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Pretreatment with antiasthmatic drug ibudilast ameliorates $A\beta_{1-42}$-induced memory impairment and neurotoxicity in mice

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ABSTRACT

Amyloid-β peptide (Aβ) is thought to be associated with the progressive neuronal death observed in Alzheimer's disease (AD). However, effective neuroprotective approaches against Aβ neurotoxicity are unavailable. Here, we investigated possible preventive effects of ibudilast, as a pharmacologic phosphodiesterase inhibitor, currently used for treatment of inflammatory diseases such as asthma, on $A\beta_{1-42}$-induced neuroinflammatory, apoptotic responses and memory impairment. We found that pretreatment with ibudilast (4 or 12 mg/kg, i.p.) significantly ameliorated impaired spatial learning and memory in intracerebroventricularly (ICV) $A\beta_{1-42}$-injected mice, as evidenced by decrease in escape latency during acquisition trials and increase in exploratory activities in the probe trial in Morris water maze (MWM) task, and by increase in the number of correct choices and decrease in latency to enter the shock-free compartment in Y-maze test. Further study showed that ibudilast prevented generation of pro-inflammatory cytokines such as NF-κB p65 and TNF-α as well as pro-apoptotic molecule caspase-3 activation and anti-apoptotic protein Bcl-2 downregulation in both hippocampus and cortex of ICV $A\beta_{1-42}$-injected mice. Taken together, our findings suggest that ibudilast has preventive effects on Aβ-induced cognitive impairment *via* inhibiting neuroinflammatory and apoptotic responses.

1. Introduction

Alzheimer's disease (AD) is a multifaceted neurodegenerative disorder of the central nervous system (CNS), featured by neuronal loss, neuroinflammation and progressive memory and cognitive impairment (Campbell and Gowran, 2007). The molecular pathogenesis of the disease encompasses extracellular accumulation of the amyloid β peptide (Aβ) as amyloid deposits in different regions of the brain, especially in the hippocampus. Aβ can interact with various cellular components to trigger signal transduction cascades that prompt free-radical generation, Ca^{2+} dysregulation, inflammatory and apoptotic response (Inestrosa et al., 2005), which results in the degeneration of hippocampal neurons and the progression of AD (Hoozemans et al., 2008; Choi and Bosetti, 2009). Thus, therapeutic efforts aiming at interruption of Aβ-induced neuroinflammation and apoptosis may be beneficial for AD.

Ibudilast is a relatively non-selective cyclic AMP phosphodiesterase (PDE) inhibitor that has been used for decades in Japan to treat bronchial asthma and post-stroke dizziness (Rolan et al., 2009; Ledeboer et al., 2007). Curiously, ibudilast is receiving increased attention as its novel pharmacological effects have emerged, especially in the CNS. *In vitro* studies have demonstrated that ibudilast possesses potential anti-inflammatory effect in the CNS, as it can inhibit lipopolysaccharide (LPS)-induced cytokine production in microglial (Deree et al., 2008; Suzumura et al., 1999), attenuate rat experimental autoimmune encephalomyelitis (Fujimoto et al., 1999), reduce white matter damage after chronic cerebral hypoperfusion (Wakita et al., 2003) and the number of TNF-α labeled cells in a genetic model of Krabbe's disease (Kagitani-Shimono et al., 2005). In multiple rat models, ibudilast has shown promise as a treatment for neuropathic pain *via* attenuating glial cell activation (Ledeboer et al., 2006). Ibudilast is also being tested under clinical trials as a treatment for multiple sclerosis, opioid withdrawal, and neuropathic pain, all of which are conditions involving aberrant microglial activation and CNS inflammation (Rolan et al., 2009; Barkhof et al., 2010; Beardsley et al., 2010). Recent studies have shown that ibudilast can inhibit TNF-α production induced by neurotoxin Tat in microglial cells, as a potential novel adjunctive therapy for the management of human immunodeficiency virus-1(HIV-1)-associated neurocognitive disorders (Kiebala and Maggirwar, 2011). In view of the multifaceted effects of ibudilast in the CNS, we hypothesized that it has preventive or therapeutic potential for memory deficits associated with AD. Thus, we firstly observed preventive effects of ibudilast on spatial learning and memory in bilateral intracerebroventrical (ICV)

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$A\beta_{1-42}$-injected mice, and further investigated its possible underlying mechanisms by detecting pro-inflammatory cytokines and the proteins associated with apoptosis in the brain.

2. Materials and methods

2.1. Animals

Male ICR mice (approx. 3 months old, weighing 25–30 g; Yangzhou University Medical Center Yangzhou, China) were used for the experiments. The experimental protocol was approved by the Institutional Review Committee for the use of Animal Subjects of China Pharmaceutical University and experimental procedures are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All animals were maintained on a 12-h light/12-h dark cycle with free access to water and standard laboratory chow.

2.2. Materials

Ibudilast was purchased from Beijing HWRK Chem Co., Ltd (Beijing, China. Lot: HW13D1209-1). $A\beta_{1-42}$ was purchased from Sigma Aldrich (St-Louis, Mississippi, USA). Antibodies were purchased from different companies: anti-TNF-α was from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany), anti-NF-κB p65 from Cell Signaling Technology, Inc. (Boston, Massachusetts, USA), anti-pro- or cleaved caspase-3 and anti-Bcl-2 from Cell Signaling Technology, Inc. (Boston, Massachusetts, USA), anti-β-actin and secondary antibodies from Bioworld Technology Co., Ltd (Minneapolis, Minnesota, USA). All other chemicals were of analytical grade and commercially available. $A\beta_{1-42}$ was reconstituted in phosphate-buffered saline (pH 7.4) at the concentration of 410 pmol/5 μL and aggregated by incubation at 37 °C for 7 days prior to administration, as described previously (Tang et al., 2014).

2.3. Drug treatment and stereotaxic ICV $A\beta_{1-42}$ injection

Fifty-three mice were randomly assigned into 4 groups: (1) vehicle plus PBS (namely sham group), (2) vehicle plus $A\beta_{1-42}$ (Veh + $A\beta_{1-42}$), (3) ibudilast (4 mg/kg) plus $A\beta_{1-42}$ (Ibu 4 mg/kg + $A\beta_{1-42}$), and (4) ibudilast (12 mg/kg) plus $A\beta_{1-42}$ (Ibu 12 mg/kg + $A\beta_{1-42}$). Animal number of each group referred to 13 or 14 mice. Mice were intraperitoneally injected with ibudilast dissolved in 0.9% saline once daily during the whole experimental process. On the fifteenth day, mice were anesthetized with the intraperitoneal injection of 350 mg/kg chloral hydrate at 1 h after administration and then immobilized on a stereotactic frame (SR-5, Narishige, Tokyo, Japan). The dura overlying the parietal cortex was exposed, and a glass micropipette connected to a microinjection pump (Dakumar machinery, Sweden) was inserted into the left and right parietal cortices situated at a site of 0.5 mm caudal to bregma, 1.0 mm from the midline, and 2.5 mm below the dural surface (Paxinos and Franklin, 2003). In the sham group, 5 μL sterile PBS (0.1 M, pH 7.4) was injected bilaterally through a micropipette; in the vehicle plus $A\beta_{1-42}$, ibudilast (4 mg/kg) plus $A\beta_{1-42}$, and ibudilast (12 mg/kg) plus $A\beta_{1-42}$ groups, PBS (5 μL) containing $A\beta_{1-42}$ (410 pM) was bilaterally injected into the cerebroventricles (1 μL/min for all the infusions). The micropipettes were left in place for 5 min to minimize back-flux of liquid. 5 days after ICV $A\beta_{1-42}$ injection, one part of the mice was submitted to behavioral tests and mice were administered 1 h before the first trial on each day, and the other part was sacrificed by cervical dislocation, and the brain tissues were taken out for assays of NF-κB p65, TNF-α, caspase-3, and Bcl-2.

2.4. Morris water maze test

Spatial memory was assessed by the MWM, which consisted of 5-day training (visible and invisible platform training sessions) and a probe trial on day 6. This was carried out as described previously (Tang et al., 2013). Mice were individually trained in a circular pool (120 cm diameter, 50 cm height) filled up to a depth of 30 cm with water maintained at 25 °C. The maze was located in a lit room with visual cues. A platform (9 cm diameter) was placed in the center of one quadrant of the pool. The platform's position was unchanged throughout the visible and hidden-platform training sessions; the starting points were pseudo-randomized for each trial, with the animals facing toward the wall. Each mouse was individually trained in both visible-platform (days 1–2) and hidden-platform (days 3–5) versions. Visible-platform training was performed for baseline differences in vision and motivation; the platform was placed 1 cm underneath the surface of the water and was indicated by a small flag (5 cm in height). The hidden-platform version evaluates spatial learning and was used to determine the retention of memory to find the platform. During the training, the platform was placed 1 cm underneath the surface of the water and the flag was removed. The platform was always in the same place. On each day, the animals were subjected to four trials with a 1-h interval between trials. Each trial lasted for 90 s unless the animal reached the platform first. If an animal failed to find the platform within 90 s, the test was ended and the animal was gently navigated to the platform by hand for 30 s. On day 6, the platform was removed and the probe trial was started, during which animals had 90 s to search for the platform. The time spent in the target quadrant and the number of target crossings (*i.e.*, the quadrant where the platform was previously located) were recorded. Data of the escape latency, the time spent in the target quadrant, the number of target crossings and swimming speed were collected by the video tracking equipment and processed by a computer equipped with an analysis-management system (Viewer 2 Tracking Software, Ji Liang Instruments, China).

2.5. Y-maze test

This was performed as descried previously (Jiang et al., 2012). The Y-maze was constructed of black plastic walls (10 cm high), consisting of three compartments (10 cm × 10 cm) connected with passages (4 × 5 cm), with the floor of 3.175 mm stainless steel rods (8 mm apart). There is a same light in each compartment. The test was conducted for 2 consecutive days. On day 1 (learning trial), each mouse was placed in one of the compartments and allowed to move freely for 5 min (habituation) before moving to the next session with electric power on. During the training, electric shocks (2 Hz, 125 ms, 10 V) were available through the stainless steel grid floor in two of the compartments and the light was on in the shock-free compartment. Each mouse was trained by turning on the light in each compartment in turn (adding up to 10 times). The training was stopped once the mouse entered the shock-free compartment and stayed for 30 s, which was recorded as a correct choice. If the mouse did not enter this compartment, it was gently navigated to the compartment and allowed to stay for 30 s. On day 2 (testing trial), each mouse was also tested for 10 times following the same procedures as on day 1. The numbers of correct choices out of 10 and the latency to enter the shock-free compartment on day 2 were recorded manually.

2.6. Open field test

The general locomotor activity of the mice associated with each treatment was evaluated by an open field test. The apparatus was made of Plexiglas, with a black-painted floor of 50 cm × 50 cm and transparent 40 cm-high walls. The experiments were conducted in a sound-attenuated room under low-intensity light (7 lx). At the beginning of the test, the mouse was gently placed at the center of the open field and the total distance traveled (m) was registered for 5 min with the ANY Maze® video tracking (Dos Santos et al., 2013). After each trial the apparatus was cleaned with ethanol solution (10% v/v) and dried with paper towels after each trial in order to