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PDE2A Modulation Attenuates A β -Induced Neuronal Injury and Associated
Oxidative, Inflammatory, and Apoptotic Responses

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Abstract

Background: Alzheimer's disease (AD) involves progressive neuronal injury driven in part by A β -associated oxidative stress, inflammatory activation, and apoptotic signaling. Cyclic nucleotide dysregulation has emerged as a contributing factor in these processes. This study evaluated the neuroprotective effects of BML-288, a phosphodiesterase-2A (PDE2A) inhibitor, in A β -induced injury models.

Methods: HT-22 neuronal cells were exposed to A β_{1-42} with or without BML-288 co-treatment. A hippocampal CA1 A β microinjection mouse model was used to assess in vivo effects. Oxidative stress markers, inflammatory cytokines, apoptosis-related proteins, and cAMP/cGMP levels were measured using biochemical assays and Western blotting. PDE2A overexpression and knockdown were used to further examine the involvement of PDE2A in A β -induced cellular responses. Cognitive performance was evaluated using the Morris water maze, Y-maze, and novel object recognition tests.

Results: A β exposure increased PDE2A expression and reduced intracellular cAMP and cGMP levels in HT-22 cells, whereas BML-288 partially reversed these changes. BML-288 also reduced A β -related increases in ROS and MDA, restored SOD activity, and decreased IL-1 β and TNF- α levels in both cell cultures and hippocampal tissue. Bax and total Caspase-3 expression were increased after A β exposure and attenuated by BML-288, accompanied by modulation of Bcl-2 expression. PDE2A overexpression enhanced oxidative and inflammatory responses, whereas PDE2A knockdown attenuated A β -associated cellular stress. In vivo, BML-288 improved performance across spatial learning and memory tasks.

Conclusions: Across cellular and animal A β injury models, BML-288 attenuated oxidative and inflammatory disturbances, modulated apoptosis-related protein expression, and improved cognitive outcomes. These findings suggest that PDE2A inhibition may help counteract cyclic nucleotide dysregulation associated with A β -induced neuronal stress and support further investigation of PDE2A-related signaling and BML-288 in A β -associated neurotoxicity.

Keywords: Alzheimer's disease; amyloid- β ; phosphodiesterase 2A; BML-288; cAMP/cGMP signaling; neuroprotection

1 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by extracellular deposition of amyloid- β (A β) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau, ultimately leading to synaptic failure and neuronal dysfunction^[1]. Clinically, AD manifests as gradual cognitive decline, memory loss, and impairment of daily living abilities^[2, 3]. Although extensive research has advanced our understanding of AD, available treatments remain largely symptomatic. Elucidating the molecular mechanisms that drive disease progression is therefore essential for developing effective disease-modifying strategies.

The etiology of AD is multifactorial. Current hypotheses emphasize A β toxicity, tau pathology, mitochondrial dysfunction, oxidative stress, and neuroinflammatory pathways, among others^[4]. Growing evidence indicates that oxidative stress is critically involved in the pathogenesis of AD. The substantial oxygen consumption and metabolic activity of the brain increase the vulnerability of neurons to oxidative injury^[5, 6]. In AD, oxidative stress reduces α -secretase activity while enhancing β - and γ -secretase expression and activation, thereby promoting A β generation^[6]. It also facilitates tau phosphorylation and aggregation through lipid peroxidation, impaired antioxidant defenses, and activation of kinases such as p38 MAPK^[7, 8]. These events accelerate A β accumulation, tau pathology, synaptic loss, and neuronal degeneration. Oxidative stress is tightly linked to neuroinflammation: A β -activated microglia and astrocytes release cytokines, chemokines, and reactive oxygen species (ROS), establishing a feed-forward cycle that amplifies neuronal injury^[9-12]. Elevated oxidative and inflammatory mediators in postmortem AD brains further support their involvement in disease progression^[13, 14].

Among the pathways implicated in oxidative and inflammatory regulation, the cGMP/cAMP signaling axis has received growing attention^[15-17]. These second messengers play key roles in neurogenesis, synaptic plasticity, circuit integration,

memory consolidation, emotional regulation, and neuronal survival^[18-29]. Their concentrations are tightly controlled by cyclic nucleotide phosphodiesterases (PDEs), which hydrolyze cAMP and cGMP to maintain signaling homeostasis.

PDE2A is a dual-substrate enzyme capable of degrading cAMP and cGMP with similar affinity and catalytic effectiveness^[30, 31]. By shaping intracellular second messenger dynamics, PDE2A regulates multiple processes relevant to cognition and neuronal resilience. Emerging evidence suggests that PDE2A expression is elevated in various pathological conditions, leading to blunted cyclic nucleotide signaling and impaired neural function; conversely, PDE2A knockdown has been shown to improve cognitive performance in preclinical models^[32-34]. BML-288 is a selective PDE2 inhibitor with an IC₅₀ of approximately 40 nM, but its potential neuroprotective effects in AD-related contexts remain largely unexplored. The present study therefore aimed primarily to determine whether BML-288 could attenuate A β ₁₋₄₂-induced neuronal injury and cognitive impairment in cellular and mouse models. We further investigated the molecular changes associated with these protective effects, focusing on cAMP/cGMP signaling, oxidative stress, neuroinflammation, apoptosis, and mitochondrial function. In addition, PDE2A overexpression and knockdown were used to further examine the involvement of PDE2A in A β -induced cellular injury.

2 Materials and Methods

2.1 Chemicals and materials

BML-288 and amyloid- β ₁₋₄₂ (A β ₁₋₄₂) were purchased from Tribioscience (Sunnyvale, CA, USA). BML-288 and A β ₁₋₄₂ were separately dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions and were subsequently diluted in phosphate-buffered saline (PBS) to the required working concentrations. Freshly prepared A β ₁₋₄₂ working solutions were used without an additional oligomerization incubation step. All final working solutions contained less than 0.1% DMSO. Corresponding vehicle solutions contained PBS with the same final concentration of DMSO as that used in the respective treatment solutions. Adenoviral vectors for PDE2A overexpression (PDE2A-OE) and knockdown (PDE2A-KD) were constructed and obtained from VectorBuilder (Chicago, IL, USA).

2.2 Cell Culture and Amyloid-Induced Injury

HT-22 mouse hippocampal neuronal cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, high glucose; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37 °C in a humidified incubator containing 5% CO₂. Cells were seeded into 96-well plates at a density of 1×10^4 cells/well and allowed to adhere overnight. A β -induced neuronal injury was established by exposing cells to A β _{1–42} at final concentrations of 0.5, 1, 3, or 6 μ M, each prepared in complete DMEM containing 10% FBS, for 24 h. Based on the dose–response assay, 6 μ M A β for 24 h was selected as the optimal injury condition for subsequent experiments. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) and Calcein-AM/Propidium Iodide (PI) live/dead staining (Elabscience, Wuhan, China) according to the manufacturers' instructions.

2.3 BML-288 intervention in A β -injured HT-22 cells

HT-22 cells were seeded into 96-well plates at a density of 1×10^4 cells/well and allowed to adhere overnight. To evaluate the dose-dependent neuroprotective effects of BML-288, cells were co-treated with 6 μ M A β _{1–42} and BML-288 at final concentrations of 1, 10, 20, or 40 nM for 24 h. Cell viability following BML-288 treatment was assessed using the CCK assay. Based on this dose–response analysis, 10 nM BML-288 was selected as the optimal working concentration for subsequent experiments.

2.4 Experimental groups for HT-22 cell assays

HT-22 cells were randomly assigned to four groups: (1) Control, receiving no treatment; (2) A β , treated with 6 μ M A β ; (3) A β + BML-L, treated with 6 μ M A β and 1 nM BML-288; and (4) A β + BML, treated with 6 μ M A β and 10 nM BML-288. All treatments followed the procedures described in Sections 2.2 and 2.3.

2.5 Protein extraction from HT-22 cells

Following the indicated treatments, cells grown in 6-well plates were washed twice with ice-cold PBS. Ice-cold RIPA lysis buffer containing protease inhibitors was added directly to each well, and cells were detached using a cell scraper. Lysates

were collected, incubated on ice for 30 min, and briefly sonicated. Samples were centrifuged at $12\,000 \times g$ for 15 min at 4 °C, and the supernatants were collected as total protein extracts and stored at –80 °C until analysis.

2.6 Animals, A β model induction, and BML-288 treatment

Eight-week-old male C57BL/6J mice (18–23 g) were obtained from the institutional animal facility and housed under controlled conditions (22 ± 2 °C; 12-h light/dark cycle) with ad libitum access to food and water. This study is reported in accordance with the ARRIVE guidelines. All animal procedures were approved by the Rutgers University Institutional Animal Care and Use Committee (IACUC; Protocol No. PROTO202100135) and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

A β_{1-42} was administered according to Laursen and Belknap^[35] and Prediger et al^[36], with the injection dose referenced from M. Figueiró et al^[37]. After one week of acclimatization, mice were randomly assigned to four groups: Control, A β model, A β + low-dose BML-288 (0.3 mg/kg), and A β + high-dose BML-288 (1 mg/kg). A β_{1-42} was bilaterally microinjected into the hippocampal CA1 region of mice in the A β model and BML-288 treatment groups. To induce the A β model, mice were anesthetized with isoflurane and placed in a stereotaxic frame. Preemptive analgesia (bupivacaine 2 mg/kg and carprofen 5 mg/kg, s.c.) was administered, and ophthalmic ointment was applied to prevent corneal drying. Following scalp disinfection, A β_{1-42} (1 μ L of 0.9 mg/mL per side) was bilaterally injected into the CA1 region (AP –1.7 mm, ML $\pm$ 0.8 mm, DV –2.0 mm) using a 30-gauge injector connected to a Hamilton syringe driven by an infusion pump at a rate of 0.1 μ L/min; the injector was maintained in place for 5 min to minimize reflux before being withdrawn. One week after A β microinjection, mice in the treatment groups received daily intraperitoneal injections of BML-288 at 0.3 or 1 mg/kg for 7 consecutive days, whereas mice in the Control and A β model groups received equal volumes of the corresponding vehicle (PBS containing the same final concentration of DMSO as the BML-288 treatment solution). Behavioral and biochemical assessments were performed following the completion of the treatment period.

2.7 Behavioral Assessments

Cognitive performance of mice was evaluated one week after completion of BML-288 treatment using the Morris water maze (MWM), Y-maze, and novel object recognition (NOR) tests. All behavioral procedures were conducted during the light phase (09:00–17:00) in a quiet, temperature-controlled room under dim illumination. Animal behavior was recorded and analyzed using the ANY-maze tracking system.

2.7.1 Morris Water Maze

Spatial learning and memory were assessed in a circular pool filled with opaque water maintained at 25 °C. A hidden-platform acquisition phase was conducted over four consecutive days, followed by a probe trial on day five. During acquisition, a submerged escape platform (1.5 cm below the water surface) remained in a fixed quadrant. Mice were released from pseudorandom start locations and given 60 s to locate the platform. Latency to reach the platform was recorded, and animals failing to locate it within 60 s were assigned a latency of 60 s. After each trial, mice were allowed to remain on the platform for 15 s and were then returned to their home cage for a 20 min intertrial interval. For the probe trial, the platform was removed. Each mouse was placed in the quadrant opposite the original platform location and allowed to swim for 60 s. The latency to reach the former platform site and the percentage of time spent in the target quadrant were recorded to evaluate memory retention.

2.7.2 Y-Maze Test

Short-term working memory was assessed using the spontaneous alternation paradigm. The Y-maze consisted of three identical arms arranged at 120° angles. Each mouse was placed at the end of one arm and permitted to explore freely for 5 min. The sequence of arm entries was recorded, and spontaneous alternation was calculated as: $\text{alternation (\%)} = [\text{number of triad alternations} \div (\text{total arm entries} - 2)] \times 100\%$.

2.7.3 Novel Object Recognition (NOR)

Recognition memory was evaluated in an open-field arena. During the training session, mice were allowed to freely explore two identical objects for 30 min. Twenty-four hours later, one familiar object was replaced with a novel object, and mice were again allowed to explore freely. Exploration time devoted to each object

was recorded, and the recognition index (RI) was calculated as: $RI (\%) = [\text{time exploring the novel object} \div \text{total exploration time}] \times 100\%$.

2.8 Tissue Collection

Following completion of behavioral testing, mice were deeply anesthetized using an inhalation anesthesia system (RWD Life Science Co., China) with oxygen (flow rate: 0.3–0.5 L/min) and isoflurane (Pivotal, China) delivered at a concentration of 2–3.5%. Anesthesia depth was confirmed by the absence of a pedal reflex. While under deep anesthesia, mice were euthanized via cervical dislocation. Death was confirmed by the absence of a heartbeat and corneal reflex. Brains were rapidly removed and placed on ice. The hippocampus and cortex were dissected, rinsed with ice-cold PBS to remove residual blood, and processed immediately for protein extraction. Tissues were homogenized in ice-cold RIPA buffer containing protease and phosphatase inhibitors using a motorized homogenizer on ice. Homogenates were incubated on ice for 30 min and centrifuged at $12\,000 \times g$ for 15 min at 4 °C. Supernatants were collected as total protein extracts, aliquoted, and stored at –80 °C until Western blotting and ELISA.

2.9 Cell Viability and Cytotoxicity Assays

Cell viability was assessed using the CCK-8 assay. HT-22 cells were seeded into 96-well plates, allowed to adhere for 24 h, and subjected to the designated treatments. After incubation, CCK-8 solution was added to each well and plates were incubated for 1.5 h at 37 °C. Absorbance at 450 nm was measured using a microplate reader, and viability was calculated relative to the control group. Cytotoxicity was further assessed using the Calcein-AM/propidium iodide (PI) live/dead assay. HT-22 cells plated in 24- or 96-well plates were treated as described above and stained with Calcein-AM and PI for 30 min at 37 °C. Fluorescence intensity was quantified using a fluorescence microplate reader, and representative images were captured using a fluorescence cell imaging system under 494/517 nm (Calcein) and 535/617 nm (PI) channels.

2.10 Western Blot Analysis

Protein concentrations from HT-22 cells and brain tissue homogenates were determined using the BCA method and normalized with lysis buffer to ensure equal loading. Samples were mixed with loading buffer, heated at 98 °C for 6 min, and separated on 10% SDS–polyacrylamide gels. Proteins were then transferred onto PVDF membranes. Membranes were washed with TBST and blocked with 5% BSA for 1 h at room temperature. Primary antibodies against PDE2A (1:1000), PKA (1:1000), PKG (1:800), Caspase-3, Bax, Bcl-2 (1:1000 each), NF- κ B, TNF- α , and IL-1 β (1:1000 each) were applied for 2 h at room temperature, followed by TBST washes. HRP-conjugated secondary antibodies were incubated for 1 h, and protein signals were visualized using enhanced chemiluminescence reagents.

2.11 cAMP and cGMP Levels

Intracellular cAMP and cGMP levels in HT-22 cell lysates and brain tissue homogenates were measured using ELISA kits (Cayman Chemical, USA) according to the manufacturer's instructions. HT-22 samples were processed with hydrochloric acid extraction, whereas brain tissue samples underwent trichloroacetic acid precipitation followed by neutralization. Absorbance was recorded at the indicated wavelength, and analyte concentrations were calculated based on the standard curves provided in each kit.

2.12 Inflammatory cytokine measurement

TNF- α and IL-1 β concentrations in brain tissue homogenates were quantified using ELISA kits (Abcam, USA) following the manufacturer's instructions. Absorbance at 450 nm was measured using a microplate reader, and cytokine levels were calculated from standard curves generated using the supplied standards.

2.13 Determination of oxidative stress markers

Following the designated treatments, intracellular ROS, MDA, and SOD levels in HT-22 cells were assessed using assay kits according to the manufacturer's instructions. ROS fluorescence was recorded at Ex/Em = 485/535 nm. MDA fluorescence was detected at Ex/Em = 532/553 nm, and SOD activity was measured by absorbance at 440 nm. Representative ROS images were captured using a fluorescence cell imaging system.

2.14 Transfection experiment in vitro

HT-22 cells were seeded at a density of 1×10^5 cells/mL and allowed to adhere overnight. Cells were infected with PDE2A-overexpressing adenovirus at a multiplicity of infection (MOI) of 50. Viral volumes were calculated based on the manufacturer-provided titer (2×10^9 PFU/mL). After 24 h of viral exposure, cells were treated with 10 nM BML-288 for an additional 24 h. For A β -related assays, 6 μ M A β_{1-42} was applied for 24 h following viral infection. Protein expression, oxidative stress markers, inflammatory cytokines, and apoptosis-related indicators were measured according to the experimental design.

2.15 Statistical analyses

Data are presented as mean $\pm$ SEM. Statistical analyses were performed using GraphPad Prism 9. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using the Brown–Forsythe test. Comparisons between two groups were performed using unpaired two-tailed Student's *t*-tests. Comparisons involving more than two groups were analyzed using ordinary one-way ANOVA followed by Dunnett's multiple-comparisons test when the assumptions of normality and homogeneity of variance were met. All tests were two-sided, and $P < 0.05$ was considered statistically significant. The number and experimental unit of independent biological replicates are specified in the corresponding figure legends.

3 Results

3.1. BML-288 attenuates A β -induced cytotoxicity in HT-22 cells

To establish the A β injury model, HT-22 cells were exposed to increasing concentrations of A β_{1-42} . Cell viability decreased in a concentration-dependent manner, with 6 μ M A β producing the most pronounced reduction compared with the control group (Fig. 1A). Live/dead staining further confirmed this injury pattern: A β exposure markedly increased PI-positive cells, whereas control cultures remained predominantly Calcein-AM-positive (Fig. 1B–C). To assess the protective capacity of BML-288, HT-22 cells were co-treated with A β and BML-288 for 24 h. BML-288 improved cell viability in a concentration-dependent pattern only within a limited range, with 10 nM representing the lowest concentration that produced a measurable

increase in viability (Fig. 1D). This concentration was therefore selected for subsequent experiments.

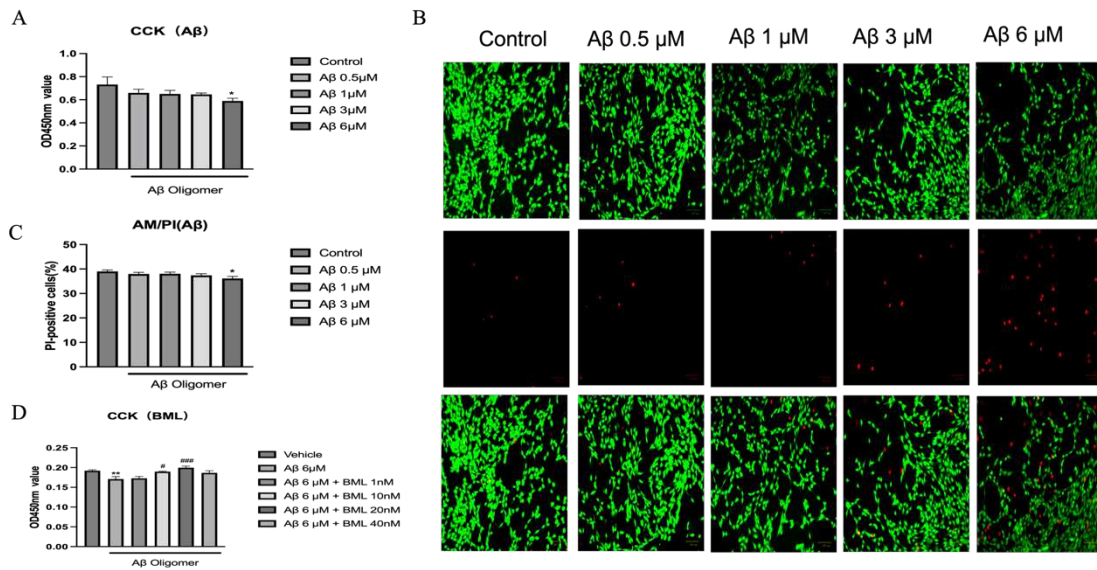


Figure 1. BML-288 protects HT-22 cells from Aβ-induced injury. (A) Cell viability of HT-22 cells after 24 h exposure to increasing concentrations of Aβ. (B) Representative AM/PI fluorescence images showing live (Calcein-AM, green) and dead (PI, red) HT-22 cells after Aβ treatment. Scale bar = 100 μm. (C) Quantification of AM/PI fluorescence intensity. (D) Cell viability of HT-22 cells treated with Aβ and increasing concentrations of BML-288 for 24 h. Data are mean ± SEM (n = 8). * p < 0.05, ** p < 0.01 vs. Control; # p < 0.05, ## p < 0.01 vs. Aβ.

3.2 PDE2 expression, cAMP/cGMP levels and PKA/PKG signaling in HT-22 cells

Western blot analysis showed that PDE2A protein levels were higher in the Aβ group than in the control group. The BML-288(1nM) group did not show a noticeable change compared with the Aβ group, whereas the BML-288(10 nM) group exhibited lower PDE2A expression (Fig. 2A–B). Consistent with this pattern, intracellular cAMP and cGMP levels were reduced following Aβ exposure, while both BML-288 groups showed increases in these analytes, with the BML-288(10 nM) group demonstrating a more apparent elevation (Fig. 2C–D). Aβ exposure produced a downward trend in PKA protein levels relative to the control group, although the change did not reach statistical significance. The BML-288(1nM) group showed no evident difference from the Aβ group, whereas the BML-288(10 nM) group exhibited a mild upward trend, which also remained non-significant (Fig. 2E–F). In contrast,

PKG expression was lower in the A β group than in controls, and the BML-288(10 nM) group showed higher PKG levels than the A β group, while the BML-288(1nM) group displayed only a modest increase (Fig. 2G–H).

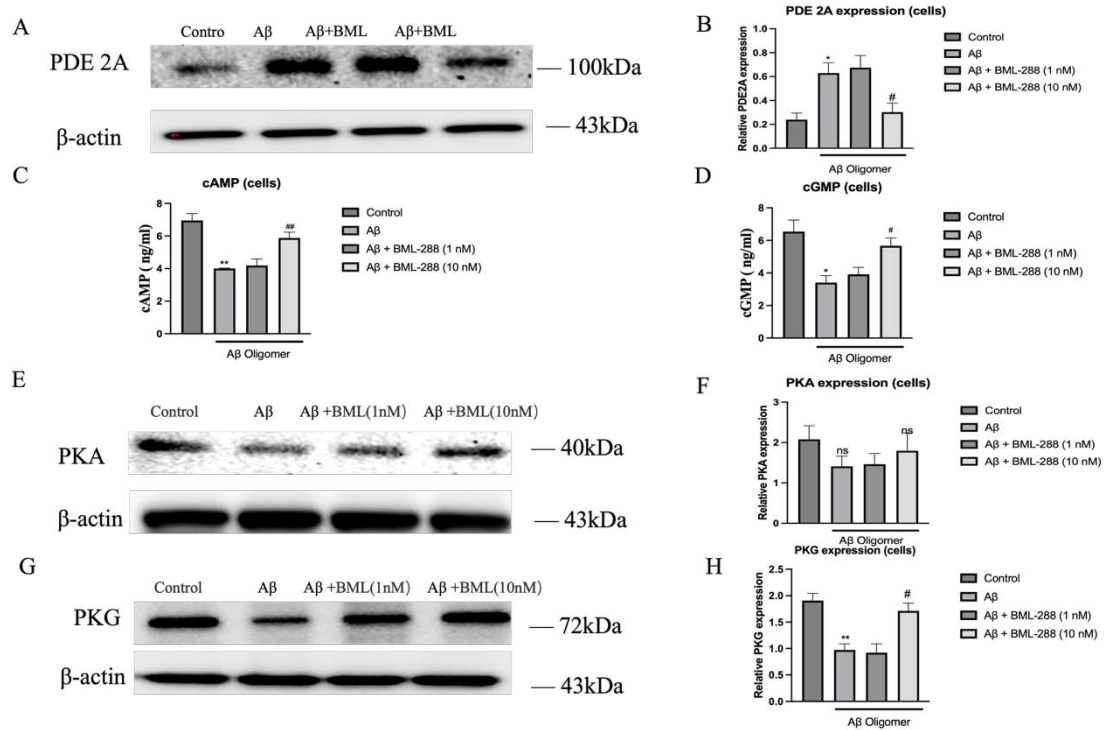


Figure 2. Effects of BML-288 on PDE2A expression, cAMP/cGMP levels, and PKA/PKG expression in HT-22 cells. (A–B) PDE2A protein expression and quantification after A β exposure with or without BML-288 pretreatment. (C–D) Intracellular cAMP and cGMP levels. (E–F) PKA protein expression and quantification. (G–H) PKG protein expression and quantification. Data are mean $\pm$ SEM (n = 3). * p < 0.05, ** p < 0.01 vs. Control; # p < 0.05, ## p < 0.01 vs. A β .

3.3 Inflammatory cytokines levels in HT-22 cells

Intracellular inflammatory cytokine levels were examined to assess the effects of BML-288 on A β -induced inflammatory responses in HT-22 cells. A β exposure markedly increased IL-1 β and TNF- α protein expression compared with the control group. In the BML-288(1nM) group, cytokine expression showed a modest reduction relative to the A β group, whereas the BML-288(10 nM) group showed a more evident decrease (Fig. 3A–B). NF- κ B-related proteins were further evaluated. A β treatment increased the level of the p50 NF- κ B subunit, while p105 precursor levels remained unchanged. BML-288(10 nM) reduced p50 levels compared with the A β group, while

the BML-288(1nM) group showed only a slight decline (Fig. 3C). Quantification of all proteins is shown in Fig. 3D–F.

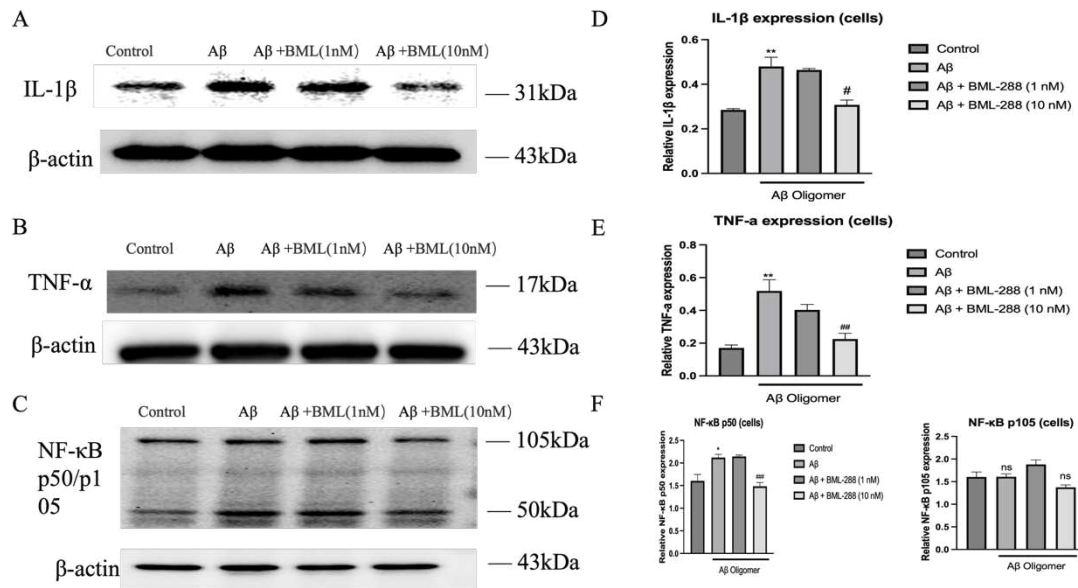


Figure 3. BML-288 reduces pro-inflammatory cytokines and NF-κB activation in HT-22 cells. (A–C) Western blot images of IL-1β, TNF-α, and NF-κB p50/p105. (D–F) Quantification of IL-1β, TNF-α, and NF-κB protein levels. Data are mean ± SEM (n = 3). * p < 0.05, ** p < 0.01 vs. Control; # p < 0.05, ## p < 0.01, ### p < 0.001 vs. Aβ.

3.4 BML-288 alleviated oxidative stress in HT-22 cells

Measurements of oxidative stress markers showed that Aβ exposure was associated with lower SOD activity and higher MDA content and ROS levels compared with the control group (Fig. 4A–C). In the BML-288(1nM) group, SOD activity exhibited a partial increase and both MDA and ROS showed modest reductions relative to the Aβ group. In the BML-288(10 nM) group, these markers showed more pronounced changes, with SOD levels higher and MDA/ROS levels lower than those in the Aβ group. Representative ROS fluorescence images are shown in Fig. 4D.

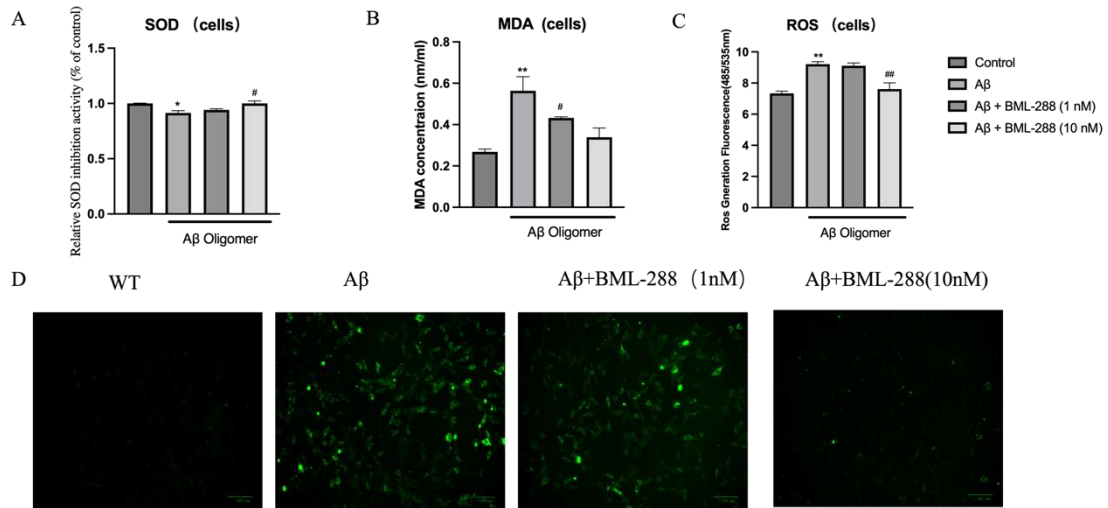


Figure 4. BML-288 attenuates oxidative stress in HT-22 cells. (A) SOD activity. (B) MDA content. (C) ROS fluorescence intensity. (D) Representative ROS fluorescence images. Data are mean $\pm$ SEM (n = 3). * $p < 0.05$, ** $p < 0.01$ vs. Control; # $p < 0.05$, ## $p < 0.01$ vs. A β .

3.5 Apoptosis-related protein expression in HT-22 cells

Apoptosis-related proteins were examined in HT-22 cells across the four experimental groups. A β exposure increased Bcl-2, Bax, and Caspase-3 expression compared with the control group (Fig. 5A–C). Treatment with 1 nM BML-288 did not noticeably alter the A β -induced elevations. In contrast, 10 nM BML-288 attenuated the increases in Bax and Caspase-3 expression and reduced the A β -induced rise in Bcl-2 relative to the A β group (Fig. 5A–C).

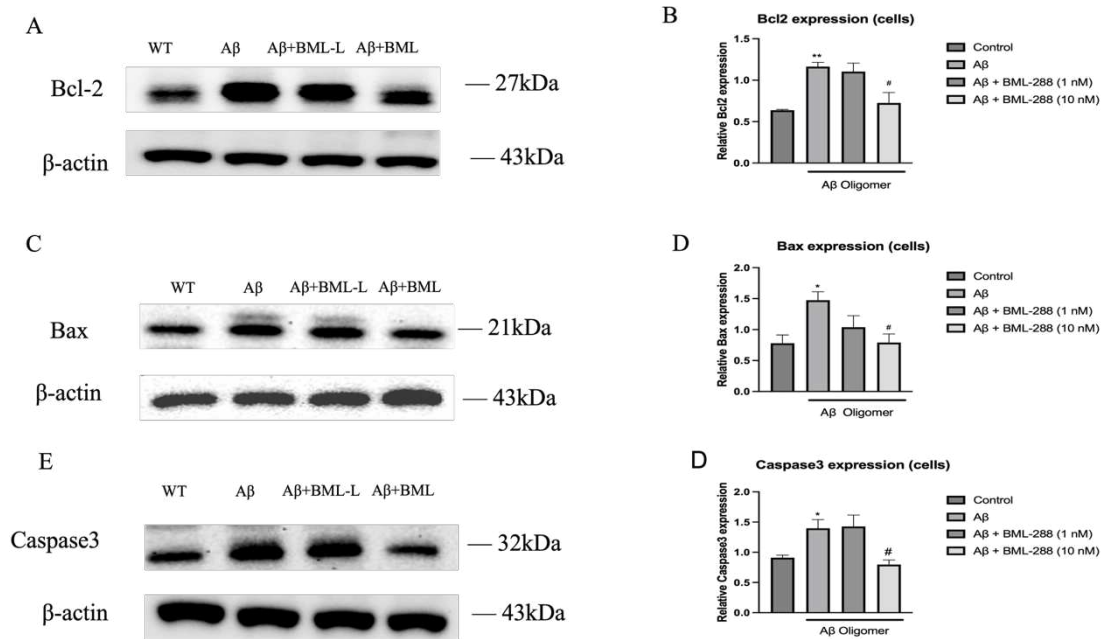


Figure 5. Effects of BML-288 on apoptosis-related protein expression in HT-22 cells. (A–C) Representative Western blot images of Bcl-2, Bax, and total Caspase-3, respectively. (D–F) Densitometric quantification of Bcl-2, Bax, and total Caspase-3 expression, normalized to the corresponding β -actin levels. Data are presented as mean $\pm$ SEM from three independent biological experiments ($n = 3$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. Control; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ vs. A β .

3.6 BML-288 improved cognitive performance in A β -treated mice

Behavioral performance was evaluated across the four experimental groups (Fig. 6A). In the water maze, representative heatmaps (Fig. 6B) showed that A β -treated mice exhibited less directed swimming patterns compared with controls. Quantitative analysis demonstrated that A β significantly increased escape latency during the acquisition phase and reduced cumulative time spent in the target quadrant during the probe trial. BML-288 at 0.3 mg/kg did not noticeably alter either parameter, whereas BML-288 at 1 mg/kg reduced escape latency and increased target-quadrant occupancy relative to the A β group (Fig. 6C). In the novel object recognition test, heatmaps (Fig. 6D) showed reduced interaction with the novel object in A β -treated mice, and the recognition index was significantly lower than in controls; BML-288 at 0.3 mg/kg did not improve performance, while BML-288 at 1 mg/kg increased the recognition index relative to the A β group (Fig. 6E). In the Y-maze test, representative activity traces

(Fig. 6F) indicated reduced arm exploration in A β -treated mice, and quantitative analysis confirmed decreased spontaneous alternation percentages; BML-288 at 0.3 mg/kg produced no detectable change, whereas BML-288 at 1 mg/kg increased alternation performance compared with the A β group (Fig. 6G).

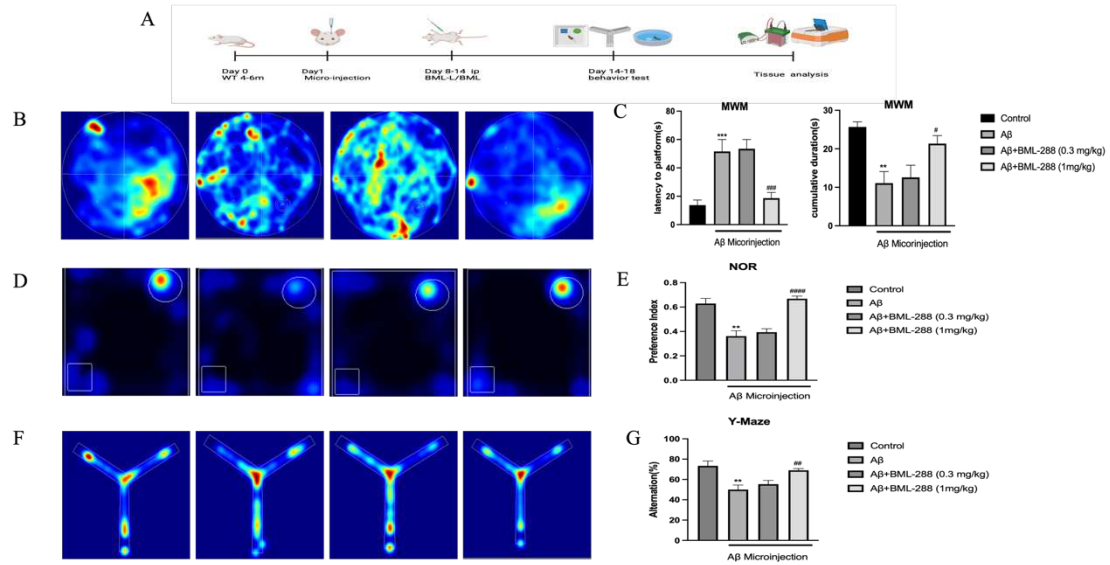


Figure 6. BML-288 improves cognitive performance in A β -treated mice. (A) Experimental timeline of A β administration and BML-288 treatment. (B) Representative swimming path heat maps in the Morris water maze (MWM). (C) Latency to platform and cumulative duration in the target quadrant in the MWM. (D) Representative heat maps in the novel object recognition (NOR) test. (E) Preference index for the novel object in the NOR test. (F) Representative Y-maze movement paths. (G) Spontaneous alternation percentage in the Y-maze. Data are mean $\pm$ SEM ($n = 5$). * $p < 0.05$, ** $p < 0.01$ vs. Control; # $p < 0.05$, ## $p < 0.01$ vs. A β .

3.7 biochemical analysis of mouse hippocampus

Biochemical analysis of hippocampal tissue was performed across the four experimental groups (Control, A β , A β + BML-288 (0.3 mg/kg), and A β + BML-288 (1 mg/kg)). A β microinjection reduced cAMP and cGMP levels compared with controls, whereas BML-288 increased both analytes relative to the A β group, with 1 mg/kg BML-288 showing a more pronounced effect (Fig. 7A–B). ELISA results showed that IL-1 β and TNF- α concentrations were elevated in hippocampal tissue following A β exposure; 0.3 mg/kg BML-288 produced minimal changes, whereas 1 mg/kg BML-288 reduced cytokine levels relative to the A β group (Fig. 7C–D).

Western blot analysis further demonstrated that A β increased Bax expression and decreased Bcl-2 expression, leading to a higher Bax/Bcl-2 ratio, while 1 mg/kg BML-288 attenuated these A β -induced alterations (Fig. 7E–F).

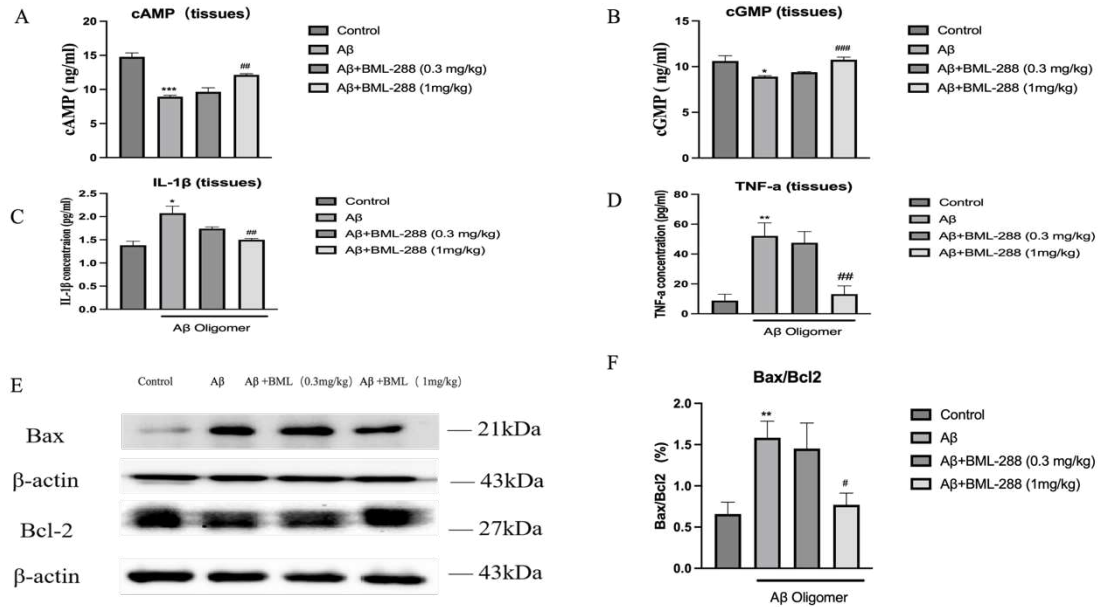


Figure 7. Effects of BML-288 on cAMP/cGMP signaling and inflammation- and apoptosis-related markers in hippocampal tissue. (A–B) cAMP and cGMP levels in hippocampal tissue. (C–D) IL-1 β and TNF- α concentrations measured by ELISA. (E) Representative Western blot images of Bax and Bcl-2. (F) Bax/Bcl-2 ratio based on densitometric quantification of Bax and Bcl-2 expression normalized to the corresponding β -actin levels. Data are presented as mean $\pm$ SEM. For cAMP, cGMP, IL-1 β , and TNF- α measurements, n = 3 mice per group; for Western blot analysis, n = 5 mice per group. * p < 0.05 and ** p < 0.01 vs. Control; # p < 0.05 and ## p < 0.01 vs. A β .

3.8 PDE2A overexpression induced oxidative stress and inflammation in HT-22 cells

To examine the cellular effects of elevated PDE2A expression, HT-22 cells were assigned to three groups: Control, PDE2A-overexpression (OE), and OE + BML-288. Western blot analysis confirmed markedly increased PDE2A protein levels in the OE group, which were reduced by BML-288 treatment (Fig. 8A–B). PDE2A overexpression increased intracellular ROS accumulation based on fluorescence imaging, whereas BML-288 reduced ROS intensity relative to the OE group (Fig. 8C). IL-1 β expression showed a similar pattern, increasing in the OE group and decreasing following BML-288 treatment (Fig. 8D–E). For apoptosis-related proteins,

PDE2A overexpression caused a mild, non-significant elevation in Bcl-2 levels, and BML-288 reduced this upward trend (Fig. 8F-G).

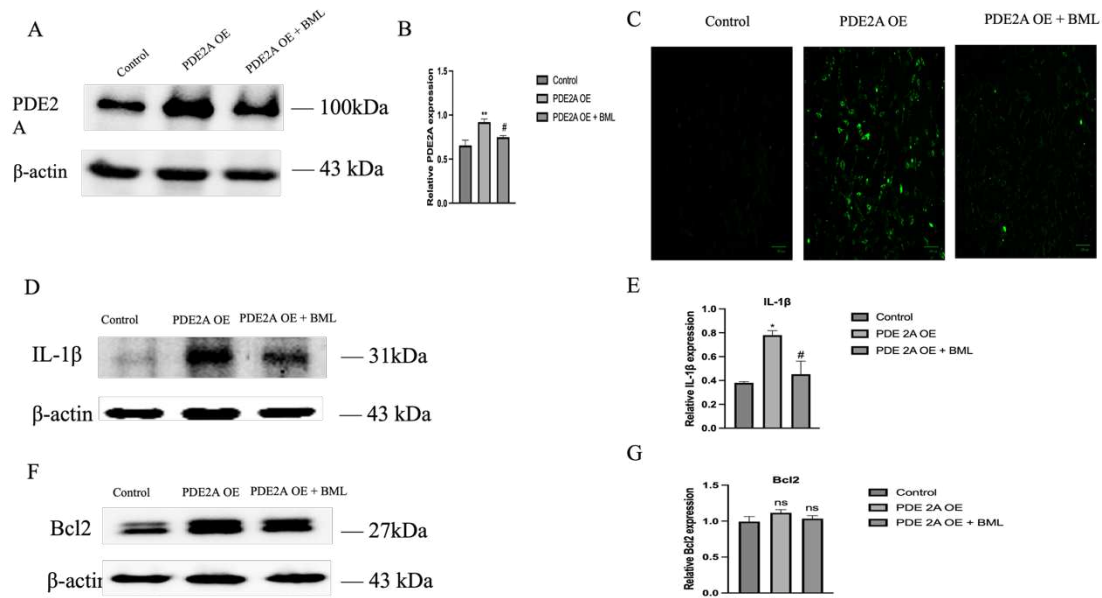


Figure 8. Effects of PDE2A overexpression on oxidative stress, inflammation, and apoptosis-related proteins in HT-22 cells. (A–B) PDE2A protein expression and quantification in Control, OE, and OE + BML-288 groups. (C) Representative ROS fluorescence images. (D–E) IL-1 β expression and quantification. (F) Bcl-2 protein expression. Data are mean $\pm$ SEM (n = 3). * $p < 0.05$, ** $p < 0.01$ vs. Control; # $p < 0.05$ vs. OE.

3.9 PDE2A knockdown mitigated A β -induced oxidative stress, inflammation, and pro-apoptotic signaling in HT-22 cells

HT-22 cells were divided into four groups (Control, PDE2A-KD, A β , and A β + KD) to investigate the effects of PDE2A knockdown. Western blot analysis confirmed reduced PDE2A protein expression in the KD group compared with controls (Fig. 9A–B). Under basal conditions, PDE2A knockdown did not substantially alter ROS levels, IL-1 β expression, or Bax abundance. In contrast, A β treatment markedly increased ROS accumulation, IL-1 β levels, and Bax expression, whereas PDE2A knockdown significantly attenuated these A β -induced changes (Fig. 9C–G).

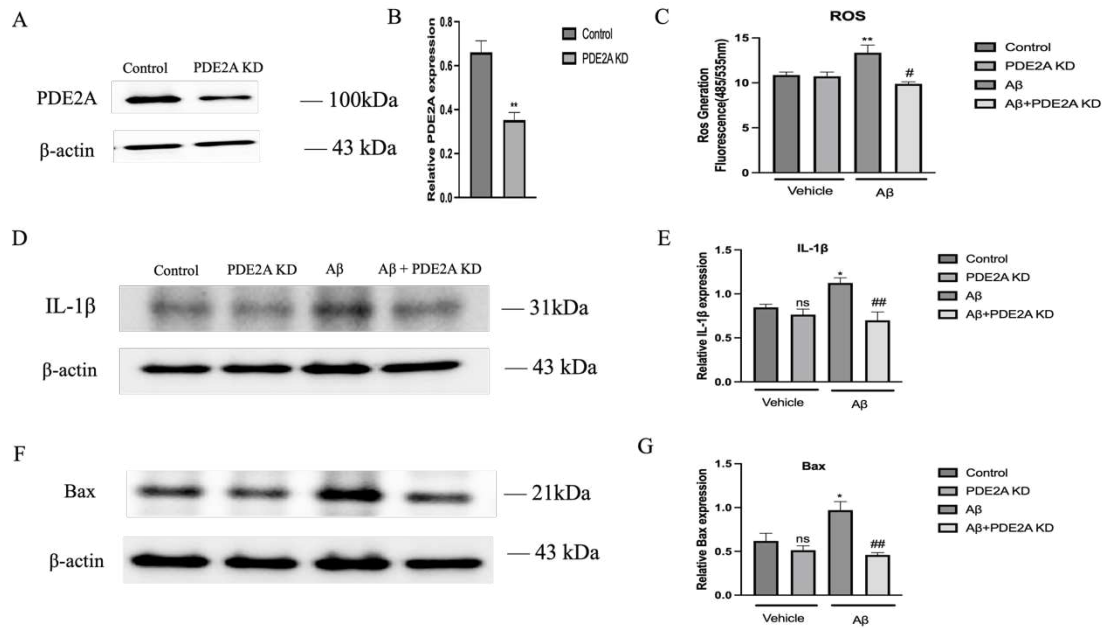


Figure 9. Effects of PDE2A knockdown on oxidative stress, inflammation, and apoptosis-related proteins in HT-22 cells. (A–B) PDE2A protein expression and quantification. (C) ROS quantification under basal and A β -treated conditions. (D–E) IL-1 β expression and quantification. (F–G) Bax expression and quantification. Data are mean $\pm$ SEM (n = 3). * $p < 0.05$, ** $p < 0.01$ vs. Control; # $p < 0.05$, ## $p < 0.01$ vs. A β .

4 Discussion

AD is characterized by progressive synaptic dysfunction, oxidative injury, neuroinflammation, and neuronal loss triggered by A β accumulation. In this study, A β -induced neuronal injury models were used to examine how BML-288 exposure relates to molecular and behavioral outcomes in vitro and in vivo. Across these models, BML-288 was associated with changes consistent with reduced oxidative stress, lower inflammatory marker levels, and altered expression of apoptosis-related proteins. These cellular and biochemical findings were accompanied by improved performance in cognitive behavioral tests. These observations suggest that modulation of cyclic nucleotide signaling may contribute to the actions of BML-288 in A β -related injury.

Our findings align with a growing body of evidence indicating that cyclic nucleotide dysregulation is involved in AD pathophysiology. Prior studies have reported that A β can influence intracellular cAMP and cGMP levels^[38–40], which may

affect synaptic plasticity^[41], and contribute to oxidative and inflammatory stress responses^[42-45]. Research indicates that PDE inhibitors which target PDE2A and PDE4 and PDE5 enzymes produce cognitive benefits and neuroprotective effects in models of cognitive decline^[46-48]. In the present study, A β exposure was associated with reductions in both cAMP and cGMP, whereas BML-288 pretreatment was associated with higher levels of these cyclic nucleotides. Although PKA expression exhibited only a modest upward tendency, PKG expression showed a more evident increase. These patterns suggest that cGMP-responsive signaling may be more sensitive to PDE2A modulation in this experimental context, although the relative contribution of cAMP-PKA and cGMP-PKG pathways likely varies across models and experimental conditions^[49].

Importantly, activation of these cyclic nucleotide pathways has been implicated in regulating redox balance and inflammatory signaling, which are important downstream contributors to A β -induced neurotoxicity. The neurotoxic effects of A β lead to excessive oxidative stress and neuroinflammation^[50-52] which research shows that anti-inflammatory and antioxidant treatments can help reduce cognitive decline^[53-56]. The study shows A β exposure leads to increased ROS and MDA levels and decreased SOD activity but pretreatment corresponded with biochemical profiles indicative of lower oxidative stress. Similarly, BML-288 exposure was associated with lower TNF- α and IL-1 β protein levels in both cell and tissue samples. Moreover, Manipulation of PDE2A expression also showed patterns consistent with altered stress susceptibility: PDE2A overexpression increased oxidative stress and inflammatory responses under basal conditions, and these effects were alleviated by BML-288. In contrast, PDE2A knockdown attenuated oxidative and inflammatory changes in A β -treated cells. These observations suggest that PDE2A levels may influence neuronal responses to oxidative and inflammatory challenges, although the specific mechanisms remain to be clarified.

A β -mediated neurotoxicity is closely associated with the activation of apoptotic pathways^[57]. In the present study, A β exposure increased Bax and total Caspase-3 expression in HT-22 cells and hippocampal tissue, whereas BML-288 attenuated

these changes. PDE2A knockdown did not significantly alter Bax expression under basal conditions but reduced the A β -associated increase in Bax, suggesting that PDE2A may be involved in the cellular response to A β -induced stress. However, because total rather than cleaved Caspase-3 was measured, these findings should be interpreted as changes in apoptosis-related protein expression rather than direct evidence of Caspase-3 activation. Bcl-2 showed different response patterns in the cellular and animal models. In PDE2A-overexpressing cells, Bcl-2 showed a modest, non-significant upward trend that was attenuated by BML-288. Similarly, acute A β exposure moderately increased Bcl-2 expression in HT-22 cells. By contrast, Bcl-2 expression was reduced in hippocampal tissue from A β -treated mice, accompanied by an increased Bax/Bcl-2 ratio. These apparently divergent responses may reflect differences in the duration and biological context of A β -induced stress. Previous studies have reported increased neuronal Bcl-2 immunoreactivity in the hippocampus of patients with AD ^[58, 59], and increased Bcl-2 expression in transgenic AD models ^[60]. These findings suggest that Bcl-2 upregulation may occur as a compensatory response in surviving neurons. Conversely, A β exposure has also been reported to reduce Bcl-2 expression in primary human neuronal cultures, potentially increasing neuronal susceptibility to cellular stress ^[61]. Thus, the direction of Bcl-2 regulation appears to depend on the experimental model, duration of injury, and cellular context. In the acute HT-22 model used here, the concurrent increases in Bax and Bcl-2 may reflect simultaneous pro-apoptotic signaling and a compensatory anti-apoptotic response; therefore, the Bax/Bcl-2 ratio was not emphasized because it provided limited additional discrimination in this setting. In contrast, the opposing changes in Bax and Bcl-2 observed in hippocampal tissue produced a more pronounced alteration in the Bax/Bcl-2 ratio, making this measure more informative in the *in vivo* model. Taken together, these findings suggest that BML-288 is associated with modulation of apoptosis-related responses following A β exposure, although additional studies using direct measures of apoptosis, including cleaved Caspase-3, would be required to establish its effects on apoptotic pathway activation.

Behavioral assessments confirmed that these cellular and molecular effects were accompanied by better performance. BML-288-treated mice showed shorter escape latency and increased target quadrant occupancy in the Morris water maze test, enhanced recognition memory in the novel object recognition test, and improved spontaneous alternation behavior in the Y-maze test. These data collectively indicate that BML-288 ameliorates A β -induced cognitive deficits *in vivo*.

Based on the overall data, a conceptual working model is proposed (Fig. 10). In this model, PDE2A modulation by BML-288 may influence cAMP and cGMP availability, which could in turn affect downstream signaling nodes such as PKA and PKG. These alterations may contribute to changes observed in oxidative stress markers, inflammatory mediators, and apoptosis-related proteins, ultimately aligning with improved behavioral outcomes. This framework is presented as a hypothesis-generating model and requires further validation through targeted mechanistic experiments.

Several limitations of this study should be acknowledged. First, Although PDE2A overexpression and knockdown experiments supported the involvement of PDE2A in A β -induced cellular responses, the causal relationships between PDE2A inhibition, cyclic nucleotide signaling, and downstream oxidative, inflammatory, and apoptosis-related changes remain to be fully established. Additional pathway-specific experiments, such as pharmacological inhibition or genetic manipulation of PKA/PKG signaling, would help clarify these relationships. Second, The specific binding properties of BML-288 for PDE2A remain unclear which makes it difficult to determine the exact molecular mechanisms at play. Third, The research used short-term A β exposure in neuronal cultures and brief animal treatment which does not match the extended progression of Alzheimer's disease. In addition, the absence of a scrambled A β peptide control and a matched sham-operated control group represents a limitation of the experimental design, as nonspecific peptide- or procedure-related effects cannot be completely excluded. Future studies incorporating these controls would help more specifically distinguish A β -related effects from nonspecific experimental responses.

A β exposure in this study was associated with increased ROS and MDA levels and lower SOD activity. BML-288 pretreatment corresponded with biochemical profiles indicative of lower oxidative stress.

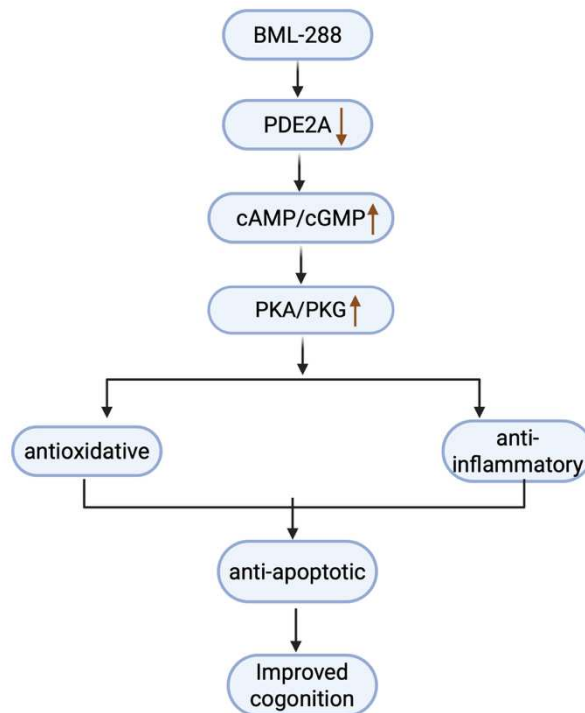


Figure 10. BML-288 exerts its protective effects against A β -induced neuronal damage through PDE2A blockade which produces elevated cAMP and cGMP levels that activate PKA and PKG pathways. The resulting changes in the system decrease oxidative damage and inflammatory responses and apoptosis which leads to better cognitive performance in A β -treated mice.

5 Conclusion

This study suggests that exposure to BML-288 was associated with changes in cyclic nucleotide signaling and molecular markers of oxidative stress, inflammation, and apoptosis in A β -induced neuronal injury models. These findings provide preliminary support for considering PDE2A-related pathways in future investigations and indicate that BML-288 may hold potential value for further exploration in Alzheimer's disease research and early-stage therapeutic development.

Abbreviations

A β : Amyloid- β

AD: Alzheimer's disease

AM/PI: Calcein-acetoxymethyl ester/Propidium iodide

ANOVA: Analysis of variance

cAMP: Cyclic adenosine monophosphate

cGMP: Cyclic guanosine monophosphate

CCK-8: Cell Counting Kit-8

ELISA: Enzyme-linked immunosorbent assay

IL-1 β : Interleukin-1 beta

KD: Knockdown

MDA: Malondialdehyde

MWM: Morris water maze

NF- κ B: Nuclear factor kappa-B

NOR: Novel object recognition

OE: Overexpression

PBS: Phosphate-buffered saline

PDE2A: Phosphodiesterase 2A

PKA: Protein kinase A

PKG: Protein kinase G

ROS: Reactive oxygen species

SEM: Standard error of the mean

SOD: Superoxide dismutase

TNF- α : Tumor necrosis factor-alpha

Declarations

Ethical Approval and Consent to Participate

All animal procedures were approved by the Rutgers University Institutional Animal Care and Use Committee (IACUC; Protocol No. PROTO202100135) and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Consent to participate is not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. Additional raw datasets (including numerical values for ELISA, biochemical assays, and behavioral tests) are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Hailiang Li and Deqin Geng designed the study and wrote the manuscript.

Hailiang Li, Baomin Dou, Fu Xu and Zhutao Sheng performed the in vitro and in vivo experiments.

Xiaoqian Lin, Bo Du and Yu Feng analyzed the data.

Yanbo Cheng provided experimental suggestions and laboratory materials.

All authors reviewed and approved the final manuscript.

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