In Vitro Antioxidant Activity of Vietnamese Ginseng Saponin and Its Components

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To elucidate the antioxidant action of Vietnamese ginseng saponin against free radial-mediated cellular damage, we examined the effect of Vietnamese ginseng saponin on lipid peroxidation in the mouse brain, liver, and liver microsomes by using two in vitro free radical generating systems (iron ferrous+ascorbic acid and iron ferrous+hydrogen peroxide). Free radical-mediated lipid peroxidation was determined by measuring the endogenous and stimulated accumulation of thiobarbituric acid reactive substance (TBA-RS). Vietnamese ginseng saponin (0.05—0.5 mg/ml), as well as vitamin E, significantly inhibited the formation of TBA-RS in tissue homogenates. Panax ginseng saponin, at the same concentration range as Vietnamese ginseng saponin, also had inhibitory action on free radical-mediated lipid peroxidation. However, majonoside-R2, ginsenoside-Rg1, and ginsenoside-Rb1, the main saponin components of Vietnamese ginseng saponin fraction, had no effect on lipid peroxidation. These results suggest that Vietnamese ginseng exerts a protective action against free radical-induced tissue injury and that this effect is attributable to minor components rather than the main saponin components tested.

Key words Vietnamese ginseng saponin; Panax ginseng saponin; free radical-induced lipid peroxidation

Materials and Methods

Materials Total Vietnamese ginseng saponin (VG saponin) and majonoside-R2 were isolated from VG root and rhizome (yields: 13.2% and 5.29% of dry material, respectively) as previously described. Briefly, powdered VG root and rhizome (5-year old) were extracted 4 times with aqueous 96%, 48%, 24% (v/v) ethanol, and water, successively. The combined extracts were evaporated and lyophilized to yield VG crude extract. Following extraction with ethyl ether, water-saturated n-butanol was added. The n-butanol extract was evaporated to yield the total saponin fraction. Quantitative analysis using high performance liquid chromatography revealed that the contents of the major saponin components majonoside-R2, ginsenoside-Rg1, and ginsenoside-Rb1 in total VG saponin were 22.67, 7.38 and 3.7% (w/w), respectively. Panax ginseng saponin (total PG saponin) was used as a standard and was composed mainly of ginsenoside-Rb1 (13.5%), -Rb2 (8.1%), -Re (9%), -Rd (4.5%), -Re (6.5%), -Rf (3.2%) and -Rg1 (4.3%) as previously described. Other drugs, ginsenosides-Rg1, ginsenoside-Rg1 and 6-α-tocopherol (vitamin E), were purchased from Nacalai Tesque, Inc. Kyoto, Japan. Malonaldehyde bis(dimethylacetal) (MDA) was from Tokyo Kasei, Japan.

Preparation of Tissue Homogenates Male Balb/c mouse brain or liver was removed and washed with ice-cold 0.9% saline. The brain was homogenized in 10 vol. of ice-cold 5 mM potassium phosphate buffer (pH 7.4) using a glass homogenizer. The liver was homogenized in 9 vol. of ice-cold 1.15% KCl. To prepare the liver microsomal fraction, the liver was rapidly homogenized in ice-cold 0.25 M sucrose and centrifuged at 9000 g at 4 °C for 20 min. The supernatant was centrifuged at 105000 g at 4 °C for 60 min. The microsomal pellets were washed 3 times with ice-cold 0.15 M KCl, and then stored at −20 °C until the experiments.

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tein content of tissue homogenates was measured by the Biuret method.\textsuperscript{[12]}

**Determination of Antioxidant Activity** The antioxidant activity was determined by quantification of thiobarbituric acid reactive substance (TBA-RS) using a slightly modified method of Buege and Aust.\textsuperscript{[13]} The reaction mixture was composed of 0.5 ml tissue homogenate, 0.9 ml phosphate buffer (50 mm, pH 7.4), 0.5 ml of one of the chemical systems generating free radicals: 0.01 mm FeSO$_4$+0.1 mm ascorbic acid (Fe-VC) or 0.01 mm FeSO$_4$+30 mm H$_2$O$_2$ (the Fenton reagent\textsuperscript{[14]}), and 0.1 ml of vehicle or an aqueous solution of test agents (total VG saponin, total PG saponin or each main saponin component). The reaction mixtures were incubated at 37°C for 30 min and the reaction was terminated by adding 1 ml of 10% (w/v) trichloroacetic acid to the mixture. After centrifugation at 8000 g for 10 min, the supernatants were incubated with 1 ml of 0.8% (w/v) TBA at 100°C for 15 min. After a cooling period, TBA-RS generated was spectrophotometrically determined at 532 nm (Beckman DU 640 Spectrophotometer) using MDA as a standard.

**Statistic Analysis** Data were expressed as the concentration of MDA nmol/mg protein/ml tissue homogenate and analyzed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test for multiple comparison among groups. Differences of \( p < 0.05 \) were considered statistically significant.

**RESULTS**

As shown in Fig. 1, total VG saponin, at the concentration range of 0.25—0.5 mg/ml reaction mixture, significantly inhibited the formation of TBA-RS in the brain tissue caused by two types of free radical-generating systems, Fe-VC and the Fenton reagent. Total PG saponin, at the same concentration range, also showed an inhibitory effect on the Fenton reaction-induced formation of TBA-RS, but it had no effect on TBA-RS formation caused by Fe-VC.

On the other hand, both total VG saponin and total PG saponin produced an inhibitory action on the Fenton reagent- and Fe-VC-induced TBA-RS production in the liver homogenate and the liver microsomal preparation (Fig. 2). The

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**Fig. 1.** Effect of Vietnamese Ginseng Saponin (Total VG Saponin) and *Punica ginseng* Saponin (Total PG Saponin) on Lipid Peroxidation in the Brain Homogenate

Lipid peroxidation in brain homogenate was catalyzed by incubating the homogenate at 37°C for 30 min in the presence of the free radical generating systems, (I) 0.01 mm FeSO$_4$+0.1 mm ascorbic acid and (II) the Fenton reagent (0.01 mm FeSO$_4$+30 mm H$_2$O$_2$). TBA-RS, the product of lipid peroxidation, was determined by using the MDA-TBA reaction test. \( *p < 0.05 \) vs. vehicle (one-way ANOVA followed by the Student-Newman-Keuls test, \( n = 6—8 \)).

**Fig. 2.** Effect of Vietnamese Ginseng Saponin (Total VG Saponin) and *Punica ginseng* Saponin (Total PG Saponin) on Lipid Peroxidation in the Liver Homogenate (A) and Liver Microsomal Fraction (B)

Lipid peroxidation was catalyzed by incubating the homogenate or microsome suspension at 37°C for 30 min in the presence of the free radical generating systems, (I) 0.01 mm FeSO$_4$+0.1 mm ascorbic acid and (II) the Fenton reagent (0.01 mm FeSO$_4$+30 mm H$_2$O$_2$). TBA-RS, the product of lipid peroxidation, was determined by using MDA-TBA reaction test. \( *p < 0.05 \) vs. vehicle (one-way ANOVA followed by the Student-Newman-Keuls test, \( n = 6—8 \)).
standard antioxidant drug vitamin E, at 10—100 μM, also significantly inhibited the TBA-RS production in the brain tissue homogenate stimulated by the Fenton reagent and Fe-VC (Table 1). In contrast, majonoside-R2 and ginsenoside-Rb1 and -Rg1 saponin components of total VG saponin, exhibited no effect on the enhanced TBA-RS production in the brain and liver homogenates (Table 2).

DISCUSSION

The present results demonstrated that total VG saponin, as well as total PG saponin, exhibited a suppressive action on the lipid peroxidation caused by radical generating systems in the brain and liver tissue preparations and that the effect of total VG saponin was more potent than that of total PG saponin when compared at the same concentration range. Recent studies have shown that ginseng extract and its active components, ginsenosides, exhibit protective action against free radial-induced damage\(^1\) and that the total PG saponin attenuates lipid peroxidation in the rat liver homogenate.\(^2\) Taken together, the present results raise the possibility that total VG saponin also exerts a protective action against free radial-induced tissue damage in vivo.

Stress exposure of experimental animals has been reported to lead to oxidative damage to lipid in the brain.\(^6\) In our previous studies,\(^8\) majonoside-R2, a major saponin component accounting for over 50% of the total VG saponin fraction, played a key role in the pharmacological action of VG and attenuated behavioral and pathophysiological changes caused by psychological stress exposure in rodents. Thus, it could be hypothesized that this saponin may also contribute to the suppressive action of total VG saponin on lipid peroxidation in the mouse brain and liver homogenates. However, in this study, neither majonoside-R2 nor the other main saponins, ginsenoside-Rg1 and -Rb1, had any effect on the stimulated production of TBA-RS. These findings suggest that the suppressive action of the total VG saponin fraction on lipid peroxidation is attributable to components other than the saponins tested here.

Activation of the function of endogenous antioxidant enzyme systems in tissues or inhibition of metal ion-dependent hydroxyl radical formation or both are characteristic of antioxidant compounds.\(^9\) For example, total PG saponin has been reported to exert an indirect scavenging effect on free radical-induced tissue injury by activating the function of endogenous antioxidant enzyme systems.\(^9\) Moreover, plant phenolics, especially flavonoids, are known to inhibit lipid peroxidation in vitro by chelating metal ions.\(^9\) Thus, it is likely that the antioxidant effect of the total VG saponin fraction is due to not only radical scavenging but also to the metal ion chelating activity of this fraction. Nevertheless, further investigation will be required to identify the exact total VG saponin component(s) involved in the antioxidant activity and to clarify the mechanism underlying the suppressive action of total VG saponin fraction on the stimulated TBA-RS production in vitro. It will be also interesting to test if total VG saponin fraction and its saponin components are able to exert antioxidant action in in vivo models such as transient ischemia of the brain and kidney, tissues susceptible to severe radical lesions. These investigations are now in progress in this laboratory.

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REFERENCES


