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Glycoside fraction of peony root improves the scopolamine-induced disruption of spatial cognition in rats

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Abstract

Effects of the water and less-polar fractions separated from the methanol extract of peony root on scopolamine (0.3 mg/kg, IP)-induced spatial working memory impairment were investigated using an eight-arm radial maze task in rats. The methanol extract of peony root dose-dependently attenuated the scopolamine-induced disruption of the choice accuracy. The water-soluble fraction of methanol extract, which contains glycosides, also significantly attenuated the scopolamine disruption, whereas the less-polar fraction of methanol extract failed to affect. Moreover, intraperitoneally injected paeoniflorin (0.1 mg/kg), a major component of this water soluble fraction, improved the scopolamine-induced impairment of the choice accuracy. These results support our previous findings that the aqueous extract of peony root ameliorates spatial working memory deficits in rats, and give a further evidence that such a beneficial effect is attributed mainly to the water-soluble glycosides such as paeoniflorin but not less-polar constituents of peony root extract.

Key words glycoside fraction, less-polar fraction, paeoniflorin, peony root, radial maze, scopolamine, working memory.

Abbreviations Fr., fraction; IP, intraperitoneal; PO, per os; ref., reference.

Introduction

Peony root (*Paeonia lactiflora* PALLAS) has been used as a component of traditional Kampo prescriptions for treatment of dementia. This herb is known to improve blood stagnancy, and to relax spasms of smooth and skeletal muscle. Recent studies using an eight-arm radial maze task[1,2] have demonstrated that the aqueous extract of peony root and its major constituent paeoniflorin dose-dependently improve working memory deficit produced by scopolamine. Working memory is analogous to recent memory in humans and this type of memory is known to be more severely impaired than remote memory in early stage of senile dementia.[3,4] Taken together, these findings strongly suggest the possible utility of peony root and its constituents in the treatment of dementia. However, whether such a beneficial effect of peony root is only derived from the aqueous fraction containing paeoniflorin and other glycosides still remains unclear. In the present study we investigated the effect of the glycosides-containing fraction on scopolamine-induced disruption of radial maze performance and compared it with that of the fraction containing less-polar compounds such as essential oil, benzoic acid, monoterpenes, *etc.*.[5,6]

Meterials and Methods

Animals : Male Wistar rats (Japan SLC, Shizuoka), weighing 290-390 g were used. Four or five rats were housed in a cage with free access to water in air-conditioned room. Housing conditions were thermos-

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tatically maintained at 23±1°C with 60 % humidity, in 12h light/dark cycle (light on : 0730-1930). Rats were maintained on a restricted feeding schedule designed to keep their body weight at about 85 % of the free-feeding level. All trials were performed between 09.00-14.00 h.

Apparatus : Each arm (50×12 cm) of the eight-arm radial maze extended from an octagonally shaped central hub (30 cm across). The platform was elevated 40 cm above the floor. Black Plexiglas cups (3 cm diameter, 1 cm deep), were placed at the end of each arm as receptacles for reinforcers (45 mg food pellet ; Bio-Serv, NJ, USA). Guillotine doors surrounded the hub.

Procedures : Animals were trained as detailed in our previous reports.[1,2,7] Briefly, one daily training trial was performed with rats using food reinforcement. The trial was judged to be completed when the rat had visited all 8 arms or had spent 10 min on the maze. Entry into an arm that the animal had not previously visited was recorded as a correct response and re-entry as an error. The number of correct responses before committing the first error (the number of initial correct responses) and the running time were used as the indices of radial maze performance. To calculate the running time, the total running time in each session was divided by the total number of choices. Only the rats that made no errors, or only one error at the eighth choice, for 5 consecutive days were used for drug tests. Since effects of scopolamine on the radial maze performance are known to vary depending on the level of training,[8] we employed this very high criterion to get a stable performance.

Drugs : Dried peony root (*Paeonia lactiflora* PALLAS) was purchased from Tochimoto Co. Ltd. (Osaka, Japan). To prepare fractions containing glycosides or less-polar substances, the dried herb was macerated with methanol and refluxed at 60-70°C for 1 hr. After filtration, the filtrate was evaporated to yield methanol extract (Fr. A). The extract was suspended in excess water, and then extracted with chloroform. The chloroform-soluble layer and water-soluble layer were separated and evaporated to yield Fr. AI and AII, respectively. Paeoniflorin was further purified from the fraction AII according to the method described by Shibata *et al.*.[9] Yields of fractions A, AI, AII and paeoniflorin were 25.6, 0.6, 25 and 1 % of dried herb weight, respectively. Fractions A and AII were dissolved in distilled water and Fr. AI suspended in 0.5 % CMC solution just before the experiments. Each fraction obtained from peony root extract was orally (PO) administered 90 min before testing. Doses of each fraction were expressed as dried herb weight per kg body weight. Scopolamine HBr (Nacalai Tesque, Inc., Kyoto, Japan) dissolved in physiological saline was intraperitoneally (IP) injected 30 min before testing. Paeoniflorin was dissolved in physiological saline and IP injected immediately before scopolamine. Drugs were tested in a counterbalanced order to exclude the possible involvement of order effects. Animals treated with scopolamine alone were always present in any pharmacological trials.

Statistical Analysis : Data were analyzed with the Kruskal-Wallis analysis of variance followed by the Mann-Whitney U-test for multiple comparison between groups. Differences with $p<0.05$ were considered statistically significant.

Results

As summarized in Fig 1, 0.3 mg/kg scopolamine (IP) significantly decreased the initial correct responses in the radial maze performance in rats. However, pretreatment of animals with 0.04-0.25 g/kg Fr. A, a methanol extract of peony root, dose-dependently attenuated the scopolamine-induced disruption of the choice accuracy. The minimal effective dose of this fraction (0.1 g/kg, PO) was smaller than that previously reported using the aqueous extract of peony root (0.25 g/kg, PO ; see ref. 2). Fr. AI, a less-polar fraction separated from Fr. A, failed to improve the disruption of the choice accuracy caused by 0.3 mg/kg scopolamine. In contrast to Fr. AI, pretreatment with Fr. AII (0.04 and 0.1 g/kg) also significantly and dose-dependently attenuated the scopolamine-induced disruption of the radial maze performance. The effective doses of Fr. AII were lower than those of Fr. A.

Intraperitoneal injection of paeoniflorin, a major constituent purified from the water-soluble glycoside fraction of peony root, significantly attenuated the scopolamine disruption of the choice accuracy. Paeoniflorin at 0.1 mg/kg (IP) reversed the initial

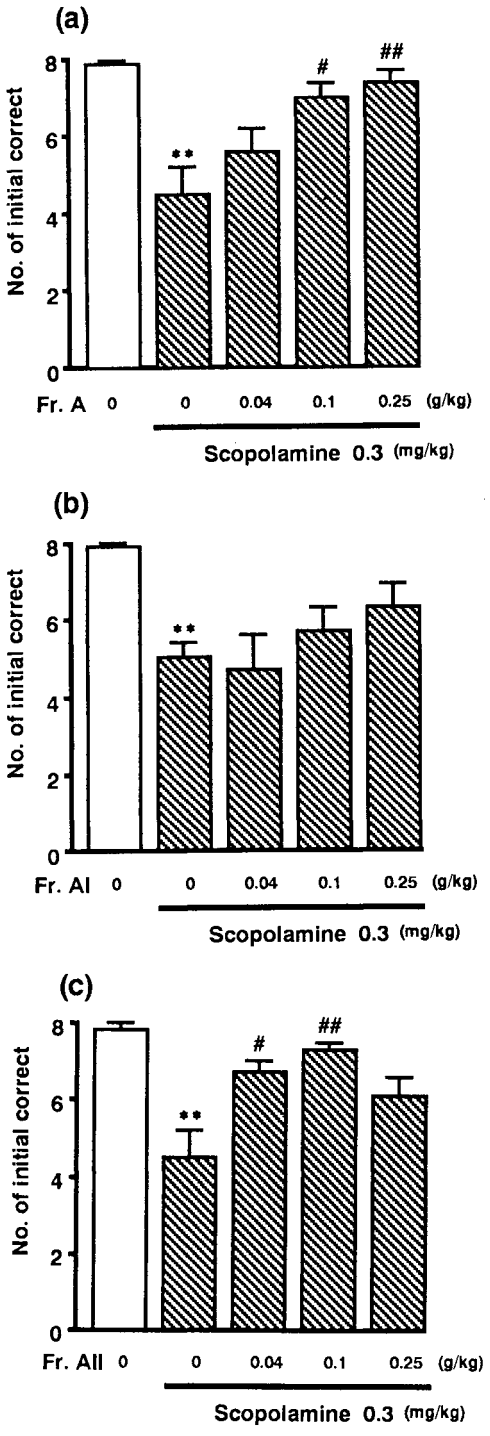

Fig. 1 Effects of Fr. A, AI and AII of methanol extract on scopolamine-induced disruption of radial maze performance. Saline or scopolamine was IP injected 30 min before testing. Fractions A (a), AI (b) and AII (c) were PO administered 60 min before scopolamine injection. Each datum represents the mean number of initial correct responses with the S.E.M. (n=7-8). $^{**}p<0.01$, compared with saline control. $\#p<0.05$, $\#\#p<0.01$ compared with scopolamine alone.

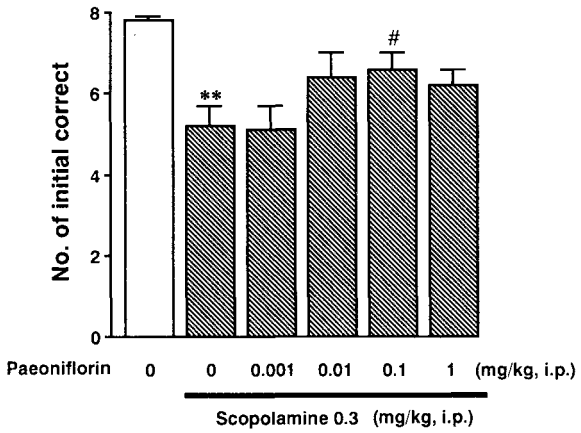

Fig. 2 Effect of paeoniflorin on scopolamine-induced disruption of radial maze performance. Scopolamine (0.3 mg/kg) was IP injected 30 min before testing. Paeoniflorin was IP injected immediately before scopolamine. Each datum represents the mean number of initial correct responses with the S.E.M. (n=10-13). $^{**}p<0.01$ compared with saline control. $\#p<0.05$ compared with scopolamine alone.

Table I Effects of fractions extracted from peony root on the running time prolonged by scopolamine.

Treatment (g/kg)	N	Running time (sec)
Exp. I		
saline	8	12.7± 1.6
scopolamine	8	31.5± 8.4*
+ Fr. A 0.04	7	32.6± 4.9
+ Fr. A 0.1	7	36.8± 8.6
+ Fr. A 0.25	7	22.5± 1.5
Exp. II		
saline	7	10.4± 1.0
scopolamine	7	32.6± 7.1**
+ Fr. AI 0.04	7	50.8± 9.3
+ Fr. AI 0.1	7	22.4± 2.3
+ Fr. AI 0.25	8	30.4± 4.8
Exp. III		
saline	8	15.1± 1.8
scopolamine	8	38.2±11.1*
+ Fr. AII 0.04	7	28.3± 3.2
+ Fr. AII 0.1	7	22.4± 3.1
+ Fr. AII 0.25	8	40.5±11.5
Exp. IV		
saline	13	11.0± 1.0
scopolamine	13	35.4±10.8**
+ paeoniflorin † 0.001	10	30.5± 5.1
+ paeoniflorin † 0.01	10	22.7± 2.6
+ paeoniflorin † 0.1	10	29.4± 3.1
+ paeoniflorin † 1.0	10	25.3± 2.8

Saline or scopolamine (0.3 mg/kg) was IP injected 30 min before testing.
Each fraction of peony root except paeoniflorin was PO administered 60 min before scopolamine. Paeoniflorin was IP injected immediately before scopolamine administration. $^{*}p<0.05$, $^{**}p<0.01$ compared with saline control. †: mg/kg

correct responses decreased by 0.3 mg / kg scopolamine (Fig. 2).

As summarized in Table I, scopolamine (0.3 mg/kg, IP) significantly prolonged the running time. However, Fr. A, AI, AII or paeoniflorin did not affect the running time prolonged by scopolamine.

Discussion

The present results confirmed our previous data that the aqueous extract of peony root ameliorates spatial working memory deficits in rats,[2] and also give a further evidence that such a beneficial effect is attributed mainly to the water- but not less-polar-fraction of peony root extract. In the present study, the methanol extract of peony root at doses of 0.1 and 0.25 g/kg dose-dependently attenuated spatial working memory deficit caused by scopolamine, but the effective doses of the extract were lower than those of the aqueous extract (0.25-1 g of peony root/kg ; see ref. 2). This discrepancy between present and previous data may be explained by difference in the efficiency of the solvent used to extract constituents from peony root, and/or the difference in the level of less-polar substances which may be involved in amelioration of spatial working memory deficits. However, the latter possibility does not seem to be the case, since further purification from the methanol extract demonstrated that a beneficial effect of the methanol extract of peony root on spatial cognition was attributed mainly to the water-soluble glycoside fraction (Fr. AII) but not less-polar fraction (Fr. AI).

None of the fractions significantly affected the running time prolonged by scopolamine. These results are consistent well with our previous observation[1, 2, 10] and suggest that both Fr. A and Fr. AII may antagonize the central but not peripheral effect of scopolamine, since methylscopolamine, a peripherally acting anticholinergic drug, can also prolong the running time without affecting the choice accuracy in the radial maze task.

The water - soluble fraction of peony root is known to be rich in glycosides such as paeoniflorin,[11] and of these glycosides, paeoniflorin is the main constituent and accounts for about 2-3% of the dried root weight.[12, 13] Previous experiments in this laboratory demonstrated that orally administered paeoniflorin attenuates spatial working memory deficit produced by scopolamine.[2] In the present study we found that intraperitoneal injection of paeoniflorin also improved the scopolamine disruption of the choice accuracy without affecting the running time. These observations suggest that this glycoside is one of the potent active constituents of peony root extract. This idea seems to be further supported by the fact that the water - soluble glycoside fraction played important roles in beneficial effects of peony root extract. Moreover, the present finding is of interest in terms of bioavailability of paeoniflorin, since although paeoniflorin is reportedly metabolized by intestinal bacteria in human[14, 15] there is no information available on its bioavailability in rats. The finding that the effective dose of intraperitoneal paeoniflorin (0.1 mg/kg) was almost the same as that of oral paeoniflorin[2] raises a possibility that paeoniflorin metabolism by intestinal bacteria is not always essential to produce the ameliorating effect on working memory deficit in rats.

Although the water - soluble glycoside fraction and its major constituent paeoniflorin improved the scopolamine-induced disruption of radial maze performance, the present results do not exclude a possibility that other minor constituents such as albiflorin, oxypaeoniflorin and benzoylpaeoniflorin in the fraction also contribute to the beneficial effects of peony root extract on deficits in the spatial working memory. Therefore, investigation of the effect of other minor glycosides on spatial learning deficits will be very interesting and useful to develop new compounds effective for the treatment of dementia. Such experiments are currently in progress in this laboratory.

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和文抄録

芍薬メタノール抽出エキスより分離して得られる水溶性分画及び低極性分画のスコポラミン誘発作業記憶障害に対する効果を8方向放射状迷路課題を用いて検討し

た。芍薬メタノールエキスは用量依存的にスコポラミン誘発空間認知障害を改善した。配糖体を含有する水溶性分画もメタノールエキスと同様に空間認知障害を改善したが，メタノールエキスの低極性分画は無効であった。更に，水溶性分画の主要成分であるペオニフロリン (0.1 mg/kg) の腹腔内投与はスコポラミン誘発空間認知障害を改善した。これらの結果は，芍薬の水抽出エキスが放射状迷路課題におけるラットの作業記憶障害を改善することを報告した我々の先の知見を支持するとともに，このような芍薬の効果に主にペオニフロリンをはじめとする配糖体が寄与することを示す。

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