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Full paper

Tokishakuyakusan ameliorates spatial memory deficits induced by ovariectomy combined with β -amyloid in rats[☆]

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ABSTRACT

Previously, we reported that ovariectomy (OVX) combined with β -amyloid peptide (A β) impaired spatial memory by decreasing extracellular acetylcholine (ACh) levels in the dorsal hippocampus. Here, we investigated the effect of tokishakuyakusan (TSS), a *Kampo* medicine, on the impairment of spatial memory induced by OVX combined with A β in rats. Repeated administration of TSS (300 mg/kg, p.o.) significantly decreased the number of errors in the eight-arm radial maze test. Though TSS had no effect on extracellular ACh levels at baseline, TSS significantly increased extracellular ACh levels in the dorsal hippocampus. These results suggest that TSS improves the impairment of spatial memory induced by OVX combined with A β by (at least in part) increasing extracellular ACh levels in the dorsal hippocampus.

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Introduction

Alzheimer's disease (AD) is characterized by: clinical evidence of cognitive failure in association with cerebral amyloidosis; cerebral intraneuronal neurofibrillary disease; neuronal and synaptic loss; and neurotransmitter deficits.¹ β -Amyloid peptide (A β) aggregate is one of the major constituents of the senile plaques in the brain regions serving memory and cognition, and plays a causative part in the neuronal degeneration and memory loss seen in AD patients.² In transgenic mice, a 14-fold increase in A β _(1–42/43) has been reported to accompany the appearance of behavioral deficits.³ Moreover, A β _{1–42} is neurotoxic,⁴ and intracerebroventricular infusion of A β _{1–42} impairs learning and memory deficits in rats.⁵

AD burden falls more heavily on women than men. It has been hypothesized that plummeting levels of circulating estrogens after

the menopause increase a woman's risk for this disorder.⁶ In animals, prolonged ovariectomy (OVX) has been shown to result in uterine atrophy and reduced serum levels of 17 β -estradiol, and to be associated with a pronounced increase in brain levels of A β . Total brain A β in ovariectomized (OVX) guinea pigs has been shown to increase by 1.5-fold on average as compared with intact controls.⁷ Yamada et al.⁸ reported A β -induced working memory deficits to be potentiated significantly in OVX rats compared with sham-operated rats. We also investigated the effects of OVX and A β _{1–42}, separately and in combination, on performance in the radial arm maze. We found that OVX combined with A β impaired spatial memory by decreasing extracellular acetylcholine (ACh) levels and alpha7 nicotinic acetylcholine receptor expression without inducing apoptosis in the dorsal hippocampus.⁹ Thus, OVX seems to potentiate the A β -induced impairment of spatial memory and neurotransmitter in rats.

Decades ago, postmenopausal hormone replacement was considered a panacea for middle-aged women. Prevention of age-related cognitive decline was among the major alleged benefits of this therapy. However, a recent systematic review and prospective cohort study showed that postmenopausal hormone therapy is not associated with a risk of all-cause dementia or AD.^{10,11}

[☆] Subject categories: Natural medicine materials (NMM) with significant biological activities; Central nervous system pharmacology.

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Tokishakuyakusan (TSS) is a *Kampo* medicine, which are Japanese traditional medicines. TSS has been used for the treatment of mainly gynecologic symptoms and cognitive disorders in elderly women due to its estrogen-secreting qualities. TSS can be recommended as anti-dysmenorrhea therapy for women with endometriosis or adenomyosis who wish to become pregnant.¹² TSS has also been used in AD treatment, especially in postmenopausal women.¹³ We have reported that TSS: improves scopolamine-induced impairment of spatial memory in rats¹⁴; protects A β _{25–35}-induced neuronal damage and lipid peroxidation in cultured rat cortical neurons¹⁵; prevents impairment of spatial memory induced by repeated cerebral ischemia in rats.¹⁶ However, the effect of TSS on the impairment of spatial memory induced by OVX combined with A β has not been explored.

The present study was conducted to investigate the effect of TSS on the impairment of spatial memory induced by OVX combined with A β in rats. We also investigated the effects of TSS on extracellular ACh levels in the dorsal hippocampus, which is an important brain site with regard to spatial memory.

Materials and methods

Animals

Experiments were undertaken on 110 female Wistar rats (230–270 g; Kyudo, Tosu, Japan). Rats were housed in groups of 5 per cage (30 × 35 × 17 cm) in a room with controlled temperature (23 ± 2 °C), relative humidity (60 ± 2%) and a 12-h light–dark cycle with the light period starting at 7 am. Animals scheduled to undergo the eight-arm radial maze test (8ARMT) were placed on restricted (10–12 g per day) food (CE-2; Clea Japan, Tokyo, Japan) intake and maintained at ≈80% of their free-feeding body weight (which was determined during the experimental period). All animals had free access to drinking water in their home cages. All animal care and use procedures were performed in compliance with the regulations established by the Experimental Animal Care and Use Committee of Fukuoka University followed the Guidelines of the Science Council of Japan (approved no. 205460 of institutional review board).

OVX. Rats were anesthetized with halothane (Takeda Chemical Industries, Osaka, Japan) using a small animal anesthetizer (TK-4; Bio Machinery, Tokyo, Japan) and laid on a ventral surface with the tail pointing towards the experimenter. The skin was incised (2 cm) midline halfway between the hump and base of the tail, and retracted to the side where the ovary was to be removed. The abdominal muscle two-thirds of the way down the side was incised. The ovary was pulled out through the muscle incision by grasping the periovarian fat and removed.¹⁷ An identical procedure was done on the other side to remove the other ovary. These rats were referred to as “OVX rats”. Rats subjected to the same procedure but where only skin and muscles were cut, but the ovaries were spared, were referred to as “sham rats”.

8ARMT. The behavioral experiment was started on postoperative day (POD)1. The behavioral testing was conducted as reported previously⁹ using a modified version of the 8ARMT (Neuroscience, Tokyo, Japan) of the original maze developed by Olton and Samuelson.¹⁸ The trial continued until the rat had entered all eight-arms or 10 min had elapsed.

The performance of each animal in every trial was assessed using three parameters: (i) the number of correct choices in the initial eight chosen arms; (ii) the number of errors (defined by choosing arms that had been visited already); (iii) the time elapsed before the animal consumed all eight pellets. One training trial was given per day, Monday to Saturday. Only the rats that made no

errors, or only one error for 3 consecutive days, were selected for the study.

Stereotaxic procedure. Rats were anesthetized (sodium pentobarbital, 50 mg/kg, i.p.; Tokyo Kasei, Tokyo, Japan) and placed in a stereotaxic frame (Narishige Scientific Instruments, Tokyo, Japan) with the upper incisor bar 3.4-mm below the level of the interaural line. Guide cannulae were implanted according to the stereotaxic coordinates employed by Paxinos and Watson.¹⁹

In microdialysis studies, guide cannulae (length = 13 mm; outer diameter (o.d.) = 0.85 mm; inner diameter (i.d.) = 0.75 mm; Eicom, Kyoto, Japan) were implanted into the dorsal hippocampus at the following coordinates (mm): anteroposterior (AP) = –4.0 posterior to the bregma; lateral (L) = 3.3 from the midsagittal line; dorsoventral (DV) = 1.8 relative to the skull surface. For intracerebroventricular infusion, guide cannulae (o.d. = 0.71 ± 0.02 mm; i.d. = 0.41 ± 0.02 mm; length = 13 mm) were implanted bilaterally into the lateral cerebral ventricles at AP = –0.8, L = 1.3 and H = 3.3.

Drugs and experimental paradigm. A β _{1–42} HCl (Anaspec, San Jose, CA, USA) was dissolved in sterile distilled water, and allowed to aggregate for 7 days at 37 °C. A β _{1–42} was injected once daily for 7 days starting 3 weeks after OVX. A β _{1–42} was administered bilaterally (300 pmol/10 μ L) using an injection cannula (o.d. = 0.35 ± 0.01 mm; i.d. = 0.17 ± 0.02 mm; length = 14 mm) connected by polyethylene tubing (o.d. = 1.09 mm; i.d. = 0.38 mm; Intramedic; Becton Dickinson, Franklin Lakes, NJ, USA) to a perfusion pump (CMA/100; Microdialysis, Stockholm, Sweden) driven at a rate of 1 μ L/min.

TSS (Lot. No. 2010023010) was a generous gift from Tsumura & Co (Tokyo). TSS was a dried extract of the following raw materials: *Alismatis tuber* (*Alismataceae*, *Alisma orientale* Juzepczuk, 4.0 g), *Angelicae acutilobae radix* (*Umbelliferae*, *Angelica acutiloba* Kitagawa, 3.0 g), *Actractylodis lanceae rhizoma* (*Compositae*, *Actractylodes lancea* De Candolle, 4.0 g), *Cnidii rhizoma* (*Umbelliferae*, *Cnidium officinale* Makino, 3.0 g), *Paeoniae radix* (*Paeoniaceae*, *Paeonia lactiflora* Pallas, 4.0 g), and *Poria* (*Polyporaceae*, *Poria cocos* Wolf, 4.0 g). Each plant material was authenticated by identification of external morphology and marker compounds of plants specimens, according to the methods of the Japanese Pharmacopoeia and Tsumura & Co's standard. The six medical herbs were extracted with purified water at 95 °C for 1 h, and the extraction solution was separated from the insoluble waste and concentrated by removing water under reduced pressure. Spray drying was used to produce a dried extract powder. The yield of the extract was about 17.5%. They were manufactured in compliance with the Japanese Pharmacopoeia (Seventeenth Edition, JP17) under Good Manufacturing Practice (GMP). The product information can be acquired in “KCONSORT” (<http://kconsort.umin.jp>).

Donepezil hydrochloride (DPZ) was obtained from Eisai, Tokyo, Japan. DPZ and TSS were dissolved in distilled water. TSS was orally administered using a plastic syringe with a stainless-steel tube immediately after injection of A β _{1–42} for 7 days. DPZ was administered (p.o.) immediately before the last injection of A β _{1–42}. DPZ and TSS doses were chosen based on reports.^{9,16,20} Control animals received oral injections with the drug vehicle (distilled water). The injection volume of vehicle, DPZ or TSS was 1 mL/kg. The 8ARMT was carried out 60 min after the final injection of A β _{1–42}. Different groups were used in HPLC study. All experiments in this study were carried out between 7 am and 7 pm.

Brain microdialysis. On day-7 of A β injection, we carried out brain microdialysis as described previously.⁹ On POD3, extracellular levels of ACh were measured in the dorsal hippocampus of non-anesthetized, freely moving rats by microdialysis. Dialyzate aliquots (20 μ L) were collected every 20 min, injected directly into a HPLC system and assayed further for ACh. After a settling period (at least 2–3 h), samples were collected over a 40-min period (baseline, BL-1 and BL-2) before A β injection for the following 120 min.

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