Protective effect of pranlukast on Aβ<sub>1-42</sub>-induced cognitive deficits associated with downregulation of cysteinyl leukotriene receptor 1

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Abstract

Deposition of extracellular amyloid-β (Aβ) peptide is one of the pathological hallmarks of Alzheimer’s disease (AD). Accumulation of Aα is thought to associate with cognition deficits, neuroinflammation and apoptosis observed in AD. However, effective neuroprotective approaches against Aβ neurotoxicity are unavailable. In the present study, we analysed the effects of pranlukast, a selective cysteinyl leukotriene receptor 1 (CysLT1R) antagonist, on the impairment of learning and memory formation induced by Aβ and the probable underlying electrophysiological and molecular mechanisms. We found that bilateral intra hippocampal injection of Aβ<sub>1-42</sub> resulted in a significant decline of spatial learning and memory of mice in the Morris water maze (MWM) and Y-maze tests, together with a serious depression of in vivo hippocampal long-term potentiation (LTP) in the CA1 region of the mice. Importantly, this treatment caused significant increases in CysLT1R expression and subsequent NF-κB signaling, caspase-3 activation and Bcl-2 downregulation in the hippocampus and prefrontal cortex. Oral administration of pranlukast at 0.4 or 0.8 mg/kg for 4 wk significantly reversed Aβ<sub>1-42</sub>-induced impairments of cognitive function and hippocampal LTP in mice. Furthermore, pranlukast reversed Aβ<sub>1-42</sub>-induced CysLT1R upregulation, and markedly suppressed the Aβ<sub>1-42</sub>-triggered NF-κB pathway, caspase-3 activation and Bcl-2 downregulation in the hippocampus and prefrontal cortex in mice. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay confirmed its presence in the brain after oral administration of pranlukast in mice. These data disclose novel findings about the therapeutic potential of pranlukast, revealing a previously unknown therapeutic possibility to treat memory deficits associated with AD.

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Introduction

Alzheimer disease (AD) is the most common form of neurodegenerative dementia. AD has a complex and progressive pathological phenotype characterized by hypometabolism and impaired synaptic function, followed by pathological burden (Zawia et al., 2009). Amyloid-β (Aβ) deposition and neurofibrillary tangle formation are the major pathogenic mechanisms that act in concert to produce memory dysfunction and decline of cognition. The amyloid deposits accumulate first in isocortical areas, followed by limbic and allocortical structures including the entorhinal cortex and the 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 have shown 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 the Y-maze and the water maze (Maurice et al., 1996), which is a commonly used experimental model of AD. However, effective neuroprotective approaches against Aβ neurotoxicity are unavailable up to now.

To date, numerous studies have demonstrated that neuroinflammatory processes contribute to the pathogenesis of AD (Wyss-Coray, 2006; Cuenca-López et al., 2010; Krause and Mueller, 2010; Gorelick, 2010; Medeiros et al., 2010; Zotova et al., 2010). It has been shown that inflammatory cytokines chemokines, proteases and reactive oxygen species can augment Aβ formation (Guo et al., 2002; Giunta et al., 2008; Jimenez et al., 2008; Krause and Mueller, 2010). Treatment that blocks the inflammatory responses to Aβ reverses Aβ-induced memory deficits (O’Hare et al., 2011; Wang et al., 2011;
Cysteinyl leukotrienes (including LTD4, LTA4 and LTE4), 5-lipoxygenase (5-LO) metabolites of arachidonic acid, are potent inflammatory mediators involved in astrocyte proliferation (Ciccarelli et al., 2004; Huang et al., 2008), brain cryoinjury, brain tumours and acute neuronal injury after focal cerebral ischemia in mice (Zhang et al., 2004, 2006; Dinq et al., 2007). The pro-inflammatory actions of cysteinyl leukotrienes are mediated by two subtypes of cysteinyl leukotriene receptors (CysLT1 and CysLT2 receptors) (Singh et al., 2010). In the central nervous system (CNS), the role of 5-LO in the pathogenesis of AD has been demonstrated (Chu and Praticò, 2011a, b; Manev et al., 2011; Chu et al., 2012). We recently reported that intracerebral infusions of LTD4 impaired memory, with obvious expression of CysLT1R in the hippocampus and cortex of mouse brains, and LTD4 also induced generation of Aβ by a CysLT1R-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 CysLT1R plays in Aβ neurotoxicity. It is of interest to know whether Aβ-induced memory deficit is involved in CysLT1R, and whether pharmacological blockade of CysLT1R prevents or reverses Aβ-induced memory deficit, neuronal inflammation or apoptosis.

Materials and methods

Animals

Male ICR mice (c. 2 months old, weighing 22–25 g; Yangzhou University Medical Center, China) were used for the experiments. All experiments were carried out according to NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised, 1996). The procedures were approved by the Animal Care and Use Committee of China Pharmaceutical University. All animals were maintained on a 12 h light/dark cycle with free access to water and standard laboratory chow.

Materials

Aβ1-42 was purchased from Sigma Aldrich (USA). Pranlukast was purchased from Jiangsu Hengrui Medicine Co. Ltd (China). Antibodies were purchased from different companies: anti-CysLT1R from Santa Cruz Biotechnology, Inc. (Germany), anti-NF-κB p65, anti-pro or cleaved caspase-3 and anti-Bcl-2 from Cell Signaling Technology, Inc. (USA), anti-β-actin, anti-Histone H3 and secondary antibodies from Bioworld Technology Co. Ltd (USA). All other chemicals were of analytical grade and commercially available. Aβ1-42 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 d prior to administration, as described previously (Russo et al., 2012).

Stereotoxic intrahippocampal Aβ1-42 infusion and drug treatments

ICR Mice were anesthetized with the intraperitoneal injection of 350 mg/kg chloral hydrate and then immobilized on a stereotactic frame (SR-5, Narishige, Japan). The dura 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 2.0 mm caudal to bregma, 1.5 mm from the midline, and 2.0 mm below the dural surface (Paxinos and Franklin, 2003). PBS (5 μl) with or without Aβ1-42 (410 pmol/mouse) was bilaterally injected into the hippocampus (1 μl/min for all the infusions). The micropipettes were left in place for 5 min to minimize back-flux of liquid. After surgery, mice were housed individually and allowed to recover for 3 d.

Three days after the surgery, the mice were orally administered with pranlukast (0.4 and 0.8 mg/kg; 0.2 ml/10 g body weight) or 0.5% sodium carboxymethyl cellulose solution (0.5%CMC-Na) every day for 4 wk. After treatment, one group of mice was submitted to the Morris water maze (MWM) task for 6 d, a further group was tested for hippocampal LTP and a third group was sacrificed by cervical dislocation and the brain tissues were taken out for assays of CysLT1R, NF-κB p65, caspase-3 and Bcl-2.

Morris water maze task

Spatial memory was assessed by the MWM test, which consisted of 5 d 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 centre 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 (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 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 unless the animal reached the platform first. The time (escape latency) that elapsed until the mouse reaches the platform was noted. 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 time spent in the target quadrant, the number of platform location crossings and swim 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).

Y-maze test
This was performed as described previously (Tang et al., 2013). The Y-maze was constructed of black plastic walls (10 cm high), consisting of three compartments (10×10 cm) connected with passages (4×5 cm), with the floor of 3.175 mm stainless steel rods (8 mm apart). The test was conducted for two 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 10 times following the same procedures as on day 1. The number of correct choices out of 10 and the latency to enter the shock-free compartment on day 2 were recorded manually.

In vivo hippocampal long term potentiation (LTP) recording
Mice were anesthetized with an intraperitoneal injection of 1.5 g/kg urethane and then immobilized on a stereotactic frame (SR-5, Narishige, Japan). The electrophysiological recording procedure was performed as described in the previous study (Yang et al., 2011). A stimulating electrode was placed in the perforant path (PP) and a recording glass pipette was inserted into the DG of the dorsal hippocampus. Electrodes (tungsten with Teflon coating; Bilaney) were implanted at coordinates 2 mm caudal to bregma, 1.4 mm from the midline and 1.5 mm below the dural surface for the recording electrode, and for the stimulating electrode, 3.8 mm caudal to bregma, 3.0 mm from the midline and 1.5 mm below the dural surface. Evoked responses were amplified, filtered at 1/60 Hz, 100 μs pulses of 0.3 mA until the appearance of a population spike (PS). Before each experiment, input–output curves were generated to determine the maximal amplitude of the PS, and the intensity of stimulus was set at a level that evoked a PS at 55–65% of the maximum amplitude. The amplitude of the PS was measured and averaged every 5 min. LTP was induced by applying high-frequency stimulation (HFS) of 8 sessions of 400 μs stimuli at 400 Hz repeated three times at a 10 s interval. All recording and stimulation was performed using an on-line computerized oscilloscope–stimulator and data analysis interface system. The percentage of the ratio of absolute PS amplitude to basal value was used to represent the level of PS amplitude. LTP was measured as normalized PS amplitude (%).

Western blot analysis
Mouse hippocampus and prefrontal cortex were chopped into small pieces and homogenized in ice-cold RIPA buffer containing 0.1% phenylmethylsulfonyl fluoride. The dissolved proteins were collected from the supernatant after centrifugation at 12000 g for 15 min. Protein concentrations were determined using Coomassie blue-based assay reagent and then assessed for expression of CysLT1R, pro- or cleaved caspase-3 and Bcl-2 proteins. Protein extracts were separated by a 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-CysLT1R antibody (1:1000), anti-pro or cleaved caspase-3 antibody (1:1000), anti-Bcl-2 antibody (1:1000), or β-actin (inner control, 1:2000). After washing with tris buffered saline–Tween 20 (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 enhanced chemiluminescence detection reagents and a gel imaging system (Tanon Science & Technology Co., Ltd., China).

Nuclear extracts were prepared using nucleoprotein extraction kit (Sangon Biotech, China). Briefly, mouse hippocampus and prefrontal cortex were chopped into small pieces, homogenized in ice-cold hypotonic buffer containing 0.5% phosphatase inhibitor, 1% phenylmethylsulfonyl fluoride and 0.1% DL-dithiothreitol, then centrifuged at 4 °C, 3000 g for 5 min. The precipitate was washed with hypotonic buffer and centrifuged at 4 °C, 5000 g for 5 min. Finally, 0.2 ml lysis buffer containing 0.5% phosphatase inhibitor, 1% phenylmethylsulfonyl fluoride and 0.1% DL-dithiothreitol were add into the precipitate, chilled for 20 min and centrifuged at 4 °C, 15000 g for 10 min. The supernatant nuclear protein extract was subjected to Western blot for assay of NF-xB p65, and histone H3 was used as a loading control.
Reverse transcription-polymerase chain reaction (RT-PCR) assay

Total RNA was extracted from the mouse hippocampus and prefrontal cortex using Trizol reagents following the procedures described in the manufacturer’s instructions. For cDNA synthesis, aliquots of total RNA (2 μg) were mixed with 0.2 μg random hexamer primer, 20 U RNasin, 1 mmol/l dNTP, and 200 UM-MuLV reverse transcriptase in 20 μl of the reverse reaction buffer. The mixture was incubated at 25 °C for 10 min, 42 °C for 60 min and then at 72 °C for 10 min to deactivate the reverse transcriptase. PCR was performed on an Eppendorf Master Cycler (Eppendorf, Germany). The mixture contained: 1 μl RT-cDNA template dissolved in 20 μl reaction mixture containing 1× PCR buffer, 200 mmol/l dNTP, 1.5 mmol/l MgCl2, 20 pmol of each primer and 0.5 U Taq DNA polymerase. Cycling parameters were as below: 94 °C for 1 min, followed by 33 cycles of 94 °C for 30 s, 63 °C for 30 s and 72 °C for 1 min, with a final extension step of 72 °C for 7 min. The abundance of transcripts in cDNA samples was measured by RT-PCR with the following primers: mouse CysLT1R forward 5′-ATTCCTGGAACATGAAATGG-3′ and reverse 5′-CATTGTCTGCACGTAGATGAG-3′ (1062 bp, nucleotides 419–1480 in NM_021476.4, GeneBank), β-actin forward 5′-TCTTGGTATGGAATCCTGTG-3′ and reverse 5′-ATCTCCTTCTGCATCCTGTCA-3′ (154 bp, nucleotides 876–1029 in NM_007393.3, GeneBank). The amplification products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide and photographed. The optical density of the bands was determined by an image analysis system (Tanon Science & Technology Co. Ltd, China).

Determination of pranlukast concentration in brain or blood samples by the LC-MS/MS

Mouse brain tissue, hippocampus or prefrontal cortex was chopped into small pieces and homogenized in H2O (1:3, g/ml). The homogenate or plasma was mixed with acetonitrile containing chlorzoxazone (1:3, ml/ml), and then centrifuged at 16000 r/min at 4 °C for 10 min. The supernatant was provided for LC-MS/MS assay. The LC-MS/MS system consisted of an API4000 and an HPLC system. The HPLC system included a LC20AD binary pump system, a SIL20AD autosampler and a CTO 40A oven (Shimadzu, Japan). The analytical column was Shimadzu Shim-pack VP-ODS (150×2.0 mm). The mobile phase consisted of water containing 0.01% ammonia water (mobile phase A) and methanol (mobile phase B) with a flow rate at 0.30 ml/min. An aliquot of 5 μl of sample solution was injected. The analytes were separated by gradient elution. The initial composition was 5% (mobile phase B) for 0.5 min, then increased to 80% in 0.5 min, and maintained at 80% from 1.0 to 3.5 min, followed by change back to the initial condition in 0.5 min and re-equilibrated at 5% (mobile phase B) for 1.0 min. Each run time was 5.0 min. The temperature of the column was set at 40 °C. Mass spectra were acquired by the API 4000 MS/MS system equipped with an electro-spray ionization (ESI) interface with a turbo spray ion source. The ion spray voltage was set at 4500 V (negative) and the temperature was maintained at 400 °C. The nebulizing gas was high purity nitrogen, and gas 1 and gas 2 were set at 35 and 30, respectively. Curtain gas and CAD were 10 and 5, respectively. The quantification of pranlukast was performed in MRM mode with a dwell time of 0.2 s for each transition. Target ions were monitored at m/z 479.8 for pranlukast and m/z 167.9 for chlorzoxazone (internal standard), the corresponding product ions were at m/z 423.8 and 132.0, respectively. The collision energy was set at −30 eV, while the declustering potential was −50 eV. The peak area ratio of drug to internal standard, and the concentrations, were calculated by analyst software (v.1.5.1, AB SCIEX, USA). The lower limit of quantification was 1 ng and the linear range was 1–1×10^3 ng. The results were expressed as ng/ml for plasma samples or ng/g for brain samples. The analysis method has been verified by methodology, with specific and high sensitivity, and the analysis result accords with requirements of biological sample analysis.

Statistical analysis

All data were normally distributed and are presented as means±SEM. In the case of single mean comparison, data were analysed by a Student’s test. In the case of multiple mean comparisons, the data were analysed by ANOVA and the Newman–Keuls post test, or two-way repeated measures ANOVA, followed by Bonferroni multiple comparison tests. p values less than 0.05 were regarded to reflect a significant difference.

Results

Pranlukast improves Aβ42-induced cognitive deficits in mice

To determine effects of pranlukast on Aβ42-induced cognitive deficit, the MWM tests were performed for evaluation of cognitive function in mice. We first assessed the performance of mice in a non-spatial visible-platform variant of the MWM to test for baseline differences in vision and motivation among treatment groups. Mice in each group exhibited similar escape latency in the visible-platform test, suggesting no influence of Aβ42 or CysLT1R antagonist pranlukast on vision or basal motivation of mice (F5,383=1.231, p=0.294; Fig. 1(a)). We then tested the mice in the spatial hidden-platform variant; the data showed that Aβ42 without drug treatment increased escape latencies compared to the corresponding controls (p<0.05; Fig. 2(b)), these were reversed by pranlukast (0.4 or 0.8 mg/kg) (p<0.05 or p<0.001; Fig. 2(b)) (4 trials/d for 3 d; F5,575=4.188, p=0.001). In the probe trial, a putative measure of spatial learning and memory
retention, all the mice showed preference for the target quadrant, with the exception of the mice in the Aβ1–42 plus vehicle group, which displayed a significant decrease in the time in the target quadrant ($F_{1,15} = 101.060, p<0.001$; Fig. 1(c)) and the number of platform location crossings ($F_{1,15} = 16.162, p=0.001$; Fig. 1(d)) compared to the control. In contrast, mice in the Aβ1–42 plus pranlukast (0.4 or 0.8 mg/kg) group showed significant increases in both indices compared to Aβ1–42 alone ($p<0.05$ for the time and $p<0.05$ for the number; Fig. 1(c, d)). In addition,
increased the number of correct choices compared to latency to enter the shock-free compartment and min post-HFS was summarized as the column in (±16.61% and 200.23±11.56%, respectively; mice treated with pranlukast at 0.4 or 0.8 mg/kg (200.95β). Pranlukast reverses Aβ4.81%) after the tetanic stimulation persisting for at enter the shock-free compartment; improved Aβ42-induced learning and memory impair-

Since our behavioural analysis identified that pranlukast improved Aβ1-42-induced learning and memory impairment, we next examined the hippocampal LTP, a well-established form of synaptic plasticity and the most intensely studied cellular model for memory (Malenka and Nicoll, 1999; Malenka, 2003). The LTP recording showed that hippocampal LTP was significantly inhibited in Aβ1-42-injected mice treated with vehicle (141.76±4.81%) after the tetanic stimulation persisting for at least 60 min compared to the controls (204.25±14.03%) (p<0.001) and markedly increased in Aβ1-42-induced mice treated with pranlukast at 0.4 or 0.8 mg/kg (200.95±16.61% and 200.23±11.56%, respectively; p<0.001; Fig. 2(a, b)). These results were consistent with the enhanced hippocampus-dependent learning and memory in these mice revealed by the behaviour tests.

**Pranlukast reverses Aβ1-42-induced CysLT1R upregulation**

Next, we considered whether the protective effects of pranlukast on Aβ1-42-induced memory deficits are involved in CysLT1R. We detected CysLT1R protein and mRNA levels in the hippocampus and prefrontal cortex using Western blot and RT-PCR, respectively. Interestingly, both protein and mRNAs of CysLT1R were significantly increased in both hippocampus and prefrontal cortex in bilateral intrahippocampal Aβ1-42-injected mice, whereas chronic treatment of pranlukast at 0.4 or 0.8 mg/kg completely reversed CysLT1R upregulation, but treatment of pranlukast alone did not influence basal expression of CysLT1R (F5.17=9.723, p<0.001 for hippocampus and F5.17=31.154, p<0.001 for prefrontal cortex in Western blot; F5.17=21.612, p<0.001 for hippocampus and F5.17=22.727, p<0.001 for prefrontal cortex in RT-PCR; Fig. 3(a-d)).

**Pranlukast suppresses Aβ1-42-activated NF-κB signaling**

Recent studies have showed that CysLT1R mediates inflammatory response via the NF-κB pathway (Hashimoto et al., 2009), and Aβ1-42 activated the NF-κB pathway by selectively inducing the nuclear translocation of p65 and p50 subunits (Valerio et al., 2006). Here, we are curious about whether Aβ1-42-activated NF-κB is involved in CysLT1R. Consistent with previous studies, Aβ1-42 activated the NF-κB pathway, characterized by a p65 subunit increment in the nuclear. Interestingly, CysLT1R antagonist pranlukast (0.4 or 0.8 mg/kg) treatment was able to block Aβ1-42-activated NF-κB signaling in the brain (F5.17=7.638, p=0.002 for hippocampus and F5.17=7.801, p=0.002 for prefrontal cortex; Fig. 4(a, b)).

**Pranlukast inhibits Aβ1-42-triggered caspase-3 activation and Bcl-2 downregulation**

Apoptosis is a tightly regulated process which involves pathological changes in AD (Awasthi et al., 2005). To evaluate whether pranlukast play an anti-apoptotic role in Aβ-induced neurotoxicity, we examined caspase-3, which is known to be a crucial mediator of apoptosis through its protease activity, and the anti-apoptotic protein, Bcl-2, by Western blot analysis. Quantification of Western blot, based on the ratio of caspase-3 fragment to procaspase-3, revealed that Aβ1-42 led to caspase-3 activation in the hippocampus and prefrontal cortex of mice as compared to vehicle. However, treatment with pranlukast (0.4 or 0.8 mg/kg) led to an attenuation in caspase-3 activation as compared with Aβ1-42 alone (F5.17=41.915, p<0.001 for hippocampus and F5.17=29.829, p<0.001 for prefrontal cortex; Fig. 5(a, b)). In addition, the level of Bcl-2 was significantly decreased in the hippocampus...
It is well known that the accumulation of Aβ in the brain induces neurotoxicity, which has been considered as the
The process of AD (Hardy and Allsop, 1991; Karran et al., 2011) is considered the central disease-causing and disease-promoting event in the disease process of AD (Tang et al., 2013; Wang et al., 2013). Here we demonstrated that a single microinfusion of Aβ1-42, a more neurotoxic Aβ species, into the hippocampus produced deficits of learning and memory accompanied by inflammatory and apoptotic responses, as evidenced by increased NF-κB p65, activated caspase-3 and decreased Bcl-2 in key regions involved in short- and long-term memories, namely the hippocampus and the frontal cortex; it also concurrently increased CysLT1R expression in both of those regions. Blockade of CysLT1R by repeated treatment with pranlukast reversed these effects of Aβ1-42. These results suggest that the CysLT1R antagonist pranlukast reverses Aβ1-42-induced cognitive deficits, which are due, at least partially, to neuronal inflammation and apoptosis mediated by CysLT1R signaling.

The CysLT1R is a high affinity G protein-coupled receptor (GPCR) for the pro-inflammatory and pro-amyloidogenic inflammatory mediator LTD4 that is implicated in many inflammatory conditions (Serhan et al., 1996; Funk, 2001). CysLT1R antagonist, pranlukast, is currently used as a treatment for asthma. CysLT1R mRNA has been found to be upregulated in neurons, microphages and proliferated astrocytes (Ciccarelli et al., 2004; Fang et al., 2006), suggesting a possible regulatory role in the mediation of different phases of neuro-inflammation. It was also reported that CysLT1R antagonists, such as pranlukast and montelukast, showed neuroprotective potential against acute and chronic ischemic brain injury in rats and mice.
Pranlukast reversed both Aβ1–42-induced upregulation of CysLT1R and the CysLT1R-induced inflammatory response, as indicated by increased NF-κB p65 in the hippocampus and prefrontal cortex. Since NF-κB signaling enhances apoptosis (Tusi et al., 2010) and inhibition of NF-κB not only protracts inflammatory responses but also prevents apoptosis (Lawrence et al., 2001), it was of interest to know whether pranlukast altered apoptotic responses induced by Aβ1–42. A variety of molecules have been shown to be involved in the cellular apoptosis machinery; these include p53 (Haupt et al., 1997; Ryan et al., 2001), the Bcl-2 family (Dimmeler et al., 1999; Cory and Adams, 2002), caspase members (Suzuki et al., 2001) and p38 MAP kinase (Wang et al., 2005). Among them, proapoptotic protein caspase-3 and anti-apoptotic protein Bcl-2 are the most important molecules in regulating apoptosis. Aβ1–42 enhanced caspase-3 activation, indicated by the ratio of caspase-3 fragments to pro-caspase-3, and increased Bcl-2, leading to increases in apoptotic responses in the hippocampus and prefrontal cortex. At the same doses that blocked Aβ1–42-induced deficits of memory, CysLT1R signaling and subsequent NF-κB signaling, pranlukast also reversed the Aβ1–42-induced Bcl-2 decrease and caspase-3 activation. Therefore, pranlukast may exhibit a potent anti-apoptotic effect, which contributes to the blockade of apoptotic responses induced by Aβ1–42. In addition, the anti-inflammatory property of pranlukast may also play a role in this process, given the close link between inflammation and apoptosis (Bradl and Hohlfeld, 2003; Dorr et al., 2005) and the involvement of NF-κB signaling in apoptosis (Lawrence et al., 2001; Tusi et al., 2010).

In conclusion, this study underlines the importance of the CysLT1R and its modulation by pranlukast in Aβ1–42-induced cognitive dysfunction. The effective neuroprotective action of pranlukast against neurotoxic Aβ strongly suggests that blockade of CysLT1R signaling in the brain, such as by the application of pranlukast, may be a novel and promising strategy to ameliorate the learning and memory deficits in neurodegenerative diseases such as AD.

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Statement of Interest
None.

References


