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YOKUKANSAN, A TRADITIONAL JAPANESE MEDICINE, AMELIORATES MEMORY DISTURBANCE AND ABNORMAL SOCIAL INTERACTION WITH ANTI-AGGREGATION EFFECT OF CEREBRAL AMYLOID β PROTEINS IN AMYLOID PRECURSOR PROTEIN TRANSGENIC MICE

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Abstract—The deposition of amyloid β protein (Aβ) is a consistent pathological hallmark of Alzheimer's disease (AD) brains. Therefore, inhibition of Aβ aggregation in the brain is an attractive therapeutic and preventive strategy in the development of disease-modifying drugs for AD. An *in vitro* study demonstrated that yokukansan (YKS), a traditional Japanese medicine, inhibited Aβ aggregation in a concentration-dependent manner. An *in vivo* study demonstrated that YKS and Uncaria hook (UH), a constituent of YKS, prevented the accumulation of cerebral Aβ. YKS also improved the memory disturbance and abnormal social interaction such as increased aggressive behavior and decreased social behavior in amyloid precursor protein transgenic mice. These results suggest that YKS is likely to be a potent and novel therapeutic agent to prevent and/or treat AD, and that this may be attributed to UH.

Key words: Alzheimer's disease, aggression, amyloid β proteins, traditional medicine, Uncaria hook, yokukansan.

Alzheimer's disease (AD), the most prevalent cause of dementia, is characterized by loss of memory and cognition in the elderly. One of the pathological characteristics of AD is the progressive deposition of insoluble amyloid β protein (Aβ) as a form of senile plaques (Wirths et al., 2004). This protein comprises peptides of approximately 39–43 amino acid residues derived from the transmembrane amyloid precursor protein (APP) (Selkoe, 2002). Aβ can exist as monomers and form a variety of different aggregate morphologies including dimers, small soluble oligomers, protofibrils, diffuse plaques, and the fibrillar deposits seen in senile plaques. Protofibrils, diffuse plaques, and fibrillar deposits seem to have a predominant β-sheet structure (Tierney et al., 1988; Barrow and Zagorski, 1991), while oligomers are believed to be more globular (Barghorn et al., 2005). Abundant evidence showing that formation of these aggregates causes primary neurodegeneration in AD has led to the amyloid hypothesis, which states that the accumulation of Aβ in the CNS is highly neurotoxic and degrades synaptic function (Selkoe, 2002; Wirths et al., 2004). Therefore, it is hypothesized that the formation, deposition, and aggregation of Aβ in the brain should be primary targets for amelioration of dementia. Currently, drugs available for dementia such as acetylcholinesterase inhibitors exert only a temporary effect on cognitive dysfunction (Millard and Broomfield, 1995; Park et al., 2000; Darreh-Shori et al., 2004), and they do not prevent or reverse the formation of Aβ deposits. Among the potentially promising strategies for developing more effective anti-dementia drugs are the inhibition of Aβ fibril formation, destabilization of aggregated Aβ, or a combination of both.

In patients with AD, not only core symptoms such as cognitive impairment, but also behavioral and psychological symptoms of dementia (BPSD) such as aggression, anxiety, and hallucinations often emerge. BPSD is a serious problem for caregivers, and because its severity and the care burden show a positive correlation, therapy for BPSD is considered to be as important as therapy for the core symptoms (Nagaratnam et al., 1998; Tanji et al., 2005). To date, anti-psychotic medicines have been used for treatment of BPSD. However, the drugs induce extrapyramidal symptoms and other adverse events, and in consequence, they decrease the quality of life and increase the difficulty of maintaining activities of daily living. Thus, new remedies without adverse effects have been sought.

Herbal remedies are used worldwide and have a long history of use to alleviate a variety of symptoms of many

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Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid β protein; BPSD, behavioral and psychological symptoms of dementia; DLB, dementia with Lewy bodies; TBS, tris–buffered saline; Tg(+), transgenic; Tg(−), non-transgenic; UH, Uncaria hook; YKS, yokukansan.

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different conditions and diseases. Recently, clinical trials in patients with AD have also shown that some traditional Japanese medicines called kampo improved Mini-Mental State Examination scores (Iwasaki et al., 2004) and blood flow in the cerebral cortex (Maruyama et al., 2006). We have reported that several traditional herbal medicines such as kamiuntanto (*Formula lienalis angelicae compositae*) (Wang et al., 2000; Nakagawasai et al., 2004) and hachimijiogan (*Pilulae octo-medicamentorum rehmanniae*) (Iwasaki et al., 2004) ameliorated symptoms of dementia.

Yokukansan (YKS, *Pulvis depressionis hepatis*) is a traditional Japanese medicine approved by the Ministry of Health, Labour and Welfare of Japan as a remedy for neurosis, insomnia, and irritability in children. Recently, we reported that it improved such BPSD as hallucinations, agitation, and aggression in patients with Alzheimer's disease, dementia with Lewy bodies (DLB), and other forms of senile dementia (Iwasaki et al., 2005a,b). Recently, to clarify the improving effect of YKS, various basic studies have been performed (Ikarashi et al., 2009; Kawakami et al., 2009, 2010; Terawaki et al., 2010). We also previously demonstrated that Uncaria hook (UH), a constituent herb of YKS, inhibited Aβ aggregation *in vitro* (Fujiwara et al., 2006), suggesting that YKS containing UH may possess anti-aggregation activity toward Aβ, and that it may improve memory disturbance and BPSD. However, sufficient animal experiments to confirm this hypothesis have not been performed yet.

The APP transgenic [Tg(+)] mouse expressing the human form of APP695SWE is known as a model of AD. Aβ accumulates in the brain of the mice with aging (Hsiao et al., 1996; Ikarashi et al., 2004). In addition, not only cognitive dysfunction but also BPSD-like symptoms such as disinhibition, hyperactivity, and impulsive behavior have been observed in Tg(+) mice (Lalonde et al., 2003; Stackman et al., 2003; Ognibene et al., 2005; Dong et al., 2005; Adriani et al., 2006; Quinn et al., 2007). These findings suggest that the Tg(+) mouse is a valuable tool for developing new drugs for dementia and BPSD.

To clarify the hypothesis described above, in the present study, we first examined the effect of YKS on Aβ aggregation *in vitro* as well as UH. Next, the effects of YKS and UH on accumulation of Aβ in the brain and phenotypes such as memory disturbance and BPSD-like behaviors such as the increase in aggressive behavior and decrease in social behavior in the Tg(+) mice were investigated.

EXPERIMENTAL PROCEDURES

Animals

Male APP Tg(+) mice, who overexpress a 695-amino acid splice form (Swedish mutation K670N M671I) of the human amyloid β precursor protein (APP695), and non-transgenic [Tg(−)] mice were purchased from Taconic Farms Inc. (Germantown, NY, USA). Each animal was housed individually in a plastic cage ($230\times155\times155$ mm^3) and allowed free access to water and standard laboratory food in a facility with the temperature controlled at 24 ± 1 °C and relative humidity at 55 ± 5% and with lights on from 7:00 to 19:00 h daily until the animals were used in the experiments. Experimental protocols were approved by the Animal Care and Use Committee of Tohoku University Graduate School of Medicine and complied with the procedures outlined in the Guide for the Care and Use of Laboratory Animals of Tohoku University.

Drugs and reagents

YKS is composed of seven dried medicinal herbs: *Atractylodes lancea* rhizome (4.0 g, rhizome of *Atractylodes lancea* De Candolle), Poria sclerotium (4.0 g, sclerotium of *Poria cocos* Wolf), Cnidium rhizoma (3.0 g, rhizome of *Cnidium officinale* Makino), Japanese Angelica root (3.0 g, root of *Angelica acutiloba* Kitagawa), Bupleurum root (2.0 g, root of *Bupleurum falcatum* Linné), glycyrrhiza (1.5 g, root and stolon of *Glycyrrhiza uralensis* Fisher), and UH (3.0 g, thorn of *Uncaria rhynchophylla* Miquel). The dry powdered extracts of YKS and UH were supplied by Tsumura & Co. (Tokyo, Japan).

Aβ peptides (1-40 and 1-42) and thioflavin-T were obtained from the Peptide Institute (Osaka, Japan) and Sigma (St. Louis, MO, USA), respectively. Other reagents (analytical grade) used for analysis were purchased from commercial sources.

In vitro study to evaluate effect of YKS on Aβ aggregation

Measurement of thioflavin-T to evaluate Aβ aggregation was performed using the method described by Suemoto et al. (2004) with slight modifications. Aβ (20 μM) dissolved in 50 mM potassium phosphate buffer (pH 7.4) with YKS was incubated at 37 °C for 96 h (A$\beta_{1\text{-}40}$) or 24 h (A$\beta_{1\text{-}42}$). At the end of the incubation, 3 μM thioflavin-T dissolved in 100 mM glycine buffer (pH 8.5) was added to the mixture. After incubation for 30 min at room temperature, the fluorescence of thioflavin-T bound to Aβ aggregates was measured using a microplate reader (Spectramax Gemini XS, Molecular Devices, Sunnyvale, CA, USA) with excitation at 442 nm and emission at 485 nm. The percentage inhibition was calculated by comparing the fluorescence values of test samples with those of control solutions without YKS.

In vivo study to evaluate behaviors and accumulation of Aβ

Ten-month-old Tg(+) mice were randomly divided into five groups: Tg(+) (n=10), Tg(+)+0.3% YKS (n=10), Tg(+)+1.0% YKS (n=10), Tg(+)+0.1% UH (n=10), and Tg(+)+1.0% UH (n=10). Tg(−) mice (n=10) were set as the control group. The mice in both the Tg(−) and Tg(+) groups were given normal powdered chow for 5 months from 10 to 15 months old. The mice in the Tg(+)+0.3% YKS and Tg(+)+1.0% YKS groups were given the powdered chow including 0.3% or 1.0% of YKS for 5 months. The mice in the Tg(+)+0.1% UH and Tg(+)+1.0% UH groups were given the powdered chow including 0.1% or 1.0% of UH for 5 months.

Step-through passive-avoidance tests were performed to evaluate learning ability from the age of 11 months to 14 months. Social interaction tests were performed at the age of 15 months. All behavioral tests were performed between 10:00 and 17:00 h.

After completion of behavioral tests, all mice were decapitated, and the dissected cerebral cortex was used for determination of Aβ levels.

Step-through passive-avoidance test

The apparatus (TK402D model, Neuroscience, Inc., Tokyo, Japan) for the step-through passive-avoidance test consisted of two compartments, one illuminated [$100\times120\times100$ mm^3; light at the top of compartment (27 W, 3000 lx)] and the other dark ($100\times170\times100$ mm^3). The compartments were separated by a guillotine door. During the learning stage, a mouse was placed in the illuminated safe compartment. While this compartment was lit,