# Neuroprotective effects of CysLTR antagonist on *Streptococcus pneumoniae*-induced meningitis in rats

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**Abstract.** Cysteinyl leukotrienes (CysLTs) modulate central nervous system inflammatory responses via their receptors, $CysLT_1R$ and $CysLT_2R$. It has been demonstrated that CysLTR participates in the infection process of *Streptococcus pneumoniae* (SP)-induced meningitis. In the present study, the effects and possible underlying mechanisms of CysLTR antagonists (pranlukast and HAMI 3379) on SP meningitis were further determined. SP meningitis was induced by intracerebroventricular injection of serotype III SP in Sprague-Dawley rats which were administrated intraperitoneally with 0.1 mg/kg antagonists. The clinical disease status of rats was evaluated by body weight and behavioral changes with neurological scoring. Survival neuron density, activated microglial and astrocytes were assessed by Nissl staining and immunohistochemical staining. The expression levels of inflammatory cytokines and NLRP3 inflammasome were detected by reverse transcription-quantitative PCR and western blotting, respectively. Pranlukast and HAMI 3379 treatment markedly alleviated the clinical disease status, which was manifested by improving body weight loss and neurological deficit. Furthermore, pranlukast and HAMI 3379 treatment ameliorated neuronal injury and inhibited microgliosis and astrogliosis. In addition, significant downregulation of inflammatory cytokines and NLRP3 expression was observed in pranlukast and HAMI 3379-treated rats. These *in vivo* findings indicated the neuroprotective effects of CysLTR antagonists against experimental SP-induced meningitis, and the mechanism of anti-inflammatory effects may partly be by inhibiting NLRP3 inflammasome overactivation.

## Introduction

Bacterial meningitis (BM) is an impacting infectious disease of the central nervous system. The annual incidence of BM in China ranged from 6.95 to 22.30 cases in children under five, and 1.84 to 2.93 cases per 100,000 population overall between 2006 and 2009 (1). BM is commonly caused by *Streptococcus pneumoniae* (SP) in 50 to 70% of cases (2). Pneumococcal meningitis has a high mortality rate, and almost half of the survivors suffer from long-term disabling sequelae, such as bilateral hearing loss cognitive and motor deficit (3). Therefore, it is of considerable clinical significance to investigate pathological mechanisms and discover therapeutic molecular targets.

The process of craniocerebral injury mediated by SP infection is regulated by multiple pattern recognition receptors (TLRs, NLRs and G-protein-coupled formyl peptide receptors), inflammatory factors and inflammatory mediators (4-6). Cysteinyl leukotrienes (CysLTs; $LTC_4$, $LTD_4$ and $LTE_4$) regulate the inflammatory responses in the 5-lipoxygenase (5-LOX) pathway through the arachidonic acid metabolism. It is predominantly produced by microglia, astrocytes and leukocytes. CysLTs modulate central nervous system inflammatory diseases primarily via $CysLT_1R$ and $CysLT_2R$; these are two G-protein-coupled receptors that have a role in the development of various inflammatory diseases of the central nervous system (7-9). $CysLT_1R$ has been revealed-*in vivo* and *in vitro*- to contribute to the HBMEC monolayer invasion and the blood-brain barrier penetration by bacteria that cause meningitis such as *Escherichia coli* (*E. coli*) and *group B streptococcus* (10,11). The $CysLT_1R$ antagonist montelukast effectively inhibits *Cryptococcus neoformans* (*C. neoformans*) penetration of the blood-brain barrier (BBB) and has neuroprotective effects on *C. neoformans* meningoencephalitis (12). A previous study revealed that CysLTR expression is upregulated with SP-induced meningitis (13). Taken together, these findings indicated that CysLTR may be critical in the pathogenesis of SP meningitis. Pyroptosis is a newly discovered type of inflammatory programmed cell death that is mediated by inflammasome and is dependent on the caspase-1 activation (14). The NLR family pyrin domain-containing 3 (NLRP3) inflammasome mediates the maturation of caspase-1 and secretion of interleukin (IL)-1β and IL-18 in the murine meningitis model induced by SP (15). Thus, the NLRP3 inflammasome plays an essential role in systemic inflammation regulation and brain injury development in pneumococcal meningitis.

In the present study, the effects of the $CysLT_1R$ antagonist pranlukast and the selective $CysLT_2R$ antagonist HAMI 3379 on the injury and inflammatory responses in a rat model of

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bacterial meningitis were evaluated by injecting SP into the posterior cistern of rats. Furthermore, the NLRP3 and caspase-1 expression changes in response to antagonists were determined. The present results provided a theoretical basis for further exploring the possible inflammatory regulation mechanism of CysLTR in SP-caused meningitis.

## Materials and methods

*Chemicals and bacterial strain*. Pranlukast (purity 99.89%) and HAMI 3379 (purity 98%) were purchased from Cayman Chemical Company. Cresyl violet was purchased from Sigma-Aldrich; Merck KGaA. All other reagents used in the experiment were of analytical grade.

The Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) provided the SP serotype III standard strain. The bacteria were cultured overnight at 37˚C on sheep blood agar plates under anaerobic conditions ($H_2$ and $CO_2$; 95:5, v/v). Then, selected single colonies were cultured overnight to logarithmic phase in broth at 37˚C and harvested by centrifugation at 2,500 x g for 10 min at 4˚C. Bacteria suspension was adjusted to $10^7$ colony forming units (CFU)/ml with sterile saline for intracisternal injection.

*Animal preparations*. A total of 80 male Sprague-Dawley rats (3 weeks-old, weighing 50-60 g) were provided by the Experimental Animal Center, Zhejiang Academy of Medical Sciences. All animals were kept in regular environmental conditions (20-24˚C, 12/12 h light/dark cycle) prior to testing, with free access to water and food. All experimental protocols were approved by the Laboratory Animal Care and Ethics Committee of Hangzhou Children's Hospital (Hangzhou, China; approval number 2021-04) and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize suffering of animals and reduce the number of animals used.

*Induction of SP meningitis rat models and drug administration*. The rats were divided into the following 4 groups (20 animals per each group): i) phosphate-buffered saline control (PBS group), ii) SP model (SP group), iii) pranlukast (SP + Pran at 0.1 mg/kg) and iv) HAMI 3379 (SP + HAMI 3379 at 0.1 mg/kg)-treated group. The 80 rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and their heads were fixed on the brain stereotaxic apparatus. Cerebrospinal fluid (CSF; 10 $\mu$l) was removed from all rats and a direct intracisternal injection of an equal volume of solution was conducted. PBS group rats were injected with sterile PBS and sacrificed on the fifth day after injection. Rats in the SP group were inoculated with SP ($1x10^7$ CFU/ml) and sacrificed at 5 days after inoculation. Pranlukast and HAMI 3379 were injected intraperitoneally to the rats 1 h following inoculation with SP on the first day, then once daily until the time of euthanasia. The same volume of saline (2 ml/kg) was injected intraperitoneally into PBS and SP rats at the same time points. To assess clinical disease status, the animals were monitored daily by measuring weight and evaluating neurological deficit score. The following scores were used to evaluate disease severity as previously described (16): 1 represented coma; 2 represented the rat did not turn upright when positioned on its back; 3 represented the rat turned upright within 30 sec; 4 represented the spontaneous rat activity decreased, turned upright within 5 sec; and 5 represented normal. At the designated endpoint, rats were anesthetized with sodium pentobarbital (50 mg/kg) and then euthanized by cervical dislocation.

*Histopathology and immunohistochemistry*. After neurological examination, rats were anesthetized as aforementioned, perfused transcardially with saline, then 4% paraformaldehyde in PBS and euthanized by decapitation. Brains were post-fixed in 4% paraformaldehyde for 24 h at 4˚C, incubated in 30% sucrose for 3 days at 4˚C and embedded in paraffin. Subsequently, 10 $\mu$m-thick slices of the coronal tissues were sliced on a CM 1900 cryomicrotomy (Leica Microsystems GmbH). The sections were stained with 1% cresyl violet for 20 min at room temperature to assess the neuronal organization of the brain as previously described (17). The stained specimens were observed and images were captured using a fluorescence microscope (BX51; Olympus Corporation). The neurons in hippocampus and cortex were calculated using ImageJ 2.0 software (National Institutes of Health).

To investigate the activation of microglial and astrocyte, immunohistochemistry was performed for Iba-1 (1:200; cat. no. 10904-1-AP), a biomarker of macrophages/microglia, and GFAP (1:200; cat. no. 16825-1-AP, both from ProteinTech Group, Inc.), a biomarker of astrocytes. The brain cryo-sections were prepared and immunohistochemical assays were performed as previously described (13). The numbers of Iba-1 and GFAP positive cells were counted using a light microscope and analyzed by ImageJ 2.0 software (National Institutes of Health).

*Reverse transcription-quantitative (RT-q) PCR analyses*. Using TRIzol® reagent (Takara Bio, Inc.), total RNA was extracted from CSF cells following the manufacturer's protocol. Subsequently, total RNA was reverse transcribed to cDNA using the Prime Script RT reagent kit (Takara Bio, Inc.), according to the manufacturer's protocol. The specific primers used for qPCR are listed in Table I. RT-qPCR was performed using SYBR Green fluorophore (Thermo Fisher Scientific, Inc.) on an Applied Biosystems 7500 System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions for qPCR were as follows: Initial denaturation at 95˚C for 10 min; then, 40 denaturation cycles at 95˚C for 15 sec; annealing and elongation at 60˚C for 60 sec. Cytokine mRNA relative expression level was analyzed using the $2^{-\Delta\Delta Cq}$ method (18) and normalized to the internal reference gene GAPDH.

*Western blot analysis*. Homogenized brain tissues were centrifuged at 12,000 x g for 30 min at 4˚C in ice-cold RIPA lysis buffer (cat. no. G2002; Wuhan Servicebio Technology Co., Ltd.). Protein concentration was determined using a BCA protein assay kit. Equal protein quantities (80 $\mu$g) were separated on 10% SDS-PAGE gels and then transferred onto polyvinylidene difluoride membranes. The membranes were incubated with the following primary antibodies: NLRP3

Table I. Primer sequences for quantitative PCR.

| Gene name | Primer sequence (5'→3') | Length (base pairs) |
|---|---|---|
| IL-1β | F: TGACCTGTTCTTTGAGGCTGAC<br>R: CATCATCCCACGAGTCACAGAG | 272 |
| TNF-α | F: CCAGGTTCTCTTCAAGGGACAA<br>R: GGTATGAAATGGCAAATCGGCT | 80 |
| IL-6 | F: AGGATACCACCCACAACAGACC<br>R: TTGCCATTGCACAACTCTTTTC | 109 |
| IL-10 | F: CACTGCTATGTTGCCTGCTCTT<br>R: GTCTGGCTGACTGGGAAGTGG | 100 |
| IL-18 | F: TCAGACCACTTTGGCAGACTTC<br>R: GATTCGTTGGCTGTTCGGTC | 134 |
| IFN-γ | F: CCAGGCCATCAGCAACAACATAA<br>R: CACCGACTCCTTTTCCGCTTC | 213 |
| GAPDH | F: CTGGAGAAACCTGCCAAGTATG<br>R: GGTGGAAGAATGGGAGTTGCT | 138 |

F, forward; R, reverse; IL, interleukin.

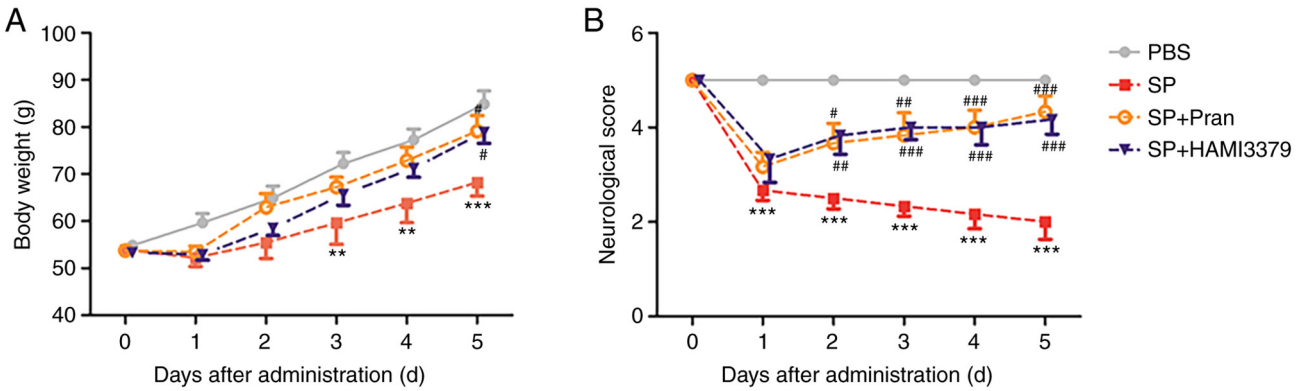


Figure 1. Effects of Pran and HAMI 3379 on body weight loss and neurological deficit scores. (A) The body weight and (B) neurological deficit scores of experimental rats were measured daily. Values are presented as the mean ± SEM (n=8). $^{**}$P<0.01 and $^{***}$P<0.001 vs. the PBS group and $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. the SP group. SP, *Streptococcus pneumoniae*; Pran, pranlukast.

(1:1,000; cat. no. 19771-1-AP; ProteinTech Group, Inc.), caspase-1 (1:300; cat. no. 22915-1-AP; ProteinTech Group, Inc.) and β-actin (1:2,000; cat. no. GB12001; Beyotime Institute of Biotechnology) overnight at 4˚C after blocking for 1 h at room temperature in 5% non-fat dry milk. Following the primary incubation, membranes were incubated with HRP-conjugated secondary antibody (13,000; cat. no. GB23302; Beyotime Institute of Biotechnology) for 1 h at room temperature. Odyssey infrared imaging system was utilized to detect the immunoblot (LI-COR Biosciences). Quantity One v4.6.2 analysis software was utilized to quantify the protein bands (Bio-Rad Laboratories, Inc.).

*Statistical analysis*. All values are presented as the mean ± SEM. One-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc analysis was performed using the Prism version 5 (GraphPad Software, Inc.) software to determine statistical significance. In all results, P<0.05 was considered to indicate a statistically significant difference.

## Results

*Effects of pranlukast and HAMI 3379 on the body weight and neurological deficit*. As revealed in Fig. 1A, the body weight of rats in the PBS group grew steadily while the rate of weight increase in the SP group significantly decreased from day 3 to day 5 after infection compared with the PBS group (P<0.01). The body weight loss was significantly improved after treatment with pranlukast or HAMI 3379 (0.1 mg/kg) on the fifth day compared with the SP group (P<0.05).

It was demonstrated that the neurological deficit scores were significantly decreased at 1 day after infection (Fig. 1B; P<0.001). The neurological deficit score of the pranlukast-treated group was significantly increased compared with the SP group from the second day (day 2, P<0.05; day 3, P<0.01; days 4 and 5, P<0.001). A significant improvement was also observed in the HAMI 3379-treated group compared with that in the SP group from the second day (day 2, P<0.01; days 3 to 5, P<0.001). These results indicated that pranlukast and

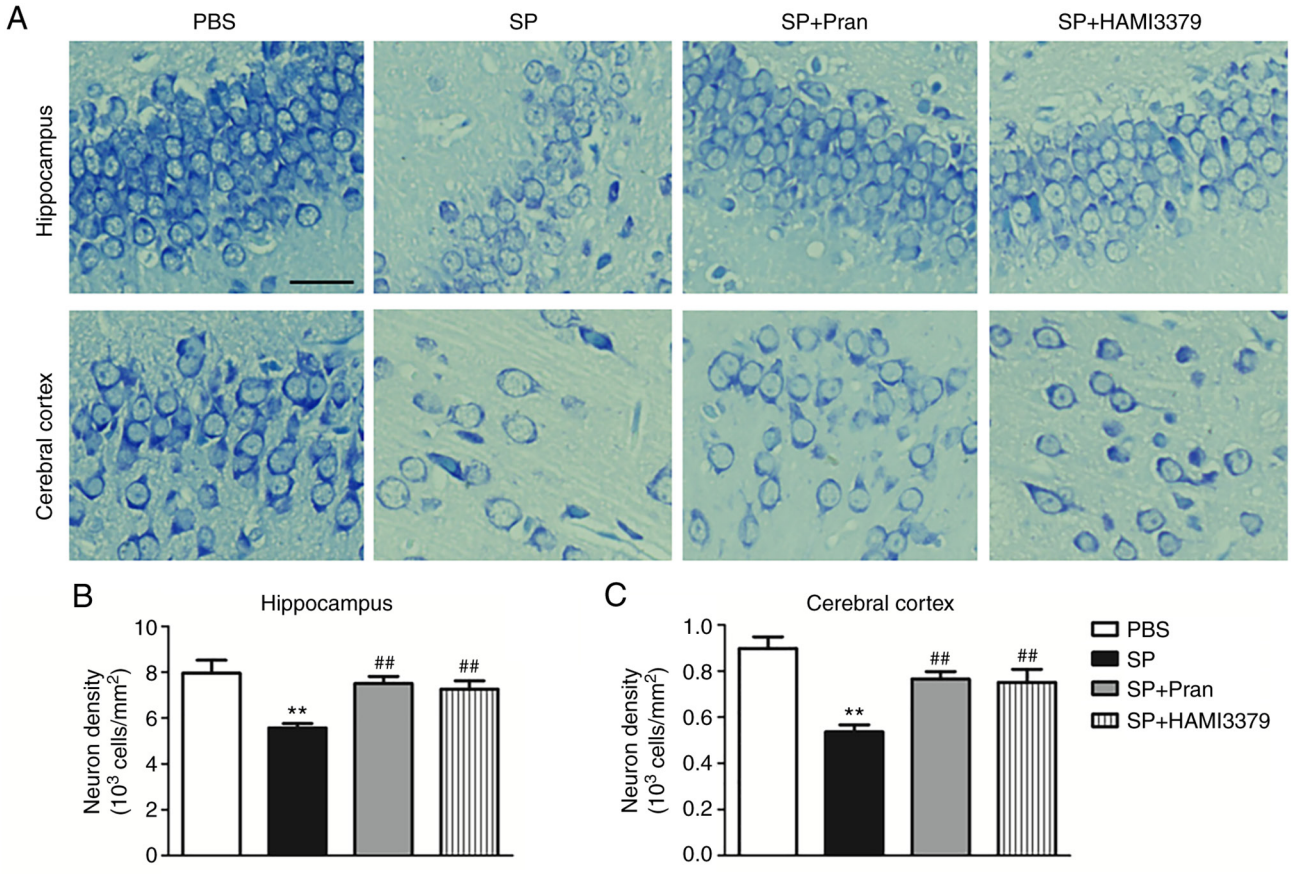


Figure 2. Effects of Pran and HAMI 3379 on neuronal density in the hippocampus and the cerebral cortex following intracisternal injection of SP. (A) Representative pictures of cresyl violet staining of hippocampus and cortex showing the morphology and density of the neurons. (B and C) Quantitative analysis of the density of cresyl violet staining in the hippocampus and the cortex. Values are presented as the mean ± SEM (n=8). $^{**}$P<0.01 vs. the PBS group and $^{\#\#}$P<0.01 vs. the SP group. Scale bar=50 $\mu$m. SP, *Streptococcus pneumoniae*; Pran, pranlukast.

HAMI 3379 had similar protective effects against SP-induced brain infection.

*Effects of pranlukast and HAMI 3379 on neuronal damage*. To assess the effect of pranlukast and HAMI 3379 on neuronal injury, the changes of neuronal damage in the hippocampus and cortex after treatment were observed (Fig. 2). Nissl staining revealed significant neuronal damage in the hippocampus and cortex of the SP group. Representative images showed that the neurons were disorderly arranged, cell spacing was widened and the Nissl bodies were shrunken or completely disappeared (Fig. 2A). Pranlukast and HAMI 3379 treatment significantly ameliorated the neuronal loss in the hippocampus and cortex at 5 days following administration of SP compared with the SP group (Fig. 2B; both P<0.01).

*Effects of pranlukast and HAMI 3379 on glial cells*. To determine whether pranlukast and HAMI 3379 affected SP-induced neuroinflammation, an analysis of microglia activation and astrogliosis response in the cerebral cortex was performed by staining with Iba-1 (Fig. 3) and GFAP (Fig. 4). As revealed in images captured using a light microscope, there were remarkable increases in the number of ramified Iba-1-positive microglial cells in the rats of the SP group in comparison with the PBS group (Fig. 3B; P<0.01). By contrast, pranlukast and HAMI 3379 administration caused a notable decrease in the number of Iba-1-positive cells (P<0.05) compared with the SP group and indicated a reduction of microglial activation (Fig. 3A). Meanwhile, GFAP-positive astrocytes in the SP group were activated with hyperplasia/hypertrophy (Fig. 4A); pranlukast and HAMI 3379 significantly inhibited the GFAP-positive astrocyte number increase in the cortex compared with the SP group (Fig. 4B; P<0.05).

*Effects of pranlukast and HAMI 3379 on the expression of inflammatory cytokines*. As revealed in Fig. 5, the mRNA expression of inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-10, IL-18 and IFN-γ) in CSF cells greatly increased after SP meningitis infection (P<0.05). Compared with the SP group, pranlukast significantly reduced the upregulation of mRNA expression of IL-1β and TNF-α, while HAMI 3379 markedly decreased mRNA expression of IL-1β, TNF-α and IFN-γ (P<0.05). The expression of IL-6, IL-10 was not affected by neither agent.

*Effects of pranlukast and HAMI 3379 on the expression of the NLRP3 inflammasome*. Western blot analysis revealed that the protein expression of NLRP3 and caspase-1 was significantly increased in the SP group compared with the PBS group (Fig. 6; P<0.05). Pranlukast diminished the expression of NLRP3. In addition, a significant decrease in the NLRP3 expression and caspase-1 was observed in HAMI 3379

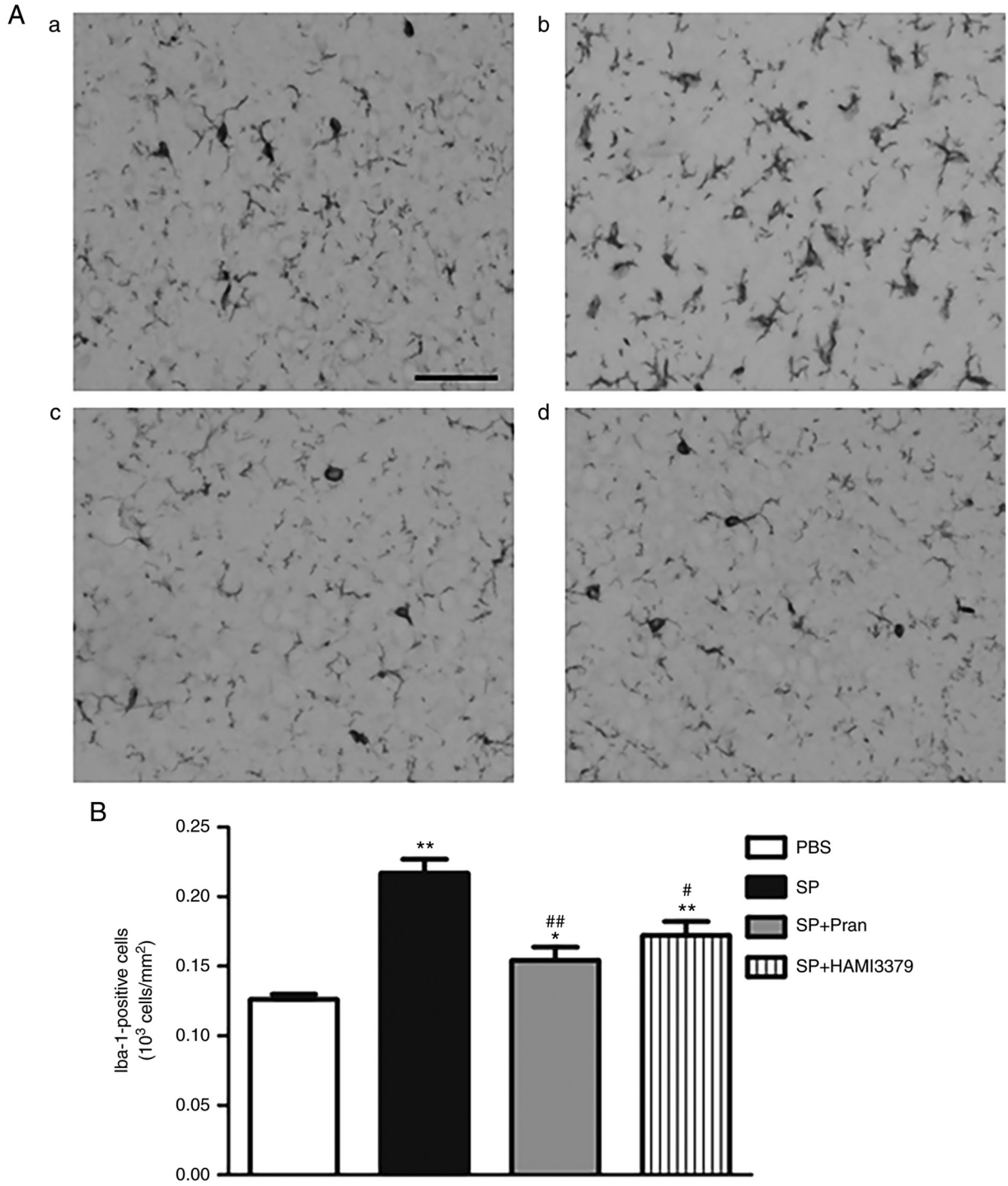


Figure 3. Effects of Pran and HAMI 3379 on microglia following intracisternal injection of SP. (A) Typical representative images revealing Iba1-immunopositive microglia in the cerebral cortex: (a) PBS group, (b) SP group, (c) SP + pranlukast group and (d) SP + HAMI3379 group. (B) The number of Iba1-immunopositive cells was calculated. Values are presented as the mean ± SEM (n=8). Scale bar=50 $\mu$m. $^{*}$P<0.05 and $^{**}$P<0.01 vs. the PBS group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the SP group. SP, *Streptococcus pneumoniae*; Pran, pranlukast.

treatment group compared with the SP group (P<0.05). These results suggested that the NLRP3 inflammasome activation in rats was suppressed by CysLT antagonists.

## Discussion

SP meningitis is a common central nervous system infectious disease in the pediatrics (19). The effects of CysLTR antagonists were assessed in a rat model of pneumococcal meningitis in the present study. The results showed that intraperitoneal injection of pranlukast and HAMI 3379 have neuroprotective effects on brain injury following SP meningitis in rats, which was manifested by improvement of neurological deficit function and body weight loss. Furthermore, these antagonists attenuated neuronal loss and inhibited microglia activation and astrocyte proliferation.

Numerous studies on human cases and pneumococcal meningitis model demonstrated that the development of

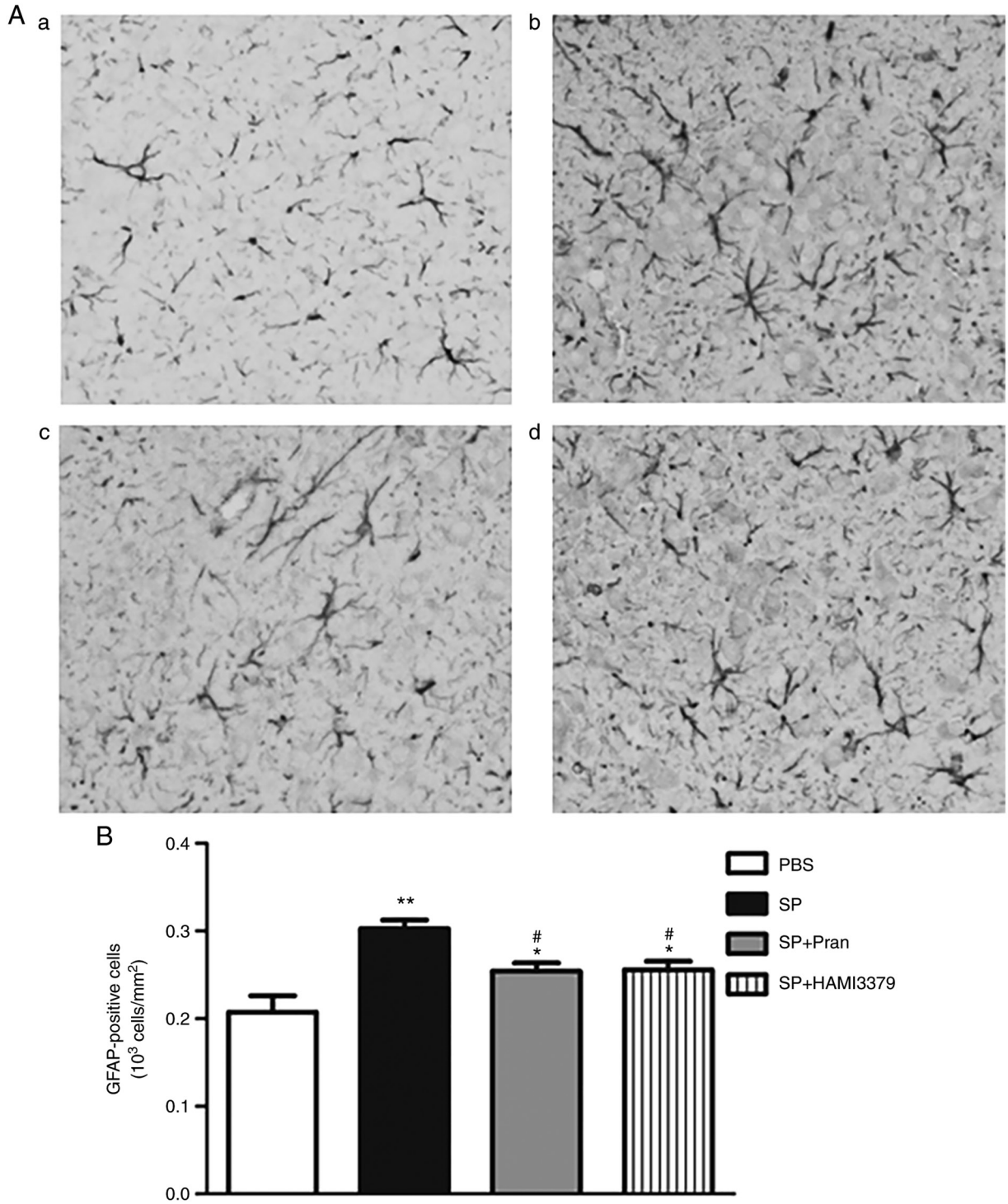


Figure 4. Effects of Pran and HAMI 3379 on astrocytes following intracisternal injection of SP. (A) Typical representative images demonstrating GFAP-positive astrocytes in the cerebral cortex: (a) PBS group, (b) SP group, (c) SP + pranlukast group, and (d) SP + HAMI3379 group. (B) The GFAP-positive cells number was calculated. Values are presented as the mean ± SEM (n=8). Scale bar=50 $\mu$m. $^{*}$P<0.05 and $^{**}$P<0.01 vs. the PBS group; $^{\#}$P<0.05 vs. the SP group. SP, *Streptococcus pneumoniae*; Pran, pranlukast.

cortical brain injury was strongly associated with diminished cerebral blood flow and cerebral blood volume (20-22). Cerebral ischemia secondary to intracranial infection is closely correlated with neurological sequelae or death in children (23). Previous studies showed that intraperitoneally-injected pranlukast and HAMI 3379 reduced the brain damage following acute and chronic cerebral ischemia with an effective dose of 0.1 mg/kg (24,25), thus 0.1 mg/kg was selected as the effective dosage in the present experiment. Based on a preliminary study, the pranlukast and HAMI 3379 time-dependent effect was examined. It was identified that the therapeutic time window was within 1 h after infection (data not shown). Continuous administration for 5 days after infection is to ensure the role of pranlukast and HAMI 3379 in the peak inflammatory phase of the brain. Using this regimen, the neurological deficit score was used to quantitatively reflect the

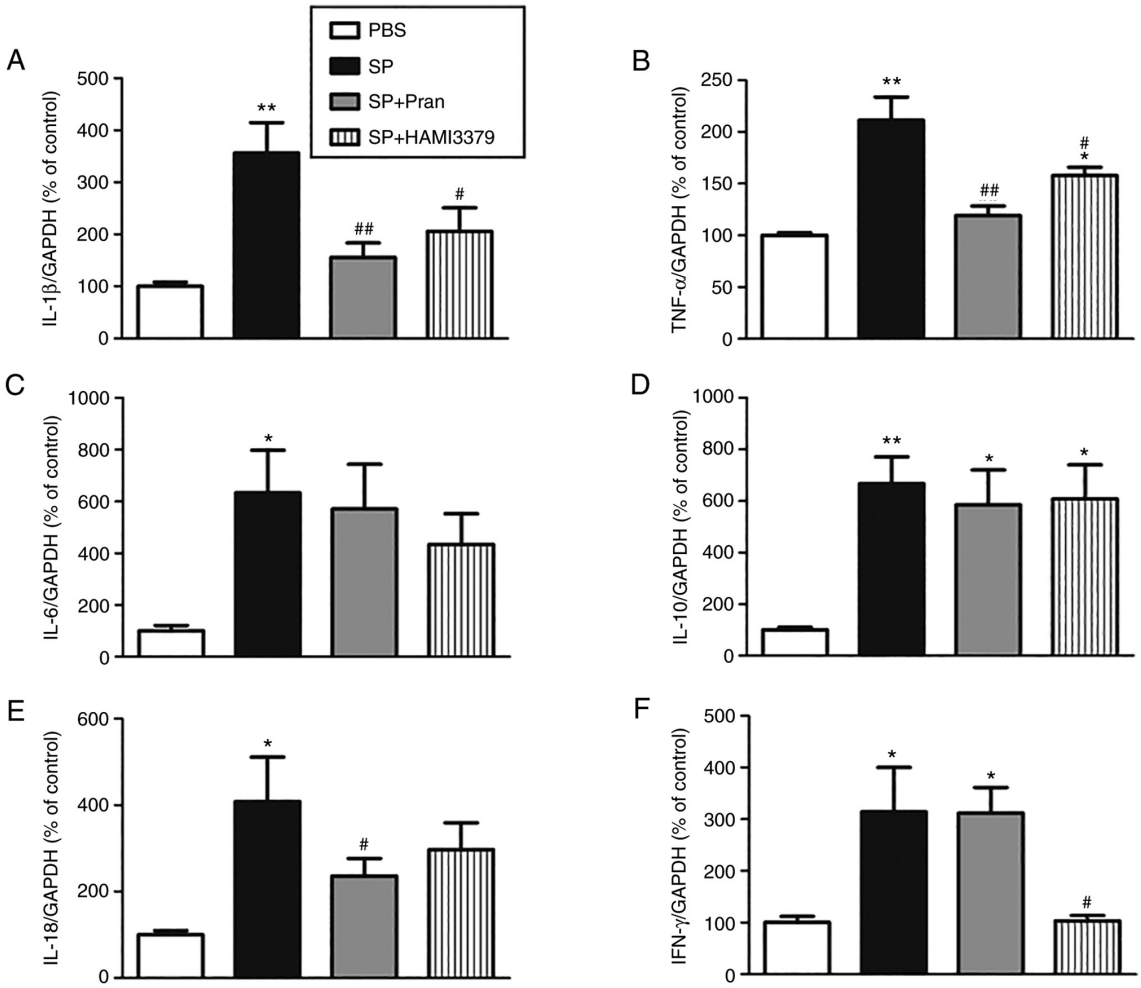


Figure 5. Effects of Pran and HAMI 3379 on the mRNA expression of inflammatory cytokines in brain tissue. (A) IL-1β, (B) TNF-α, (C) IL-6, (D) IL-10, (E) IL-18 and (F) IFN-γ. Values are presented as the mean ± SEM (n=8). $^{*}$P<0.05 and $^{**}$P<0.01 vs. the PBS group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the SP group. SP, *Streptococcus pneumoniae*; Pran, pranlukast.

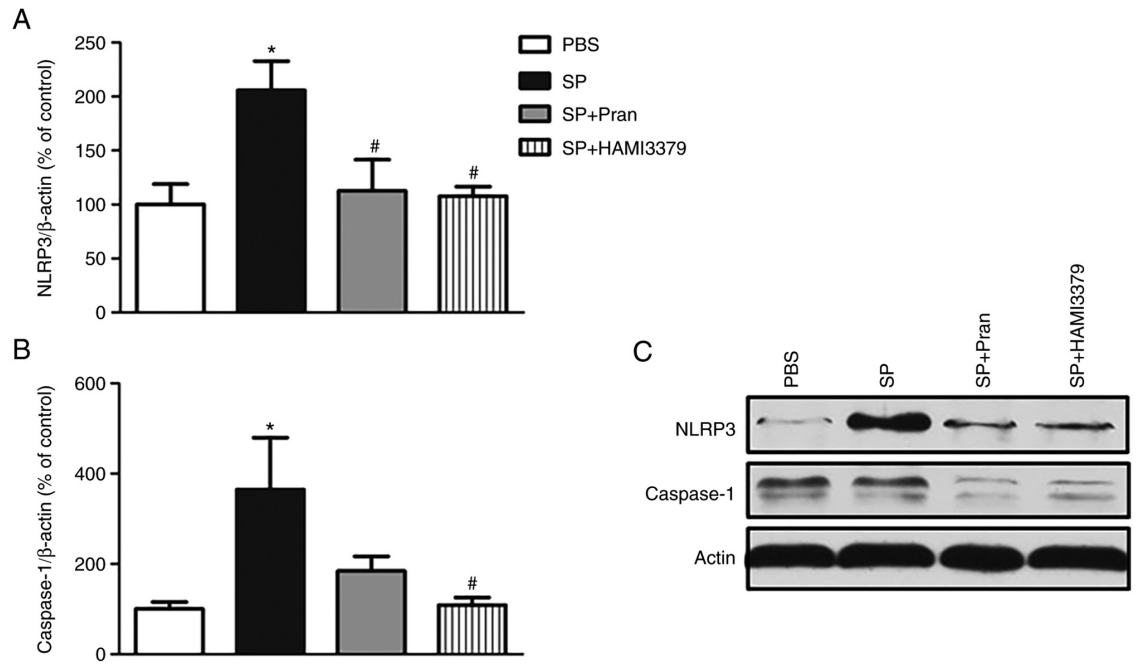


Figure 6. Effects of Pran and HAMI 3379 on the expression of NLRP3 inflammasome in brain tissue. (A and B) Representative images of western blot analysis for NLRP3 and caspase-1 expression. (C) Quantitative analysis of densitometries of western blot band. Values are presented as the mean ± SEM (n=8). $^{*}$P<0.05 vs. the PBS group and $^{\#}$P<0.05 vs. the SP group. SP, *Streptococcus pneumoniae*; Pran, pranlukast.

degree of nerve damage caused by meningitis. It was observed that pranlukast and HAMI 3379 could significantly ameliorate neurological deficits. Additionally, the histopathological characteristic of experimental pneumococcal meningitis is extensive neuronal injury, which predominantly manifested as acute neuronal necrosis in the cortex and apoptosis in the dentate gyrus of the hippocampus (26,27). It has been identified that the $CysLT_1R$ antagonist montelukast exhibited less neuronal injury of animals with E. coli meningitis (28). The present results are consistent with the aforementioned study, as more survival neurons may directly contribute to the improved neurological function recovery. In primary cultures of neurons (microglia and mixed cortical cells), a previous study revealed that CysLTs and their receptors are not directly involved in ischemic injury of neurons in rats; HAMI3379 indirectly protects neurons by inhibiting microglia activation (29). These *in vitro* findings indicated that SP-induced neuronal injury is a comprehensive effect with multicellular interaction and protective effect of CysLTR antagonists on neurons may be mediated by microglia or astrocytes.

In the present study, it was also found that pranlukast and HAMI 3379 attenuate microgliosis and astrogliosis in response to an inflammatory insult. As it has been previously reported, gliosis is a hallmark feature of brain injury (30). Glial cells such as microglia and astrocytes represent the first line of defense against SP and they participate in the initiation and/or progression of inflammation (31). Persistent or excessive activation of microglia and astrocytes may be partially contributing to cognitive decline in meningitis survivors (32). In the first stage of gliosis, activated microglia transform from ramified to an amoeboid morphology, then play pivotal roles in initiation and modulation of astrogliosis; inhibition of microglia activation can also reduce the number of ascrocytes (33,34). Therefore, it can be hypothesized that microglia are an important regulator of activation of astrocytes. Astrocytes themselves also secrete certain factors to achieve either self-regulation or feedback regulation of microglia. Meanwhile, activated microglia and astrocytes can release inflammatory cytokines such as TNF-α and IL-1β and mediators such as 5-LOX and CysLTs. In addition to increasing the susceptibility of neurons, these inflammatory mediators may cause the impairment of the BBB, which may eventually lead to focal ischemia and necrosis of brain tissue (30). Previous studies demonstrated that $CysLT_1R$ and $CysLT_2R$ mediate neuronal damage in the acute phase, but microgliosis and astrogliosis in the chronic/subacute phase following focal cerebral ischemia (35-37). Both pranlukast and HAMI 3379 inhibited BBB disruption, indicating that CysLTR is involved in regulating the permeability of BBB. Compared with pranlukast, the inhibitory effect of HAMI 3379 on astrogliosis is more dependent on the regulation of microglia (25). Collectively, it is hypothesized that $CysLT_1R$ is a master regulator of astrogliosis proliferation and glial scar formation, while $CysLT_2R$ plays a major role in earlier stage of microglial activation during SP-induced neuroinflammation. Nevertheless, there are certain limitations to the present study. It needs to be further clarified how both antagonists act on the cells and the crosstalk between different cell types in a brain inflammation model caused by gram-positive pneumococci *in vitro*.

In relation to the inflammatory cytokines, the present results showed that both pranlukast and HAMI 3379 significantly inhibit the increased expression of typical pro-inflammatory cytokines TNF-α and IL-1β during murine pneumococcal meningitis, indicating that they exert neuroprotective effects partly through their general anti-inflammatory properties. However, they had different effects on the expression of IFN-γ; HAMI 3379 significantly decreased the expression of IFN-γ. IFN-γ is produced by natural killer cells and type 1 T helper (TH1) cells, also involved in the pathology of pneumococcal meningitis by inhibiting bacterial clearance, as well as modulating myeloid recruitment and activation (38). The findings of the present study are in accordance with the results of others, confirming that pranlukast did not affect the release of IFN-γ in the asthmatic mice lung (39). Fujii *et al* (40) found that IFN-γ led to the increased $CysLT_2R$ expression on eosinophils in patients with asthma. Moreover, inhibiting the expression of $CysLT_2R$ was shown to attenuate the apoptosis of human umbilical vein endothelial cells by altering the IFN-γ secretion. The mechanisms underlying the effects of CysLTR antagonists on cytokine expression after SP meningitis require further investigation.

The NLRP3 inflammasome is a multi-protein complex composed of NLRP3, ASC adaptor (apoptosis-associated speck-like protein) and caspase-1. It plays an important role in the immune defense pathology of SP meningitis by promoting the occurrence of pyroptosis (15). Meanwhile, the arachidonic acid anabolic pathway is also closely related to inflammatory immune response. Therefore, it was wondered if there was an interrelationship between these two seemingly independent immune responses. In the present study, the results revealed that both pranlukast and HAMI 3379 significantly reduced NLRP3 expression; HAMI 3379 could additionally reduce the upregulated expression of caspase-1. It has been found that blockade of enzymes involved in arachidonic acid metabolism could inhibit macrophages M1 polarization and activation of NLRP3 inflammasome in monosodium urate crystal (MSU)-induced inflammation (41). Upon stimulation with MSU, $LTB_4$, a metabolite of 5-LOX, was associated with the assembly of the NLRP3 inflammasome and caspase 1-dependent IL-1β production *in vitro* and *in vivo* (42). Zhang *et al* further verified that the selective NLRP3 inhibitor MCC950 can significantly reduce the level of $LTC_4$ in serum of allergic rhinitis mice (43). The aforementioned findings imply a complex interaction between CysLTs and pyroptosis mediated by the NLRP3 inflammasome. Consequently, it was hypothesized that CysLTR antagonists may ameliorate detrimental inflammatory responses in murine pneumococcal meningitis by inhibiting pyroptosis.

In conclusion, the results of the present study first revealed that intraperitoneal injection of CysLTR antagonists could improve neurological deficit, interfering neuronal injury, microgliosis and astrogliosis, decrease the expression of inflammatory cytokines and inhibit over-activation of NLRP3 inflammasome, resulting in the amelioration of immune-inflammatory reaction in a rat model of SP meningitis. CysLTR antagonists may be a novel therapeutic strategy for SP meningitis. However, the neuroprotective effects of CysLTR antagonists were only proved in rats, thus the precise mechanisms underlying the effects of antagonists in SP meningitis require further elucidation.

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**Availability of data and materials**

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

**Authors' contributions**

SYY, XJC and JY designed the research. SYY, XJC, XYL and YYJ performed the experiments and data collection. SYY and JY analyzed the data. SYY and XJC confirmed the authenticity of all the raw data and wrote the manuscript. All authors read and approved the final manuscript, and ensure the integrity of the work..

**Ethics approval and consent to participate**

All animal experiments were approved by the Animal Care and Use Committee of Hangzhou Children's Hospital (Hangzhou, China) and conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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