Food and Chemical Toxicology 48 (2010) 1516–1520

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

The effects of ginseng total saponin, panaxadiol and panaxatriol on ischemia/reperfusion injury in isolated rat heart

Tae-Hoon Kim, Sun-Mee Lee *

School of Pharmacy, Sungkyunkwan University, 300 Cheoncheon-dong, Jangan-gu, Suwon, Gyeonggi-do 440-746, Republic of Korea

ARTICLE INFO

Article history:
Received 8 October 2009
Accepted 15 March 2010

Keywords:
Cardiac function
Ischemia/reperfusion
Langendorff
Oxidative stress
Panax ginseng

ABSTRACT

The aim of the present study was to evaluate the protective effect of ginseng total saponin, panaxadiol and panaxatriol, which are the major components of *Panax ginseng*, against myocardial ischemia/reperfusion (I/R) injury in isolated rat hearts. Rats were orally administered once a day with total saponin (20 mg/kg), panaxadiol (5 mg/kg) and panaxatriol (5 mg/kg) for consecutive 7 days. On day 8, the hearts were isolated and perfused with Krebs-Henseleit bicarbonate buffer solution using Langendorff apparatus. After 30 min of global ischemia, hearts were reperfused for 30 min. Myocardial function, coronary flow and biochemical parameters, such as lactate dehydrogenase (LDH), creatine kinase (CK), adenosine triphosphate (ATP), malondialdehyde (MDA) and reduced glutathione (GSH) were measured. Total saponin and panaxatriol significantly improved I/R-induced myocardial dysfunction by increasing left ventricular development pressure, (−dP/dt)/(+dP/dt) and time to contracture. Moreover, the increases in the levels of LDH, CK and MDA and the decrease in the levels of GSH were attenuated by total saponin and panaxatriol. However, the ATP levels did not affected by total saponin, panaxadiol and panaxatriol pretreatment. Our findings suggest that pretreatment with ginseng total saponin, especially panaxatriol, ameliorates I/R-induced myocardial damage and this protection is caused by reducing oxidative stress.

1. Introduction

Myocardial ischemia and reperfusion (I/R) is clinically relevant in situations such as myocardial infarction, coronary angioplasty, thrombolytic therapy, coronary revascularization and heart transplantation (Rao and Viswanath, 2007). Although the nature of this I/R injury has been extensively studied, the mechanisms by which organ damage occurs are not clear.

The production of reactive oxygen species (ROS) from various cellular metabolic processes caused by the restoration of coronary flow after cardiac ischemia is thought to contribute to the observed myocardial damage (Bolli, 1991; Gross et al., 1986; Zweier et al., 1987). These ROS cause non-specific damage to lipids, proteins and DNA, leading to an alteration or loss of the cellular function. The abrupt rise in ROS as a result of the reoxygenation of ischemic or hypoxic cardiac muscle has been associated with a partial irreversible inhibition of mitochondrial respiration (Xie et al., 1996). Alterations in generation and/or use of energy are thought to be important contributing factors to the observed dysfunction caused by I/R (Taniguchi et al., 2001). Rapid resumption of oxidative phosphorylation is critical for the restoration of adequate energy metabolism (ATP and creatine phosphate production) and cellular survival.

Panax ginseng C.A. Mayer (Araliaceae) is one of the most popular natural tonics and has been shown to possess various biological activities such as a protein anabolic effect, anti-tumor activities and an inhibitory effect of tumor angiogenesis and metastasis (Sato et al., 1994). Also, ginseng has been used for treatment of heart failure and to protect tissues from damage when an organism is in stress (Wagner and Liu, 1987). Moreover, ginseng has the advantage that it is free from harmful side effects. Ginsenosides, which are glycosides containing an aglycone (protopanaxadiol or protopanaxatriol), are the major effective components of ginseng and have been shown to have a wide variety of biological activities including immunomodulatory effects, antioxidant, anti-inflammatory and anti-tumor activity (Attele et al., 1999; Kenarova et al., 1990; Park et al., 2003; Shibata, 2001). However, it is still unknown which ginsenosides have beneficial effects on the cardiac injury caused by I/R.

Abbreviations: ATP, adenosine triphosphate; ANOVA, analysis of variance; CK, creatine kinase; DP, double product of heart rate×left ventricular developed pressure divided by 1000; EDP, end-diastolic pressure; GSH, reduced glutathione; HR, heart rate; LDH, lactate dehydrogenase; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; LVP, left ventricular pressure; LVSP, left ventricular peak systolic pressure; MDA, malondialdehyde; ROS, reactive oxygen species; TTC, time to contracture.

* Corresponding author. Tel.: +82 31 290 7712; fax: +82 31 292 8800.
E-mail address: sunmee@skku.edu (S.-M. Lee).

0278-6915/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2010.03.018

Accordingly, we designed this study to investigate the effects of ginseng total saponin, panaxadiol and panaxatriol on post-ischemic heart injury, particularly on the oxidative stress and energy metabolism.

2. Materials and methods

2.1. Materials

Total saponin, panaxadiol, panaxatriol were isolated from 6 years old red ginseng by thin layer chromatography and high performance liquid chromatography, and supplied by Korea Ginseng and Tobacco Research Institute. Sodium pyruvate, lactate dehydrogenase (LDH), creatine kinase (CK) and ATP kits were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and heparin sodium was supplied from Choongwae Pharmaceutical Co. (Suwon, Korea). All other chemicals were of the reagent grades commercially available locally.

2.2. Treatment of animals

Male Sprague–Dawley rats weighing 250–300 g (Jeil Animal Breeding Company, Korea) were used for the study. The animals were housed in cages in temperature controlled rooms with a 12:12 h light–dark cycle and received water and food *ad libitum*. All animals were treated humanely according to the Sungkyunkwan University Animal Care Committee guidelines. Total saponin, panaxadiol and panaxatriol were dissolved in phosphate-buffered saline (pH 7.4, vehicle). The animals were orally administered once a day with total saponin (20 mg/kg), panaxadiol (5 mg/kg) and panaxatriol (5 mg/kg) for consecutive 7 days. In pre-ischemic period, there were no significant differences in biochemical parameters, coronary flow and time to contracture among treatment groups; thus, the data from rats were pooled to one pre-ischemic group.

2.3. Langendorff heart preparation

The rats were anesthetized with sodium pentobarbital (100 mg/kg, i.p.). The femoral vein was injected with heparin (1000 U/kg) and the isolation of heart was performed according to the method of Grover et al. (1995). While the trachea of rats was intubated and mechanically ventilated, their hearts were perfused *in situ* with oxygenated Krebs-Henseleit bicarbonate buffer solution (pH 7.4) by retrograde aortic cannulation. The hearts were then excised and quickly moved to Langendorff apparatus, where they were perfused with oxygenated Krebs-Henseleit solution containing (in mM) NaCl, 112; $NaHCO_3$, 25; KCl, 5; $MgSO_4$, 1.2; KH_2PO_4, 1; $CaCl_2$, 1.25; glucose, 11.5; and pyruvate, 2 at a constant perfusion pressure (75 mm Hg). A water-filled latex balloon attached to a metal cannula was inserted into the left ventricle and connected to Statham pressure transducer (Gould Inc., Oxnard, CA) for measurement of left ventricular pressure (LVP).

2.4. Experimental protocols

The hearts were allowed to equilibrate for 15 min, at which time end-diastolic pressure (EDP) was adjusted to 5 mm Hg; this balloon volume was maintained for the duration of the experiment. Pre-ischemia or predrug contractile function, heart rate (HR) and coronary flow (volume of buffer overflowed out of isolated heart chamber during 1 min) were then measured (Grover et al., 1995). Cardiac contractile function was determined using the double product (DP) of HR × left ventricular developed pressure (LVDP) divided by 1000. LVDP was calculated from the difference between left ventricular peak systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP). In addition, the first derivative of LVDP, the rates of left ventricular maximal pressure development (+dP/dt) and relaxation (−dP/dt) were monitored by polygraph differentiator (Model 7P20, Grass instrument Co., Quincy, MA, USA). Cardiac temperature was maintained throughout the experiment by submerging the hearts in 37 °C buffer which was allowed to accumulate in a stoppered, heated chamber. The hearts were then subjected to global ischemia by completely shutting off the perfusate for 30 min. Time to contracture (TTC) during global ischemia was the calculated. TTC was the time (in seconds) from onset of global ischemia in which the first 5 mm Hg increase in LVEDP is observed. The hearts were reperfused and, 30 min later, contractile function [LVDP, DP, HR and (−dP/dt)/(+dP/dt)] and coronary flow were monitored. The timing of I/R were selected based on previous reports (Lee et al., 1999; Ko et al., 1997).

2.5. Analytical procedures

The reperfusion effluent was collected for cumulative lactic acid dehydrogenase (LDH) and creatine kinase (CK) releases. LDH and CK concentrations in perfusate and ATP levels in the heart were determined by standard spectrophotometric procedures using diagnostics kits (Sigma Chemical Co.). Lipid peroxide was assayed by the method of Buege and Aust (1978), and 1,1,3,3-tetraethoxypropane [malondialdehyde (MDA) tetraethyl acetal] was used as the standard. Total glutathione was determined in liver homogenates after precipitation with 1% picric acid and using yeast-glutathione reductase, 5,50-dithio-bis(2-nitrobenzoic acid), and NADPH, at 340 nm. Glutathione disulfide (GSSG) was determined by the same method in the presence of 2-vinylpyridine and reduced glutathione (GSH) calculated from the difference between total glutathione and GSSG (Anderson, 1985).

2.6. Statistical analysis

All values are expressed as means ± S.E.M. Date were analyzed by the paired Student's *t*-test between two values and one-way analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparisons. All statistical differences were determined at $P < 0.05$ and $P < 0.01$ level.

3. Results

3.1. Cardiac contractility function

As shown Table 1, cardiac contractile function and heart rate were similar for all experimental groups before ischemia. Total saponin, panaxadiol and panaxatriol alone did not affect pre-ischemic cardiac function and heart rate (data not shown). In the vehicle-treated group, cardiac contractile function and heart rate significantly depressed after 30 min reperfusion, indicating severe I/R damage. Total saponin and panaxatriol significantly improved cardiac function as shown in LVDP, DP and (−dP/dt)/(+dP/dt), indicating marked cardioprotection.

3.2. Coronary flow and time to contracture

Baseline values of coronary flow and time to contracture before ischemia were similar between all the treatment groups. The baseline value of coronary flow was 14.1 ± 1.21 ml/min, and which was slightly decreased after 30 min reperfusion. Treatments of total saponin, panaxadiol and panaxatriol did not affect the decrease of coronary flow, only showing the tendency to increase without statistical significance (Fig. 1A). Time to contracture is the most important parameter that determines the anti-ischemic effect of a substance for cardiac muscles. In the vehicle-treated group, severe contracture failure was observed and TTC was

Table 1
Effects of ginseng total saponin, panaxadiol and panaxatriol on cardiac function before and after global ischemia and reperfusion.

Parameter	Pre-ischemia	Post-reperfusion
LVDP (mmHg)		
Vehicle-treated	73.3 ± 1.3	19.6 ± 3.5**
Total saponin	72.1 ± 2.0	42.7 ± 5.5++
Panaxadiol	72.9 ± 0.5	20.9 ± 5.0
Panaxatriol	71.4 ± 0.9	68.1 ± 1.8++
HR (beats/min)		
Vehicle-treated	278.0 ± 13.8	212.7 ± 22.3*
Total saponin	267.8 ± 10.9	247.6 ± 10.3
Panaxadiol	273.3 ± 10.1	231.8 ± 6.5
Panaxatriol	229.0 ± 15.9	206.7 ± 6.3
DP(LVDP × HR/1000)		
Vehicle-treated	20.3 ± 0.6	4.1 ± 0.9**
Total saponin	19.3 ± 1.0	10.4 ± 1.1++
Panaxadiol	19.9 ± 0.8	4.8 ± 1.0
Panaxatriol	16.4 ± 1.2	12.8 ± 1.0++
(−dp/dt)/(+dp/dt)		
Vehicle-treated	0.95 ± 0.03	0.65 ± 0.02*
Total saponin	0.95 ± 0.03	0.82 ± 0.05+
Panaxadiol	0.96 ± 0.03	0.77 ± 0.05
Panaxatriol	0.94 ± 0.04	0.92 ± 0.02++

Values are means ± S.E.M. for 6–8 rats per group.
* Significantly different ($P < 0.05$) from pre-ischemia values.
** Significantly different ($P < 0.01$) from pre-ischemia values.
+ Significantly different ($P < 0.05$) from vehicle-treated group.
++ Significantly different ($P < 0.01$) from vehicle-treated group.