Selective inhibition of PCAF suppresses microglial-mediated β-amyloid neurotoxicity

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Abstract. Recent studies have emphasized the functional role of the P300/CBP-associated factor (PCAF) enzyme in resistance to β-amyloid (Aβ)-mediated neurotoxicity; however, the underlying mechanisms through which PCAF regulates inflammation and neurotoxicity have not yet been elucidated. In this study, we used computer-based molecular docking simulations to perform structure-based artificial screening for PCAF-specific inhibitors. Our results revealed that one of the compounds from the screened library, compound C-11, selectively inhibited PCAF, but not p300 or GCN5, with a half-maximal inhibitory concentration (IC_{50}) of approximately 0.25 μM. Furthermore, C-11 had no effects on the activities of other epigenetic enzymes. Western blot analysis using an antibody against acetyl-nuclear factor-κB (NF-κB) demonstrated that PCAF mediated the Aβ-induced activation of NF-κB by acetylation at Lys-122. We also found that the knockdown of PCAF completely inhibited Aβ-induced cytokine production in BV-2 cells in a similar manner to C-11 treatment. Finally, PCAF inhibition suppressed both Aβ-induced cytokine production and Aβ-mediated neuronal cell death. Therefore, our results suggest that in neuronal cells, PCAF is a promising therapeutic target for alleviating the inflammatory progression of Alzheimer's disease.

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Introduction

Chronic inflammation is associated with the pathogenesis of several human diseases, including cancer and neurodegenerative diseases (1,2). Nuclear factor-κB (NF-κB) is the most well-known regulator of inflammatory signaling (3), and activated NF-κB is commonly observed in inflammatory lesions during pathogenesis (4). Evidence to date demonstrates that the functions of NF-κB are modulated by diverse post-transcriptional modifications (5). Among these modifications, acetylation has been shown to enhance the nuclear localization of NF-κB and lead to the transcription of NF-κB target genes (6,7). NF-κB is mainly acetylated 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, activating its transcriptional activity (7). On the other hand, PCAF specifically acetylates only Lys-122 of NF-κB (9). The difference between acetylation by PCAF and p300 on the regulation of NF-κB function, however, is largely unknown. Conversely, the blocking of NF-κB acetylation by histone acetyl transferase (HAT) inhibitors diminishes the nuclear retention and transcriptional activity of NF-κB (10-12). Thus, the pharmacological inhibition of HAT activity may be a useful method for the treatment of chronic inflammation.

Neuroinflammation is a hallmark of neurodegenerative diseases that is linked to glial cell activation (13-15). The abnormal activation of microglia promotes neuronal injury through the release of pro-inflammatory and cytotoxic factors, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), nitric oxide and reactive oxygen species that contribute to localized and more widespread central nervous system injuries (16-18). NF-κB is also involved in neuroinflammation and neuronal cell death induced by β-amyloid (Aβ) (19-21). Consistent with this, the immunoreactivity of the

RelA (p65) subunit of NF-κB in the brains of patients with Alzheimer's disease is stronger in the neurons, astrocytes and microglial cells surrounding amyloid plaques (22). In a recent study, we reported the inhibitory effects of gallic acid, a HAT inhibitor, on NF-κB activity and the activation of microglial inflammation (10). In addition, we have previously shown that gallic acid inhibits multiple HAT enzymes, including p300 and PCAF (23). Of note, it has recently been demonstrated that PCAF knockout mice develop resistance to amyloid toxicity and have altered memory capacities, suggesting the importance of the PCAF enzyme in the development of neurodegenerative diseases (24,25). However, the detailed mechanisms through which PCAF promotes Aβ-induced microglial inflammation and neurotoxicity remain unclear.

In this study, we used computer-based molecular docking simulations to screen for PCAF-specific inhibitors. We demonstrate that the inhibition of PCAF abrogates lipopolysaccharide (LPS)-induced NF-κB activation and the acetylation of NF-κB at Lys-122. Finally, we used co-culture analysis, demonstrating that a PCAF inhibitor efficiently prevented neuronal cell death caused by Aβ-induced neurotoxicity. Collectively, our results demonstrate that the selective inhibition of NF-κB acetylation by a PCAF inhibitor shows promise for the treatment of neurodegenerative diseases.

Materials and methods

Molecular docking analysis and chemicals. Structure-based artificial screening with computer-based molecular docking simulations was conducted by InnoPharmaScreen Inc. (Asan, Korea). The X-ray crystal structure of the HAT domain of human PCAF bound to coenzyme A (CoA; Protein Data Bank ID: 1CM0) was used for the initial structure of the target protein. CoA was removed and atomic hydrogens were added to produce a complete molecular model of the target protein for use in the molecular docking simulations. Docking simulations were performed between the target model and the ChemBridge chemical library that consists of 450,000 unique compounds. Binding efficiency was based on the binding force between the target molecule and the test compound as calculated by the Gold 4.0.1 software package (Cambridge Crystallographic Data Centre, Cambridge, UK). The top 10 docking positions for each compound were calculated, and the library search efficiency was 100%.

Cell culture, reagents and antibodies. Murine BV-2 cells and Neuro-2A cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA; CRL no. 2270). Fetal bovine serum (FBS), trypsin-EDTA, penicillin and 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 (St. Louis, MO, USA). $A\beta_{1-42}$ was purchased from Bachem (Bubendorf, Switzerland). Other chemicals used were purchased from Sigma-Aldrich. BV-2 cells were cultured in Dulbecco's modified Eagle's medium (Gibco-BRL) containing 5% heat-inactivated endotoxin-free FBS, 2 mM glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin in a humidified 5% CO_2 atmosphere at 37˚C. Neuro-2A cells were cultured in modified Eagle's medium (Gibco-BRL) containing 10% heat-inactivated endotoxin-free FBS, 2 mM glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin in a humidified 5% CO_2 atmosphere at 37˚C. Aβ peptides were dissolved in phosphate-buffered saline (PBS) and pre-incubated at 37˚C for 5 days to allow for fibril formation. The peptides were stored at -20˚C until use. Compound C-11 was dissolved in dimethyl sulfoxide (DMSO) and later diluted with distilled water (the final concentration of DMSO was <0.5%). For the co-culture experiments, BV-2 cells were treated with various concentrations of C-11 (0.1-1.0 μM final concentration) for 12 h prior to stimulation with 5 μM aggregated $A\beta_{1-42}$ (the Aβ + C-11 group), 5 μM non-aggregated $A\beta_{1-42}$, or medium only (control) for 24 h. Conditioned medium (CM) from the BV-2 cells and primary microglial 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 the control. Following incubation, cell viability was measured by MTT assay and western blot analysis was performed.

HAT, histone deacetylase (HDAC), histone methyltransferase (HMT) and sirtuin 1 (SIRT1) activity assays. HeLa cell nuclear extract was prepared as previously described (26). HAT and HDAC activity assays were performed with nuclear extracts using commercially available kits according to the manufacturer's instructions (BioVision, Inc., Milpitas, CA, USA). For *in vitro* HAT activity assays, recombinant HAT proteins were incubated with HAT assay buffer [50 mM HEPES pH 8.0, 10% glycerol, 1 mM DTT, 1 mM phenylmethanesulfonyl fluoride (PMSF), 10 mM sodium butyrate and 1 μl [^{3}H]-acetyl-CoA], 5 μg histone H4 tail peptides, and/or indicated concentration of inhibitors at 30˚C for 1 h. Samples were separated on a 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and analyzed by autoradiography. HMT activity assays were performed using the assay buffer and core histones from the HMT Assay Reagent kit (Upstate Biotechnology, Inc., Lake Placid, NY, USA) according to the manufacturer's instructions. Core histones were incubated for 1 h at 30˚C in methyltransferase buffer (final concentration 50 mM Tris pH 8.0, 1 mM PMSF, and 0.5 mM DTT), 1 μl (0.55 μCi) S-adenosyl-L-[methyl-^{3}H]-methionine (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA) and 10 μg/ml HeLa nuclear extract. The total volume of the reaction mixture was 30 μl. The reaction was spotted on P-81 paper for scintillation counting. The P-81 paper was washed 3 times with 10% trichloroacetic acid (Upstate Biotechnology, Inc.) for 15 min, washed with 95% ethanol for 5 min at room temperature and allowed to dry. Dry P-81 papers were counted with a multi-purpose LS 6500 scintillation counter (Beckman Coulter, Indianapolis, IN, USA). SIRT1 activity was assayed using a SIRT1/Sir2 Deacetylase Fluorometric assay kit (CycLex Co., Ltd., Nagano, Japan) according to the manufacturer's instructions.

Lentiviral shRNAs. For silencing PCAF expression, 2 pairs of oligonucleotides that encoded PCAF-specific shRNA were purchased from MISSION shRNA (Sigma-Aldrich). Lentiviral particles were prepared using pLKO.1-PURO PCAF shRNA via a 3 plasmid co-transfection according to the instructions of the manufacturer (Invitrogen, Carlsbad, CA, USA). The BV-2 cells were transfected with the lentivirus. After 3 days of incubation, the lentivirus from the culture medium was collected

and concentrated in a Centricon-plus-20 centrifugal filter device (Millipore, Billerica, MA, USA). Lentivirus PURO shRNA was generated as the control.

Cell viability assay (MTT assay). Cell viability was measured to determine the cytotoxicity of the Aβ peptides on neuronal cells. Neuro-2A cells were seeded at $1x10^4$ to $1x10^5$ cells in a 96-well plate. Following 12 h of incubation, the medium was replaced with CM from the BV-2 cell cultures treated with or without Aβ and different concentrations of C-11. After 24 h, the CM was replaced with non-CM, 15 μl MTT solution (2 mg/ml final concentration) was added for 90 min at 37˚C, and the absorbance was recorded at 570 nm. A reference was recorded at 630 nm using a microplate reader (Model 550; Bio-Rad, Hercules, CA, USA).

Western blot analysis. The treated cells were washed with cold PBS, scraped off the culture dishes and harvested. The cells were incubated for 20 min in lysis buffer containing 0.5% Triton X-100, 20 mM HEPES (pH 7.4), 150 mM NaCl, 2 mM DTT and 1 mM PMSF. The lysates were centrifuged at 20,000 x g for 10 min at 4˚C. The protein concentrations in the clarified lysates were determined with the Bradford assay using bovine serum albumin as a reference. Total cell lysate proteins were separated on 8 or 12% SDS-PAGE gels and transferred onto nitrocellulose membranes. The membranes were blocked by incubating 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 (1:1,000 dilution), COX-2 (1:1,000), IL-1β (1:500), NF-κB (p65; 1:500) or β-actin (1:5,000). The antibody against acetyl-NF-κB (K122) was raised against the synthetic peptide, CIHSFQNLGIQCV_{AC}KKRDLE, by GenScript (Piscataway, NJ, USA). After washing 3 times with PBS and 0.1% Tween-20, the membranes were incubated with secondary horseradish peroxidase-conjugated antibody (1:1,000) for 1 h. The bands were detected using the enhanced chemiluminescence system (Amersham Pharmacia Biotech, Inc.) according to the manufacturer's instructions.

RNA extraction and real-time PCR analysis. Total RNA from the Neuro-2A, BV-2 and primary microglial cells was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The mRNA levels of iNOS, COX-2 and IL-1β were determined by real-time PCR (ABI PRISM 500 Sequence Detection System, Applied Biosystems, San Jose, CA, USA). cDNA amplification was performed in duplicate in 20-μl reaction mixtures containing 2X SYBR-Green Master Mix (Roche, Indianapolis, IN, USA) and 10 pM forward and reverse primers. The initial denaturation step was 5 min at 95˚C followed by 40 amplification cycles of 30 sec at 95˚C, 30 sec at 58˚C and 30 sec at 72˚C with a final 10 min extension at 72˚C. The results were analyzed using ABI sequence detector software version 2.3. Relative mRNA expression of the target genes was calculated after normalizing to GAPDH expression and was expressed as the fold induction. The primers used for amplification were as follows: human IL-1β gene forward, 5'-GTTGACGGACCCCAAAAGAT-3' and reverse, 5'-AAGG TCCCACGGGAAAGACAC-3'; human COX-2 gene forward, 5'-GAGTGGGAGGCACTTGCATT-3' and reverse, 5'-TGGA GGCGAAGTGGGTTTTA-3'; human iNOS gene forward, 5'-TCTTGGAGCGAGTTGTGGAT-3' and reverse, 5'-GGGT GGTAATGTCCAGGAAGT-3'; and human GAPDH gene forward, 5'-GTGTTCCTACCCCCAATGTGT-3' and reverse, 5'-AGGAGACAACCTGGTCCTCAGT-3'. All reactions were performed in triplicate. Relative expression levels and standard deviations (SDs) were calculated using the comparative value method.

Table I. Gold fitness score for each compound.

No.	Score	No.	Score	No.	Score	No.	Score
C-1	85.55	C-26	73.43	C-51	72.12	C-76	70.86
C-2	84.11	C-27	73.42	C-52	71.94	C-77	70.81
C-3	81.48	C-28	73.33	C-53	71.89	C-78	70.77
C-4	81.00	C-29	73.29	C-54	71.87	C-79	70.65
C-5	80.75	C-30	73.18	C-55	71.76	C-80	70.58
C-6	80.58	C-31	73.14	C-56	71.69	C-81	70.53
C-7	80.21	C-32	73.10	C-57	71.59	C-82	70.42
C-8	77.79	C-33	73.05	C-58	71.51	C-83	70.40
C-9	77.56	C-34	72.97	C-59	71.51	C-84	70.39
C-10	77.45	C-35	72.95	C-60	71.38	C-85	70.32
C-11	76.83	C-36	72.92	C-61	71.37	C-86	70.28
C-12	76.35	C-37	72.90	C-62	71.36	C-87	70.28
C-13	76.27	C-38	72.84	C-63	71.36	C-88	70.20
C-14	75.87	C-39	72.83	C-64	71.36	C-89	70.20
C-15	75.42	C-40	72.80	C-65	71.33	C-90	70.19
C-16	74.93	C-41	72.64	C-66	71.30	C-91	70.16
C-17	74.73	C-42	72.55	C-67	71.28	C-92	70.15
C-18	74.33	C-43	72.46	C-68	71.15	C-93	70.07
C-19	74.20	C-44	72.42	C-69	71.09	C-94	70.04
C-20	74.10	C-45	72.37	C-70	71.06	C-95	69.98
C-21	74.04	C-46	72.36	C-71	71.03	C-96	69.94
C-22	73.91	C-47	72.30	C-72	71.00	C-97	69.94
C-23	73.71	C-48	72.21	C-73	71.00	C-98	69.93
C-24	73.62	C-49	72.19	C-74	70.98	C-99	69.92
C-25	73.47	C-50	72.16	C-75	70.86	C-100	69.84

Results

Structure-based artificial screening of PCAF-specific inhibitors. To develop PCAF-specific inhibitors, we performed structure-based artificial screening using computer-based molecular docking simulations (Fig. 1A). We initially selected 50 compounds with a binding force of at least 70 after the molecular docking simulation (Table I). Among these, compound number 11 (C-11) displayed the most significant inhibitory effect against the PCAF enzyme (Fig. 1B). Furthermore, C-11 selectively inhibited PCAF, but not p300 and GCN5 (Fig. 1C). Thus, we selected C-11 as the putative PCAF inhibitor for our experiments.

To examine the enzyme specificity of C-11, we examined whether C-11 inhibits other epigenetic enzymes, including HDAC, HMT and SIRT1. As shown in Fig. 2A, C-11 failed to

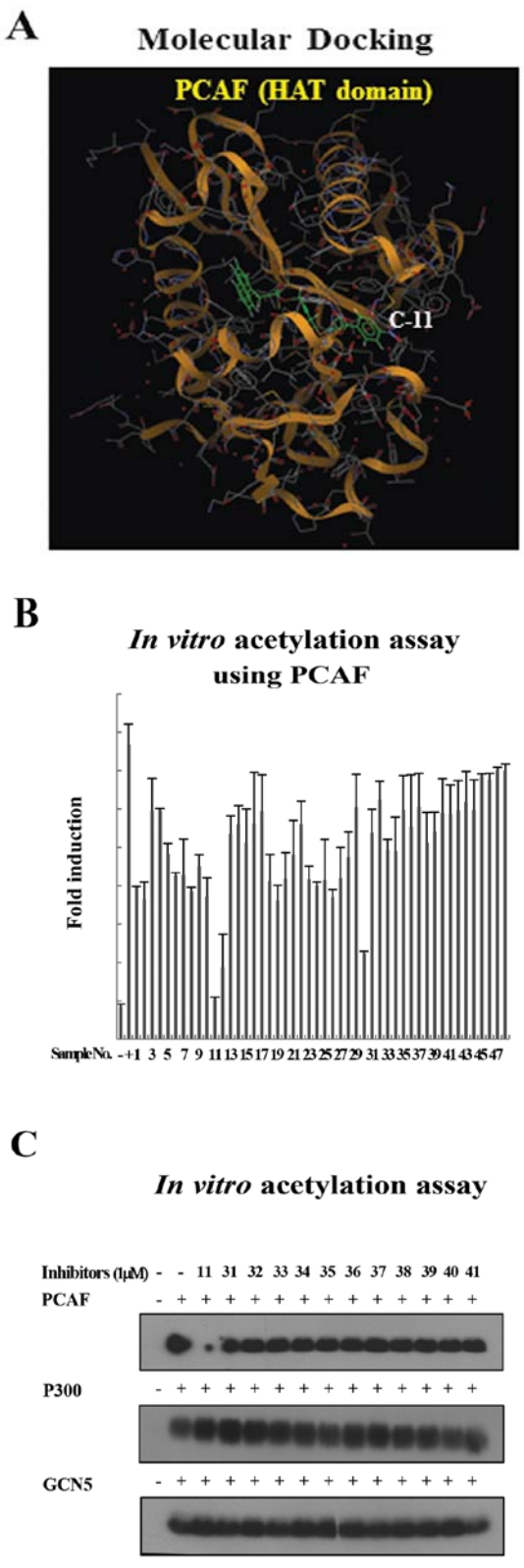

Figure 1. Identification of P300/CBP-associated factor (PCAF) inhibitors by structure-based virtual screening. (A) Molecular docking analysis was performed between the 450,000 compounds of the ChemBridge chemical library and the molecular model of PCAF. The Gold 4.0.1 software package was used to score the binding forces between molecules. (B) C-11 possesses the highest anti-PCAF activity among the putative PCAF inhibitors. Histone acetyl transferase (HAT) activity was assayed with a colorimetric assay kit with recombinant PCAF proteins as the source of HAT enzymes. The results are shown as the means ± SD of 3 independent experiments. (C) C-11 selectively inhibits PCAF activity. *In vitro* HAT activity assays were performed with the indicated HAT enzymes in the presence of chemical compounds (1 μM) and a synthetic histone 4 tail peptide.

inhibit the enzyme activities of HDAC and SIRT1. Furthermore, C-11 had no effect on HMT activity (Fig. 2B). These results demonstrate that C-11 specifically inhibits PCAF acetyltransferase activity but not the activity of other epigenetic enzymes. As several HAT inhibitors (HATi) have recently been shown to possess anti-PCAF activity (12,23,27), we compared the relative anti-PCAF activity of C-11 with other HATi. As shown in Fig. 2C, C-11 had the highest anti-PCAF activity among the reported HATi with a half-maximal inhibitory concentration of approximately 0.25 μM (Fig. 2C and D).

Inhibition of PCAF suppresses Aβ-induced NF-κB acetylation at Lys-122. Since PCAF was found to activate NF-κB-mediated transcription in response to cytokines (6), we first examined whether the inhibition of PCAF by C-11 suppresses NF-κB activity by inhibiting the acetylation of NF-κB. Since PCAF is known to acetylate NF-κB at Lys-122 (6), we first generated an acetyl-specific NF-κB antibody that recognizes acetylated NF-κB at Lys-122. Polyclonal antibodies were generated against the NF-κB acetyl-peptide, ^{109}CIHSFQNLGIQCV$_{AC}$KKRDLE^{127}, and the antisera were examined by western blot analysis following *in vitro* acetylation reactions with recombinant PCAF enzyme and GST-p65 substrate (Fig. 3A, upper panel). A peptide competition assay clearly demonstrated the specificity of the NF-κB Lys(Ac)-122 antibody, as the complete blocking of NF-κB acetylation was observed by the antibody raised against the acetyl-peptide, but not by an antibody raised against a non-acetyl-peptide (Fig. 3A, lower panel). In addition, non-acetyl-NF-κB antibody failed to detect the acetylation that resulted from an *in vitro* HAT reaction and thus confirmed the specificity of the acetyl-specific NF-κB antibody for the PCAF-mediated acetylation of NF-κB at Lys-122 (Fig. 3A, lower panel).

We then examined whether Aβ treatment induces NF-κB activation through the acetylation of NF-κB at Lys-122. Aβ treatment efficiently induced the activation of NF-κB, and this was inhibited by the knockdown of PCAF (Fig. 3B and C). Importantly, Aβ treatment also increased the acetylation of NF-κB at Lys-122, which was verified by 2 antibodies that recognize acetylated NF-κB at Lys-122 (Fig. 3D). The acetylation of NF-κB at Lys-122 was specifically mediated by PCAF, as shRNA against PCAF, but not control shRNA, abrogated the Aβ-induced acetylation of NF-κB. Collectively, these data suggest that PCAF mediates the Aβ-induced activation of NF-κB through the acetylation of NF-κB at Lys-122.

Pharmacological inhibition of PCAF abrogates the Aβ-induced activation of NF-κB. We then investigated the inhibitory effects of PCAF on NF-κB activity and cytokine production. We first assessed the effect of knocking down PCAF on cytokine production by BV-2 cells. Consistent the results from our previous study (10), Aβ treatment significantly increased the expression of cytokine genes in BV-2 cells (Fig. 4A). Of note, treatment with shRNA against PCAF markedly diminished the Aβ-induced cytokine production in BV-2 cells when compared with control shRNA (Fig. 4A). Importantly, PCAF inhibition by C-11 markedly reduced the Aβ-induced cytokine production in BV-2 cells. Furthermore, we also observed similar results with LPS treatment (Fig. 4B). These data collectively demonstrate that the inhibition of

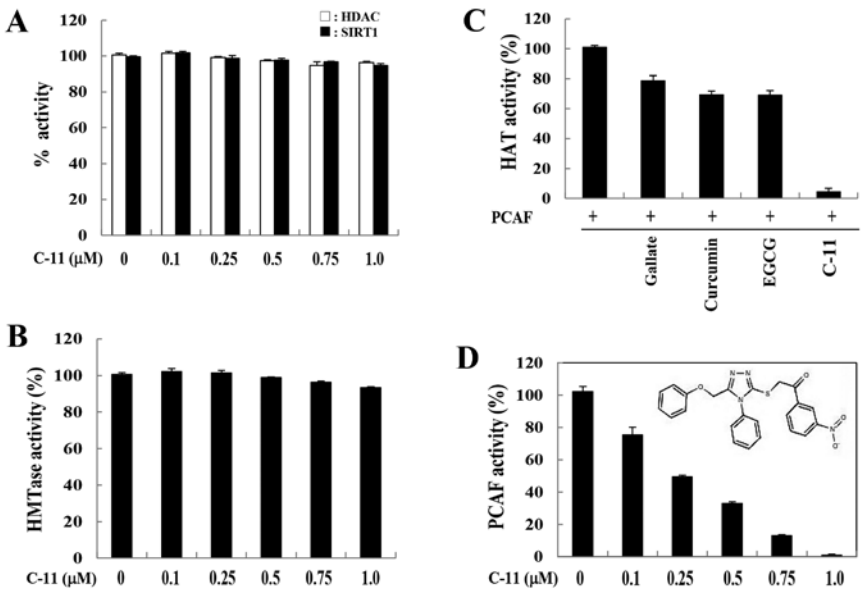

Figure 2. Compound C-11 selectively inhibits P300/CBP-associated factor (PCAF) activity. (A) C-11 treatment had no effect on histone deacetylase (HDAC) activity as assayed with a colorimetric assay kit. Sirtuin 1 (SIRT1) activity was assayed with a SIRT1/Sir2 deacetylase fluorometric assay kit. Deacetylase enzyme activity was assessed by measuring the change in fluorescence intensity. The results represent the means ± SD of 3 independent experiments. (B) Histone methyltransferase (HMT) filter-binding assays were performed in 30-μl reaction volumes in the presence or absence of C-11 with HeLa cell nuclear extracts as the enzyme source. (C) C-11 has potent anti-PCAF activity. PCAF activity was assayed with a histone acetyl transferase (HAT) activity colorimetric assay kit with recombinant PCAF as the source of HAT enzymes. The results represent the means ± SD of 3 independent experiments. (D) C-11 inhibits PCAF activity in a dose-dependent manner. PCAF activity was assessed with the indicated concentrations of C-11.

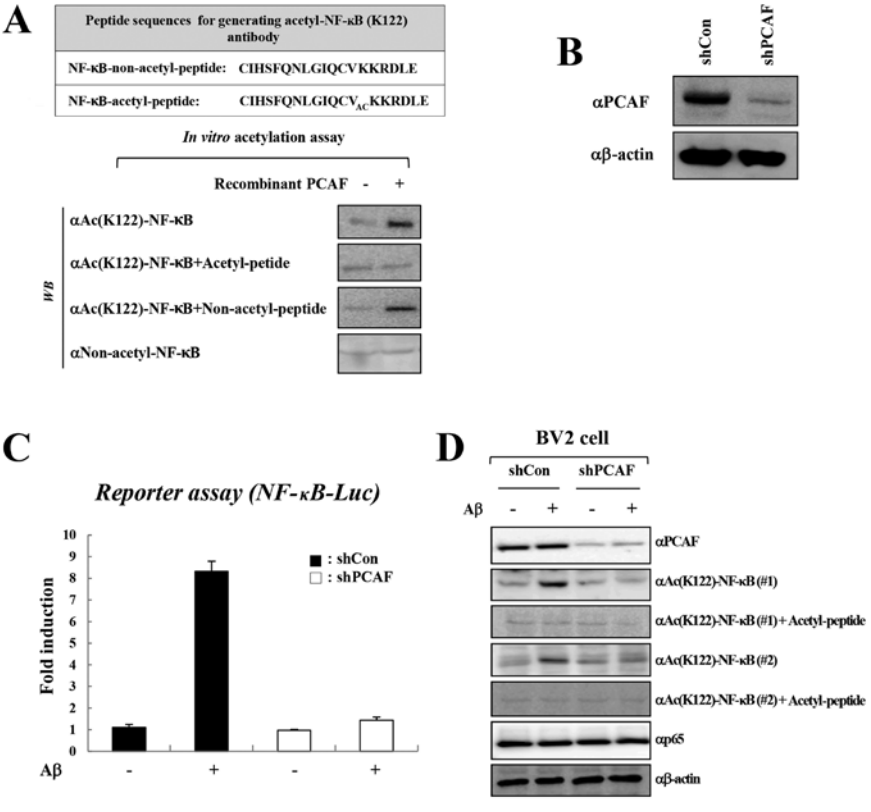

Figure 3. C-11 inhibits the P300/CBP-associated factor (PCAF)-mediated acetylation of NF-κB on Lys-122. (A) Peptide sequences for the generation of the acetylation-specific NF-κB antibody. *In vitro* acetylation assays were performed with recombinant PCAF enzyme and the indicated GST-p65 proteins. Western blot analysis was performed with acetyl-NF-κB (K122) antibodies and/or 1 μg/ml of non-acetyl-peptide or acetyl-peptide. (B) Validation of the effect of shRNA against PCAF. Western blot analysis was performed with the indicated antibodies. BV-2 cells were transfected with lentiviral shRNA against PCAF, and lentivirus PURO shRNA was generated as the control. (C) Knockdown of PCAF reduced Aβ-induced NF-κB activity in BV-2 cells. BV-2 cells were transfected with an NF-κB binding site-driven luciferase reporter plasmid and treated with 1 μM Aβ. Whole cell extracts were used in the luciferase assay, and the results are presented as the means of 2 independent experiments performed in triplicate. (D) Knockdown of PCAF prevented Aβ-induced p65 acetylation at Lys-122 *in vivo*. BV-2 cells were treated with lentiviral shRNA against PCAF and/or Aβ. The level of p65 acetylation was assayed by western blot analysis using an antibody against acetylated p65 at Lys-122.

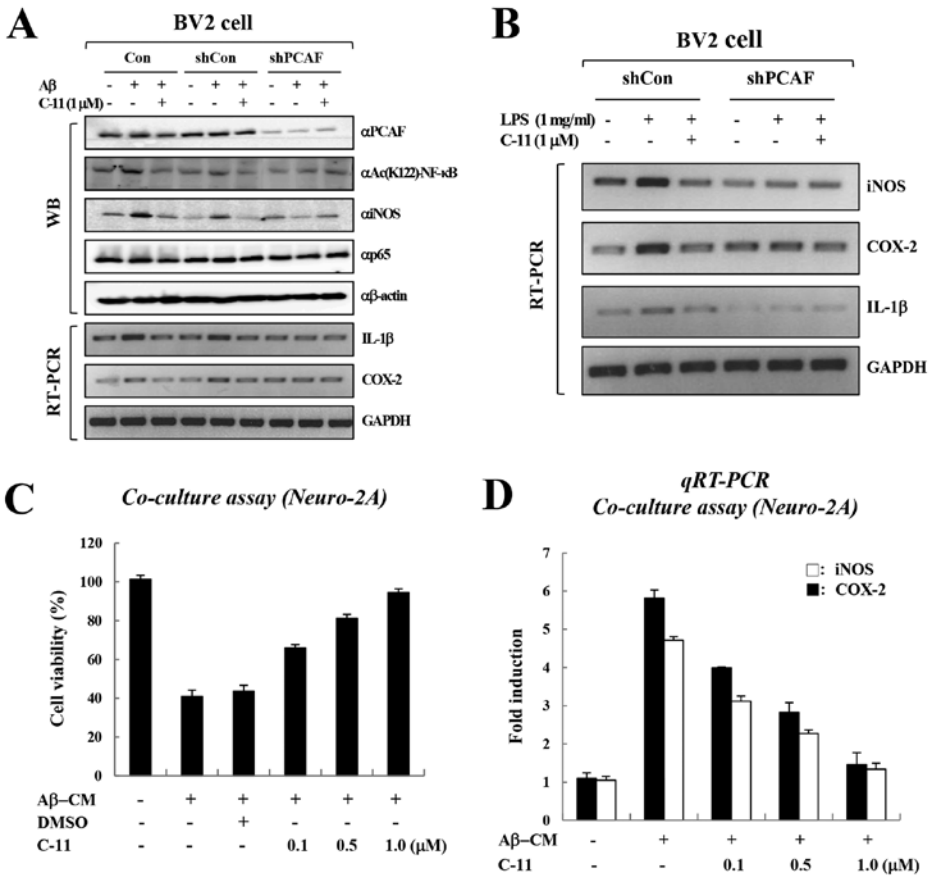

Figure 4. Selective inhibition of P300/CBP-associated factor (PCAF) abolishes microglial-mediated cytokine production and Aβ-mediated neurotoxicity. (A) PCAF inhibition suppresses the Aβ-induced expression of cytokine genes. BV-2 cells were transfected with shRNA against PCAF and after 48 h the cells were treated with Aβ and/or C-11. The protein levels were assayed by western blot analysis (WB) using the indicated antibodies. (B) PCAF inhibition prevented LPS-induced cytokine production. Total RNA was prepared from each sample and used in RT-PCR reactions to measure the expression of the NF-κB target genes. (C) Treatment with a PCAF inhibitor prevented Aβ-induced neuronal cell death. The conditioned medium from BV-2 microglial cells was transferred to the Neuro-2A cells, and cell viability was assessed with the MTT assay. (D) Effect of a PCAF inhibitor on the expression of cytokines in Neuro-2A cells was analyzed with real-time PCR. Columns represent the averages of 3 independent experiments and error bars represent the standard deviations.

PCAF abolishes NF-κB-mediated cytokine production by blocking the acetylation of NF-κB at Lys-122.

Inhibition of PCAF prevents Aβ-induced neuronal cell death. Since PCAF inhibition suppresses the expression of pro-inflammatory cytokines, we examined the neuroprotective effects of PCAF inhibition on BV-2 microglial-mediated Aβ neurotoxicity. Consistent with the results from our previous study showing the dose-dependent cytotoxicity of gallic acid in the same system (10), treatment with Aβ-conditioned medium (CM) from BV-2 microglial cells reduced the viability of Neuro-2A cells, and C-11 reversed the Aβ-CM-mediated neuronal cell death in a dose-dependent manner (Fig. 4C). Real-time PCR demonstrated that C-11 consistently suppressed the Aβ-CM-induced cytokine production in Neuro-2A cells (Fig. 4D). Collectively, these data demonstrate that the inhibition of PCAF by C-11 inhibits NF-κB acetylation, which in turn suppresses Aβ-induced neuroinflammation and Aβ-mediated neurotoxicity.

Discussion

In this study, we demonstrated a role for PCAF in microglial inflammation through acetylation-dependent NF-κB activation, and provide a rationale for the use of PCAF as a promising therapeutic target for the treatment of Alzheimer's disease. Previous studies have shown that PCAF acetylates NF-κB at Lys-122 in response to cytokine signaling and that this in turn, leads to increased NF-κB activity (8). However, due to the unavailability of an acetyl-specific NF-κB antibody, it is still unclear as to whether PCAF indeed acetylates NF-κB at Lys-122. In the current study, we utilized an acetyl (Lys-122)-specific NF-κB antibody, clearly demonstrating that PCAF mediates the Aβ-induced activation of NF-κB through the acetylation of NF-κB at Lys-122. Furthermore, we did not observe any change in the acetylation of NF-κB at Lys-122 after knocking down p300 (data not shown). Thus, our study confirms the specific acetylation of NF-κB at Lys-122 by PCAF.

Accumulating evidence suggests that microglial inflammation by cytokines aggravates Aβ-induced neurotoxicity (28,29). Furthermore, we have previously demonstrated that the inhibition of NF-κB acetylation with a HAT inhibitor, such as gallate significantly reduces Aβ-mediated neuronal cell death through the suppression of inflammatory signaling (10). The pharmacological inhibition of NF-κB acetylation may, therefore, prove useful as a therapeutic intervention in Alzheimer's disease. In this context, a previous studies have demonstrated that PCAF

knockout mice are insensitive to Aβ neurotoxicity (24,25), suggesting a pivotal role for PCAF in the regulation of Aβ-induced neurotoxicity and the memory capacity of the brain. In support of these findings, to our knowledge, in this study, we demonstrate for the first time that the pharmacological inhibition of PCAF prevents Aβ-induced cytokine production and toxicity in neuronal cells. The most promising compound selected by our molecular docking analysis was compound C-11, a PCAF-specific inhibitor. C-11 treatment specifically inhibited the cytokine-induced activation of NF-κB by preventing the acetylation of NF-κB at Lys-122. Importantly, C-11 had no effect on the activity of any of the other epigenetic enzymes examined. More importantly, the knockdown of PCAF with shRNA had a similar effect on cytokine production and the viability of neuronal cells as did C-11 treatment.

In conclusion, our data suggest that the selective inhibition of NF-κB acetylation by PCAF inhibition is a possible therapeutic approach for alleviating the inflammatory progression of Alzheimer's disease.

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