Selective PCAF inhibitor ameliorates cognitive and behavioral deficits by suppressing NF-κB-mediated neuroinflammation induced by Aβ in a model of Alzheimer's disease

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Abstract. Several recent studies have reported an association between neurodegeneration and histone modifications, such as acetylation, deacetylation and methylation. In addition, questions have been raised regarding a potential functional role for the histone acetylation enzymes in β-amyloid (Aβ)-mediated neurotoxicity, particularly the p300/CBP-associated factor (PCAF) enzyme. We recently reported the potential utility of a PCAF inhibitor in the suppression of Aβ-induced neuronal cell death, although the in vivo effectiveness of the PCAF inhibitor remained unclear. In this study, we modified the PCAF inhibitor by chemical derivatization and selected compound C-30-27 as the most potent PCAF inhibitor. We demonstrated that C-30-27 selectively inhibited acetylation-dependent nuclear factor-κB (NF-κB) at Lys-122 and suppressed the NF-κB-mediated inflammatory response induced by lipopolysaccharide (LPS) or Aβ in both BV2 and Neuro-2A (N2A) cells. Finally, we demonstrated that C-30-27 improved cognitive deficits, as well as the capacity for locomotion and the damaged cholinergic system in the Aβ-treated rats. In conclusion, our results demonstrate that this selective PCAF inhibitor has the potential to reduce the neuroinflammatory response induced by Aβ.

Introduction

Neuroinflammation is a hallmark of neurodegenerative diseases that is linked to glial cell activation (1,2). This activation is induced by nuclear factor-κB (NF-κB) (3), and the abnormal activation of microglia promotes neuronal injury through the release of pro-inflammatory and cytotoxic factors, thus also promoting central nervous system injuries (4,5).

NF-κB/Rel designates a family of transcription factors participating in the activation of a wide range of genes that are crucial players in immune and inflammatory function. Over the years, the NF-κB/Rel system has been recognized as a central mediator of the rapid and coordinated induction of genes in response to external, primarily pathogenic stimuli (6). NF-κB has also been shown to control the induction of the transcription of pro-inflammatory mediators, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-6 (7). The transcriptional activity of NF-κB is activated by acetylation, a task primarily performed by p300/CBP and p300/CBP-associated factor (PCAF) (8). p300/CBP acetylates multiple lysine residues of NF-κB, including Lys-122, -123, -218, -221 and -310 (9). However, PCAF specifically acetylates only Lys-122 of NF-κB (10). The blocking of NF-κB acetylation by histone acetyl transferase (HAT) inhibitors diminishes the nuclear retention and transcriptional activity of NF-κB (11-13). Thus, the pharmacological inhibition of HAT activity may be a useful method for the treatment of inflammation.

Previous studies have demonstrated that PCAF knockout mice develop resistance to amyloid toxicity and do not have reduced memory impairment, suggesting the importance of PCAF in the development of neurodegenerative disease (14). In our previous studies, we reported that gallic acid, which inhibits multiple HAT enzymes, including p300 and PCAF, reduced...
NF-κB acetylation and activity, and suppressed microglial inflammation (12,15). In addition, we screened PCAF-specific inhibitors using a computer-based molecular docking simulation and found that a PCAF inhibitor that selectively blocked the acetylation of NF-κB at Lys-122 effectively prevented neuronal cell death caused by Aβ-induced microglial inflammation and neurotoxicity (16). However, the in vivo effectiveness of this PCAF inhibitor remains unclear.

In this study, we further developed this class of PCAF inhibitors by performing chemical derivatization of the parent compound C-30 identified in a previous study (16) and evaluating the activities of the derivatives. We selected C-30-27 as the most potent PCAF inhibitor and evaluated its effects on neuroinflammation, cognitive impairment and damage to the cholinergic system resulting from treatment with Aβ in vitro or in vivo.

Materials and methods

Synthesis. The compounds were synthesized using the 4-hydroxy-benzaldehyde analogues. Benzaldehyde was O-benzylated with proper benzyl chloride under basic conditions. Subsequent condensation with 4-(2-aminoethyl)oropholine or 1-(2-amino-ethyl)piperidine yielded imine compounds. Finally, the imine compounds were reduced using sodium borohydride (NaBH₄) to produce the desired compounds. All the compounds prepared showed consistent spectroscopic data corresponding to the desired structures.

Chemistry (general). The majority of the chemicals and reagents used were obtained from Sigma-Aldrich (St. Louis, MO, USA) and others were from companies, such as Acros Organics (Waltham, MA, USA) and Tokyo Chemicals (Tokyo, Japan). Melting points were measured without correction in open capillaries using the Barnstead Electrothermal melting point apparatus, Manual Mel-Temp (model no. 1202D; Thermo Fisher Scientific Inc., Waltham, MA, USA). Chromatographic separations were monitored by thin-layer chromatography using a commercially available pre-coated Merck Kieselgel 60 F₂₅₄ plate (0.25 mm; Sigma-Aldrich) and detected by visualization under UV light at 254 and 365 nm. Silica gel column chromatography was carried out using the Merck Kieselgel 60 plate (0.040-0.063 mm). All solvents used for chromatography were directly used without distillation. NMR spectra were recorded on a Varian AS 400 spectrometer (¹H-NMR at 400 MHz and ¹³C-NMR at 100 MHz) (Agilent Technologies, Santa Clara, CA, USA) with tetramethylsilane as an internal standard. Chemical shift (δ) values are expressed in parts per million (ppm) and coupling constant (J) values in hertz (Hz). Mass spectral investigations were run by liquid chromatography-electrospray ionization-time of flight mass spectrometry (LC-ESI-TOF-MS; Agilent Technologies) in a positive mode.

General synthetic method. A mixture of substituted 4-hydroxy-benzaldehyde (1 equivalent), corresponding benzyl chloride (1 equivalent) an potassium carbonate (K₂CO₃; 1 equivalent) in dimethylformamide (DMF) was refluxed for 1 day. The reaction mixture was cooled to room temperature and extracted with ethyl acetate. Organic solvent was washed with water, sodium bicarbonate (NaHCO₃) and brine, and dried over magnesium sulphate (MgSO₄). The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography to yield O-benzylated compounds. The O-benzylated compound (1 equivalent) and 4-(2-aminoethyl)morpholine (1 equivalent) or 1-(2-aminoethyl)piperidine in ethanol was refluxed for 2 h and cooled to room temperature. The solvent was removed under reduced pressure to yield imine compounds. Finally the imine (1 equivalent) and NaBH₄ (1.2 equivalent) in ethanol was stirred at room temperature under N₂ for 2 h. The reaction mixture was extracted using ethyl acetate. The combined organic solvent was washed with water and dried over MgSO₄. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography to yield the final compound, C-30.

In vitro PCAF activity assay. PCAF activity assay was performed using commercially available kits according to the manufacturer's instructions (BioVision, Inc., Milpitas, CA, USA). For in vitro PCAF activity assays, recombinant PCAF proteins (Sigma-Aldrich) were incubated with in vitro acetylation assay buffer [50 mM HEPES pH 8.0, 10% glycerol, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM sodium butyrate and 1 μl [³²P]acetyl-CoA], 5 μg histone H4 tail peptides and/or the indicated concentration of inhibitors at 30°C for 1 h. The samples were separated on a 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and analyzed by autoradiography.

Western blot analysis. Total cell lysate protein was separated with 8% or 12% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked by incubation for 12 h in 5% (w/v) non-fat Difco™ skim milk blocking buffer. The blocked membranes were incubated overnight at 4°C with primary antibodies that recognize iNOS (Cat. no. 160862) (Cayman, AnnArbor, MI, USA), IL-1β (Cat. no. sc-7884) (Santa Cruz Biotech, Dallas, TX, USA), NF-κB (Cat. no. sc-8008) (p65; Santa Cruz Biotech) and β-actin (Cat. no. A2228) (Sigma-Aldrich). Antibody against Ac(K122)NF-κB was as previously described (16). After extensive washing 3 times with PBS/0.1% Tween-20, the membranes were incubated with secondary horseradish peroxidase-conjugated antibody (1:1,000) for 1 h. The bands were detected with the Enhanced Chemiluminescence System (GE Healthcare, Uppsala, Sweden) according to the manufacturer's instructions.

Cell culture and reagents. BV-2 murine microglial cells and Neuro-2A neuroblastoma cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Fetal bovine serum (FBS), trypsin-EDTA and penicillin-streptomycin were purchased from Gibco-BRL™ (Gaithersburg, MD, USA). 3-(4,5-Dimethylthiazol-2-ly)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich. Aβ₁-₄₂ was purchased from Bachem (Bubendorf, Switzerland). Other chemicals were purchased from Sigma-Aldrich. The BV-2 cells were cultured in DMEM (HyClone, Logan, UT, USA) containing 5% heat-inactivated endotoxin-free FBS, 2 mM glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin in a humidified 5% CO₂ atmosphere at 37°C. The Neuro-2A cells were cultured in modified Eagle's medium (MEM; HyClone) containing 10% heat-inactivated endotoxin-free FBS, 2 mM glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin in a humidified 5% CO₂ atmosphere at 37°C. For the co-culture
experiments, the BV-2 cells were treated with various concentrations of C-30-27 (0-100 μM) for 12 h prior to stimulation with Aβ1-42 (5 μM; Aβ + C-30-27), Aβ1-42 (5 μM) alone or medium only (control) for 24 h. Conditioned medium (CM) from the BV-2 cells was collected, centrifuged and transferred to the Neuro-2A cells for a further 24 h. CM from the medium-only treated cells was used as a control. Following incubation, cell viability was measured by MTT assay and reverse transcription-quantitative PCR (RT-qPCR) was performed.

Cell viability assay (MTT assay). Cell viability was measured using the conventional MTT reduction assay to determine the cytotoxic effects on the BV2 and Neuro-2A cells. Briefly, 5x10^4-1x10^5 BV-2 cells were seeded in a 96-well plate. After 12 h of incubation, the cells were pre-incubated for 24 h with or without C-30-27, and then incubated with 1 mM of lipopolysaccharide (LPS; Sigma-Aldrich) or Aβ1-42 (5 μM) for a further 24 h. The cells were then treated with 15 ml MTT (Sigma-Aldrich) solution (2 mg/ml) for 90 min at 37°C and the absorbance was recorded at 570 nm, and a reference was recorded at 630 nm using a microplate reader (Model 550; Bio-Rad Laboratories, CA, USA).

RNA isolation and RT-qPCR. Total RNA from the BV-2 cells was extracted using TRizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer’s instructions. The levels of iNOS, COX-2 and IL-1β mRNA were determined by qPCR (ABI PRISM 500 Sequence Detection system; Applied Biosystems, San Jose, CA, USA). cDNA amplification was performed in duplicate in 20-ml reaction mixtures containing 2X SYBR-Green master mix (Roche, Indianapolis, IN, USA) and 10 pM forward and reverse primers. The amplification mixtures contained 2X SYBR-Green master mix (Roche, Indianapolis, IN, USA) and 10 pM forward and reverse primers. A 2X SYBR-Green master mix (Roche, Indianapolis, IN, USA) was added to the reaction mixture. Following 40 amplification cycles: 30 sec at 95°C, 30 sec at 58°C, 30 sec at 72°C, with a final 10-min extension at 72°C.

The results were analyzed using ABI sequence detection software version 2.3. The relative mRNA expression of the target genes was calculated following normalization to GAPDH expression and expressed as the fold induction. The primers used for amplification were as follows: iNOS, 5’-ACCAGGAGTGTGGAATCCTTGGT and 5’-GTTGCAGTTAACAGTCCAGG AAGT; COX-2, 5’-AAGGTGGAGCAGCAATGGCAT T and 5’-GAGGAGCCAAGTGAGGTTTTA; TNF-α, 5’-TTCTCCTATTCTGCTGTTGGC and 5’-AAGGTGGAGCAGCAATGGCAT T; IL-1β, 5’-GTTGCGACGGGACCCCCAAAGAT and 5’-AAGGT GGAGCAGCAATGGCAT T; and GAPDH, 5’-GTTGCCCTACC CCAAATGTTG and 5’-AGGAGACACCCCTGGTTCCAGT.

Animals. Male Sprague-Dawley rats (weight range, 220-240 g) were purchased from the Central Laboratory Animal, Inc. (Seoul, Korea) and housed 7 per cage in a light-controlled room (lights on from 8:00 a.m. to 8:00 p.m.) at a temperature of 22±2°C and a humidity of 50±5% with food and water available ad libitum. All experiments were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals of the Yonsei University College of Medicine, Seoul, Korea.

Experimental groups. The rats were randomly assigned into one of 5 groups as follows: i) the vehicle-injected plus saline-administered group (control group); ii) the Aβ1-42-injected plus saline-administered group (β-amyloid group); iii) the Aβ1-42-injected plus donepezil (Eisai Co., Ltd., Tokyo, Japan)-administered group (donepezil group); iv) the Aβ1-42-injected plus low concentration [15 mg/kg body weight (BW)/day] of C-30-27 PCAF inhibitor-administered group (27-L group) and; v) the Aβ1-42-injected plus high concentration (20 mg/kg BW/day) of C-30-27 PCAF inhibitor-administered group (27-H group). For the induction of AD, the rats were anesthetized with isoflurane [3%, intra-arterial (i.a.); Abbott Animal Health, Wiesbaden, Germany], and placed in a stereotaxic instrument (Harvard Apparatus, Holliston, MA, USA). An incision was made on the scalp of each rat, and the skull was adjusted to position bregma and lambda on the same horizontal plane. Small burr holes were drilled, and Aβ1-42 (4 μg) or the vehicle were injected into the hippocampus (-3.0, 2.2 and -2.8 mm) using a 5-μl Hamilton syringe (Sigma-Aldrich) at a rate of 1 μl/min. The hole was then covered with dental acrylic cement and the scalp was closed with sutures. Following surgery, each rat was injected with penicillin in the hindquarter muscle (100,000 units) and individually housed following surgery. All behavioral tests commenced on day 2 following surgery using unanesthetized freely moving rats. Donepezil (1.5 mg/kg BW/day) was administered intragastrically once daily for 7 days before and after the injection of Aβ into the hippocampus. The C-30-27 PCAF inhibitor was administered during the test period. Artificial cerebrospinal fluid (145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl2, 1.0 mM MgCl2) was used as the vehicle.

Morris water maze test. The water maze test was performed according to standard methods (23) in a circular pool (diameter, 180 cm; height, 75 cm; Iwo Scientific Co., Seoul, Korea) filled to a depth of 25±1 cm. The transparent platform (24 cm) was submerged approximately 1 cm below the surface of the water, and non-fat milk was added to the water to make it opaque. The first day of the experiment was a swim training day during which the rats swam for 60 sec in the absence of the platform. On each day following the first training day, the rats were subjected to 4 sessions, with an intertrial interval of 5 min. The starting point changed for each session, but the location of the platform was fixed during the entire test period.

The time from being placed into the pool to reaching the platform was measured. The time after the final training session, each rat was subjected to a probe trial (90 sec) in which no platform was present. The time spent in the target quadrant (the quadrant in which the platform had previously been located) was taken as a measure of spatial memory retention. All data were collected and analyzed using the Smart video tracking system (Smart version 2.5.19; Panlab, Barcelona, Spain).

Passive avoidance test. The rats were tested for memory retention deficits using a passive avoidance apparatus (Iwo Scientific Co.,) as previously described (17). The apparatus consisted of a two-compartment dark/light shuttle box with a guillotine door separating the compartments. The dark compartment had a stainless steel shock grid floor. During the acquisition trial, each rat was placed in the light chamber. After a 60-sec habitation period, the guillotine door was opened, and the latency for the rat to enter the dark chamber (hereafter termed ‘initial latency’) was recorded. Rats with an initial latency of >60 sec were excluded from further experiments. Immediately after the rat entered the dark chamber, the
A guillotine door was closed and an electric foot shock (75 V, 0.2 mA, 50 Hz) was delivered through the floor grid with a stimulator for 3 sec. Five seconds later, the rat was removed from the dark chamber and returned to its home cage. Eight hours later, this test was repeated and the latency to enter the dark chamber was recorded (hereafter termed ‘retention latency’).

Rotarod test. Performance on a rotarod task (five-lane accelerating rotarod; Jeung Do Bio & Plant, Seoul, Korea) was used to measure motor balance and coordination as previously described (17). The rats were placed on a horizontal rotating rod (diameter, 8 cm; rotation speed, 20 rpm) and were left on the rod for 5 min or until they fell off. Falling off the rod activated a switch that automatically stopped a timer. Five rats separated by large disks were tested simultaneously. On the testing day, each rat was submitted to 3 trials with an inter-trial interval of 10 min.

Vertical pole test. The vertical pole test (Iwoo Scientific Co.) was performed as previously described (17). The rats were tested for measuring grip strength and sensorimotor performance on a vertical pole (2 cm in diameter, 60 cm high). The rats were placed on the center of the pole, which was fixed in a vertical position. Rats with deficits in grip strength and sensorimotor performance fell off the pole. Rats were habituated to the task by undergoing 2 trials/day for 2 days. On the testing day (3rd day), 3 measures were taken over 3 trials/rat.

Measurements of acetylcholine (ACh) content and acetylcholinesterase (AChE) activity. The ACh content was determined according to the method of Vincent (18). Aliquots (20 µl) of brain homogenate and 50 µl of 1% hydroxylamine were added to a 96-well plate and incubated for 15 min at 25°C, followed by the addition of 250 µl iron(III) chloride (FeCl₃) (in 0.1 N HCl, pH 1.2±0.2). The absorbance was read at 540 nm and calibrated with a blank. AChE activity was determined by the colorimetric method described by Ellman (19). A total of 20 µl of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB, 0.01 mM; Sigma-Aldrich) and 10 µl of brain homogenate in 0.1 mM sodium phosphate buffer (pH 8.0) were added to a 96-well plate and incubated for 5 min at 25°C. The reaction was then initiated by the addition of 10 µl of 0.1 M acetylthiocholine (acetylthiocholine chloride; Sigma-Aldrich). The hydrolysis of acetylthiocholine was monitored by the formation of yellow 2-nitro-5-sulfidobenzene-carboxylate anion resulting from the reaction of DTNB with thiocholine for 10 min at a wavelength of 412 nm.

Statistical analysis. The results are expressed as the means ± SD. The data were statistically evaluated using the Student’s t-test or one-way analysis of variance (ANOVA) followed by Duncan’s
multiple range test to compare significant differences between the groups at \( P \leq 0.05 \).

**Results**

**Inhibitory effect of the PCAF inhibitor, C-30-27, against the acetylation of NF-κB on Lys-122.** In our previous study, we screened several PCAF inhibitors using computer-based molecular docking simulation (16). Among those candidates, we selected C-30 for chemical derivatization and synthesized 10 derivatives (Fig. 1A and B). We then assessed the inhibitory activities of these derivatives against the PCAF enzyme by an *in vitro* acetylation assay. We selected compound 25 (C-30-25), compound 26 (C-30-26), compound 27 (C-30-27) and compound 29 (C-30-29), all of which showed greater inhibition against PCAF activity than the other compounds tested (Fig. 1C). We then determined the inhibitory effects on the expression of cytokines, such as COX-2, iNOS, IL-1β and TNF-α induced by LPS in BV2 cells by RT-qPCR (Fig. 2A). Of the tested compounds, C-30-27 showed the highest inhibitory effects on cytokine expression. Therefore, we selected C-30-27 as our lead PCAF inhibitor candidate.

To examine the effects of our compounds on cell cytotoxicity, we treated the BV-2 cells with 0-100 µM C-30-27 for 24 h and evaluated cell viability by MTT assay. Treatment with 0-25 µM C-30-27 did not have an effect on cell viability; however, treatment with 50-100 µM of C-30-27 induced cell cytotoxicity (Fig. 2B). Based on these results, we selected a C-30-27 concentration of 10 µM for further experiments in which we determined whether the inhibition of PCAF blocks the acetylation of NF-κB and suppresses NF-κB activity in LPS-treated BV2 cells. As either treatment with LPS or with antibody (Ab) has been shown to commonly induce the PCAF-mediated acetylation of NF-κB on Lys-122 (12,16), we then examined whether the PCAF inhibitor, C-30-27, suppresses the LPS-induced NF-κB acetylation at Lys-122. As expected, treatment with LPS robustly induced the acetylation of NF-κB at Lys-122. However, treatment with C-30-27 substantially reduced the level of NF-κB acetylation at Lys-122. Moreover, the levels of NF-κB, IL-1β and iNOS were consistently decreased by treatment with C-30-27 (Fig. 2C). Collectively, we selected C-30-27 as a potent PCAF inhibitor for the suppression of the NF-κB-mediated inflammatory response.

The *PCAF inhibitor, C-30-27, prevents amyloid β-induced neuronal cell death and suppresses cytokine production.* We previously demonstrated that treatment with Aβ effectively induced the activation of NF-κB by increasing the acetylation of NF-κB at Lys-122, leading to the upregulation of cytokines (11,16). Therefore, we then evaluated the inhibitory effects of C-30-27 against cytotoxicity and cytokine production induced by Aβ (Fig. 3A). First, we examined the effects of C-30-27 on the viability of BV-2 microglial cells and Neuro-2A neuroblastoma cells by MTT assay. Treatment with C-30-27 (0-100 µM) did not affect the viability of either cell line (Fig. 3A, left panel). However, the high concentration of C-30-27 (100 µM) was slightly toxic to both the BV-2 and Neuro-2A cells, suggesting that an appropriate concentration for further testing is below 50 µM. We then examined the effects of
Aβ-induced microglial activation on neuronal cell survival. For this experiment, Aβ1-42-treated conditioned medium (Aβ-CM) from the Aβ-treated BV-2 microglial cells was applied to the Neuro-2A cells, and cell viability was determined by MTT assay. C-30-27 suppressed the Aβ-induced Neuro-2A cell death in a dose-dependent manner without affecting the survival of the BV-2 cells, demonstrating the neuroprotective effects of C-30-27 (Fig. 3A, right panel).

As activated microglia-derived cytokines are responsible for neuronal cell death, we then examined whether C-30-27 suppresses cytokine production following Aβ-induced microglial activation. Upon treatment with Aβ, cytokine production in both the BV-2 cells and Aβ-CM-treated Neuro-2A cells was substantially increased (Fig. 3B). As expected, treatment with C-30-27 markedly suppressed the expression of pro-inflammatory cytokines in a dose-dependent manner. These results indicated that C-30-27 prevented Aβ-induced neuronal cell death and cytokine production in the BV-2 and Neuro-2A cells.

Effects of C-30-27 on the Aβ1-40-induced impairment of memory acquisition and retention. To evaluate the in vivo efficacy of C-30-27 as an inhibitor of neuroinflammation, we carried out several in vivo experiments (Fig. 4A). The effects of C-30-27 on spatial memory were investigated using the Morris water maze test in trials 1-4 on day 4 (Fig. 4B). The escape latencies in the Aβ-treated group were significantly delayed compared to the vehicle-treated control group and showed similar patterns during all 4 days, suggesting that treatment with Aβ effectively induced memory impairment. As expected, donepezil, a palliative medicine for the treatment of AD, improved the spatial memory impairment induced by Aβ. The escape latencies in the C-30-27 low-dose group (27-L) and the C-30-27 high-dose group (27-H) were significantly shorter than the escape latency in the Aβ-treated group in the final trial on day 4 (Fig. 4C).

We also performed spatial probe trials to further evaluate the effects of C-30-27, and the results are depicted in Fig. 5A. The time spent in the target quadrant (where the platform was previously placed) was significantly decreased in the Aβ-treated group compared to the control group. However, the time spent in the target quadrant following treatment with C-30-27 was significantly increased. Finally, we evaluated the effects of treatment with C-30-27 on retention latency using the passive avoidance test; the results are depicted in Fig. 5B. In the Aβ-treated group, the retention latencies were significantly
decreased in comparison with the control group. However, the decrease in retention latency resulting from Aβ treatment was alleviated by treatment with donepezil and treatment with the higher dose of C-30-27.

**Effects of the PCAF inhibitor, C-30-27, on Aβ-induced behavioral deficits.** The effects of C-30-27 on the behavioral deficits induced by Aβ were determined using the rotarod and vertical pole tests. In the rotarod test, treatment with Aβ led to a significant decrease in time spent on the rotarod compared to the controls, and treatment with C-30-27 increased the time spent on the rotarod, suggesting that C-30-27 may be effective for the partial recovery of balance and coordination (Fig. 5C). Through the vertical pole test, the times for grip strength and sensorimotor performance were measured. As a result of this test, we found that both the times for grip strength and sensorimotor performance were markedly lower in the animals treated with Aβ; however, treatment with C-30-27 markedly improved the time spent on the vertical pole (Fig. 5D).

**Effects of C-30-27 PCAF inhibitor on AChE activity and ACh content in Aβ-injected rats.** AD is accompanied by synaptic dysfunction in the cholinergic system. Increased AChE activity and a significant loss in the ACh content correlate with cognitive impairments. In addition, it has been shown that the cholinergic system in the brain, which includes ACh receptors, ACh and AChE, is closely related to the condition of microglial cells in the brain (20). Therefore, we measured ACh and AChE activity in the Aβ- and C-30-27-treated rats.

Following treatment with Aβ, the ACh content was decreased, but AChE activity was significantly elevated in the Aβ-treated group (Fig. 6). Importantly, treatment with C-30-27 increased the ACh content, leading to the attenuation of AChE activity in the brains of Aβ-treated rats. Therefore, these results suggest that the blocking of neuroinflammation by PCAF inhibition ameliorates Aβ-induced cognitive dysfunction, at least in part, which leads to the improvement of function of the cholinergic system.

**Discussion**

The present study was conducted to elucidate the biological effects of C-30-27, an inhibitor of acetylation-dependent NF-kB activation, on microglial-mediated neuroinflammation and the restorative effects on Aβ-induced cognitive dysfunction. NF-kB activation is a central event in inflammation and a common feature of a number of neurodegenerative diseases (21). In
PARK et al: SELECTIVE PCAF INHIBITOR SUPPRESSES NF-κB-MEDIATED NEUROINFLAMMATION

the brains of patients with AD, NF-κB activation is observed predominantly in neurons and glial cells (22,23). Furthermore, Aβ has also been shown to activate NF-κB, which leads to increased cytokine production in neurons and glial cells (24). Therefore, the inhibition of NF-κB activation may be a useful method of blocking Aβ-induced neuroinflammation in patients with AD.

As previously demonstrated, HAT enzyme-mediated NF-κB acetylation is required for the nuclear translocation and subsequent activation of NF-κB signaling. Thus, HAT-mediated NF-κB acetylation is believed to be a critical step in the NF-κB-mediated inflammatory response, which is presumably correlated with the development of a number of pathological states, particularly those involving acute inflammation, such as...
Furthermore, there is evidence from animal studies that cholinergic system causes neuroinflammation and ACh can activation and local inflammatory processes are known to play has the capacity to improve the function of the cholinergic nism to improve cognitive deficits. 

In conclusion, the results from this study suggest that the selective inhibition of NF-κB acetylation by the PCAF inhibit or, C-30-27, relieves the inflammatory response in LPS- and Aβ-treated BV2-cells and prevents cognitive deficits associated with Aβ treatment. Therefore, this approach has potential for use as a therapeutic strategy in patients with AD.

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