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Research article

Ginseng total saponin attenuates myocardial injury via anti-oxidative and anti-inflammatory properties

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ABSTRACT

Background: Ginseng total saponin (GTS) contains various ginsenosides. These ginsenosides are widely used for treating cardiovascular diseases in Asian communities. The aim of this study was to study the effects of GTS on cardiac injury after global ischemia and reperfusion (I/R) in isolated guinea pig hearts.
Methods: Animals were subjected to normothermic ischemia for 60 minutes, followed by 120 minutes of reperfusion. GTS significantly increased aortic flow, coronary flow, and cardiac output. Moreover, GTS significantly increased left ventricular systolic pressure and the maximal rate of contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$). In addition, GTS has been shown to ameliorate electrocardiographic changes such as the QRS complex, QT interval, and RR interval.
Results: GTS significantly suppressed the biochemical parameters (i.e., lactate dehydrogenase, creatine kinase-MB fraction, and cardiac troponin I levels) and normalized the oxidative stress markers (i.e., malondialdehyde, glutathione, and nitrite). In addition, GTS also markedly inhibits the expression of interleukin-1β (IL-1β), IL-6, and nuclear factor-κB, and improves the expression of IL-10 in cardiac tissue.
Conclusion: These data indicate that GTS mitigates myocardial damage by modulating the biochemical and oxidative stress related to cardiac I/R injury.

1. Introduction

According to the World Health Organization, myocardial infarction (MI) is the leading cause of mortality worldwide [1]. The primary manifestations of myocardial ischemia and reperfusion (I/R) are myocyte death and contractile dysfunction [2,3]. Numerous studies have suggested that treatment with cardioprotective drugs can significantly reduce MI [4,5]. Recently, traditional herbs have been suggested to improve heart disease [6,7].

Panax ginseng Meyer belongs to the Araliaceae family and is a perennial herbal medicine [8]. Ginseng has been used as a folk medicine for several thousand years: *P. ginseng* is extensively used for preventing cardiovascular diseases in Asian countries [9,10]. We had previously reported that ginseng total saponin (GTS) protects against I/R-induced injury in isolated rat hearts [11]. However, to our knowledge, there is no study that evaluated the effects of GTS in guinea pigs. Therefore, this study was designed to examine the effects of GTS on I/R injury in isolated guinea pig hearts. The primary aim of this study was to evaluate hemodynamic functions such as aortic flow, coronary flow, and cardiac output and, in particular, the left ventricular systolic pressure (LVSP) and the maximal rate of contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$). We also aimed to determine whether GTS reduces abnormal electrocardiographic (ECG) changes such as QRS complex, QT interval, and RR interval, and whether GTS inhibits the levels of biochemical markers such as lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), cardiac troponin I (cTnI), and malondialdehyde (MDA). In addition, we established whether GTS increases antioxidant parameters, such as glutathione (GSH) and nitrite, and protects cardiomyocytes through the regulation of

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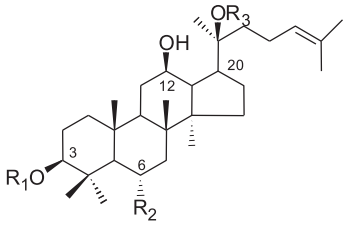

Ginsenosides	R_1	R_2	R_3
Rb_1	-Glc_2-Glc	-H	-Glc_6-Glc
Rb_2	-Glc_2-Glc	-H	-Glu_6-Ara(pyr)
Rc	-Glc_2-Glc	-H	-Glc_6-Ara(fur)
Rd	-Glc_2-Glc	-H	-Glc
Re	-H	-O-Glc_2-Rha	-Glc
Rf	-H	-O-Glc_2-Glc	-H
Rg_1	-H	-O-Glc	-Glc
Rg_2	-H	-O-Glc_2-Rha	-H
Rg_3	-Glc_2-Glc	-H	-H

Fig. 1. Structures of the nine representative ginsenosides. They differ at three side chains attached to the common steroid ring. Superscript numbers indicate the carbon in the glucose ring that links the two carbohydrates. Ara (pyr), arabinopyranoside; Glc, glucopyranoside; Rha, rhamnopyranoside.

inflammatory cytokines and nuclear factor-kappa B (NF-κB) activity.

2. Materials and methods

2.1. Animals

Forty-five male Duncan-Hartley guinea pigs weighing 300–350 g were purchased from Samtako (Seoul, Korea) and used in this study. All animals were housed in colony cages at an ambient temperature of 22 ± 2°C and humidity of 45 ± 10°C with alternating 12-hour light and dark cycle. All animals had access to standard food and water *ad libitum* for 1 week prior to the experiment. All animal experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

2.2. Test drugs

GTS was obtained from Korea Ginseng Corporation (Daejeon, Korea; Fig. 1). Ginsenosides Rg1, Re, Rf, Rh1, Rb1, Rc, Rb2, Rd, Rg3s, and Rg3r were purchased from Chromadex Co. (Irvine, CA, USA) and ginsenosides Rg2s and Rg2r were obtained from Embo Laboratory (Seoul, Korea; Fig. 2). Modified Krebs–Henseleit bicarbonate (KH) solution consisted of NaCl 120.0mM, $NaHCO_3$ 25mM, KCl 4.8mM, KH_2PO_4 1.2mM, $CaCl_2$ 1.25mM, $MgSO_4$ 1.2mM, and glucose 11.0mM. All reagents were guaranteed grade, and high-performance liquid chromatography-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany).

2.3. Preparation of GTS and liquid chromatography

The ultraperformance liquid chromatography was performed using a Waters ACQUITY system equipped with a binary solvent delivery pump, an auto sampler, and a photodiode array detector. The mobile phase consisted of acetonitrile and water at a flow rate of 0.6 mL/min. Gradient elution was as follows: isocratic elution with 15% for 0.5 minutes, followed by a 14-minute gradient to 30%, 2 2-minute gradients to 40%, 3.5-minute gradient minutes to 90%, then isocratic elution with 90% in for 2 minutes, and then finally returned to 15% in 4 minutes.

2.4. Experimental protocols

The animals were divided into the following five groups ($n = 9$ in each group): Normal control (N/C), guinea pigs orally received 0.2% (vol/vol) starch in tap water once daily for 14 days and their hearts were not subjected to I/R; GTS control, animals were fed with 200 mg/kg GTS orally once per day for 14 days and their hearts were not subjected to I/R; I/R control, animals received 0.2% starch for 14 days and then I/R was induced for 60 minutes and 120 minutes, respectively; 100GTS + I/R, animals were treated with 100 mg/kg GTS for 14 days and then I/R was induced for 60 minutes and 120 minutes, respectively; 200GTS + I/R, animals received 200 mg/kg GTS and then I/R was induced for 60 minutes and 120 minutes, respectively. At the end of reperfusion, the coronary effluents and left ventricle tissues were quickly frozen and fixed in 10% formalin (−80°C) for biochemical examination, respectively.

2.5. Preparation of isolated heart

After GTS administration for 14 days, animals were anesthetized with pentobarbital (30 mg/kg) intraperitoneally. The heart was excised as described previously [12]. In brief, standard perfusion was carried out at 37°C with a modified KH solution. The veins entering the right atrium were ligated, so that coronary sinus effluent passed into the right ventricle and was ejected through the pulmonary artery. Coronary flow was continuously recorded with a flow meter. The left atrium was cannulated through an opening by uniting the pulmonary orifices. This allowed for the natural filling and contraction of the left atrial appendage [13].

2.6. Measurement of hemodynamic parameters

To evaluate the effects of GTS, hemodynamic data after reperfusion were compared for changes in aortic flow, coronary flow, cardiac output, and LVSP. Aortic flow was measured by the flow volume ejected from the cannula located 100 cm above the heart. Coronary flow was measured by the collection of perfusate from the pulmonary trunk (mL/min). Cardiac output was evaluated by summing the aortic and coronary effluents. LVSP was evaluated by a pressure transducer connected to the aortic cannula. In this study, the maximal rate of contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) are considered indices of ventricular contractility [14]. The $+dP/dt_{max}$ and $-dP/dt_{max}$ values were analyzed at 30-minute intervals for reperfusion.

2.7. Preparation of ECG recording

For ECG recording, electrodes were placed on the epicardial surface and signals from electrodes were amplified by an electric amplifier (AB-621G; Nihon Kohden, Tokyo, Japan), evaluated on a computer (PC-9801VX; NEC, Tokyo, Japan) through an A/D converter (Analog-Pro Jr., Canopus Electric, Kobe, Japan), and analyzed with WAVE MASTER II and WM Read (Canopus Electric) as described previously [15,16]. If rhythm is irregular during the equilibration, the heart was discarded.

2.8. Biochemical, oxidative stress, and anti-inflammatory assays

The coronary effluent was collected after reperfusion. Myocardial injury was assessed using the LDH level [17], CK-MB activity