Effect of yokukansan on memory disturbance in an animal model of cerebrovascular dementia

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Abstract

Yokukansan (YKS) is a traditional Japanese herbal medicine, which was reported to improve the behavioral and psychological symptoms of dementia (BPSD). However, the effect of YKS on memory dysfunction remains unknown. In this study, we examined the effect of YKS on impaired spatial memory in rats subjected to repeated cerebral ischemia, a well-established animal model for cerebrovascular dementia. Additionally, we compared the effect of YKS and donepezil (DPZ) on cholinergic dysfunction and hippocampal CA1 neuronal death in rats subjected to repeated cerebral ischemia. Spatial memory, as assessed using the eight-arm radial maze task, was impaired by repeated cerebral ischemia and significantly improved following administration of YKS (100, 300, 1000 mg/kg per day, p.o. for 14 days before and after ischemia treatment. Furthermore, a significant improvement was observed following additional 7-day treatment with YKS (1000 mg/kg per day, p.o.) after ischemia. YKS treatment was comparable to DPZ (10 mg/kg per day, p.o.) after ischemia. Microdialysis studies showed that spontaneous release of acetylcholine (ACh) from the dorsal hippocampus was significantly reduced following repeated cerebral ischemia. However, treatment with YKS or DPZ increased spontaneous ACh release following repeated cerebral ischemia. In contrast, hippocampal apoptosis, which developed after repeated ischemia, was suppressed by YKS, but not by DPZ. Overall, we found that YKS improves spatial memory disturbance via its unique character of having both an increasing effect on ACh release and a neuroprotective effect, which will be useful not only for BPSD but also memory dysfunction in cerebrovascular dementia patients.

Key words Dementia, Memory, Yokukansan, Acetylcholine, Apoptosis.

Introduction

Patients with cerebrovascular dementia (VaD) or Alzheimer's disease (AD) often show cognitive dysfunction as a core symptom; other common behavioral and psychological symptoms of dementia (BPSD) include hallucinations, paranoia, depression, aggressiveness, and wandering. Memory disturbance is a core symptom of dementia. Recent studies based on the molecular causes of the disease have been widely undertaken, and treatment methods and curative medicines for its improvement have been actively carried out throughout the world. For dementia patients, reduction of hippocampal cholinergic function is known to play an important role in memory loss, and the use of central acetylcholinesterase inhibitors such as donepezil (DPZ) to increase this function has already begun. Another problem is that

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BPSD occurs comparatively early and causes a reduction in daily activities, which places a heavy burden on those providing nursing care. However, clinical and not pharmacologic treatment for BPSD, the mechanism of which is still unclear, has been established. In actuality, antipsychotic drugs\(^1\) as a curative drug for schizophrenia, and acetylcholinesterase inhibitors\(^2\) as a curative drug for dementia, are used to treat these symptoms.

Yokukansan (YKS, yi-gan san in Chinese), a traditional Japanese herbal medicine, has been reported to be clinically effective for BPSD in Alzheimer’s dementia patients.\(^3\)\(^,\)\(^4\) Egashira et al. reported that YKS shows a dose-dependent inhibition of ambulation, rearing and aggressive behaviors in mice,\(^7\) suggesting that the prefrontal serotonin neurons, especially serotonin receptor (5-HT\(_{2A}\)) function, are strongly related to these behavioral changes. Terawaki et al. reported that YKS shows a partial agonistic action by binding to 5-HT\(_{1A}\) receptors.\(^5\) In terms of serotonergic involvement, we speculate that 5-HT\(_{2A}\) receptor down-regulation by YKS may be the result of an intracellular response through the partial agonistic effect on 5-HT\(_{1A}\) receptors.

The core symptoms of AD and VaD are memory and cognitive dysfunction. Progression of these core symptoms are further exacerbated by BPSDs. The efficacy of YKS for BPSD in dementia has been presented in both AD and VaD animal studies\(^7\)\(^,\)\(^8\) and in clinical applications.\(^9\) Core symptoms and BPSD in VaD patients do not occur separately; usually they occur concurrently. Thus an effective medicine for both core symptoms and BPSD is needed. DPZ, a cholinesterase inhibitor, nimodipine, a calcium antagonist, and nicergoline, a cerebral metabolic enhancer, are used as symptomatic therapies for the core symptoms of VaD\(^7\); however, there is no effective drug for only BPSD in VaD.

In this study, we examined the effect of YKS on spatial memory in rats subjected to repeated cerebral ischemia using the eight-arm radial maze task. The effect of YKS on acetylcholine nerve terminals, which project from the septum to the hippocampus, was examined using the intracerebral microdialysis method. In addition, we tested whether YKS has a neuroprotective effect by examining hippocampal apoptosis in rats with experimental cerebral ischemia.

**Materials and Methods**

**Animals:** Male Wistar rats weighing 300-350 g (KYUDO Co., LTD, Saga, Japan) were kept in a room at a controlled temperature (23 ± 2°C), with a relative humidity of 60 ± 5%, and a 12-h light/dark cycle. Food and water were available *ad libitum*, except that the rats used in the eight-arm radial maze task were subjected to a restricted feeding schedule. Animal care and the experimental procedures were based on the regulations of the Animal Care and Use Committee of Fukuoka University.

**Eight-arm radial maze task:** The radial maze apparatus used in this study (Neuroscience Co., Tokyo, Japan) was a modified version\(^12\) of that originally adopted by Olton and Samuelson.\(^13\) It consists of eight equally spaced, transparent acrylic arms (50 cm long, 10 cm wide, with a transparent 50 cm high side wall) extending from a central octagonal hub (24 cm across), surrounded by an opaque guillotine door at the entrance of each arm. The maze was elevated 50 cm from the floor. Food cups (3 cm diameter, 1 cm depth, black acrylic), mounted at the end of each arm, 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.

The schedule started at the beginning, and continued throughout the experiment. The schedule was achieved by reducing the daily consumption of ration (10-12 g/day; CE-2, Clea Japan, Tokyo, Japan) so that the body weight of each rat was maintained at 80-90 % of the free-feeding level. Water was always available *ad libitum*.

**Assessment of maze performance:** The rats were pretrained in groups (five rats/group) with the apparatus and reinfocer food pellets for 3 days, three times daily for 10 min with repetition after 60 min. The training began 1 day after pretraining and was performed three times/day for 14 days to allow the rats to learn how to perform the maze task. In the training and drug test trials, each rat was placed in the central platform, then the guillotine was lifted after 1 min and the rats were allowed to move freely in the maze to the baited arms.
The trial continued until the test animal had entered all eight arms and consumed the bait, or until 10 min had elapsed. If the test animals proceeded in the eight-arm radial maze task using sequential routes consisting of repeating a given angular direction (such as 45°, 135°) to the neighboring arm, then such animals were excluded from the present experiment. Only rats that made no errors or only one error in 3 consecutive days were selected for the study.

Performance assessment: The following two parameters were considered criteria for radial maze performance: 1) the number of correct choices in the initial eight chosen arms (entry into an arm that the animal had not previously visited); 2) number of errors (reentry into an arm that the rat had previously visited).

Four-vessel cerebral ischemia: Four-vessel occlusion was performed according to the method described by Pulsinelli et al.,14 and according to our previous study. Briefly, rats were anesthetized with 50 mg/kg sodium pentobarbital intraperitoneally (i.p.) and immobilized in a stereotaxic apparatus. The bilateral vertebral arteries were electrocauterized with a bipolar coagulator (MICRO-3D; Mizuo Industrial Co., Tokyo, Japan). Repeated ischemia was induced for 10 min by occluding both the common carotid arteries using aneurysm clips, and this was repeated once after 1 h. These rats were referred to as repeated cerebral ischemic rats. Body temperature was maintained at 37°C using a heating pad and heating lamp until recovery from anesthesia after surgical operation, or until the righting reflex reappeared following occlusion of carotid arteries. The rats that did not demonstrate loss of their righting reflex during arterial occlusion were excluded from the subsequent experiment. The rats that only underwent cauterization of the vertebral arteries and then were fitted with occluders on the common carotid arteries without occlusion were used as sham-operated controls.

Microdialysis procedures: The extracellular ACh levels were measured in the dorsal hippocampus by in vivo microdialysis in non-anesthetized freely moving rats. At 7 days after repeated cerebral ischemia, the extracellular ACh levels were measured before the last administration of YKS, vehicle, or DPZ. The microdialysis probes were 13 mm long, 0.5 mm i.d., and 0.6 mm o.d. (Eicom, Kyoto, Japan), with an active dialysis membrane exposed at the tip. The dialysis membrane was a U-shaped tube (each arm 2 mm long, 0.22 mm i.d., 3.14 mm o.d., total length of both arms 4.5 mm) made of hollow cellulose fiber with a molecular weight cut-off value of 50 kDa. Ringer-primed probes were implanted through the guide cannulae with the U-shaped dialysis membrane parallel to the longitudinal axis of the brain, protruding 2 mm into the dorsal hippocampus through the tip of the guide cannulae, and were secured by caps. Rats were then placed in a Plexiglas chamber (50 cm × 35 cm × 35 cm). The inlet of the probe was connected to the descending limb of the spatial two-way tubing that was, in turn, connected via a pair-ring swivel system to another Teflon tube connected to a micro syringe driven by a pump (Eicom SP-64, Eicom). The outlet of the probe was connected to the ascending limb of the swivel system, connected by another Teflon tube to an autoinjector (Eicom-EAS-20). The tubes were 0.1 mm i.d., 0.4 mm o.d., and 50 cm long (Eicom). The implanted probe was perfused with Ringer’s solution or high-potassium Ringer’s solution (147 mM NaCl, 4.02 mM KCl or 100 mM KCl for high-K⁺, and 2.2 mM CaCl₂·2H₂O containing 0.1 mM serine, at a flow rate of 1 μL/min. Dialysis aliquots of 20 μL were collected every 20 min. The collection was then subjected to high-performance liquid chromatography (HPLC). The HPLC system (HTEC-500; Eicom) consisted of a degasser, an HPLC pump system, a column temperature controller, and an electrochemical detector with a platinum electrode. A pre-column (3.0 × 40 mm) (Eicom) and an enzyme column (φ3.0 mm × 40 mm) (AC-Enzympak; Eicom) were placed before and after the analytical column (φ2.0 × 150 mm) (Eicompack AC-GEL; Eicom), respectively. Following its elution from the analytical column, ACh was converted to hydrogen peroxide inside the enzyme column. The analytical and enzyme columns and the electrode were kept at 33°C by a column temperature controller. Phosphate buffer (0.05 M, pH 8.2) containing 300 mg/L sodium 1-decanesulfonate and 5 mg/L ethylenediamine-N,N,N′,N′-tetraacetic acid, disodium salt, dihydrate was used for the mobile phase. The flow rate of the mobile phase was 150 μL/min.

We examined the time course of ACh release and its
total amount from the dorsal hippocampus. Five fractionated samples from each group were combined to determine the amount of ACh release.

**Histochemical studies:** Rats were sacrificed by decapitation 7 days after repeated cerebral ischemia. Fat and water were removed from brains in an autoclaving unit (RH-12, Sakura Seiko Co., Tokyo, Japan) and then embedded in paraffin. Subsequently, 5 μm-thick sections were mounted on slides and dried at 37°C for a day. Hematoxylin and eosin (HE) staining was carried out. After deparaffinization and rehydration, sections were incubated in proteinase K buffer for 10 min. The sections were then incubated with terminal deoxynucleotidyl transferase incubation buffer for 60 min, standard saline citrate for 15 min and propidium iodide for 15 min. Coverslips were mounted onto slides and sections were analyzed by fluorescence microscopy. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells (green; Fluorescein-4-isothiocyanate (FITC)) in sections that included the dorsal hippocampus were counted.

**Caspase-3 measurement:** The expression of caspase-3 protein was evaluated by western blotting, following sample extraction and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Rats were sacrificed after the behavioral test. Hippocampi were separated by dissection and homogenized in T-PER Tissue Protein Extraction Reagent (Thermo Fisher Scientific K.K., Waltham, MA, USA) with protease inhibitor cocktail (Nacalai Tesque, Kyoto, Japan), and centrifuged (4°C, 20,400 × g, 30 min) to extract the protein. Protein concentrations were determined using the Bradford method. Equal amounts of protein were separated on 15% (w/v) SDS-PAGE (Bio-Rad, Hercules, CA, USA), and then transferred to 0.22 μm polyvinylidene difluoride membranes using a Trans-Blot semi-dry system (Bio-Rad). The membranes were blocked in 5% (w/v) skim milk in Tris-buffered saline with Tween 20 for 1 h and then incubated overnight at 4°C with the following primary antibodies: anti-caspase-3 (1:1000; Cell Signaling Technology, Inc., Danvers, MA, USA) and anti-β-actin (1:5000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Next, the membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibody (bovine anti-rabbit IgG, 1:10000; Cell Signaling Technology, Inc.) for 2 h at room temperature. The blots were developed using a chemiluminescence kit (GE Healthcare, Hertfordshire, UK) and were exposed to film. The bands on the film were scanned and analyzed with Image J.

**Treatment and schedule:** YKS provided by Tsumura & Co. Yokukansan was dissolved in distilled water and orally administered 1 h before testing in the eight-arm radial maze task. YKS was orally administered for 7 days before, during and after ischemia treatment, for a total of 14 days (14-day group). For cases of administration after ischemia, YKS was administered for 7 days after ischemia treatment (7-day group). For the control group, distilled water was orally administered at the same schedule. DPZ provided by Eisai Co., Ltd. DPZ was dissolved in distilled water and orally administered for 7 days after ischemia.

**Statistical analysis:** Performance in the eight-arm radial maze was analyzed by the Mann Whitney U-test. For HPLC, statistically significant differences between groups, extracellular ACh levels and their variation with repetition were evaluated using ANOVA-for repeated measures. Post hoc comparisons were performed by Dunnett’s test whenever ANOVA revealed a significant difference.

**Results**

**Effect of YKS on spatial memory disturbance:** Spatial memory in the eight-arm radial maze was significantly impaired following 10-min repeated cerebral ischemia with 1-h intervals. When YKS (100-1000 mg/kg/day) was administered for a total of 14 days before and after the ischemia treatment (14-day group), a significant decrease in the number of errors was observed at a dose of 1000 mg/kg when compared with the vehicle-administered group. The tendency toward an increased number of correct choices was also observed at the same dose (Fig. 1). Seven days after the ischemia treatment, YKS (7-day group) significantly decreased the number of errors and tended to increase the number of correct choices (Fig. 2). DPZ, which has been
**Figure 1** Effect of 14-day pre- and post-ischemic treatment with YKS on spatial memory impairment following cerebral ischemia.

(A) Schedule of eight-arm radial maze and treatment with YKS. (B) The number of correct choices in the eight-arm radial maze task in rats. (C) The number of errors in the eight-arm radial maze task in rats. Values are expressed as the mean ± S.E.M. of five to eight rats. **P<0.01 vs. Vehicle group, Dunnett test.**

**Figure 2** Effect of 7-day post-ischemic treatment with YKS and DPZ on spatial memory impairment following cerebral ischemia.

(A) Schedule of eight-arm radial maze and treatment with YKS. (B) The number of correct choices in the eight-arm radial maze task in rats. (C) The number of errors in the eight-arm radial maze task in rats. Values are expressed as the mean ± S.E.M. of six to eight rats. **P<0.01 vs. Vehicle group, Dunnett test.**
commonly used in AD treatment, also significantly reversed the reduction in the number of correct choices, and increased the number of errors in repeated ischemia rats at a dose of 10 mg/kg (Fig. 2).

**Effect of YKS on ACh release from the dorsal hippocampus:** ACh release from the dorsal hippocampus was observed following repeated cerebral ischemia in rats using the brain microdialysis method. Following repeated cerebral ischemia, there was a significant reduction in basal release of ACh from the dorsal hippocampus 7 days after ischemia treatment (Fig. 3). Furthermore, the significant increase in depolarization-evoked release by high-K⁺ was not observed (Fig. 3). In contrast, in both the 14-day and 7-day treatment groups, YKS (1000 mg/kg, p.o.) significantly increased the basal release and high-K⁺-evoked ACh release from the dorsal hippocampus (Figs. 3, 4). There was also a significant increase in the basal release and high-K⁺-evoked ACh release from the dorsal hippocampus when 10 mg/kg of DPZ was administered for 7 days after ischemia. However, the degree of change was smaller than for YKS (Fig. 4). In addition, ACh release from the dorsal hippocampus before the last administration of YKS was significantly increased (Figs. 3, 4), but did not increase in the DPZ treatment group.

**Effect of YKS on neuronal cell death in the hippocampus:** Seven days after repeated cerebral ischemia treatment, HE staining revealed pyknosis, eosinophilia, karyorrhexis, and chromosome condensation in the CA1 pyramidal neurons of the vehicle-treated repeated ischemia group when compared with the sham group (Fig. 5: A, B, C) (Fig. 6: A, B). The number of TUNEL-positive cells in the hippocampus CA 1 region was increased (Fig. 5: E, F, G) (Fig. 6: E, F). The suppression of neuronal cell death by HE staining and the disappearance of TUNEL-positive cells were suppressed in the YKS 14-day group (Fig. 5: D, H). Likewise, neuronal cell death was suppressed in the YKS 7-day group (Fig. 6: C, G). In contrast, DPZ did not affect the ischemia-induced neuronal cell death (Fig. 6: D, H). In repeated cerebral ischemia rats, caspase-3 was significantly increased when compared with sham-operated

![Figure 3](https://example.com/figure3.png) **Figure 3** Effect of 14-day pre- and post-ischemic treatment with YKS on ACh release from the dorsal hippocampus. Extracellular ACh levels are expressed as fmol/μl. Rats were perfused with Ringer’s solution or high-potassium Ringer’s solution. Vehicle or YKS was administered for a total 14 days before and after ischemia. The extracellular ACh levels before the last administration of Vehicle or YKS are expressed. Extracellular ACh levels were continuously monitored after administration of Vehicle or YKS. (A) Time courses of ACh release from the dorsal hippocampus. (B) Integrated amount of ACh release from the dorsal hippocampus. Values are expressed as the mean ± S.E.M. of four to six rats. *P<0.05, **P<0.01 vs. Vehicle group, Dunnett test.
Figure 4 Effect of 7-day post-ischemic treatment with YKS and DPZ on ACh release from the dorsal hippocampus. Extracellular ACh levels are expressed as fmol/μl. Rats were perfused with Ringer's solution or high-potassium Ringer's solution. Vehicle, YKS or DPZ were administered for a total of 7 days after ischemia. At 7 days after repeated cerebral ischemia, extracellular ACh levels before the last administration of Vehicle, YKS or DPZ were monitored. Extracellular ACh levels after administration of Vehicle, YKS or DPZ were monitored. (A) Time courses of ACh release from the dorsal hippocampus. (B) Integrated amount of ACh release from the dorsal hippocampus. Values are expressed as the mean ± S.E.M. of four to six rats. *P<0.05, **P<0.01 vs. Vehicle group, Dunnett test.

Figure 5 Effect of 14-day pre- and post-ischemic treatment with YKS on neuronal cell death in the hippocampal CA1 region. Histological sections of the hippocampal CA1 field showing HE staining (A-D) and TUNEL staining (E-H). Sham (A, B, E, F), cerebral ischemia treated with vehicle (C, G), cerebral ischemia treated with YKS 1000 mg/kg (D, H). Red cells: Propidium iodide-stained cells, Green cells: TUNEL-positive FITC-stained cells. Calibration bar = 20 μm.
rats (Fig. 7: A, B). In contrast, in both the 14-day and 7-day treatment groups, YKS (1000 mg/kg, p.o.) decreased the amount of caspase-3 in the hippocampus (Fig. 7: A, B). On the other hand, this change was not observed with the administration of 10 mg/kg of DPZ (Fig. 7: B).

Figure 6 Effect of 7-day post-ischemic treatment with YKS and DPZ on neuronal cell death in the hippocampal CA1 region. Histological sections of the hippocampal CA1 field showing HE staining (A-D) and TUNEL staining (E-H). Sham (A, E), cerebral ischemia treated with vehicle (B, F), cerebral ischemia treated with YKS 1000 mg/kg (C, G), cerebral ischemia treated with DPZ 10 mg/kg (D, H). Red cells: Propidium iodide-stained cells, Green cells: TUNEL-positive FITC-stained cells. Calibration bar = 20 μm.

Figure 7 Effect of YKS and DPZ on caspase-3 expression in the hippocampus. Samples for measurement of caspase-3 expression were obtained 6 days after ischemic treatment. (A) Effect of 14-day pre- and post-ischemic treatment with YKS. (B) Effect of 7-day post-ischemic treatment with YKS. Values are expressed as the mean ± S.E.M. of five to nine rats. **p<0.01 vs. Vehicle group, Dunnett test.

Discussion

In this study, we showed the efficacy of YKS for BPSD, using repeated cerebral ischemia, a well-established animal model of VaD in rats. Our results suggest that YKS is effective for spatial memory disturbance via its unique character of both increasing ACh release from the dorsal hippocampus and preventing neuronal apoptosis following ischemic injury. The
effective dosage for the improvement of spatial memory was the same as the effective dosage for BPSD. YKS is thought to be effective not only for BPSD but also for core symptoms such as memory loss in cerebrovascular dementia patients.

Furthermore, an effect was observed with administration of YKS for 7 days after ischemia treatment. This effect was also observed with 7-day administration of DPZ, which is reported to be effective for AD. Recently, efficacy has been reported in clinical tests for VaD using DPZ. In addition to the improvement in core symptoms following YKS treatment, YKS also improved ischemia-induced depression. Therefore, YKS may be clinically useful. Furthermore, YKS has the advantage of suppressing BPSD, which DPZ does not, and so can be considered a curative drug with a very high use value.

On the other hand, ACh nerve terminals project from the septum to the hippocampus and are known to be related to memory and the core symptom of dementia. In a previous study from our laboratory, we reported that the repeated cerebral ischemia-induced impairment of spatial memory is due to dysfunction of the hippocampal and cortical ACh systems. Our results suggest that ACh release from the hippocampus is reduced by repeated cerebral ischemia. However, administration of YKS increased the basal rate of spontaneous ACh release, and also increased the release of ACh induced by high-K+. Our finding that YKS enhances ACh release by neurons, especially in the hippocampus in animals, is a new discovery. We previously reported that YKS significantly restored the expression of dynamin 1, an important factor in synaptic vesicle endocytosis in the hippocampus of rats treated with Aβ oligomers and cerebral ischemia, returning them to levels seen in sham-treated rats. YKS may have increased ACh release by acting on presynaptic terminals.

It is interesting that YKS significantly increased not only ACh release after the last administration of YKS, but also spontaneous ACh release before the last administration of YKS. This result means that YKS improves spontaneous ACh release function. The ameliorative effect of YKS on spatial memory impairment in our rat model of VaD may be mediated by activation of ACh release and improvement of spontaneous ACh release function. In contrast, DPZ could not improve spontaneous ACh release function. DPZ increased the amount of ACh in the synaptic cleft through inhibition of acetylcholinesterase (AChE).

We previously reported that the repeated cerebral ischemia-induced impairment of spatial memory may be due to dysfunction of hippocampal neurons. In the present study, we found that YKS prevented neuronal apoptosis following ischemic injury. We have reported that repeated cerebral ischemia changes the composition ratio of glutamate receptor 1 and glutamate receptor 2, which consist of α-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors and divalent ions such as Ca2+, which usually do not pass through, but started to enter cells here and induce apoptosis. This Ca2+-overload may cause cellular toxicity and induce spatial memory disturbance. In the present study, we report that YKS decreased the amount of caspase-3 and TUNEL-positive cells. Basal levels of ACh release (before final administration) were increased in the 14-day YKS treatment group compared with those of the 7-day YKS treatment group. Neuroprotective effects may have been involved in this difference. Compared with the 7-day YKS treatment group, the 14-day YKS treatment group had the tendency to decrease the amount of caspase-3 in the hippocampus (data is not shown). Therefore, ACh release from cells of the 14-day YKS treatment group may be more sustained than that of the 7-day YKS treatment group. YKS and DPZ activated the AChergic system and improved spatial memory deficit despite their differing mechanisms of action. Our results suggest that YKS maintains ACh function via its neuroprotective action. Furthermore, ACh release was shown to be increased by restoring the expression of dynamin1. Thus, the expression of dynamin1 may play a role in YKS-mediated activation of AChergic neurons. In contrast, DPZ has been shown to enhance the AChergic system via the inhibition of AChE and elevation of the ACh concentration in the synapse. To the best of our knowledge, this is the first study demonstrating the ameliorative effects of YKS on impaired spatial memory and a suppressive effect on neuronal apoptosis in the VaD rat model. A relationship of nerve cell death via glutamate and YKS was reported by Kawakami et al. They reported that YKS significantly improves the activity of glutamic acid transporters (GLAST and GLT-1).
in cultured astrocytes under thiamine-deficient conditions. YKS has also been shown to bind to NMDA receptors, and this is closely related to the glutamatergic mechanism.

In clinical settings, there is no evidence regarding the effectiveness of YKS on memory impairment. There are two reasons why clinical research does not correlate with our laboratory studies. Most clinical research on YKS has been performed in short studies (4-12 weeks), because it has the purpose of examining the effect of YKS on BPSD. To clarify the effect of YKS for memory impairment, a longer-term study may be necessary. Because the effective dosage for the improvement of spatial memory was the same as that for BPSD, there is thus a possibility to improve memory impairment in the clinical setting.

There are several studies that have revealed the usefulness of other herbal medicines in the treatment of cognitive disorders. Chotosan was demonstrated to have an ameliorating effect on cognitive dysfunction in stroke patients, and tokishakuyakusan inhibited the worsening of impairment and independence in post-stroke patients. Chotosan and tokishakuyakusan contain constituent herbs that are similar to those in YKS. The main extract of chotosan is Uncaria, which is also one of the main extracts in YKS. Tokishakuyakusan is a mixture of six medical plants; four of these are also in YKS. Our data suggest that the herbal components of YKS have similar ameliorating effects on cognitive dysfunction, consistent with previous work on similar plant-derived medicines. Tokishakuyakusan and chotosan are useful for stroke patients. Therefore, YKS may be effective for the clinical improvement of core symptoms based on such criteria as targeted in VaD patients. Furthermore, by combining crude drugs, commonly found in herbal medicine, which may be efficacious against cognitive disorders, a specific mechanism of action (e.g. activation of the AChergic system) may be found and thus, the active substance discovered.

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