absorbance was read at 490 nm using a 96-well plate spectrophotometric reader (Bio-Rad).

ASSAYS FOR SOD, CAT, GSH

Cardiomyocytes were washed twice with Hank's solution containing 0.05 mM EDTA and were sonicated at 4°C. After centrifugation (800*g*, 5 min), the cell supernatants were immediately assayed for activities of antioxidant enzyme and level of GSH. Total superoxide dismutase (SOD) activity was determined by measuring the blue color of formed formazan with a spectrophoto-meter (measuring absorbance at 560 nm). Superoxide anion ($O_2^{\bullet -}$) formed in the Xanthine-XOD system reduces NBT into formazan. The rate of the reduction with $O_2^{\bullet -}$ were linearly related to the xanthine oxidase (XO) activity, and was inhibited by SOD [Goering et al., 2002]. Results were expressed as U/mg protein. CAT activity was determined according to Aebi's method [Aebi, 1984]. The principles of the assay are based on the determination of the H_2O_2 decomposition rate at 240 nm. Results were expressed as U/mg protein. GSH was determined according to Rotruck et al. [1973], calculated on the basis of GSH calibration curve. Results were expressed as mg/mg protein. Protein content in the cell lysate was determined by the Bradford assay using bovine serum albumin (BSA) as standard.

MEASUREMENT OF INTRACELLULAR ROS

Intracellular reactive oxygen species (ROS) was assessed using the cell-permeable probe 5(6)-carboxy-2′,7′-dichloro-fluorescein diacetate (cDCFH-DA). Cardiomyocytes in 96-well plates were incubated with DCFDA (10 μM) for 20 min at 37°C. The cells were washed by PBS solution three times. DCF fluorescence levels were measured at 0, 30, 60, 90, 120, 150, and 180 min after the initiation of reoxygenation using a multiwell fluorescence plate reader (Bio-Rad). The excitation and emission were set at 488 and 530 nm, respectively. Data (mean ± s.e.m., n = 6) are expressed as arbitrary units of cDCF. The cells in culture bottle pretreated with drug were dissociated after 1 h of reoxygenation and incubated with 10 μM of cDCFH-DA for 20 min at 37°C. The cells were washed by PBS solution twice. PI (10 μM, Molecular Probes) was added to stain for nonviable cells before data acquisition [Yamawaki et al., 2004] The data were analyzed by flow cytometry (FACSCalibur cell sorter, Becton Dickinson) and Cell quest soft. Fluorescence was determined as the emission intensity at 530 nm for cDCF and 670 nm for PI.

MEASUREMENT OF INTRACELLULAR CALCIUM LEVEL

After 1 h of reoxygenation, intracellular calcium measurements were made with the calcium sensitive fluorescent probe, fluo-4-AM as described [Sato et al., 2005]. Cardiomyocytes in 96-well plates were incubated with fluo-4-AM (10 μM) for 40 min at 37°C. The cells were washed by PBS solution three times. Fluorescence levels were measured at 0, 30, 60, 120, and 180 min after the initiation of reoxygenation using a multiwell fluorescence plate reader (Bio-Rad). The excitation and emission were set at 488 and 530 nm, respectively. Data (mean ± s.e.m., n = 6) are expressed as arbitrary units of Fluo-4. The cells in culture bottle were exposed to 10 μM fluo-4-AM for 40 min at 37°C After 1 h of reoxygenation. Washed by PBS solution, the cells were dissociated. PI was added just before data acquisition. During flow cytometry, about 1×10^4 cells were analyzed in each sample. Data were collected for emission at 530 nm for fluo-4 and 670 nm for PI and were plotted simultaneously. Only those cells with low fluorescent intensity at 670 nm (PI-negative) were included in the comparative analysis of $[Ca^{2+}]_i$.

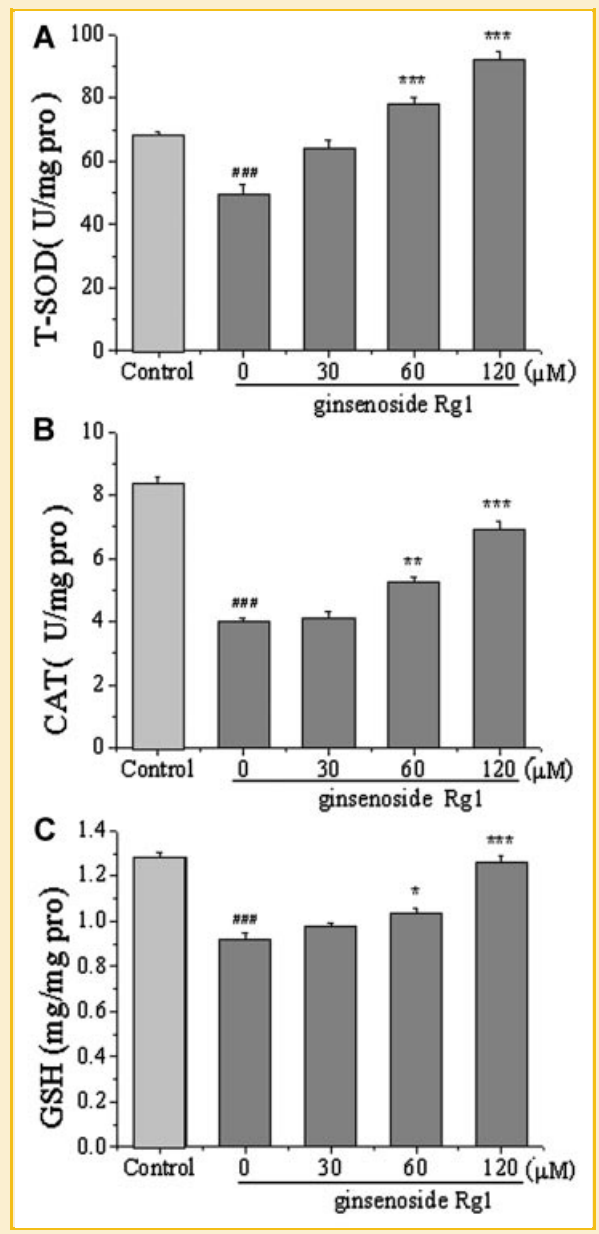

Fig. 3. Pretreatment with ginsenoside Rg1 increases antioxidant activities of cardiomyocyte. Cardiomyocytes were pretreated for 48 h with ginsenoside Rg1. The cells were subjected to H/R as described in Methods Section. The cells lysate was centrifugated, and then the cell supernatant was assayed for activities of SOD (A), CAT (B), and GSH (C). The results are expressed as mean ± s.e.m. (n = 6). $^{###}P < 0.001$ as compared with control without H/R. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with the drug-free control.

STATISTICAL ANALYSES

Data are expressed as mean values with standard error of mean (s.e.m.). One-way ANOVA followed by *t*-test with Bonferroni's correction for multiple comparisons. A difference of $P < 0.05$ was considered significant.

RESULTS

PRETREATMENT WITH GINSENOSIDE Rg1 REDUCES H/R DAMAGE

LDH leakage from cells is widely known as a marker of cellular injury and necrotic cell death. Cadiomyocytes subjected to H/R damage had a 26-fold increase in LDH leakage compared with the without H/R control cells. In cadiomyocytes subjected to H/R damage after being pretreated with ginsenoside Rg1 48 h, LDH leakage was reduced in a dose-dependent manner from 85.8% (60 μM) to 70.4% (120 μM) of the drug-free control (Fig. 2A). After treatment 72 h with ginsenoside Rg1 up to 1,200 μM, cardiomyocytes did not show cytotoxicity, and there were insignificant differences in the total LDH activities compared with the without H/R control (data not shown). Pretreatment with ginsenoside Rg1 (60–120 μM) also increased the cell viability (27.54% and 31.88%, respectively) of the cadiomyocytes subjected to H/R compared with the drug-free control, as shown in MTS (Fig. 2B).

PRETREATMENT WITH GINSENOSIDE Rg1 INCREASED ANTIOXIDANT ACTIVITIES

A number of reports have shown that there is a cellular adaptive response against oxidative stress by strengthening the antioxidant defense system [Kaminski et al., 2002; Zhao, 2004]. The effects of ginsenoside Rg1 on the activity of the antioxidant enzymes and the GSH level in the cadiomyocytes were determined. Pretreatment of

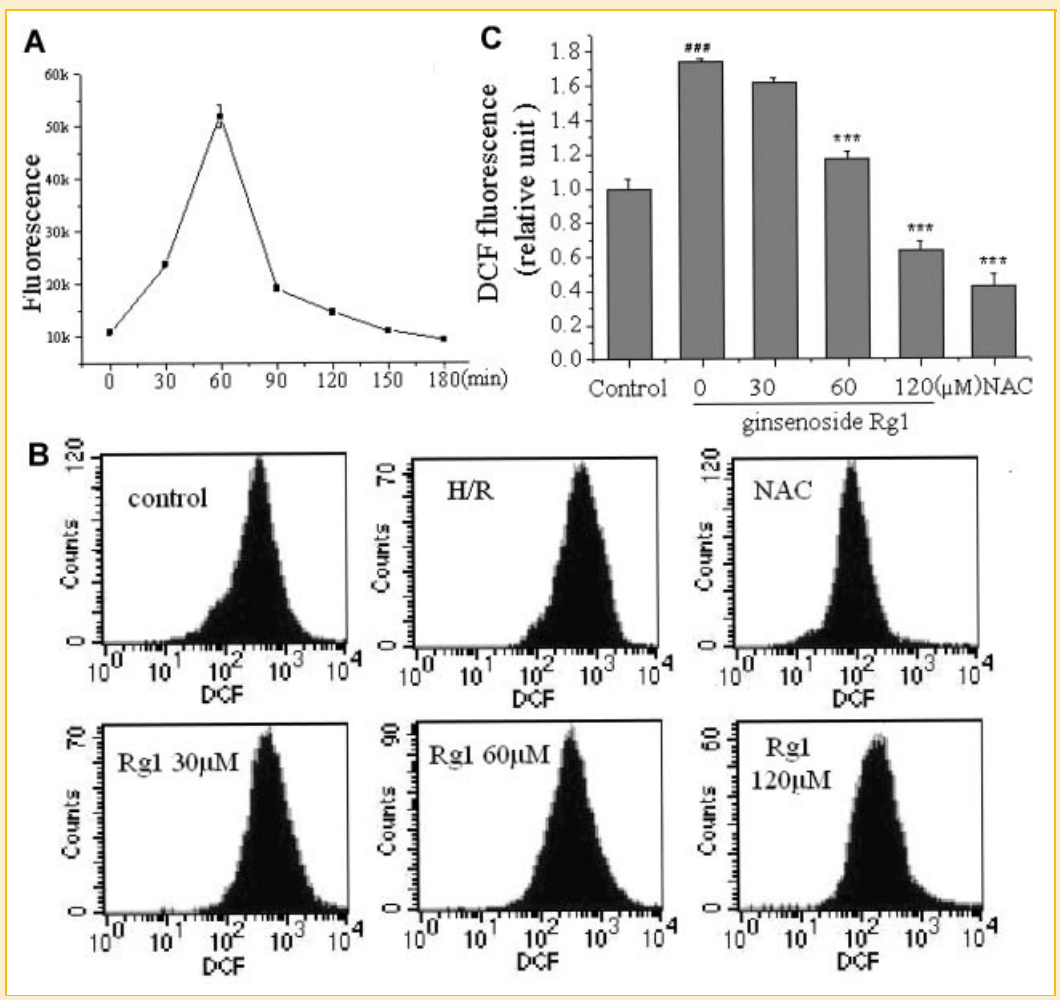

Fig. 4. Pretreatment with ginsenoside Rg1 attenuates intracellular ROS level in cardiomyocytes. Fluorescence of cardiomyocytes loaded DCF was detected at 0, 30, 60, 90, 120, 150, and 180 min of reoxygenation. Fluorescence value peaked at 60 min of reoxygenation and then began to decline quickly. The data were expressed as the mean ± s.e.m. (n = 8) (A). Pretreatment respectively with ginsenoside Rg1 (30–120 μM) 48 h, 10 mM NAC 20 min significantly decreases the elevation of intracellular ROS of cardiomyocytes undergoing H/R (B). Results are expressed as mean ± s.e.m. (n = 3), $^{###}P < 0.001$ as compared with control without H/R; $^{***}P < 0.001$ as compared with the drug-free control in the same treatment group (C).

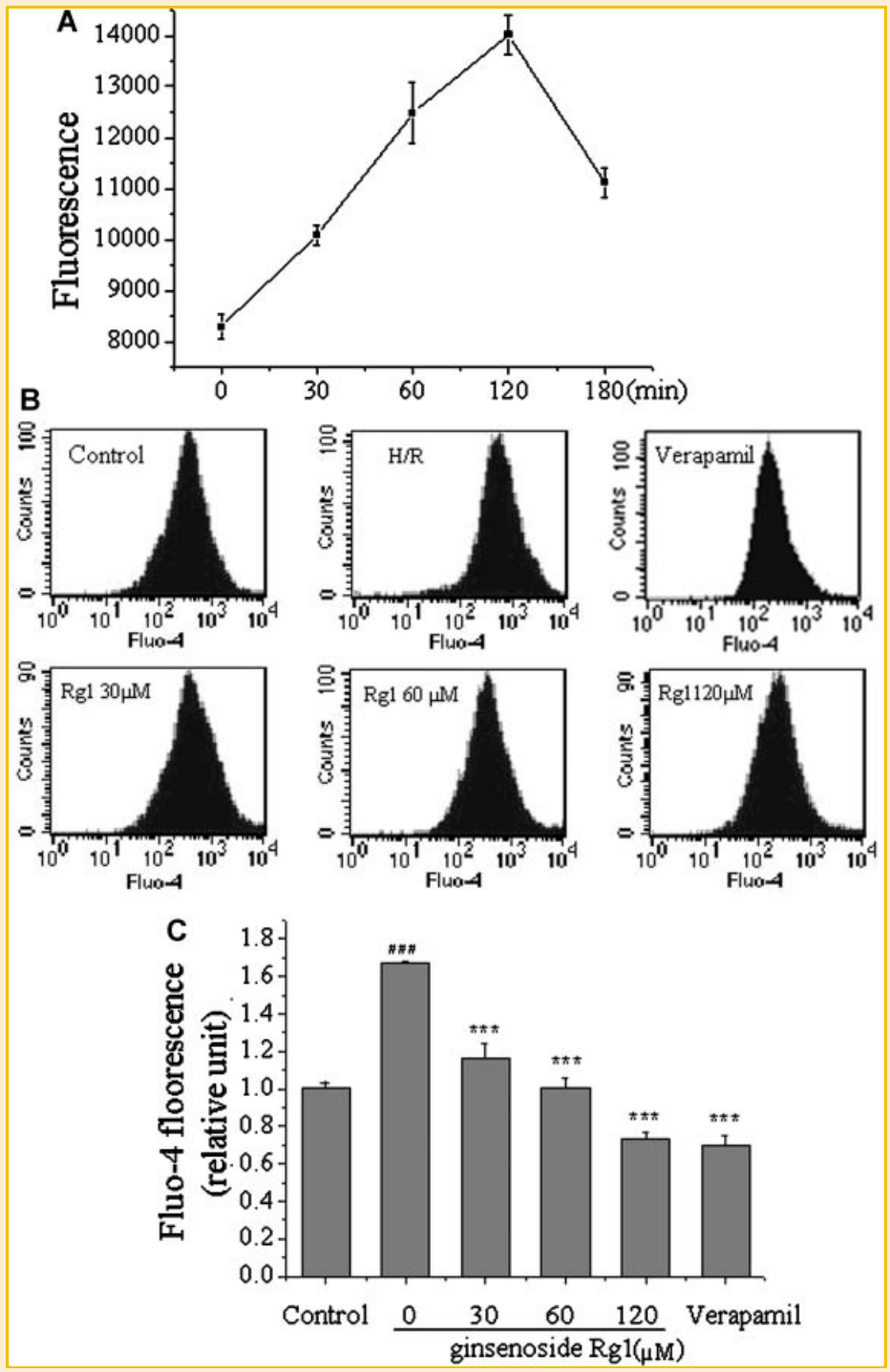

Fig. 5. Pretreatment with ginsenoside Rg1 suppresses $[Ca^{2+}]_i$ overload of cardiomyocytes undergoing H/R Fluorescence of cardiomyocytes loaded Fluo-AM was detected at 0, 30, 60, 120, and 180 min of reoxygenation. Fluorescence value reached maximum at 60 min of reoxygenation and slowly declined during 120–180 min of reoxygenation. The data were expressed as the mean ± s.e.m. (n = 6) (A). Pretreatment respectively with ginsenoside Rg1 (30–120 μM) 48 h, 10 μM verapamil 15 min significantly decreased the elevation of $[Ca^{2+}]_i$ compared with drug-free control in the same treatment group (B). The data were expressed as mean ± s.e.m. (n = 3), $^{###}P < 0.001$ as compared with control without H/R; $^{***}P < 0.001$ as compared with the drug-free control in the same treatment group (C).

the cadiomyocytes with ginsenoside Rg1 (120 μM) 48 h the T-SOD (Fig. 3A) and CAT (Fig. 3B) activities, respectively, were significantly upregulated 85.8% and 73.1% of the drug-free control cells. While the GSH (Fig. 3C) level of the cadiomyocytes was slightly increased 35.9% of the drug-free control cells. These data suggest that the inducted activity of these antioxidant enzymes is one of the cardioprotective effects of ginsenoside Rg1.

PRETREATMENT WITH GINSENOSIDE Rg1 ATTENUATES THE INTRACELLULAR ROS LEVEL

The ROS level increases rapidly during reperfusion (IR) of the ischemic myocardium [Garlick et al., 1987; Doweney, 1990]. The data showed that the intracellular ROS of the cadiomyocytes subjected to H/R increased significantly and reached maximum after 1 h of reoxygenation (Fig. 4A), about 10-fold compared with the control without H/R. Then the intracellular ROS level declined with lengthening reoxygenation time and reached the status of initiation of reoxygenation. Therefore 1 h of reoxygenation was chosen as the end of the H/R in the later experiment. Pretreatment of the cadiomyocytes with ginsenoside Rg1 (60–120 μM) 48 h, respectively, attenuated the intracellular ROS to 67.2% and 36.2% of the drug-free control cells ($P < 0.001$; Fig. 4B,C). NAC, a general antioxidant was chosen for positive drug control. NAC (10 mM) significantly attenuated the intracellular ROS to 24.7% of the drug-free control cells ($P < 0.001$; Fig. 4B,C).

PRETREATMENT WITH GINSENOSIDE Rg1 SUPPRESSES $[Ca^{2+}]_i$ OVERLOAD

The intracellular Ca^{2+} overload as a consequence of oxidative stress may play a crucial role in the transition of cardiac hypertrophy to heart failure. Investigation was employed to determine whether the cardioprotective effect of ginsenoside Rg1 is related to the suppression of intracellular calcium accumulation. Figure 5A showed an increase in $[Ca^{2+}]_i$ peak to about twofold of control after 60 min of reoxygenation (Fig. 5A). $[Ca^{2+}]_i$ of the cadiomyocytes pretreated with ginsenoside Rg1 30–120 μM was markedly suppressed from 69.5% to 44.3% of the control drug-free cells ($P < 0.001$; Fig. 5B,C). Verapamil, a calcium channel blocker, 10 μM significantly inhibited increase of $[Ca^{2+}]_i$ to 41.9% of control drug-free cells ($P < 0.001$; Fig. 5B,C).

DISCUSSION

Based on PNS's multiple pharmacological and therapeutic effects, through investigation, we want to know a major active saponin, ginsenoside Rg1, play a crucial role. In the present study, we have demonstrated that ginsenoside Rg1 exerted protective effects on the cadiomyocytes subjected to hypoxia/reoxygenation injury. It was confirmed by reducing LDH leakage and increasing cell viability.

Myocardial ischemic injury is mediated in part by ROS produced during the ischemic process and is exacerbated by IR [Dhalla et al., 2000; Lefer and Granger, 2000]. ROS generated from metabolic processes are normally scavenged by antioxidants that are part of physiological cellular defense mechanisms. In disease states such as sudden hypoxia, however, overproduction and consumption of ROS results in oxidative cellular damage [Halliwell and Gutteridge, 1999]. Rapid restoration of blood flow increases the level of tissue oxygenation, but there is a second burst of ROS generation that leads to the syndrome of IR injury [Maxwell and Lip, 1997]. ROS-mediated cell death occurs in cells exposed to hypoxia and hypoxia/reoxygenation both in vitro and in vivo [Fliss and Gattinger, 1996; Dhalla et al., 2000]. ROS cause oxidation of proteins, nucleic acids, and lipids, affect critical signal transduction pathways, and finally result in alterations of cardiac function, cellular injury, and death [Duranteau et al., 1998; von Harsdorf et al., 1999; Hensley et al., 2000]. In the heart, ROS-induced abnormalities can include cytotoxicity, cardiac stunning, arrhythmias, alterations of Ca^{2+} homeostasis, and intracellular ATP depletion [Das and Maulik, 1994; Lefer and Granger, 2000]. Cellular antioxidants are critical in combating ROS-induced cell death and tissue damage. They scavenge free radicals and minimize a variety of insults resulting from the oxidative stress [Ferrari et al., 1991; Frei, 1999; Hayes and McLellan, 1999; Wang et al., 2002].

The present results exhibited that ginsenoside Rg1 obviously reduced H/R-induced ROS generation through increasing the activity of endogenous antioxidants, including T-SOD, CAT, and GSH. Recently two reports showed similar results that ginsenoside Rg1 exerts antioxidative effects in lung injury [Shen et al., 2007] and in WI-38 diploid fibroblast cells [Chen et al., 2008].

ROS generated by H/R may promote the entry of Ca^{2+} into vascular myocytes and results in intracellular Ca^{2+} concentration overload. $[Ca^{2+}]_i$ overload is also widely believed to be a precipitating cause of myocardial injury upon ischemia and IR based on the observation that $[Ca^{2+}]_i$ overload is always associated with myocardial injury upon IR [Boston et al., 1998]. Therefore, reduction of $[Ca^{2+}]_i$ during ischemia can greatly alleviate calcium overload during IR.

Some investigations reported that PNS inhibited Ca^{2+} influx into cardiomyocyted [Li and Shi, 1990], and acted as a calcium channel blocker in rat liver cells [Peng et al., 1997; Lu et al., 1999]. The present result shows that ginsenoside Rg1 significantly suppressed intracellular Ca^{2+} overload induced by H/R in cadiomyocytes. A new investigation showed that ginsenoside Rg1 inhibited Ca^{2+} influx through NMDA receptors and L-type voltage-dependent Ca^{2+} channels in neurons. Therefore, we suppose that ginsenoside Rg1 directly suppresses Ca^{2+} channel to prevent intracellular Ca^{2+} overload in cardiomyocytes. Future experiments are being designed to address this issue but are beyond the scope of this report.

More and more studies have been conducted to determine the variety of biological functions associated with the PNS. Penetrating investigation of this mechanism is of benefit for clinic treatment of PNS.

SUMMARY

The present study suggests that ginsenoside Rg1 has shown a cardioprotective effect by scavenging ROS and intracellular Ca^{2+}

homeostasis during H/R. Ginsenoside Rg1 scavenges ROS by increasing the activity of endogenous antioxidants.

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REFERENCES

Aebi H. 1984. Catalase in vitro. Method Enzymol 105:121–126.

Boston DR, Koyama T, Rodriguez-Larrain J, Zou A, Su Z, Barry WH. 1998. Effects of angiotensin II on intracellular calcium and contracture in metabolically inhibited cardiomyocytes. J Pharmacol Exp Ther 285:716–723.

Chen HW, Chien CT, Yu SL, Lee YT, Chen WJ. 2002. Cyclosporine A regulate oxidative stress-induced apoptosis in cardiomyocytes: Mechanisms via ROS generation, iNOS and Hsp70. Br J Pharmacol 137:771–781.

Chen XC, Zhu YG, Zhu LA, Huang C, Chen Y, Chen LM, Fang F, Zhou YC, Zhao CH. 2003. Ginsenoside Rg1 attenuates dopamine-induced apoptosis in PC12 cells by suppressing oxidative stress. Eur J Pharmacol 473:1–7.

Chen X, Zhang J, Fang Y, Zhao C, Zhu Y. 2008. Ginsenoside Rg1 delays tert-butyl hydroperoxide-induced premature senescence in human WI-38 diploid fibroblast cells. J Gerontol A Biol Sci Med Sci 63(3):253–264.

Cheng Y, Shen LH, Zhang JT. 2005. Anti-amnestic and anti-aging effects of ginsenoside Rg1 and Rb1 and its mechanism of action. Acta Pharmacol Sin 26:143–149.

Das DK, Maulik N. 1994. Antioxidant effectiveness in ischemiareperfusion tissue injury. Methods Enzymol 233:601–610.

Dhalla NS, Temsah RM, Netticadan T. 2000. Role of oxidative stress in cardiovascular diseases. J Hypertens 18:655–673.

Doweney JM. 1990. Free radicals and their involvement during long-term myocardial ischemia and reperfusion. Annu Rev Physiol 52:487–504.

Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT. 1998. Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. J Biol Chem 273:11619–11624.

Ferrari R, Ceconi C, Curello S, Cargnoni A, Alfieri O, Pardini A, Marzollo P, Visioli O. 1991. Oxygen free radicals and myocardial damage: Protective role of thiolcontaining agents. Am J Med 91:95S–105S.

Fliss H, Gattinger D. 1996. Apoptosis in ischemic and reperfused rat myocardium. Circ Res 79:949–956.

Frei B. 1999. On the role of vitamin C and other antioxidants in atherogenesis and vascular dysfunction. Proc Soc Exp Biol Med 222:196–204.

Garlick PB, Davies MJ, Hearse DJ, Slater TF. 1987. Direct detection of free radicals in the perfused rat heart using electron spin resonance spectroscopy. Circ Res 61:757–760.

Goering PL, Morgan DL, Ali SF. 2002. Effects of mercury vapor inhalation on reactive oxygen species and antioxidant enzymes in rat brain and kidney are minimal. J Appl Toxicol 22:167–172.

Halliwell B, Gutteridge JMC. 1999. Free radicals in biology and medicine, 3rd edition. Oxford: Clarendon Press.

Han JA, Kuang YQ, Zhou HT, Yang LB, Zeng FJ, Sun ZH, Hu WY, Wang XL. 2000. Effects of panax notoginseng saponins on malondialdehyde content of the brain following brain injury in rabbits. Chin J Pathophysiol 16:269–271.

Hayes JD, McLellan LI. 1999. Glutathione and glutathionedependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radic Res 31:273–300.

Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. 2000. Reactive oxygen species, cell signaling, and cell injury. Free Radic Biol Med 28:1456–1462.

Kaminski KA, Bonda TA, Korecki J, Musial WJ. 2002. Oxidative stress and neutrophil activation—The two keystones of ischemia/reperfusion injury. Int J Cardiol 86:41–59.

Lefer DJ, Granger DN. 2000. Oxidative stress and cardiac disease. Am J Med 109:315–323.

Li HT, Shi L. 1990. Effects of total saponins of Panax notoginseng on Ca^{2+} Influx into myocardial cells. Acta Pharmacol Sin 11(3):213–217.

Li X, Chen JX, Sun JJ. 1990. Protective effects of Panax notoginseng Saponin on experimental myocardial injury induced by ischemia and reperfusion in rat. Acta Pharmacol Sin 11(1):26–29.

Liao B, Newmark H, Zhou R. 2002. Neuroprotective effects of ginseng total saponin and ginsenosides Rb1 and Rg1 on spinal cord neurons in vitro. Exp Neurol 173:224–234.

Lin W. 2006. Progression of clinical application of notoginseng saponins. Appl J Gen Pract 4(5):590–592.

Liu JH, Feng Y, Wang T, Xie XD, Yao B. 2002. Effect of notoginsenosides on Ca^{2+} excitory amino acid content in rat brain of cerebral ischemia and reperfusion. Chin J Clin Pharmacol Ther 7(1):33–34.

Liu J, Kam KW, Zhou JJ, Yanm WY, Chen M, Wu S, Wong TM. 2004. Effects of heat shock protein 70 activation by metabolic inhibition preconditioning or—Opioid receptor stimulation on Ca^{2+} homeostasis in rat ventricular myocytes subjected to ischemic insults. J Pharmacol Exp Ther 30:606–612.

Lu MD, Huang JL, Xiao DZ, Huang XH, Guan YY. 1999. Effects of total saponins of panax notoginseng on Ca^{2+} influx into rat hepatocytes loaded with Fura-2. Chin Pharmacol Bull 15(2):150–152.

Ma LY, Xiao PG. 1998. Efects of saponins of Panax notoginseng on intracellular free Ca^{2+} concentration in dissociated neurons. Chin Pharm J 33(8):467–469.

Maxwell SR, Lip GY. 1997. Reperfusion injury: A review of the pathophysiology, clinical manifestations and therapeutic options. Int J Cardiol 58: 95–117.

Ng TB. 2006. Pharmacological activity of sanchi ginseng(Panax notoginseng). J Pharm Pharmacol 58:1007–1019.

Peng BG, Lu MD, Xiao DZ, Guan YY. 1997. Inhibitory effect of panax notoginseng saponins on Ca influx in rat liver cells. Chin Pharmacol Bull 13(1):70–73.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science 179:588–590.

Sato T, Saito T, Saegusa N, Nakaya H. 2005. Mitochondrial Ca^{2+}-activated K^{+} channels in cardiac myocytes: A mechanism of the cardioprotective effect and modulation by protein kinase A. Circulation 111:198–203.

Shen L, Han JZ, Li C, Yue SJ, Liu Y, Qin XQ, Liu HJ, Luo ZQ. 2007. Protective effect of ginsenoside Rg1 on glutamate-induced lung injury. Acta Pharmacol Sin 28(3):392–397.

Shi R, Liu L, Huo Y, Cheng YY. 2007. Study on protective effects of Panax notoginseng saponins on doxorubicin-induced myocardial damage. Chin J Chin Materia Medica 32(24):2635–2635.

Simpson P, Savion S. 1982. Differentiation of rat myocytes in single cell cultures with and without proliferating nonmyocardial cells. Crossstriations, ultrastructure, and chronotropic response to isoproterenol. Circ Res 50:101–116.

von Harsdorf R, Li PF, Dietz R. 1999. Signaling pathways in reactive oxygen species-induced cardiomyocyte apoptosis. Circulation 99:2934–2941.

Wang QD, Pernow J, Sjoquist PO, Ryden L. 2002. Pharmacological possibilities for protection against myocardial reperfusion injury. Cardiovasc Res 55:25–37.

Wang CZ, McEntee E, Wicks S, Wu JA, Yuan CS. 2006. Phytochemical and analytical studies of Panax notoginseng (Burk.) F.H. Chen. J Nat Med 60: 97–106.

Wu JX, Chen JX. 1988. Depressant action of Panax notoginseng saponins on vascular smooth muscles. Acta Pharmacol Sin 9(2):147–152.

Yamawaki M, Sasaki N, Shimoyama M, Miake J, Ogino K, Igawa O, Tajima F, Shigemasa C, Hisatome I. 2004. Protective effect of edaravone against hypoxia-reoxygenation injury in rabbit cardiomyocytes. Br J Pharmacol 142:618–626.

Yuan WY, Ye QF, Jiang WQ, Jiang WL, Shen RS. 2005. Elimination of oxygen free radicals in rat liver mitochondria by total saponins of panax notoginseng during ischemia reperfusion. Chin J Mod Med 15(20):3076–3078.

Zhao ZQ. 2004. Oxidative stress-elicited myocardial apoptosis during reperfusion. Curr Opin Pharmacol 4:159–165.

Zhu LQ, Fan JP, Huang QF, Sun SL, Gao Y, Zou YH, Zhang Z, He LY, Zheng H. 2003. Study on the anti-apopotosis induced by hypoxia/hypoglycemia and reoxygenation of panax notoginseng saponins in cultured rat hippocampal neurons. Chin J Chin Materia Medica 28(1):52–55.