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Montelukast targeting the cysteinyl leukotriene receptor 1 ameliorates $A\beta_{1-42}$-induced memory impairment and neuroinflammatory and apoptotic responses in mice

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ABSTRACT

Montelukast, known as a cysteinyl leukotriene receptor 1 ($CysLT_1R$) antagonist, is currently used for treatment of inflammatory diseases such as asthma. Here, we investigated effects of montelukast on neuroinflammatory, apoptotic responses, and memory performance following intracerebral infusions of amyloid-β (Aβ). The data demonstrated that intracerebroventrical infusions of aggregated $A\beta_{1-42}$ (410 pmol/mouse) produced deficits in learning ability and memory, as evidenced by increase in escape latency during acquisition trials and decreases in exploratory activities in the probe trial in Morris water maze (MWM) task, and by decrease in the number of correct choices and increase in latency to enter the shock-free compartment in Y-maze test, and caused significant increases in pro-inflammatory cytokines such as NF-κB p65, TNF-α and IL-1β as well as pro-apoptotic molecule caspase-3 activation and anti-apoptotic protein Bcl-2 downregulation in hippocampus and cortex. Interestingly, this treatment resulted in upregulation of protein or mRNA of $CysLT_1R$ in both hippocampus and cortex. Blockade of $CysLT_1R$ by repeated treatment with montelukast (1 or 2 mg/kg, ig, 4 weeks) reduced $A\beta_{1-42}$-induced $CysLT_1R$ expression and also suppressed $A\beta_{1-42}$-induced increments of NF-κB p65, TNF-α, IL-1β and caspase-3 activation, and Bcl-2 downregulation in the hippocampus and cortex. Correspondingly, montelukast treatment significantly improved $A\beta_{1-42}$-induced memory impairment in mice, but had little effect on normal mice. Our results show that montelukast may ameliorate $A\beta_{1-42}$-induced memory impairment via inhibiting neuroinflammation and apoptosis mediated by $CysLT_1R$ signaling, suggesting that $CysLT_1R$ antagonism represents a novel treatment strategy for Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD), the most common form of irreversible dementia, is placing a considerable and increasing burden on patients, caregivers and society, as more people live long enough to become affected. AD is clinically characterized by a progression from episodic memory problems to a slow global decline of cognitive function (Querfurth and LaFerla, 2010). One critical event in the pathogenesis of AD is abundant deposits of senile plaques composed of amyloid-β (Aβ). The amyloid deposits accumulate first in isocortical areas, followed by limbic and allocortical structures including entorhinal cortex and hippocampus (Arnold et al., 1991; Thal et al., 2002). The neurotoxicity of Aβ peptides has been well documented (Deshpande et al., 2006), and the Aβ hypothesis of AD has been widely accepted. Numerous studies showed that prolonged infusion of synthetic Aβ into the brain can cause learning and memory deficits in animals (Nitta et al., 1997), including impairment of working memory and place learning in Y-maze, and water maze (Maurice and Lockhart, 1996). However, effective neuroprotective approaches against Aβ neurotoxicity are unavailable yet.

Increasing evidence from human and mouse models suggests that inflammation is an important player in the pathogenesis of AD (Wyss-Coray, 2006) and pro-inflammatory cytokines may result in neuronal damage and Aβ accumulation in brain (Daniela and Norbert, 2010; Gorelick, 2010). Cysteinyl leukotrienes (CysLTs) including LTC4, LTD4, and LTE4 are inflammatory lipid mediators derived from the 5-lipoxygenase (5-LO) pathway of the arachidonic acid metabolism (Singh et al., 2010). Pharmacological

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characterizations have suggested the existence of at least two types of cysteinyl leukotriene receptors, designated as cysteinyl leukotriene receptor 1 ($CysLT_1R$) and $CysLT_2R$, based on potency of the agonists and antagonists (Singh et al., 2010). Modifiers of the leukotriene signaling pathways, including 5-LO inhibitor (zileuton) and $CysLT_1R$ antagonists (montelukast, zafirlukast, and pranlukast), are the only orally administered anti-asthmatic drugs (Rao et al., 2007; Nishio et al., 2007; Schelfhout et al., 2008). In more recent years, the focus on $CysLT_1R$ has been intensified as its novel pathophysiological role has emerged in several types of brain damage, such as cerebral ischemia, traumatic brain injury, experimental autoimmune encephalomyelitis, and *etc* (Yu et al., 2005; Zhang et al., 2006a,b; Yuan et al., 2009; Ciccarelli et al., 2004; Huang et al., 2008; Ding et al., 2007; Zhang et al., 2004; Wang et al., 2011). We recently reported that intracerebral infusions of LTD4 impaired memory, with obvious expression of $CysLT_1R$ in hippocampus and cortex of mouse brains, and LTD4 also induced Aβ generation by $CysLT_1R$-mediated β-secretase pathway *in vivo* and *in vitro* (Tang et al., 2013; Wang et al., 2013). However, it is not completely understood what role $CysLT_1R$ plays in Aβ neurotoxicity. Montelukast is a commonly used $CysLT_1R$ antagonist that blocks the proinflammatory action of LTD4, and approved for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. It acts by inhibiting neutrophil infiltration, balancing oxidant-antioxidant status, and regulating the generation of inflammatory mediators (Tugtepe et al., 2007). In this study, we firstly observed the effects of montelukast targeting $CysLT_1R$ on spatial learning and memory in bilateral intracerebroventrical $A\beta_{1\text{-}42}$-injected mice. Further, we investigated its possible underlying mechanisms by detecting proinflammatory cytokines and the proteins related to apoptosis in brain.

2. Materials and methods

2.1. Materials

Montelukast(2-[1-[[(1R)-1-[3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanylmethyl]cyclopropyl] acetic acid) was purchased from Mudanjiang Hengyuan Pharmaceutical Co., Ltd (Heilongjiang, China. Lot: 2011R000188). $A\beta_{1\text{-}42}$ was purchased from Sigma Aldrich (St-Louis, Missouri, USA). Antibodies were purchased from different companies: anti-$CysLT_1R$, anti-TNF-α and anti-IL-1β 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 Beyotime Institute of Biotechnology Co., Ltd (Haimen, China), anti-β-actin from Boster Biotechnology Co., Ltd (Wuhan, China), secondary antibodies from Bioworld Technology Co., Ltd (Minneapolis, Minnesota, USA). Trizol was purchased from Invitrogen (Carlsbad, California, USA). All the other chemicals were of analytical grade and commercially available. $A\beta_{1\text{-}42}$ 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 (Russo et al., 2012).

ICR male mice (approximately 3 months old, weighing 20–25 g) were purchased from Medical Center of Yangzhou University (Yangzhou, China). All animal procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of China. Mice were housed in with population of eight per cage and allowed access to diet and autoclaved water. Animal housing rooms were maintained at a constant room temperature (25 °C) in a 12-h light (7:00 A.M.)/dark (7:00 P.M.) cycle.

2.2. Stereotaxic intracerebroventricular (ICV) $A\beta_{1\text{-}42}$ injection

Mice were randomly assigned to 4 groups: (1) vehicle plus vehicle (Veh + Veh), (2) $A\beta_{1\text{-}42}$ plus vehicle ($A\beta_{1\text{-}42}$ + Veh), (3) $A\beta_{1\text{-}42}$ plus montelukast (1.0 mg/kg) ($A\beta_{1\text{-}42}$ + Mon 1.0 mg/kg), (4) $A\beta_{1\text{-}42}$ plus montelukast (2.0 mg/kg) ($A\beta_{1\text{-}42}$ + Mon 2.0 mg/kg). During the surgery, mice were anesthetized with the intraperitoneal injection of 350 mg/kg chloral hydrate 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 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 vehicle plus vehicle group, 5 μL sterile PBS (0.1M, pH7.4) was injected bilaterally through the micropipette; in the $A\beta_{1\text{-}42}$ plus vehicle, $A\beta_{1\text{-}42}$ plus montelukast 1.0 mg/kg and $A\beta_{1\text{-}42}$ plus montelukast 2.0 mg/kg groups, PBS (5 μL) with $A\beta_{1\text{-}42}$ (410 pmol) was injected bilaterally 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. Mice started receiving intragastric administration 72 h after ICV infusion. Montelukast was dissolved in 0.5% sodium carboxymethyl cellulose (CMC-Na). After 4 weeks of drug treatment, one part of the mice was submitted to behavioral tests, while the rests were sacrificed by cervical dislocation, and the brain tissues were taken out for assays of $CysLT_1R$, NF-κB p65, TNF-α, IL-1β, caspase-3, and Bcl-2.

2.3. Morris water maze (MWM) task

Spatial memory was assessed by the MWM task, which consisted of 5-days training (visible and invisible platform training sessions) and a probe trial on day 6. This was carried out as described previously (Jiang et al., 2012). Mice were individually trained in a circular pool (120 cm diameter, 50 cm height) filled 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 fixed throughout the 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 below the surface of the water and was indicated by a small flag (height, 5 cm). 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 below the surface of the water and the flag was removed. The platform was always in the same place. On each day, the animal was subjected to four trials with a 1-h interval between trials. Each trial lasted for 90 s or 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 started, during which animals had 90 s to search for the platform. The time spent in the target quadrant (i.e., the quadrant where the platform was previously located) and the number of platform location crossings was recorded. Data of the escape latency, the percentage of time spent in the target quadrant and the number of platform location 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).

2.4. Y-maze test

The Y-maze was performed as descried previously (Tang et al., 2013). It was constructed of black plastic walls (height, 10 cm), consisting of three compartments (10 cm × 10 cm) connected with passages, with the floor of 3.175 mm stainless steel rods (8 mm apart). 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 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 number of correct choices during the 10 trials and the latency to enter the shock-free compartment were recorded manually.

2.5. Western blot analysis

Mouse hippocampus and cortex were chopped into small pieces, homogenized in 0.5 ml of RIPA buffer (50 mM Tris–HCl (pH7.4), 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS). The dissolved proteins were collected from the supernatant after centrifugation at 12,000 *g* for 15 min. Protein concentrations were determined using Coomassie blue-based assay reagent and then assessed for expression of $CysLT_1R$, NF-κB p65, TNF-α, IL-1β, caspase-3 and Bcl-2 proteins. Protein extracts were separated by an SDS-polyacrylamide gel electrophoresis and then transferred onto a PVDF membrane. The membrane was blocked with 5% skim milk in Tris buffer saline and then incubated at 4 °C overnight with respective primary antibodies for anti-$CysLT_1R$ antibody (1:1000), anti-NF-κB p65(1:1000), anti-TNFα (1:500), anti-IL-1β (1:1000), anti-pro-caspase-3 or cleaved caspase-3 (1:1000), anti-Bcl-2(1:1000), or β-actin (inner control, 1:3000). After washing with TBST, the membranes were incubated with a horseradish peroxidase conjugated secondary antibody (1:5000) for 2 h at room temperature. The antibody-reactive bands were visualized by using the enhanced chemiluminescence detection reagents by a gel imaging system (Tanon Science & Technology Co., Ltd., Shanghai, China).