Journal of Ethnopharmacology 122 (2009) 157–162

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Ameliorative effects of *yokukansan*, a traditional Japanese medicine, on learning and non-cognitive disturbances in the Tg2576 mouse model of Alzheimer's disease

Masahiro Tabuchi*, Takuji Yamaguchi, Seiichi Iizuka, Sachiko Imamura, Yasushi Ikarashi, Yoshio Kase

Tsumura Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan

ARTICLE INFO

Article history:
Received 19 September 2008
Received in revised form
20 November 2008
Accepted 16 December 2008
Available online 25 December 2008

Keywords:
Yokukansan
Alzheimer's disease
Non-cognitive defects
BPSD
Tg2576

ABSTRACT

Aim of this study: Aim of the present study is to clarify the effects of *yokukansan* (TJ-54) on learning and non-cognitive disturbances in the Tg2576 mouse expressing the human form of the APP695SWE (APP-Tg mice), which is considered to be an animal model of Alzheimer's disease.
Materials and methods: Powdered diets containing 0.5 and 1.0% TJ-54 were given to the mice for 10 months (from 5 to 15 months old). The Morris water-maze test, elevated plus-maze test, and open-field test were performed for evaluation of learning and non-cognitive disturbances.
Results: Treatment with 1.0% TJ-54 for 5 months shortened the time it took for APP-Tg positive (+) mice to reach the platform in the Morris water-maze test. In the elevated plus-maze test, treatment with 1.0% TJ-54 for 2 months significantly reduced the increased number of entries and the time spent in open arms observed in APP-Tg(+) mice. In an open-field test, treatment of 1.0% TJ-54 for 9 months significantly suppressed the increase in locomotion observed in APP-Tg(+) mice.
Conclusion: These results suggest the possibility that TJ-54 ameliorates learning deficits and non-cognitive defects including a decrease in the anxiety (or disinhibition) and an increase in locomotor activity (hyperactivity) observed in APP-Tg(+) mice.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with memory dysfunction; it is frequently accompanied by neuropsychiatric symptoms including excitation, aggressiveness, hallucination, delusion, apathy, disinterest, anxiety or disinhibition, and depression (Finkel et al., 1996; Cha et al., 2001; Blennow et al., 2006). These symptoms have received the designation behavioral and psychological symptoms of dementia (BPSD). Because the severity of BPSD and the care burden show a positive correlation, therapy for BPSD is considered to be as important as that for the core symptom, cognitive dysfunction (Tanji et al., 2005). While treatments with cholinesterase inhibitors (for example, donepezil) and an *N*-methyl-D-aspartate antagonist (memantine) for cognitive impairment in patients with AD have been partially successful (Findeis, 2007), antipsychotic drugs used to treat BPSD are generally known to cause adverse events such as drug-induced extrapyramidal symptoms. In addition, the U.S. Food and Drug Administration warned that mortality in elderly patients with dementia was increased by using atypical antipsychotic medicines (www.fda.gov/cder/drug/advisory/antipsychotics.htm., 2005). Wang et al. (2005) reported that conventional antipsychotics also involve the same risk as the atypical antipsychotics. Thus, new remedies without adverse effects have been sought.

Recently, TJ-54, a traditional Japanese medicine that has been used for the treatment of neurosis, insomnia, and irritability in children was reported to improve BPSD such as agitation/aggression and irritability in patients with dementia without any serious adverse events (Iwasaki et al., 2005). However, basic studies of TJ-54 for dementia and BPSD using animals have not yet been performed.

The Tg2576 [APP-Tg(+)] mouse expressing the human form of APP695SWE is known as a model of Alzheimer's disease; it has been demonstrated that β-amyloid (Aβ), which is thought to be a causative protein in Alzheimer's disease, accumulates in the brain of these mice with aging (Hsiao et al., 1996). In addition, not only cognitive dysfunction but also non-cognitive BPSD-like symptoms such as disinhibition, hyperactivity, and impulsive behavior have been observed in APP-Tg(+) mice (Lalonde et al., 2003; Stackman et al., 2003; Ognibene et al., 2005; Dong et al., 2005; Adrian et al., 2006; Quinn et al., 2007). These findings suggest that APP-Tg(+) mice are a valuable tool for developing new drugs for dementia and BPSD.

* Corresponding author. Tel.: +81 29 889 3850; fax: +81 29 889 3872.
E-mail address: tabuchi_masahiro@mail.tsumura.co.jp (M. Tabuchi).

0378-8741/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2008.12.010

In the present study, we investigated the effect of TJ-54 on leaning and non-cognitive disturbances in APP-Tg(+) mice.

2. Materials and methods

2.1. Animals

Tg(HuAPP695/SWE)2576 transgene-positive female mice [APP-Tg(+) mice] and their corresponding transgenic negative female controls [APP-Tg(−) mice] were purchased from Taconic Farms Inc. (Germantown, NY, USA). APP-Tg(+) mice are reported to have the human transgene containing a double mutation (Lys670 → Asn, Met671 → Leu) of amyloid precursor protein, which is expressed under the control of the hamster prion protein cosmid vector in APP-Tg(+) mice. Deposition of Aβ in the brain is observed from 6 to 7 months of age, and it increases with aging (Kawarabayashi et al., 2001).

In the present study, APP-Tg mice were housed 3–4 mice to a cage in plastic cages at a temperature of 23 ± 2 °C, relative humidity of 55 ± 10%, and a 12-h light/dark cycle with lights on from 07:00 to 19:00 h daily, and allowed free access to water and standard laboratory food (MF, Oriental Yeast Co. Ltd., Tokyo, Japan). All experimental procedures in this study were approved by the laboratory animal committee of Tsumura & Co. (Ibaraki, Japan) and met the guidelines of the Japanese Association for Laboratory Animal Science.

2.2. Drugs

Yokukansan (TJ-54), manufactured by Tsumura & Co. (Tokyo, Japan) and approved for ethical use by the Ministry of Health, Labor, and Welfare of Japan, is a dried extract of the following raw materials: 4.0 parts Atractylodis Lanceae Rhizoma (rhizome of *Atractylodes lancea* De Candolle, Compositae), 4.0 parts Poria (sclerotium of *Poria cocos* Wolf, Polyporaceae), 3.0 parts Cnidii Rhizoma (rhizome of *Cnidium officinale* Makino, Umbelliferae), 3.0 parts Angelicae Radix (root of *Angelica acutiloba* Kitagawa, Umbelliferae), 2.0 parts Bupleuri Radix (root of *Bupleurum falcatum* Linné, Umbelliferae), 1.5 parts Glycyrrhizae Radix (root and stolon of *Glycyrrhiza uralensis* Fisher, Leguminosae), and 3.0 parts Uncaria Uncis Cum Ramulus (hook of *Uncaria rhynchophilla* Miquel, Rubiaceae). 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 our company's standard. The seven 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 15.9%.

2.3. Experimental procedures

Five-month-old APP-Tg mice were randomly divided into four groups: Tg(−) control (n = 10), Tg(+) (n = 10), 0.5% TJ-54 (n = 10), and 1.0% TJ-54 (n = 10). The Tg(−) control group was given powdered standard laboratory food (MF) to Tg(−) mice for 10 months from 5 to 15-month-old. The Tg(+) group was given the same powdered chow to Tg(+) mice for 10 months. The 0.5 and 1.0% TJ-54 Tg(+) groups were given the powdered chow including 0.5 or 1.0% of TJ-54 to Tg(+) mice for 10 months.

Morris water-maze tests were performed to evaluate learning ability at the age of 11 months. Elevated plus-maze tests were performed to evaluate anxiety at the ages of 7 and 10 months. Open-field tests were performed to evaluate locomotor activity at the ages of 7, 10, and 14 months. All behavioral tests were performed between 10:00 and 17:00 h. After completion of behavioral tests, in order to elucidate the effect of TJ-54 on Aβ level accumulated in the brain of Tg(+) mice, the mice were decapitated at 15 months old, and the brains were used for histological (n = 3) and biochemical (n = 7) assessments of Aβ.

2.3.1. Morris water-maze

The apparatus used in Morris water-maze test consisted of a blue plastic circular pool 100 cm diameter enclosed by a 25-cm high wall. The pool was filled with water kept at a temperature of 19 °C. A transparent plastic platform (8 cm in diameter) was placed 0.5 cm below the water surface and 18 cm from the wall of the pool. Visual cues for navigation were provided by four posters on the room walls.

In training trials for the Morris water-maze test, a mouse was placed at an area opposite the platform set in the pool and allowed to swim for a maximum of 60 s. The behavior was recorded by video. The time required to reach the platform (the latency period) and the distance of swimming were measured by using the analysis software LimeLight (Neuroscience Inc., Tokyo, Japan). When a mouse did not find the platform within 60 s, it was allowed to stay on the platform for 15 s. Such training trials were repeated for 5 days (1 trial/day). Swimming speed in each animal was determined as an index of spontaneous motor activity on the 5th day in the training trials.

A probe trial of the Morris water-maze test was performed on the day after the 5-day training was finished. The probe test was performed without the platform. The mouse was placed in the pool and swam for 60 s looking for a platform. In this test, we counted the number of times that the animal crossed the place where platform had been in the pool to verify the maintenance of spatial memory.

2.3.2. Elevated plus-maze

The elevated plus-maze was made of plastic and consisted of two opposite open arms (30 cm × 5 cm), and two opposite equal-sized arms enclosed by 20 cm high walls. The arms were connected by a central 5 cm × 5 cm square, and thus the maze formed a 'plus-sign' shape. The maze was elevated 40 cm from the floor and lit by dim light. A video camera was mounted vertically over the maze. A mouse was placed in the central square of the elevated plus-maze, and the behavior was monitored with a video camera (video tracking system) for 5 min. The behavioral data were saved directly on a computer. From the saved data, the number of entries into the open arms and the time spent in the open arms on the plus-maze in 5 min were measured by using the analysis software LimeLight.

2.3.3. Open-field test

An open-field test for evaluation of locomotor activity was performed 24 h after the elevated plus-maze test. The apparatus (Neuroscience, Inc.) consisted of a square floor (50 cm × 50 cm) enclosed by walls 25 cm high. A mouse was placed in the center of the apparatus and monitored with a video camera (video tracking system) for 5 min, and the data were saved on a computer. From the saved data, the total distance travelled (cm) in the open field for 5 min were determined by using the analysis software LimeLight.

2.3.4. Histological and biochemical assessments of Aβ

After completion of behavioral tests, three mice (15 months old) in Tg(+) or 1% TJ-54 group were used for histological assessment of Aβ: the animal was decapitated, and the brains were removed immediately. The whole brains were fixed in 4% paraformaldehyde in phosphate-buffered saline, dehydrated, and embedded in paraffin. Coronal serial sections including the cortex and hippocampus were stained with Congo red and counterstained with hematoxylin. The dimension (μm^2) of 50 number of Aβ plaques in each brain section were measured using Image J (NIH) software.

Other seven mice in Tg(+), 0.5 and 1% TJ-54 group were used for determination of soluble or insoluble forms of Aβ. They were