Prevention of amyloid ß-induced memory impairment by fluvastatin, associated with the decrease in amyloid ß accumulation and oxidative stress in amyloid ß injection mouse model

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Abstract. Alzheimer's disease (AD), the most common cause of dementia in the elderly, is characterized by amyloid ß (Aß)-containing plaques and neurofibrillary tangles, and synaptic and neuronal loss, along with progressive cognitive impairment. Although growing evidence suggests the beneficial effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) on AD, this notion is still controversial. To evaluate the efficacy of statins for Aß-induced cognitive impairment, we employed an Aß injection model. Using this model, the present study demonstrated that pretreatment with fluvastatin, but not post-treatment just after Aß exposure, prevented Aß-induced memory impairment. We also observed that fluvastatin significantly decreased Aß accumulation and oxidative stress after Aß injection. Mice treated with simvastatin, but not fluvastatin, did not demonstrate the prevention of Aß-induced memory impairment, and showed no significant decrease in oxidative stress. More importantly, fluvastatin significantly prevented the loss of neurons in the basal forebrain induced by Aß.

Overall, the present study demonstrated that fluvastatin significantly prevented memory impairment induced by Aß. The beneficial effects of fluvastatin might be explained by the preservation of neurons through a significant decrease in Aß accumulation and oxidative stress. In clinical practice, the timing of the start of fluvastatin treatment might be critical in achieving a beneficial effect on cognitive function.

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Introduction

Alzheimer's disease (AD), the most common cause of dementia in the elderly, is characterized by amyloid ß (Aß)-containing plaques and neurofibrillary tangles, and synaptic and neuronal loss, along with progressive cognitive impairment. Aß, a 38-43 amino acid peptide, is the primary component of senile plaques (1). Aß deposited in senile plaques is considered to be primarily involved in the pathogenesis of AD, because i) familial AD (FAD) has been linked to mutations in the amyloid precursor protein (APP) (2-4), and ii) FAD-linked mutations in the amyloid precursor protein (APP) and presenilin genes (5-7) result in the increased production of Aß42 (8,9), which is the predominant form found in senile plaques (10). Moreover, levels of total Aß40 and Aß42 are elevated in early dementia and levels of both peptides are strongly correlated with cognitive decline (11).

Although growing evidence suggests the beneficial effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) for AD, this notion is still controversial. It was firstly reported that there is a lower prevalence of diagnosed AD in patients taking the statins, lovastatin and pravastatin, in the US (12). Another group reported that patients in the UK, receiving statins, had a lowered risk of developing dementia (13). Furthermore, it was recently reported that atorvastatin produced significant positive effects on cognitive function (14,15). Consistent with clinical reports, the effect of statins on Aß metabolism has been reported *in vitro* and *in vivo* (16-18). Simvastatin reduces Aß levels in cell cultures and in guinea pig brain homogenate (16). Atorvastatin has been reported to activate α-secretase, and subsequently reduce the production of Aß (17). However, other cohort studies indicated that lipid levels and the use of lipid-lowering agents do not seem to be associated with the risk of AD (19). Thus, there is an apparent discrepancy among studies regarding the effectiveness of statins for AD. In this study, we hypothesized that the timing of the start of treatment with a statin, or the kind of statin, might be critical in achieving beneficial efficacy. Thus, we employed an Aß1-40 injection model to evaluate the effects of statins

on memory impairment at different timings. The present study demonstrated that fluvastatin, but not simvastatin, significantly prevented memory impairment induced by Aß through a significant decrease in Aß accumulation and oxidative stress, and the prevention of neuronal loss.

Materials and methods

Animals. Male ddY mice (6-8 weeks old) were obtained from CLEA Japan, and housed in specific pathogen-free facilities under a standard 12/12-h light/dark cycle. All experiments were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals of Osaka University School of Medicine.

Peptide and chemicals. Aß1-40 was purchased from Peptide Institute. Aß1-40 solution was prepared for each experiment as described previously (20). Briefly, 0.55 mg Aß1-40 peptide was dissolved in 3250 μl PBS with 35% acetonitrile and 0.1% trifluoroacetic acid. To remove the remaining undissolved Aß1-40, centrifugation was performed at 15,000 x g for 3 min before Aß1-40 solution was aliquoted. The control peptide Aß40-1 was also prepared in the same way as Aß1-40. Fluvastatin was provided by Novartis Pharma AG, and simvastatin was purchased from Sigma-Aldrich.

Aß1-40 injection model. To evaluate the effects of the statins on Aß-induced cognitive impairment, we employed a mouse model produced by previously reported methods with modification (21,22). We injected Aß1-40 into the cerebral ventricle by single injection. It has been reported that the levels of total Aß40 and Aß42 are elevated early in dementia, and the levels of both peptides are strongly correlated with cognitive decline (11), and that the level of Aß was increased 80-fold compared with control (11). Thus, we calculated the quantity of Aß to be injected. As the basal level of Aß in the mouse brain is normally in the low nanomolar range, and in transgenic mouse models of brain amyloidosis it varies from 40 to 250 nM/kg body weight from 3 to 12 months of age (23), we determined the dose of Aß1-40 to be injected as 200 pmol/5 μl. Although the quantity of Aß injected was relatively low as compared to that in a previous report (21,22), our preliminary study revealed memory impairment induced by Aß, as assessed by a water-finding task to evaluate spacial reference memory (data not shown).

Intracerebroventricular (i.c.v.) administration was carried out in accordance with a procedure described previously (22). Briefly, the mice were anesthetized with isoflulane gas and intraperitoneal xylazine and ketamine, Aß1-40 (200 pmol/5 μl) was injected i.c.v. into the mice, aimed at 1 to 1.5 mm lateral to the midline, 0.5 mm posterior to the bregma, and 3 mm deep, using a 100 μl Hamilton syringe with a 27 gauge needle.

Immunohistochemical detection of Aß. Two days after Aß injection, the mice were sacrificed by transaortic perfusion-fixation with cold saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The brains were removed, and postfixed for 12 h in the same fixative. The brains were left in 15% sucrose in sodium phosphate buffer for 24 h, and 30% sucrose for 48 h at 4˚C. The frozen brains were cut at 10-μm thickness with a cryostat (Leica CM3050 S, Leica Microsystems). A mouse anti-human Aß protein monoclonal antibody (6E10; Sigma) was used at a 1:200 dilution. The tissue sections were incubated overnight at 4˚C with the primary antibody. After washing in PBS three times, the tissues were incubated for 2 h at room temperature with the second antibody (goat anti-mouse antibody, Alexa488 conjugate). Images were obtained by a using fluorescence microscope (Nikon Eclipse TE300, Nikon).

Water-finding task. Three weeks after Aß injection, the water-finding task was performed (24). Briefly, the apparatus consisted of an open field (50x30 cm with walls 15 cm high) with an alcove (10x10x10 cm with ceiling) in the middle of one of the longer sides of the wall. A water bottle with a tube tip was inserted into the ceiling of the alcove. In the training trial, the mice were placed individually into one corner of the opposite side of the alcove and allowed to explore freely for 3 min. The frequency of touching, sniffing, or licking the water tube in the alcove (number of approaches) was counted. Mice that could not find the water tube within 3 min were excluded from the test. The mice were immediately returned to their home cages after the training trial, and were then deprived of water for 48 h to be motivated to look for water. In the retention trial, the mice were placed individually in the same corner of the apparatus. To evaluate memory acquisition, drinking latency (DL) was measured as the time from placement in the corner to first touching the water tube.

Measurement of Aß. The brain homogenates were analyzed by Aß40 ELISA kit (Wako Pure Chemical Industries). To extract soluble cerebral Aß, 150 mg of fresh frozen tissue was homogenized with a Teflon-glass homogenizer (6 strokes) in 1 ml of 70% formic acid. Homogenates were centrifuged at 100,000 x g for 1 h to remove particular material. The supernatant was neutralized with a 20-fold dilution in 1 M Tris base. After neutralization, the sample was diluted with the standard diluent in the Aß40 ELISA kit and measured as directed in the package insert.

Detection of superoxide anion in brain sections. Superoxide anion was detected as described previously (25). In brief, frozen, enzymatically intact, 10-μm sections were prepared from mouse brain two days after Aß injection and immediately incubated with dihydroethidium (DHE) (10 mol/L; Molecular Probes Inc.) in PBS for 30 min at 37˚C in a light-protected humidified chamber. DHE is oxidized on reaction with superoxide to ethidium, which binds to DNA in the nucleus and fluoresces red. Images were obtained using a Bio-Rad Radiance 2100 laser scanning confocal microscope (Bio-Rad Laboratories, Inc.). The intensity of the fluorescence was analyzed and quantified using ImageJ.

Aß degradation assay and neprilysin assay. The mouse brain samples were homogenized on ice in 3 ml of 0.25 M sucrose in 50 mM Tris-HCl (pH 7.4) without protease inhibitors by a Teflon-glass homogenizer for 8 strokes. The samples were centrifuged at 1300 x g for 5 min at 4˚C, and then the supernatant fractions were centrifuged at 100,000 x g for 1 h to

collect membrane pellets. After washing with 100 mM Na_2CO_3 (pH 11.3), the membrane fraction was re-suspended in 50 mM Tris-HCl (pH 7.4), and sonicated by ultrasonic disruptor UD-201 (Tomy) at minimum power. Protein concentration was determined by a detergent compatible protein assay kit (Bio-Rad). The brain membrane fraction was used for Aß degradation and neprilysin assays.

The Aß degradation assay was performed as previously described (26). Briefly, 100 pM synthetic human ^{125}I-Aß (2000 μCi/mmol of specific activity; GE Healthcare) was incubated with 50 μg/ml brain membrane fraction in 50 mM Tris-HCl (pH 7.4) at 37˚C for up to 4 h. At each time point, aliquots of the reaction products were treated with 15% trichloroacetic acid (TCA) to precipitate undegraded Aß. Following centrifugation at 16,000 x g for 20 min, the radioactivity of the supernatant (degradation products) and pellet (intact peptides) were measured.

Neprilysin (NEP) activity was quantified using the fluorogenic peptide substrate for NEP, N-dansyl-D-Ala-Gly-p-(nitro)-Phe-Gly (DAGPNG; Sigma) (7). Briefly, 50 μg brain membrane fraction was incubated with 100 μM DAGPNG and 200 μM enalapril (Sigma) in 0.5 ml of 50 mM Tris-HCl (pH 7.4) at 37˚C for up to 2 h. At each time point, 100 μl aliquots were collected, and immediately biled to terminate proteolytic activity. Following centrifugation at 3000 x g for 5 min, the fluorescence activity of the supernatant was read on a Mithras LB 940 (Berthold Technologies). The assay specificity was confirmed by the NEP inhibitor, thiorphan (data not shown).

Gel electrophoresis and Western blotting of IDE and NEP. SDS-PAGE was carried out on 4-20% Tris/glycine gradient gel (Invitrogen). The mouse brain membrane fraction was mixed with SDS sample buffer and boiled for 5 min immediately prior to electrophoresis. The samples were transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore). For immunoblotting, the membranes were probed with antibodies raised to insulin-degrading enzyme (IDE) (Calbiochem) or NEP (56C6; Novocastra).

Histological analysis of choline acetyltransferase-positive cells. Two days after Aß injection, sections of mouse brain were prepared according to the method of Aß immunohistochemical analysis described above. The brain sections were incubated with 0.4% Triton in PBS at room temperature for 30 min. Then, immunohistochemical detection of choline acetyltransferase (ChAT) was carried out with goat anti-ChAT polyclonal antibody (1:100 dilution; Chemicon). The brain sections were incubated overnight at 4˚C with the primary antibody. After washing in PBS five times, the sections were incubated for 2 h at 4˚C with rabbit anti-goat IgG (1:200 dilution; Chemicon). After washing in PBS five times, the sections were incubated with goat peroxidase-anti-peroxidase (PAP) (1:400 dilution; Chemicon) for 2 h at 4˚C. Then they were washed in PBS five times and incubated in 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution (Wako Pure Chemical Industries) for 10 min. The tissues were reacted in 0.05% DAB and 0.01% H_2O_2 in TBS. Images were obtained by using a Nikon Eclipse TE300 optical microscope. Average numbers of ChAT-positive cells in the basal forebrain were obtained by combining the data of three consecutive coronal sections.

Statistical analysis. All values are expressed as mean + SEM. Data were statistically analyzed by Student's t-test or ANOVA. Values of $p<0.05$ were considered significant.

Results

Behavioral analysis of Aß-injected mice treated with fluvastatin before or after Aß injection. To test the hypothesis that the timing of the start of treatment with a statin is critical to improve the memory impairment induced by Aß injection, we initially investigated the prevention of memory impairment by statin pretreatment, in which a statin was administered from 2 weeks before Aß injection, or post-treatment, in which a statin was administered from just after Aß injection (Fig. 1A). In the previous report on the Aß1-40 infusion model, Aß accumulation was observed in the brain (21). In the present study, we observed Aß accumulation in the cortex and hippocampus (Fig. 1B). Consistent with the previous reports, as assessed by the water-finding task, memory was significantly impaired in Aß injection model mice. Unexpectedly, pretreatment with fluvastatin prevented the memory deficit induced by Aß, although post-treatment with fluvastatin did not improve memory deficit (Fig. 1C). In the training trial, the number of approaches did not vary among groups, suggesting that the opportunity for the mice to learn about the apparatus was not different among the groups (data not shown). To rule out the possibility that this difference was due to the duration of fluvastatin treatment, we also treated mice with fluvastatin for 5 weeks, from just after Aß injection. However, fluvastatin treatment for 5 weeks from just after Aß injection did not improve Aß-induced memory impairment (data not shown). Thus, the present study suggests that the beneficial effects of fluvastatin might be dependent on the time of starting the drug. To elucidate whether this beneficial effect of fluvastatin is due to the specific structure of fluvastatin or not, we tested another statin, simvastatin, in the model. Unexpectedly, mice treated with simvastatin did not perform the water-finding task better than control (Fig. 1D).

Molecular mechanisms of the improvement of memory impairment by fluvastatin. In light of the above, we examined why pretreatment with fluvastatin significantly improved memory impairment. Initially, we focused on the effects of fluvastatin on Aß accumulation. When Aß is administered into the caudate nucleus, Aß is metabolized and eliminated from the brain relatively rapidly (23). Thus, we measured Aß levels in brain homogenates at several time points after Aß injection by Aß sandwich ELISA. Interestingly, we found that pretreatment with fluvastatin significantly decreased Aß accumulation after Aß injection (Fig. 2A). Fluvastatin also significantly reduced Aß levels in the brain as compared with no treatment at 3 weeks after injection (Fig. 2B).

To further qualify the favorable effects of fluvastatin on Aß accumulation, we examined whether the decrease in Aß accumulation by fluvastatin might be due to an increase in Aß degradation activity, since Aß is mainly degraded by

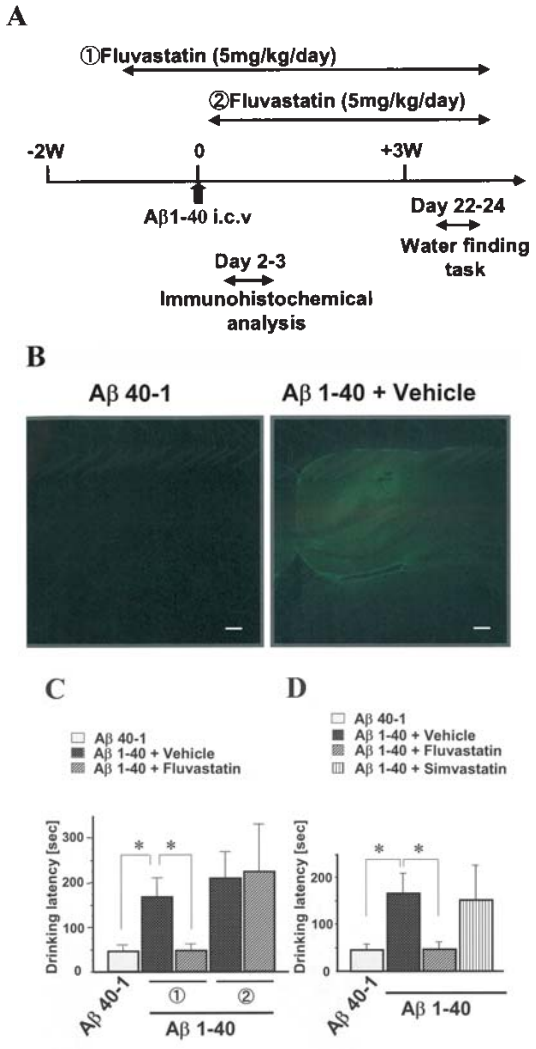

Figure 1. Behavioral analysis of Aß-injected mice treated with or without fluvastatin before or after Aß injection. (A) Time course of fluvastatin treatment. Fluvastatin (5 mg/kg/day) was administered for 2 weeks before (①) or just after (②) Aß injection. After Aß1-40 or Aß40-1 was injected i.c.v., mice were treated for a further 4 weeks with fluvastatin. A water-finding task were performed 3 weeks after Aß injection. (B) Detection of Aß in the coronal section of mouse brain by immunohistochemical staining with monoclonal anti-Aß antibody (6E10). Aß40-1, coronal section of mouse brain injected with Aß40-1; Aß1-40, coronal section of mouse brain injected with Aß1-40. Scale bar, 200 μm. (C) Water-finding task. Drinking latency, i.e. the time (sec) from the start to the first touch of the water bottle tube, was used for evaluation of learning. Aß40-1, mice injected with Aß40-1 (n=10); Aß1-40, mice injected with Aß1-40. Mice were treated with fluvastatin starting 2 weeks before (①) or just after (②) Aß injection (n=10 per group). $^{*}p<0.05$. (D) Comparison of effects of fluvastatin and simvastatin on Aß-induced memory impairment in the water-finding task. Mice were treated with fluvastatin, simvastatin, or vehicle starting 2 weeks before Aß injection. Aß40-1, mice injected with Aß40-1 (n=10); Aß1-40, mice injected with Aß1-40 (n=10 per group). $^{*}p<0.05$.

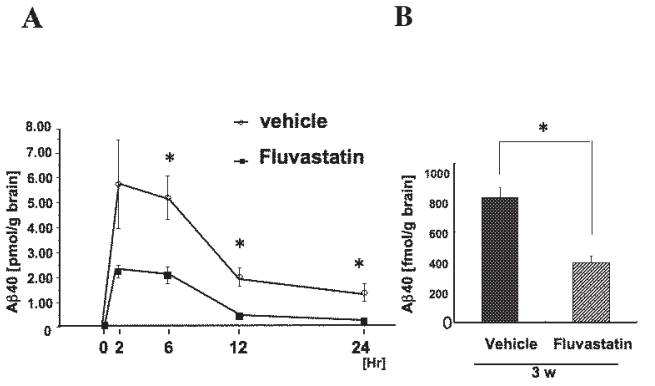

Figure 2. Effect of fluvastatin on Aß accumulation. (A) Quantitative analysis of Aß by Aß sandwich ELISA. Aß level in the mouse brain was measured just before, and 2, 6, 12, and 24 h after Aß injection in mice with or without fluvastatin treatment (n=4 per group at each time point). $^{*}p<0.05$. (B) Aß level in the mouse brain at 3 weeks after Aß injection in mice with or without fluvastatin treatment (n=3 per group). $^{*}p<0.05$ vs. vehicle.

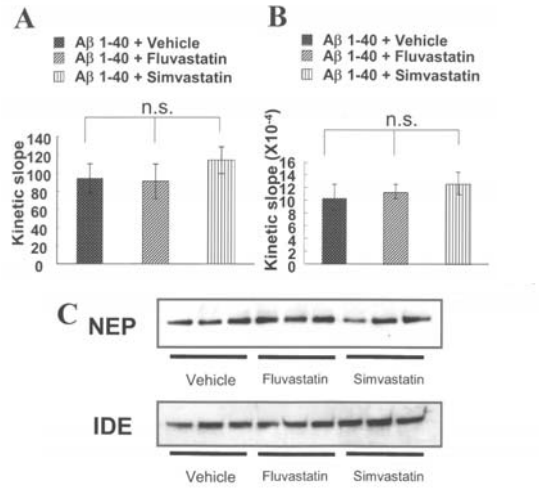

Figure 3. Effects of pretreatment with fluvastatin/simvastatin on Aß degradation. (A) NEP activity in mice treated with fluvastatin or simvastatin as determined by DAGPNG degradation. Vertical axis shows the kinetic slope, i.e., DAGPNG degradation per min. (B) IDE activity in mice treated with fluvastatin or simvastatin as determined by ^{125}I-Aß degradation. Vertical axis shows the kinetic slope, i.e., ^{125}I-Aß degradation per min. (C) Western blots of NEP (top) and IDE (bottom) from the brains of mice. Each group of three lanes corresponds to a set of three mice per mouse group. Mice were treated with either vehicle, fluvastatin, or simvastatin.

neprilysin (28) and insulin degrading enzyme (IDE) (29). An Aß degradation assay was mainly employed to assess IDE activity (30,31) while a neprilysin assay was employed to evaluate neprilysin (27,29). Importantly, pretreatment with fluvastatin affected neither IDE activity nor neprilysin activity (Fig. 3A and B). Moreover, pretreatment with fluvastatin did not change the expression of NEP and IDE (Fig. 3C). These results indicate that the decrease in Aß accumulation by fluvastatin was not through an increase in Aß degradation.

We then focused on oxidative stress, since Aß is well known to induce oxidative stress *in vivo*, both in the Aß injection model (32,33) and in APP transgenic mice (34). Moreover, it has been reported that fluvastatin has potent anti-oxidative effects in various models, unlike other statins (35-38). Using DHE, an oxidative fluorescent dye, to detect superoxide in brain sections (25), the present study revealed that Aß injection significantly induced oxidative stress in the hippocampus (Fig. 4A and B). Importantly, fluvastatin significantly reduced the oxidative stress induced by Aß (Fig. 4A and B). These results suggest that the anti-oxidative effects of fluvastatin may have contributed to its beneficial

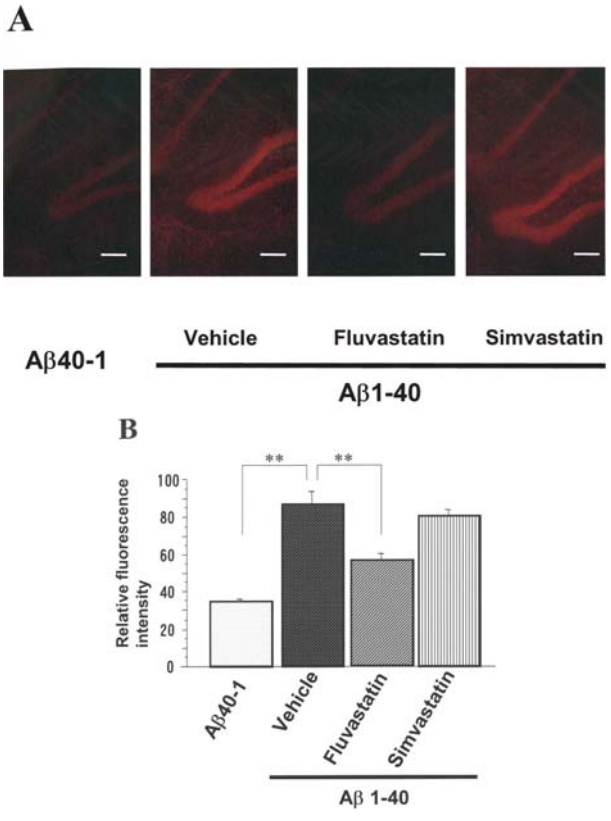

Figure 4. Effects of pretreatment with fluvastatin or simvastatin on oxidative stress induced by Aß. (A) DHE staining of hippocampus and dentate gyrus of Aß/vehicle-, Aß/fluvastatin-, or Aß/simvastatin-pretreated mouse brains. Aß40-1, mice injected with Aß40-1; Aß1-40/vehicle, mice treated with vehicle and injected with Aß1-40; Aß1-40/fluvastatin, mice treated with fluvastatin and injected with Aß1-40; Aß1-40/simvastatin, mice treated with simvastatin and injected with Aß1-40. Scale bar, 200 μm. (B) Quantitative analysis of DHE staining in dentate gyrus. Y axis shows the ratio of fluorescence intensity in brain sections to the mean of that in brain sections of Aß40-1-injected mice. n=5 per group. *p<0.001.

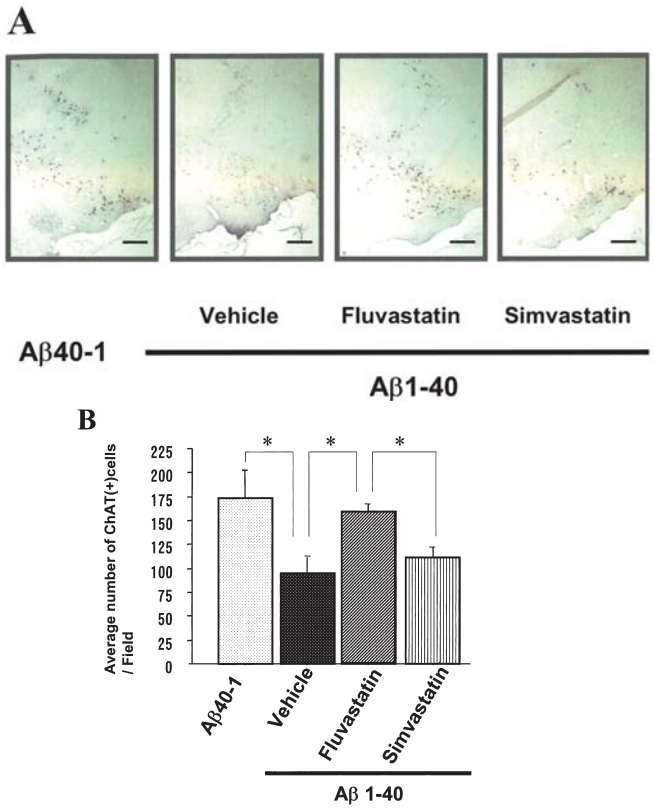

Figure 5. Effects of pretreatment with fluvastatin/simvastatin on cholinergic neuronal loss in basal forebrain induced by Aß. (A) Immunohistochemical analysis of the basal forebrain region of Aß/statin-pretreated mice with anti-ChAT antibody. Aß40-1, mice injected with Aß40-1; Aß1-40/vehicle, mice treated with vehicle and injected with Aß1-40; Aß1-40/fluvastatin, mice treated with fluvastatin and injected with Aß1-40; Aß1-40/simvastatin, mice treated with simvastatin and injected with Aß1-40. Scale bar, 200 μm. (B) Average number of ChAT-positive cells in medial septal region of Aß/vehicle-, Aß/fluvastatin-, or Aß/simvastatin-pretreated mouse brains. Y axis shows the average number of ChAT-positive cells obtained by combining the data in three consecutive coronal sections. n=5 per group. *p<0.05.

effects on cognitive function. Importantly, simvastatin did not reduce oxidative stress induced by Aß, unlike fluvastatin (Fig. 4A and B).

Basal forebrain cholinergic neuronal loss is a common feature of AD (39). Moreover, this phenomenon is recapitulated in APP transgenic mice (40) and the Aß injection model (22). Different effects of fluvastatin and simvastatin were also confirmed by measurement of cholinergic neurons. Fluvastatin, but not simvastatin, significantly prevented basal forebrain cholinergic neuronal loss induced by Aß, whereas Aß injection induced cholinergic neuronal loss in the basal forebrain (Fig. 5A and B).

Discussion

Treatment of dementia is now becoming a social problem. Among numerous possible treatments, statins might be an attractive candidate. In a French cohort study, the Three-City Study, statins were associated with decreased risk of dementia (41). However, in the Cardiovascular Health Study, a cohort study in the US, statin therapy was not associated with a decreased risk of dementia (42). The results varied in these studies, due to the limited number of subjects. On the other hand, an early report indicated that atorvastatin produced a change in the slope of deterioration of MMSE in the treatment of mild-to-moderate AD (14). These discrepancies among clinical trials indicate the need for further studies to elucidate the role of statins in the treatment of dementia. Our present study revealed two important aspects: the timing of treatment with statins, and the selection of statin. First, we clearly demonstrated that treatment with fluvastatin before the injection of Aß significantly prevented memory impairment induced by Aß. Thus, the timing of administration of statins might need to be more carefully considered in clinical situations. The second question appears to be more sensitive and crucial. Our present study indicated that fluvastatin significantly improved memory impairment through a significant decrease in Aß accumulation and oxidative stress and a significant increase in cholinergic neurons, while simvastatin did not affect memory impairment. It has been reported that oxidative mechanisms are involved in the cell loss and other neuropathology associated with AD (43,44). As anti-oxidant effects of fluvastatin have been reported in patients with type 2 diabetes and hyperlipidemia as compared to simvastatin (45), the clinical comparison of anti-oxidant effects between fluvastatin and

simvastatin might be important to elucidate the pathological significance of oxidative stress in AD. It has been suggested that the beneficial effects of statins on AD might not be only through lipid-lowering effects, but also through pleiotropic effects. One of the important mechanisms to improve memory impairment is considered to be through anti-oxidative effects, although further studies are necessary.

We observed that fluvastatin significantly decreased Aß accumulation after Aß injection in the model. It has been reported that cholesterol-lowering drugs and cholesterol-extracting resins strongly reduce intracellular and secretory neuronal Aß42 and Aß40 levels *in vitro*, and that administration of simvastatin to guinea pigs strongly reduced cerebral Aß levels, including that of the Aß42 isoform (16). Another group also reported that statins inhibited the dimerization of ß-secretase via both isoprenoid- and cholesterol-mediated mechanisms, and then reduced Aß production (46). Thus, previous studies suggested that the effects of statins were through the down-regulation of Aß production and/or secretion. In the present study, Aß was exogenously injected and therefore, the decrease in Aß accumulation by fluvastatin was not through the direct inhibition of Aß production and/or secretion, but maybe through a novel action of statins on Aß metabolism. The possibility that statins might affect Aß degradation is not evident from the data in this study (Fig. 3). One possible explanation is related to the transport of Aß across the microvascular endothelium (i.e. the blood-brain barrier), since the transport of Aß is essential to control Aß levels in the brain (47). Indeed, statins have pleiotropic action on endothelial cells through the up-regulation of eNOS transcription (48), an increase in cerebral blood flow and improvement of neurological function in mice (49). Thus, these favorable actions of statins on the endothelium might up-regulate Aß metabolism, including the clearance of Aß.

We also demonstrated that fluvastatin, but not simvastatin, rescued basal forebrain cholinergic neuronal loss induced by Aß, visualized as ChAT immunoreactivity. Several studies in humans indicate that basal forebrain cholinergic pathways, especially those projecting from Meynert's nucleus to the cortex, play a crucial role in conscious awareness and mnemonic processes (50). In AD patients, a loss of brain cholinergic functions significantly contributes to the cognitive decline (51). In APP transgenic mice, neuronal damage and cholinergic dysfunction *in vivo* underlie the impairment of learning and memory function (52). In this study, the prevention of cholinergic neuronal loss by fluvastatin might underlie the protection against Aß-induced memory impairment. The prevention of basal forebrain cholinergic neuronal loss by fluvastatin might be due to a decrease in Aß accumulation and/or inhibition of oxidative stress.

Overall, the present study demonstrated that fluvastatin, but not simvastatin, significantly prevented memory impairment induced by amyloid ß. The beneficial effects of fluvastatin might be explained by the prevention of cholinergic neuronal loss through a significant decrease in Aß accumulation and oxidative stress. In clinical settings, the timing of the start of treatment and the selection of statin might be critical in achieving a beneficial effect on cognitive function.

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