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Red Ginseng Saponin Fraction A Isolated from Korean Red Ginseng by Ultrafiltration on the Porcine Coronary Artery

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Red ginseng saponin fraction-A (RGSF-A) contains a high percentage of panaxadiol saponins that were isolated from Korean red ginseng by ultrafiltration. The aim of this study was to elucidate the effects of RGSF-A on the porcine distal left anterior descending (LAD) coronary artery. The relaxant responses to RGSF-A were examined during contractions induced by 100 nM U46619 (9,11-dideoxy-9a,11a-methanoepoxy-prostaglandin F2a), a stable analogue of thromboxane A2. RGSF-A dose-dependently induced biphasic (fast- and slow-) relaxation in the distal LAD coronary artery in the presence of an intact endothelium. The fast-relaxation was quickly achieved in a minute, and then the slow-relaxation was slowly developed and sustained for more than thirty minutes after the administration of RGSF-A. The slow-relaxation had a tendency to be bigger than the fast-relaxation. Fast relaxation induced by RGSF-A was almost blocked by N_{ω}-Nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase synthase inhibitor and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a guanylate cyclase inhibitor. However slow relaxation induced by RGSF-A was only partially inhibited by L-NAME and ODQ. In the endothelium-removed ring, RGSF-A evoked only slow-relaxation to a certain extent. These data suggest that RGSF-A induced both endothelium dependent fast- and slow-relaxation and endothelium independent slow-relaxation in the porcine distal LAD coronary artery. The endothelium dependent fast-relaxation is mediated by the nitric oxide (NO)-cGMP pathway, and the endothelium dependent slow-relaxation is at least partially mediated by the NO-cGMP pathway. However, the endothelium-independent slow-relaxation remains to be elucidated.

Keywords: Ginseng, Red ginseng saponin fraction-A, Porcine coronary artery, Endothelium, Nitric oxide

INTRODUCTION

Korean red ginseng (*Panax ginseng*) has been used for more than two thousand years in traditional medicine in the Far Eastern Asian regions for a variety of disorders. Ginsenosides, which have a four ring steroid-like structure with attached sugar moieties, are considered a biologically active component of *P. ginseng* [1-3]. The ginseng root contains more than 30 types of ginsenosides divided into two major groups based on their chemical structure: panaxadiols with sugar moieties at the C-3 and C-21 positions of the sterol structure and panaxatriols with sugar moieties at positions C-6 and C-21 [4]. Ginsenoside Rb_1, Rb_2, Rb_3, Rc, Rd, Rg_3, Rh_2 and Rs_1 represent the panaxadiols, whereas ginsenoside Re, Rf, Rg_1, Rg_2 and Rh_1 represent panaxatriols. It has been reported that panaxadiol and panaxatriol have different effects in various tissue [5-8].

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It has been reported that cardiovascular protection effects of the ginseng root and ginsenosides are closely associated with vasodilation and promotion of endothelium-derived nitric oxide which enhances the accumulation of cGMP [9-13]. However, these results on vasodilation induced by ginseng were mainly obtained from experiments using aortic rings *in vitro*. Although there are several reports indicating that ginseng or ginsenosides can dilate the coronary artery of the heart [14-18], there are few reports that state the effects of ginseng or ginsenosides on the coronary artery are mediated by the nitric oxide (NO) pathway [19]. The aim of this study was to investigate whether ginsenosides relax the porcine coronary artery by the NO pathway *in vitro*. In the present study, we used red ginseng saponin fraction A (RGSF-A) as the ginsenoside mixture.

MATERIALS AND METHODS

Materials

RGSF-A is a fraction containing a high percentage of panaxadiols, which was isolated from Korean red ginseng by ultrafiltration. RGSF-A was kindly obtained from the Korea Ginseng Corporation (Daejeon, Korea). Nine ginsenosides, i.e., Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2 and Rg_3 were identified from RGSF-A through a comparison of the retention times with authentic compounds. The contents of the ginsenosides were 9.94, 4.22, 4.29, 1.72, 1.66, 1.27, 1.52, 0.695 and 0.695% of the dry extract, respectively (Table 1). The following compounds were used: U46619 (Cayman, Ann Arbor, MI, USA), sodium nitroprusside (Sigma, St. Louis, MO, USA), Nω-Nitro-L-arginine methyl ester (L-NAME, Sigma) and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; Calbiochem-Novabiochem, La Jolla, CA, USA). ODQ was prepared in dimethyl sulfoxide. The other compounds were prepared in distilled water. Further dilutions to the desired concentrations were made with physiological salt solution (PSS).

Preparation

Porcine hearts were obtained from a local slaughterhouse and transported in ice-cold oxygenated PSS. The composition of PSS was as follows (in mM): NaCl 118, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 10.0. The distal part of the left anterior descending (LAD) coronary artery (outer diameter, 0.8-1.5 mm) was dissected from the heart in oxygenated PSS. The isolated artery was trimmed of fat and connective tissues under a dissecting microscope and cut into rings, 4 mm in length. Care was taken to ensure that the endothelium was not damaged during the processing of the tissue preparation. Where indicated, the endothelial cells were removed by gently rubbing the inner surface of the vessel with a moistened cotton thread moistened with PSS. The endothelium-removed rings were confirmed if substance P (10 nM) did not induce relaxation. Substance P induces endothelium-dependent vasorelaxation in the porcine coronary artery [20].

Measurement of isometric tension

The arterial ring was suspended by a pair of stainless steel stirrups in a water-jacketed bath filled with 10 mL of PSS. The solution in the bath was gassed with 95% O_2 and 5% CO_2, and its temperature was maintained at 37°C. The upper end of the strip was connected to the isometric force transducer (FT-03; Grass-Telefactor, West Warwick, RI, USA). The output of the transducer was processed through Powerlab 2/25 and Chart 5.2 (AD Instruments, Castlehill, Australia). The ring was stretched until an optimal tension of 2 g was loaded and then allowed to equilibrate for at least 60 min before the start of the experiments. The RGSF-A or other drugs were administered after the contraction had reached a plateau level by U46619 (100 nM , 9,11-dideoxy-9a,11a-methanoepoxy-prostaglandin F2a), a stable analogue of thromboxane A_2. Rings that failed to produce a contraction greater than 3 g with U46619 or relaxed by less than 50% with substance P (5 nM) were discarded except when using the endothelium-removed rings.

Statistical analysis

All values were expressed as the mean±SEM. Statistical assessment of the data was calculated made by student's *t*-test. A *p*-value of less than 0.05 was taken to be statistically significant.

Table 1. Percentage of ginsenosides present in RGSF-A

Composition of RGSF-A (dry extract, %)												
Rb_1	Rb_2	Rc	Rd	Re	Rf	Rg_1	Rg_2	Rg_3	Total	Diol	Triol	PD/PT
9.94	4.22	4.29	1.72	1.66	1.27	1.52	0.695	0.925	26.24	21.10	5.15	4.10

RGSF-A, red ginseng saponin fraction-A; PD/PT, panaxadiol/panaxatriol.

RESULTS

Red ginseng saponin fraction-A induced biphasic relaxation

RGSF-A was administered into the bath in a cumulative method (1-300 μg/mL) when contraction of the porcine distal LAD coronary artery by U46619 (100 nM) reached the plateau. RGSF-A dose-dependently induced biphasic (fast- and slow-) relaxation in the distal LAD coronary artery in the presence of intact endothelium. Fast-relaxation was quickly achieved in a minute, and then the slow-relaxation was slowly developed and sustained for more than thirty minutes after administration of RGSF-A (Fig. 1A). The percentage of fast-relaxation of RGSF on the swine distal LAD coronary artery was 1.4±1.11, 4.9±2.19, 15.9±3.65, 32.1±8.48, 58.3±6.79 and 74.3±5.43 % at doses of 1, 3, 10, 30, 100 and 300 μg/mL, respectively. In addion, the percentage of slow-relaxation of RGSF on swine distal LAD coronary artery was 3.2±1.15, 7.34±2.47, 22.6±9.44, 41.90±8.69, 67.8±8.05 and 87.8±4.40% at doses of 1, 3, 10, 30, 100 and 300 μg/mL, respectively. The IC_{50} of RGSF-A in fast- and slow-relaxation were 43.3 and 64.4 μg/mL, respectively. The slow-relaxation had a tendency to be bigger than the fast-relaxation (Fig. 1B).

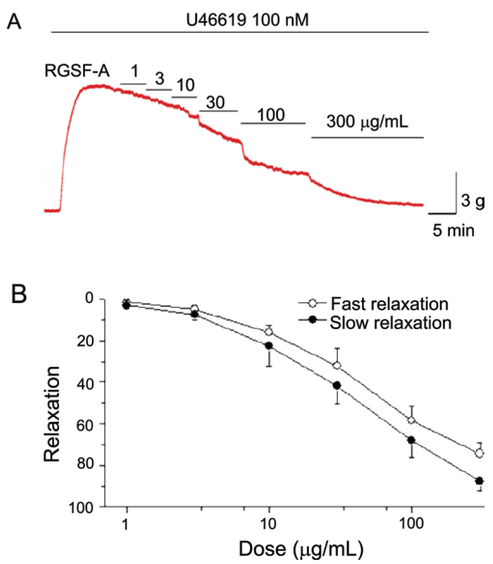

Fig. 1. Red ginseng saponin fraction-A (RGSF-A) dose-dependently induced biphasic (fast- and slow-) relaxation in the distal left anterior descending coronary artery with the endothelium contracted by U46619. The fast-relaxation was quickly achieved in a minute, and then the slow-relaxation was slowly developed and sustained for more than thirty minutes after the administration of RGSF-A (A). The slow-relaxation had a tendency to be bigger than the fast-relaxation (B) (*n*=6).

Involvement of NO pathway in RGSF-A induced vasorelaxation

Administration of RGSF-A (100 μM) alone evoked fast- and slow- relaxation in the porcine distal LAD coronary artery (Fig. 2A). The slow relaxation was much bigger than the fast-relaxation (Figs. 2A and 3A). With the pretreatment of L-NAME (100 μM), a nitric oxide synthase inhibitor, the fast-relaxation by RGSF-A was almost abolished, and the slow- relaxation by RGSF-A was significantly attenuated by L-NAME (Fig. 2B, C). However, with the pre-incubation of L-NAME, the RGSF-

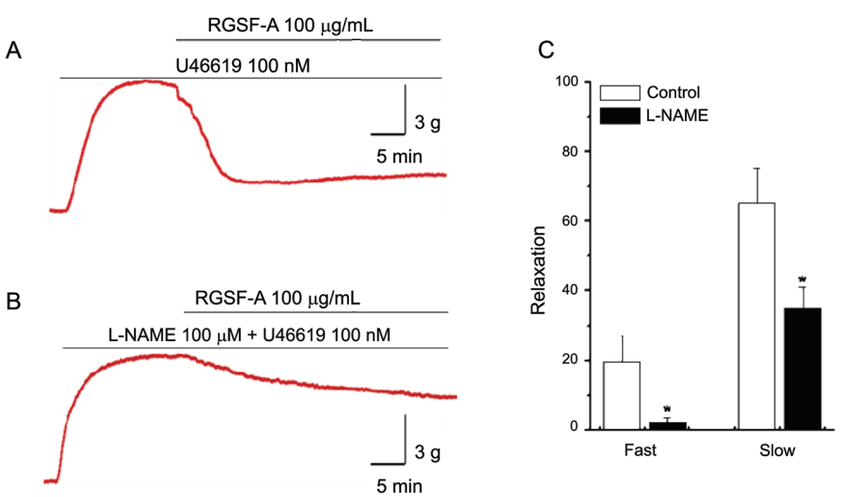

Fig. 2. Effects of red ginseng saponin fraction-A (RGSF-A) on the porcine coronary artery were inhibited by Nω-Nitro-L-arginine methyl ester (L-NAME). Administration of RGSF-A (100 μg/mL) induced fast- and slow- relaxation in the porcine distal left anterior descending coronary artery (A). Fast-relaxation by RGSF-A was almost abolished by L-NAME (100 μM) and slow- relaxation by RGSF-A was significantly attenuated by L-NAME (B,C). Both tracings were recorded from the same tissue (*n*=5, *p<0.05 *vs*. control).

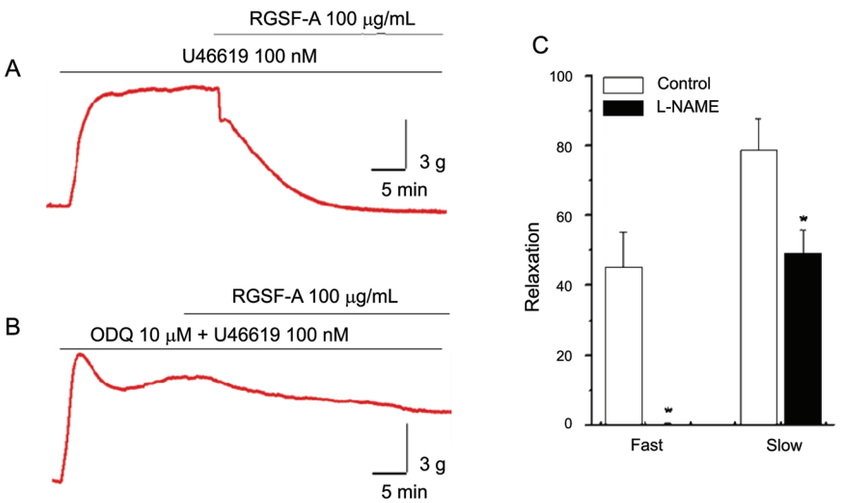

Fig. 3. Effects of red ginseng saponin fraction-A (RGSF-A) on the porcine coronary artery were inhibited by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). Fast- and slow- relaxation were induced by RGSF-A in the distal left anterior descending coronary artery (A). Fast- relaxation evoked by RGSF-A was almost abolished by ODQ (10 μM) and the slow-relaxation was significantly attenuated by ODQ (B,C). Both tracings were recorded from the same tissue (n=5, *p<0.05).

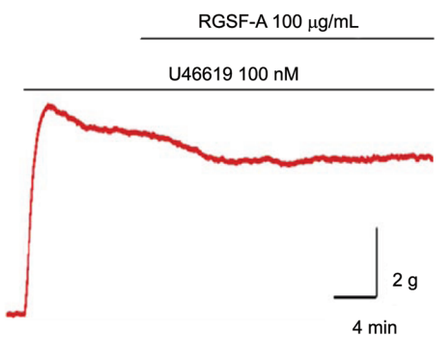

Fig. 4. Effects of red ginseng saponin fraction-A (RGSF-A) on the porcine distal left anterior descending (LAD) coronary artery without an endothelium. RGSF-A induced slow relaxation to a small degree but did not evoke fast relaxation in the distal LAD coronary artery without an endothelium.

A induced slow-relaxation still remained to a degree. The effects of RGSF-A in the absence or presence of L-NAME were recorded in the same rings. ODQ, soluble guanylate cyclase inhibitor, also inhibited the effects of RGSF-A on the distal LAD coronary artery (Fig. 3B). Fast-relaxation by RGSF-A was completely abolished and slow-relaxation by RGSF-A was significantly attenuated by ODQ (10 μM). However, slow-relaxation by RGSF-A still remained to a degree in the pretreatment of ODQ (Fig. 3B, C).

Effects of RGSF-A on an endothelium-removed coronary artery ring

Administration of RGSF-A induced a slowly developed relaxation in the endothelium-removed coronary artery ring. Typical tracings indicated that RGSF-A evoked a slow relaxation to a certain extent but not a fast-relaxation in an endothelium-denuded coronary artery (Fig. 4).

DISCUSSION

In this study, RGSF-A dose-dependently induced biphasic (fast- and slow-) relaxation in the porcine distal LAD coronary artery. RGSF-A is the fraction containing a high percentage of panaxadiols, which was isolated from the Korean red ginseng by ultrafiltration. Fast-relaxation was quickly achieved in a minute, and then slow-relaxation was slowly developed and sustained for more than thirty minutes after the administration of RGSF-A. In a cumulative addition of RGSF-A, the extent of fast-relaxation was close to that of the slow-relaxation. However, a single administration of RGSF-A induced a much bigger slow-relaxation than fast-relaxation in porcine distal LAD coronary artery. We think that the small difference between the fast- and slow-relaxation in a cumulative addition of RGSF-A is due to the administration of higher concentrations of RGSF-A after slow relaxation has developed by the previously added low concentrations of RGSF-A.

Recently, it was reported that total ginsenosides have cardioprotective effects and can enhance the coronary artery flow against ischemia/reperfusion injury in isolated rat hearts [6,19]. This increasing effect of total ginsen-

osides on coronary artery flow is mediated, at least partially, by the NO pathway. The relaxing effect of ginsenosides on the coronary artery has been reported in rabbits [15] and swine [14,21] *in vitro*. However, these reports did not consider whether the effect of the ginsenosides on the coronary artery was mediated by NO.

In this experiment, the RGSF-A induced fast-relaxation was almost blocked by L-NAME (nitric oxide synthase inhibitor) and completely blocked by ODQ (sGC inhibitor) in the porcine distal LAD coronary artery with an intact endothelium. In addition, the slow-relaxation was significantly attenuated by L-NAME and ODQ. In the endothelium-removed rings, RGSF-A induced only a slow-relaxation to a certain extent without any fast-relaxation. These data suggest that the fast-relaxation induced by RGSF-A is mediated by the NO-cGMP pathway and is endothelium-dependent. The slow-relaxation is partially mediated by the endothelium-dependent NO-cGMP pathway. However, these data could not exclude the possibility that the slow-relaxation induced by RGSF-A is also involves another endothelium-dependent mechanism like as endothelium-derived hyperpolarizing factor [19,22].

In the present study, our data suggest that the slow relaxation induced by RGSF-A consists of both endothelium-dependent and independent mechanisms, and currently, our data cannot explain the mechanism for the endothelium-independent slow relaxation induced by RGSF-A. It was reported that ginsenosides induced endothelium-independent relaxation in the swine coronary artery [21,23] and rat aortic rings [24]. Increasing data show that ginsenosides have effects on the ion channels of vascular smooth muscle cells. It was reported that ginsenosides activate large-conductance Ca^{2+}-activated K^+ channels in rabbits [15] and rat aortic artery smooth muscle cells [25]. On other hand, it is known that ginsenosides inhibit L-type Ca^{2+}-current in porcine coronary artery smooth muscle cells [21]. These data might explain the endothelium-independent relaxation of RGSF-A.

In conclusion, RGSF-A induced both endothelium dependent fast- and slow-relaxation and endothelium independent slow-relaxation in the porcine distal LAD coronary artery. The endothelium dependent fast-relaxation is mediated by the NO-cGMP pathway and the endothelium dependent slow-relaxation is at least partially mediated by the NO-cGMP pathway. However, the endothelium-independent slow-relaxation remains to be elucidated.

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