Glycation exacerbates the neuronal toxicity of \( \beta \)-amyloid

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Accumulation evidence shows that \( \beta \)-amyloid (\( \beta \)-amyloid) is a neurotoxic and accumulation of \( \beta \)-amyloid is responsible for the pathology of Alzheimer’s disease (AD).\(^1\text{,}^2 \) The major component in the plaques is \( \beta \)-amyloid (\( \beta \)-amyloid), a peptide of 39–43 amino acids, produced from amyloid precursor protein (APP) by \( \beta \)-secretase pathway.\(^3 \) Numerous studies show that the \( \beta \)-amyloid-induced neurotoxicity is responsible for the pathology of AD.\(^4 \) However, what makes \( \beta \)-amyloid more toxic and which forms of \( \beta \)-amyloid are more toxic are elusive.

The plaques in the AD brains are colocalized with the advanced glycation endproducts (AGEs), and the plaque-enriched fractions contain approximately threefold higher AGE adducts than that of the age-matched controls,\(^5 \) suggesting that \( \beta \)-amyloid may be glycated. The long-live proteins are preferentially modified to form AGEs and the stability of \( \beta \)-amyloid makes it an ideal substrate for non-enzymatic glycation and formation of AGEs. Although in vitro studies show that \( \beta \)-amyloid can be glycated and the glycated \( \beta \)-amyloid contribute to the \( \beta \)-amyloid accumulation,\(^5\text{,}^6 \) it is currently not characterized whether \( \beta \)-amyloid is also glycated in vivo to form \( \beta \)-amyloid-AGEs and the role of \( \beta \)-amyloid-AGE in the pathogenesis of AD.

Senile plaques (SP) and neurofibrillary tangles are hallmark pathologies in the brains of Alzheimer’s disease (AD).\(^1\text{,}^2 \) The major component in the plaques is \( \beta \)-amyloid (\( \beta \)-amyloid), a peptide of 39–43 amino acids, produced from amyloid precursor protein (APP) by \( \beta \)-secretase pathway.\(^3 \) Numerous studies show that the \( \beta \)-amyloid-induced neurotoxicity is responsible for the pathology of AD.\(^4 \) However, what makes \( \beta \)-amyloid more toxic and which forms of \( \beta \)-amyloid are more toxic are elusive.

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Accumulation of AGEs in the brains may impair neural cells through direct covalent crosslinking to the substrates\(^7\text{,}^8 \) or binding to the surface AGE receptors, namely RAGEs.\(^9 \) The ligand–receptor interaction may perturb cell functions by activating receptor-mediated signal transduction pathways.\(^10 \) Interestingly, \( \beta \)-amyloid has been identified as a ligand of RAGE.\(^11 \) RAGE is overexpressed in the AD brains and acts as a binding site for \( \beta \)-amyloid at the plasma membrane of neurons, microglial cells, and endothelial cells of the vessel wall.\(^11 \) Uptregulation of RAGE mediates \( \beta \)-amyloid oxidative stress,\(^12 \) activation of nuclear factor\(-\)\( \kappa \)B,\(^11 \) neuronal expression of macrophage colony-stimulating factor,\(^13 \) and cell death.\(^14 \) Recent studies show that RAGE-dependent signaling contributes to an impaired learning/ memory in AD-like transgenic models.\(^15 \) It is currently unknown whether RAGE mediates neurotoxicity induced by glycated \( \beta \)-amyloid.

Formation of AGEs involves nonenzymatic reactions of reducing sugars or dicarbonyl compounds, such as methylglyoxal (MG) and glyoxal, and abnormal glucose metabolism or oxidative stress can lead to the formation of the reactive dicarbonyl compounds.\(^16 \) Aminoguanidine (AG) is a prototype scavenging agent that prevents the formation of AGEs from...
the dicarbonyl precursors both in vitro and in vivo. AG reduces tissue AGEs accumulation and inhibits the vascular and renal manifestations induced by experimental diabetes or AGEs administration. Recent studies show that AG prevents hippocampal alterations in streptozotocin-induced dementia in rats and protected neuroblastoma cells against the neurotoxic effects of MG.

Based on the previous findings, we hypothesized that glycated Aβ in vitro could exacerbate the neurotoxicity of Aβ, and in vivo inhibition of AGEs partially constituted by Aβ-AGE could restore early cognitive decline of AD.

Results

Glycation exacerbates neurotoxicity of Aβ in hippocampal neurons. To synthesize Aβ-AGE in vitro, Aβ1-42 was incubated with MG for 1 month. The production of Aβ-AGE was identified with fluorescence spectrophotometer measuring AGE-specific fluorescence at emission of 440 nm and excitation of 370 nm. We observed that the fluorescence of Aβ-AGE was about seven times as much as Aβ1-42 (data not shown), suggesting Aβ-AGE had been successfully produced in vitro.

To explore whether Aβ-AGE is more toxic than authentic Aβ, 8-DIV embryonic hippocampal neurons were treated with Aβ or Aβ-AGE for 24 h. We found that Aβ-AGE was more toxic than Aβ in decreasing cell viability, increasing cell apoptosis, inducing tau hyperphosphorylation, and reducing synaptic proteins (Figures 1a–f). By circular dichroism (CD) spectra analysis, we found that Aβ-AGE displayed a significantly different profile (secondary structure) from Aβ (Figure 1g), which may underlie exacerbating toxicity of Aβ-AGE.

Activation of RAGE and glycogen synthase kinase-3 (GSK-3) mediates Aβ-AGE-exacerbated neurotoxicity in hippocampal neurons and Tg2576 mice. RAGE is the common receptor of Aβ and AGEs. To verify whether Aβ-AGE induces the neurotoxicity through RAGE, we first measured the level of RAGE after Aβ or Aβ-AGE treatment. We found that both Aβ-AGE and Aβ increased RAGE level, but the level of RAGE was even higher in Aβ-AGE group (Figures 2a–c). By using RAGE antibody to block the receptor, we found that blockage of RAGE almost abolished the Aβ-AGE-induced reduction of cell viability (Figure 2d), elevation of apoptotic rate (Figure 2e), tau hyperphosphorylation (Figures 2f and g) and deficits of synaptic proteins (Figures 2h and i). These data indicate that Aβ-AGE may be a more suitable ligand for RAGE than Aβ in exacerbating the Aβ-induced neurotoxicity.

We have recently reported that AGEs induces cognitive impairment in SD rats through RAGE/GSK-3 pathway. To explore the involvement of GSK-3 in Aβ-AGE-induced neural impairments, we measured the activity-dependent phosphorylation of GSK-3β at Ser9 by western blot (Figures 3a and b) and immunofluorescence (Figure 3c). The phosphorylated GSK-3β at Ser9 decreased significantly in Aβ-AGE group than in Aβ group, suggesting that higher GSK-3 activity in Aβ-AGE group than the Aβ group. These data indicate that upregulation of GSK-3 may be involved in Aβ-AGE-induced toxicities. To further verify the role of GSK-3, we used inhibitor of GSK-3. We found that simultaneous inhibition of GSK-3 by LiCl attenuated the Aβ-AGE-induced reduction of cell viability (Figure 3d), elevation of apoptosis rate (Figure 3e), tau hyperphosphorylation (Figures 3f and g) and decline of synaptic proteins (Figures 3h and i). These data suggest that GSK-3 may mediate the Aβ-AGE-induced exacerbation of neural impairments.

It is well known that Akt can phosphorylate GSK-3β at Ser9 and thus inhibit the kinase. Therefore, we measured the activity-dependent phosphorylation level of Akt. We found that phosphorylation of Akt at Thr473 was remarkably decreased

![Figure 1](image-url)  
Figure 1  Aβ-AGE with a special CD profile is more toxic to hippocampal neurons than the authentic Aβ. (a-f) The hippocampal neurons cultured 8 DIV were treated with Aβ or Aβ-AGE for 24 h, then the viability of the neurons was analyzed by using CCK-8 kit (a); the apoptosis rate was examined by flow cytometry (b); the level of tau phosphorylation at Thr231 and Ser396 (c and d) and synaptic proteins (e and f) were analyzed by western blot. Levels of phosphorylated tau and synaptic proteins were normalized, respectively, to Tau-5 and DM1A. (g) Aβ or Aβ-AGE was prepared in vitro as described in the methods and the structural property was measured by CD spectra analysis. n = 3, **P < 0.01 versus Aβ group.
after Aβ-AGE treatment (Figures 3j and k). These data suggest that inhibition of Akt may be upstream of the GSK-3 activation by Aβ-AGE.

Aβ is glycated to form Aβ-AGE with an age-dependent elevation of AGEs in the brains of Tg2576 mice. By western blot, we observed that the level of AGEs indeed increased significantly in hippocampus of a 9-month-old Tg2576 mice (Figures 4a and b). To verify whether Aβ is glycated, we analyzed the component of AGEs in a 9-month-old Tg2576 mice by coimmunoprecipitation and western blot. We found that Aβ was co-immunoprecipitated with an antibody against AGEs and vice versa (Figures 4c and d), suggesting that the glycated Aβ (Aβ-AGE) may be one of the major component of AGEs in Tg2576 mice.

Early inhibiting the Aβ-AGE formation rescues cognitive impairment in Tg2576 mice. To verify the toxic roles of Aβ-AGE, we injected subcutaneously AG, an inhibitor of AGE formation, into Tg2576 mice for 3 months, started at 6-months old, and then measured the level of AGEs, Aβ, and Aβ-AGE by dot blot, ELISA and immunoprecipitation, respectively. We found that AG treatment decreased the levels of AGEs compared with normal saline (NS) group (Figure 5a) and Aβ in both of the cortex and the hippocampus (Figures 5b and c), simultaneously, the levels of AGE-associated Aβ and the Aβ-associated AGEs were reduced remarkably (Figures 5d–g). These data confirm that Aβ is glycated and AG inhibits the formation of Aβ-AGE in Tg2576 mice glycation.

To test whether inhibition of Aβ-AGE by AG could rescue the cognitive impairments, we trained 9-month-old Tg2576 mice administrated with AG or NS for 3 months in water maze for 6 days, and tested the memory retention. We found that AG treatment improved the learning and memory of the mice, demonstrated by decreased latency (Figure 6a), increased platform crossings (Figure 6b) and increased time spent in target quadrant (Figure 6c). These data suggest that inhibition of Aβ-AGE can rescue learning and memory in Tg2576 mice.

We also found that both pre-synaptic (synapsin I, synaptophysin, and VAMP2) and post-synaptic proteins (NR2A, NR2B, PSD93, and PSD95), as well as memory-associated proteins (c-fos and arc) were downregulated in 9-month-old Tg2576 mice, and administration of AG restored the levels of the synaptic and memory-associated proteins (Figures 6d and e). In addition, tau was hyperphosphorylated at Thr231 and Ser396 but not at Ser404, Thr205, and Ser198/199/202 (Tau-1 epitope) in 9-month-old Tg2576 mice, and AG treatment attenuated tau hyperphosphorylation (Figures 6f–h). These data suggest that inhibiting formation of AGEs, which included Aβ-AGE, attenuates the neuropathology, which can contribute to the improved cognition of the mice. These in vivo data...
partially demonstrated the enhanced neurotoxicity of \( \alpha \beta \)-AGE observed during in vitro experiments.

In view that the involvement of RAGE/GSK3 pathway in \( \alpha \beta \)-AGE-induced exacerbation of neurotoxicity was found in hippocampal neurons, we also examined the level alteration of RAGE and activity of GSK-3 in Tg2576 mice after treatment with AG. We found that the level of RAGE increased and GSK-3 was activated in 9-month-old Tg2576 mice, whereas treatment of AG attenuated the upregulation of RAGE and GSK-3 in the mice (Figure 7). These in vivo data further support that RAGE and GSK-3 are participated in \( \alpha \beta \)-AGE-induced neurotoxicity.

**Discussion**

In type 2 diabetes mellitus (T2D) patients, the consequence of the elevated blood glucose leads to the generation of AGEs. Previous study showed that the increased AGEs contribute to the failure of sensory nerve regeneration in diabetes, and administration of exogenous AGE-modified proteins modulates the maturation and functions of peripheral blood dendritic cells and neural stem cells. Epidemiological studies have shown that diabetes mellitus is an independent risky factor of AD. However, the molecular mechanism is not fully understood. As the therapeutics advances for diabetes, the T2D patients will most likely live longer and thus the world may soon be facing the daunting challenge of dealing with a new population of AD sufferers with T2D. One of the hallmark lesion observed in AD brain is the formation of SPs, which are composed of \( \alpha \beta \), derived from APP proteolysis. Studies suggest that accumulation of \( \alpha \beta \), is responsible for the age-related memory decline in AD model, however, it is not fully understood what may lead to \( \alpha \beta \) accumulation and in which form \( \alpha \beta \) may exert its toxic effects. Formation and accumulation of AGEs has been proposed to be involved in the evolution of AD. The level of
AGEs is increased in the AD brains and the glycated Aβ accumulation accelerates Aβ deposition.5,34 The proteins with prolonged turnover, such as tau in paired helical filament and Aβ in the AD brains,35 are favorable substrates for the formation of AGEs. Therefore, we speculate that the over-produced Aβ in Tg2576 mice may be glycated and the glycation may exacerbate the toxicity of Aβ.

To investigate the toxicity of glycated Aβ, we synthesized glycated Aβ (Aβ-AGE) in vitro and treated the hippocampal neurons cultured 8 days in vitro. We observed that the Aβ-AGE was more toxic than the authentic Aβ in decreasing the cell viability, increasing apoptosis, causing tau hyperphosphorylation and damaging the synapses. By CD spectra analysis, we also observed that the glycated Aβ showed an un-ordered secondary structure, which favors protein aggregation.36

RAGE, a cell surface binding site for Aβ and AGEs, is upregulated in affected cerebral vessels, neurons, and microglia37,38 when Aβ increases. RAGE mediates Aβ transport across the blood–brain barrier and accumulation in the brain.39 Transgenic mice overexpressing mutant human APP and RAGE in neurons displayed earlier stage deficits of spatial learning/memory and more serious neuro-pathologic changes.40 A growing body of evidence demonstrates that increased expression of RAGE allows for more profound RAGE-induced cellular perturbation.4142 To explore whether Aβ-AGE affects RAGE and whether RAGE is involved in the exacerbation of neural toxicity of Aβ-AGE, we measured the level of RAGE in hippocampal neurons in vitro. We found that the RAGE level was higher in Aβ-AGE-treated cells than in the Aβ-treated ones, whereas simultaneous application of RAGE antibody attenuated more significantly the neural damages induced by Aβ-AGE. These

**Figure 4** Aβ is glycated with an age-dependent increase of AGE in the brains of Tg2576 mice. (a and b) The hippocampal extracts from Tg2576 (Tg) or wild-type (WT) mice at 1, 3, 6, 9, and 12 months were analyzed by dot blot using anti-AGE antibody normalized against DM1A (b). (c and d) The hippocampal extracts from 9-month-old Tg mice were precipitated with AGE or Aβ or IgG antibody, and then the level of Aβ or AGE in the precipitate was measured by western blot using anti-Aβ (c) or anti-AGE (d) antibody, n = 5, *P < 0.01 versus WT group; **P < 0.01 versus 6 months in Tg group.

**Figure 5** Subcutaneous injection of AG prevents formation of Aβ-AGE in Tg2576 mice. Tg2576 (Tg) mice and the wild-type (WT) littermates at 6-month old were subcutaneously injected with AG (blocker of AGE formation) or NS for 3 months. (a) Level of AGE in the cortex extracts was measured by dot blot and quantitated by normalization against DM1A. **P < 0.01 versus WT + NS group; ***P < 0.01 versus Tg + NS group. (b and c) The levels of Aβ1-40 and Aβ1-42 in cortex and hippocampus were estimated by ELISA. hippo: hippocampus. *P < 0.05, **P < 0.01 versus Tg + NS group. (d–g) The cortex extracts were immunoprecipitated with IgG or AGE or Aβ antibody, and then the levels of Aβ-AGE or AGEs in precipitate were measured by western blot using anti-Aβ or anti-AGE antibody as indicated. **P < 0.01 versus Aβ-Tg + NS (e) or AGE-Tg + NS group (g).
data imply that RAGE may have an important role in exacerbating the toxic effect of Aβ.

GSK-3 has been verified to participate in the pathogenesis of AD. Upregulation of GSK-3 inhibits long-term potentiation and causes memory deficit. Based on our previous study that AGEs contribute to memory impairment through RAGE-mediated GSK-3 activation, we speculate that, with upregulation of RAGE, GSK-3 may be implicated in the exacerbated neural damages induced by Aβ-AGE. Indeed, our results showed that GSK-3 in hippocampal neurons in vitro was activated and simultaneous application of GSK-3 inhibitor attenuated the exacerbated pathological change of Aβ-AGE, suggesting the involvement of RAGE-dependent GSK-3 activation pathway. We also observed a more significant inhibition of Akt by Aβ-AGE than Aβ. The activity of GSK-3 is regulated by phosphatidyl inositol 3 kinase-Akt pathway, and upregulation of Akt attenuates the AGE-induced dysfunction of endothelial progenitor cells, which supports our data.

The in vitro results have shown that Aβ was the suitable substrate for the glycation and glycated Aβ appeared to be more neurotoxic than Aβ. Thus, we speculated that AGEs may be produced by glycating Aβ even in the early stage of Tg2576 mice, in which accumulation of Aβ contributes to the

**Figure 6** Subcutaneous injection of AG improves spatial memory and neuropathology in Tg2576 mice. (a and b) Tg2576 (Tg) mice and the wild-type (WT) littermates at 6-month old were subcutaneously injected with AG or NS for 3 months, then the spatial learning and memory was measured by Morris water maze. The mice were trained to remember the hidden platform in the maze for 6 days (learning process) and the latency (time to find platform) was recorded (a). The platform was removed at ninth day and the spatial memory was tested by measuring the time of platform quadrant crossing, the time stayed in the target quadrant and swimming paths (b and c). (d and e) The levels of presynaptic proteins (synapsin1, synaptofadin, and VAMP-2), postsynaptic proteins (NR2A, NR2B, PSD93, and PSD95) and the memory-related molecules (c-fos and arc) in hippocampus were measured by western blot and normalized against DM1A. (e and f) The phosphorylation levels of tau at Thr231, Ser396, Ser404, Thr205, and Tau-1 (Ser198/199/202) in hippocampus were measured by using phosphorylation site-specific antibodies as indicated in the blots normalized against total tau probed by Tau-5. (g) Slices of Tg mice treated with AG or NS were immunostained with antibody against phosphorylated tau at Thr231. (h) n = 8; c-g: n = 3. *P < 0.05, **P < 0.01 versus WT + NS group; # P < 0.05, ## P < 0.01 versus Tg + NS group. Scale bar in h, 50 μm.

**Figure 7** Inhibition of Aβ-AGE formation suppresses RAGE upregulation and GSK-3b activation in Tg2576 mice. Tg2576 (Tg) or wild-type (WT) mice at 6-month old were injected subcutaneously with AG or NS for 3 months. At 9 month old, the mice were killed and levels of RAGE and the phosphorylated GSK-3b at Ser9 were estimated by western blot. RAGE and pS9-GSK-3b were normalized, respectively, to DM1A and total GSK-3b. n = 3. *P < 0.01 versus WT + NS group. **P < 0.01 versus Tg + NS group.
pathology. To test this, we measured the levels of AGES and glycation of Aβ in hippocampus of Tg2576 mice. We found that the level of AGES increased age dependently started from 9-month-old onward of the mice, and Aβ was co-immunoprecipitated with AGES, suggesting Aβ is glycated to form Aβ-AGE. To verify the toxic role of AGES, we used AG, an inhibitor of AGES that can block glycation of proteins or peptides by glucose or its derivants, to suppress formation of AGES. The AG was administrated subcutaneously for 3 months, started at 6 months old when the AGES level was normal. We observed that infusion of AG decreased levels of AGES and Aβ-AGE in hippocampus with significant improvement of spatial learning and memory of the mice. Simultaneously, levels of the hyperphosphorylated tau, synaptic, and memory-related proteins were remarkably restored in the hippocampus of mice. We also carried out the above experiments in the cortex of mice, the result of which was consistent with that in hippocampus. These data suggest that formation of Aβ-AGE may exaggerate AD pathologies.

In view of observation of amplified RAGE-mediated GSK-3 activation induced by Aβ-AGE in hippocampal neurons, we also examined the expression of RAGE and GSK-3 in AG-treated Tg2576 mice. We found that inhibition of Aβ-AGE formation could attenuate upregulation of RAGE and activation of GSK-3, which provides the in vivo data supporting the participation of RAGE/GSK-3 pathway in the exacerbated neural dysfunctions induced by Aβ-AGE. We also found that the levels of Aβ1-40 and Aβ1-42 decreased with inhibition of AGES. In a recent study, the authors showed that inhibition of RAGE with RAGE antibody or RAGE knockout could downregulate β-site APP cleaving enzyme 1 (BACE1), whereas activation of RAGE upregulate BACE1, which may partially explain the arrested deposition of Aβ after AG treatment observed in the current study.

Aβ is found in extracellular SP cores and is associated with neurodegeneration in later stages of AD. In contrast, recent studies suggest that accumulation of intraneuronal Aβ may be an early event in the pathogenesis of AD. RAGE mediates intraneuronal transport of Aβ and neuronal dysfunction. The above studies demonstrate that Aβ also exits inside the neurons, especially in the early stage of AD. Oxidative stress and glucose metabolism disorder in AD lead to intracellular production of carbonyl compounds, which could modify intracellular Aβ to form Aβ-AGE. This may explain why we observed numerous AGES inside the neurons.

In summary, we have found for the first time that the formation of Aβ-AGE exacerbates the toxicity of Aβ with the mechanisms involving activation of RAGE/GSK-3 pathway, and inhibition of AGES including Aβ-AGE can restore the cognitive deficit in AD-like model mice, which reveal that the underlying mechanism of T2D to be related to AD may be through increasing formation of Aβ-AGE.

**Materials and methods**

**Antibodies and chemicals.** Mouse monoclonal antibody (mAb) AβE was from Tans Genic.Inc (Kumamoto, Japan). Rabbit polyclonal antibody (pAb) against tau phosphorylated at Ser396, Ser404, Thr231, and Thr205 were from Biosource (Camarillo, CA, USA). mAb Tau1 against tau unphosphorylated at Ser198/199/202, RAGE, and synapsin I were from Millipore (Billerica, MA, USA). mAb Tau5 against total tau was from Lab Vision Corp (Fremont, CA, USA). pAb GSK-3, pSer-9-GSK-3, and pT308-Akt, total Akt, and c-fos were from Cell Signaling Technology (Beverly, MA, USA); pAb Aβ (pT308-Akt, NR2A, NR2B, PSD93, PSD95, VAMP2, and Arc were from Abcam (Cambridge, UK). mAb DM1A against >-tubulin and mAb synapto phosphin were from Sigma (St Louis, MO, USA). Peptide Aβ(1-42) was from ChinaPeptides Co., Ltd (Shanghai, China). Anti-rabbit IRDye and anti-mouse IRDye were from Li-Cor Biosciences (Lincoln, NE, USA). BCA kit was from Pierce (Rockford, IL, USA). Peroxidase-conjugated goat anti-rabbit and goat anti-mouse secondary antibodies were from Pierce (Rockford, IL, USA). Neurobasal and B27 were from Gibco (Grand Island, NY, USA).

**Animals and treatment.** APP transgenic mice (Tg2576) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). These mice overexpress human APP695 with a double mutation KM670/671NL. All mice were produced by the Experimental Animal Center of Tongji Medical College. The genotype was confirmed by PCR analysis of tail biopsies. All mice were kept under standard laboratory conditions: 12 h light and 12 h dark; lights on at 0600 hours; temperature: 22 ± 2 °C; water and food ad libitum. All animal experiments were performed according to the ‘Policies on the Use of Animals and Humans in Neuroscience Research’ revised and approved by the Society for Neuroscience in 1995. Six-month Tg2576 or control C57BL mice were subcutaneously injected with 0.1 μl NS or AG (20 mg/ml) at 1000 hours for 3 months. Then, mice were trained for Morris water maze test.

**Preparation of Aβ-AGE.** Aβ-AGE was prepared by incubating 100 mg/ml of Aβ(1-42) with or without 0.5 mM MG in 0.1 M phosphate buffer, pH 7.2, at 37 °C for 1 month under sterile condition. After dialyzed against phosphate buffer for 48 h to remove MG, the prepared Aβ-AGE was sterilized by filtration and kept at −20 °C. The production of Aβ-AGE was identified with western blot and fluorescence spectroscopy measuring AGE-specific fluorescence at emission of 440 nm and excitation of 370 nm (Perkin-Elmer, Waltham, MA, USA).

**CD spectroscopy.** CD spectra from Aβ or glycated Aβ (0.1 mg/ml) in water were taken using Jasco J-810 spectropolarimeter (Jasco International Co. Ltd, Tokyo, Japan). The CD spectrum was recorded in the range of 190–250 nm using a 0.1 cm path length quartz cuvette at 25 °C in continuous scanning mode. The acquisition parameters were 100 nm/min, with a 1.0-s response and a 1.0 nm bandwidth. The data were accumulated over 10 runs, the presented data being the average. The results were expressed in term of molecular ellipticity (θ) in unit of deg.cm²/dmol.

**Cell culture and treatment.** Embryonic hippocampal neurons were cultured according to the procedure described previously. Primary hippocampal neurons at 8 DIV were treated with Aβ (100 nM) or Aβ-AGE (100 nM) for 24 h. Then, cells were cultured for western blot and immunofluorescent staining. For investigating underlying mechanism, 8-d hippocampal neurons were pre-incubated with or without RAGE antibody (10 μg/ml) or LiCl (4 mM) for 1 h before treatment with or without Aβ (100 nM) or Aβ-AGE (100 nM) for 24 h.

**Morris water maze test.** Morris water maze test was performed according to the procedure described previously. Briefly, the mice were trained to find a submerged platform by using a stationary array of cues outside the pool tub. The water was made opaque by using milk powder for chiascoro. Acquisition training consisted of a total of 28 trials, given as four spaced trials a day for 6 consecutive days. The probe tests were performed with the platform on the seventh day and by removing the platform on the ninth day. Swimming paths in probe test were monitored using an automatic tracking system. This system was used to record the swimming trace and calculate the latency to the platform and the time spent in each quadrant.

**Western blot and dot blot.** Hippocampus were homogenized and cells were lysed in a cooled buffer containing 10 mM Tris-HCl (pH 7.8), 50 mM NaF, 1 mM Na3VO4, 1 mM EDTA, 1 mM benzamidine, and 1 mM phenylmethylsulfonylfluoride and protease inhibitors mixture (1 mg/ml) each of a leupeptin, protinin, and pepstain A. The tissue homogenates and cell lysates were added with one-third volume of sample buffer containing Tris-HCl (pH 7.6) 200 mM, 8% SDS, 40% glycerol, and boiled in a water bath for 10 min. The lysates were centrifuged at 12,000 × g for 15 min at 4 °C. The protein concentration in the supernatant was measured by BCA kit according to manufacturer’s instruction.
For western blot, equal amounts of protein were separated by 10% SDS-PAGE or tricine-SDS-PAGE (for Aβ) 120 or 130 electronmicroscopy, and transferred to nitrocellulose membrane. The membranes were blocked with 5% nonfat milk dissolved in TBS-Tween-20 (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.2% Tween-20) for 1 h and probed with primary antibodies overnight at 4 ºC. For dot blot, 120 draw grid by pencil to indicate the region to be blotted. Using narrow-mouth pipet tip, adjust proteins to the same concentration, and spot 2 µl of samples onto the nitrocellulose membrane at the center of the grid. Let the membrane dry at 37 ºC.

Then the blots were incubated with anti-rabbit or anti-mouse IgG conjugated to IRDye (800 CW) for 1 h at room temperature and visualized using the Odyssey Infrared Imaging System (Lincoln, NE, USA).

Conflicts of Interest

The authors declare no conflict of interest.

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