Oral Administration of Synthetic Retinoid Am80 (Tamibarotene) Decreases Brain β -Amyloid Peptides in APP23 Mice

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The purpose of this study is to investigate whether a synthetic retinoid Am80 (tamibarotene) exhibits any improving effects on amyloid precursor protein (APP)23 mice, a model of Alzheimer's disease. Am80 was orally administered in feed to 20-week (5-month)-old APP23 mice at a dose of 0 (control) or 0.5 mg/kg/d for 14 weeks. The Am80 treatment reduced significantly the insoluble $A\beta$ levels in brain, in particular $A\beta_{42}$, while it gave no apparent effects on the soluble $A\beta$ levels. The results suggest that oral administration of Am80 may have potency to reduce the extracellular $A\beta_{42}$ of insoluble and possibly oligomeric or protofibril forms, which are related to the cause and/or progression of Alzheimer's disease. The Am80 treatment showed no significant effect on spatial learning and memory of APP23 mice by Morris water maze analysis. The main reason for the absence of significance seems based on the large deviation and some mice both in the treated and the non-treated groups would neither swim nor make efforts to reach the platform.

Key words retinoid; Am80; tamibarotene; Alzheimer's disease; β -amyloid

A pathological hallmark of Alzheimer's disease (AD) is the presence of senile plaque deposition of β -amyloid ($A\beta$) peptides. $A\beta$ peptides are generated by successive proteolysis of amyloid precursor protein (APP) that is initially cleaved by the β -site APP-cleaving enzyme 1 (BACE1) and subsequently by γ -secretase. Among the $A\beta$ peptides generated, $A\beta_{42}$ aggregates more easily and plays particular roles for the $A\beta$ deposition.¹⁾ Preventing, reversing, or reducing the $A\beta$ deposition, therefore, is considered to be a promising therapeutic way against AD.

Retinoic acid (RA), the active metabolite of vitamin A (retinoid), regulates gene expression through its nuclear receptors, RA receptors (RARs) and retinoid X receptors (RXRs). Deprivation of vitamin A results in $A\beta$ accumulation.²⁾ RA regulates a disintegrin and metalloprotease family 10 (ADAM10),^{3,4)} an α -secretase of APP. RA also induces the expression of tissue plasminogen activator (tPA),^{5,6)} an enzyme that cleaves plasminogen to generate plasmin, a protease that degrades $A\beta$ oligomers and monomers. Importantly,

clinically, evidence has shown defective retinoid transport and function in AD brain,⁷⁾ suggesting that increasing the availability of RA in the brain may prevent or decrease $A\beta$ -associated neurodegeneration.^{7,8)}

Am80, a synthetic retinoid with agonistic activities on RAR- α and - β , but not - γ , is one of the selective RAR ligands. Am80 has been reported to rescue the memory deficit caused by scopolamine, as a model of the main clinical symptom of AD.⁹⁾ In the present study, we examined the effect of Am80 treatment on the neurodegenerative pathology and memory deficits in APP23 mice,¹⁰⁾ a well established AD mouse model.

MATERIALS AND METHODS

Compounds Am80 (tamibarotene) was prepared as previously described.¹¹⁾ Feeding pellets were made by mixing Am80 with commercial rodent diet, MF (Oriental Yeast, Tokyo, Japan). In order to administer approximately 0.5 mg/kg/d Am80 to mice, pellets containing 75 mg Am80 in 10 kg MF were made, based on the estimate for the food consumption that mice with average body weight of 30 g would take 1.5 g food each per day. The dose was set at 0.5 mg/kg/d, based on the effective results obtained in other *in vivo* studies with Am80, such as ref. 12.

Animals Hemizygous APP23 mice, which overexpress human-type APP carrying double mutations (K670N/M671L), were generated and bred as previously described.¹⁰⁾ Animals were housed in standard mouse cages under conventional laboratory conditions (food and water available *ad libitum*, constant room temperature and humidity, 12/12-h light–dark cycle). At 20 weeks of age, male APP23 mice ($n=26$) and wild-type littermates (C57BL/6J, $n=13$) were divided into two groups by stratified random sampling in order to equalize the mean body weight of groups; 0.5 mg/kg/d Am80 and control group. Am80 was administered in feed for 14 weeks. Weight of mice was measured once a week. The animal experiments were conducted in accordance with Guidelines of the Kumamoto University Animal Committee.

Enzyme-Linked Immunosorbent Assay (ELISA) The soluble (Tris-buffered saline (TBS)-extractable) fraction and TBS-insoluble (but guanidine-HCl-extractable) fraction from the cerebral hemisphere were prepared as described previously.¹³⁾ The amounts of $A\beta_{x-40}$ and $A\beta_{x-42}$ in each fraction were determined by sandwich ELISA (294-64701 and 290-62601, Wako Pure Chemical Industries, Osaka, Japan), respectively, according to the manufacturer's instructions.

Morris Water Maze Test (MWM Test) The spatial learning and memory were evaluated by MWM test.¹⁴⁾ Acquisition training consisted of 3–5 trials a day for 4 d (day 1: 3 trials, day 2: 3 trials, day 3: 5 trials, and day 4: 4 trials). The trials were started from three different positions in a semi-random order with a 20-min intertrial interval. If a mouse failed to find the platform within 120 s, it was guided carefully to the platform and allowed to stay on it for 20 s. The acquisition phase was followed by a probe trial; the platform was removed from the maze, and animals were allowed to swim freely for 100 s. Both during acquisition and probe trials, the animals' trajectories were recorded using a computerized video-tracking system (CompACT VAS, Muro-

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machi Co., Ltd., Tokyo, Japan). During training trials, the escape latency to the platform was measured. Results of the probe trials were expressed as the percentage of time spent in each quadrant of the MWM.

RESULTS

Effect of Am80 Administration on the Levels of Brain A β Peptides in APP23 Mice To investigate whether Am80 inhibits AD development, APP23 mice were fed with feeding pellets containing Am80 (at 0.5 mg/kg/d) or normal pellets from 20 to 34 weeks of age. During the experiment period, there was no significant difference in the body weight of mice between each group (data not shown). The oral administration of Am80 for 14 weeks influenced A β accumulation in the brain of APP23 mice. On the levels of soluble A β peptide, Am80 had no effect (Figs. 1A, B). However, the insoluble A β_{40} level of Am80-group was lower than that of control-group ($p=0.081$, Fig. 1C). More importantly, the insoluble A β_{42} level of Am80-group showed significantly lower than that of control-group ($p=0.015$) (Fig. 1D). These results indicate that oral administration of Am80 decreases brain insoluble A β levels, in particular, A β_{42} , of APP23 mice. The effect of Am80 on A β levels in wild-type mice was not determined, because preliminary tests showed their levels were below the detection limit in the ELISA system (data not shown).

Effect of Am80 Administration on Morris Water Maze Performance in APP23 Mice After the Am80 treatment for 14 weeks, the spatial learning and memory was also evaluated by MWM test (Fig. 2).¹⁴⁾ The vehicle-treated APP23 mice showed impaired acquisition of spatial learning, compared with the wild-type littermates (two-way ANOVA: $p<0.001$, Fig. 2A). Under the conditions, Am80-fed APP23 mice showed a slight improvement in acquisition of spatial learning, but no significant difference was observed statistically between Am80- and control-groups of APP23 mice (two-way ANOVA: $p=0.254$, Fig. 2A). During the MWM test, some APP23 mice (3/13 and 4/13 of the Am80-treated and the control mice, respectively) showed low locomotive activity (swim speed below the 0.06-m/s threshold). On day 4, all mice were given a probe trial to evaluate their spatial memory for the platform position (Fig. 2B). On the percentage of time spent in the quadrant "trained area" where the hidden platform was previously located, there was no significant difference between the two groups ($p=0.241$, Fig. 2B), although the data variation of Am80 treated mice was much smaller than that of APP23 control. These results indicate that memory deficits in the vehicle-treated control APP23 mice were not improved by Am80 treatment under the conditions.

DISCUSSION

A recent review⁸⁾ has summarized that all-*trans* RA (ATRA) affects the production or degradation of A β , based on several *in vitro* studies. In the present study, however, we demonstrated that oral administration of Am80 decreased significantly the brain level of insoluble A β_{42} in APP23 mice.

The soluble (TBS-extractable) A β fraction is mainly localized in neurons, while the insoluble A β fraction is ac-

Fig. 1. Effect of Am80 Administration on the Levels of Brain A β Peptides in APP23 Mice

After the Am80 treatment for 14 weeks, A β_{x-40} (A, C) and A β_{x-42} (B, D) levels in both soluble (A, B) and insoluble (C, D) fractions of the mouse brains were determined by sandwich ELISA. Error bars represent mean $\pm$ S.E.M. from thirteen mice per group. The p value was calculated by Student's t test.

Fig. 2. Effect of Am80 Administration on Morris Water Maze Performance in APP23 Mice

After the Am80 treatment for 14 weeks, the spatial learning and memory was evaluated by the Morris water maze test. (A) Acquisition of spatial learning in the Morris water maze hidden-platform task. Ordinate shows the time to find a hidden escape platform. Lines represent mean $\pm$ S.E.M. from the indicated mice per group. WT, wild type. (B) Memory test in Morris water maze probe trial without platform in APP23 mice. Percent search time for each quadrant during a 100-s session was measured. Error bars represent mean $\pm$ S.E.M.

cumulated in extracellular plaques¹³⁾ that are formed *via* oligomeric or protofibril form. Therefore, it is considered that the Am80 treatment may affect the production or degra-

dation of A β s in neuron as described in the *in vitro* and *in vivo* results by retinoids through ADAM10^{3,4)} or tPA,^{5,6)} and also may affect preferentially the internalization or clearance of extracellular A β s. For reducing the A β s produced, it is of note that glial internalization of oligomeric and fibrillar A β s^{15–17)} may be critically involved in their removal from synaptic cleft, in addition to the degradation of A β in/on neurons by A β -degrading enzymes such as neprilysin.¹³⁾ Recent reports also demonstrated that anti-inflammatory cytokine IL-4 induces selective phagocytotic activity to oligomeric A β in microglia,¹⁸⁾ while pro-inflammatory cytokines decrease it.¹⁹⁾ Since Am80 was reported to increase IL-4 production in T-cell system,²⁰⁾ similar activation of Th2 cells or type-2 microglia¹⁸⁾ might be induced in our Am80-treated APP23 mice. Presently, however, we have no data in the relationship between Am80 treatment and IL-4 production in brain, and further study is needed.

Our trials of immunostaining of brain slices with anti-A β _{17–24} antibody gave no statistically significant difference between the Am80 treated and the control APP23 mice (data not shown). This may be resulted from the reason that the commercially available antibody (Anti-A β _{17–24} antibody: 4G8 from Covance, U.S.A.) has broad specificity to react with even APP and/or intermediate species before generating A β _{1–42} or A β _{1–40}. We should seek and use more specific antibodies for A β _{1–42} or A β _{1–40} to clarify the problem.

Although the oral administration of Am80 decreased the A β s, it gave no significant effect on the learning deficits of APP23 mice by the MWM test (Fig. 2). While our study was proceeding, Fan and colleagues²¹⁾ reported that ATRA effectively reduced A β accumulation and rescued the spatial learning and memory deficits by MWM test, when it was intraperitoneally administered for 8 weeks to 5-month-old APP/presenilin 1 (PS1) double-transgenic mice. There are at least two major differences between the two studies: the AD model mice used and the methods of drug administration. In A β accumulation and disease progression, APP/PS1 mice are faster than APP23.^{21,22)} Oral administration we used can be largely different in the absorption, timing and pharmacokinetic profiles from the intraperitoneal administration. Am80 has reasonable absorption characteristics and the dose (0.5 mg/kg/d) seems potentially effective, as shown in other *in vivo* studies with Am80, such as ref. 12. In our MWM test, some mice (3–4 mice among 13) would neither swim nor try to make efforts to reach the platform. Therefore, we should try other behavioral tests which are milder and less energy-consuming. Oral administration has a considerable advantage for therapeutic use, and alternative schedule (tim-

ing and period) and dosage of Am80 administration should also be considered.

In conclusion, Am80 is capable of reducing the extracellular level of A β ₄₂ in the brain.

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