

Effect of Korea red ginseng on cerebral blood flow and superoxide production

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ABSTRACT

AIM: To investigate the effects of Korea red ginseng (KRG) on the cerebral perfusion rate in the rats and the generation of superoxide anion in the endothelial cells. **METHODS:** The cerebral perfusion rate was measured using laser-doppler flowmetry before and after the administration of crude saponin (CS) and saponin-free fraction (SFF) of KRG in the anesthetized rats. The superoxide generation was measured by the method based on lucigenin-enhanced chemiluminescence in the cultured endothelial cells. **RESULTS:** The relative cerebral perfusion rate (rCBF) was significantly increased by the intraperitoneal injection of CS (100 mg/kg) in the rats, but SFF had no effect on the rCBF. Chronic treatment with CS for 7 d significantly inhibited the decrease of forebrain cerebral blood flow induced by clamping both carotid arteries in the rats. Furthermore, CS (0.1 g/L) significantly suppressed NADPH-induced superoxide generation in the human umbilical vein endothelial cells ($P < 0.01$). **CONCLUSION:** The present study demonstrated that crude saponin fraction of KRG enhanced cerebral blood flow in rats. Furthermore, crude saponin fraction of KRG abrogated the NADPH-driven superoxide generation in endothelial cells.

INTRODUCTION

Panax ginseng C A Meyer has been used for more than 2000 years as a general tonic in traditional oriental medicine. The pharmacological effects of ginseng have been demonstrated in the central nervous system, cardiovascular, endocrine, and immune system. Ginseng and its constituents have been ascribed to possess

antistress, and antioxidant activity^[1,2]. Our previous studies had demonstrated that the saponin fraction of KRG had vasorelaxing and hypotensive effects^[3-5]. The change of vasomotor tone is one of the important regulators in the cerebral circulation^[6,7]. Earlier reports indicate that KRG can influence vasorelaxation of cerebral vessels^[7,8]. Cerebral blood flow is controlled by complex factors, such as neuronal, endocrine, metabolic factors. Although the direct effect of ginseng on the cerebral blood flow has not been studied, we hypothesized that KRG might influence cerebral blood flow.

Reactive oxygen species appear to be a key mediator of cellular signaling. Potential enzymatic sources

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of superoxide in blood vessels include cyclooxygenase, xanthine oxidase, NAD(P)H oxidase^[9]. In the circulatory system, the vascular endothelial cell is an early focus of free radical injury. Recent evidence, mainly from the aorta, suggests that NAD(P)H oxidase is a major source of vascular superoxide^[9,10]. NADPH dose-dependently increased superoxide levels and induced vasoconstriction in the basilar artery^[11]. *Panax ginseng* showed a remarkable capacity to protect brain tissue proteins from oxidative damage *in vitro*^[12]. Also, it was reported that aqueous extracts of ginseng scavenged several reactive oxygen species^[13]. However, effect of saponin fraction of KRG on reactive oxygen species has not been studied so far.

In the present study, we investigated the effect of KRG on cerebral blood flow in the anesthetized rats as well as the basal and NADPH-driven superoxide production in the cultured endothelial cells.

MATERIALS AND METHODS

Materials Crude saponin (CS) and saponin-free fraction (SFF) of Korea red ginseng were kindly donated from Korea Ginseng and Tobacco Research Institute (Taejon, KOREA) as described in our previous report^[5].

Measurement of cerebral blood flow Rats, weighing 250-300 g were anesthetized with urethane (1 g/kg) and placed in a stereotaxic instrument. Relative cerebral blood flow (rCBF) was monitored continuously for all groups, using a laser Doppler flow probe (Transonic Systems, USA). A 2-mm-diameter burr hole was drilled 1 mm posterior to bregma and 2 mm from the midline on the right side. A laser Doppler probe (1-mm diameter) was placed through the burr hole to touch the underlying cortex. The probe was rigidly affixed to the skull after reliable rCBF measurements were confirmed. For rCBF determinations, steady-state values before forebrain ischemia were recorded for each rat and were considered baseline values. In some experiments, forebrain ischemia was produced by the clamping of the both common carotid artery for 2 min, ischemia was terminated by removal of the carotid clamps.

Measurement of superoxide production Human umbilical vein endothelial cells (HUVEC, Clonetics, Co) were used to examine superoxide production. Lucigenin-enhanced chemiluminescence was used to measure superoxide levels^[14]. Lucigenin (*bis-N*-methylacridinium nitrate) luminesces specifically in the

presence of superoxide. In briefly, HUVEC (1×10^5 cells) were transferred into scintillation vials containing Krebs-HEPES buffer (NaCl 100 mmol/L, KCl 4.7 mmol/L, CaCl₂ 1.9 mmol/L, MgSO₄ 1.2 mmol/L, K₂HPO₄ 1.03 mmol/L, NaHCO₃ 25 mmol/L, Na-HEPES 20 mmol/L, pH 7.4) with 5 μ mol/L lucigenin. Chemiluminescence was assessed over 10 min in 1 min intervals. The emitted light units, after subtracting a blank, were used as a measure of superoxide production. Values are expressed as relative light units per 1×10^5 cells (RLU/ 1×10^5 cells).

Statistical analysis Data were expressed as mean \pm SEM and significance was assessed by the unpaired or paired Student's *t*-test.

RESULTS

Effect of KRG on cerebral blood flow in resting state To evaluate the KRG on the cerebral blood flow in the resting state, CS (50, 100 mg/kg, ip) and SFF (50, 100 mg/kg, ip) were administrated peritoneally in the anesthetized rats. Relative cerebral blood flow was measured for 60 min after treatment with KRG. As shown in Fig 1, CS significantly increased cerebral blood flow after 30 min of treatment about 30 %-50 % compared with the initial value, but SFF and normal saline as the vehicle did not change significantly rCBF in the anesthetized rats.

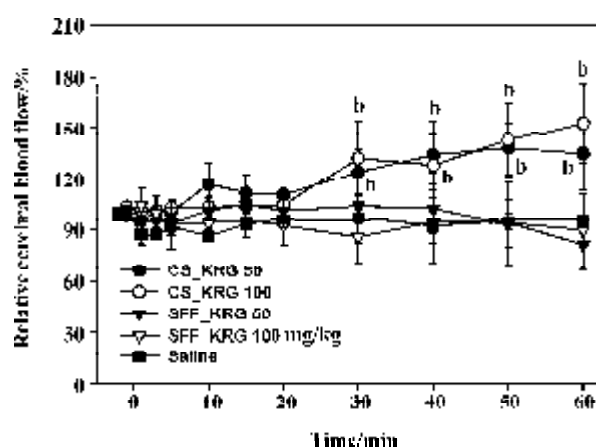


Fig 1. Crude saponin of KRG increases cerebral blood flow. Change of relative cerebral blood flow (rCBF) after the intraperitoneal injection of the crude saponin (CS) and saponin-free fraction (SFF) of Korea red ginseng (KRG) for 60 min. $n=7$. Mean \pm SEM. ^b $P < 0.05$ vs initial value.

Effect of both carotid arterial clamping on forebrain cerebral blood flow When both carotid arteries were clamped by arterial clamps for 2 min, rCBF was immediately reduced in the forebrain of rats. And then

rCBF was slightly recovered in the clamping state, however, it was maintained reduced rCBF for 2 min. In the case of un-clamping of both carotid artery, rCBF was immediately elevated, but its level was greater than initial value (Fig 2A). To evaluate the effect of KRG on the transient forebrain ischemia, we evaluated the change of cerebral blood flow when both carotid artery was clamped in the normal saline, CS (100 mg/kg, *po* for 7 d), and SFF (100 mg/kg, *po* for 7 d) -treated rats, respectively. As shown in Fig 2B, pretreatment with CS inhibited significantly the reduction of cerebral blood flow induced by both carotid arterial clamp, compared with rats pretreated with normal saline (12.90 % ± 6.13 % for CS vs 23.74 % ± 1.65 % for normal saline, $P < 0.05$). However, pretreatment with SFF did not affect the transient forebrain ischemia. (18.52 % ± 3.84 % for CS vs 23.74 % ± 1.65 % for normal saline, $P > 0.05$).

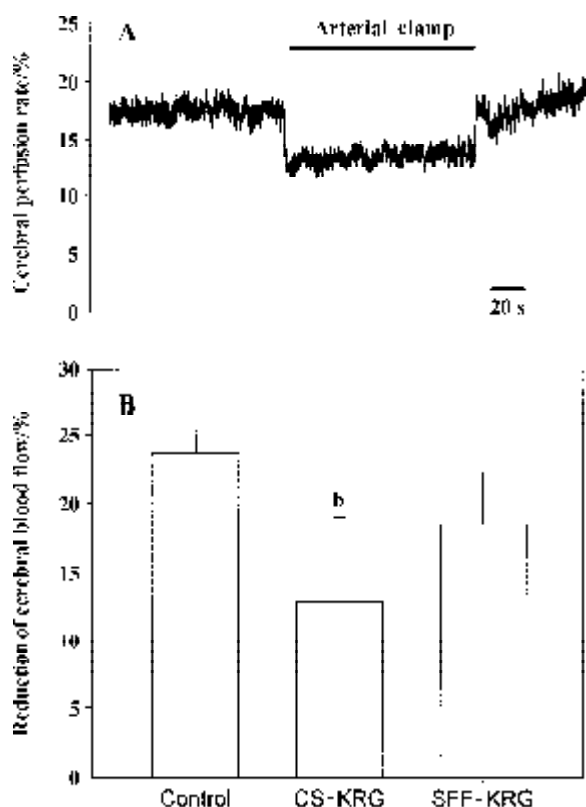


Fig 2. Crude saponin of KRG inhibits forebrain ischemia by bilateral carotid clamps. A) Typical change of forebrain cerebral blood flow by the both carotid arterial clamps in the rats. B) Effect of crude saponin, and saponin-free fraction of Korea red ginseng on the change of forebrain cerebral blood flow. Control: Normal saline-treated group. CS-KRG: Crude saponin of Korea red ginseng-treated group for 7 d (100 mg·kg⁻¹·d⁻¹), SFF-KRG: Saponin-free fraction of Korea red ginseng-treated group for 7 d (100 mg·kg⁻¹·d⁻¹). *n*=5. Mean±SEM. ^b $P < 0.05$ vs normal saline-

Effect of KRG on superoxide production To evaluate the effect of KRG on the superoxide production in the endothelial cells, HUVEC were pretreated with CS (0.1 g/L) or SFF (0.1 g/L) for 30 min. As shown as Fig 3, both CS and SFF did not affect basal superoxide production in endothelial cells [(177±28) for CS and (134±30) for SFF vs (100±25) RLU/1×10⁵ cells for no treatment]. NADPH (0.1 mmol/L) markedly increased superoxide production in the HUVEC cells [(5665±860) vs (100±25) LU/1×10⁵ cells for no treatment, $P < 0.01$]. NADPH-driven superoxide production was significantly decreased about 70 % by pretreatment with CS (0.1 g/L) [(2167±93) for CS vs (5665±860) for control RLU/1×10⁵ cells, $P < 0.01$]. On the other hand, this superoxide production was slightly inhibited by SFF (0.1 g/L) [(5023±580) for SFF vs (5665±860) for control RLU/1×10⁵ cells, $P > 0.05$].

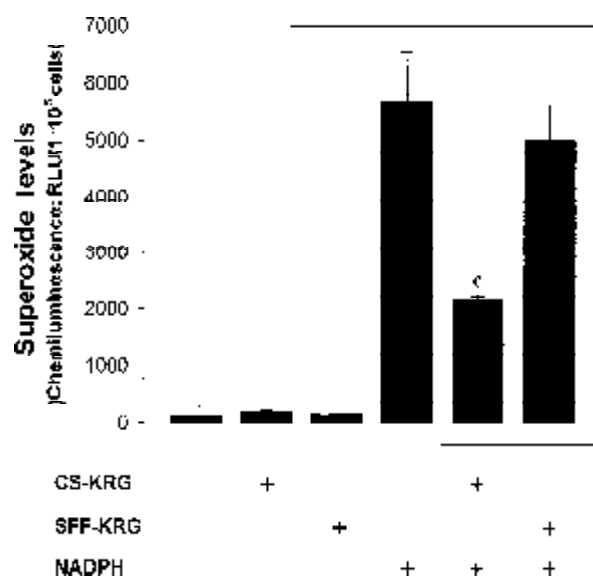


Fig 3. Crude saponin of KRG inhibits NADPH-driven superoxide production. Superoxide (measured by lucigenin chemiluminescence) after the treatment with NADPH (0.1 mmol/L) in the presence of CS (0.1 g/L) or SFF of KRG (0.1 g/L). Representative data from four independent experiments are shown. Mean±SEM. ^b $P < 0.01$ vs NADPH-treated cells as control.

DISCUSSION

The present study shows that saponin fraction of KRG increases cerebral blood flow in rats and inhibits NADPH-driven superoxide production in the endothelial cells. Cerebral circulation, like most other vascular beds (eg, coronary, mesenteric, and skeletal muscle),

but in contrast to some other vascular beds (renal and cutaneous), is characterized by “coupling” of changes in metabolism and blood flow^[15].

In our previous report, it was demonstrated that saponin fraction of KRG had an anti-hypertensive property and increased the level of nitric oxide in the endothelial cells^[4]. It has also been demonstrated that saponin fraction of KRG can act as nitric oxide donor^[4,16]. In the present study, we demonstrated that CS, but not SFF, increased cerebral blood flow in the resting state (Fig 1). It has been shown that ginseng saponin relaxed the basilar artery in a concentration-dependent and partly endothelium-dependent manner^[8]. Therefore, our results suggest that saponin fraction of KRG increased cerebral blood flow, maybe due to vasorelaxing action of KRG in the cerebral vessel in the resting state.

Unilateral common carotid arterial clamp could not affect the forebrain cerebral blood flow because of compensation from contralateral blood flow in the rats. Both carotid artery clamp model was developed to examine changes in the posterior circle of Willis and the basilar and intracranial vertebral arteries after bilateral common carotid ligation. This procedure produced a major redistribution of blood to the head, with increased flow through the vertebral and basilar arteries^[17]. Chronic treatment with CS of KRG (but not SFF of KRG) inhibited the decrease of forebrain cerebral blood flow induced by both common carotid arterial clamps (Fig 2). The protective effect in transient forebrain ischemia by CS of KRG may be mediated through a major redistribution of blood to the head, with increased flow through the vertebral and basilar arteries^[17]. It has been shown that KRG has an antioxidant activity including radical scavenging activity^[18-20], and may involve altered NO release or synthesis^[11]. Therefore, chronic treatment with CS of KRG may affect the redistribution of blood to the head via vasodilation of vertebral and basilar arteries^[17].

Ginsenosides attenuate neuronal cell damage induced by glutamate exposure^[21]. Also, *Panax ginseng* showed a remarkable capacity to protect brain tissue proteins from oxidative damage *in vitro*^[12]. It appears that vascular NADPH oxidase utilizes both NADH and NADPH as electron donors in the generation of superoxide. NADPH induces the contraction of basilar artery^[11]. The contractile response to NADPH is mediated by superoxide. Therefore, activation of NADPH oxidase, a significant source of superoxide in cerebral

arteries, may reduce cerebral blood flow. In the present study, saponin fraction of KRG inhibits NADPH-driven superoxide production in the cultured endothelial cells. However, KRG did not suppress basal superoxide production in endothelial cells. Therefore, inhibition of NADPH-driven superoxide generation by KRG as observed in the present study as well as vasorelaxing action of KRG may contribute to increase in cerebral blood flow.

Although the precise mechanism underlying neuroprotective effect of KRG is unknown, our data suggest that Korea red ginseng increases cerebral blood flow in the resting state, chronic treatment with saponin fraction of KRG may protect forebrain ischemia, maybe due to the inhibition of superoxide production in cerebral vessels.

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