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Increase in Hepatic DT-Diaphorase Activity by Chronic Administration of *Panax ginseng* Extract to Mice

Kang-Mee Lee*, Myung-Bok Wie, Dong-Keun Song,
Yong-Sik Kim** and Yung-Hi Kim

Institute of Natural Medicine, Department of Pharmacology, Hallym University, Chunchon 200-702, Korea
**Samchundang Pharmaceut. Co., Seoul 150-032, Korea*
***Department of Pharmacology, Seoul National University, Seoul 110-799, Korea*
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Abstract□Effects of chronic administration of ginseng extracts (30 or 150 mg/kg/day for 52 days, *p.o.*) to mice on the activities of DT-diaphorase and glutathione S-transferase (GST) in the liver and the brain were studied. The DT-diaphorase activity in the liver was increased over 2-fold at the dose of both 30 and 150 mg/kg/day, while there was no change in the activity of the enzyme in the brain. The GST activity in the liver was increased in a dose-dependent fashion upto 142% of the control value at the dose of 150 mg/kg/day, while there was no change in the activity of the enzyme in the brain. The ginseng-induced increase in the activities of these hepatic phase II drug-metabolizing enzymes which are involved in the detoxification of carcinogens, is suggested to underlie, at least in part, the anticarcinogenic activity of *Panax ginseng*.

Key words□*Panax ginseng*, DT-diaphorase, glutathione S-transferase, liver, mice.

Introduction

It is now widely accepted that a variety of compounds of dietary and synthetic origin can help prevent chemical carcinogenesis.[1-4] These chemoprotective compounds induce phase II drug-metabolizing enzymes such as glutathione S-transferase (EC 2.5.1.18; GST), uridine 5'-diphosphoglucuronyltransferase (EC 2.4.1.17) and DT-diaphorase [NAD(P)H : quinone oxidoreductase; EC 1.6.99.2][1-3]. As these enzymes are involved in the detoxification of carcinogens, it is believed that induction of these enzymes results in protection against the chemical carcinogenesis.[4]

The root of *Panax ginseng* C.A. Meyer (Araliaceae) has been used for thousands of years as a tonic in traditional Oriental medicine. Recently, chronic administration of *Panax ginseng* extract to rodents was reported to have protective effect against chemical carcinogenesis induced by urethane,[5] 3-methylcholanthrene,[6] benzo(a)pyrene,[7] and diethylnitrosamine.[8] Furthermore, epidemiological study suggested that high consumption of ginseng reduces the risk of developing cancer.[9]

Therefore, it would be of interest to characterize further the mechanism involved in the anticarcinogenic action of ginseng. To determine whether the anticarcinogenic effect of ginseng is accompanied by induction of such protective enzymes, we studied the effect of chronic administration of ginseng extract to mice on the activities of DT-diaphorase and glutathione S-transferase in the liver and the brain.

Materials and Methods

1. Materials

Korean white ginseng (4 years old; Keumsan) was purchased from a local supplier. Dried roots of ginseng (100 g) were cut to fine pieces and transferred into a flask equipped with a reflux conden-

ser. One liter of 50% ethanol solution was added. After boiling for 6 hours and filtering, the crude fraction was condensed in a rotary evaporator. The concentrated extract was dried under reduced pressure (40℃) and the ethanol extract powder was obtained. The overall yield was about 22% for the dry weight of the root.

2. Animals

Female ICR mice were obtained from the Experimental Animal Center (Hallym University). They were fed laboratory diet *ad libitum* and allowed free access to tap-water; they were kept in 12/12 light/dark cycle. The ginseng extract powder was dissolved in physiological saline and was administered orally at the dose of 30 or 150 mg/kg/day for 52 days (from 4 weeks to 11 weeks of age) to mice. Animals administered with only physiological saline served as control.

3. Enzyme assays

Livers or brains were homogenized with 4 volumes of 0.25 M sucrose. After centrifugation of the homogenate at 25,000 g for 30 min (4℃), the supernatant was used for enzyme assay. The dicoumarol-sensitive DT-diaphorase activity was measured by determining the differential initial velocity of reduction of 2,6-dichlorophenol indophenol.[10] The GST activity was measured by using 1-chloro-2,4-dinitrobenzene as substrate.[11] Protein concentration was determined according to Hatree.[12]

4. Statistical analysis

Data are expressed as the mean± S.E.M. The statistical significance of differences among the group means was determined with the Student's t-test. Discrepancies with $p<0.05$ was considered statistically significant.

Results and Discussion

After the administration of the ethanol extract of *Panax ginseng* to mice at the doses of 30 and 150 mg/kg/dap *p.o.* for 52 days, we measured the activities of DT-diaphorase and glutathione S-transferase in the liver and the brain. The DT-diaphorase activity in the liver was increased over 2-fold at the doses of both 30 and 150 mg/kg/day, while there was no change in the activity of the enzyme

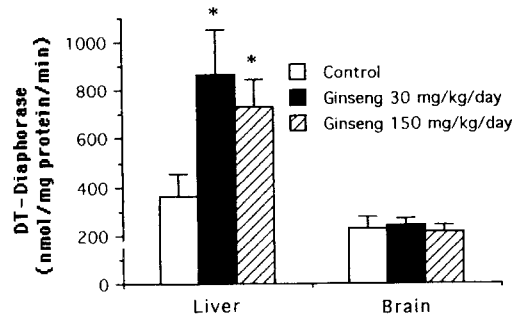

Fig. 1. Effect of chronic administration of *Panax ginseng* ethanol extract to mice on the activity of DT-diaphorase in the liver and the brain. The ginseng extract was administered orally at the dose of 30 or 150 mg/kg/day for 52 days to female ICR mice. Animals administered with only physiological saline served as control. After homogenization of livers or brains with 4 volumes of 0.25 M sucrose, the 25,000 g supernatant was used for the enzyme assay. The dicoumarol-sensitive DT-diaphorase activity was measured by determining the differential initial velocity of reduction of 2,6-dichlorophenol indophenol. Data are expressed as the mean± S.E.M. ($n=5\sim8$). *$p<0.05$ compared to control values.

in the brain (Fig. 1). The GST activity in the liver was increased in a dose-dependent fashion upto 142 % of the control value at the dose of 150 mg/kg /day, while there was no change in the activity of the enzyme in the brain (Fig. 2). There was no change in the activity of monoamine oxidase in the liver (data not shown). The organ weight of liver and brain as well as serum alanine and aspartate aminotransferase activities were not changed (data not shown).

Administration of ginseng extract to rodents has been previously reported to increase the activities of hepatic detoxifying enzymes, such as GST[13,14] and eposide hydratase,[14,15] but very little is known about the effect of administration of ginseng on the DT-diaphorase activity. The results of the present study showed that DT-diaphorase activity as well as GST activity was increased in the liver after long-term administration of ginseng extract. The increase in the hepatic DT-diaphorase activity was more marked and reached the maximum value at

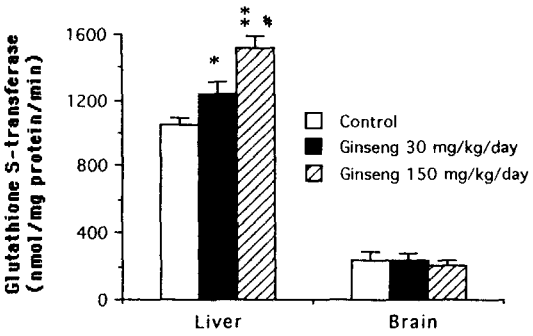

Fig. 2. Effect of chronic administration of *Panax ginseng* ethanol extract to mice on the activity of glutathione S-transferase (GST) in the liver and the brain. For details, see legend of Fig. 1. The GST activity was measured by using 1-chloro-2,4-dinitrobenzene as substrate. Data are expressed as the mean± S.E.M. (n=5~8). *p< 0.05, **p<0.01 compared to control values. #p< 0.05 compared to values of 30 mg/kg/day. group.

the lower dose of ginseng extract than in the case with the GST activity (Fig. 1 and 2). The degree of ginseng-induced increase in the hepatic GST activity is in good accordance with the previous reports.[13,14] Although significant activities of DT-diaphorase and GST were detected in the brain, there was no changes in these enzyme activities in the brain, suggesting that the inducing factor(s) probably cannot pass the blood brain barrier or that the brain enzymes are modulated differently from the liver enzymes. In the liver, the ginseng-induced increase in the detoxifying enzyme activities was selective, as there was no change in the activity of monoamine oxidase.

As DT-diaphorase prevents formation of reactive oxygen species, and GST could increase the rates of conjugation of both endogenous and exogenous electrophiles including many carcinogens, the increase in the activities of these protective enzymes by ginseng would underlie, at least partly, the anticarcinogenic activity of ginseng. And further study is needed to identify the responsible chemoprotective compound(s) of ginseng extract.

요 약

생쥐에게 인삼 엑스를 장기간 투여한 후(30 및 150 mg/kg/日, 경구 52일) 간장과 뇌에서 DT-diaphorase와 glutathione S-transferase(GST)의 활성을 측정하였다. 간장의 DT-diaphorase 활성은 30 및 150 mg/kg/日 용량에서 대조치의 2배 이상으로 증가하였으나 뇌에서는 변화가 없었다. 간장의 GST 활성은 투여된 인삼 엑스의 용량에 의존적으로 증가하여 150 mg/kg/日에서는 대조치의 142%로 증가하였으나 뇌에서는 변화가 없었다. 발암원의 해독에 관여하는 第II相 약물대사효소들이 인삼의 투여로 인하여 간장에서 증가하는 것은 인삼의 항암효과의 적어도 일부분을 설명할 수 있으리라 사료된다.

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