J. Toxicol. Pub. Health
Vol. 17, Supplement, pp. 257-261, 2001

Anti-dementia Effects of Gouteng-san and Si-Wu-Tang

Hiroshi Watanabe*

Department of Pharmacology, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama 930-0194, Japan

ABSTRACT: *Recently, a traditional medicine called Gouteng-san, which consists of eleven herbs, was reported to be effective in treating vascular dementia with a double-blind, placebo-controlled study. Gouteng-san is also used for patients with vascular dementia in combination with Si-Wu-Tang. The effect of Gouteng-san and Si-Wu-Tang on deficit of learning behavior was investigated using step-down passive avoidance task in mice. Hot-water extract of Gouteng-san (1.5 and 6 g/kg, p.o.) significantly prolonged the step-down latency shortened by scopolamine. The extract of Uncaria hook (150 mg/kg, p.o.), one of the component herb of Gouteng-san, significantly prevented the decrease in the latency after scopolamine. Hot-water extract of Si-Wu-Tang (1.5 and 6 g/kg of dried herbs, p.o.) prevented dose-dependently scopolamine-induced disruption of learning behavior. Si-Wu-Tang also prevented the ischemia-induced deficit of learning behavior. Both hot water extract of peony and angelica (1.5 g/kg, p.o.), which are component herbs of Si-Wu-Tang, prevented the scopolamine-induced learning behavior deficit. Scopolamine (10 uM) suppressed long-term potentiation (LTP) of population spike in the CA1 region of the rat hippocampal slices. Peoniflorin (0.1~1 uM) extracted from paeony root significantly ameliorated scopolamine-induced inhibition of LTP. These results suggest that improvement of deficit of learning behavior by Gouteng-san and Si-Wu-Tang is mediated by direct and/or indirect activation of the cholinergic system in the brain.*

Key Words : *Traditional medicine, Gouteng-san, Si-Wu-Tang, Dementia, Learning, Memory*

I. INTRODUCTION

In Japan, there are many reports which showed clinical efficacy of Japanese traditional medicines, which is the same as the traditional Chinese medicine, for the treatment of pateints with senile dementia, especially vascular dementia. Recently, one of the traditional medicines Gouteng-san, which consists of eleven medicinal herbs, was reported to be effective for the treatment of vascular dementia in multi-center, double-blind and placebo-controlled study (Terasawa *et al.*, 1997). Results of the clinical study showed that Gouteng-san was statistically superior to the placebo in global improvement rating and of psychiatric symptoms. There was a tendency of the improvement of subjective symptoms and of disturbance in the daily living activity. These results indicate that Gouteng-san is very useful medicine in the treatment of patients with vascular dementia.

Si-Wu-Tang is a basic traditional medicine, which has been used to treat menstrual problems, anemia and autonomic imbalances in women for several centuries in China and Japan. It is frequently applied to many diseases in combination with other traditional medicines. Recently, Si-Wu-Tang in combination with Gouteng-san was used for the treatment of senile dementia (Narita, 1991).

To demonstrate the clinical efficacy of those traditional medicines and clarify the action mechanisms, we investigated the effect of Gouteng-san and Si-Wu-Tang on scopolamine-induced amnesia in the conditioned passive avoidance task in mice.

II. MATERIALS AND METHODS

1. Animals

Male ICR mice (4 weeks old, Nippon SLC, Hamamatsu, Japan) were Used. The animals were housed in a controlled environment (25±1°C, 60±5% humidity) with a 12 h hight/dark cycle (lights on at 7:30~19:30) for at least one week before the experiment. All experiments were performed between 09:00~18:00. Food and water were given ad libitum.

*To whom correspondence should be addressed

2. Traditional medicines and agents

Uncaria hook, Citrus, Pinellia, Ophiopogon, Poria, Ginseng, Chrysanthemum, Lebedebouriella, Gypsum, Glycyrrhiza, Zingiber were mixed for Gouteng-san and Cnidium rhizome, angelica root, paeonia root and rehmannia root were mixed and extracted with hot water for 1 hr. Hot water extract of traditional medicine was freeze-dried and kept in the dissicator. The powder of the extract was dissolved in physiological saline and given orally to animals 60 min before the learning trial. Tacrine, a reference drug, was also dissolved in distilled water and administered orally 60 min before the learning trial. Scopolamine dissolved in physiological saline (1.0 mg/kg, i.p.) was injected 15 min prior to the learning trial. The solution of the agents and traditional medicines was prepared just before starting the experiments.

3. Apparatus

Learning and memory were examined using the step-down passive avoidance task in mice. The apparatus consisted of a Plexiglas box (30×30×40 cm high) with a grid floor made to 25 parallel steel rods (0.3 cm in diameter set 1 cm apart. A rubber platform (4×4×4 cm) was fixed at the center of the grid floor. The cage was illuminated with 15 W lamp during the experimental period. In the training period, each mouse was placed gently onto the rubber platform, when the animal stepped down from the platform and placed all its paws on the grid floor, an intermittent electroshock (0.4 mA, 4 s) was delivered continuously by an isolated stimulator. Twenty-four hours after training, the mouse was again placed on the platform and the time the animal took to step down onto the grid floor was recorded as the step-down latency. Upper cut-off time was set at 300 s. Number of times which the mouse stepped down the grid floor and received electric shocks errors for 5 min were recorded as the number of errors.

Spontaneous motor activity was measured using the apparatus, which consists of rectangular cage with infrared-beam and the detectors, for measuring the number of movements, vertical activity, and total distance for every ten minutes (Scanet SV-10, Toyo Sangyo, Toyama).

4. LTP measurement

The preparation of hippocampal slices from rat brain and the recording of population spikes from the slices were performed according to the method as previously reported (Tabata *et al.*, 2000). Briefly, Male Wistar rats were decapitated under anesthesia, the brains were rapidly removed, the left hippocampus was dissected and slices were prepared using a microslicer and incumbated in artificial cerebrospinal fluid. A single slice was transfered to a perfusion chamber and was perfused with artificial cerebrospinal fluid. A glass capillary filled with 2 M NaCl was placed in the pyramidal cell layer of the CA1 region. Electrical stimuli consisting of 100 microsecond square pulses were delivered via bipolar electrodes to the Schaffere-commissural fiber system (0.033 Hz). LTP was induced by delivering tetanus stimuli at 10 min after the start of drug application.

5. Staistics

The effects of the drugs on the latency were analyzed by the Kruskal-Wallis-test followed by the Mann-Whitney U-test for multiple comparisons. LTP was analyzed by the Newman-Keuls multiple comparison test. Differences with $P<0.05$ were considered statistically significant.

III. RESULTS

1. Effect of Gouteng-san and Si-Wu-Tang on scopolamine-induced learning deficit and spontaneous motor activity

Hot-water extract of Gouteng-san (0.75~6 g/kg, p.o.) significantly prolonged the step-down latency shortened by scopolamine (1.0 mg/kg, i.p.) in a dose-dependent manner (Fig. 1). The extract of Uncaria hook (150 and 600 mg/kg, p.o.), one of the component herb of Gouteng-san, significantly prevented the decrease in the latency after scopolamine (Fig. 2). Hot-water extract of Si-Wu-Tang (1.5 and 6 g/kg of dried herbs, p.o.) prevented dose-dependently scopolamine-induced decrease in step-down latency (Fig. 3). Both hot water extracts of peony root and of angelica root (1.5 g/kg each, p.o.), which are component herbs of Si-Wu-

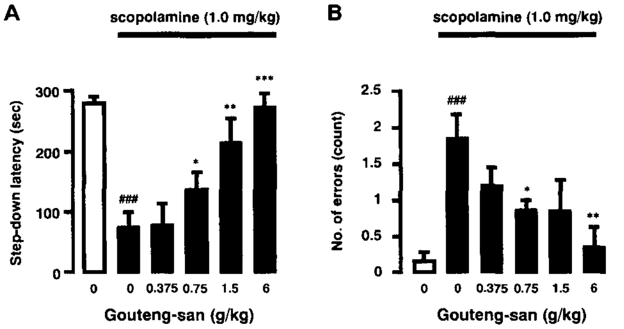

Fig. 1. Effect of Goputeng-san on learning behaviour deficit induced by scopolamine in mice. Gouteng-san and scopolamine were administered 60 min and 15 min prior to learning trial, respectively. A: step-down latency, B: number of errors. ###P<0.001 vs. normal saline, *P<0.05, **P<0.01, ***P<0.001 vs. scopolamine.

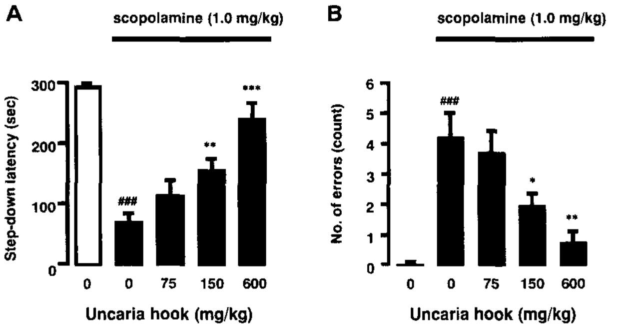

Fig. 2. Effect of Uncaria hook on learning behaviour deficit induced by scopolamine in mice. Uncaria hook and scopolamine were administered 60 min and 15 min prior to learning trial, respectively. A: step-down latency, B: number of errors. ###P<0.001 vs. normal saline, *P<0.05, **P< 0.01, ***P<0.001 vs. scopolamine.

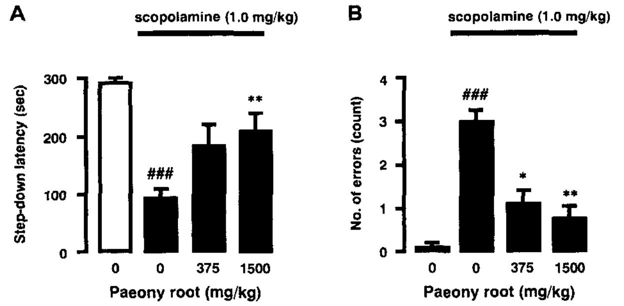

Fig. 4. Effect of paeony root on learning behaviour deficit induced by scopolamine in mice. Paeony root and scopolamine were administered 60 min and 15 min prior to learning trial, respectively. A: step-down latency, B: number of errors. ###P<0.001 vs. normal saline, *P<0.05, **P<0.01 vs. scopolamine.

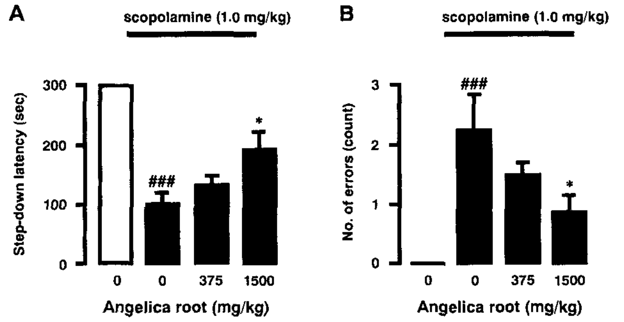

Fig. 5. Effect of angelica root on learning behaviour deficit induced by scopolamine in mice. Angelica root and scopolamine were administered 60 min and 15 min prior to learning trial, respectively. A: step-down latency, B: number of errors. ###P<0.001 vs. normal saline, *P<0.05 vs. scopolamine.

Tang, prevented the shortening of step-down latency induced by scopolamine (Figs. 4, 5). While the extracts of other component herbs of Si-Wu-Tang, cnidium rhizome and rehmannia root, did not affect the step-down latency. A reference drug, tacrine (2.5 mg/kg, p.o.), reversed the shortened step-down latency after scopolamine (Fig. 6). Number of errors in the passive avoidance test showed the similar tendency to the

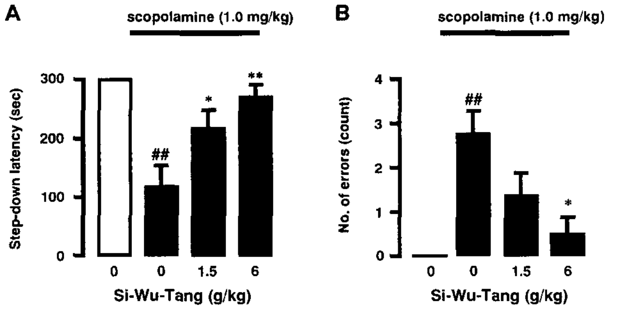

Fig. 3. Effect of Si-Wu-Tang on learning behaviour deficit induced by scopolamine in mice. Si-Wu-Tang and scopolamine were administered 60 min and 15 min prior to learning trial, respectively. A: step-down latency, B: number of errors. ##P<0.001 vs. normal saline, *P<0.05, **P<0.01 vs. scopolamine.

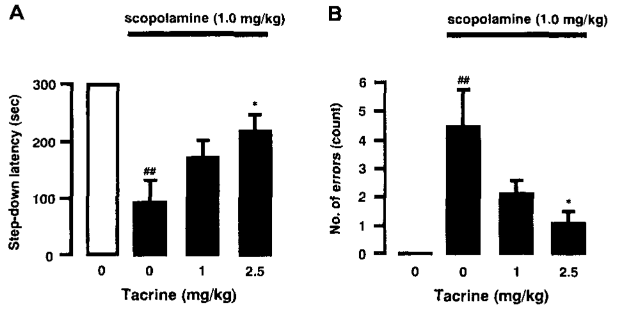

Fig. 6. Effect of tacrine on learning behaviour deficit induced by scopolamine in mice. Tacrine and scopolamine were administered 60 min and 15 min prior to learning trial, respectively. A: step-down latency, B: number of errors. ##P< 0.01 vs. normal saline, *P<0.05 vs. scopolamine.

step down latency in response to various drugs. Scopolamine produced a significant increase in the locomotor activity, whereas Gouteng-san and Si-Wu-Tang caused no change in the motor activity. Both traditional medicines did not affect scopolamine-induced increase in the motor activity.

2. Effect of paeoniflorin on LTP in the rat hippocampal slice

Bath application of scopolamine at a concentration of 10 uM significantly suppressed LTP of population spike in the CA1 region of the rat hippocampal slices. Peoniflorin (0.1~1 uM), major constituent of paeony root, did not affect LTP induction, whereas it significantly reversed suppressive effects of scopolamine (Fig. 7).

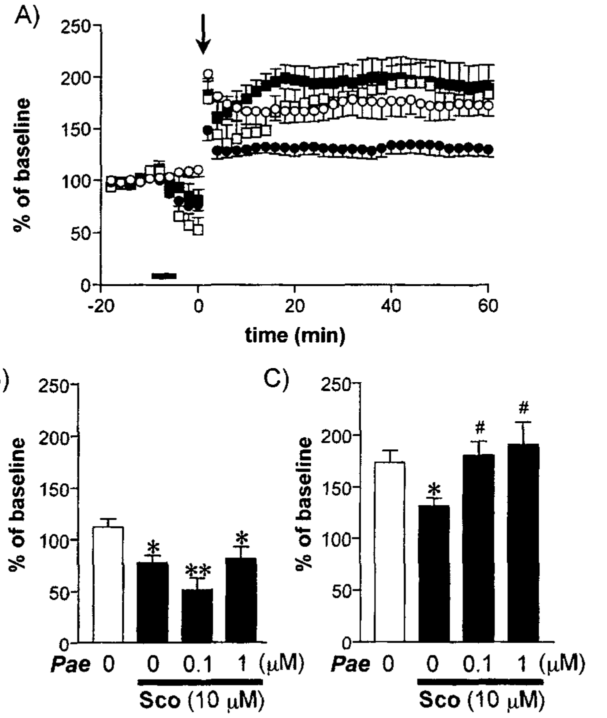

Fig. 7. Effects of paeoniflorin on scopolamine-induced suppression of LTP in the hippocampal CA1 region. A) A tetanic stimulation (100 Hz, 1 s) was delivered at the time (0 min) indicated by an arrow, and drugs dissolved artificial cerebrospinal fluid were perfused for 10 min before and during tetanic stimulation, as indicated y a solid bar. Each point is the mean±SEM of population spike amplitude expressed as a percentage of baseline value before drug application (n=8; ○, control; ●, 10 μM scopolamine; □, 10 μM scopolamine + 0.1 μM paeoniflorin; ■, 10 uM scopolamine + 0.1 uM paeoniflorin). B and C) Effect of co-application of paeoniflorin and scopolamine on the magnitude of basal population spike and LTP. In B, the population spike amplitudes recorded immediately before tetanic stimulation were averaged and compared in the presence and absence of test drugs (scopolamine: Sco, paeoniflorin: Pae). In C, the population spike amplitudes recorded from 50 to 60 min after tetanic stimulation were averaged and compared in the presence and absence of test drugs. *P<0.05, **P<0.01 compared to control, #P<0.05 compared to scopolamine alone (the Newman-Keuls multiple comparison test).

IV. DISCUSSION

The present results on Gouteng-san and Si-Wu-Tang supports the clinical efficacy of the treatment for vascular dementia in multi-center, double-blind and placebo controlled study (Terasawa *et al.*, 1997). The extract of Uncaria hook, one of the component herb of Gouteng-san, prevented the scopolamine-induced amnesia as did Gouteng-san. Effects of other component herbs on experimental amnesia are not examined yet, whereas the present result indicates that Uncaria hook is at least one of the active component herbs in Gouteng-san. Since Uncaria hook contains many indole alkaloids, so further study on indole alkaloids and component herbs are in progress.

The present study clearly showed preventive effects of Si-Wu-Tang on scopolamine-induced learning deficit. This phenomenon may not be due to changes in the motor activity, but may be specific to scoplamine-treated animals, since Si-Wu-Tang affected neither spontaneous motor activity in naive animals nor the activity induced by scopolamine. We have already reported the beneficial effects of Si-Wu-Tang on scopolamine-induced impairment in the spatial cognitive deficit (Watanabe *et al.*, 1991). These results indicate the clinical efficacy of Si-Wu-Tang in the treatment of patients with vascular dementia.

To clarify which component herbs contribute to the improving effect of SWT, we examined the effect of each herb in the present study. Both peony root and angelica root significantly attenuated the scopolamine-induced disruption in the learning process, whereas neither cnidium rhizome nor rehmannia root did it. We did not examined the effect of paeoniflorin, a major component of the peony root, in the passive avoidance task, while it improved the scopolamine-induced impairment in the spatial cognitive deficit in the rat and learning impairment of aged rats in operant brightness discrimination task (Watanabe *et al.*, 1991; Ohta *et al.*, 1994a, b). These results suggested that paeoniflorin may be one of the active constituent in the paeony root.

To analyze the mechanism of action of paeoniflorin, we investigated the long-term potentiation (LTP) induced by tetanus stimulation of Schefer collateral in the CA1 region of the hippocampal slice, since LTP is a kind of in vitro model of the memory. In the present study, paeoniflorin antagonized the scopolamine in the LTP response. We have already shown that Si-Wu-Tang and paeoniflorin antagonize the scopolamine-induced decrease in the acetylcholine content in the striatum of the rat (Ohta *et al.*, 1993). These results indicated that paeoniflorin may be a candidate of the cognitive enhancer. However, we need further study on the action mechanisms of paeoniflorin, peony root and Si-Wu-Tang, and also of Gouteng-san.

REFERENCES

Narita, H. (1991): Kampo and Brain deseases (Araki, G. ed.) Iyaku Jouranl Co., Tokyo, pp. 16-24.

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

Ohta, H., Nishi, K., Matsumoto, K., Watanabe, H. and Shimizu, M. (1994a): Paeoniflorin improves learning deficit in 4-arm baited radial maze performance in rats with unilateral nucleus basalis magnocellularis lesion, *Phytomed.*, **1**, 117-121.

Ohta, H., Matsumoto, K., Shimizu, M. and Watanabe, H. (1994b): Paeoniflorin attenuates learning impariment of aged rats in operant brightness discrimination task, *Pharmacol. Biochem. Behau*, **49**, 213-217.

Tabata, K., Matsumoto, K. and Watanabe, H. (2000): Paeoniflorin, a major constituent of peony root, reverses muscarinic M1-receptor antagonist-induced suppression of long-term potentiation in the rat hippocampal slice, *Jpn. J. Pharmacol.*, **83**, 25-30.

Terasawa, K., Shimada, Y., Kita, T., Yamamoto, T., Tosa, H., Tanaka, N., Saito, Y., Kanaki, E., Goto, S., Mizushima, N., Fujioka, M., Takase, S., Seki, H., Kimura, I., Ogawa, T., Nakamura, S., Araki, G., Maruyama, I., Maruyama, Y. and Takaori, S. (1997): Choto-san in the treatment of vascular dementia: a double-blind, placebo controlled study, *Phytomed.*, **4**, 15-22.

Watanabe, H., Ni, J.-W., Ohta, H., Ni, X.-H. and Matsumoto, K. (1991): A Kampo prescription, Shimotsu-to, improves scopolamine-induced spatial cognition deficits in rats, *Jpn. J. Psycopharmacol.*, **11**, 215-222.