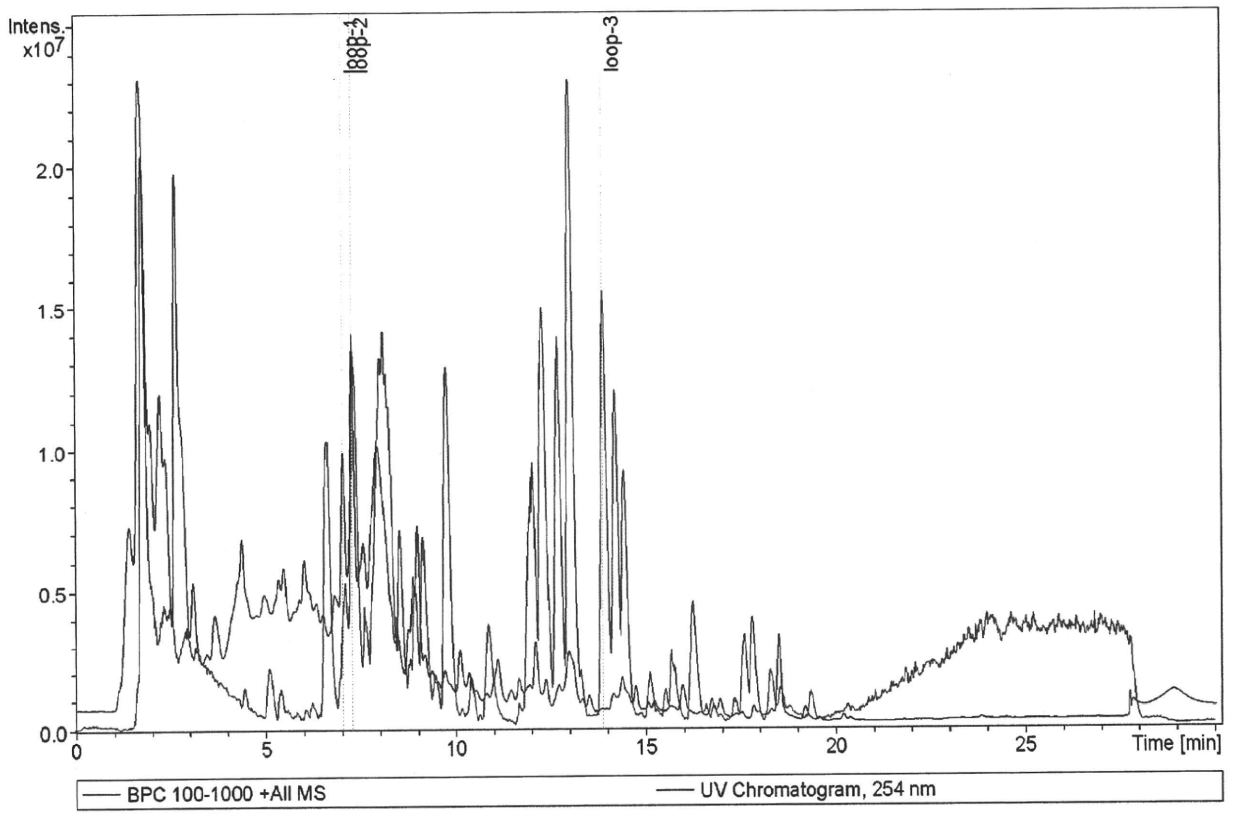

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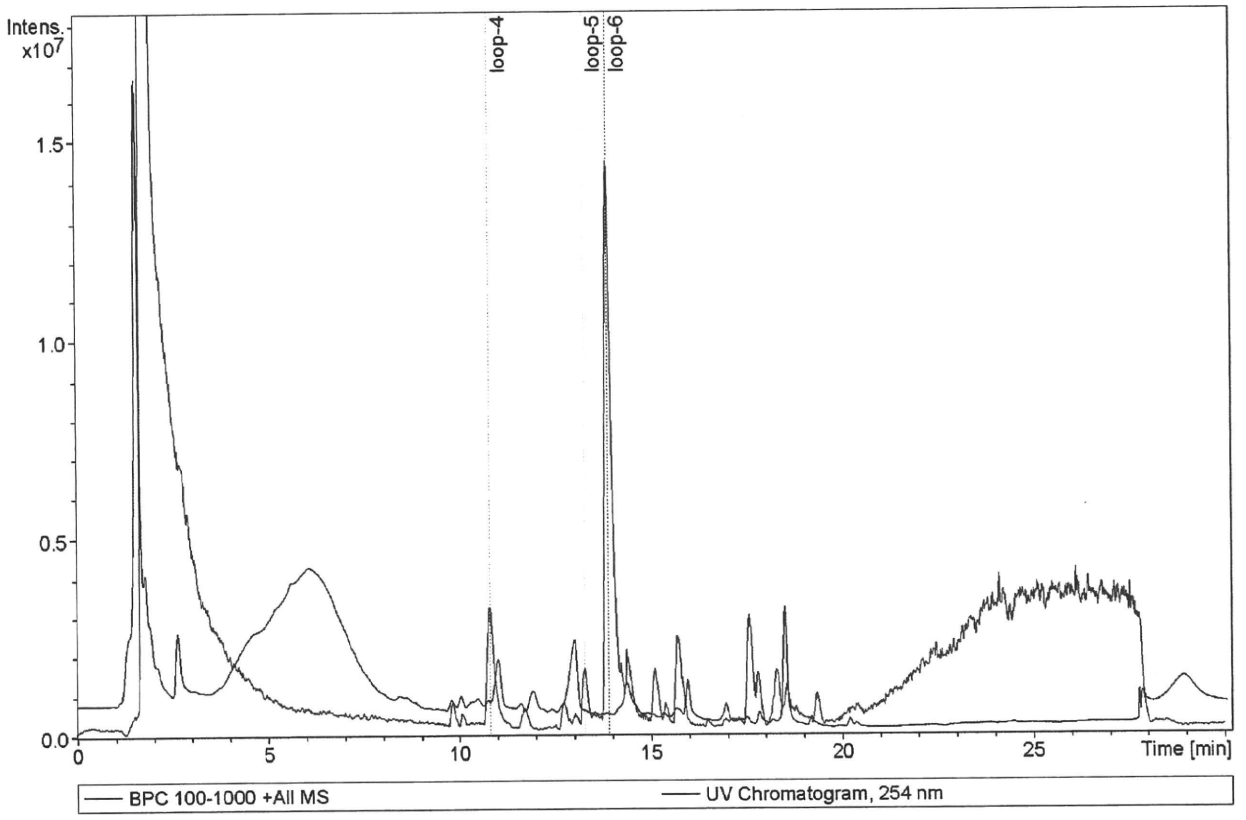

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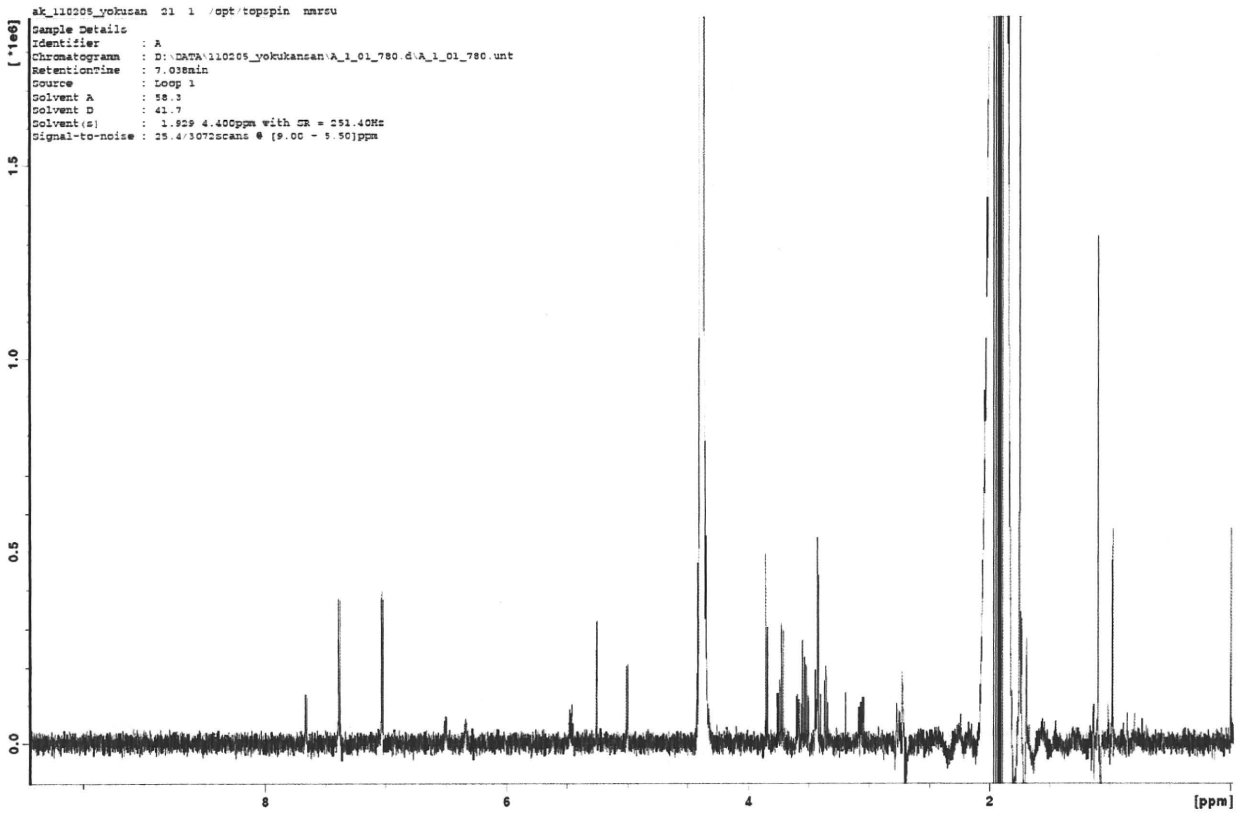
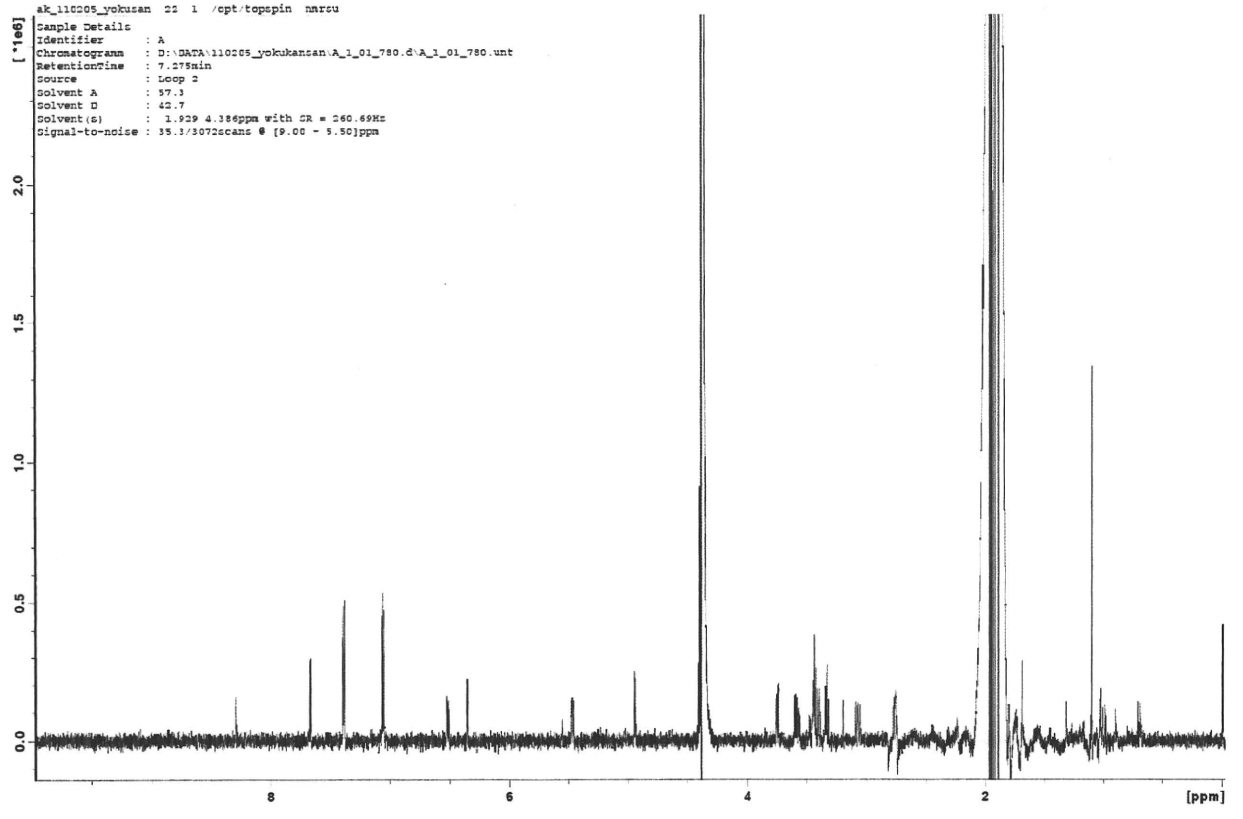


図 3 loop1 の NMR チャート



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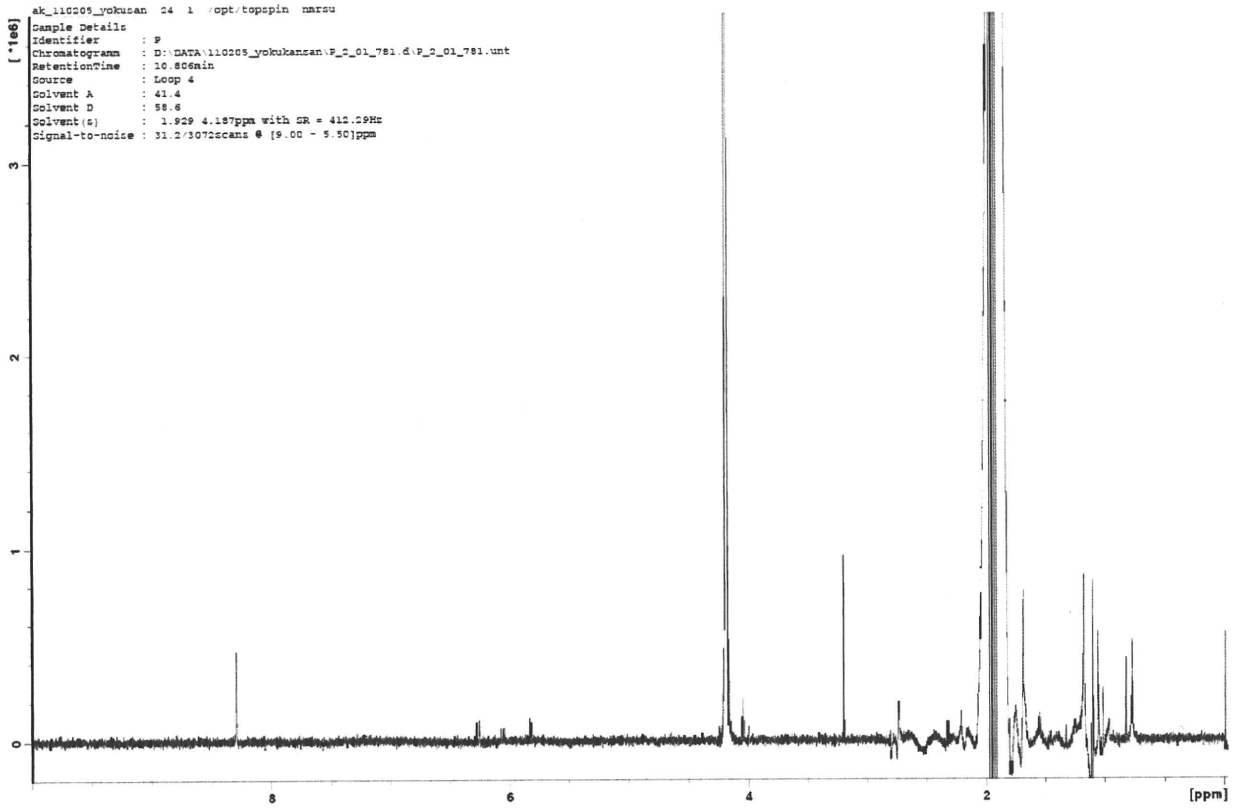

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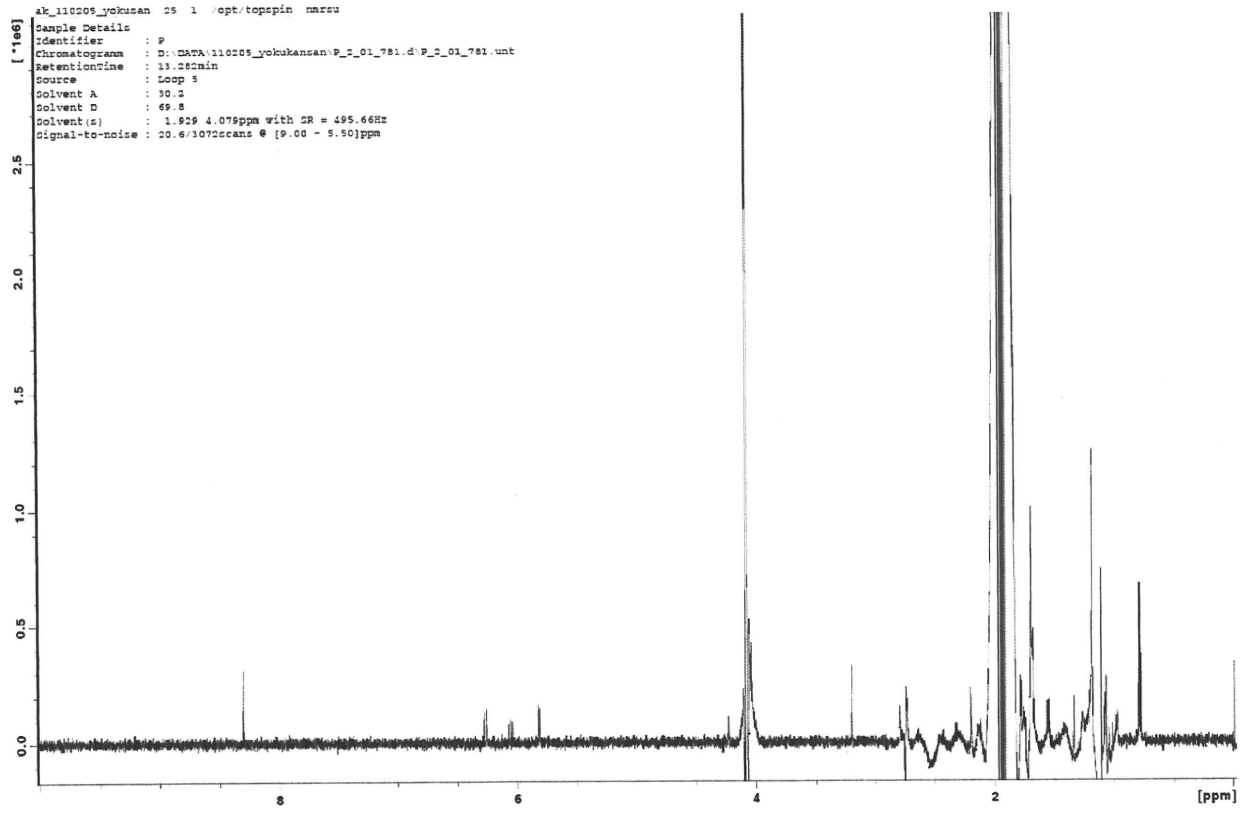

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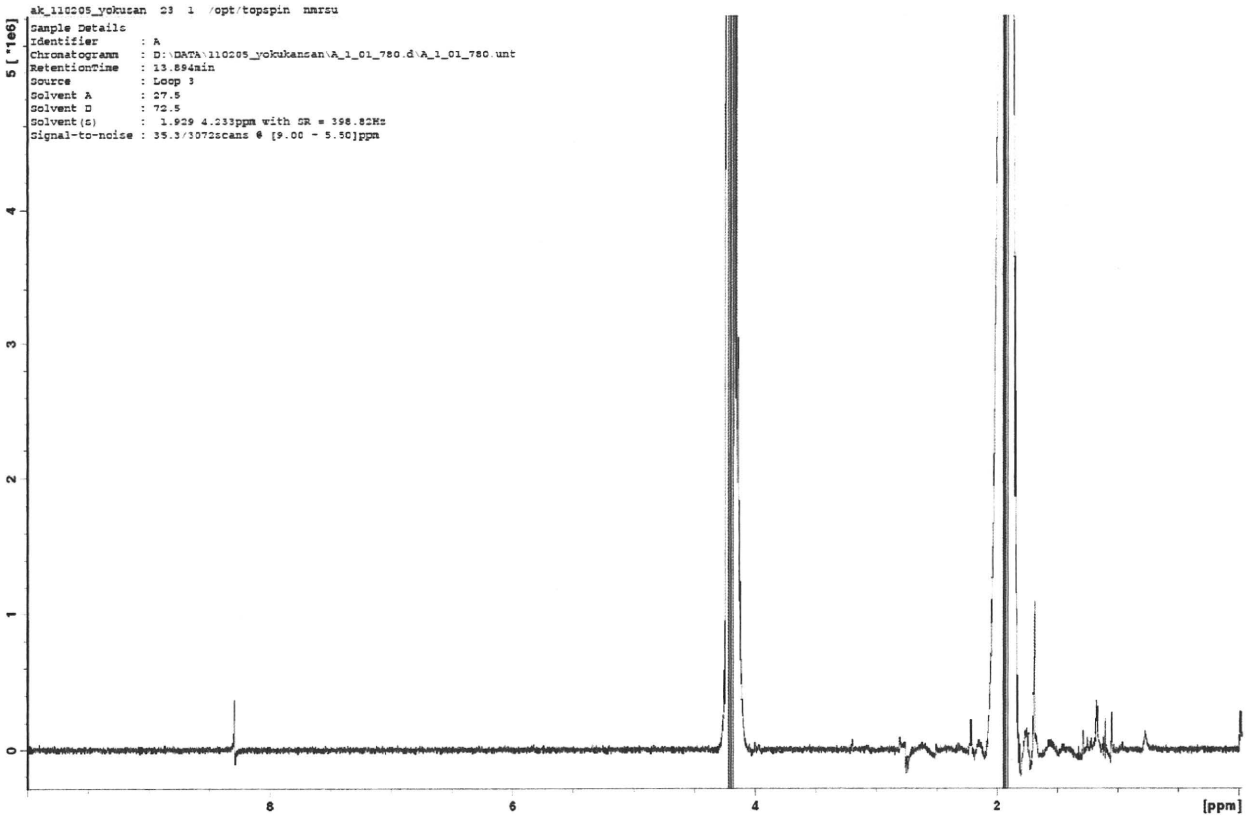

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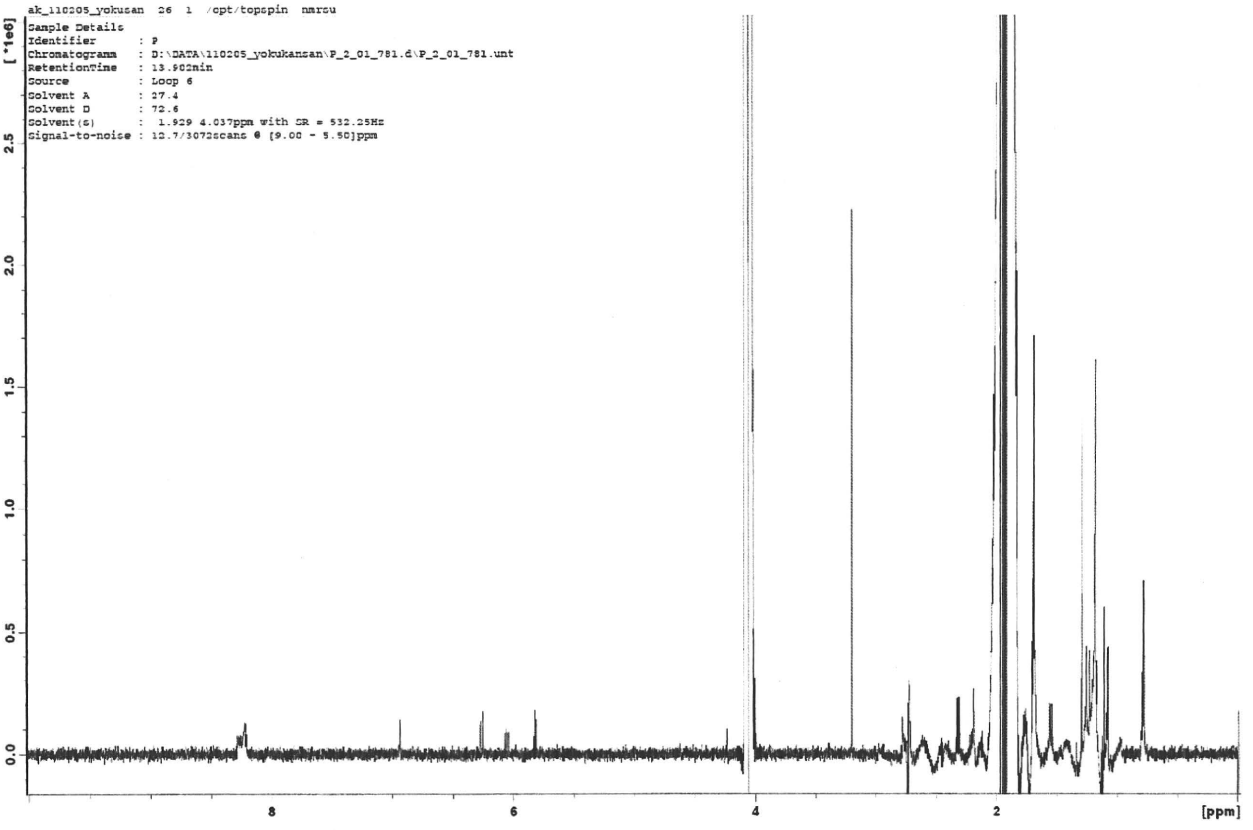

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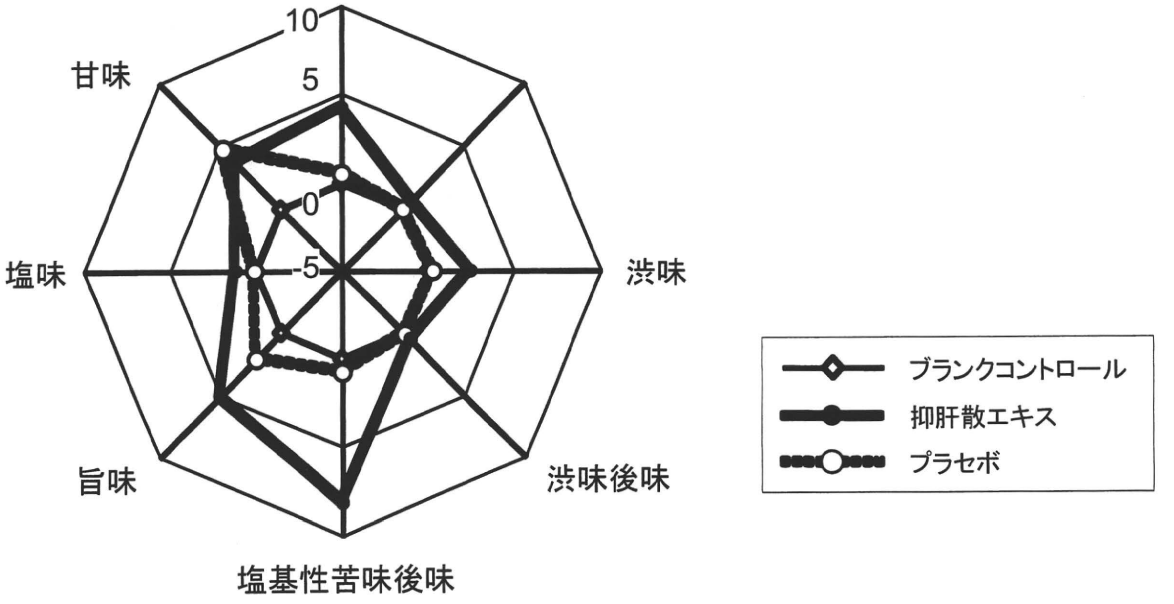

図 9 抑肝散エキスとプラセボの味パターン

別紙4

研究成果の刊行に関する一覧表

書 籍

著者氏名	論文タイトル名	書籍全体の編集者名	書 籍 名	出版社名	出版地	出版年	ページ
神﨑恒一	第3章高齢者によくある症状と生活機能の関係 Ⅶ転倒	鳥羽研二	高齢者の生活機能の総合的評価	新興医学出版社	東京	2010	115-121

雑 誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Fujiwara H, Takayama S, Iwasaki K, Tabuchi M, Yamaguchi T, Sekiguchi K, Ikarashi Y, Kudo Y, Kase Y, Arai H, Yaegashi N	Yokukansan A tradhitional Japanese medicine, Ameliorates memory disturbance and abnormal social interaction with anti-aggregation effect of cerebral amyloid precursor protein transgenic mice	Neuroscience	180	305-313	2011
Arai H, Okamura N, Furukawa K, Kudo Y.	Geriatric medicine, Japanese Alzheimer's disease neuroimaging initiative and biomarker development	Tohoku J. Exp. Med	221	87-95	2010
荒井啓行	私の認知症研究	Dementia Japan	24	115-121	2010
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神﨑恒一	高齢者の転倒予防	日老医誌	47(2)	137-139	2010

町田綾子、山田如子、木村紗矢香、神﨑恒二、鳥羽研二	認知症の周辺症状と介護負担感に対する抑肝散長期投与の効果	日老医誌	47(3)	262-263	2010
神﨑恒一	寝たきり	日老医誌	47(5)	393-395	2010
Kumiko Nagai,Koichi Kozaki,Kazuki Sonohara,Masahiro Akishita,Kenji Toba	Relationship between interleukin-6 and cerebral deep white matter and periventricular hyperintensity in elderly women	Geriatr Gerontollnt	11		2011

Neuroscience 180 (2011) 305–313

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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doi:10.1016/j.neuroscience.2011.01.064

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 (230×155×155 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 ($Aβ_{1-40}$) or 24 h ($Aβ_{1-42}$). 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 [100×120×100 mm^3; light at the top of compartment (27 W, 3000 lx)] and the other dark (100×170×100 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,

the mouse stepped through the opened guillotine door into the dark compartment. The time spent in the illuminated compartment was defined as the latency period. 3 s after the mouse entered the dark compartment, a foot shock (0.01 mA, 200 V, 50 Hz ac, for 1 s) was delivered to the floor grid in the dark compartment. The mouse could escape from the shock only by stepping back into the safe illuminated compartment. Such acquisition trials during the learning stage were carried out once a day for 5 days. The mouse was judged to have learned avoidance from the foot shock when the latency period reached 300 s. Retention trials were carried out once per week for 78 days (11–14-months-old) to evaluate the retention of avoidance memory. The latency was measured for up to 300 s without delivering a foot shock. It was judged that the mouse retained the avoidance memory when it stayed in the illuminated safe compartment for 300 s.

Social interaction test

Social interaction such as aggressive behavior and social behavior in mice were evaluated by a social interaction test (File, 1980) using a square box-type open-field apparatus (50×50×40 cm^3, Neuroscience, Inc., Tokyo, Japan). A video camera was mounted vertically over the apparatus. Two mice in each group were placed together in the open-field apparatus. The interactive behaviors between the two animals were monitored by the video camera for 10 min, and the behavioral data were saved directly on a computer. Then, the total number of aggressive behaviors (tail rattling, chasing, and attacking) as the index of aggressiveness or normal social behaviors (sniffing, following, and contacting) as the index of sociability of each animal was counted by two observers blind to the treatment. The total distance traveled (cm) of each animal was analyzed as motor activity by using software (analyzing behavior system, Viewer II, Bioserve, Bonn, Germany).

Measurement of brain Aβ levels

The dissected cerebral cortex was homogenized and sonicated in Tris–buffered saline (TBS) and 70% formic acid containing 1× protease inhibitor mixtures to obtain soluble and insoluble fractions with slight modification of the method described previously (Calon et al., 2004). The homogenate was subjected to ultracentrifugation at 200,000×g at 4 °C for 20 min. The soluble supernatant was collected and frozen. To analyze the insoluble Aβ, the insoluble pellet was sonicated in 200 μl of 70% formic acid and subjected to ultracentrifugation at 300,000×g at 4 °C for 30 min to collect the soluble supernatant.

Brain $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were measured using sandwich ELISA with a Human β Amyloid ELISA Kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan) according to the manufacturer's instructions. BAN50 is a monoclonal antibody raised against a synthetic peptide of human $A\beta_{1-16}$; it preferentially reacts with the N-terminal portion of human Aβ starting at Asp-1, but does not cross-react with N-terminal–truncated Aβ or with rodent-type Aβ. BA27 and BC05, which specifically recognize the C terminus of $A\beta_{1-40}$ and $A\beta_{1-42}$, respectively, were conjugated with horseradish peroxidase and used as detector antibodies. The insoluble mouse brain fractions described above were neutralized and subjected to BAN50/BA27 or BAN50/BC05 ELISA. The protein concentration of the fraction was measured using a protein assay kit (Bio-Rad Lab., Hercules, CA, USA). Finally, the Aβ value was expressed as pmol per g of protein.

Data analysis

Data are expressed as mean±SEM. The date of passive-avoidance test was evaluated by analysis of variance (Kruskal-Wallis) followed by a Mann-Whitney *U* test. The data of other experiments were evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni/Dunn tests. The significance level in each statistical analysis was accepted at $P<0.05$.

RESULTS

Effects of YKS on Aβ aggregation *in vitro*

The effects of YKS on $A\beta_{1-40}$ and $A\beta_{1-42}$ aggregation *in vitro* are shown in Fig. 1A, B, respectively. YKS inhibited the aggregation of $A\beta_{1-40}$ and $A\beta_{1-42}$ in a concentration-dependent manner. Significant inhibition was observed at 10 and 100 μg/ml for $A\beta_{1-40}$ and at 100 μg/ml for $A\beta_{1-42}$.

Effects of YKS and UH on memory disturbance in Tg(+) mice

Step-through passive-avoidance tests were carried out on mice at 11–14 months of age. In the first acquisition trial of the learning stage, all mice (11 months old) in the Tg(−), Tg(+), Tg(+)+YKS, and Tg(+)+UH groups entered the dark compartment immediately after being placed in the illuminated compartment. Repeating the acquisition trial increased the latency times in all groups. All mice in all

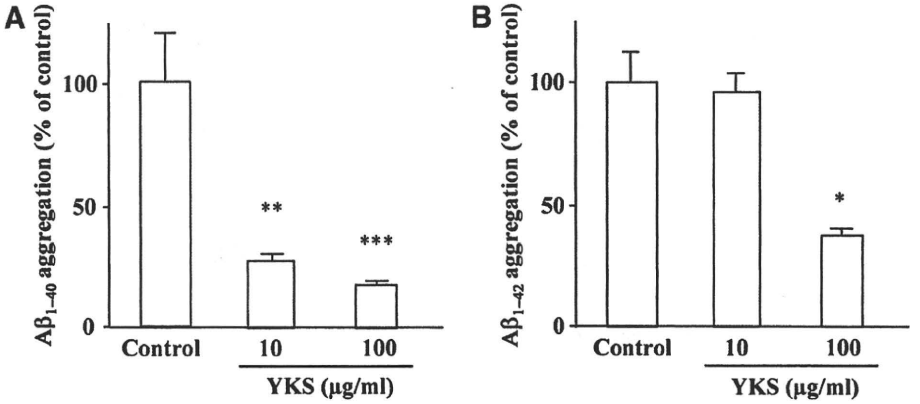

Fig. 1. Effects of YKS on $A\beta_{1-40}$ (A) or $A\beta_{1-42}$ (B) aggregation. Aβ aggregation was assessed by the thioflavin T method and expressed as the percentage of control aggregation in the absence of YKS. Values represent mean±SE from four independent experiments. Significance by Bonferroni/Dunn tests following one-way ANOVA is indicated as * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. control.

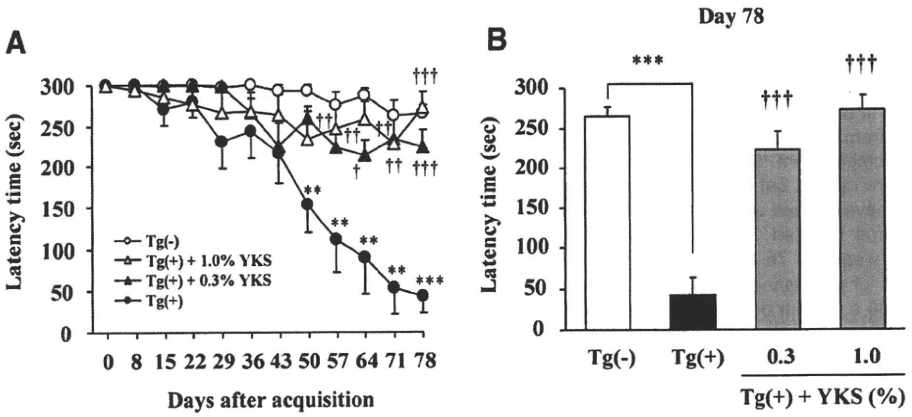

Fig. 2. Step-through latencies in the retention test of the passive-avoidance task in YKS-treated Tg mice. Changes in the latencies during retention tests for 78 d after acquisition of avoidance memory in each group are shown in (A), and the final results on day 78 are shown in (B). Values represent the means±SE (n=10 in each group). Significance by Mann-Whitney U test following analysis of variance (Kruskal-Wallis) is indicated as ** P<0.01, *** P<0.001 vs. corresponding Tg(−) control, and † P<0.05, †† P<0.01 and ††† P<0.001 vs. Tg(+) on each day.

groups acquired avoidance memory, staying in the illuminated compartment over 300 s on the fifth day. No statistically significant differences were observed in the mean latency times among all groups during the acquisition trials (data not shown).

Memory retention tests were performed once a week for 78 days after the final acquisition trial. Changes in the step-through latency in the YKS-treated groups are shown in Fig. 2A, and the results on the terminal day 78 are shown in Fig. 2B. The latency time of the Tg(+) group was significantly shorter than that of the Tg(−) group. The shorter latency was significantly prolonged by treatments with 0.3 and 1.0% YKS.

Changes in the step-through latency in UH-treated groups are shown in Fig. 3A, and then the results on the terminal day 78 are shown in Fig. 3B. The shortened latency time of the Tg(+) group was significantly prolonged by treatment with UH (0.1% and 1.0%) in a dose-dependent manner.

Effects of YKS and UH on aggressiveness and sociability in Tg(+) mice

The effects of YKS on aggressive behavior, social behavior, and motor activity are shown in Fig. 4. The aggressive behavior in the Tg(+) group increased significantly more than that in the Tg(−) group. The increase was significantly inhibited by treatment with 1.0% YKS (Fig. 4A). On the other hand, social behavior in the Tg(+) group decreased significantly more than that in the Tg(−) group. The decrease was significantly inhibited by treatment with 1.0% YKS (Fig. 4B). No significant differences of the motor activities (distance) were observed between Tg(−), Tg(+), and Tg(+)+YKS groups (Fig. 4C).

The effects of UH on aggressive behavior, social behavior, and motor activity are shown in Fig. 5. The aggressive behavior in the Tg(+) group increased significantly more than that in the Tg(−) group. The increase was

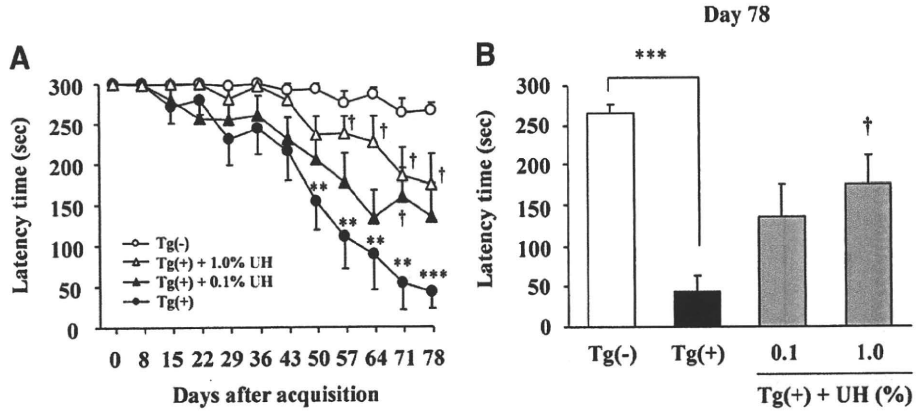

Fig. 3. Step-through latencies in the retention test of the passive-avoidance task in UH-treated Tg mice. Changes in the latencies during retention tests for 78 d after acquisition of avoidance memory in each group are shown in (A), and the final results on day 78 are shown in (B). All mice acquired avoidance memory by five repeated acquisition trials. Values represent the means±SE (n=10 in each group). Significance by Mann-Whitney U test following analysis of variance (Kruskal-Wallis) is indicated as ** P<0.01, *** P<0.001 vs. corresponding Tg(−) control, and † P<0.05 vs. Tg(+) on each day.

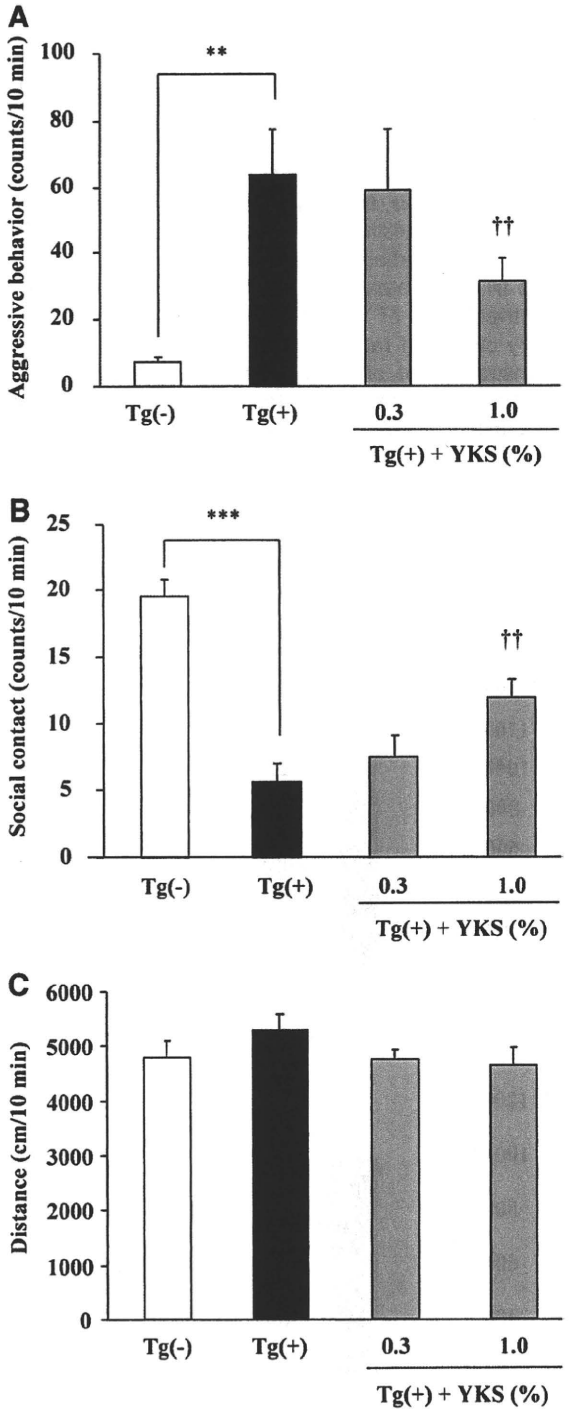

Fig. 4. Aggressive behavior (A), social contact (B), and distance as indexes of motor activity (C) of YKS-treated mice in the social interaction test. Value represents the mean±SE (n=10 in each group). Significance by Bonferroni/Dunn tests following one-way ANOVA is indicated as ** $P<0.01$, *** $P<0.001$ vs. Tg(−), and †† $P<0.01$ vs. Tg(+).

significantly inhibited by treatment with 1.0% UH (Fig. 5A). On the other hand, social behavior in the Tg(+) group decreased significantly more than that in the Tg(−) group. The decrease was significantly inhibited by treatment with 1.0% UH (Fig. 5B). No significant differences of the motor activities were observed between Tg(−), Tg(+), and Tg(+) + UH groups (Fig. 5C).

Effects of YKS and UH on cerebral Aβ levels in Tg(+) mice

To determine whether oral YKS or UH treatment affected the accumulation of Aβ in cerebral cortex, the cortical $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were measured. The results are shown in Fig. 6A, B, respectively. Large amounts of both forms of Aβ were detected in the cortex of Tg(+) mice but not in Tg(−) mice. YKS or UH treatment had no significant effect on $A\beta_{1-40}$ levels in the Tg(+) mice (Fig. 6A). However, both YKS and UH inhibited $A\beta_{1-42}$ accumulation in Tg(+) mice in a dose-dependent manner (Fig. 6B).

DISCUSSION

Aβ is thought to be a causative substance of AD (Hsiao et al., 1996; Selkoe, 2002; Wirths et al., 2004). We previously demonstrated that UH (10 and 100 μg/ml) had a potent anti-aggregation effect on Aβ proteins *in vitro*, in a concentration-dependent manner (Fujiwara et al., 2006). In the present *in vitro* study, we demonstrated that YKS (100 μg/ml) significantly inhibited Aβ aggregation as shown in Fig. 1. UH is contained 14.6% in YKS, that is, 14.6 μg/ml of UH is contained in 100 μg/ml of YKS. This 14.6 μg/ml concentration is included within the range of effective concentrations (Fujiwara et al., 2006). Therefore, the anti-aggregation effect of YKS is suggested to be attributed to UH. It is important to verify whether *in vitro* results are reflected *in vivo*. However, it is difficult to compare effective dose or concentration directly between *in vitro* and *in vivo* experiments, because the experimental conditions are different between them. In the present *in vivo* study, YKS or UH were given to animals as diets containing 0.3 and 1.0% YKS, or 0.1 and 1.0% UH. The YKS intake levels in 0.3 and 1.0% YKS groups corresponded to 240 and 800 mg/kg/d when the levels were calculated from food consumption. Similarly, the UH intake levels in 0.1 and 1.0% UH groups corresponded to 80 and 800 mg/kg/d. As shown in Fig. 6B, we found that both YKS and UH dose-dependently inhibited $A\beta_{1-42}$ accumulation in the cerebral cortex of Tg(+) mice. As 800 mg/kg/d of YKS contains 117 mg/kg/d (14.6%) which is included within the range of 80 and 800 mg/kg/d of UH, we suggest the possibility that YKS (800 mg/kg/d) containing UH (117 mg/kg/d) has the anti-aggregation effect as well as the *in vitro* study. This is a first finding demonstrating that YKS and UH inhibit the accumulation of Aβ *in vivo*.

The Tg(+) mice used in the present study are known to develop disturbance of memory or cognitive function (Hsiao et al., 1996; Wegiel et al., 2001; Barnes et al., 2004; Ikarashi et al., 2004). In the present study, we evaluated the memory disturbance in Tg(+) mice using a step-through passive avoidance test. In the retention test, the latency time of the Tg(+) mice was significantly shorter

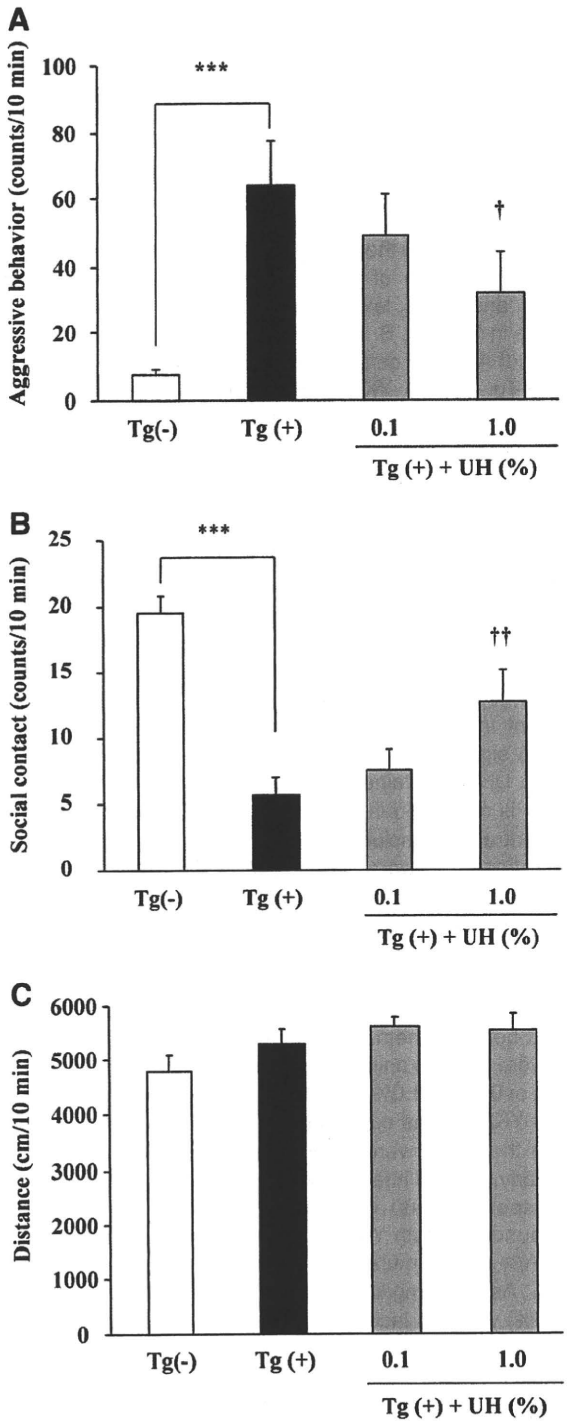

Fig. 5. Aggressive behavior (A), social contact (B), and distance as indexes of motor activity (C) of UH-treated mice in the social interaction test. Value represents the mean±SE (n=10 in each group). Significance by Bonferroni/Dunn tests following one-way ANOVA is indicated as *** $P<0.001$ vs. Tg(−), and † $P<0.05$, †† $P<0.01$ vs. Tg(+).

than that of the Tg(−) mice. The shorter latency in Tg(+) mice was significantly prolonged by treatment with YKS or UH. Though the prolongation of latency time is well-known to be affected by drug-dependent physical effects such as catalepsy and suppression of motor activity, we previously demonstrated that YKS did not induce them as haloperidol or risperidone does (Sekiguchi et al., 2009). Therefore, the ameliorative data of YKS and UH against the shorter latency in Tg(+) mice is thought to be not due to the physical disturbance, that is, these changes are selective to memory function, suggesting that YKS and UH might ameliorate the memory disturbance in Tg(+) mice.

Up till now, Tateno et al. (2008) demonstrated neuroprotective effects of YKS on Aβ-induced cytotoxicity in a primary culture of rat cortical neurons. We also recently demonstrated not only neuroprotective effects of YKS on glutamate-mediated excitotoxicity in cultured cells (Kawakami et al., 2009, 2010) but also ameliorative effects of YKS on learning and memory disturbance induced by i.c.v. injection of Aβ in mice (Sekiguchi et al., in press) and thiamine deficiency in rats (Ikarashi et al., 2009). These findings suggest that YKS has neuroprotective effects as one of the mechanisms. In addition to the mechanism, the

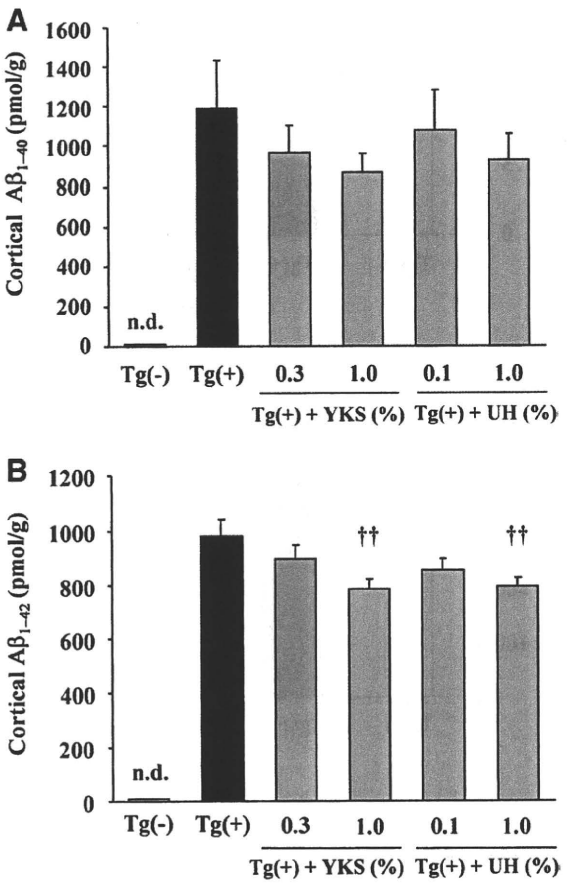

Fig. 6. Effects of YKS and UH on cortical concentrations of $A\beta_{1-40}$ (A) and $A\beta_{1-42}$ (B) in Tg mice. Large amounts of both forms of Aβ were detected in the cortex of Tg(+) mice but not in Tg(−) controls (n.d.). Values represent the mean±SE (n=10 in each group). Significance by Bonferroni/Dunn tests following one-way ANOVA is indicated as †† $P<0.01$ vs. Tg(+).

present results newly suggest a possibility that YKS ameliorates memory disturbance by preventing the aggregation of Aβ, which may be attributed to UH. To strongly support the participation of UH in the future, it will be necessary to confirm disappearance of the ameliorative effect of YKS by elimination of UH.

Kawarabayashi et al. (2001) demonstrated that Aβ deposit in the brain was started in the late stage (8–10 month old) though Aβ was detected biochemically in the early stage (4–5 month old). In the present study, YKS or UH was administered for 5 months from 10 to 15-month-old. In the acquisition trial for the learning at 11-month-old, no significant differences were observed in the latency times among all groups, suggesting that 11-month-old Tg(+) mice possess learning ability as well as Tg(−) control mice. However, the retention memory in the Tg(+) mice gradually decreased during 10- and 15-month-old during which Aβ deposits are facilitated. These data suggest close relation between Aβ deposit and memory disturbance. As YKS and UH ameliorated the memory disturbance in Tg(+) mice, these medicines are thought to have the preventing effect of memory disturbance.

On the other hand, in the present study, though it is true that YKS and UH statistically decreased Aβ accumulation, large amount of Aβ still existed in the brain: nevertheless, memory disturbance was ameliorated by treatment with YKS or UH. As a possible explanation for this ameliorative effect, synergistic effect of YKS including neuroprotective effect and inhibitory effect of Aβ aggregation is inferred. Furthermore, the improvement of cognition with YKS treatment was not demonstrated in clinical trials, most of which were evaluated in the comparatively short term of 4 weeks (Iwasaki et al., 2005a; Mizukami et al., 2009). To prove our hypothesis in the clinical trial, a long-term trial will be necessary in the future.

In patients with dementia, not only core symptoms but also BPSD often emerge. YKS has been reported to ameliorate BPSD such as hallucinations, agitation, and aggression in patients with AD, DLB, and other forms of senile dementia (Iwasaki et al., 2005a,b; Mizukami et al., 2009; Shinno et al., 2007, 2008). In the present study, the development of BPSD-like behaviors such as a marked increase in aggressive behavior and decrease in social behavior was observed in the Tg(+) mice, and YKS or UH ameliorated those abnormal behaviors. These effects, evaluated using social interaction tests, also are known to be influenced by changes in drug-dependent motor activity. In this test, we measured the traveling distance as an index of motor activity together with the interactive behavior, and confirmed that no significant difference in motor activity was observed among all groups. Therefore, the amelioration of aggression and sociability by YKS and UH are suggested to be a direct effect, not due to the secondary effect induced by the suppression of motor activity. The ameliorative effects by YKS of abnormal aggressiveness and sociability in Tg(+) mice are thought to support the finding in the clinical studies reporting that YKS ameliorated excitement, anger and decrease in activities of daily living in patients with AD (Iwasaki et al., 2005a).

Two mechanisms are inferred from the BPSD-ameliorating effect of YKS. One putative mechanism is that the effect may be obtained by inhibiting Aβ accumulation, which has already been discussed. However, it might be difficult to demonstrate this possibility by clinical studies because several clinical studies that evaluated YKS with a 4-week treatment period showed improvement in BPSD without amelioration of cognitive dysfunction in patients with dementia (Iwasaki et al., 2005a; Mizukami et al., 2009). This finding suggests another mechanism or possibility that YKS has a quicker improving effect on some neuronal function than the putative ameliorative effect on memory dysfunction and Aβ accumulation. Takeda et al. (2008a,b) reported that YKS attenuated the abnormal increase in cerebral glutamate release in zinc-deficient rats. Ikarashi et al. (2009) demonstrated YKS inhibited the increase in the cerebral extracellular concentration of glutamate in thiamine-deficient rats. Egashira et al. (2008) reported that YKS inhibited the 5-HT 2A receptor agonist-induced heat-twitch response by decreasing expression of 5-HT2A receptors in the prefrontal cortex. Terawaki et al. (2010) demonstrated *in vitro* that YKS and UH showed a partial agonistic effect on 5-HT1A receptors. This *in vitro* finding was also supported by *in vivo* experiment demonstrating that YKS ameliorated abnormal aggressiveness and sociability observed in para-chloroamphetamine-induced cerebral 5-HT-depletion rats, and the ameliorative effect was counteracted by co-administration of a 5-HT1A receptor antagonist, WAY-100635 (Kanno et al., 2009). Taken together, the ameliorative effects of YKS and UH on abnormal aggressive and social behaviors in Tg(+) mice may also relate to the glutamateric and serotonergic functions.

YKS has been used in Asian countries as a remedy for restlessness and agitation in children since it was developed by Xue Kai in 1555 (Iwasaki et al., 2005a). Recently, approximately over 100 million packages of YKS were sold during a year in Japan. Recent accumulated clinical studies reported not only the usefulness of YKS on BPSD but also information on the side effects. Mizukami et al. (2009) reported the appearance of hypokalaemia (two patients), gastrointestinal symptoms including vomiting/diarrhoea, nausea, epigastric distress (three patients), sedation (one patient), and leg edema (one patient) in a randomized cross-over study of YKS using 106 patients with dementia. In particular, the kampo medicines including glycyrrhiza, such as YKS, have been well-known to sometimes cause hypokalaemia (Ohtake et al., 2007; Makino et al., 2008). Therefore, the serum potassium concentration should be monitored.

CONCLUSION

In conclusion, the present study demonstrated that YKS inhibited accumulation of Aβ fibrils *in vitro* and *in vivo*. As a result, it improved not only memory deficits but also BPSD-like behaviors such as increased aggressive behavior and decreased social behavior in the APP transgenic

mice. Therefore, YKS may have potential as a therapeutic drug for patients with AD and mild cognitive impairment.

Acknowledgments—This work was partially supported by (1) a grant-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan (#16590554), (2) a program for the promotion of fundamental studies in Health Science of the National Institute of Biomedical Innovation (NIBIO) of Japan (#03-1) and (3) a grant-in-aid from Core Research for Evolutional Science and Technology of Japan Science and Technology Corporation.

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(Accepted 31 January 2011)
(Available online 12 February 2011)

Tohoku J. Exp. Med., 2010, **221**, 87-95

Invited Review for the 90th Anniversary

Geriatric Medicine, Japanese Alzheimer's Disease Neuroimaging Initiative and Biomarker Development

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Due to a change in disease spectrum in aged countries, the primary role of geriatricians should be directed to an appropriate management and prevention of 1) cognitive decline and dementia, 2) swallowing and aspiration pneumonia and 3) falls and fractures. Management of dementia constitutes a central part in the practice of geriatric medicine in order to support independence of life in elderly people. The current paradigm of cognitive function-based testing for the diagnosis and treatment of Alzheimer's disease (AD) is going to drastically shift to a biomarker-based test approach, a shift that will correspond to the emergence of disease-modifying drugs. In addition, a new molecular imaging technique that visualizes neuronal protein deposits or pathological features has been developed in Japan and the U.S.A. Based on these achievements, the Alzheimer's Disease Neuroimaging Initiative (ADNI) was proposed and initiated in 2005. The ADNI is a long-term observational study being conducted in the U.S.A., Europe, Australia, and Japan using identical protocols. The objectives of ADNI are: 1) to establish methodology which will allow standard values related to long-term changes in imaging data, such as MRI and PET, in patients with AD and mild cognitive impairment and normal elderly persons; 2) to obtain clinical indices, psychological test data, and blood/cerebrospinal fluid biomarkers to demonstrate the validity of image-based surrogate markers; and 3) to establish optimum methods to monitor the therapeutic effects of disease-modifying drugs for AD. Patient enrollment in the Japanese ADNI has begun in July 2008. Imaging of AD pathology not only acts as a reliable biomarker with which to assay curative drug development by novel pharmaceutical companies, but it also helps health promotion toward AD prevention.

Keywords: geriatric medicine; Alzheimer's disease; Amyloid β-peptide; Biomarker; Amyloid imaging; ADNI
Tohoku J. Exp. Med., 2010, **221** (2), 87-95.

Geriatrician's role and proposal of "Geriatric Triangle"

Geriatric medicine is an independent internal medicine division that is specialized for management of medical problems of elderly people. Despite a fact that elderly people appear healthy, a variable latent organ dysfunction may be present due to a limited residual capacity. A condition referred to as geriatric syndrome is a complex and multi-organ disease especially suffered by elderly people. The geriatric syndrome consists of more than 50 medical conditions such as dementia, depression, delirium, pneumonia, urinary incontinence, osteoporosis and fractures as well as malnutrition, sarcopenia, skin ulceration and renal failure. Importantly, these clinical conditions often occur in combination rather than separately. As illustrated in Fig.1, most important functions which support independence of life in later years are: 1) Thinking and judgments; 2) Eating and swallowing; and 3) Standing and walking. Loss of these basic functions alone or in combination will directly lead to devastating health implications and reduced quality of life. Disturbance of cognitive ability manifests as dementia. Impairment of ordered oropharyngeal functions causes a disturbed swallowing or dysphasia followed by development of aspiration pneumonia. Failure of standing and walking results in repeated falls and fractures. — all being hardly present before the age of 65 but highly prevalent over the age of 75. Moreover, these problems not merely occur in separate occasions but they also are inter-related each other. For example, people with advanced dementia are likely to develop eating problems and aspiration (Nakagawa et al. 1997; Wada et al. 2001; Mitchell et al.

Received March 23, 2010; revision accepted for publication April 5, 2010. doi:10.1620/tjem.221.87
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2009). Repeated episodes of pneumonia will develop disturbed nutrition and dehydration which leads to sarcopenia with an increased risk of falls and fractures (Lang et al. 2010). A long-term bedridden state due to hip or vertebral fractures will result in worsening of dementia (Muir et al. 2009). Conversely, demented patients who were treated by anti-psychotic drugs are associated with an increased risk of falls and fractures (Horikawa et al. 2005). A long-term bedridden state due to hip or vertebral fractures are prone to develop esophageal regurgitation and aspiration (Matsui et al. 2002). Drugs that up-regulate brain dopaminergic function are occasionally beneficial to prevent aspiration pneumonia in the elderly (Yamaya et al. 2001). Here, we propose to term such a closely-related condition as "geriatric triangle" as shown in Fig.1. Patients diagnosed as having geriatric triangle are likely to be placed on a long-term care facility due to reduced quality of life (Sasaki 2008). Therefore, the primary role of geriatricians should be directed to an appropriate management and prevention of geriatric triangle. Moreover, every single geriatrician should be capable of managing the geriatric triangle beyond a scope of each organ specialist (Sasaki 2008). Hence, primary targets of geriatric medicine may include assessment and treatment of 1) cognitive decline and dementia, 2) swallowing and aspiration pneumonia and 3) falls and fractures. On the other hand, it is unlikely as a primary role of geriatrician only to manage elderly people with diseases which are spanning entire stages of life. Such diseases, for example, hypertension and diabetes mellitus, can be taken care of by each organ specialist. Due to a change in disease spectrum in aged countries, it should be emphasized that geriatric medicine has become a separate and independent practice division from other organ-specialized fields of internal medicine.

Current scientific approach toward understanding of Alzheimer's disease (AD) pathogenesis

Alzheimer's disease (AD) deprives sufferers of variable life-supporting functions that are necessary for independence in the later years of life. Development of AD leads to parting from society. Care-taking families sacrifice their quality of life and their mental and physical burdens are immeasurable. Loss of personality due to alteration of brain function while physical appearance remains the same is horrible and miserable. As an essential domain of geriatric triangle as described in Fig. 1, prevalence of dementia (the number of people with the disease at any one time) doubles for every 5-year age group beyond the age 65. Briefly, dementia hardly develops prior to age 60. However, according to data from Ministry of Health, Labor and Welfare in Japan, the prevalence of dementia is estimated to be 1.5% for age 65-69, 3.6% for age 70-74, 7.1% for age 75-79, 14.6% for age 80-84, 27.3% beyond age 85 (http://www.mhlw.go.jp/english/index.html). The elderly population aged 65 or older is now approximately 22% of the whole population in Japan. Therefore, it is likely that dementia becomes quite common over the age of 65. According to recently conducted community survey, AD is a leading cause of dementia among elderly Japanese population (Yamada et al. 2001; Wada-Isoe et al. 2009). The rapid increase in the number of AD patients can be a consequence of a rapid increase in human life span. In Japan, an average life span in 1947 was 50.6 years for men and 53.9 years for women. Surprisingly, that was 79.3 years for men and 86.1 years for women in 2008. It is possible that AD is only encountered when the nation reaches a sufficiently aged society. Furthermore, AD is a major factor in increasing national medical expense. It is a universal desire to find a way to control AD. The U.S.A. calls the rapid increase in

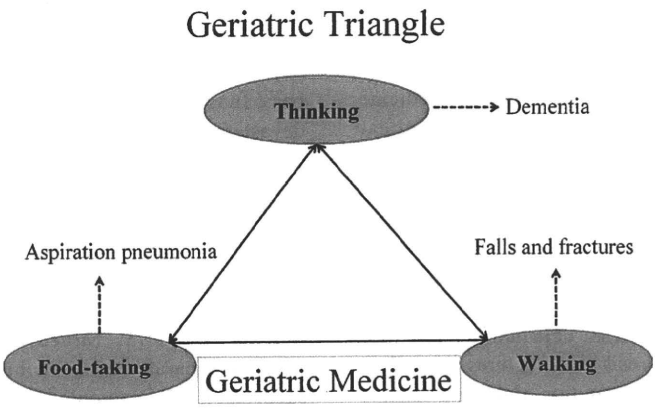

Fig. 1. Proposed concept of geriatric triangle.
At least three physical and mental functions are needed to support independence of life in elderly people. They are 1) Food-taking ability, 2) Standing and walking, and 3) Thinking and judgments. Loss of these basic functions alone or in combination will lead to devastating health implications and reduced quality of life through a vicious circle. Here, we propose to term such a vicious circle as "geriatric triangle". Geriatric triangle constitutes a major part of geriatric syndrome. Therefore, each geriatrician should be capable of managing the geriatric triangle beyond a scope of each organ specialist.

AD patients and concomitant pressure on federal budget a "National Crisis" which illustrates the seriousness of the problem (A National Alzheimer's Strategic Plan, 2009).

Understanding of pathogenesis of AD has markedly progressed in the last 3 decades. Pathological changes of AD occur gradually initially in cognitively normal people with dementia representing the end stage of many years of accumulation of amyloid β-peptide (Aβ). Aβ was first sequenced from meningeal blood vessels of AD brains (Glenner&Wang 1984). A year later, the same peptide was discovered as the primary components of senile plaques (Masters et al. 1985). Shortly after these earlier findings, cloning of the gene encoding amyloid β-peptide precursor protein (APP) and its localization to chromosome 21coupled with the recognition that Down's syndrome (trisomy 21) leads invariably to AD neuropathology set a initial hypothesis that Aβ is a primary driving force in the pathogenesis of AD. The other neuropathological features that are characteristic of AD include neurofibrillary changes and neuron death. Spatial distribution of senile plaques differs from that of neurofibrillary changes (Arriagada et al. 1992a; Arriagada et al. 1992b). A major building block of neurofibrillary changes was shown to be abnormally phosphorylated tau (Lee et al. 1991). According to the amyloid hypothesis, cortical Aβ accumulation causes all of the disease process associated with AD including microglial and astroglial activation, synaptic injury, oxidative injury followed by abnormal tau phosphorylation and eventually loss of neurons and dementia (Hardy and Selkoe 2002). The amyloid hypothesis also tells us that control of amyloid deposition would achieve success to control AD. There have been several conceptually important observations that strongly support the amyloid hypothesis. First, we occasionally see Aβ-positive but tau-negative brains from cognitively normal elderly people in autopsy samples, suggesting that Aβ deposition predates tau deposition (Arai et al. 1990). This time framework was further evidenced by the observation that Aβ-positive senile plaques occur at age 30's, whereas tau-positive neurofibrillary changes are seen only after the age of 40 in the brains afflicted with Down's syndrome (Mann et al. 1989). Thirdly, genetic mutations causing autosomal dominant familial AD were discovered in the APP gene clustering at or very near the sites that are normally cleaved by proteases called β or γ-secretases (Goate et al. 1991). These mutations enhance proteolytic processing of APP to generate amyloidogenic Aβ (Citron et al. 1992). Other AD-causing mutations in PS-1 and PS-2 gene also enhance generation of amyloidogenic Aβ by changing proteolytic processing of APP (Scheuner et al. 1996). Finally, a distinct Aβ species ending at amino acid 42 (Aβ42) is highly amyloidogenic, and there was a uniform pattern of Aβ42 deposition as an initial event of pathology either in non-demented, AD or Down's syndrome patients (Iwatsubo et al. 1994). As illustrated in Fig. 2, we can use a hypothetical assumption to think about the progression of AD. Namely, assuming that memory loss became noticeable at the age 70 followed by progression of multiple cognitive decline and behavioral problems at the age of 75. The patient was eventually diagnosed as suffering AD. In such an instance, we can assume that accumulation of cerebral Aβ may have started at around 50 years of age followed by intracellular accumulation of tau in the form of neurofibrillary changes as well as neuron death may have started at approximately 60-65 years of age. Therefore, it should be emphasized that there is an approximately 20-year time lag between the initiation of amyloid protein deposition and onset of the earliest clinical manifestations of dementia in AD. During this lag-period, individuals are cognitively normal but they are not aware of what changes are taking place in their brains. We assume that such individuals would ultimately develop AD if he or she lived long enough. Furthermore, a prodromal stage of AD often referred to as mild cognitive impairment (Petersen et al. 2009) is characterized by onset of mildest cognitive symptoms despite a massive neuron loss in vulnerable cortical areas (Gómez-Isla et al. 1997). Hence, there is an extremely high need for development of methods that simply and reliably detect amyloid and tau deposits. One such approach is a recently developed molecular imaging technique called "amyloid imaging".

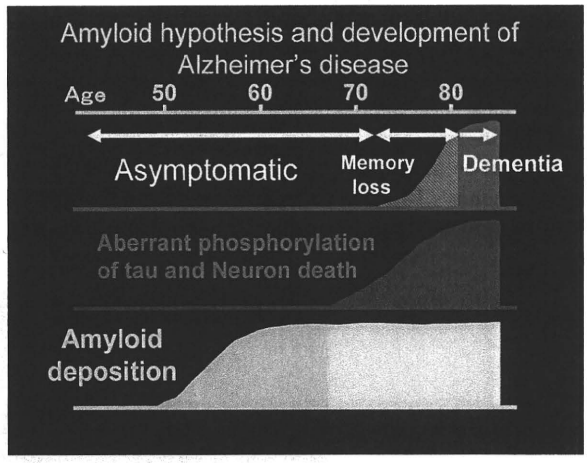

Fig. 2. Hypothetical scheme of progression of AD from amyloid deposition to development of dementia.
It is noteworthy that brain amyloid continues to be accumulating towards the onset of AD during which subjects are not aware of what changes are taking place in their brains. When subjects are first symptomatic, abundant neurofibrillary changes and a massive neuron death have already begun in vulnerable brain regions such as hippocampal or entorhinal cortex. Original description was made by Yasuo Ihara.

A paradigm shift in the diagnosis and treatment of AD

Fig. 3 illustrates a superimposition of the diagnostic and treatment framework in the context of the hypothetical amyloid cascade described above. AD has so far been diag-

nosed clinically only by demonstrating "cognitive decline" which has progressed to a stage that is sufficient enough to disturb independent social or occupational life. It is likely that cognitive decline is associated with a massive neuron death that exceeds so-called "cognitive reserve capacity" (Stern 2009). In addition to cognitive testing, two other diagnostic techniques including magnetic resonance imaging (MRI) and fluorodeoxyglucose (FDG)-PET are currently in common use to demonstrate a mass of dead nerve cells directly or indirectly. Symptomatic drugs such as donepezil hydrochloride and memantine hydrochloride are best considered at this point. However, a dramatic improvement of memory function cannot be expected since disturbance of episodic memory is based upon a massive loss of hippocampal and entorhinal cortical neurons. Accordingly, if we assume that AD represents chronic effects of a long-standing imbalance between Aβ production and Aβ clearance and this imbalance causes all existing events in the downstream of Aβ, a special attention should be directly paid to amyloid and tau depositions in the development of preventive strategies. If we are successful in developing diagnostic methodologies to detect amyloid or tau deposition before a massive neuron death occurs, such approaches will make a great contribution to developing a disease-modifying or curative treatment that directly targets amyloid and also tau. A paradigm of cognitive function-based testing for the diagnosis and treatment of AD is going to drastically shift to a biomarker-based test approach in accordance with the emergence of disease-modifying drugs. Hope for prevention of AD would be potentially carried out. As mentioned later, the Alzheimer's Disease Neuroimaging Initiatives (ADNI) will change paradigm of diagnostic and treatment of AD drastically with biomarkers as a bridging role in the paradigm shift.

Biomarkers with a bridging role in the paradigm shift

In general, biomarkers of AD are defined as indicators of specific features that characterize AD in vivo. Either biochemical or imaging biomarkers are expected to provide potentially diverse purposes as summarized elsewhere (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association & NIAWG 1998; Frank et al. 2003; Shaw et al. 2009). First, biomarkers will support preonset diagnosis. As demonstrated in Fig. 2 and 3, AD pathology has already started with abundant amyloid pathology even though individuals are otherwise normal and are still independent in their daily living activities,. This stage can be an ideal therapeutic time point in which disease-modifying or curative drugs should be indicated before neurodegenerative cascade is triggered. Such biomarkers will enable us to move from disease modification to prevention of AD. Second purpose is evaluation of disease severity. Currently, severity or clinical stage of AD is evaluated by neuropsychological testing. However, neuropsychological test results are likely to vary due to the patient's physical condition on the day of the test and experience of the examiners. In a study involving 192 AD patients performed by Jack et al., the annual change in ADAS-Cog score in mild to moderate AD was 4.25 ± 7.2 (mean ± S.D.) points, while the yearly change in hippocampal volume on MRI in the same patients was −234 ± 144 (mean ± S.D.) mm^3 (Jack et al. 2003; Petersen et al. 2005). The SD, representing variation of the values, of the hippo-

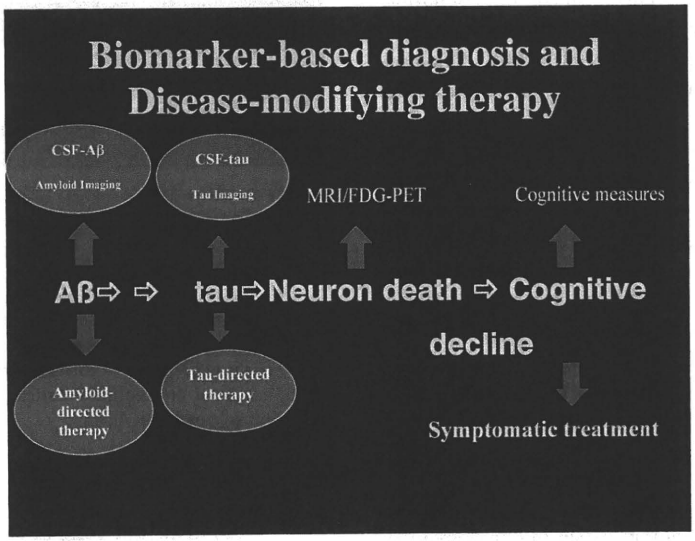

Fig. 3. Strategies for new diagnostic and therapeutic approaches for AD are presented based on amyloid hypothesis.
This figure illustrates a superimposition of the diagnostic and treatment framework in the context of the hypothetical amyloid cascade as described in Fig. 2. In the hypothesis, amyloid is located upstream probably due to a causative agent of AD. Therefore, amyloid imaging is quite attracting because this technology will facilitate both detection and intervention that targets amyloid. If tau imaging would also be possible, tau-targeting therapy might be considered.