Influence of Ginseng Saponins on the Isolated Aortic Contractile Response of the Spontaneously Hypertensive Rat

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Abstract: The present study was attempted to investigate the effects of total ginseng saponin (GTS), panaxadiol-type (PDS) and panaxatriol-type saponin (PTS) on contractile responses of vasoconstrictors in aortic smooth muscle strips of normotensive (NR) and spontaneous hypertensive rats (SHR). Phenylephrine (an adrenergic $\alpha_2$-receptor agonist) and high potassium (a membrane depolarizing agent) caused greatly contractile responses in both NR and AHR aorta, respectively. Phenylephrine and high potassium-induced contractile responses were greater in NA than those in SHR aortic smooth muscle strips. In NR, the contractile responses of high potassium ($5.6 \times 10^{-2}$ M) were not affected in the presence of GTS (300 $\mu$g/ml), PDS (300 $\mu$g/ml), and PTS (300 $\mu$g/ml), respectively whereas phenylephrine ($10^{-4}$ M)-induced contractile responses were markedly inhibited. In SHR, the contractile responses of high potassium ($5.6 \times 10^{-2}$ M) were not affected in the presence of GTS (300 $\mu$g/ml), PDS (300 $\mu$g/ml), and moderate doses of PTS (150-300 $\mu$g/ml), respectively but greatly blocked by high concentration of PTS (600 $\mu$g/ml). Phenylephrine ($10^{-6}$ M)-induced contractile responses were inhibited in a dose dependent fashion (150-600 $\mu$g/ml) by the pretreatment with PTS while not altered in the presence of GTS (300 $\mu$g/ml) and PDS (300 $\mu$g/ml), respectively. Taken together, these experimental results suggest that ginseng saponins cause vascular relaxation through blockade of adrenergic $\alpha_2$-receptors and some unknown mechanisms, and that there is some difference in sensitivity of vascular smooth muscle between NR and SHR in responses to ginseng saponins. It seems that panaxatriol type of some ginseng saponins has the greatest potency in vascular relaxation.

Key words: Contractile response, aortic smooth muscle, spontaneous hypertensive rat, phenylephrine, vascular relaxation

INTRODUCTION

Ginsenosides are found to induce endothelin-dependent relaxation and to increase tissue content of cGMP in isolated rat thoracic aorta, possibly due to the release of EDRE$^1$ Protopanaxatriol group and its purified ginsenoside Re$_1$ (Re$_2$$_3$) and Re caused endothelium-dependent relaxation, which is associated with the formation of cGMP.$^2$ It has been also reported that ginsenosides may induce vasorelaxation via activation of Ca$^{2+}$-dependent K$^+$-channels resulting in hyperpolarization of the vascular smooth muscle with subsequent inhibition of the opening of voltage-dependent Ca$^{2+}$-channels.$^3$ In experimental and clinical study of Korean Red Ginseng (KGR) treatment on hypertension, KRG is shown to have a certain effect in preventing retinopathy of hypertensive arteriosclerosis, and also well tolerated and effective on lowering blood pressure during treatment with hypertension.$^4$

Hong and his coworkers (1999)$^5$ has reported that total ginseng saponin can inhibit the releasing effect of catecholamines evoked by nicotinic receptor stimulation from the isolated perfused rat adrenal medulla, which seems to be associated to the direct inhibition of calcium influx into the rat adrenomedullary chromaffin cells. However, in the isolated perfused rabbit adrenal glands, total ginseng saponin increases a calcium-dependent secretion of catecholamines via direct action on chromaffin cells with partly mediation of muscarinic action.$^6$ Moreover, Lim and his coworkers (1988; 1989)$^7$ have also found that both of panaxadiol and panaxatriol type saponins cause the increased secretion of catecholamines (CA) in a Ca$^{2+}$-dependent fashion from the isolated perfused rabbit adrenal glands through the activation of cholinergic (both nicotinic and muscarinic) receptors and partly the direct action on the rat adrenomedullary chromaffin cells.

It has been shown that total ginseng saponin produces the pressor and depressor actions in the anesthetized normotensive rats.$^9$ It has suggested that this depressor
response is mediated in part through the blockade of adrenergic α-receptors as well as the stimulation of cholinergic muscarinic receptors, and that its pressor response is caused by stimulation so nicotinic cholinergic receptors at the sympathetic ganglia. In previous studies, it has been known that ginseng extract cause the hypotensive action\textsuperscript{10-14} while it rather produces the hypertensive action.\textsuperscript{15-16} Some studies have suggested that Ginseng extract causes a biphasic response on blood pressure, namely, transient fall followed by prolonged elevation\textsuperscript{17-19}.

Furthermore, Ginseng, when given at small dose in spontaneously hypertensive rat (SHR), cause pressor response, but at relatively large dose rather produces dose-dependent hypotensive response with decreased plasma renin activity.\textsuperscript{20-22} Sokabe and his coworkers (1984)\textsuperscript{23} have shown that administration of KRG powder for 11 weeks has no effect on blood pressure in normotensive Donryu (DON) rats, SHR and renal hypertensive rats, whereas it elevates slightly blood pressure in deoxycorticosterone salt hypertensive rats. As mentioned so far, there are many controversial reports on vascular effects of ginseng. Therefore, the present study was attempted to investigate the effects of total Ginseng saponin, panaxadiol-type and panaxatriol-type saponins on contractile responses evoked by stimulation of adrenergic α-receptors and membrane depolarization in the isolated aorta of normotensive (NR) and spontaneously hypertensive rats (SHR) as well as their underlying mechanisms.

**MATERIALS AND METHODS**

1. **Experimental procedure**

Normotensive male Sprague-Dawley rats (NR) and spontaneously hypertensive rats (SHR), weighing 150 to 350 grams, were used in the present experiment. The animals were housed individually in separate cages, and food (Cheil Animal Chow) and tap water were allowed ad libitum for at least a week to adapt to experimental circumstances. On the day of experiment, a rat was anesthetized with thiopental sodium (40 mg/kg) intraperitoneally, and tied in supine position on fixing panel. The thorax was opened by a mid-line incision, and the heart and surrounding area were exposed by placing three hook retractor. The heart and portion of the lung were not removed, but pushed over to the right side and covered by saline-soaked gauge pads in order to obtain enough working space for isolating aortic vessel.

The aorta was isolated from the proximal part to the vicinity of liver and immediately immersed in cold Krebs solution. The blood within the aorta was rapidly removed. The aorta was cut into the ring of 4-5 mm length.

2. **Recording of mechanical activity**

As shown in Fig. 1, the ring segment of aorta isolated from NR and SHR was mounted in a muscle bath by sliding the ring over two parallel stainless-steel hooks (0.15 mm in diameter). The lower hook was fixed on bottom of the bath and the upper was connected to isometric transducer (Grass FT. 03). The signal from the transducer was displayed on a polygraph (Grass Instruments Model 79). The volume of bath was 25 ml and the bath solution was saturated with 95% O\textsubscript{2} and 5% CO\textsubscript{2} at 37°C.

The composition (mM) of Krebs was: NaCl, 118.4; KCl, 4.7; CaCl\textsubscript{2}, 2.5; MgCl\textsubscript{2}, 1.18; NaHCO\textsubscript{3}, 25; KH\textsubscript{2}PO\textsubscript{4}, 1.2; glucose, 11.7. The final pH of the solution was maintained at 7.4-7.5. During equilibration period of 2 hours at 2 g, the final resting tension was adjusted to 0.5 g. After the equilibration period, the ring was challenged two times with 56 mM KCl, and if it responded with contraction, the proper experiment was started. Vasoconstrictors were

![Fig. 1. A schematic representation of the isometric contraction recording system with a vertical chamber. The chamber (15 ml) was maintained at 37°C with temperature-regulated circulator and aerated with 95% O\textsubscript{2} and 5% CO\textsubscript{2}.](image)
administered into the bath in order to obtain dose-response curves. In the subsequent experiments, under the presence of total ginseng saponin some vasoconstrictors were administered. The data were expressed as the active tension in gram.

3. Statistical analysis
All data are presented as means with their standard errors, and the significance of differences were analyzed by Student's paired t-test using the computer program of statistics system as previously described.24

4. Drugs and their sources
The following drugs were used: phenylephrine hydrochloride and potassium chloride (Sigma Chemical Co., U.S.A.). Total Ginseng saponin, panaxadiol-type and panaxatriol-type saponins were gifted from late Professor Young-Ho Kim (Sejong University, Seoul, Korea). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required. Concentrations of all drugs used are expressed in terms of molar base.

RESULTS

1. The effects of total ginseng saponin on contractile responses induced by phenylephrine and high K⁺ in the rat aortic strips isolated from NR and SHR
The resting (basal) tension from the isolated rat aortic strips reaches a steady state after the perfusion with oxygenated Krebs-bicarbonate solution for 90 min before the experimental protocol is initiated. The resting tension was adjusted to 0.5 g. The effect of total Ginseng saponin on phenylephrine- as well as potassium chloride-mediated contractile responses in the rat aorta was examined. In the present study, total Ginseng saponin itself did not produce any effect on the resting tension in both aortas isolated from NR and SHR (data not shown). However, it was found that there is difference in the contractile responses induced by phenylephrine and high potassium between NR and SHR as shown in Fig. 2.

When 10⁻⁶ M of phenylephrine was administered into the aortic bath, the active tension was 2.6±0.2 g in NR and 0.8±0.3 g in SHR from the resting tension level, respectively. However, under the pre-loading with total Ginseng saponin at a concentration of 300 µg/ml, phenylephrine-induced tensions were amounted to 1.4±0.2 g (P<0.01, n=10) in NR and 1.0±0.2 g (ns, n=5) in SHR, respectively. They were 54% and 125% of the control contractile responses (100%), respectively (Fig. 3 and 4).

Fig. 2. Comparison of contractile responses induced by high KCl and phenylephrine (PE) in the isolated aortic strips of normotensive and spontaneous hypertensive rats. The contractile responses were induced by adding 56 mM KCl and 1 mM PE into the bath, respectively after adaptation with normal Krebs solution for 2 hours prior to initiation of the experimental protocol. "NR" and "SHR" denote normotensive rats and spontaneously hypertensive rats, respectively. Vertical bars represent mean±S.E. Ordinate: the active tension (gram). Abscissa: concentrations of KCl and PE. Statistical difference was obtained by comparing the NR with the S:HR group from 6 experiments. PE: phenylephrine. **P< 0.01.

High K exerts two distinct effects on cells: (1) depolarization of cell membrane, and (2) depolarization-induced influx of calcium via voltage-dependent calcium channels.25 When added through the bath, high potassium at the concentration of 56 mM, which is membrane-depolarizing agent, caused an increase in aortic contraction. As shown in Fig. 3, high potassium-induced contractile responses before pre-loading with total ginseng saponin were 2.2±0.2 g in NR and 0.6±0.1 g in SHR, respectively, while, after pretreatment with total ginseng saponin at a concentration of 300 µg/ml, they were not altered to 0.6±0.05 g (ns, n=5) and 1.8±0.1 g (ns, n=12), which were nearly close to 100% and 82% of the corresponding control, respectively. These results are in agreement with that by the previous study.3)
Fig. 3. Influence of total Ginseng saponin (GTS) on high KCl- and phenylephrine (PE)-induced contractile responses in the isolated aortic strips of normotensive rats (NR) and spontaneously hypertensive rats (SHR). The contractile responses were induced by adding 56 mM KCl and 1 µM PE, respectively after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol. “A” and “C” denote active tension of the control induced evoked by KCl or PE in NR and SHR, respectively before adding GTS. “B” and “D” denote active tension of the control induced evoked by KCl or PE in NR and SHR, respectively after adding GTS (300 µg/ml). Number in the parenthesis indicates number of rat aorta. Statistical difference was obtained by comparing the control with the GTS-pretreated group in NR and SHR, respectively. Other legends are same as in Fig. 2. **P<0.01, ns: Statistically insignificant.

2. The effects of panaxadiol-type saponin on contractile responses induced by phenylephrine and high K⁺ in the rat aortic strips isolated from NR and SHR

Ginsenosides are a mixture of saponin from Panax ginseng, the major form of glycosides belong either to the protopanaxadiol group or the protopanaxatriol group. Therefore, it was likely interesting to compare the effects of ginseng saponins on the contractile responses induced by high potassium and phenylephrine.

In the presence of panaxadiol-type saponin (300 µg/ml), the aortic contractile response of the NR evoked by phenylephrine (13⁻⁶ M) was 2.0±0.3 g (P<0.01, 74% of the control)) from the resting tension level from 8 experiments in comparison with its corresponding control response of 2.7±0.2 g, while the aortic contractile response of the SHR evoked by phenylephrine was 0.7±0.1 g (ns, 88% of the control) from 14 rat aortae in comparison with its corresponding control response of 0.8±0.1 g as depicted in Fig. 5 and 6.

High potassium-induced contractile responses before treatment with panaxadiol-type saponin were 2.2±0.5 g in NR and 1.7±0.3 g in SHR, while after pretreatment with panaxadiol-type saponin at a concentration of 300 µg/ml they were 2.1±0.3 g (ns, n=8) and 1.8±0.4 g (ns, n=7), respectively, which were nearly close to 96% and 106% of the corresponding (Fig. 5).

3. The effects of panaxatriol-type saponin on contractile responses induced by phenylephrine and high K⁺ in the rat aortic strips isolated from NR and SHR

High potassium (56 mM)-induced aortic contractile response of the NR before pre-loading with panaxatriol-type saponin were 2.3±0.2 g while after pretreatment with panaxatriol-type saponin at a concentration of 300 µg/ml, it was 2.5±0.3 g (ns, n=8), which was 109 % of the corresponding control (Fig. 7). However, in aortic strips of the SHR, the contractile responses of high potassium (56 mM) in the presence of panaxatriol-type saponin at concentrations of 150, 300 and 600 µg/ml were 1.0±0.05 g (ns, n=6), 1.1±0.2 g (ns, n=6) and 0.4±0.1 g (P<0.01, n=6) as compared their corresponding control responses of 1.0±0.1 g, 1.2±0.4 g and 1.4±0.2 g.
Fig. 5. Influence of panaxadiol-type saponin (PDS) on high KCl- and phenylephrine (PE)-induced contractile responses in the isolated aortic strips of normotensive rats (NR) and spontaneously hypertensive rats (SHR). The contractile responses were induced by adding 56 mM KCl and 1 μM PE, respectively after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol. “A” and “C” denote active tension of the control induced evoked by KCl or PE in NR and SHR, respectively before adding PDS. “B” and “D” denote active tension of the control induced evoked by KCl or PE in NR and SHR, respectively after adding PDS (300 μg/ml). Other legends are the same as in Fig. 2 and 4. The statistical difference was obtained by comparing the control with the PDS-pretreated group in NR and SHR, respectively. *P<0.05. ns: Statistically insignificant.

respectively (Fig. 7 and 8).

The active tension of phenylephrine (10⁻⁶ M) given into the aortic bath before the treatment with panaxatriol-type saponin was 3.5±0.3 g in NR from the resting tension level. However, under the pre-loading with panaxatriol-type saponin at a concentration of 300 μg/ml, phenylephrine-induced tension was greatly inhibited to 2.4±0.3 g (P<0.01, n=8), which was 69% of the control contractile response (Fig. 9 and 11). However, in aortic strips of the SHR, the contractile responses of phenylephrine (10⁻⁶ M) in the presence of panaxatriol-type saponin at concentrations of 150, 300 and 600 μg/ml were a dose dependently.

Fig. 6. The typical tracing showing the effect of panaxadiol-type saponin on phenylephrine (PE)-induced contractile response in aortic strips of the normotensive rat. Upper: PE-induced contractile response. Lower: PE-induced contractile response in the presence of PDS (300 μg/ml). At dots, the indicated does (1 μM) of PE was added into the bath. The chart speed was 5 mm/min.

Fig. 7. Influence of panaxatriol-type saponin (PTS) on high potassium-induced contractile responses in the isolated aortic strips of normotensive rats (NR) and spontaneously hypertensive rats (SHR). High potassium (56 mM) was added into the bath before (CONTROL) and after pretreatment with PTS (150, 300 and 600 μg/ml, respectively) in SHR and NR (300 μg/ml). Other legends are the same as in Fig. 2 and 4. **P<0.01. ns: Statistically insignificant.
Fig. 8. The typical tracing showing the effect of panaxatriol-type saponin on high potassium-induced contractile response in aortic strips of the spontaneously hypertensive rat. Upper: high KCl (56 mM)-induced contractile response. Lower: KCl-induced contractile response in the presence of PTS (600 µg/ml). At dots, the indicated dose of KCl (56 mM) was added into the bath. The chart speed was 5 mm/min.

Fig. 9. Influence of panaxatriol-type saponin (PTS) on high potassium-induced contractile responses in the isolated aortic strips of normotensive rats (NR) and spontaneously hypertensive rats (SHR). High potassium (56 mM) was added into the bath before (CONTROL) and after pretreatment with PTS (150, 300 and 600 µg/ml, respectively) in SHR and NR (300 µg/ml). Other legends are the same as in Fig. 2 and 4. **P<0.01. ns: Statistically insignificant.

Fig. 10. The typical tracing showing the effect of panaxatriol-type saponin on phenylephrine (PE)-induced contractile response in aortic strips of the normotensive rat. Upper: PE-induced contractile response. Lower: PE-induced contractile response in the presence of PTS (300 µg/ml). At dots, the indicated dose (1 µM) of PE was added into the bath. The chart speed was 5 mm/min.

Fig. 11. The typical tracing showing the effect of panaxatriol-type saponin on phenylephrine (PE)-induced contractile response in aortic strips of the spontaneously hypertensive rat. Upper: PE (1 µM)-induced contractile response. Lower: PE-induced contractile response in the presence of PTS (600 µg/ml). At dots, the indicated dose of PE (1 µM) was added into the bath. The chart speed was 5 mm/min.

DISCUSSION

The present experimental results suggest that ginseng
saponins cause vascular relaxation through blockade of adrenergic $\alpha_1$-receptors and some unknown mechanisms, and that there is some difference in sensitivity of vascular smooth muscle between NR and SHR in responses to ginseng saponins. It seems that panaxatriol type of some ginseng saponins has the greatest potency in vascular relaxation.

In support of this idea, among drugs which interfere with peripheral sympathetic function, adrenergic $\alpha$-receptor blocking agents alone cause reversal of the epinephrine pressor response. When epinephrine is administered to untreated animals, its $\alpha$-agonist properties predominate, resulting in a rise in mean arterial pressure. However, in the presence of adrenergic $\alpha$-receptor blockade, the peripheral $\beta_2$-agonist properties of epinephrine predominate and a fall in arterial pressure or reversal of the pressor response is observed. In contrast, the pressor responses to norepinephrine are impaired by adrenergic $\alpha$-receptor blockade, but are not reversed as this agent processes little $\beta_2$-agonist activity. In terms of the fact that phenylephrine-evoked contractile response is greatly depressed by ginseng saponins, it is thought that ginseng saponins have vascular dilatory activity through the adrenergic $\alpha$-receptor blockade. Furthermore, It has been shown that total ginseng saponin produces the pressor and depressor actions in the anesthetized normotensive rats. It has suggested that this depressor response is mediated in part through the blockade of adrenergic $\alpha$-receptors as well as the stimulation of cholinergic muscarinic receptors, and that its pressor response is caused by stimulation of nicotinic cholinergic receptors at the sympathetic ganglia.

Generally, it well known that potassium chloride (KCl) opens voltage-dependent calcium channels by depolarizing the cell membrane of vascular smooth muscle, resulting in increased influx of extracellular $\text{Ca}^{2+}$ (Bolton, 1979; Schwartz & Taira, 1983; Dub et al., 1985; 1988). Kim and his colleagues (1989) have shown that the contractile responses of vascular smooth muscle induced by CaCl$_2$ and KCl may result most likely from increased influx of extracellular $\text{Ca}^{2+}$ through the voltage-dependent calcium channels. In terms of these results, the present findings that PTS of some ginseng saponins inhibited the contraction of rat aortic smooth muscle evoked by phenylephrine ($\alpha_1$-adrenergic receptor agonist) and KCl (membrane depolarizer) suggest strongly that PTS can facilitate the opening of potassium channels. Moreover, Kim and his colleagues (1998) have shown that ginsenosides cause a concentration-dependent relaxation of rat aortic rings without endothelium constricted with 25 mM KCl but affected only minimally those constricted with 60 mM KCl. They have also suggested that ginsenosides may induce vascular dilatation via activation of $\text{Ca}^{2+}$-dependent $K^+$ channels resulting in hyperpolarization of the vascular smooth muscle with subsequent inhibition of the opening of voltage-dependent Ca channels.

In previous studies, three cellular mechanisms have been proposed to explain relaxant response of vascular smooth muscle: (i) blockade of extracellular $\text{Ca}^{2+}$ entry into cells, (ii) increase in binding or sequestration of intracellular $\text{Ca}^{2+}$, and (iii) inhibiting the release of intracellular stored $\text{Ca}^{2+}$. In contrast, the contractions of vascular smooth muscles induced by neurohumoral agents have been composed of two components: Phasic contraction induced by the $\text{Ca}^{2+}$ released from inside the cell and tonic tension related to the $\text{Ca}^{2+}$ influx both leading to increased intracellular calcium.

In the light of these findings, it could not be ruled out that ginseng saponins can dilate the contractile responses of vascular smooth muscle evoked by phenylephrine and/or KCl through the blockade of extracellular $\text{Ca}^{2+}$ entry into the muscle cells. Thus, these effects of ginseng saponins seem to contribute at least partly to the facts that ginseng extract causes the hypotensive action, but not to the facts that it rather produces the hypertensive action. Some studies have suggested that ginseng extract causes a biphasic response on blood pressure, namely, transient fall followed by prolonged elevation.

In the present study, GTS, PDS and PTS inhibited markedly the contractile response induced by phenylephrine in NR, but not by high potassium. In SHR, PDS only inhibited a concentration-dependently phenylephrine-induced contractile response, and high concentration of PTS (600 $\mu$g/ml) also depressed high potassium-induced contraction. It has been shown that PTS of some ginseng saponin components produces the greatest potency in relaxation of aortic smooth muscle contraction.

Taken together, these experimental results suggest that ginseng saponins cause vascular relaxation through blockade of adrenergic $\alpha_1$-receptors and some unknown mechanisms, and that there is some difference in sensitivity of vascular smooth muscle between NR and SHR in responses to ginseng saponins. It seems that panaxatriol type of some ginseng saponins has the greatest potency in vascular relaxation.

요 약

본 연구에서는 인삼사포닌 성분 즉, 총인삼사포닌(GTS), panaxadiol-type saponin(PDS) 및 panaxatriol-type saponin(PTS)의 경상 혈압
REFERENCES


