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Newly developed glycogen synthase kinase-3 (GSK-3) inhibitors protect neuronal cells death in amyloid-beta induced cell model and in a transgenic mouse model of Alzheimer's disease

Min-Young Noh [a,1], Kwangwoo Chun [b,1], Byung Yong Kang [a], Heejaung Kim [a], Ji-Seon Park [b], Han-Chang Lee [b], Young-Ha Kim [b], Saekwang Ku [c], Seung Hyun Kim [a,*]

[a] Department of Neurology, Hanyang University College of Medicine, Seoul, Republic of Korea
[b] Division of New Drug Discovery Development, R&D Center, Jeil Pharmaceutical Co., Ltd., 177-1, Keungok-Ri, Baekam-Myun, Cheoin-Gu, Yongin-City, Kyunggi-Do 449-861, Republic of Korea
[c] Department of Anatomy and Histology, College of Oriental Medicine, Daegu Haany University, Gyeongsan 712-715, Republic of Korea

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ABSTRACT

Glycogen synthase kinase-3 (GSK-3) is emerging as a prominent therapeutic target of Alzheimer's disease (AD). A number of studies have been undertaken to develop GSK-3 inhibitors for clinical use. We report two novel GSK-3 inhibitors (C-7a and C-7b) showing good activity and pharmacokinetic (PK) profiles. IC_{50} of new GSK-3 inhibitors were in the range of 120–130 nM, and they effectively reduced the Aβ-oligomers induced neuronal toxicity. Also, new GSK-3 inhibitors decreased the phosphorylated tau at pThr231, pSer396, pThr181, and pSer202, and inhibited the GSK-3 activity against Aβ-oligomers induced neuronal cell toxicity. In B6;129-*Psen1*^{tm1Mpm} Tg(APPSwe, tauP301L)1Lfa/Mmjax model of AD, oral administration of C-7a (20 mg/kg, 50 mg/kg) showed increased total arm entries and spontaneous alteration of *Y*-maze which was regarded as short-term memory. In particular, 50 mg/kg C-7a treated mice significantly decreased the level of phosphorylated tau (Ser396) in brain hippocampus. We suggest that new GSK-3 inhibitor (C-7a) is potential candidates for the treatment of AD.

1. Introduction

Glycogen synthase kinase 3 (GSK-3) is a ubiquitous serine/threonine kinase, which first described as the major regulator of glycogen metabolism by phosphorylating and thereby inhibiting glycogen synthase [1]. It has two isoforms GSK-3α and GSK-3β, which are in two highly homologous forms in mammals. They are inactivated by phosphorylation of Ser21 or Ser9 in GSK-3 α/β [2]. It has been implicated in various biological processes that GSK-3 phosphorylates primed substrates and occasionally acts as its own priming kinase. These properties of GSK-3 are associated with the pathogenesis of several diseases including diabetes, neurodegeneration, cancer, and inflammation [3].

The role of GSK-3 has been implicated in AD pathogenesis, as a linker between the two histopathological hallmarks: the extracellular senile plaques composed of amyloid-beta (Aβ), and the intracellular neurofibrillary tangles (NFTs) made of hyper-phosphorylated tau protein [4,5]. In AD brain, total GSK-3 is increased and active GSK-3 is localized to pre-tangle neurons, dystrophic neuritis and NFTs [6]. There are numerous studies showing that abnormal increases in the level and activity of GSK-3β induce neuronal cell death paired with helical filament tau formation, and neurite retraction in AD [7]. A key candidate for both physiological and pathological tau phosphorylation is GSK-3β [8]. Multiple sites of phosphorylation in tau linked GSK-3β, cycline-dependent kinase-5 (CDK-5), amongst others [9]. However, substantial evidence exists to support a major role for GSK-3 in both physiological and pathological tau phosphorylation at Thr69, Thr175, Thr181, Ser184, Ser199, Ser202, Thr212, Thr217, Thr231, Ser235, Ser258, Ser262, Ser289, Ser356, Ser396, Ser400, Ser404, Ser409, and Ser413 [10]. Therefore, many pharmaceutical and academic investigators have focused on the development of a GSK-3 inhibitor as a potential therapeutic drug for treating AD, but thus far none has proved successful.

In the present study, we describe the successful development of new GSK-3 inhibitors for the treatment of AD. The new GSK-3 inhibitors were synthesized through docking studies and the

Abbreviations: Aβ, amyloid-beta; Aβ-oligomers, amyloid-beta (1-42) oligomers; AD, Alzheimer's disease; CDK-5, cycline-dependent kinase-5; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; GSK-3, glycogen synthase kinase-3; HBSS, Hank's balanced salt solution; IRs, immunoreactivities; NBM, neurobasal media; NFTs, neurofibrillary tangles; OD, optical density; PBS, phosphate buffered saline; PK, pharmacokinetic; SD, Sprague–Dawley; Ser, serine; Thr, threonine.

* Corresponding author. Address: Department of Neurology, Hanyang University College of Medicine, #17 Haengdang-dong, Seongdong-gu, Seoul 133-791, Republic of Korea. Fax: +82 2 2296 8370.
E-mail address: kimsh1@hanyang.ac.kr (S.H. Kim).

[1] These authors (Noh MY & Chun KW) contributed equally to this work.

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structure–activity relationship. The new GSK-3 inhibitors (C-7a and -7b), having desirable oral PK, was found to be potent in enzyme and cell-based assays. We examined the GSK-3 inhibitors having the highest inhibitory effects and further studied its effects in Aβ-oligomers induced neuronal cell death and in B6; 129-*Psen1*^{tm1Mpm} Tg(APPSwe,tauP301L)1Lfa/Mmjax model. And demonstrated that new compound 7a and 7b showed significant inhibitory effect on tau-hyperphosphorylation through GSK-3 inhibition not only in Aβ-oligomers induced neuronal cell injury model, but also in 3xTg AD mice model.

2. Materials and methods

2.1. Materials

The GSK-3 inhibitors used in this study and the non-specific inhibitor SB415286 was purchased from Sigma–Aldrich. All in-house compounds were synthesized at Jeil Pharmaceutical Drug Discovery Laboratories (R&D Center; Jeil Pharmaceutical Co., Ltd.). All compounds were conducted using a concentration of <0.1% DMSO in vitro study.

2.2. Synthesis of new compounds, C-7a, C-7b

Synthetic procedure of compound **7** was shown in Fig. 1. Compound **3** was obtained by cyclization of compound **2** with triphosgene, followed by reduction with $SnCl_2$ or $Pd(OH)_2$ to yield an anime **4**. Compound **6** was synthesized by reductive amination of Boc-Protected amine 5 with 3-(pyridine-4-yl) propanal [11]. Finally, *N*-alkyl amine salt 7 was prepared with 4 *N* HCl in 1,4-dioxane. And, we developed 8-amino-[1,2,4] triazolo[4,3-*a*]pyridin-3(2*H*)-one derivatives as potential GSK-3 inhibitors. Among the derivatives, C-7a and -7b showed potent enzyme and cellular activity (Table 1). Further, C-7a exhibited desirable PK, brain permeability and good water solubility. (Table 2, *Bioorg. Med. Chem. Lett.* In press).

2.3. Evaluation of IC_{50} values of new GSK-3 inhibitors

The inhibitory effect of GSK-3 inhibitors was determined using human recombinant GSK-3 enzyme (upstate, 14–306) and Phospho-Glycogen Synthase Peptide-2 (GS2, upstate, 12–241), as described previously (*Bioorg. Med. Chem. Lett.* In press). The result analysis was performed using SigmaPlot 10 (Systat Software Inc., USA) to calculate IC_{50} values of the compounds.

2.4. Evaluation of EC_{50} values of new GSK-3 inhibitors

H4IIE (ATCC, CRL154) was used for the cell-based assay [12], as described previously (*Bioorg. Med. Chem. Lett.* In press). Analysis of the results was performed using SigmaPlot 10 (Systat Software Inc., USA) to calculate EC_{50} values of the compounds.

2.5. Primary cultures and treatment of rat cortical neurons

All procedures on animals were performed in accordance with the Hanyang University guidelines for the care and use of laboratory animals. Primary cultured cortical neurons were isolated from the cerebral cortex of fetal Sprague Dawley (SD) rats (16 days of gestation) [13]. Cultures were suspended in neurobasal media (NBM) supplemented with B27. Cultures were kept at 37 °C under a humidified 5% CO_2 atmosphere. Two days after plating, non-neuronal cells were removed by adding 5 μM cytosine arabinoside for 24 h. Only mature cultures (7 days in vitro) were used for experiments. The cultures consisted of about 85% primary cortical neurons.

Cells pre-treated with several concentration of GSK-3 inhibitors (0, 0.1, 1 μM, and 10 μM) alone for 24 h were then treated with 10 μM Aβ-oligomers for 6 h an washed carefully several times with phosphate-buffered saline (PBS). Cell viability was assessed by the MTT and CCK-8 assays as described previously [14].

2.6. Aβ-oligomers preparations

Aβ42 peptide (Sigma, Spruce Street, St. Louis, USA) was resuspended in DMSO to a final concentration of 5 mM, vortexes thoroughly, and sonicated for 10 min. Diluted with ice-cooled phenol red-free Ham's F12 medium to 100 μM. Placed at 4 C overnight to form Aβ42 oligomers. The oligomer solution was centrifuged briefly, use the supernatant.

2.7. Immunoblot analysis

After chemical treatment, cells were lysed with RIPA buffer supplemented with phosphatase inhibitor. Cell lysates were sub-

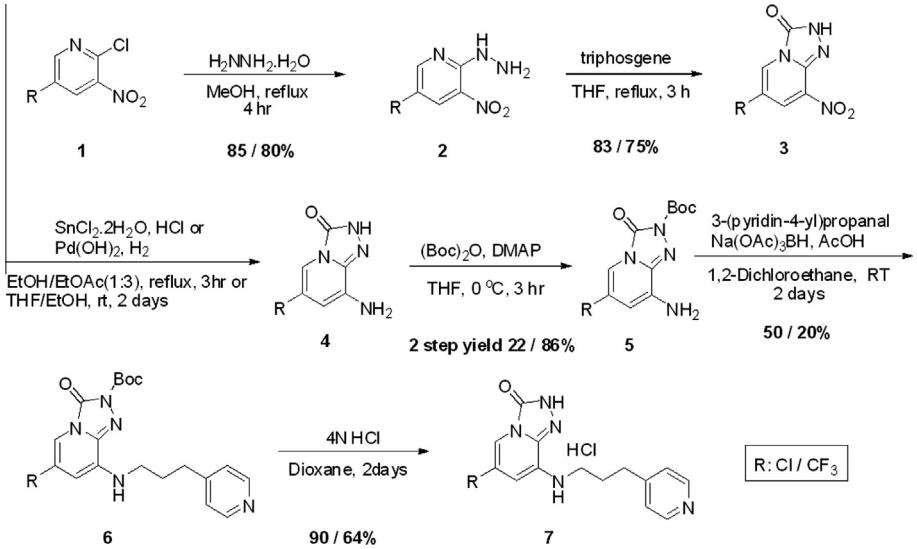
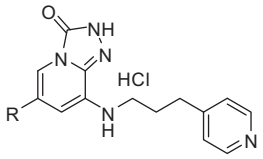


Fig. 1. Synthesis of compound **7**.

Table 1
Results of enzyme- and cell-based assays for the evaluation of inhibitory effects of newly synthetic GSK-3 inhibitors.



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Compounds	R	IC_{50} (nm) in enzyme-based assay	EC_{50} (μM) in cell-based assay
7a	Cl	111	1.78
7b	CF3	63.0	1.75

Table 2
Rat PK profile of 7a and 7b[a], $n = 3$.

Compounds	**7a**		**7b**	
Administration route	iv	po	iv	po
Dose (mg/kg)	3	5	3	5
AUO_{0-inf} (hr.ng/ml)[b]	2489.5	3455.7	1492.3	1736.0
CL (L/hr/k)[c]	1.2	–	2.0	–
$t_{1/2}$ (hr)[d]	0.40	1.2	0.20	0.40
F (%)[e]	–	83	–	70

[a] The values were determined by LC/MS/MS analysis after administration.
[b] AUC means area under the concentration–time curve.
[c] CL means clearance.
[d] $t_{1/2}$ means half-life of elimination.
[e] F means bioavailabilty.

jected to immunoblotting with antibodies; tau-5 (total tau; 1:1000, Invitrogen corporation, Camarillo, CA), p-tau (Thr231) (1:1000, abcam), p-tau (Ser396) (1:1000, Invitrogen), p-tau (Thr181) (1:500, abcam), p-tau (Ser202) (1:1,000, abcam), and GAPDH (1:2000, Santa Crus Biotechnology). Hippocampus was homogenized in RIPA buffer supplemented with phosphatase inhibitor using an electric homogenizer. Lysates were subjected to immunoblotting with antibodies; total GSK3 (1:1000, Santa Cruz Biotechnology), p-S9-GSK3β (1:500, Cell signaling, MA, USA), tau-5 (1:1000, Invitrogen), p-tau (Ser396) (1:1000, Invitrogen), and GAPDH (1:2000, Santa Cruz Biotechnology). The reactive bands were detected by ECL (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and quantified with an image analyzer (Bio-Rad, Quantity One-4, 2, 0). The same membranes were probed for GAPDH as an internal control.

2.8. Immunocytochemistry

Briefly, the cells were fixed in 4% paraformaldehyde in PBS for 15 min. The specimen was incubated with p-tau (Ser396) (1:200, Invitrogen), tau-5 (1:200, Invitrogen) in 1% BSA in PBST in a humidified chamber for overnight at 4 °C. After a wash, Allexa 488 anti-rabbit secondary antibody and TRITC anti-mouse secondary antibody (1:200, Invitrogen) was applied for 2 h in dark. Cover slip was mounted with a drop of mounting medium with DAPI [13].

2.9. GSK-3 activity assay

GSK-3 activity assay was assessed by the phospho-TauS396 targeted assay (in situ ELISA) [15], as described previously.

2.10. Triple transgenic mice model

The 3xTg-AD mice (B6;129-*Psen1*^{tm1Mpm} Tg(APPSwe,tauP301L) 1Lfa/Mmjax), with amyloid beta precursor protein (APPswe), presenilin 1 (PS1M146V), and tau P301L transgenes [16], were purchased from The Jackson Laboratory and bred in our laboratories. Mice were group-housed in an animal room maintained at 23 ± 1 °C with a 12 h light/dark cycle and free access to water and food. All experiments were approved by the institutional Animal Care and Use Committee of Hanyang University.

2.11. Drugs administration and Y-maze test

A GSK-3 inhibitor, C-7a, was dissolved in saline and administered orally twice a day for 21 days to 13-month-old mice at the indicated doses. The 3xTg-AD mice were randomly divided into three groups: a vehicle group ($n = 16$) which was treated with saline and two C-7a groups which were treated with 20 mg/kg ($n = 15$) and 50 mg/kg ($n = 16$), respectively. They were observed daily and evaluated body weight twice a week. Behavioral test was performed by *Y*-maze test at a treatment time of 21 days. The *Y*-maze has three black plastic arms at a 120° angle from each other. Mice were placed within one arm and allowed to freely explore the three arms for 8 min. The number of arm entries and the number of triads are recorded and the percentage of alternation was calculated [17,18].

2.12. Statistical analysis

All in vitro data are presented as means ± SEM of five or more independent experiments. Statistical comparisons between groups were analyzed by one-way ANOVA followed by Tukey's test. Body weight was analyzed by repeated measures ANOVA, followed by a Duncan's multiple comparisons test. Behavioral data were analyzed using one-way ANOVA with Duncan post hoc test. Two-tailed *P*-values less than 0.05 were considered statistically significant. All statistical analyses were performed using the SPSS 17.0 software package for Windows (SPSS, Seoul, Korea).

3. Results

3.1. Effect of newly developed GSK-3 inhibitors on the viability in rat cortical neurons

To examine the effect of Aβ-oligomers on neuronal viability, rat cortical neurons were treated with different concentrations of Aβ-oligomers for 6 h. As shown in Fig. 2A, the viability was reduced in a concentration-dependent manner. Based on these results, 10 μM was selected as an optimal Aβ-oligomers concentration for subsequent experiments.

To evaluate the effect of new GSK-3 inhibitors (C-7a and 7b) on cell viability, we used SB415286 as a reference. Cells were treated with various concentrations of C-7a and -7b for 24 h, and cell viability was measured. C-7a and -7b had no detrimental effect on the

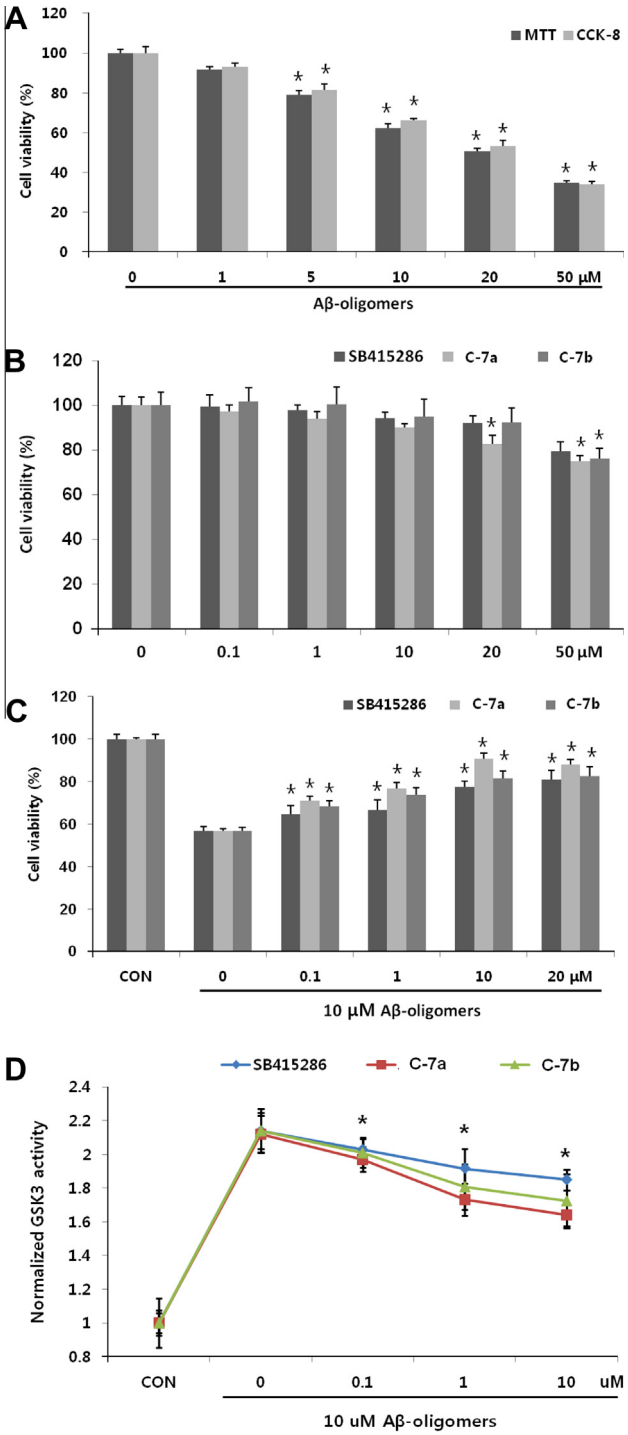

Fig. 2. Effect of newly developed GSK-3 inhibitors on the viability in rat cortical neurons A. Toxic effects of Aβ-oligomers on the viability. B. Comparative cell viability of GSK-3 inhibitors on the viability. Cell viability was quantified by MTT assays. $^{*}p < 0.01$ when compared with the non-treated group. C. Concentration-dependent effect of GSK-3 inhibitors on Aβ-oligomers induced neuronal toxicity. Cell viability was quantified by MTT assays. $^{*}p < 0.01$ when compared with the group treated with 10 μM Aβ-oligomers only. The data are represented as mean % of the non-treated group ± SEM. D. Effects of GSK-3 inhibitors on GSK-3 enzyme activity. The data are represented as mean (normalized to the non-treated group) ± SEM. $^{*}p < 0.01$ when compared with the group treated with 10 μM Aβ-oligomers only.

cell viability at 10 μM (Fig. 2B). Previous studies have demonstrated that GSK-3 inhibitors protected Aβ-induced neuronal cell death [19]. To determine the effect of new GSK-3 inhibitors on Aβ oligomers-induced neuronal toxicity, we pretreated with several concentrations of C-7a and -7b for 24 h followed by treatment with 10 μM Aβ-oligomers for 6 h, and measured the cell viability. C-7a and -7b protected the cells against the loss of viability due to Aβ oligomers treatment (Fig. 2C). These results suggest that new GSK-3 inhibitors have neuroprotective effects against Aβ-oligomers neurotoxicity.

3.2. Effects of C-7a and C-7b on GSK-3 enzyme activity in rat cortical neurons

To provide direct evidence that the new GSK-3 inhibitors decreased GSK-3 enzyme activity against 10 μM Aβ oligomers-induced toxicity on stably expressed human 4R tau-HEK293 cell, we treated the cells with 10 μM Aβ oligomers-treated cells and measured the enzyme activity in the absence and the presence of the new GSK-3 inhibitors against Aβ-oligomers induced neurotoxicity. When cells were treated with 10 μM Aβ oligomers-treated cells, the enzyme activity was markedly increased. In the presence of the inhibitors (1, 10 μM of C-7a and -7b) against Aβ-oligomers induced neurotoxicity, GSK-3 enzyme activity was significantly decreased (Fig. 2D). These findings suggest that the new GSK-3 inhibitors are more potent inhibitors of GSK-3 enzyme activity compared to the reference compound.

3.3. C-7a and C-7b effectively decreased Aβ-oligomers induced tau phosphorylation

We examined the effects of new GSK-3 inhibitors on substrate of GSK-3: a phosphorylated tau at different sites (pThr231, pSer396, pThr181, and pSer202), using SB415286 as a reference (Fig. 3A).

When normalization to total levels of tau, 10 μM Aβ-oligomers only treated cells showed increased levels of phosphoryled tau of different sites (pThr231, pSer396, pThr181, and pSer202). Shown as Fig 3, ratios of phosphorylated tau to total tau were more significantly decreased with dose dependent pattern after treatment of C-7a, -7b and SB415286 when compared with the 10 μM Aβ-oligomers only treated group ($p < 0.01$, respectively). Which findings were more remarkable at GSK-3-mediated tau-phosphorylatiion sites including pThr231, pSer396, and pSer202 than pThr181. Moreover, effect of newly developed C-7a, -7b on reducing tau phosphorylation was more significant than reference material, SB 415286.

In addition, immunocytochemical studies were performed using antibodies for p-tau (Ser396) and Tau-5 to evaluate and compare the effect of new GSK-3 inhibitors and SB 415286 on phosphorylation tau at S396. Only Aβ-oligomers treated cells increased tau phosphorylation (Ser396) immunoreactivity in cortical neurons compared with controls. Pretreatment with new GSK-3 inhibitors (C-7a, -7b) and SB 415286 decreased immunoreactivity of S396 pTau, but these findings were more notable in C-7a and -7b than SB415286 (Fig. 3B). Western blot and immunocytochemical data suggest that the new GSK-3 inhibitors (C-7a and -7b) effectively reduced phosphorylation of tau on pThr231, pSer396, pThr181, and pSer202 on Aβ-oligomers induced neurotoxicity.

Based on above findings and PK data shown Table 2, C-7a was selected for further animal study.

3.4. Effects of C-7a in B6; 129-Psen1^{tm1Mpm} Tg(APPSwe,tauP301L)1Lfa/Mmjax animal model

To evaluate the effect of new GSK-3 inhibitor, C-7a, using 3xTg mice (B6; 129-*Psen1*^{tm1Mpm} Tg(APPSwe,tauP301L)1Lfa/Mmjax), in the aspects of behavior change and level of tau phosphorylation in hippocampus. There were no significant differences in body weight among groups. *Y*-maze test was performed at a treatment time of 21 days to evaluate short-term memory. It measures the

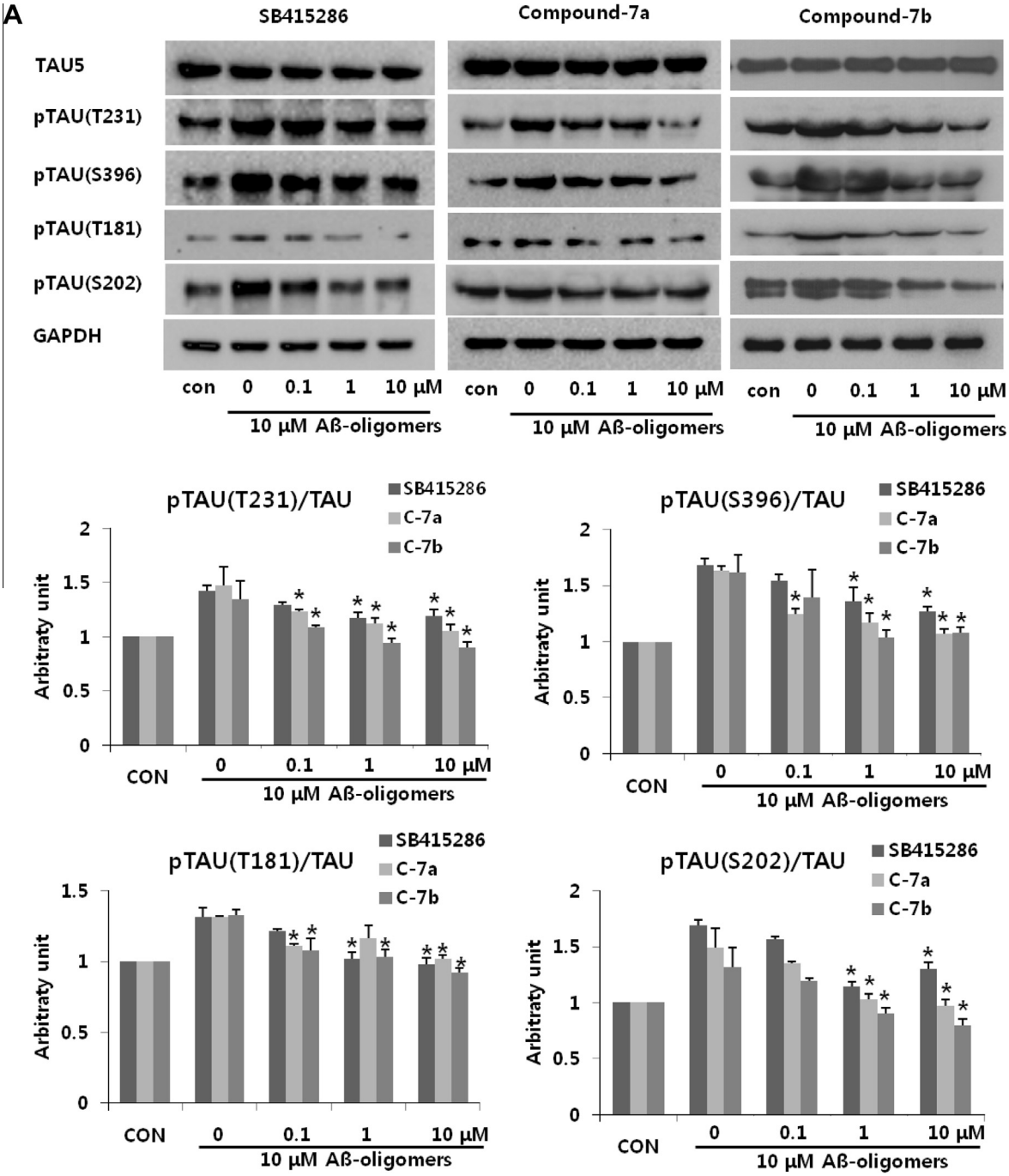

Fig. 3. C-7a and 7b effectively decreased Aβ-oligomers induced tau phosphorylation A. Effects of GSK-3 inhibitors on Aβ-oligomers induced tau phosphorylation by western blotting. Data were expressed as a ratio of the simultaneously assayed control group's value and were compared using Tukey's test after a one-way ANOVA ($n = 5$). The data are represented as mean (normalized to the non-treated group) ± SEM. $^{*}p < 0.01$ when compared with the group treated with 10 μM Aβ-oligomers only. B. Immunofluorescent imaging of phosphorylated tau (Ser396) after treatment of 1 μM GSK-3 inhibitors is shown. DAPI was used to counter stain the nucleus. Scale bars = 50 μm.

willingness of rodents to explore new environment. C-7a treated groups showed increased total arm entries compared with vehicle group, which served as an indicator of activity (Fig. 4A). The mice treated with 50 mg/kg C-7a showed significantly increased spontaneous alternation which was regarded as short-term memory (Fig. 4B).

When the levels of p-GSK-3β (Ser9) and total GSK-3 β protein were assessed by western blot analysis (Fig. 4C), there were no significant differences in quantification of p-GSK-3β levels to total GSK protein among groups. However, when p-tau (Ser396) was surveyed (Fig. 4D), phosphorylation ratio to total-tau of 50 mg/kg C-7a treated mice significantly decreased compared to the vehicle group. Taken together, the 3xTg-AD mice suggest that treatment with new GSK-3 inhibitor, C-7a is able to prevent short-term memory deficits and to modulate hyperphosphorylation of tau protein at downstream of GSK-3 signaling.

4. Discussion

GSK-3 is an important target for the treatment of Alzheimer's disease (AD) where abnormal increases in GSK-3 levels and activity

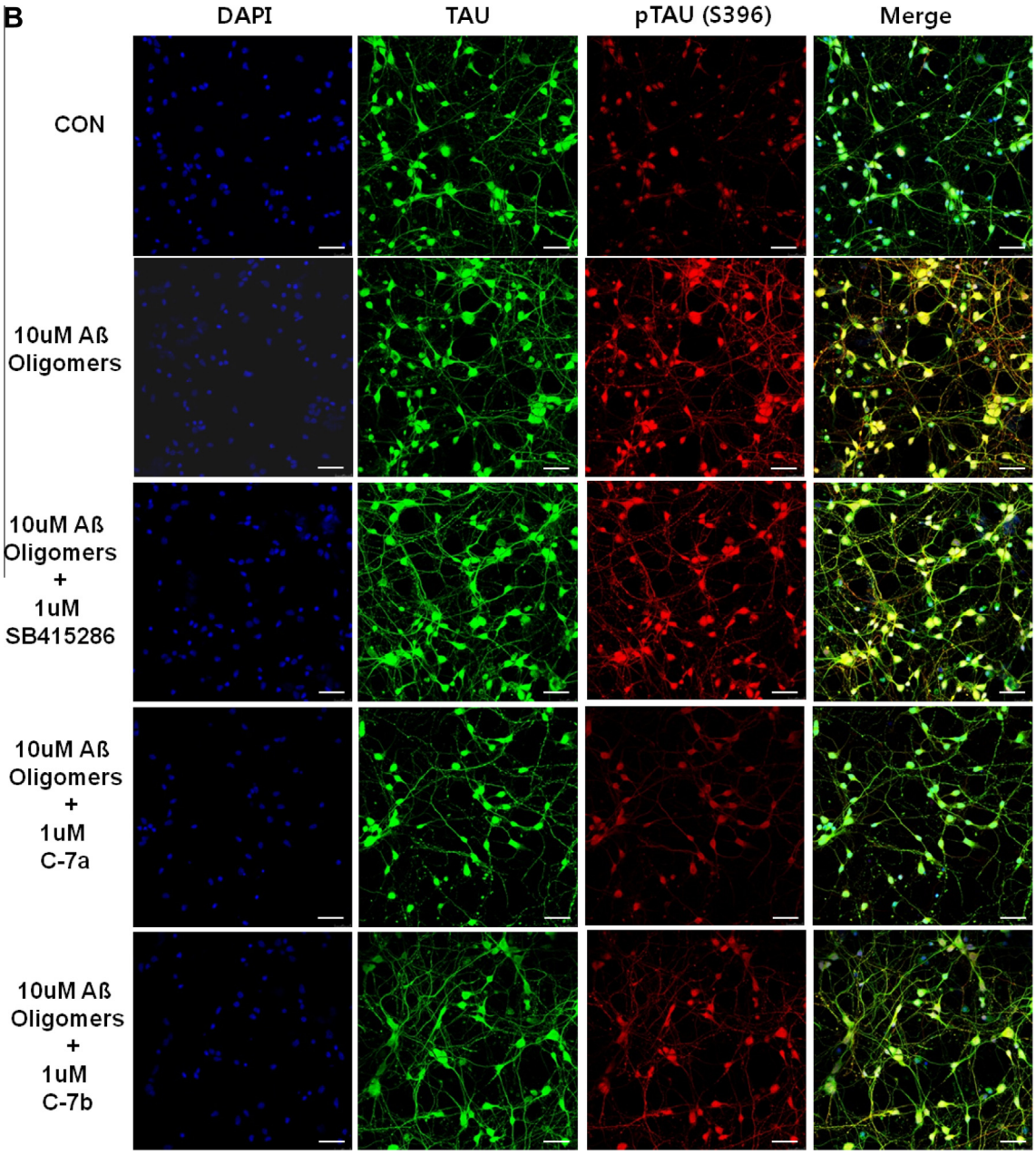

Fig. 3. (*continued*)

have been associated with neuronal death, paired helical filament tau formation and neuritis retraction as well as a decline in cognitive performance [20,21]. Abnormal tau phosphorylation plays an important role on axonal assembly associated with synaptic transmission, neuronal dysfunction, degeneration, and neurofibiliary pathology in AD [9]. As a dynamic process, tau phosphorylation represents a balance of kinase and phosphatase activity through addition and removal of phosphate groups, where induction of tau phosphorylation involved increased kinase and/or decreased phosphatase activity [22]. Reducing tau phosphorylation through GSK-3 inhibition has therefore emerged as a target for drug development [1,23].

Aβ peptide can form oligomers that bind to certain cell receptors, preventing the activation of PI3 kinase, and subsequently that of Akt. GSK-3 increases its activity in the absence of activated Akt [24]. In addition, Aβ peptide activates GSK-3 resulting in phosphorylation of tau protein [25]. It is well known that GSK-3 inhibition reduces Aβ oligomers-induced neuronal cell death, reduces tau phosphorylation, and inhibits GSK3 activity [19,26]. Our new GSK-3 inhibitors (C-7a, -7b) reduced tau phosphorylation in Aβ oligomers-induced neuronal cell death may involve these mechanisms (Figs. 2 and 3).

Recently, normalization of GSK-3 activity inhibition emerges as promising therapy for treatment of neurodegenerative and behavior disorders. Inhibition of GSK-3 results in beneficial outcomes in multiple in AD animal model, the neurotherapeutic effects of GSK-3 inhibition are ameliorates Aβ pathology in FTDP-17 tau transgenic mice, reduces tau phosphorylation in APP/PS1 mice, improves learning and memory in hAPP mice, and indicates neuroprotective activity in 3xTg mice and GSK-3β mice [27–29]. We confirmed that the effects of C-7a were improved memory and reduced tau phosphorylation at hippocampus in 3xTg-AD mice

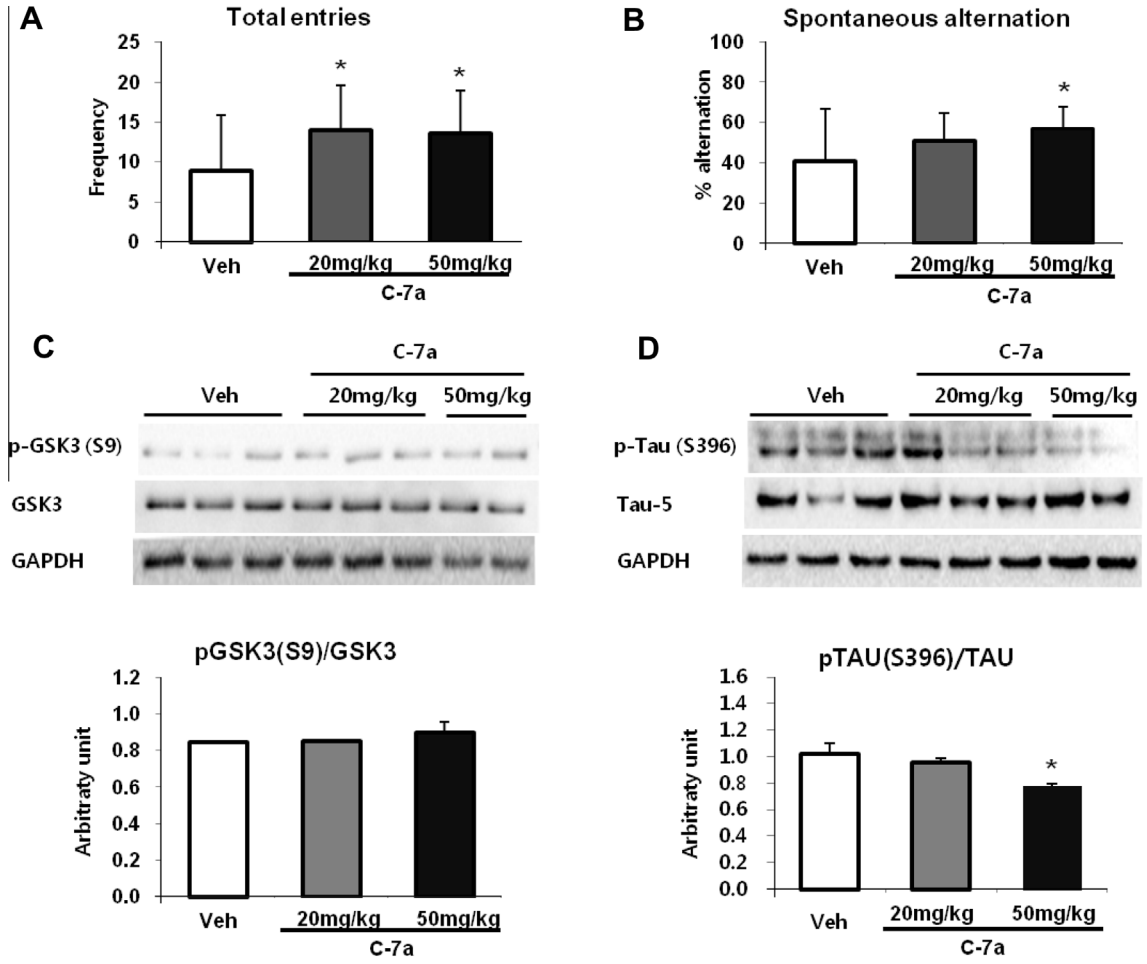

Fig. 4. Effects of GSK-3 inhibitor (C-7a) in 3xTg-AD mice (B6; 129-*Psen1*^{tm1Mpm} Tg(APPSwe, tauP301L)1Lfa/Mmjax). *Y*-maze test after C-7a administration. C-7a (20 and 50 mg/kg) or vehicle was administered orally twice a day for 21 days to 13-month-old mice, at 21 days to the *Y*-maze test. Data are presented as total frequency of arm entries (A) and % alternation rate (B) during an 8 min exploration. Western blot analysis in hippocampus; p-GSK-3 (Ser9) and GSK-3(C), p-tau (Ser396) and TAU-5 (D). Data were expressed as mean ± SEM from five independent experiments.

(B6; 129-*Psen1*^{tm1Mpm} Tg(APPSwe, tauP301L)1Lfa/Mmjax) (Fig. 4), the resulting in the development of both Aβ plaque and tau neurofibrillary tangle pathology in AD-relevant brain regions, including hippocampus [16].

Based on these results, numerous efforts have been made to develop GSK-3 inhibitors [30,31]. The roles of GSK-3 in both AD pathological conditions and in dysfunctional neurons, it is important to identify a GSK-3 inhibitor that can be tested in the clinic for its therapeutic potential in the treatment of AD. We recently developed novel GSK-3 inhibitors (Fig. 1). Our new GSK-3 inhibitors (C-7a, -7b) showed potent inhibitory effects on GSK-3 in the enzyme- and cell-based assay (Table 1). C-7a and -7b showed that they more effectively reduced Aβ oligomers-induced neuronal cell death than the other inhibitor, SB-415286 (Fig. 2). We confirmed that new GSK-3 inhibitors (C-7a, -7b) inhibited direct enzyme activity of GSK-3 (Fig. 2D) and reduced tau phosphoration at various sites (Fig. 3A and B). C-7a had beneficial effects in 3xTg-AD mice (B6; 129-*Psen1*^{tm1Mpm} Tg(APPSwe, tauP301L)1Lfa/Mmjax). 50 mg/kg of C-7a treated groups showed significantly increased total arm entries, spontaneous alternation compared with vehicle group (Fig. 4A and B), and reduced tau phosphoryration at Ser396 (Fig. 4D). These effects of C-7a were similar to or better than the effects of the previously reported other GSK-3 inhibitors in animal models. Further studies using C-7a need to be performed for the development of new GSK-3 inhibitors in pre-clinically and in humans.

5. Conclusion

In summary, we have discovered novel GSK-3 inhibitors (C-7a, -7b) and demonstrated pharmacological validation by its capacity to interfere with Aβ oligomers-induced neuronal cell death and tau phosphorylation in vitro. Especially, C-7a was reduced the tau phosphorylation and the short-term memory in vivo; events that are believed to play an important role in the pathogenesis of AD. Based on these data, C-7a may have both disease-modifying and symptomatic potential in the treatment of AD and related tauopathies. These results were suggested that C-7a might be potent candidates for AD.

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