# Effects of paeoniflorin and paeoniflorin-related glycosides on scopolamine-induced disruption of radial maze performance in rats

Ko-hei AKAZAWA,[a] Kinzo MATSUMOTO,[a] Mineo SHIMIZU,[b] Hiroyuki ICHIKI,[c] Masao MARUNO[c] and Hiroshi WATANABE*[a]

[a] *Department of Pharmacology, Research Institute for Wakan-Yaku,* [b] *Laboratory of Pharmacognosy, Department of Medicinal Resources, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University*
[c] *Central Research Laboratory, Kampo and Pharmacognosy Laboratory, Tsumura & Co.*

(*Received July 17, 1996. Accepted September 20, 1996.*)

## Abstract

Effects of glycoside constituents of peony root, paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin and albiflorin, on scopolamine-induced spatial cognitive impairment were investigated using an eight-arms radial maze task in rats. Paeoniflorin (0.01–0.1 mg/kg, *p.o.*), oxypaeoniflorin (0.01–0.1 mg/kg, *p.o.*) and benzoylpaeoniflorin (0.3–3 mg/kg, *p.o.*), constituents which have a cage-like skeleton with acetal and hemiketal structure, dose-dependently attenuated the scopolamine (0.3 mg/kg, *i.p.*)-induced decrease in the choice accuracy. The ameliorative effects of these constituents decreased at large doses. On the other hand, albiflorin (0.01–1 mg/kg, *p.o.*), which has a lactone ring in the pinane skeleton, had no effect on the maze performance disrupted by scopolamine. These results suggest that not only paeoniflorin but also oxypaeoniflorin and benzoylpaeoniflorin ameliorate spatial cognitive impairment, and that a pinane skeleton including acetal and hemiketal structure may play an important role in the ameliorating effect.

**Key words** paeoniflorin, benzoylpaeoniflorin, oxypaeoniflorin, albiflorin, spatial cognition, scopolamine.

## Introduction

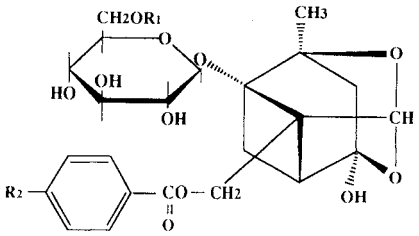
Peony root (*Paeonia lactiflora* PALLAS) has long been used to treat certain types of dementia as a component of traditional Chinese herbal prescriptions. The previous reports from this laboratory have demonstrated that peony root extract and the prescription Shimotsu-to (Si-Wu-Tang), which includes peony root as a component, improve spatial cognitive deficits caused by the muscarinic acetylcholine receptor antagonist scopolamine in rats,[1,2] and that peony root plays a dominant role in the ameliorative effect of Shimotsu-to on the spatial cognitive impairment.[1] Moreover, Nishi *et al.* have implicated the glycoside fraction of peony root in such a beneficial effect of peony root extract.[3] In fact, paeoniflorin, a major constituent of this glycoside fraction, has been shown to ameliorate not only spatial memory deficit caused by scopolamine but also learning impairment of aged rats in operant brightness discrimination task. These findings suggest that peony root may be useful in the treatment of dementia and age-related loss of cognitive function.

Other glycoside constituents such as oxypaeoniflorin, benzoylpaeoniflorin and albiflorin which have similar chemical structure to paeoniflorin have been isolated.[4] Oxypaeoniflorin and benzoylpaeoniflorin, as well as paeoniflorin, have a cage-like pinane skeleton including acetal and hemiketal structure, whereas albiflorin has a lactone ring in the pinane skeleton (Fig. 1). In the present study, to obtain preliminary

*〒930-01 富山市杉谷2630
富山医科薬科大学和漢薬研究所 生物試験部門 渡辺裕司
2630 Sugitani, Toyama 930-01, Japan

和漢医薬学雑誌 13, 243–247, 1996

| | R1 | R2 |
|---|---|---|
| paeoniflorin | H | H |
| oxypaeoniflorin | H | OH |
| benzoylpaeoniflorin | $COC_6H_5$ | H |



albiflorin

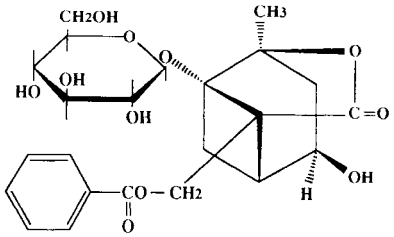


Fig. 1 Chemical structures of peony glycosides, paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin and albiflorin

information on whether paeoniflorin and paeoniflorin-related glycosides may be available as a lead compound for the treatment of senile dementia, we investigated the effect of paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin and albiflorin on the scopolamine-induced impairment of radial maze performance in rats. We used tacrine, a potent cholinesterase inhibitor, as a reference drug, since this drug reportedly exhibits a beneficial effect on Alzheimer type senile dementia.[5]

## Materials and Methods

*Animals* : Male Wistar rats (Japan SLC, Shizuoka), weighing 290-390 g were used. Animals were housed in groups of 4 per cage with free access to water. Housing conditions were thermostatically maintained at 23 ± 1°C with a constant humidity (60 %) and 12 h light/dark cycle (lights on : 07:30-19:30). 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 09:00-18:00.

*Apparatus* : An eight arm radial maze was used. Each arm (50 cm × 12 cm) 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* : The experimental procedures are the same as those described in previous reports.[1,6-8] Briefly, prior to the maze training, each animal was handled for 5-10 min daily for 2 days and was also given 3 days to adapt to the maze. Following the adaptation period, one daily training trial was conducted for each rat. The trial was judged complete when the rats had visited all 8 arms or had spent 10 min in the maze. Entry into an arm that the rat 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 (No. of initial correct responses) was used as the index of radial maze performance. Only the rats that made no errors, or only one error at the eighth choices, for 5 consecutive days were used for drug tests.

*Drugs* : Scopolamine HBr (Nacalai tesque, Kyoto, Japan) was dissolved in saline and injected *i.p.* 30 min before the start of the experiments. Peony glycosides, paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin and albiflorin, were purified from the methanol extract of peony root according to the method described previously.[4] Paeoniflorin, oxypaeoniflorin and albiflorin were dissolved in distilled water and benzoylpaeoniflorin was suspended in 0.5 % carboxymethylcellulose Na (CMC). Oxypaeoniflorin and albiflorin were administered *p.o.* 30 min before scopolamine injection. Paeoniflorin and benzoylpaeoniflorin were administered *p.o.* 60 min before scopolamine. Tacrine, a reference drug, was also dissolved in distilled water and administered orally 30 min before scopolamine injection. These drug solutions were prepared just before starting the experiments.

*Statistics* : The effects of the drugs on the number of initial correct responses were analyzed by the Kruskal-Wallis test followed by the Mann-Whitney U-test for multiple comparisons. Differences with $p < 0.05$ were considered statistically significant.

## Results

As shown in Fig 2A, administration of scopolamine (0.3 mg/kg, *i.p.*) significantly decreased the number of initial correct responses in the radial maze performance. Pretreatment of animals with the reference drug tacrine significantly attenuated the scopolamine-induced decrease in the choice accuracy. Moreover, consistent with our previous data,[1,7,8] oral administration of paeoniflorin (0.1 mg/kg) attenuated the scopolamine-induced decrease in the choice accuracy (Fig. 2B). Oxypaeoniflorin (0.1 mg/kg, *p.o.*) and benzoylpaeoniflorin (3.0 mg/kg, *p.o.*) also exhibited antagonistic activities against the scopolamine-induced decrease in the choice accuracy (Fig. 3). In contrast, albiflorin (0.01-0.1 mg/kg, *p.o.*) did not sig-

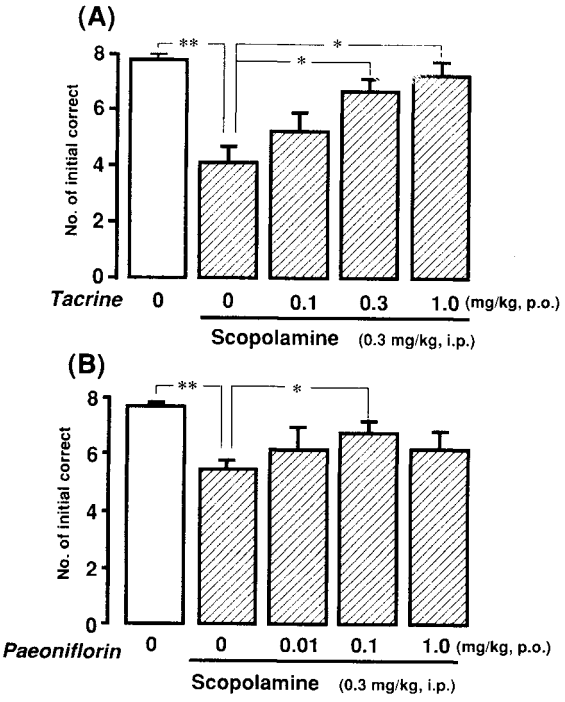



Fig. 2 Effect of tacrine and paeoniflorin on scopolamine-induced disruption of radial maze performance. Saline (n=9) or scopolamine was intraperitoneally injected 30 min before the start of experiments. (A) Tacrine [0 (n=9), 0.1 (n=9), 0.3 (n=9) or 1.0 mg/kg (n=4)] was orally administered 30 min prior to scopolamine injection. (B) Paeoniflorin [0 (n=12), 0.01 (n=7), 0.1 (n=11) or 1.0 mg/kg (n=11)] was orally administered 60 min prior to scopolamine injection. $^{**}p<0.01$ and $^{*}p<0.05$.

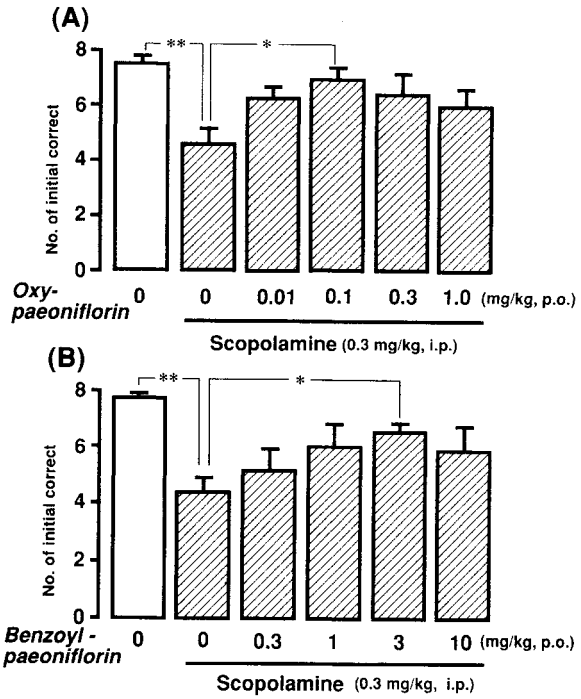



Fig. 3 Effect of oxypaeoniflorin and benzoylpaeoniflorin on scopolamine-induced distuption of radial maze performance. Saline (n=13) or scopolamine was intraperitoneally injected 30 min before the start of experiments. (A) Oxypaeoniflorin [0 (n=13), 0.01 (n=10), 0.1 (n=9), 0.3 (n=9) or 1.0 mg/kg (n=9)] was orally administered 30 min prior to scopolamine injection. (B) Benzoylpaeoniflorin [0 (n=14), 0.3 (n=7), 1.0 (n=7), 3.0 (n=11) or 10.0 mg/kg (n=8)] was orally administered 60 min prior to scopolamine injection. $^{**}p<0.01$ and $^{*}p<0.05$.

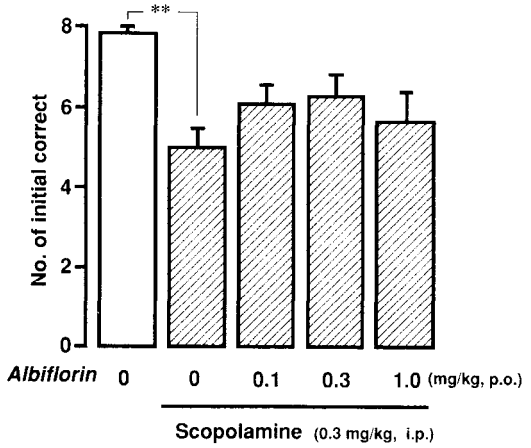



Fig. 4 Effect of albiflorin on scopolamine-induced disruption of radial maze performance. Saline (n=7) or scopolamine was intraperitoneally injected 30 min before testing. Albiflorin [0 (n=10), 0.1 (n=10), 0.3 (n=10) or 1.0 mg/kg (n=9)] was orally administered 30 min prior to scopolamine injection. $^{**}p<0.01$.

nificantly improve the disruption of the radial maze performance caused by 0.3 mg/kg scopolamine (Fig. 4).

## Discussion

The previous reports from this laboratory have indicated that the glycoside fraction of peony root and its major constituent paeoniflorin dose-dependently attenuate spatial working memory impairment caused by scopolamine in the radial maze task of rats.[1,3] In the present study, we confirmed such an ameliorative effect of paeoniflorin using the same task, and found that benzoylpaeoniflorin, oxypaeoniflorin and the reference drug tacrine, as well as paeoniflorin, exhibited ameliorative effects on the scopolamine-induced disruption of radial maze performance, while albiflorin failed to reverse the effect of scopolamine.

The effective dose of oxypaeoniflorin to improve the disrupted radial maze performance was almost the same as that of paeoniflorin. Taking into account the difference in the chemical structure between these two compounds, the present results indicate that the hydroxy group at the R2-position of paeoniflorin structure does not play an important role in the ameliorative effect of oxypaeoniflorin. Benzoylpaeoniflorin also antagonized the decrease in the choice accuracy caused by scopolamine but it required about 30-fold larger doses than paeoniflorin and oxypaeoniflorin for exhibiting an ameliorative effect on the maze performance. Although very little information is available on the pharmacokinetics of glycoside constituents of peony root, it could be speculated that the benzoyl group at the R1 position of benzoylpaeoniflorin may decrease the affinity of this compound for the site of action of paeoniflorin.

It is of interest to note that albiflorin had no effect on the scopolamine-induced impairment of spatial cognition in rats. Paeoniflorin homologs have a cage-like pinane skeleton including acetal and hemiketal structure in their molecules, whereas albiflorin has a lactone ring in the pinane skeleton (Fig. 1). Sugaya *et al.*[9] have demonstrated that albiflorin exhibits anticonvulsant activity at smaller doses than paeoniflorin, and suggested that albiflorin is the important component in the anticonvulsant action of the peony root. This difference in the pharmacological activity of albiflorin between their and out studies remains unclear but it may be due to difference in the experimental models used to elucidate the effect of this compound. Taking into account the difference in the chemical structure between paeoniflorin and albiflorin, the pinane skeleton including acetal and hemiketal structure may be essential to the ameliorative effect of paeoniflorin on the spatial cognitive impairment caused by scopolamine. Nevertheless, to clarify the structure-activity relationship of glycoside constituents of peony root require further investigation.

Recent evidence suggests that the peripheral system partly contributes to the memory-improving effects of several drugs.[10-12] In fact, our previous reports demonstrate that the centrally and peripherally acting $\beta$-antagonist propranolol and the peripherally acting $\beta$1-antagonist atenolol augment the scopolamine-induced deficit in radial maze performance in rats, suggesting the important role of the interaction between central cholinergic and peripheral $\beta$-adrenergic systems in the radial maze performance.[6] Moreover, not only $\alpha$1- but also $\beta$-adrenoceptors, especially peripheral $\beta$-adrenoceptors, appear to be involved in the antagonistic effect of paeoniflorin on this scopolamine-induced deficit.[7,8] Thus, it is very likely that the ameliorative effects of oxypaeoniflorin and benzoylpaeoniflorin, are also mediated by these receptor mechanisms.

In conclusion, the present results suggest that the cage-like pinane skeleton including acetal and hemiketal structure plays an important role in the antagonistic effects of paeoniflorin on the scopolamine-induced disruption of radial maze performance, and that paeoniflorin may be useful as a lead compound to develop a new anti-amnesic drug.

## Acknowledgment

This study was supported in part by the Proposal-Based Advanced Industrial Technology R&D Program from the New Energy and Industrial Technology Development.

## 和文抄録

スコポラミンで誘発したラットの空間認知障害に対する芍薬中の配糖体成分の影響について，8方向放射状迷路課題を用いて検討した。Pinane 骨格上にアセタールおよびヘミケタール構造をもつペオニフロリン (0.01-0.1 mg/kg, *p.o.*)，オキシペオニフロリン (0.01-0.1 mg/kg, *p.o.*)，およびベンゾイルペオニフロリン (0.3-3 mg/kg, *p.o.*) は用量依存的にスコポラミン誘発の迷路行動障害に拮抗したが，高用量では拮抗作用の減弱が認められた。一方，Pinane 骨格にラクトン環を有するアルビフロリンは (0.01-0.1 mg/kg, *p.o.*) はこの迷路行動障害に影響を与えなかった。以上の成績はペオニフロリンのみならず，オキシペオニフロリンおよびベンゾイルペオニフロリンにも空間認知障害改善作用があること，およびこれらの成分中のアセタールおよびヘミケタール構造を有する "cage-like" pinane 骨格が改善効果発現に重要であることが示唆された。

## References


1) Ohta, H., Ni, J.-W., Matsumoto, K., Watanabe, H., Shimizu, M.: Peony and it major constituent, paeoniflorin, improve radial maze performance impaired by scopolamine in rats. *Pharmacol. Biochem. Behav.* **45**, 719-723, 1993.

2) Watanabe, H., Ni, J.-W., Ohta, H., Ni, X.-H., Matsumoto, K.: A Kampo prescription, Shimotsu-to, improves scopolamine-induced spatial cognitive deficits in rats. *Jpn. J. Psychopharmacol.* **11**, 1991.

3) Nishi, K., Matsumoto, K., Ohta, H., Shimizu, M., Watanabe, H.: Glycoside fraction of peony root improves the scopolamine-induced disruption of spatial cognition in rats. *J. Trad Med.* **11**, 118-122, 1994.

4) Kitagawa, I., Yoshikawa, M., Tsunaga, K., Tani, T.: Studies on Moutan Cortex (II) on the chemical constituents. *Shoyakugaku Zasshi* **33**, 171-177 (abstract in English), 1979.

5) Summers, W.K., Majovski, L.V., Marsh, G.M., Tachiki, K., Kling, A.: Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. *New Eng. J. Med.* **315**, 1241-1245, 1986.

6) Ohta, H., Matsumoto, K., Watanabe, H.: The interaction between central cholinergic and peripheral $\beta$-adrenergic systems on radial maze performance in rats. *Brain Res.* **622**, 353-356, 1993.

7) Ohta, H., Matsumoto, K., Watanabe, H., Shimizu, M.: Involvement of $\beta$-adrenergic systems in the antagonizing effect of paeoniflorin on the scopolamine-induced deficit in radial maze performance in rats. *Jpn. J. Pharmacol.* **62**, 345-349, 1993.

8) Ohta, H., Matsumoto, K., Watanabe, H., Shimizu, M.: Involvement of $\alpha$1- but not $\alpha$2-adrenergic systems in the antagonizing effect of paeoniflorin on scopolamine-induced deficit in radial maze performance in rats. *Jpn. J. Pharmacol.* **62**, 199-202, 1993.

9) Sugaya, A., Suzuki, T., Sugaya, W., Yuyama, N., Yasuda, K., Tsuda, T.: Inhibitory effect of peony root extract on pentylenetetrazol-induced EEG power spectrum changes and extracellular calcium concentration changes in rat cerebral cortex. *J. Ethnopharmacol.* **33**, 159-167, 1991.

10) Mondadori, C., Petschke, F.: Do piracetam-like compounds act centrally via peripheral mechanisms? *Brain Res.* **435**, 310-314, 1987.

11) Schindler, A.M., Kapp, B.S., Sherman, W.M.: The effects of centrally and peripherally acting cholinergic drugs on the short-term performance gradient following passive avoidance training. *Psychopharmacologia* **18**, 77-81, 1970.

12) Wenk, G.L.: An hypothesis on the role of glucose in the mechanism of action of cognitive enhancers. *Psychopharmacology* **99**, 431-438, 1989.