

# Cholinergic involvement in the improving effects of Yoku-kan-san-ka-chimpi-hange (Yi-Gan-San-Jia-Chen-Pi-Ban-Xia) on the disruption of spatial cognition and the electroconvulsive shock-induced immobilization

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(Received October 17, 2000. Accepted February 8, 2001.)

## Abstract

We investigated the effect of Yoku-kan-san-ka-chimpi-hange (Yi-Gan-San-Jia-Chen-Pi-Ban-Xia; 抑肝散加陳皮半夏) on an experimentally-induced disruption of spatial cognition using an eight arm radial maze task and the electroconvulsive shock (ECS)-induced immobilization in comparison with that of tetrahydroaminoacridine (THA) in rats. Yoku-kan-san-ka-chimpi-hange (10–100 mg/kg, *p.o.*) improved the scopolamine (0.5 mg/kg, *i.p.*) and the  $\Delta^9$ -tetrahydrocannabinol (THC; 6 mg/kg, *i.p.*)-induced disruption of spatial cognition in rats. THA (0.5–20 mg/kg, *p.o.*) also improved the scopolamine- and THC-induced disruption of spatial cognition in rats. Yoku-kan-san-ka-chimpi-hange (30–100 mg/kg) and THA (5 mg/kg) suppressed the ECS (90 mA, 0.2 s)-induced immobilization, which is a behavioral model of a disturbance of consciousness. On the other hand, Yoku-kan-san-ka-chimpi-hange had no effect on the immobility time in a rat forced swimming test, a model of depression in experimental animals. Furthermore, Yoku-kan-san-ka-chimpi-hange (10–100 mg/kg, *p.o.*) and THA (5–20 mg/kg, *p.o.*) enhanced oxotremorine-induced tremors in mice. These results suggest that Yoku-kan-san-ka-chimpi-hange may indirectly enhance the cholinergic neurons and also improve an experimentally-induced disruption of spatial cognition and consciousness.

**Key words** Yoku-kan-san-ka-chimpi-hange (Yi-Gan-San-Jia-Chen-Pi-Ban-Xia; 抑肝散加陳皮半夏), Electroconvulsive shock, Forced swimming test, Spatial cognition, Tetrahydroaminoacridine, Acetylcholine.

**Abbreviations** Yoku-kan-san-ka-chimpi-hange (Yi-Gan-San-Jia-Chen-Pi-Ban-Xia), 抑肝散加陳皮半夏; ACh, Acetylcholine; AD, Alzheimer's disease; DA, Dopamine; ECS, electroconvulsive shock; M, muscarinic; 5-HT, Serotonin; THC,  $\Delta^9$ -tetrahydrocannabinol; THA, tetrahydroaminoacridine.

## Introduction

Memory impairment is a cardinal symptom of Alzheimer's disease (AD) and is thought to be secondary, at least to some degree, to the central cholinergic

neuron pathology.<sup>1,2)</sup> Donepezil and tetrahydroaminoacridine (THA), cholinesterase inhibitors, have been shown to improve memory disorders in AD patients.<sup>3,4)</sup> From these facts, scopolamine, a non-selective muscarinic (M) receptor antagonist, has thus been used as a model for memory disturbance in

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experimental animals.<sup>5-7)</sup> Our previous report suggested that the scopolamine-induced disruption of spatial cognition was improved by the administration of not only cholinergic drugs but also of noradrenergic drugs in an 8-arm radial maze task.<sup>8,9)</sup> This finding suggested the involvement of multiple neurotransmitter systems on the spatial cognition. Furthermore, our recent study demonstrated a close relationship between the induction of disruption in spatial cognition and the degree of acetylcholine (ACh) release in the ventral hippocampus in scopolamine-treated rats, suggesting that scopolamine impairs spatial cognition by blocking not only the postsynaptic M<sub>1</sub> receptor but also by blocking the presynaptic M<sub>2</sub> receptor.<sup>10)</sup>

In addition, we reported that  $\Delta^9$ -tetrahydrocannabinol (THC), one of the major constituents of marihuana, also disrupted the spatial cognition in the 8-arm radial maze task.<sup>6,11)</sup> Sullivan suggested that the cognitive alterations induced by THC may be attributable to interference with the cholinergic and glutaminergic neurons through cannabinoid receptor in the hippocampus.<sup>12)</sup> On the other hand, our previous study showed the THC-induced disruption of spatial cognition to be improved by either cholinergic drugs or noradrenergic drugs.<sup>11)</sup> Furthermore, it was likely the THC-induced disruption of spatial cognition is thought to likely be associated with a dysfunction of the serotonergic neurons since small doses of serotonergic agents markedly improved the THC-induced disruption of spatial cognition. From these findings, the THC-induced disruption of spatial cognition was thought to involve not only the cholinergic neurons but also the monoamine neurons.

A traditional Kampo medicine, Yoku-kan-san-ka-chimpi-hange (Yi-Gan-San-Jia-Chen-Pi-Ban-Xia; 抑肝散加陳皮半夏), consisting of *Pinelliae tuber* (5 g), *Atractylodis lanceae rhizoma* (4 g), *Hoelen* (4 g), *Cnidii rhizoma* (3 g), *Aurantii nobilis pericarpium* (3 g), *Angelicae radix* (3 g), *Uncariae ramulus et uncus* (3 g), *Bupleuri radix* (2 g) and *Glycyrrhizae radix* (1.5 g) has been clinically used for the treatment of neurosis and insomnia. In experimental animals, Yoku-kan-san-ka-chimpi-hange has been reported to increase both the choline acetyltransferase activity and ACh contents.<sup>13,14)</sup> We also previously reported the ameliorative effects of Yoku-kan-san-ka-chimpi-

hange on the scopolamine- and THC-induced disruption of spatial cognition using the 8-arm radial maze task.<sup>15)</sup> As a result, Yoku-kan-san-ka-chimpi-hange appears to activate the central cholinergic neuron. However, it has yet to be determined whether or not the improving effects of Yoku-kan-san-ka-chimpi-hange on the disruption of spatial cognition involve the central cholinergic neuron. Therefore, in order to investigate whether Yoku-kan-san-ka-chimpi-hange possesses a central cholinergic activity, we examined the effect of Yoku-kan-san-ka-chimpi-hange on the scopolamine- and THC-induced disruption of spatial cognition in the 8-arm radial maze task, and on the tremors induced by oxotremorine, an M receptor agonist, in comparison with THA, a cholinesterase inhibitor.

In general, patients with dementia are known to demonstrate not only memory impairment but also a disturbance of consciousness. Our previous study reported that electroconvulsive shock (ECS)-induced immobilization was effectively suppressed by the injection of amantadine,<sup>16)</sup> which has been clinically used to treat disturbance of consciousness,<sup>17)</sup> and this immobilization also improved with the injection of nootropic drugs.<sup>16)</sup> We therefore examined the effect of Yoku-kan-san-ka-chimpi-hange on ECS-induced immobilization in a behavioral model for the disturbance of consciousness.

Moreover, to investigate whether the ameliorative effects of Yoku-kan-san-ka-chimpi-hange on the disruption of spatial cognition are due to its antidepressant activity, we examined the effect of Yoku-kan-san-ka-chimpi-hange on the duration of immobility during a forced swimming test, which is used as a model of depression in experimental animals.

## Materials and Methods

**Animals :** Male Wistar rats weighing 200-250 g and male ddY mice weighing 20-25 g were obtained from the KYUDO Co., Ltd. (Saga, Japan); they were housed in groups of 4 to 5 per cage for rats, and of 8 to 10 per cage for mice, in a room with a controlled temperature ( $23 \pm 2^\circ\text{C}$ ) and a relative humidity of  $60 \pm 10\%$  with lights on from 7:00 to 19:00. The animals

scheduled to undergo the 8-arm radial maze task were placed under a restricted food intake (10-12 g/day, CE-2, Clea Japan, Tokyo, Japan). Their body weights were maintained at approximately 80 % of the free feeding level during the experimental period. Water was freely available in their home cages. All animal care and procedures were based on the regulations stipulated by the Animal Care and Use Committee at Fukuoka University.

**Drugs :** Yoku-kan-san-ka-chimpi-hange (Tsumura Co., Tokyo, Japan) and THA (Aldrich Chem. Co., USA) were dissolved in water. Scopolamine, oxotremorine and amantadine (Sigma Chem. Co., USA) were dissolved in 0.9 % physiological saline. THC, isolated from cannabis by Prof. Y. Shoyama (Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Kyushu University), was suspended in 1 % Tween 80.

**Eight-arm radial maze task :** As previously reported,<sup>15,18)</sup> behavioral testing was conducted on an 8-arm radial maze (Neuroscience Co., Tokyo, Japan), a modified one originally developed by Olton and Samuelson.<sup>19)</sup> The test animals were trained in a group to be habituated to the apparatus and the food pellets for 3 days before each test animal's actual training began. This 10-min period of habituation was repeated 3 times a day. The interval time between habituations was more than 1 h. For each training session, the test animal was placed in a circular plastic wall on a platform in the middle of the 8-arm radial maze. Next, after a 1 min waiting period, the ring was lifted and the test animal was allowed to move freely in the maze. The trial continued until the test animal had either entered all 8 arms or 10 min had elapsed. If the test animals proceeded in the 8-arm radial maze task by using sequential routines consisting of repeating a given angular direction (e.g. 45° or 135°) by repeated training, then such animals were excluded in the present experiment. The performance of the test animal in each trial was assessed by three parameters; the number of correct choices in the initial 8 chosen arms, the number of errors which was defined as choosing arms that had already been visited, and the time that elapsed before the animal ate all 8 pellets. If the test animals made 7 or 8 correct choices and less than one error in three successive

sessions, they were then used for the drug evaluation the next day. Yoku-kan-san-ka-chimpi-hange and THA were orally administered 60 min before each session. Scopolamine and THC were intraperitoneally administered 30 min and 60 min, respectively, before the session. Wilcoxon's rank sum test was used for the data analysis in these experiments.

**ECS-induced immobilization :** ECS (90 mA, 0.2 s) was administered via saline-moistened earclip electrodes using a E.C. Stimulator ; model MK-80 (Neuroscience Co., Tokyo, Japan). A mild clonic convulsion was observed in each rat receiving ECS. After 15 min they were removed and allowed to put up both forelimbs over a cork (height: 12 cm; diameter : 2.5 cm), which was placed in the middle of a Hall's open-field apparatus (bottom diameter : 30 cm). This unnatural stretched posture was considered to be ECS-induced immobilization. The duration of immobilization was measured for a 60s-observation period. Amantadine, a reference drug, was intraperitoneally administered 15 min before the ECS-treatment. THA and Yoku-kan-san-ka-chimpi-hange were orally administered 45 min before the ECS-treatment. All data were analyzed using a one-way analysis of variance (ANOVA) followed by the Dunnett's test for parametric multiple comparisons.

**Forced swimming test :** The procedure used in this study was basically the same as that described by Porsolt *et al.*<sup>20)</sup> Briefly, the rats were individually forced to swim inside cylinders (height : 40 cm ; diameter : 15 cm) containing water (20 cm deep) maintained at 25°C. They were given 2 trials for 15 min. There was a 24 h interval between the first and second trial. The duration of immobility during the first 5 min test of the second trial was measured by using a Video Image Motion Analyzer ; model AXIS 30 (Neuroscience Co., Tokyo, Japan). Yoku-kan-san-ka-chimpi-hange was orally administered 24, 4 and 1 h (3 injections) before the second trial. Significant differences for the data analysis were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's test for parametric multiple comparisons.

**Oxotremorine-induced tremor :** Oxotremorine (0.3 mg/kg) was administered intraperitoneally. Yoku-kan-san-ka-chimpi-hange and THA were orally administered 50 min before the injection of oxotremor-

ine. The tremor observations were made at 5 min intervals starting 30 min after the injection of oxotremorine. The severity of tremors was scored according to 5 grades: 0: absent, 1: mild, 2: moderate, 3: marked, and 4: severe. All data were analyzed by Wilcoxon's rank sum test.

## Results

### Eight-arm radial maze task

Scopolamine (0.5 mg/kg, *i.p.*) significantly reduced the number of correct choices and also increased the

number of errors (N=24), thus indicating that a disruption of the spatial cognition had occurred. Yoku-kan-san-ka-chimpi-hange (10 mg/kg, *p.o.*) significantly increased the number of correct choices and decreased the number of errors in scopolamine-treated rats, thus suggesting that the disruption in spatial cognition was improved (N=8, Fig. 1-A). On the other hand, THA (1, 10 and 20 mg/kg, *p.o.*) also improved the disruption in spatial cognition (N=6-8, Fig. 1-B).

In the next experiment, THC (6 mg/kg, *i.p.*) markedly produced a disruption of spatial cognition

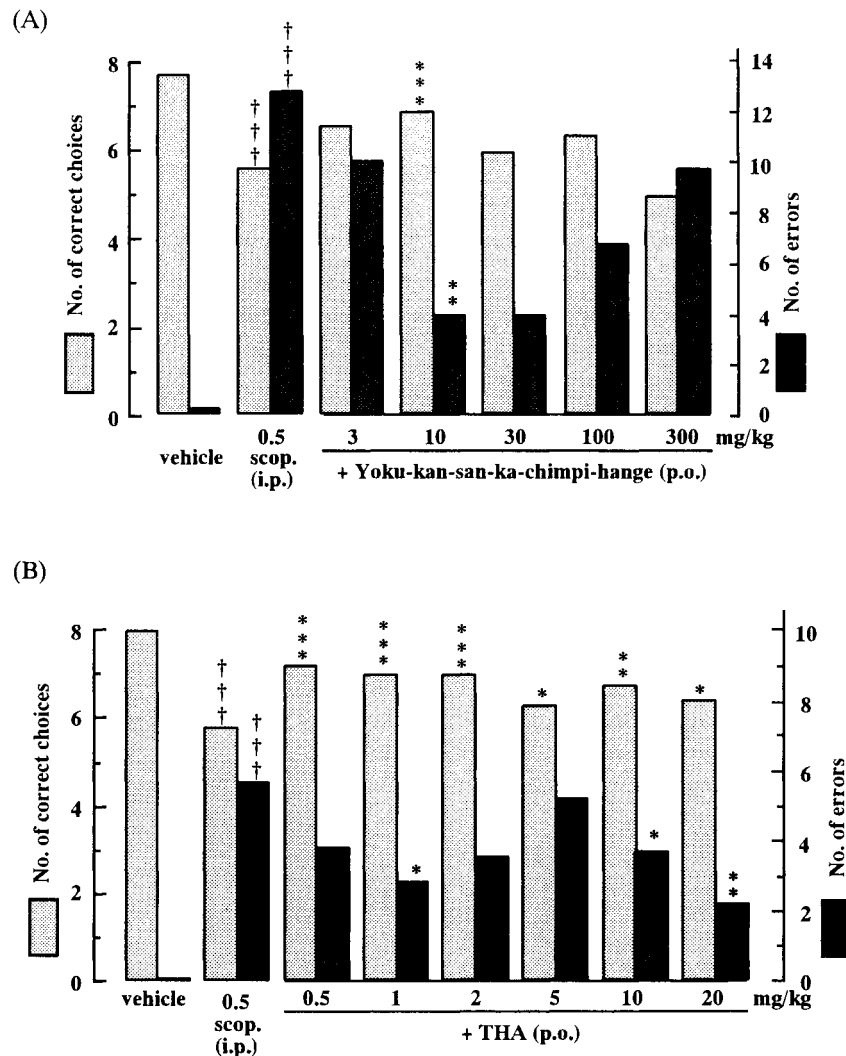


Fig. 1 Effects of Yoku-kan-san-ka-chimpi-hange (A) and THA (B) on the scopolamine-induced disruption of spatial cognition in the 8-radial arm maze. Yoku-kan-san-ka-chimpi-hange and THA were administered *p.o.*, 30 min. before scopolamine injection, respectively. Each value is a mean (N=6-24).

†††  $p < 0.001$  vs vehicle-treated group; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs scopolamine-treated group (Wilcoxon's rank sum test). Scop.: scopolamine.

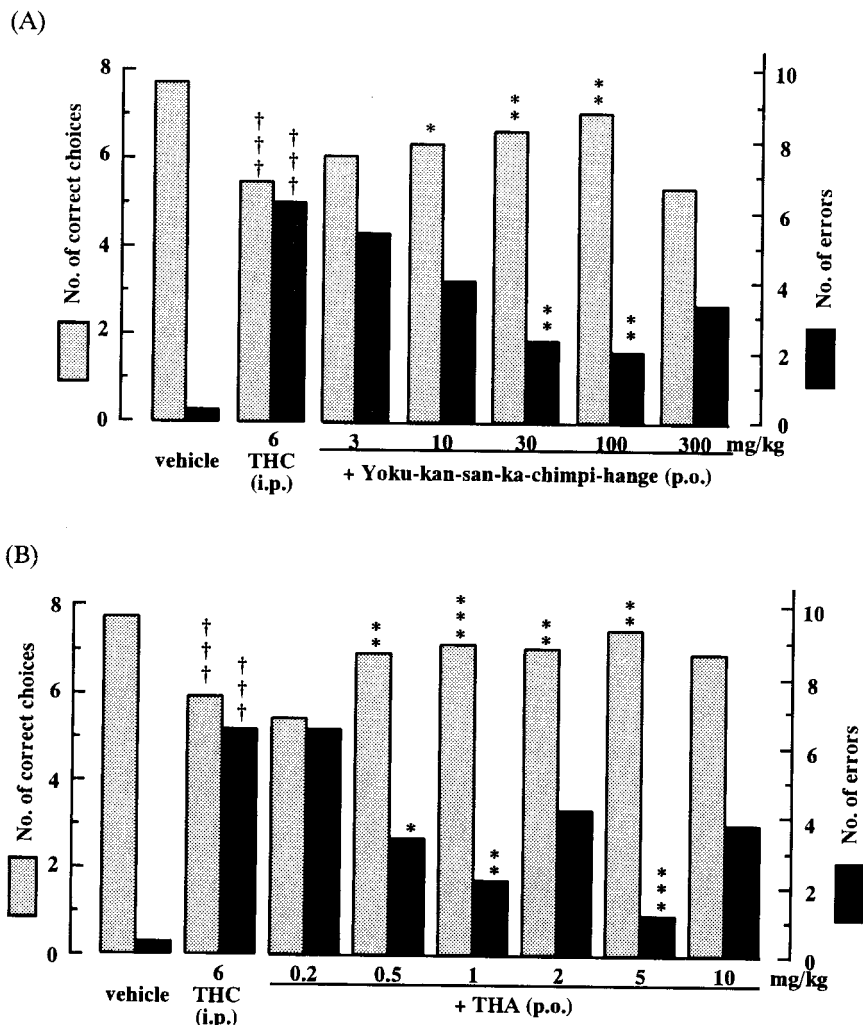


Fig. 2 Effects of Yoku-kan-san-ka-chimpi-hange (A) and THA (B) on the THC-induced disruption of spatial cognition in the 8-radial arm maze. Yoku-kan-san-ka-chimpi-hange and THA were administered *p.o.*, immediately before THC injection, respectively. Each value is a mean (N=8-30).

†††  $p < 0.001$  vs vehicle-treated group; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs THC-treated group (Wilcoxon's rank sum test).

and therefore THC significantly reduced the number of correct choices and increased the number of errors (N=30). The THC-induced disruption of spatial cognition was different from the scopolamine-induced one, namely some rats hesitated to enter the first arm, while others obtained a food pellet and then returned to the food cup without a pellet on the way to the center platform. Yoku-kan-san-ka-chimpi-hange (30 and 100 mg/kg, *p.o.*) improved this disruption in spatial cognition (N=8, Fig. 2-A). On the other hand, THA (0.5, 1 and 5 mg/kg, *p.o.*) also improved this

disruption in spatial cognition (N=8-9, Fig. 2-B).

#### *ECS-induced immobilization*

The duration of immobilization was approximately 70 % of the time during the 60s-observation period in vehicle-treated rats (N=34). Amantadine (10 mg/kg, *i.p.*) effectively suppressed the ECS-induced immobilization (N=7, Fig. 3-B). Yoku-kan-san-ka-chimpi-hange (30 and 100 mg/kg, *p.o.*) and THA (5 mg/kg, *p.o.*) also suppressed the ECS-induced immobilization (N=8, Fig. 3-A, -B).

#### *Forced swimming test*

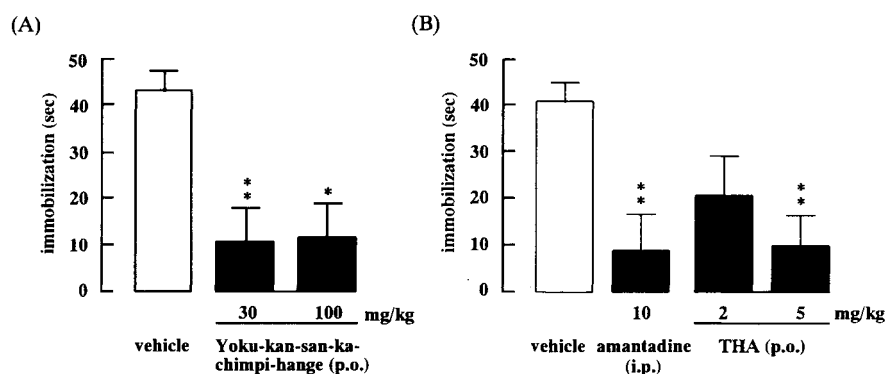


Fig. 3 Effects of Yoku-kan-san-ka-chimpi-hange (A), amantadine and THA (B) on the ECS-induced immobilization in rats. Yoku-kan-san-ka-chimpi-hange and THA were administered *p.o.*, 45 min. before the ECS-treated, respectively. Amantadine administered *i.p.*, 15 min. before the ECS-treated. Each value is a mean and S.E.M. (N=7-34).  
\**p* < 0.05, \*\**p* < 0.01, vs vehicle-treated group (Dunnett's test).

The vehicle-treated rats which were placed in the cylinder initially exhibited an escape-directed behavior such as climbing the wall or jumping, gradually became immobile and the total duration of immobility was approximately 68 % of the time during the first 5 min of the second trial. Yoku-kan-san-ka-chimpi-hange (10-500 mg/kg, *p.o.*) had no effect on the duration of this immobility (N=7-8, Fig. 4).

#### Oxotremorine-induced tremors

The mice that were intraperitoneally treated with oxotremorine (0.3 mg/kg, *i.p.*) developed tremors which became most marked 10 min after injection and

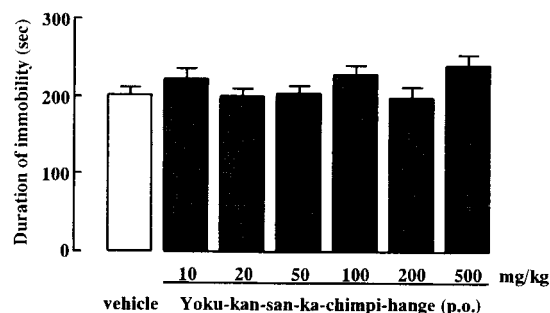


Fig. 4 Effect of Yoku-kan-san-ka-chimpi-hange on the duration of immobility in a forced swimming test. Yoku-kan-san-ka-chimpi-hange was administered *p.o.*, 24, 4 and 1 h (3 injections) before the second trial. Each value is a mean and S.E.M. (N=7-8).

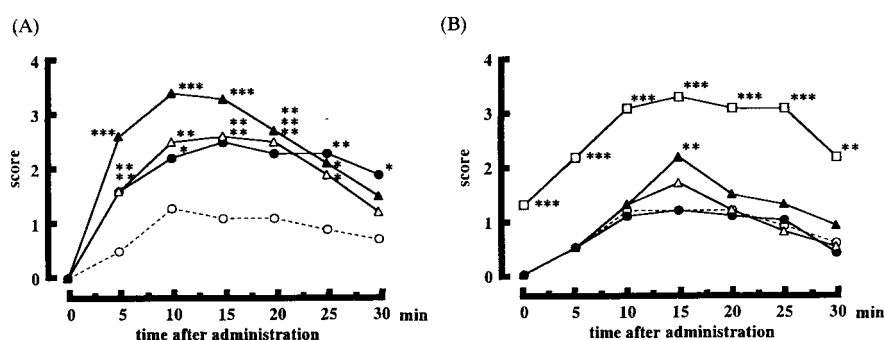


Fig. 5 Effects of Yoku-kan-san-ka-chimpi-hange (A) and THA (B) on oxotremorine-induced tremors in mice.

- (A); (○) oxotremorine 0.3 mg/kg, *i.p.* + vehicle; (●) oxotremorine + Yoku-kan-san-ka-chimpi-hange 10 mg/kg;  
(△) oxotremorine + Yoku-kan-san-ka-chimpi-hange 30 mg/kg;  
(▲) oxotremorine + Yoku-kan-san-ka-chimpi-hange 100 mg/kg;  
(B); (○) oxotremorine 0.3 mg/kg, *i.p.* + vehicle; (●) oxotremorine + THA 0.5 mg/kg;  
(△) oxotremorine + THA 1 mg/kg; (▲) oxotremorine + THA 5 mg/kg;  
(□) oxotremorine + THA 20 mg/kg;

Yoku-kan-san-ka-chimpi-hange and THA were administered *p.o.*, 50 min. before oxotremorine injection, respectively. Each value is a mean (N=10). \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001 vs the vehicle-treated mice (Wilcoxon's rank sum test).

then later subsided over time ( $N=10$ ). Yoku-kan-san-ka-chimpi-hange (10, 30 and 100 mg/kg, *p.o.*) markedly potentiated the oxotremorine-induced tremor ( $N=10$ , Fig. 5-A). However, Yoku-kan-san-ka-chimpi-hange alone did not cause tremors or salivation. On the other hand, THA (5 mg/kg, *p.o.*) significantly potentiated the oxotremorine-induced tremor ( $N=10$ ). Furthermore, THA (20 mg/kg, *p.o.*) alone did cause tremors, salivation and sometimes even death ( $N=10$ , Fig. 5-B).

### Discussion

Donepezil and THA, cholinesterase inhibitors, have been shown to improve memory disorders in AD patients.<sup>3,4)</sup> Furthermore, recent studies have shown that donepezil and THA have a beneficial effect on memory and learning in experimental animals.<sup>21-23)</sup> Our previous report demonstrated that the scopolamine-induced disruption of spatial cognition was improved not only by the administration of cholinergic drugs but also by the injection of noradrenergic drugs.<sup>8)</sup> In addition, our previous study also showed the THC-induced disruption of spatial cognition to improve after the administration of either cholinergic drugs or noradrenergic drugs.<sup>11)</sup> Furthermore, the THC-induced disruption of spatial cognition was associated with a dysfunction of the serotonergic neuron, because a small dose of serotonergic agents markedly improved the THC-induced disruption in spatial cognition (unpublished data). We therefore studied the effects of Yoku-kan-san-ka-chimpi-hange on the scopolamine- and THC-induced disruption of spatial cognition and compared them with those of THA.

THA reversed both the scopolamine- and THC-induced disruption in spatial cognition. THA had a more ameliorative effect on the THC-induced disruption of spatial cognition than on the scopolamine-induced disruption of spatial cognition. As a result, THA may not be a pure cholinesterase inhibitor. THA apparently has an anticholinesterase action. However, it is also a potent monoamine oxidase inhibitor.<sup>24)</sup> Therefore, THA can inhibit the oxidation of serotonin (5-HT). Furthermore, recent studies have reported that THA inhibited the uptake rates of both

5-HT and dopamine (DA) and stimulated the release of both 5-HT and DA in the rat brain.<sup>25,26)</sup> These facts suggest that the ameliorative effect of THA may involve not only the cholinergic neuron but also the serotonergic neuron.

Yoku-kan-san-ka-chimpi-hange also improved both the scopolamine- and THC-induced disruption of spatial cognition in the 8-arm radial maze task. Based on these findings, Yoku-kan-san-ka-chimpi-hange was thus considered to potentially be a cognitive enhancer for senile dementia. However, the ameliorative effect of Yoku-kan-san-ka-chimpi-hange was different from that of THA. Yoku-kan-san-ka-chimpi-hange had a more ameliorative effect on the scopolamine-induced disruption of spatial cognition than on the THC-induced disruption of spatial cognition. Furthermore, the effective doses of Yoku-kan-san-ka-chimpi-hange in the 8-arm radial maze markedly enhanced the oxotremorine-induced tremors. However, Yoku-kan-san-ka-chimpi-hange alone did not cause tremors and salivation. These results suggested that the effect of Yoku-kan-san-ka-chimpi-hange was due to indirect stimulation of the cholinergic neuron.

The positive effect of Yoku-kan-san-ka-chimpi-hange on the disruption of spatial cognition had an inverted-U dose response function. This mode of action was also observed in the treatments with noradrenergic, serotonergic and cholinergic agents.<sup>8,11)</sup> This finding suggests that the multiple neurotransmitter balance plays an important role on spatial cognition. As a result, higher doses of Yoku-kan-san-ka-chimpi-hange may thus cause an imbalance in multiple neurotransmitter interactions. Furthermore, a recent study has reported that Yoku-kan-san-ka-chimpi-hange showed a bell-shaped dose-response curve regarding the ChAT activity in rat embryo septal cultures.<sup>13)</sup>

In general, patients with dementia are known to demonstrate not only memory impairment but also a disturbance of consciousness. Our previous study showed that the ECS-induced immobilization was effectively suppressed by the injection of amantadine,<sup>16)</sup> which has been clinically used to treat a disturbance of consciousness,<sup>17)</sup> and that this immobilization was also improved by the injection of

some nootropic drugs.<sup>16)</sup> The immobilized state is induced by light ECS-treatment. Such ECS-induced immobilization can be easily discontinued by air blowing, sound, tactile stimuli and so on (unpublished data). ECS-induced immobilization is therefore widely different from catalepsy which is induced by the large-dose administration of major tranquilizers. From these findings, this immobilized state may be thought to represent a decreased level of consciousness. Therefore, ECS-induced immobilization is thought to be a good behavioral model for the disturbance of consciousness. In the next experiment, we examined the effects of THA and Yoku-kan-san-ka-chimpi-hange on the ECS-induced immobilization in rats. THA suppressed this immobilization at a dose of 5 mg/kg, *p.o.* The same dose of THA significantly potentiated the oxotremorine-induced tremors. In addition, anticholinergic drugs, such as atropine and scopolamine, exacerbated the degree of ECS-induced immobilization (data not shown). Spingnol *et al.* found a decrease in the hippocampal ACh level which was still statistically significant at 30 min after the ECS application.<sup>27)</sup> Furthermore, in a microdialysis study, Zis *et al.* showed that ACh release increased within 10 min in the rat striatum followed by a decrease below the baseline values for approximately 30 min following a single ECS.<sup>28)</sup> Based on these findings, the immobilization induced by ECS was considered to be related to the cholinergic neuron. In the present study, Yoku-kan-san-ka-chimpi-hange also suppressed the ECS-induced immobilization at doses of 30 and 100 mg/kg, *p.o.* Therefore, Yoku-kan-san-ka-chimpi-hange improved not only the disruption of spatial cognition but also the disturbance of consciousness. The same doses of Yoku-kan-san-ka-chimpi-hange greatly enhanced the occurrence of oxotremorine-induced tremors. These results suggest that Yoku-kan-san-ka-chimpi-hange improves both the disruption of spatial cognition and the ECS-induced immobilization by enhancing the cholinergic neuron.

In addition, we previously reported that Toki-shakuyaku-san (Dang-Gui-Shao-Yao-San; 当归芍药散), a traditional Kampo prescription, improved the scopolamine-induced disruption of spatial cognition,<sup>18)</sup> and *Angelicae radix* (Toki), a medicinal herb contained in Toki-shakuyaku-san, was the

most effective of all component herbs with Toki-shakuyaku-san.<sup>29)</sup> In addition, *Angelicae radix* is one of medicinal herbs contained in Yoku-kan-san-ka-chimpi-hange. *Angelicae radix* may be the most effective of all the component herbs with Yoku-kan-san-ka-chimpi-hange.

Moreover, to investigate whether the effects of Yoku-kan-san-ka-chimpi-hange on the disruption of spatial cognition and the ECS-induced immobilization are due to its antidepressant activity, we investigated whether or not Yoku-kan-san-ka-chimpi-hange had an antidepressant effect on the duration of immobility in the forced swimming test, a model of depression in experimental animals. However, Yoku-kan-san-ka-chimpi-hange had no effect on the duration of immobility. It is thus unlikely that the ameliorative effect of Yoku-kan-san-ka-chimpi-hange was related to the antidepressant activity.

Finally, a higher dose of THA alone caused such side effects as tremors, salivation and even death. In clinical trials for AD, THA also showed signs of liver damage.<sup>30)</sup> In contrast, Yoku-kan-san-ka-chimpi-hange alone did not cause any tremors or salivation. Based on these findings, Yoku-kan-san-ka-chimpi-hange might therefore be a useful drug for the treatment of dementia without any detrimental side effect.

## Conclusions

Yoku-kan-san-ka-chimpi-hange (10–100 mg/kg, *p.o.*) improved both the scopolamine- and THC-induced disruption of spatial cognition in rats. Yoku-kan-san-ka-chimpi-hange (30–100 mg/kg) suppressed the ECS-induced immobilization, a behavioral model of a disturbance of consciousness. On the other hand, Yoku-kan-san-ka-chimpi-hange had no effect on the immobility time in a rat forced swimming test, a model of depression in experimental animals. Furthermore, Yoku-kan-san-ka-chimpi-hange (10–100 mg/kg, *p.o.*) enhanced oxotremorine-induced tremors in mice. These results suggest that Yoku-kan-san-ka-chimpi-hange may indirectly enhance the cholinergic neurons and also improve an experimentally-induced disruption of spatial cognition and consciousness.



## 和文抄録

ラットを用いて抑肝散加陳皮半夏の scopolamine ならびに THC による空間認知記憶障害に対する作用について THA と比較検討した。また、意識障害モデルと考えられる ECS による不動状態に対する抑肝散加陳皮半夏ならびに THA の作用についても行動薬理的に検討を行った。さらに、実験的うつ病モデルである強制水泳による不動時間に対する抑肝散加陳皮半夏の作用について検討を行った。一方、抑肝散加陳皮半夏の中樞 ACh 神経に対する賦活作用を調べるためにマウスを用いた oxotremorine による振戦を指標にして増強作用を観察した。抑肝散加陳皮半夏ならびに THA は空間認知記憶障害ならびに ECS による不動状態に対して改善作用を有することが判った。また、抑肝散加陳皮半夏は強制水泳による不動時間に対して有意な作用は認められず、抑肝散加陳皮半夏の改善作用は抗うつ作用によるものでないことが明らかとなった。一方、抑肝散加陳皮半夏は oxotremorine による振戦に対して著明な増強作用を示した。しかし、THA と異なり、それ単独では振戦、流涎および死亡は認められなかった。このことから、抑肝散加陳皮半夏は中樞 ACh 神経に対して間接的に賦活作用をもつことが考えられた。また、この ACh 神経の賦活作用が本方剤の空間認知記憶障害ならびに ECS による不動状態に対する改善作用に関与しているものと推察された。

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