Effect of crude saponin extracted from the leaf and stem of *Panax ginseng* on scopolamine-induced memory disruption in rats

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Abstract

Effects of crude saponin fraction extracted from the leaf and stem of *Panax ginseng* (PG) on scopolamine-induced working memory disruption were examined using the T-maze delayed alternation task and three-panel runway task in rats. Scopolamine (0.2 mg/kg, *i.p.*) significantly decreased the percentage of correct responses in the T-maze task. Not only the crude saponin extracted from the PG root (12.5–50 mg/kg, *p.o.*) but also the saponin from the leaf and stem of PG (12.5–50 mg/kg, *p.o.*) improved the disrupting effect of scopolamine in the T-maze task. In the three-panel runway test, the crude saponin from the leaf and stem of PG (12.5–50 mg/kg, *p.o.*), as well as physostigmine (0.4 mg/kg, *i.p.*), significantly reduced the scopolamine (0.5 mg/kg, *i.p.*)-induced increase in errors. These results suggest that the crude saponin fraction of the leaf and stem of PG, as well as that of the PG root, possesses beneficial effects on working memory deficit in rats.

Key words crude saponin, the leaf and stem of *Panax ginseng*, scopolamine, working memory, three-panel runway task, delayed alternation T-maze task.

Abbreviations PG, *Panax ginseng*; IRI, inter run interval.

Introduction

Panax ginseng root (*Panax ginseng* C.A. MEYER) has been widely used as a tonic in Asian countries. Recent reports demonstrated that *Panax ginseng* root extract and ginsenosides produced anti-amnesic effect in passive or conditioned avoidance task.[1,2] Moreover, in the Morris water-maze task, chronic intake of ginseng root extract improves the impaired maze performance of aged rats.[3] Generally the root of *Panax ginseng* is used for medicinal purposes and it is well known to include ginsenosides.[4] On the other hand, the leaf and stem of ginseng have not been used for medicinal purposes and only a little information is available on the pharmacological activity of the extract prepared from these parts of ginseng, although they contain saponins such as ginsenoside Rg.[4]

We previously reported that the water extract of *Panax ginseng* root improved scopolamine-induced working memory deficit in the T-maze delayed alternation task in rats.[5] In the present study, we investigated the effect of crude saponin extracted from the leaf and stem of *Panax ginseng* on the working memory performance impaired by scopolamine in the T-maze delayed alternation task and three-panel runway task in rats.

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Materials and Methods

Experimental animals : Male Wistar rats (Harbin Railroad General Hospital Animal Center, China) weighing 200 - 250 g were used. Animals were maintained on a restricted feeding schedule to keep their weight about at 85 % of the free-feeding level. Water was given *ad libitum*.

Apparatus

1) *T-maze*

The T-maze was made of wood with a center stem and two side arms. The center stem (72 cm long, 12 cm wide) was equipped with a guillotine door 20 cm apart from the end of the stem (start box). Each side arm was 60 cm long and 12 cm wide. The side walls of the maze were 15 cm high. The floor was made of black Plexiglas, and a black food cap, 3.5 cm in diameter, was placed at the end of each arm.

2) *The three-panel runway apparatus*

The apparatus described by Furuya *et al.*[6] was modified and used for the experiments. Briefly, it was made of wood and consisted of a start box (20 cm long, 12 cm wide and 25 cm high), four consecutive choice boxes (A-D : 30 cm long, 36 cm wide and 25 cm high, each) and a goal box (45 cm long, 36 cm wide and 25 cm high). Each choice box had three panel gates (a-c). Animals were prohibited from passing through two of the three panel gates in each choice box by a front stopper and also prohibited from coming back to the previous box by a stopper.

Procedure

1) *The T-maze delayed alternation task*

Prior to the experiments, animals were handled 15 min daily over 3 days and were given 3 days adaptation to the maze. Pretraining and testing were performed as detailed in our previous reports.[5,7] Briefly, all rats were trained to run to the goal boxes for over 5 days. Each trial of the delayed alternation task consisted of one forced run and one choice run. In the forced run, one of the two side arms was blocked by lowering a guillotine door to allow a rat to enter the other arm with a food reward. The subsequent choice run was carried out without closing the guillotine doors. Thus, the animal was allowed to choose the arm. When the rat entered the arm opposite to the one rewarded in the forced run, the performance was recorded as a correct response and the animal was further rewarded with the three pellets. If the rat chose the incorrect arm, the animal was confined in the arm for about 10 sec. Six trials daily were carried out for each rat. The number of right-correct and left-correct trials were equal for each day, but their sequences were randomized. Only the rat that made no more than one incorrect response during each session for 5 consecutive days was used for the experiments. After the pretraining was completed, a delay interval of 0, 40, 100 and 300 sec (inter run interval : IRI) was interposed between the forced run and the choice run. Eight daily trials, which consisted of two each IRI trials in randomized sequences, were carried out over 5 days. Data obtained during this period were collected and analyzed.

When testing drugs, a 40-sec delay was used as the IRI. Ten trials, five left- and five right-correct trials were conducted daily for each rat in a randomized sequence, and the percentage of correct trials was calculated. To evaluate the effect of scopolamine hydrobromide, rats were first tested 20 min after saline injection (1 ml/kg, *i.p.*) and the next day they were retested 20 min after scopolamine injection (0.2 mg / kg, *i.p.*) and were divided into four balanced groups with the same score level. To evaluate the effects of test agents on scopolamine-induced performance disruption, each group was *p.o.* administered with either distilled water or crude saponin extracted from the root or the leaf and term of ginseng 60 min before the test. After 40 min, scopolamine (0.2 mg/kg) was *i.p.* injected. The delayed alternation test was carried out 20 min after scopolamine.

2) *The three-panel runway task*

This task was carried out according to the method reported by Furuya *et al.*.[6]

Acquisition training : Rats were handled daily over 3 days and habituated to the apparatus, as described above. They were preliminary trained to run within 20 sec from the start box to the goal box. During this training period, all the front stoppers were removed, and rats could pass through all panel gates. Then, they were trained with one session of 6 consecutive trials under the condition that one of three panel gates in each choice box could be passed through. The pattern

of correct panel gate position was fixed during each session but it was changed every day. After completing 6 sessions, the number of errors in each session was summed and the animals were divided into 4 balanced groups with the same score level. When testing agents, crude saponin extracted from the leaf and stem of ginseng was *p.o.* administered 60 min before the test. After 40 min, scopolamine (0.5 mg/kg) was *i.p.* injected. The maze performance was examined 20 min after scopolamine. Physostigmine (0.4 mg/kg, *i.p.*) was administered immediately after scopolamine.

Drugs : Crude saponin fraction was prepared from the root or the leaf and stem of ginseng according to the method described by Lin.[8] Briefly, the dried and powdered leaf and stem of ginseng (1 kg ; *Panax ginseng* C.A. MEYER) was boiled in 6 l distilled water for 1 hr and filtered. The residue was boiled again in 4 l distilled water for the same period. The filtrates were combined. The filtrate obtained from the leaf and stem of ginseng was adjusted to pH 8.5 by adding $Ca(OH)_2$ solution. After precipitation, the supernatant was neutralized with 1N-HCl and concentrated (to about 1 l). The filtrate obtained from the root of ginseng was concentrated without adding $Ca(OH)_2$ solution and 1 N-HCl. The concentrated solution was applied to the Diaion column (8×100 cm ; Chemical reagent factory of Nankai University, Tianjin, China). After washing the column with excess distilled water, ginsenosides were eluted by 95 % ethanol. The combined ethanol fractions were evaporated to dryness to yield crude ginsenoside. Yields of the fraction from the root or the leaf and stem of *Panax ginseng* were 1.8% and 3.0%, respectively. Scopolamine hydrobromide and physostigmine salicylate were purchased from Merck (Darmstadt, Germany).

Statistical analysis : The effects of drugs on the percentage of correct responses in the T-maze delayed alternation task were analyzed by the Kruskal-Wallis analysis of variance followed by the Mann-Whitney U-test for multiple comparisons. The data obtained in the three-panel runway task were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test. Differences with a $p<0.05$ were considered statistically significant.

Results

Effects of crude saponin extracted from the root and the leaf and stem of ginseng on scopolamine-induced performance disruption in the T-maze delayed alternation task

As shown in Fig. 1, the choice accuracy markedly

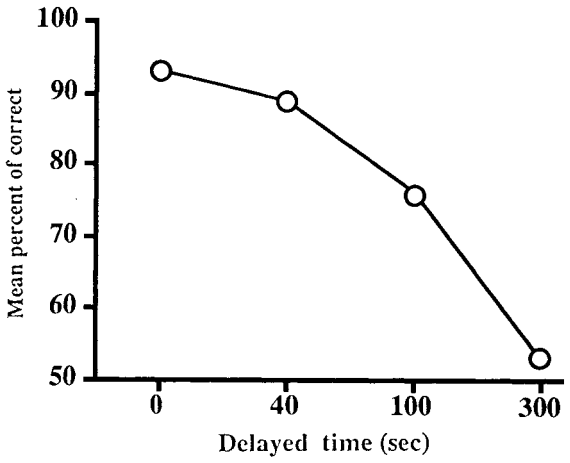

Fig. 1 Delay-dependent performance of the delayed alternation T-maze task in rats. Each datum represents the mean value of 30 rats.

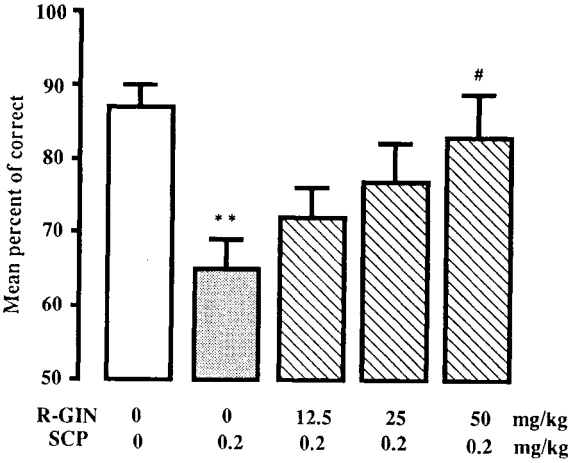

Fig. 2 Effects of crude ginsenoside extracted from ginseng root on the 40 sec-delayed alternation T-maze task of rats treated with scopolamine. Each column represents the mean (±S.E.M.) percentage of correct responses (N=14). $^{**}p<0.01$ compared with control. $\#p<0.05$ compared with scopolamine alone. R GIN : crude ginsenoside extracted from ginseng root. SCP :scopolamine

decreased as the IRI increased. The percentage of correct responses at 300 sec of the IRI (about 53 %) was quite near the random level. Scopolamine (0.2 mg/kg, *i.p.*) significantly decreased the correct responses at 40 sec of the IRI (Fig. 2). Crude saponin extracted from *Panax ginseng* root (12.5-50 mg/kg, *p.o.*) significantly suppressed scopolamine-induced decrease in correct responses in a dose-dependent manner. Crude saponin from the leaf and stem of ginseng, at doses of 12.5-50 mg/kg (*p.o.*), also significantly increased the correct responses decreased by scopolamine, although dose-dependency was not observed clearly (Fig. 3).

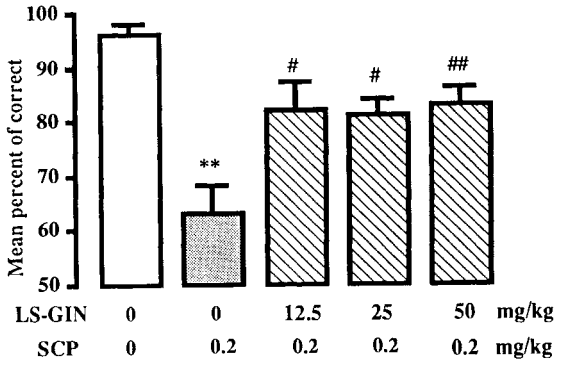

Fig. 3 Effects of crude ginsenoside extracted from ginseng leaf and stem on the 40 sec delayed alternation T-maze task of rats treated with scopolamine. Each column represents the mean (±S.E.M.) precentage of correct responses (N=14). $^{**}p<0.01$ compared with control. $\#p<0.05$ and $\#\#p<0.01$ compared with scopolamine alone. LS-GIN: crude ginsenoside extracted from the leaf and stem of ginseng. SCP: scopolamine

Effects of crude saponin extracted from the leaf and stem of Panax ginseng on scopolamine-induced maze deficit in the three-panel runway task

The number of errors in this task markedly decreased as the number of trials and sessions increased (Fig. 4). As shown in Fig 5, scopolamine (0.5 mg/kg, *i.p.*) significantly increased the number of errors in each trial, and this maze performance deficit caused by scopolamine was significantly improved by 0.4 mg / kg physostigmine (*i.p.*). Crude saponin extracted from the leaf and stem of ginseng (12.5 and 50 mg / kg, *p.o.*) significantly improved the scopolamine-induced deficit of the maze performance in a dose-dependent manner (Fig. 6).

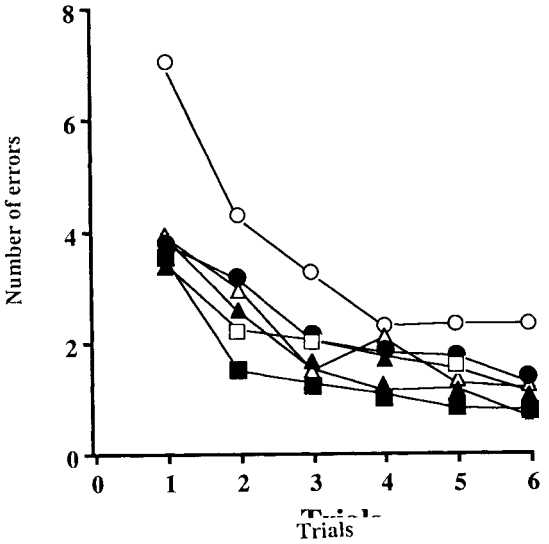

Fig. 4 Training trial and training session-dependent decrease in number of errors in three-panel runway test. Each training session consisted of 6 consecutive trials. Each line represents the mean number of errors (N=11). The pattern of correct panel gate position was fixed during each session, but was changed every day in each trial. Each symbol represents the session number (○: 1; △: 2; □: 3: ●: 4; ▲: 5; ■: 6).

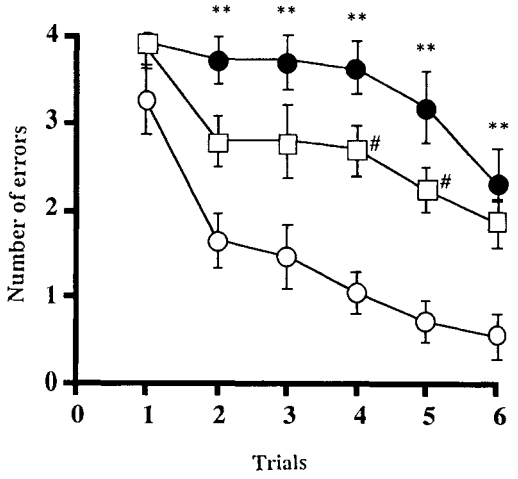

Fig. 5 Effects of physostigmine on working memory disruption induced by scopolamine in three-panel runway task in rats. Each point represents the mean (±S.E.M.) number of errors (N=11). Rats were given six consecutive trials in each session. $^{**}p<0.01$ compared with the respective control. $\#p<0.05$ compared with the respective scopolamine-treated group.
○: saline; ●: scopolamine (0.5); □: physostigmine (0.4) + scopolamine (0.5) (mg/kg)

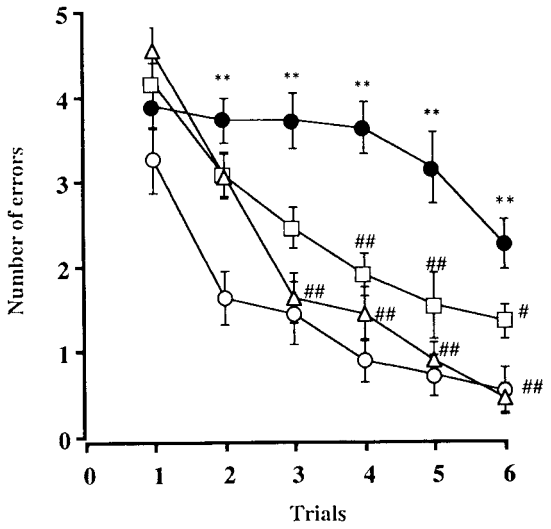

Fig. 6 Effects of crude ginsenoside extracted from the leaf and stem of ginseng on working memory deficit induced by scopolamine in three-panel runway task in rats. Each point represents the mean (±S.E.M.) number of errors (N = 11). Rats were given six consecutive trials in each session. $^{**}p<0.01$ compared with the respective control. $\#p<0.05$ and $\#\#p<0.01$ compared with the respective scopolamine-treated group. ○ : saline ; ● : scopolamine (0.5) ; □ : crude ginsenoside from the leaf and stem of ginseng (12.5) + scopolamine (0.5) ; △ : crude ginsenoside from the leaf and stem of ginseng (50) + scopolamine (0.5) (mg/kg)

Discussion

The present study demonstrated that crude saponin extracted from the leaf and stem of *Panax ginseng*, as well as those extracted from the ginseng root, significantly improved scopolamine - induced working memory deficit in the T-maze delayed alternation task and in the three-panel runway task in rats. The T-maze task is known to be operationally analogous to the delayed matching or non-matching to sample tasks used in human dementia[9] and to be useful to evaluate the spatial working memory in rats.[10] We previously reported that the aqueous extract from *Panax ginseng* root improved the spatial working memory deficit.[5] The present results agree with our previous data and indicate that although the leaf and stem of *Panax ginseng* have not been used for medicinal purposes, they contains saponins improving the impairment of working memory in rats.

In the T-maze task, the minimum effective dose of crude saponin extracted from *Panax ginseng* root was 50 mg/kg (*p.o.*), while that of crude ginsenoside from the leaf and stem of ginseng was 12.5 mg/kg (*p.o.*), suggesting that the crude saponin from the leaf and stem of ginseng more effectively improves scopolamine-induced memory deficit than that from *Panax ginseng* root. Soldati and Sticher[4] demonstrated that the ginsenosides (yield: 5.188%) extracted from the leaf of *Panax ginseng* contains ginsenoside-Rb (0.731%), -Rc (0.736%), -Rd (1.113%), -Re (1.524 %) and -Rg (1.078%), and that the contents of ginsenoside-Re and -Rg in the crude saponin fraction of *Panax ginseng* leaves are about 10 and 3 times higher than those in the fraction of *Panax ginseng* root. Thus, taking into account the data that *Panax ginseng* root saponins, ginsenoside-Rg1 and -Rb1, improve acquisition or retrieval process in passive avoidance[1,11] and conditioned avoidance tests,[2] the more effective action of crude ginsenoside from the leaf and stem of ginseng may be due to the higher level of ginsenoside Rg and -Re existing in these parts of *Panax ginseng*. Nevertheless, further investigations are needed to clarify the exact component(s) implicated in the effect of ginsenosides from the leaf and stem of *Panax ginseng*.

In the three-panel runway task, the pattern of correct panel gate position was changed every session so that the rats had to perform according to the working memory available only in each session. Consistent with the data reported by Furukawa *et al.*,[12] scopolamine injection significantly disrupted working memory in this task and the effect of scopolamine was abolished by physostigmine, a cholinestrase inhibitor, indicating the involvement of cholinergic dysfunction in the performance impairment in this task. Thus, the finding that the crude saponin fraction extracted from the leaf and stem of *Panax ginseng* blocked the effect of scopolamine in a dose-dependent manner, indicates that this fraction improves the working memory deficit in the three-panel runway task in rats. Central cholinergic system may be involved in such beneficial effects of the saponin, but further investigations are needed to elucidate the exact mechanisms which underlie these effects.

In conclusion, the present results suggest that the crude saponin fraction extracted from the leaf and

stem of *Panax ginseng*, as well as the fraction from *Panax ginseng* root, possesses a beneficial effect on the working memory deficit in rats.

和文抄録

薬用人参葉茎部より抽出した粗サポニン分画のスコポラミン誘発作業記憶障害に対する影響をT-迷路遅延交替課題および3パネル走行課題を用いて検討した。スコポラミン（0.2 mg/kg, *i.p.*）はT-迷路課題における正反応率を低下させた。薬用人参の根から抽出した粗サポニン分画（12.5-50 mg/kg, *p.o.*）および葉茎部から抽出した粗サポニン分画（12.5-50 mg/kg, *p.o.*）は何れもT-迷路課題におけるスコポラミン誘発記憶障害を改善した。3パネル走行課題では，フィゾスチグミン（0.4 mg/kg, *i.p.*）と同様に薬用人参葉茎部から抽出した粗サポニン分画（12.5-50 mg/kg, *p.o.*）はスコポラミン（0.5 mg/kg, *i.p.*）で増加したエラー数を有意に減少させた。これらの成績は薬用人参葉茎部の粗サポニンは根部の粗サポニンと同様にラットにおける作業記憶障害に対し改善効果を有することを示唆する。

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