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Effects of Ginseng on the Metabolism of Enflurane and Methoxyflurane

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Ginseng has been believed to be a powerful tonic by oriental people for a long time and is one of the most popular folk medicine in oriental countries. Intraperitoneal injection of ginseng into rats and mice has been reported to Increase the rates of hepatic RNA and protein synthesis, increase proliforation of rough RES of liver, and enhance alcohol metabolism. We have carried out a study to see the effects of red ginseng powder and extract on in vivo and in vitro metabolism of enflurane and methoxyflurane in male Fisher 344 rats. Red ginseng powder was dissolved in deionized water and dosed for two weeks ad libitum in rats. Hepatic microsomes were prepared and oxidative defluorination of enflurane and methoxyflurane were measured in vitro. Using red ginseng extract, studies were done of both acute and chronic treatment in rats. In chronic experiments, they were dosed with several dosages three times a day for three days; on the fourth day enflurane was administered i.p. and one hour later fluoride levels were mesured in plasma and hepatic microsomes were prepared for in vitro studies as above. In the acute experiment, enflurane was administered intraperitoneally eighteen hours after single oral dosage of ginseng and plasma defluorination was measured. There were no statistically significant differences in hepatic microsomal cytochrome P-450 content or defluorination of enflurane and methoxyflurane between control and experimental groups using either red ginseng extract or powder. The results showed that ginseng ingestion did not affect the metabolism of enflurane and methoxyflurane.

Key Words: Red ginseng powder, red ginseng extract, enflurane, methoxyflurane, microsomes, defluorination, cytochrome P-450

Ginseng has been used by man for thousands of years, and oriental folk medicine describes it as both a tonic for restoration of strength and a *panacea* (the genus PANAX, meaning all healing). The active principle of ginseng is reported as a mixture of glycosides, consisting of steroidal saponins, called ginsenosides (Choi *et al.* 1980a; Choi *et al.* 1980b; Chen and Staba 1987).

Ginseng has been used as a detoxicant of alcohol and there have been many reports that ginseng enhances alcohol metabolism by alcohol dehydrogenase and the microsomal cytochrome P-450 enzyme system (Joo *et al.* 1982; Kim *et al.* 1983; Joo 1984).

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Ethanol treatment of rats has been characterized by increased rates of metabolism of alcohol (Ohnishi and Lieber 1977), and of the fluorinated ether anesthetics, enflurane, methoxyflurane and isoflurane (Rice *et al.* 1980; Van Dyke 1984; Ryan *et al.* 1986; Pantuck *et al.* 1985).

Now-a-days, ginseng is one of the most popular natural tonics used in oriental countries and manufactured in many commercial forms. Therefore, there are many of opportunities to encounter patients who are taking ginseng. We have carried out studies in rats to determine whether red ginseng increase cytochrome P-450 content and oxidative defluorination of enflurane and methoxyflurane.

MATERIALS AND METHODS

Experimental animals

Male Fischer 344 rats from Charles River Laboratories, Cambridge, Massachusetts were used for all studies. They were bedded on ground corn-

cob and fed Purina rat chow (Ralston Purina, St. Louis, Missouri) and allowed free access to deionized water instead of tap water to obviate the trace amount of fluoride in tap water.

Ginseng

Red ginseng powder and red ginseng extract (water extract of steamed dried red ginseng powder) supplied by the Office of Monopoly, Republic of Korea were used.

Treatment of animals

A. Red ginseng powder administration:

Twenty rats weighing 110-120g were divided into 4 groups (n=5) as follows: I-control; II-ginseng 15 mg/kg/day; III-ginseng 75 mg/kg/day; IV-ginseng 200 mg/kg/day. The dosage were based on Office of Monopoly ROK's prescription and the method of Yun *et al.* (1983). According to a pilot study, though there was a few drops of spillage, rats consumed an average of 20 ml of water a day. Red ginseng powder was dissolved in deionized water based on 20 ml/rat/day. After two weeks of drinking ad libitum they were killed by cervical dislocation and liver microsomes were prepared by the method of Levin *et al.* (1974) as described below. The microsomes were stored at −90°C until used for measurement of protein content, cytochrome P-450 content and anesthetic defluorination activity as described below.

B. Red ginseng extract administration:

1) Chronic experiment; Twenty four rats weighing 45-50g were divided into four groups (n=6) as follows: I-control; II-ginseng 62.5 mg/kg/day; III-ginseng 125 mg/kg/day; IV-ginseng 250 mg/kg/day. Each dose was dissolved in 0.9% saline (0.5 ml) and administered with a 22G gavage needle three times a day for three days. In the morning of the fourth day, rats were administered enflurane (Anaquest, Madison, Wisconsin) 0.6 μl/g i.p.. Following enflurane administration the rats were permitted no further food or water. One hour later, they were anesthetized with ether and, after laparotomy, blood was obtained by vena caval puncture. Heparinized blood was centrifuged and the plasma was used immediately for measurement of fluoride content. As soon as withdrawal of blood was completed, livers were excised, weighed and stored at −90°C until used for preparation of microsomes as described below. The microsomes were assayed for cytochrome P-450 content and anesthetic defluorination activity as described below.

2) Acute experiment; Twenty four rats divided into four groups (n=6) as follows: I-control; II-ginseng 62.5 mg/kg; III-ginseng 125 mg/kg; IV-ginseng 250 mg/kg. Rats were dosed 18 hours before administration of enflurane 0.6 μl/g i.p.. Blood samples were obtained and studied as in the chronic experiment.

Preparation of microsomes

Livers were homogenized in 2 volumes of Tris-1.15% KCl, pH 7.4. The homogenate was centrifuged at 9,000 xg for 30 minutes in a refrigerated centrifuge and the resulting supernatant fraction was sedimented at 100,000 xg for 1 hour. The microsomal pellet so obtained was suspended in 1.15% KCl and resedimented at 100,000×g for 1 hour. This pellet was suspended in 0.01M Tris-0.25M sucrose-0.01M EDTA buffer and stored at −90°C until used.

Assays

Fluoride concentration in plasma was determined using an ion specific electrode (Orion Research Inc., Cambridge, Massachusetts as described by Fry and Taves (1970). Standard curves were prepared by adding known amounts of fluoride to plasma from control rats.

The protein concentration in rat liver microsomes was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Cytochrome P-450 content in rat liver microsomes was determined spectrally from the carbon monoxide difference spectrum of the reduced hemoprotein by the method of Omura and Sato (1964).

The rates of enflurane and methoxyflurane defluorination by rat liver microsomes were determined by incubation of 10 μl of each anesthetic with 5 mg of microsomal protein as described by Rice *et al.* (1980). Inorganic fluoride released during 30 minutes incubation was measured with an ion-specific electrode as described above. Incubations using heat-inactivated microsomes were used as blanks. Standard curves were prepared by adding known amounts of fluoride to blanks.

Each rat could be used for both the *in vivo* and the *in vitro* studies since the administration enflurane (0.6 μl/g i.p.) to rats one hour prior to preparation of microsomes did not enflurence either the level of fluoride in blanks or the rates of defluorination of the anesthetics (Pantuck *et al.* 1987).

Statistical analysis

When multiple comparisons were made, results

Table 1. Effect of Chronic Treatment with Red Ginseng Powder on Hepatic Microsomal Cytochrome P-450 Content and Defluorination of Enflurane and Methoxyflurane *in Vitro* by Hepatic Microsomes

Treatment group	Body weight (g)	Ratio of liver weight to body weight	Hepatic microsomal cytochrome P-450 (nmol/mg protein)	Fluoride concentration (nmol F^-/mg protein/min)	
				Enflurane	Methoxyflurane
I . Control	178.4± 4.7	0.046±0.0016	0.74±0.07	0.052±0.013	0.089±0.034
II . Ginseng-15 mg/kg/day	172.6±14.1	0.050±0.0018	0.70±0.04	0.058±0.013	0.090±0.036
III . Ginseng-75 mg/kg/day	171.0± 7.6	0.047±0.0002	0.74±0.09	0.054±0.016	0.097±0.034
IV. Ginseng-200 mg/kg/day	155.2± 6.0	0.045±0.0013	0.73±0.07	0.048±0.016	0.099±0.029

Values are means±SD of 5 rats

There is no significant difference between control and each experimental group.

Table 2. Effect of Chronic Treatment with Red Ginseng Extract on Plasma Defluorination of Enflurane *in vivo*

Treatment group	Fluoride concentration (nmol F^-/ml plasma)
I . Control	13.09±2.21
II . Ginseng 62.5 mg/kg/day	11.98±2.59
III . Ginseng 125 mg/kg/day	8.76±0.71
IV. Ginseng 250 mg/kg/day	12.29±3.40

Values are means ± SD of 6 rats

There is no significant difference between control and each experimental group.

Table 3. Effect of chronic Treatment with Red Ginseng Extract on the Hepatic Microsomal Cytochrome P-450 content, and Defluorination of Enflurane and Methoxyflurane *in Vitro* Hepatic Microsome

Treatment	Liver weight	Hepatic microsomal cytochrome P-450 content (nmol/mg protein)	Fluoride concentration (nmol F^-/mg protein/min)	
			Enflurane	Methoxyflurane
I . Control	2.50±0.21	0.46±0.12	0.040±0.007	0.066±0.027
II . Ginseng 62.5 mg/kg/day	2.60±0.22	0.46±0.09	0.046±0.012	0.078±0.011
III . Ginseng 125 mg/kg/day	2.68±0.20	0.45±0.08	0.040±0.007	0.070±0.018
IV. Ginseng 250 mg/kg/day	2.80±0.34	0.44±0.10	0.047±0.011	0.078±0.018

Values are means ± SD of 6 rats

There is no significant difference between control and each experimental group.

were analyzed using analysis of variance followed by the Bonferroni method described by Wallenstein *et al.* (1980). All other data were analyzed using the unpaired Student's t-test. Differences in mean values were considered statistically significant if P was less than 0.05 using a two-tailed test.

RESULTS

A. Red ginseng powder administration

No statistically significant differences were observed in body weight, ratio of liver to body weight, hepatic microsomal cytochrome P-450 content or defluorination of enflurane and methoxyflurane *in vitro* between control and experimental groups (Table 1, P>0.05).

B. Red ginseng extract administration

1) Chronic experiment: No differences were found between control and treated rats in cytochrome P-450 content or defluorination of enflurane and methoxyflurane *in vivo* and *in vitro* study (Table 2,3, P>0.05).

Table 4. Effect of a Single Dose of Red Ginseng Extract on Plasma Defluorination of Enflurane *in Vivo*

Treatment group	Fluoride concentration (nmol F^-/ml plasma)
I . Control	10.62±3.60
II . Ginseng 62.5 mg/kg	9.97±2.24
II . Ginseng 125 mg/kg	13.47±2.30
IV. Ginseng 250 mg/kg	11.88±1.86

Values are means ± SD of 6 rats
There is no significant difference between control and experimental group.

2) Acute experiment: There were no difference in plasma fluoride concentration between control and every experimental group (Table 4, $P>0.05$).

DISCUSSION

Cytochrome P-450, the terminal electron transport system of the hepatic microsomal mixed function oxidase system, catalyzes the metabolism of xenobiotics as well as certain endogenous compound (Conney 1967). Hepatic microsomes contain multiple cytochrome P-450 isozymes, possessing broad and overlapping substrate selectivity, which contributes to the metabolic versatility of the system (Conney 1986).

There is considerable evidence that the oxidative metabolism of ethanol, enflurane, and methoxyflurane occurs, at least to a substantial extent, by a single ethanol-inducible isozyme of cytochrome P-450, cytochrome P-450j (Ohnishi and Lieber 1977; Van Dyke 1984; Rice *et al.* 1983; Ryan *et al.* 1986; Pantuck *et al.* 1985).

Joo *et al.* (1982) reported that study of the effect ginseng saponin on rat hepatic MEOS (microsomal ethanol oxidizing system) *in vitro* as well as *in vivo* showed that the oxidation of ethanol was significantly accelerated in the presence of the saponin. For this reason, substances that induce the oxidative metabolism of ethanol must be considered as potentially capable of inducing the oxidative metabolism of these fluorinated ether anesthetics.

Much of the literature dealing with ginseng research is done in abstract form (Lee *et al.* 1978; Clifford *et al.* 1978; Choi *et al.* 1980a; Choi *et al.* 1980b; Chen and Staba 1978) making it hard to duplicate the methods used by others. Joo *et al.* (1982) & Kim *et al.* (1983) used a methanol extract of white ginseng. We did our initial experiments with red ginseng extract (water extract form), but none of our studies showed an increase in the defluorination of ether anesthetics *in vivo* or *in vitro*. To be sure that the lack of effect found in these studies was not due to removal of active substances during water extraction, we repeated some of our experiments using powdered whole ginseng, but no differences could be found in body weight, hepatic microsomal P-450 content or defluorination of ether anesthetics, enflurane and methoxyflurane, between control and treated rats.

Also no differences between control and treated rats were found when baby rats (40-50g) were substituted for grown up rats (110-120g) in red ginseng extract experiment.

The metabolism of methoxyflurane is very markedly induced by phenobarbital (Greenstein *et al.* 1975), phenytoin (Dooley *et al.* 1979), and ethanol and isoniazid (Rice *et al.* 1980; Van Dyke 1984; Pantuck *et al.* 1985; Ryan *et al.* 1986; Pantuck *et al.* 1987). This suggests that substantial metabolism of methoxyflurane can occur by more than one isozyme of cytochrome P-450. But in our study, no differences in defluorination of either methoxyflurane or enflurane could be found suggesting that these isozymes of cytochrome P-450 were unaffected by prior treatment of rats with ginseng.

In summary, there was no increase in defluorination of enflurane or methoxyflurane with red ginseng powder as well as red ginseng extract. We have found the ordinary red ginseng product did not affect the metabolism of fluorinated ether anesthetics.

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