

Full Paper

Determination of the Effectiveness of Components of the Herbal Medicine *Toki-Shakuyaku-San* and Fractions of *Angelica acutiloba* in Improving the Scopolamine-Induced Impairment of Rat's Spatial Cognition in Eight-Armed Radial Maze TestIzzettin Hatip-Al-Khatib^{1,2}, Nobuaki Egashira³, Kenichi Mishima², Katsunori Iwasaki^{2,3}, Kiyo Iwasaki³, Kouji Kurauchi³, Keiichiro Inui³, Tomoaki Ikeda⁴, and Michihiro Fujiwara^{2,3,*}¹Department of Pharmacology, Division of Internal Medicine, Faculty of Medicine, Pamukkale University, Denizli 20070, Turkey²Advanced Material Institute, Fukuoka University, 8-19-1 Nanakuma, Jonan-Ku, Fukuoka 814-0180, Japan³Department of Neuropharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-Ku, Fukuoka 814-0180, Japan⁴Department of Obstetrics and Gynecology, Miyazaki Medical College, University of Miyazaki, Miyazaki 889-1692, Japan

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Abstract. The improving effects of various components of *Toki-Shakuyaku-San* (TSS) and fractions isolated from *Angelica acutiloba* Radix (*Toki*) on scopolamine-induced spatial memory impairment were investigated in eight-armed radial maze. The scopolamine-induced memory impairment was characterized by prominent increase of error choices in addition to decreased correct choices. *Toki*, *Cnidium officinale* Rhizoma (*Senkyu*), *Poria cocos* Hoelen (*Bukuryo*), *Alisma orientale* Rhizoma (*Takusha*), and *Atractylodes lancea* Rhizoma (*Sojutsu*) increased the correct choices, while only the *Toki*, *Sojutsu*, and *Takusha* decreased the error choices. No effect was produced by *Paeonia lactiflora* Radix (*Shakuyaku*). Investigation of effects of fractions isolated from *Toki* revealed that its activity mainly resided in the butanol layer and its contents of *N*-methyl- β -carboline-3-carboxamide and amines. Moreover, the alkaloid, internal and external solutions (containing poly-, di-, and monosaccharides) obtained by dialysis with Visking cellophane tubing also improved the memory. However, no improving properties were detected for methanol and hexanol layers, L(-)-tryptophan, L-arginine, L(-)-lysine, and choline chloride. The results showed that the TSS components could improve the reference and working memory impaired by scopolamine. The improving effect of TSS is produced greatly by the *Toki* component, the activity of which was greatly produced by the fraction extracted by butanol.

Keywords: *Toki-Shakuyaku-San*, *Angelica acutiloba*, *N*-methyl- β -carboline-3-carboxamide, scopolamine, spatial memory

Introduction

Toki-Shakuyaku-San (TSS), a Chinese medicine (*Danggui-Shaoyao-San*), is a mixture of 6 medicinal plants: *Alisma orientale* Rhizoma (*Takusha*), *Angelica acutiloba* Radix (*Toki*), *Atractylodes lancea* Rhizoma (*Sojutsu*), *Cnidium officinale* Rhizoma (*Senkyu*), *Paeonia lactiflora* Radix (*Shakuyaku*), and *Poria cocos*

Hoelen (*Bukuryo*) at a ratio of 4:3:4:3:4:4, respectively. It has been reported that TSS increases NGF (1), has an antioxidant action, and has a prophylactic effect against free radical-mediated neurological disease associated with aging (2, 3). Recent pharmacological studies revealed that TSS differentially enhances release of T helper 1 cytokines from peripheral blood mononuclear cells but not from decidual mononuclear cells (4). Additionally, it has been reported that TSS provides neuroprotection against a variety of insults like hypo-

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glycemia/hypoxia (5) and glutamate (6) by inhibiting the inordinated increase of cytosolic Ca^{2+} levels (7).

The various components of TSS has been used for the treatment of a variety of diseases. *Toki* is used as CNS depressant, analgesic, antipyretic, skeletal muscle relaxant, and vasodilator. *Shakuyoku* and *Senkyu* are used as a depressant, analgesic, and vasodilator. Moreover, some components of TSS are prescribed as hypoglycemic (*Sojutsu* and *Bukuryo*), diuretic (*Takusha* and *Bukuryo*), and anticoagulant (*Toki* and *Bukuryo*). TSS has also been widely used as tonic for both men and women and in treatment of a variety of diseases such as anemia, fatigue, circulatory disorders, and acne (8). Due to its estrogenic properties, TSS has been used in oriental medicine for treatment of ovarian dysfunction, infertility, and postmenopausal Alzheimer's-type dementia (9).

The behavioral studies have shown that TSS displays an effect on the cholinergic and aminergic systems. TSS potentiates tremors induced by direct stimulation of acetylcholine receptors with oxotremorine (10). Neurochemical studies revealed that TSS could increase nicotine acetylcholine receptor number and norepinephrine content in the cerebral cortex, could suppress the decrease of cortical and hippocampal choline acetyltransferase activity and norepinephrine content, and improve memory related behavior in ovariectomized mice (11).

Because of its cholinergic activating and circulation-improving effects, TSS is being used in the treatment of neural dysfunctions such as Alzheimer's disease, senile dementia, memory loss, and other cognitive disorders. Experimentally, it has been reported that TSS improves scopolamine-induced disruption of memory (12). However, no study of the cognition improving effect of the components of TSS or the fractions of the most effective component is available. Accordingly, the present study was conducted to determine the effects of various components and fractions of TSS on scopolamine-induced spatial memory impairment. The scopolamine-induced memory impairment was chosen because this type of impairment is produced by impairment of the cholinergic activity mainly in the hippocampus, and TSS improves memory by mechanisms including enhancement of the cholinergic activity.

Materials and Methods

Animals

The experiments were performed on eight-week-old male Wistar rats weighing 200 ± 10 g (Kyudo, Saga). The rats were housed in groups of five per cage in a room with controlled temperature ($23 \pm 2^\circ\text{C}$), relative humidity ($60 \pm 10\%$), and 12-h light/dark cycle, light

period starting at 7:00 am. Food and water were available ad libitum. The experiments were carried out in compliance with the guidelines stipulated by the Animal Care and Use Committee of Fukuoka University.

Eight-arm radial maze (RAM)

Apparatus: The RAM test was conducted according to our previous study (13). The RAM apparatus used in this study (Neuroscience Co., Tokyo) was consisted of equally spaced transparent plexiglas eight arms (50-cm-long, 10-cm-wide with transparent 50-cm-high side walls) extended from a central octagonal hub (24-cm-across, surrounded by opaque guillotine doors at the entrance of each arm). The maze was elevated 50 cm from the floor. Food cups (3-cm diameter, 1-cm depth, black plexiglas) were mounted at the end of each arm and served as receptacles for the reinforcers (two lumps, 50–60 mg crystallized sugar) in the baited arms. The experiments were conducted, in a room containing many fixed extra-maze visual cues, between 07–19.0 o'clock.

Procedures: 1. *Restricted feeding schedule:* The schedule that was applied during this study was achieved by reducing the daily consumption of ration (10–12 g/day, CE-2; Clea Japan, Tokyo) so that body weight of each rat was maintained at 80–90% of the freely feeding level. Water was available ad libitum.

2. *Pretraining, training, and assessment of RAM performance and drug effects:* In the pretraining, the animals were acclimatized in groups of 5 rats to the apparatus and the reinforcer food pellets daily (each session of 10 min repeated three times at intervals of 60 min) for three days before training. The training phase was started one day after the pretraining and was performed three times/day for 14 days in order to allow the rats to learn how to perform the RAM task. In the training and drug tests trials, each rat was placed in the central platform, then the guillotine was lifted after 1 min, and the rats were allowed moving freely in the apparatus to the baited arms. The trial continued until the test animal had either entered all eight arms and consumed the baits or 10 min had elapsed. If the test animals proceeded in by using sequential routes consisting of repeating a given angular direction (e.g., 45°) to the neighboring arm, then such animals were excluded from the present experiment, since such a repetitive entrance to the neighboring arms indicates poor working memory. Only the rats that made no errors or only one error for three consecutive days were selected for the study.

Performance assessment: The following parameters were considered the criteria for radial maze performance: 1) the number of correct choices (CC) in the initial 8 chosen arms (entry into an arm that the animal

had not previously visited and avoidance of nonbaited arm) and 2) number of error choices (EC) (reentry into an arm that the rat had previously visited and subsequent visit to nonbaited arm during the same trial). The CC reflect the extent to which the arrangement of area baited during the predelay phase was retained across the delay (reference memory), whereas the EC reflect impaired performance accuracy across successive choices during the postdelay phase reflecting working memory (14). The RAM performance was observed by a Video Image

Motion Analyzer (AXIS 30; Neuroscience Co., Tokyo).

Drugs

The plants and the fractions of *Toki* were supplied by Tsumura & Co., Tokyo. The six plants constituting TSS with their active ingredients are shown in Table 1. The fractions of *Toki* used in this study, the method applied for their isolation and their active ingredients are depicted in Table 2. Scopolamine (Sigma Chem. Co., St. Louis, MO, USA) was dissolved in 0.9% physio-

Table 1. The six components of *Toki-Shakuyaku-San*, their ingredients, and the effective doses in radial maze task

Plant name	Contents
<i>Alisma orientale</i> Rhizoma (<i>Takusha</i>)	Alisol A-B-C, Alisol A-B-C monoacetate, D-Glucose, D-Fructose, Sucrose, β -Sitosterol, Lecithin, Choline
<i>Angelica acutiloba</i> Radix (<i>Toki</i>)	Ligustilide, <i>n</i> -Butylidene-phthalide, Sedanoic acid, Saffrole, Palmitic acid, Linoleic acid, Bergaptene, Scopoletin, Falcarinol, Falcarindiol, Cyanocobalamine, Nicotinic acid
<i>Atractylodes lancea</i> Rhizoma (<i>Sojutsu</i>)	β -Eudesmol, Hinesol, Elemol, Atractylodin
<i>Cnidium officinale</i> Rhizoma (<i>Senkyu</i>)	Ligustilide, Cnidilide, Neocnidilide, Butylphthalide, Butylidenephthalide
<i>Paeonia lactiflora</i> Radix (<i>Shakuyaku</i>)	Paeoniflorin, Oxypaeoniflorin, Benzoyl paeoniflorin, Albiflorin, Paeonol
<i>Poria cocos</i> Hoelen (<i>Bukuryo</i>)	Pnchyman, Eburicoic acid, Pachymic acid, Dehydroeburicoic acid, Ergosterol, 3 β -O-Acetyltumulosic acid, 3 β -O-Acetyldehydrotumulosic acid

Table 2. Properties of *Angelica acutiloba* (*Toki*) fractions

Fractions	% Value of total methanol part	Composition
Methanol part* [Extract with CHCl ₃ :MeOH:H ₂ O (3:2:1)]		
A. Upper part [Extract with BUOH:H ₂ O (1:1)]		
Butanol layer	1.2	β -Carboline, Nucleoside, Amines
Water layer	17	Monosaccharides, Disaccharides
B. Lower part [Extract with Hexane:Methane:H ₂ O (10:5:1)]		
Hexane layer	0.5	Lipid, Glyceraldehyde
Methanol layer	0.6	Phthalides, Coumarine, Polyacetylene derivatives
C. Alkaloid	1	
Residue [Extraction with H ₂ O]		
A. Water part [Elude with H ₂ O:EtOH (1:3)]		
Precipitate**		
Internal dialysis solution	0.5	Polysaccharides
External dialysis solution***	4.7	Monosaccharides, Disaccharides
Supernatant		
B. Residue: Discarded		

Toki* was firstly extracted with methanol. **Dialysis with Visking cellophane tubing. *Combination of the fraction outside the dialysis membrane and the supernatant. The values are the percentage of crude *Angelica acutiloba* plant material.

logical saline and injected (0.5 mg/kg) intraperitoneally (i.p.) 30 min before the session. TSS and the fractions isolated from *Toki* were administered p.o. 60 min prior to each session except for FG-7142 (*N*-methyl- β -carboline-3-carboxamide), and amines were injected i.p. because the amines are subject to gastric inactivation, and a plethora of literature indicate activity of FG-7142 after i.p. injection. Moreover, the amines and FG-7142 were injected concomitantly with scopolamine because of their rapid absorption following i.p. injection.

Statistical analyses

The effects on maze performance were evaluated using one way ANOVA for determining the statistically significant differences among the groups (vehicle, scopolamine, and various doses of TSS components or fractions of the *Toki* in each experimental set) followed by Tukey's multiple comparison (*post hoc*) test. All data are expressed as the mean \pm S.E.M.

Results

Scopolamine-induced memory impairment and effects of TSS components

According to the method of selection of rats for the maze test, the results of 12 different sets of experiments on 120 rats showed that the CC of the selected naive rats was 7.6 ± 0.16 to 7.8 ± 0.1 , whereas their EC was 0.3 ± 0.1 to 0.5 ± 0.16 . On the other hand, scopolamine at 0.5 mg/kg, i.p. significantly decreased ($P < 0.001$) the CC to 5.3 ± 0.18 to 5.8 ± 0.1 , whereas it increased ($P < 0.01$) the EC to 7.1 ± 0.7 to 10.3 ± 1.1 in the maze test.

The improving effects produced by TSS components and the fractions extracted from *Toki* were generally independent of the dose. ANOVA revealed a significant group (treatment) effect of the *Takusha* on the CC ($F_{5,73} = 20.95$, $P < 0.001$). Figure 1 shows that *Takusha* increased ($P < 0.05$) the CC (16–18%) at 10 and 100 mg/kg. Moreover, a significant group effect was also obtained for the effect on the EC ($F_{5,73} = 16.56$, $P < 0.01$). *Takusha* reduced the EC at 50 mg/kg ($P < 0.01$) and 100 mg/kg ($P < 0.05$). No effect was detected for *Takusha* at 20 mg/kg.

A significant group effect was detected for the effect of the *Toki* on the CC ($F_{7,122} = 26.17$, $P < 0.001$). *Toki* increased the CC at 0.1 mg/kg ($P < 0.01$), 1 mg/kg ($P < 0.001$), 5 mg/kg ($P < 0.002$), and 10 mg/kg ($P < 0.05$). On the other hand, *Toki* reduced the EC only at 1 mg/kg ($P < 0.01$). At the other doses, the reduction of the EC did not reach a significant level. No effect was obtained for higher doses of *Toki* at 30 and 50 mg/kg (data not shown for the latter dose).

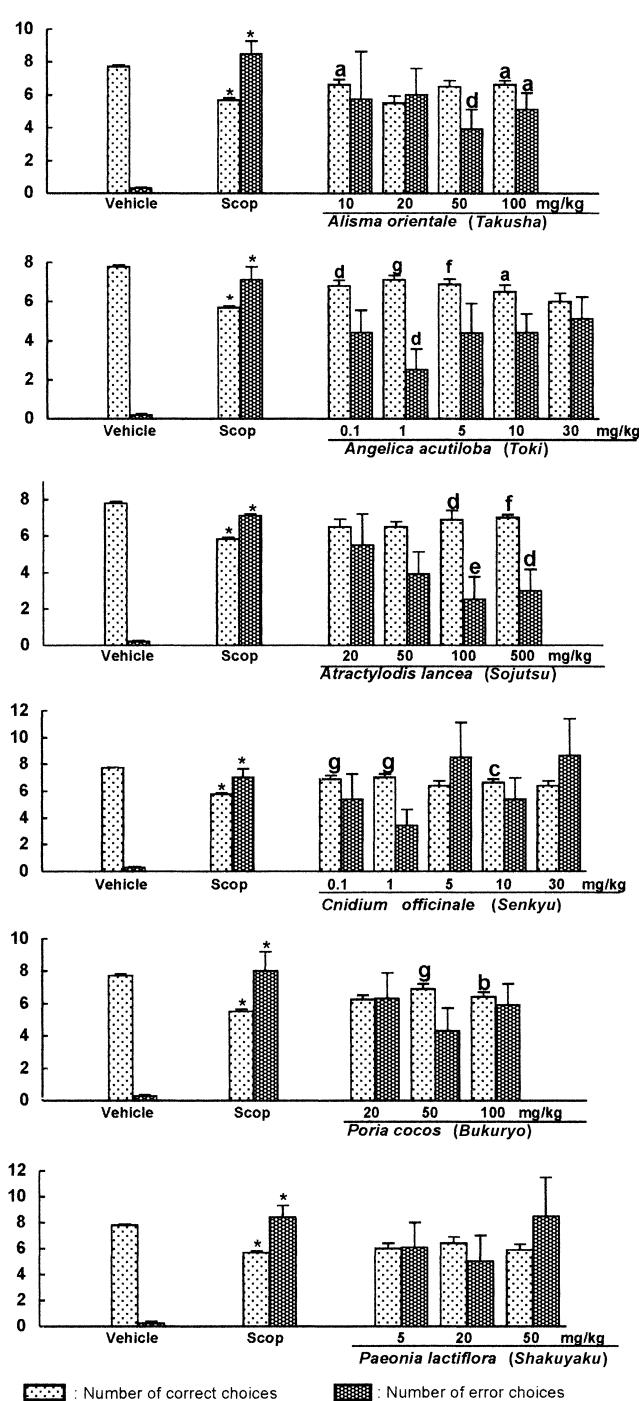


Fig. 1. Effects of the plants comprising *Toki-Shakuyaku-San* on the scopolamine (Scop)-induced memory impairment assessed in the 8-arm radial maze as the numbers of correct or error choices. Scop, 0.5 mg/kg, was injected i.p. 30 min before, and the components of *Toki-Shakuyaku-San* were administered p.o. 60 min prior to each session at the doses indicated at the bottom of each column. Data are means \pm S.E.M. * $P < 0.001$: compared to vehicle (distilled water); ^a $P < 0.05$, ^b $P < 0.03$, ^c $P < 0.02$, ^d $P < 0.01$, ^e $P < 0.003$, ^f $P < 0.002$, and ^g $P < 0.001$: significant differences compared to Scop (Tukey's multiple comparison test). The numbers of rats were 10 (Vehicle and Scop) and 8 for each dose of the *Toki-Shakuyaku-San* components.

It was found that *Sojutsu* displayed no improving activity at 20 and 50 mg/kg, whereas it improved the maze performance that was impaired by scopolamine at 100 and 500 mg/kg. The significance of group effect for the effect of *Sojutsu* on the CC was $F_{5,83} = 20.88$, $P < 0.01$. The levels of significance of effects on the CC were $P < 0.01$ and $P < 0.002$ for 100 and 500 mg/kg, respectively. A significant group effect was also obtained for the effect of *Sojutsu* on the EC ($F_{5,83} = 15.55$, $P < 0.001$). *Sojutsu* at 100 and 500 mg/kg reduced the EC at $P < 0.003$ and $P < 0.01$, respectively. The reduction produced by 50 mg/kg did not reach a significant level ($P < 0.08$).

Senkyu significantly increased the CC ($F_{7,125} = 26.82$, $P < 0.001$). The results showed that *Senkyu* increased the CC at 0.1 mg/kg ($P < 0.001$), 1 mg/kg ($P < 0.001$), and 10 mg/kg ($P < 0.02$), but without significantly changing the EC. At 5 and 30 mg/kg, *Senkyu* produced no change in either CC or EC.

Bukuryo increased the CC ($F_{4,57} = 26.37$, $P < 0.001$). *Bukuryo* at 20 mg/kg did not display any significant effect. On the other hand, significant effects were produced by 50 mg/kg ($P < 0.001$) and 100 mg/kg ($P < 0.03$). No effect was produced by the *Bukuryo* on the EC.

The results of the present study showed that *Shakuyaku* did not improve the maze performance.

Effects of various fractions extracted from the *Angelica acutiloba* on Scopolamine-induced memory impairment

Figure 2 shows that the butanol layer at 1 and 2 mg/kg increased the CC ($P < 0.05$ and $P < 0.01$, respectively) and decreased the EC ($P < 0.001$ and $P < 0.003$, respectively). No effect on either CC or EC was obtained for the doses of 0.01 and 0.1 mg/kg.

The results showed that FG-7142 increased the CC only at 0.01 mg/kg ($P < 0.03$). Moreover, FG-7142 decreased the EC at 0.01 mg/kg ($P < 0.001$) and 1 mg/kg ($P < 0.05$).

Figure 2 also shows that choline, lysine, and L-tryptophan isolated from the *Toki* did not display any significant effect on maze performance when examined at 0.1 mg/kg. However, L-tryptophan increased the EC ($P < 0.05$) instead of decreasing it. On the other hand, at 0.1 mg/kg, arginine only decreased the EC ($P < 0.05$), whereas L-ornithine increased the CC and decreased the EC ($P < 0.05$). L-Ornithine at 0.01 mg/kg did not improve the scopolamine-induced memory impairment.

The results showed that when examined at low doses (0.01 – 1 ng/kg), the alkaloid fraction increased the CC at 0.1 and 1 mg/kg, whereas it decreased the EC only at 0.1 ng/kg ($P < 0.05$). Moreover, at higher doses (0.01 – 1 μ g/kg), the alkaloid fraction only decreased the EC

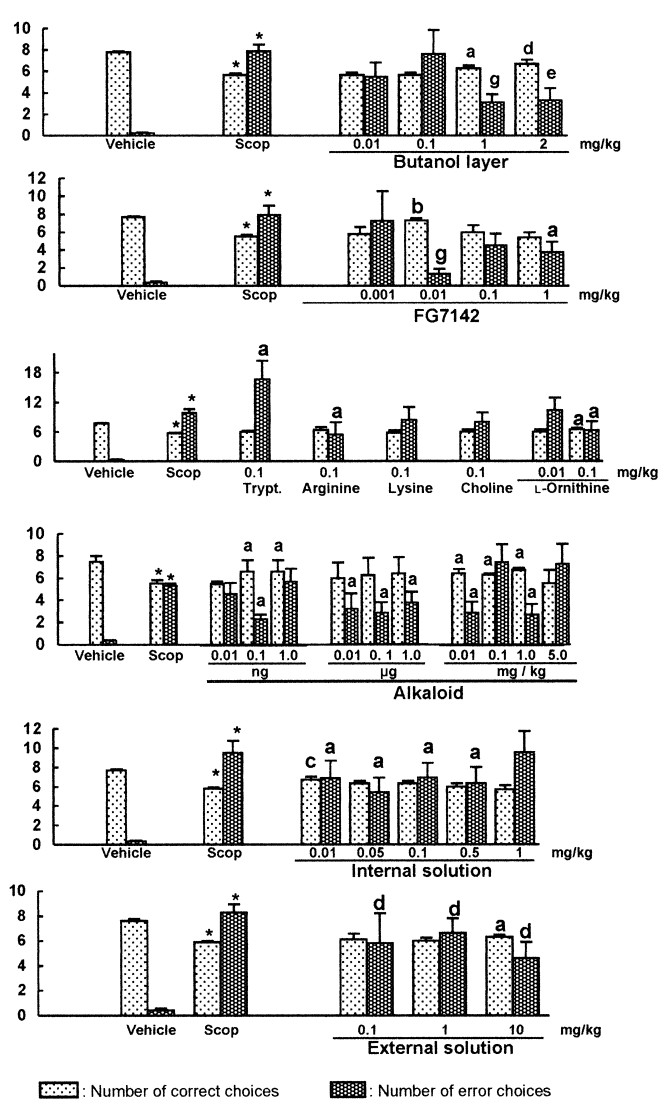


Fig. 2. Effects of various fractions isolated from *Angelica acutiloba* on the scopolamine (Scop)-induced memory impairment assessed in the 8-arm radial maze as the numbers of correct or error choices. Scop, 0.5 mg/kg, was injected i.p. 30 min before, and the fractions were administered p.o. 60 min prior to each session (except FG7142 and amines were injected i.p. and concomitantly with Scop). Data are means \pm S.E.M. * $P < 0.001$: compared to vehicle (distilled water); ^a $P < 0.05$, ^b $P < 0.03$, ^c $P < 0.02$, ^d $P < 0.01$, ^e $P < 0.003$, and ^g $P < 0.001$: significant differences compared to Scop (Tukey's multiple comparison test). The numbers of rats were 10 (Vehicle and Scop) and 8 for each dose of the fraction isolated from the *Toki*. Distilled water was the vehicle for Visking dialysates (internal and external solution) and the water layer, whereas 1% Tween 80 was the vehicle for the other fractions. FG7142: *N*-methyl- β -carboline-3-carboxamide; Trypt.: L-Tryptophan.

($P < 0.05$). On the other hand, at more higher doses, 0.01 – 1.0 mg/kg, the alkaloid fraction increased the CC and decreased the EC at 0.01 and 1 mg/kg ($P < 0.05$). The alkaloid fraction at 5 mg/kg did not improve the maze performance but instead increased the EC,

although nonsignificantly.

Examination of the effects of fractions isolated from the residue that was not soluble in methanol showed that the internal solution, obtained by dialysis with Visking cellophane tubing, increased the CC ($P < 0.02$) only at 0.01 mg/kg, whereas it decreased the EC at 0.01–0.5 mg/kg ($P < 0.05$). No effect was produced by internal solution at 1 mg/kg. On the other hand, the external solution of the dialysis decreased the EC ($P < 0.01$) at 0.1–10 mg/kg, but increased the CC ($P < 0.05$) only at 10 mg/kg. The effect of the water layer was similar to that of the external solution (data not shown).

The present results revealed that MeOH fraction did not display any significant effect, whereas the hexane fraction increased only number of the CC without improving the EC (data not shown).

Discussion

The radial maze used in this study involves no aversive stimuli and is considered suitable for evaluating memory. Moreover, drugs that are clinically used in treatment of dementia have also displayed effectiveness in the radial-maze (15). The scopolamine-induced memory impairment has been used as a model to evaluate drugs that improve memory. The dose of scopolamine used in this study (0.5 mg/kg) is enough to impair the spatial memory, but lower than the 2–3 mg/kg effective in passive avoidance (16). It is noteworthy that scopolamine decreased the CC and increased the EC, suggesting that scopolamine disrupts both reference and working memory. However, the rate of impairment of the CC exceeded that of the EC, indicating greater impairment of the working memory than the reference memory. The effect of scopolamine on the memory is possibly a central type because the peripherally acting anticholinergic methylscopolamine can prolong the running time without affecting the choice accuracy in the radial maze task (17) and elevated plus-maze test (18). The scopolamine-induced performance impairment is related to muscarinic acetylcholine receptor blockade, but not to choline acetyltransferase activity (19). However, the scopolamine-induced memory disruption could also involve other mechanisms as noncholinergic drugs like low dose of amantadine and *L-threo*-DOPS (noradrenergic enhancer) improve the spatial memory disrupted by scopolamine (20).

There are several reports on the usefulness of herbal drugs in the treatment of cognitive disorders. It has been reported that TSS could elongate the life span and median survival by preventing the senility (21). Moreover, treatment with TSS has also been shown to

enhance the cognitive function of post-menopausal women with Alzheimer's disease (9) and to improve the daily life of Alzheimer's disease patients (22) by increasing the cholinergic activity, the dysfunction of which is implicated in Alzheimer's disease (23).

TSS produces different behavioral and biochemical effects depending on frequency of administration (single or repeated) and the dose. It has been reported that single administration of TSS inhibits vertical and horizontal locomotor activities and inhibited the scopolamine-induced increase in locomotor activities (24). Moreover, single administration of TSS also produces different neurochemical effects such as stimulation of the function of the dopaminergic system and inhibition of that of the adrenergic nervous system (25). In this study, single administration of TSS improved the scopolamine-induced memory disturbance. Our results are in line with those reporting the memory improving effect for the TSS (12, 26). It is noteworthy that three components of the TSS (*Toki*, *Takusha*, and *Sojutsu*) increased the CC and decreased the EC, whereas two components (*Senkyu* and *Bukuryo*) only increased the CC, and one component (*Shakuyaku*) did not display any improving effect on either CC or EC. Accordingly, it could be suggested that the TSS, by virtue of five herbal components, improves the reference and working memory, with greater improvement of the former. The effect of TSS is a central one since TSS did not affect neuromuscular transmission in the frog sartorial muscle in spite of slightly depolarizing the membrane potential and strongly decreasing the peak heights of the Na^+ and Ca^{2+} current components of the action potential in the order *Sojutsu* \gg *Shakuyaku*, *Takusha*, *Toki*, *Senkyu* (27). The mechanism underlying the memory-enhancing effect of TSS is displayed on cholinergic neurons either indirectly without induction of choline acetyltransferase activity (28) or directly by increasing acetyl choline synthesis (29) and release (our unpublished data) and inhibiting scopolamine-induced decrease in acetylcholine levels (24). However, the memory improving effect of TSS could also involve mechanisms other than enhancement of the cholinergic activity because TSS stimulates the dopaminergic function in the hippocampus (25) and olfactory bulbs (1); it increases concentrations of γ -aminobutyric acid (GABA), alanine, and glycine in the cortex, hippocampus, and striatum of senescence accelerated mice (30); and dopaminergic and noradrenergic agents have also been reported to improve the scopolamine-induced memory impairment (20).

One study concerning *Shakuyaku* is available in the literature reporting that the water soluble fractions, containing the glycoside paeoniflorin, attenuate spatial working memory deficit caused by scopolamine at the

dose of 0.3 mg/kg, i.p. injected 30 min before testing and 60 min after the *Shakuyaku* fractions (17). However, our study showed that the whole *Shakuyaku* did not improve the memory. This discrepancy could possibly be attributed to the dose of scopolamine, injection time, employment of the whole or fraction of *Shakuyaku*, and different effects of gut floral metabolism on the whole plant or its fraction.

In this study we investigated the effect of *Toki* and its various fractions on scopolamine-induced memory impairment. The effect of *Toki* on the EC took on an inverse bell-shape, whereas that on the CC was increased at the doses of 0.1–5 mg/kg, then decreased as the dose was increased beyond 10 mg/kg (bell-shaped). This result is similar to that reported for purified ginsenosides (31) and cholinergic drugs (32, 33). The present results revealed that the fraction extracted by butanol displayed memory improving activity, because the other parts extracted with hexane and methanol were inactive. The activity of the butanol fraction could be attributed to its contents of β -carboline, nucleoside, and amines. We do not think that acute intake of nucleosides participate in the memory improving effects of *Toki* because only chronic oral administration of nucleoside, but not the acute one, is reported to be associated with a reduction in the age-related deterioration of brain morphology and memory (34). This was also the reason for not investigating the effect of nucleosides in this study. One of the active ingredients participating in the memory improving effect of *Toki* and rendering it distinctively efficient in this regard is FG7142 in the butanol layer. FG7142 is a partial inverse agonist of benzodiazepine that could indirectly increase the mnemonic function of cholinergic neurons by reducing the GABA-ergic inhibition. FG7142 has been reported to reverse scopolamine-induced mistakes (35) and to improve memory when injected into the nucleus basalis prior to training in the double Y-maze (36, 37) and intralaminar thalamic nuclei in delayed matching-to sample (38). It should be noted that FG7142 improved the memory at 0.01 mg/kg by increasing the CC and decreasing the EC. It produced no effect at 0.1 mg/kg, whereas at 1 mg/kg it decreased the EC without affecting the CC. This result may be attributed to the biphasic property of the effect of FG7142 due to increasing performance at low dose and decreasing it at high dose (38, 39).

L-Ornithine is one of the amines present in the butanol fraction from the *Toki*. L-Ornithine is a member of the “glutamate family” of amino acids, which also includes glutamine, glutamate, proline, histidine, and arginine. L-Ornithine metabolism is very important in brain function. It has been reported that inhibition of the

catabolism of L-ornithine provides additional protection against electroshock-induced seizures (40). Moreover, L-ornithine decarboxylase disruption leads to abnormal expression of nicotinic acetylcholine receptors and is involved in the adverse neurobehavioral effects of numerous neuroteratogens (41). Although the exact role of L-ornithine in regulation of memory is not adequately investigated yet, L-ornithine and the enzymes involved in its metabolism concentrate in brain regions associated with memory control and regulation. Arginase II protein, synthesizing L-ornithine from L-arginine, shows an extremely high expression in the brain with the highest level in the dentate gyrus (42). Moreover, the cerebral cortex, hypothalamus, and hippocampus exhibit a high activity of L-ornithine- δ -aminotransferase, a proline biosynthetic enzyme (43). The present results provide evidence that arginine, although it is a precursor of L-ornithine, only decreased the EC but L-ornithine additionally increased the CC. Accordingly, it could be suggested that L-ornithine could be essential for preventing memory impairment, at least the one caused by cholinergic dysfunction. A plethora of data suggest a memory improving effect of the amino acid L-tryptophan as a precursor of serotonin. However, L-tryptophan depletion is also reported to be not sufficient for induction of memory impairment (44) or even that L-tryptophan may itself cause memory deficit (45). In this study, L-tryptophan increased the EC. This result may be associated with an increased activity level rather than specific error increasing property of L-tryptophan. Moreover, the present results also showed that the saccharides, especially polysaccharides in the internal solution, are also involved in the effect of *Toki* on the EC.

The present study revealed the phasic nature of the memory improving effect shared by the alkaloid in the activity of the *Toki*. The results showed that the alkaloid fraction increased the CC at 0.1 and 1 ng/kg level, decreased the EC at 0.01–1 μ g/kg level, and improved maze performance by increasing the CC and decreasing the EC at 0.01–1 mg/kg, whereas no effect was obtained with the higher dose of 5 mg/kg. However, in this study we did not determine the type and structure of the alkaloid, which remain to be determined by further studies.

In conclusion, the present results revealed that TSS could improve the reference and working memory impaired by scopolamine. The activity of the *Toki* is produced mainly by alkaloid and the fraction extracted by butanol containing β -carboline and L-ornithine. The polysaccharides share the working memory (EC-decreasing) improving effect of *Toki*.

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