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NLRP3 inflammasome activates interleukin-23/interleukin-17 axis during ischaemia-reperfusion injury in cerebral ischaemia in mice

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ABSTRACT

Aims: NLRP3 inflammasome has been reported associated with some inflammatory and autoimmune diseases. We previously researches showed that interleukin-23 (IL-23) and interleukin-17 (IL-17) aggravates the ischaemic injury of the brain tissue. However, it is poorly understood whether the NLPR3 inflammasome was involved in regulating and activating the IL-23/IL-17 axis in ischaemic stroke. We aimed to delineate whether the NLRP3 inflammasome signalling provokes the IL-23/IL-17 axis and interleukin-23 receptor (IL-23R) inducing the ischaemia-reperfusion injury of the brain in mice.

Main methods: The male C57/BL6 mice with experimental transient middle cerebral artery occlusion (tMCAO) were established for cerebral ischaemia-reperfusion injury. MCC950 was utilized as a selective NLRP3 inflammasome inhibitor. NLRP3 inflammasome associated protein, IL-23/IL-17 and IL-23R were detected to investigate their changes in the brain tissue after tMCAO.

Key findings: MCC950 inhibited the NLRP3 inflammasome, which alleviated the neurological ischaemia-reperfusion injury. Inhibition the NLRP3 inflammasome signalling by treatment with MCC950 decreased the activation of IL-23/IL-17 axis and the expression of IL-23R.

Significance: The NLRP3 inflammasome facilitated the injury effect of the IL-23/IL-17 axis, which contributed to the cerebral ischaemia-reperfusion injury. This process was associated with IL-23R. Furthermore, this indicated that the NLRP3 inflammasome, as an important therapeutic target for ischaemic stroke, involves multiple mechanisms in ischaemia-reperfusion injury, and MCC950 is a promising way for clinical treatment.

1. Introduction

Stroke is a devastating illness threatening human health everyday worldwide. The number of stroke patients is about 30 million annually [1], and ischaemic stroke accounts for approximately 87% [2]. One of the most effective treatments of ischaemic stroke is thrombolytic therapy within a narrow therapeutic window to restore the blood flow of the cerebral artery. However, cerebral blood flow reperfusion causes ischaemia-reperfusion injury [1]. After the ischaemic stroke, immune cells are activated and produce pro-inflammatory factors, inducing further injury to the brain tissue. Both the innate and adaptive immune system is associated with the inflammatory injury [3].

The innate immune response is important in the pathological mechanisms of central nervous system after tissue injury or pathogen infection [4]. Pathogen-associated molecular patterns (PAMPs) is linked to pathogens infection. While damage-associated molecular patterns (DAMPs) is associated with tissue-based injury. The NOD-like receptors (NLRs) interact with PAMPs and DAMPs [5]. Stimulated by PAMPs or DAMPs, NLRs form the inflammasome by combination of the adaptor protein knowns as ASC [6]. The inflammasome initiates the activation of pro-caspase-1 to the mature caspase-1. Subsequently, the inactive of pro-IL-1β and pro-IL-18 converts into the active form IL-1β and IL-18 [7]. The inflammasome is found in the diseases of Alzheimer's disease, stroke, and type 2 diabetes and so on [1]. And the NLRP3 inflammasome is the best known inflammasome. Inflammasome were upregulated in stroke animal models [8]. However, the specific inflammatory mechanisms underlying ischaemic stroke are not entirely clear. Effective anti-inflammatory targets have not been identified. MCC950 is an inhibitor of NLRP3 inflammasome which exhibits the high selective potency for suppressing the signalling pathway of NLRP3 inflammasome. It ameliorates the neurological injury and improves the survival in experimental autoimmune encephalomyelitis (EAE) [9]. Thus it is suggested that MCC950 is a tool used for the studies involving the NLRP3 inflammasome signalling pathway. MCC950 is also suggested as

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a treatment strategy for NLRP3 inflammasome associated diseases [9].

Interleukin-17 (IL-17) is an important factor of the adaptive immune system, mainly released by Th17 cells [10]. Interleukin-23 (IL-23) is produced by several innate and adaptive immune cells [11]. IL-23 generates and expands T cells of IL-17-producing, which participates in the injury after ischaemic stroke [12,13]. The interleukin-23 receptor (IL-23R) is mainly expressed on Th17 cells [11]. In the skin inflammation, IL-1β stimulates Th17 cells to produce IL-17, and the activation of inflammasome causes the production of IL-1β [14]. The lack of IL-18 reduced the IL-17 response in EAE [15]. The NLRP3 inflammasome adaptor molecule ASC plays an important part in the Th17-mediated pathological mechanism of EAE [16]. Therefore, the effect of NLRP3 inflammasome signalling on IL-23/IL-17 axis in ischaemia-reperfusion injury of the brain tissue is a crucial question in revealing the further inflammatory mechanism during ischaemic stroke. In the present study, we hypothesized that activated NLRP3 inflammasome signalling participates in the activation of IL-23/IL-17 axis and regulation of IL-23R in cerebral ischaemia-reperfusion injury.

2. Materials and methods

2.1. Animals

Male C57/BL6 mice (weighting 25 ± 2 g, Beijing Vital River Laboratory Animal Technology Co, Ltd., China) were used in our study. All the experimental procedures were conducted according to the guidance for the care and use of experimental animals by the National Institute of Health. Each effort was made to minimize the suffering and number of mice. Mice were randomly arranged to each group.

2.2. Experimental transient middle cerebral artery occlusion model (tMCAO)

The animal model of tMCAO was considered the cerebral ischaemia-reperfusion injury. The mice were subjected to fasting overnight with free access to water. Animals were anaesthetized with 10% chloral hydrate (350 mg/kg, i.p.). The right common carotid artery, external carotid artery, and internal carotid artery were surgically exposed and isolated. The operation was then conducted by inserting monofilament with poly-L-lysine-coated (A_3-1620, Cinontech Co., LTD, Beijing, China) into the right internal carotid artery and gently pushing it to occlude the middle cerebral artery. The monofilament was withdrawn from the internal carotid artery after one-hour of occlusion. The mice in sham group were subjected to the same procedure but without occlusion. To determine the changes of NLRP3 inflammasome signalling and IL-23/IL-17 axis in cerebral ischaemia-reperfusion injury, mice in the tMCAO groups were euthanized at 6 h, 24 h, 72 h, 5 d and 7 d after reperfusion, respectively. In the subsequent experiments, to explore whether inhibition of NLRP3 inflammasome signalling with MCC950 reduces the cerebral ischaemia-reperfusion injury and the activation of IL-23/IL-17 axis, mice were randomly assigned to tMCAO + MCC950 group, tMCAO + PBS group, sham + MCC950 group and sham + PBS group. Next, the brain tissues were collected from each group. Prior to euthanasia, the neurological scores were determined by a blinded examiner. The score system is as follow: 0 score, no neurological deficits; 1 score, difficulty in fully extending the left forelimb; 2 score, unable to extend the left forelimb; 3 score, circling to the left side; 4 score, falling to the left; and 5 score, featuring a depressed level of consciousness and failing to walk spontaneously. Mice after tMCAO were excluded from this study: 1, those died before euthanasia; 2, those with a subarachnoid haemorrhage or intraparenchymal haemorrhage; and 3, those that with a 0 score or 5 score.

2.3. Drug administration

MCC950 (SM-PZ0280-25 mg, Sigma, USA) was dissolved in phosphate- buffered saline (PBS) and administrated by intraperitoneal injection [9]. Mice in the tMCAO + MCC950 groups and sham + MCC950 groups were injected with MCC950 (20 mg/kg body weight, i.p) dissolved in 0.2 ml PBS. Mice in the tMCAO + PBS groups and sham + PBS groups were injected with 0.2 ml PBS as control groups. Two time points, 24 h and 72 h, were selected for this study. In the groups with the time point of 24 h, the C57BL/6 mice were pretreated with MCC950 or an equal volume of vehicle control (PBS) 1 h before operation and were euthanasia 24 h after reperfusion. In the groups with the time point of 72 h, mice were treatment with MCC950 or an equal volume of PBS 1 h before operation of tMCAO, then treated with MCC950 or PBS at 24 h and 48 h (every 24 h) after reperfusion, and sacrificed at 72 h after reperfusion. The brain tissues from tMCAO + MCC950 groups, tMCAO + PBS groups, sham + MCC950 groups and sham + PBS groups (24 h- and 72 h-) were utilized for infarction volume measurement, Nissl staining, western blotting and immunohistochemical staining.

2.4. 2, 3, 5-Triphenyltetrazolium chloride staining (TTC)

We tested the infarction volume in mice after tMCAO with MCC950 or PBS in the 24 h-groups and 72 h-groups. The brain infarct volume was evaluated as the previous methods [17]. After mice were euthanized, the brain tissue was rapidly collected and sliced into 2-mm thick coronal sections. All slices were stained with 2% TTC (Sigma, USA) at 37 °C for 15–20 min kept in dark. The brain slices were photographed with digital camera for further analysis with software of the Image-Pro-Plus. The total infarction volume was calculated by adding up the infarction volume of each slice. The infarction size was calculated as described previously [18]. Infarction size (%) = [(contralateral hemisphere area) − (ipsilateral hemisphere non-infarct area)] × 100%/contralateral hemisphere. Total infarction volume(%) = $\Sigma\{[(\text{the above infarction size}) + (\text{the below infarction size})]_1 \ldots [(\text{the above infarction size}) + (\text{the below infarction size})]_n\} \times 2/2$.

2.5. Nissl staining

After euthanasia, mice were perfused with saline and 4% paraformaldehyde subsequently. The brain tissue was collected and fixed with 4% paraformaldehyde for 24 h. The brains tissue were embedded in paraffin and sliced into 4-μm thick coronal sections. After dewaxed, the brain sections were stained with 1% methyl violet for approximately 15 min. After rinsing several times, they were stained with Nissl differentiation liquid for 4–8 s. The brain sections were observed and photographed under the light microscope to observe the Nissl bodies.

2.6. Western blotting

Protein in the brain were extracted from the right cerebral hemisphere in each group. Protein were separated by 12% SDS-PAGE and transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked in non-fat milk (5%) for 1 h at room temperature. Membranes were incubated with antibodies specific for NLRP3 (1:1000 dilution, TA336883, OriGene Technologies, Inc., USA), ASC (1:2000 dilution, clone2EI-7, Merck Millipore, Germany), caspase-1 (1:1000 dilution, 22915-1-AP, Proteintech Group, Inc., USA), IL-1β (1:1000 dilution, 16806-1-AP, Proteintech Group, Inc., USA), IL-18 (1:1000 dilution, ab71495, Abcam, USA), IL-23 (1:1000 dilution, ab45420, Abcam, USA), IL-17 (1:1000 dilution, ab79056, Abcam, USA), IL-23R (1:1000 dilution, bs-1460R, Bioss, USA), and Bcl-2 (1:1000 dilution, 12789-1-AP, Proteintech Group, Inc., USA) overnight at 4 °C. Next, the membranes were incubated with secondary antibodies (1:5000 dilution, ZB-2305 and ZB-2301, ZSGB-BIO, China) for 1 h at room temperature. The protein bands were observed with a Protein Simple imaging system (FluroChem E, Protein Simple, USA). β-Tubulin (1:1000 dilution, TA-10, ZSGB-BIO, China) was used as an internal

contrast. Quantified of the protein bands were measured the band intensity with Gel-Pro Application software. The expression of protein ratios were normalized to β-tubulin.

2.7. Immunohistochemical staining and haematoxylin-eosin (HE) staining

After euthanasia, mice were perfused with 100 ml of 0.9% saline subsequently by 4% paraformaldehyde. The brain tissue were fixed with 4% paraformaldehyde for 24 h and then embedded with paraffin. The brain sections were dewaxed and rehydrated. After blocked in 3% H_2O_2, the sections were subjected to antigen retrieval. The brain sections were incubated with primary antibody against NLRP3 (1:100 dilution, TA323326, OriGene Technologies, Inc., USA), IL-23 (1:200 dilution, ab45420, Abcam, USA), and IL-17 (1:200 dilution, ab79056, Abcam, USA) antibodies overnight at 4 °C. Next, the sections were incubated secondary antibody at room temperature for 1 h. After washed three times in PBS, the brain sections were stained with diaminobenzidine tetrahydrochloride (DAB). Deparaffinized tissue sections stained with haematoxylin and eosin were used for HE staining. The sections were photographed by 400 magnification with a light microscope. The size of each picture was the same as the total observed area. The integrated optical density (IOD) of the total observed area was analyzed by the software of Image-Pro Plus 6.0.

2.8. Statistical analysis

Data are expressed as the mean ± SEM. The statistical analysis was performed with GraphPad Prism software 8.0 (GraphPad Software, US). We performed one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to analyze the difference and multiple-comparisons among groups. *t*-Test was used to analyze the difference in infarct volume and neurological scores. $p < .05$ was taken as statistically significant.

3. Results

3.1. The NLRP3 inflammasome was activated after tMCAO

To investigate the NLRP3 inflammasome signalling is activated after tMCAO, we firstly examined the protein associated with the NLRP3 inflammasome, including NLRP3, ASC, caspase-1, IL-1β and IL-18. Brain morphological structure was examined after tMCAO (Fig. 1A). HE staining revealed that the brain tissue was loose, the nucleus of neurons was shrunk and trachychromatic, water was accumulated in the injury tissue and microglial cells were increased after tMCAO. Immunohistochemical staining was performed with antibody against NLRP3 to detect the expression of NLRP3 protein. In Fig. 1B, rare positive staining for NLRP3 was observed in sham group and the 6 h-tMCAO group, while greater NLRP3-positive staining was observed in the other tMCAO groups. The IOD analysis of NLRP3-positive staining indicated a greater NLRP3 expression in the 24 h-, 72 h- and 5 d-tMCAO groups compared with the sham group ($p < .0001$, $p < .0001$ and $p = .0007$, respectively) (Fig. 1C). Western blotting revealed that the NLRP3 protein was increased in the 24 h-tMCAO ($p = .0430$ vs. the sham group) and 72 h-tMCAO groups ($p = .0013$ vs. the sham group) (Fig. 1D). It also indicated that the level of NLRP3 protein was higher at 5 days after tMCAO than that in the sham group, but no statistical significance was obtained ($p = .0793$) (Fig. 1D). Additionally, the protein expression of ASC, caspase-1, IL-1β and IL-18 examined by western blotting are displayed in Fig. 2. All the four protein were elevated after tMCAO. The protein levels of ASC were significantly higher in 24 h- and 72 h-tMCAO groups compared to the sham group ($p = .0005$ and $p = .0062$, respectively) (Fig. 2A). The caspase-1 protein were elevated at 24 h after tMCAO ($p = .0451$ vs. the sham group) (Fig. 2B), while the protein levels of IL-18 were increased at 72 h after tMCAO ($p = .0117$ vs. the sham group) (Fig. 2C). The IL-1β protein expressions were elevated at 24 h, 72 h and 5 days ($p = .0360$, $p = .0002$ and $p = .0037$, respectively, vs. the sham group) (Fig. 2D). Since the protein expressions of NLRP3 inflammasome signalling pathway were significantly increased at 24 h and 72 h after tMCAO, the two time points were selected in the subsequent experiments.

3.2. IL-23/IL-17 axis was upregulated after tMCAO

To explore the changes of IL-23/IL-17 axis in ischaemia-reperfusion of the brain tissue after tMCAO, immunohistochemistry and western blotting were performed after tMCAO. In Fig. 3A–B, rare IL-23 and IL-17 positive staining was observed in the sham group, and the IOD analysis of positive staining indicated that the IL-23 levels significantly increased for 24 h after tMCAO ($p = .0012$ vs. sham group) (Fig. 3C) and that the IL-17 protein levels were significantly increased in 24 h-, 72 h-, and 5d-tMCAO groups ($p = .0114$, $p = .0162$ and $p = .0006$ vs. sham group, respectively) (Fig. 3D). Additionally, western blot analysis of IL-23 and IL-17 also indicated that the protein levels after tMCAO were increased compared with that in the sham groups (Fig. 3E–F). As displayed in Fig. 3E, the protein levels of IL-23 were significantly elevated in 6 h-, 24 h- and 72 h-tMCAO groups ($p = .0043$, $p = .0021$ and $p = .0038$ vs. sham, respectively). The protein levels of IL-17 were elevated in 24 h-, 72 h- and 5d-tMCAO groups significantly ($p = .0044$, $p = .0013$ and $p = .0206$ vs. sham group, respectively) (Fig. 3F). These findings indicated that the IL-23/IL-17 axis associated protein expression were significantly increased at 24 h and 72 h after tMCAO, and the subsequent experiments explored at the two time points.

3.3. NLRP3 inflammasome was suppressed by MCC950 after tMCAO

MCC950 has been reported to inhibit the activity of the NLRP3 inflammasome signalling pathway selectively in mice after EAE or intracerebral haemorrhage (ICH) animal model [9,19]. The present study investigated whether MCC950 could inhibit the NLRP3 inflammasome signalling pathway after tMCAO. Immunohistochemical staining with the antibody against NLRP3 demonstrated that MCC950 treatment reduced the positive staining for NLRP3 compared to the PBS groups in the 24 h and 72 h groups after tMCAO (Fig. 4A, B). However, semi-quantitative analysis of the results indicated that the NLRP3 protein expression showed no statistical significance between the 24 h-tMCAO + MCC950 group and the 24 h-tMCAO + PBS group ($p = .1975$) (Fig. 4C). But semi-quantitative analysis between the 72 h-tMCAO + MCC950 group and the 72 h-tMCAO + PBS group indicated that NLRP3 expression significantly decreased in 72 h-tMCAO + MCC950 group ($p = .0001$) (Fig. 4D). Consistent with these findings, western blot analysis showed the same tendency for NLRP3 protein expression as the immunohistochemical staining (Fig. 4E, F). Western blotting demonstrated that ASC and IL-18 were reduced in the 24 h- and 72 h-tMCAO groups treated with MCC950 after tMCAO ($p = .0344$ and $p = .0193$, vs. 24 h-tMCAO + PBS groups; $p = .0111$ and $p = .0163$, vs. 72 h-tMCAO + PBS groups) (Fig. 5A, B, G, H). A sharp decrease in caspase-1 protein expression was observed in comparison between the 72 h-tMCAO + MCC950 group and 72 h-tMCAO + PBS treatment group ($p = .0385$) (Fig. 5D), while caspase-1 protein levels showed no statistical significance between the 24 h-tMCAO + MCC950 group and the 24 h-tMCAO + PBS group ($p = .8303$) (Fig. 5C). The protein expression of IL-1β was compared between the 24 h-tMCAO + MCC950 group and the 24 h-tMCAO + PBS group (Fig. 5E), as well as between the 72 h-tMCAO + MCC950 group and the 72 h-tMCAO + PBS group (Fig. 5F). The results showed a similar tendency as caspase-1 ($p = .4256$ for 24 h groups and $p = .0065$ for 72 h groups). These data indicated that MCC950 suppressed the signalling pathway of NLRP3 inflammasome after tMCAO in mice.

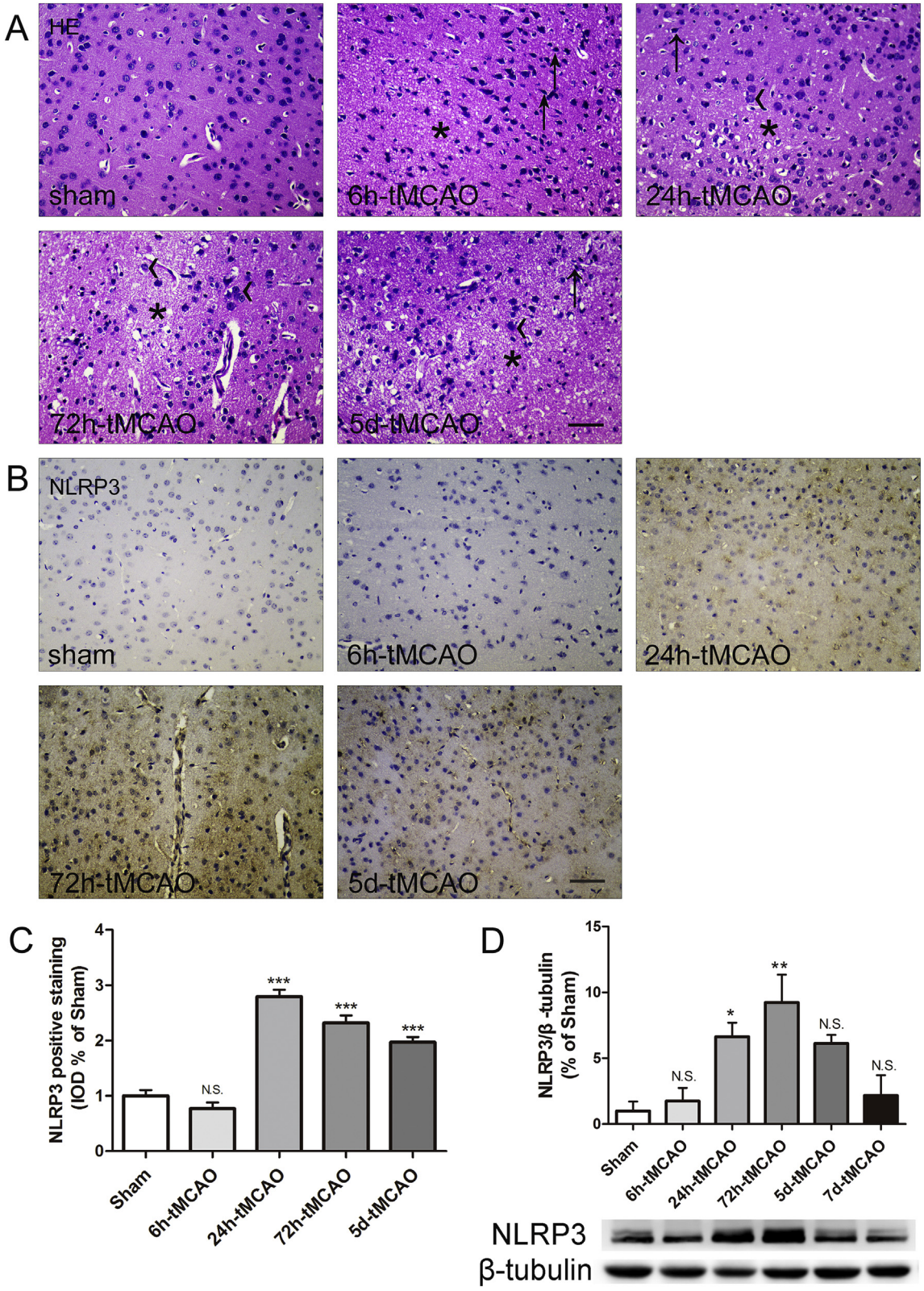

Fig. 1. The activation of NLRP3 in brain tissues after tMCAO in mice. (A) Haematoxylin-and-eosin-stained slides of the mouse brain tissue in the groups of 6 h-, 24 h-, 72 h- and 5 d-tMCAO. In the tMCAO operation groups, the brain tissue was loose and water was accumulated (*), the nucleus of neurons was shrunk and trachychromatic (↑), and microglial cells were increased (ς). (B) Immunohistochemical staining of NLPR3 expression in the sham and tMCAO groups (n = 4). (C) The integrated optical density (IOD) of NLRP3-positive staining among groups (n = 4). Data are analyzed as percentage ratio of the values from sham group. (D) Western blot analysis of the NLRP3 protein among sham operation group and tMCAO groups (n = 4). Analysis of the grey value obtained via western blotting, normalized to β-tubulin. Data are expressed as a percentage of the values from sham group. *$p < .05$, **$p < .01$, ***$p < .001$ and N.S. not significant vs. sham group. Scale bar = 50 μm.

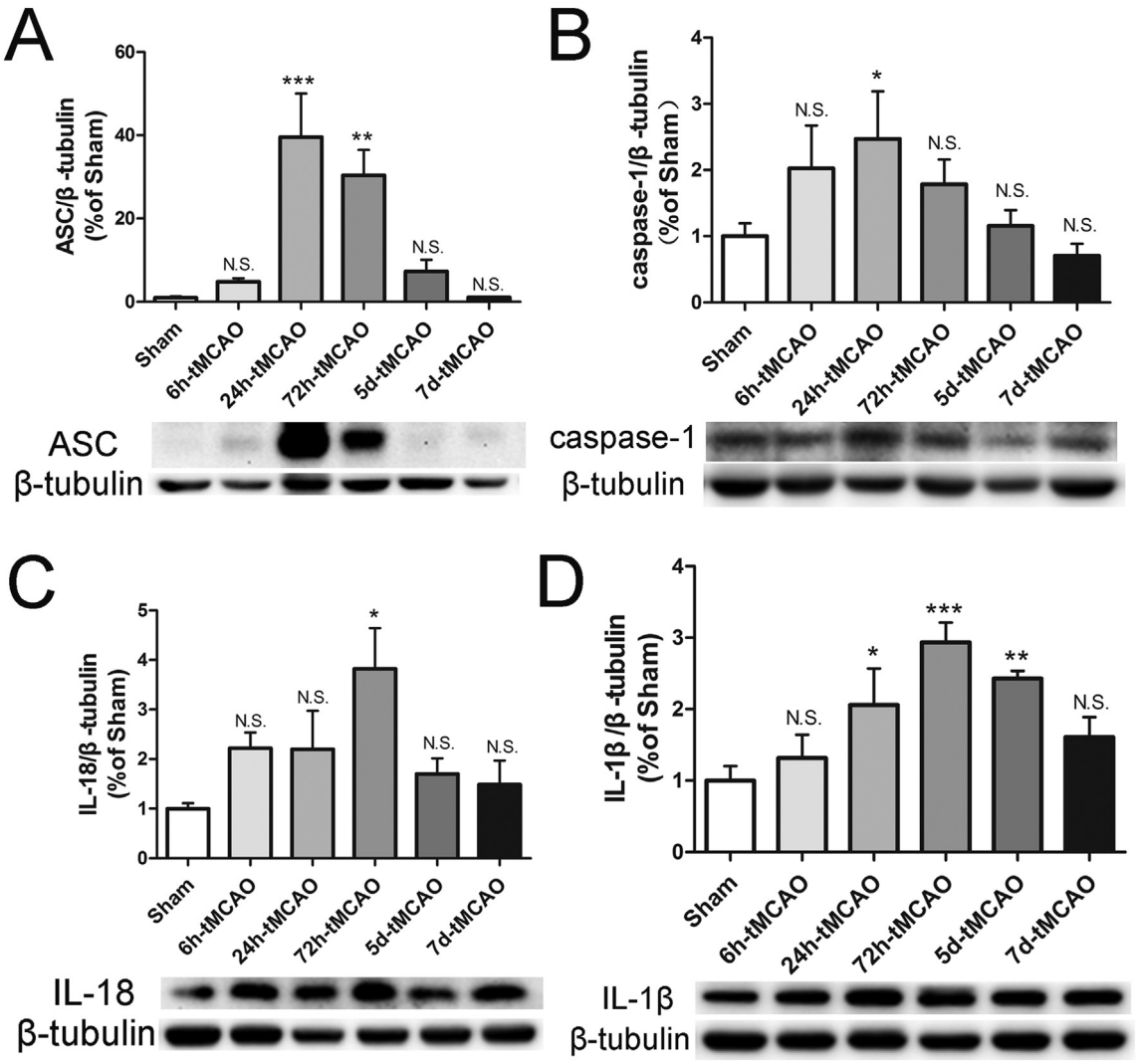

Fig. 2. The NLRP3-inflammasome-associated protein in brain tissues of mice after tMCAO. (A) ASC protein levels were evaluated by western blot analysis among groups (n = 4). (B) The protein expressions of caspase-1 were evaluated by western blot analysis among the sham and tMCAO groups (n = 4). (C) IL-18 protein expression levels were evaluated by western blot analysis among the sham and tMCAO groups (n = 4). (D) IL-1β protein experssion levels were evaluated by western blot analysis among the sham and tMCAO groups (n = 4). Analysis of the grey value obtained via western blotting, normalized to β-tubulin. Data are expressed as a percentage of the values from the sham group. *$p < .05$, **$p < .01$, ***$p < .001$ and N.S. not significant vs. sham group.

3.4. MCC950 attenuated the cerebral ischaemia-reperfusion injury after tMCAO

To assess whether MCC950 induces a neurological protective effect in mice after tMCAO, we evaluated the infarction volume by TTC staining, the cerebral injury by Nissl staining, the Bcl-2 protein level evaluated by western blotting of the brain tissue and the neurological score. Infarct volumes of the brain tissue in mice were sharply reduced in 24 h- and 72 h-tMCAO + MCC950 groups compared to the 24 h- ($p = .0224$) and 72 h-tMCAO + PBS groups ($p = .0026$) (Fig. 6A, B), respectively. Nissl staining of the mice after tMCAO demonstrated more Nissl bodies in neurons in the 24 h- and 72 h-tMCAO + MCC950 groups compared with that in PBS groups (Fig. 6C). The neurological score improved in 24 h- and 72 h-tMCAO + MCC950 groups compared to the tMCAO + PBS groups ($p < .0001$), and the score in 72 h group was lower than that in the 24 h group ($p = .0370$) (Fig. 6D). The Bcl-2 protein levels increased in the 24 h- and 72 h-tMCAO + MCC950 groups compared with the 24 h- ($p = .0202$) and 72 h-tMCAO + PBS groups($p = .0244$), respectively (Fig. 6E, F). All data demonstrated that treatment with MCC950 attenuated the cerebral ischaemia-reperfusion injury.

3.5. MCC950 reduced the activation of IL-23/IL-17 axis after tMCAO

It is believed that IL-23/IL-17 axis has a vital pro-inflammatory role in the ischaemic stroke. To assess whether inhibition the signalling pathway of NLRP3 inflammasome by MCC950 affects the IL-23/IL-17 axis in ischaemia-reperfusion brain tissue, immunohistochemical staining and western blot analysis were performed. Immunohistochemical staining showed that MCC950 attenuated the activation of IL-23/IL-17 axis in the 24 h- and 72 h-tMCAO + MCC950 groups compared with that in 24 h- and 72 h-tMCAO + PBS groups, respectively (Fig. 7A, B, C, D). The IOD of positive staining analysis indicated that IL-23 levels were decreased in 24 h- and 72 h-tMCAO + MCC950 groups compared with that in the tMCAO + PBS groups ($p < .0001$ for 24 h group and $p = .0215$ for 72 h group) (Fig. 7E, F). The IL-17 protein levels also decreased in the 24 h- and 72 h-tMCAO + MCC950 groups compared with the levels in the 24 h- and 72 h-tMCAO + PBS groups ($p = .0203$ and $p = .0472$, respectively) (Fig. 7G, H). Consistent with these findings, western blotting showed the same tendency as the immunohistochemical staining results. The IL-23 protein levels were significantly decreased in 24 h- and 72 h-tMCAO + MCC950 groups ($p = .0011$ for 24 h and $p = .0200$ for 72 h,

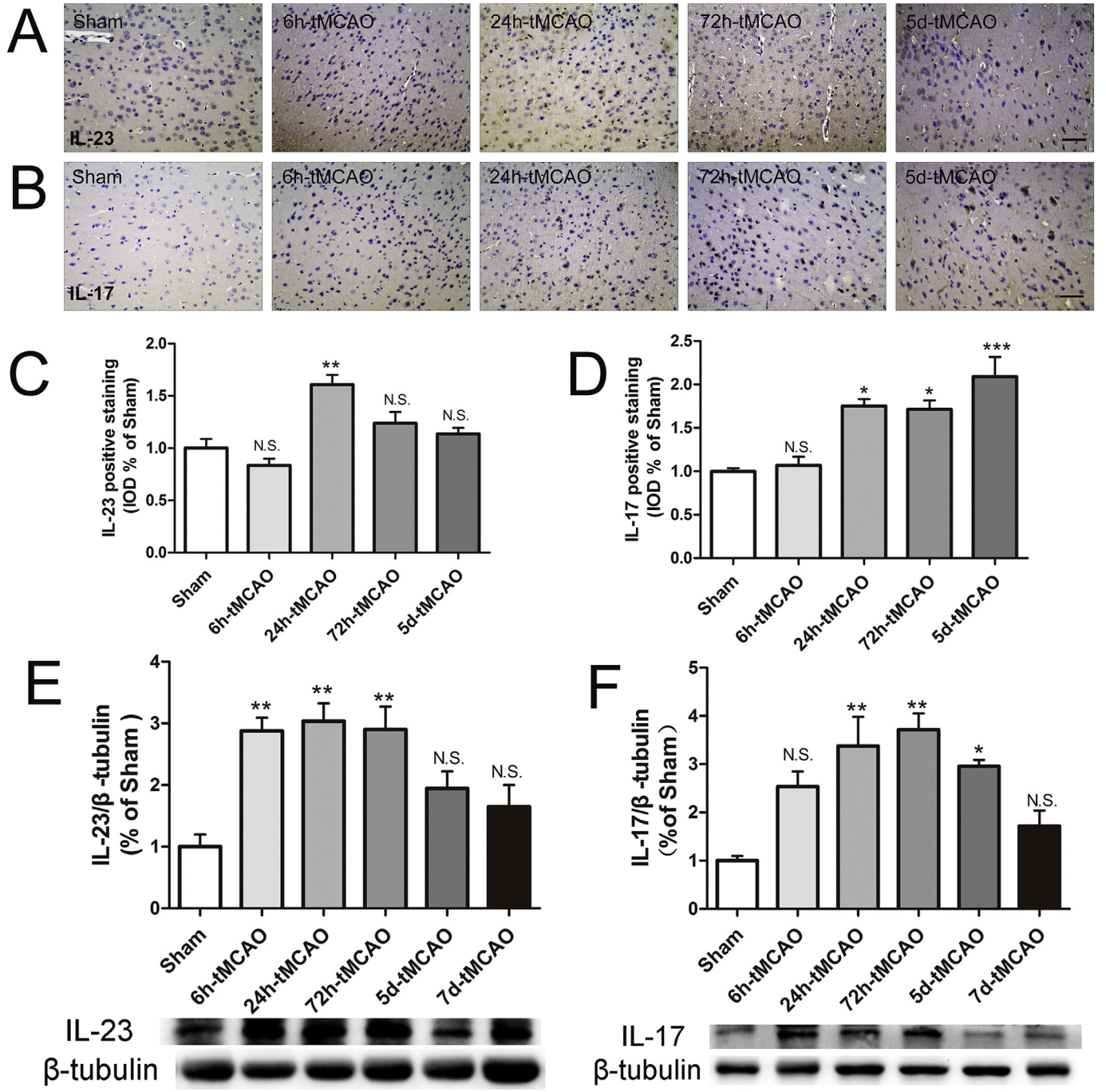

Fig. 3. The activation of IL-23/IL-17 axis in the brain tissues after tMCAO. (A, B) Immunohistochemical staining of IL-23 and IL-17 in the sham and tMCAO groups (n = 4). (C, D) The IOD value of IL-23/IL-17-positive staining among tMCAO groups (n = 4). Data are expressed as the percentage ratio of values from the sham group. (E, F) The protein of IL-23/IL-17 expression levels were evaluated by western blotting among groups (n = 4). Analysis of the grey value obtained via western blotting, normalized to β-tubulin. Data are expressed as a percentage of the values from the sham group. *$p < .05$, **$p < .01$, ***$p < .001$ and N.S. not significant vs. sham group. Scale bar = 50 μm.

vs. tMCAO + PBS groups) (Fig. 8A, B). The protein levels of IL-17 were decreased in 24 h- and 72 h-tMCAO + MCC950 groups compared with that in the tMCAO + PBS groups ($p = .0180$ for 24 h and $p = .0080$ for 72 h) (Fig. 8C, D). These data indicated that inhibitor of the NLRP3 inflammasome signalling decreased the IL-23/IL-17 axis activation after tMCAO in mice.

3.6. MCC950 reduces the expression of IL-23R after tMCAO

We investigated the IL-23R protein levels of the mouse brain tissue after tMCAO with MCC950 or PBS treatment. The protein levels were reduced in the 24 h- and 72 h-tMCAO + MCC950 groups compared with that in the tMCAO + PBS groups ($p = .0205$ for 24 h and $p = .0053$ for 72 h group) (Fig. 9A, B). These data indicated that MCC950 decreased the expression of IL-23R after tMCAO in mice.

4. Discussion

Evidence suggests that effective regulation or inhibition of NLRP3 may help to protect against the injury of ischaemic stroke [20].

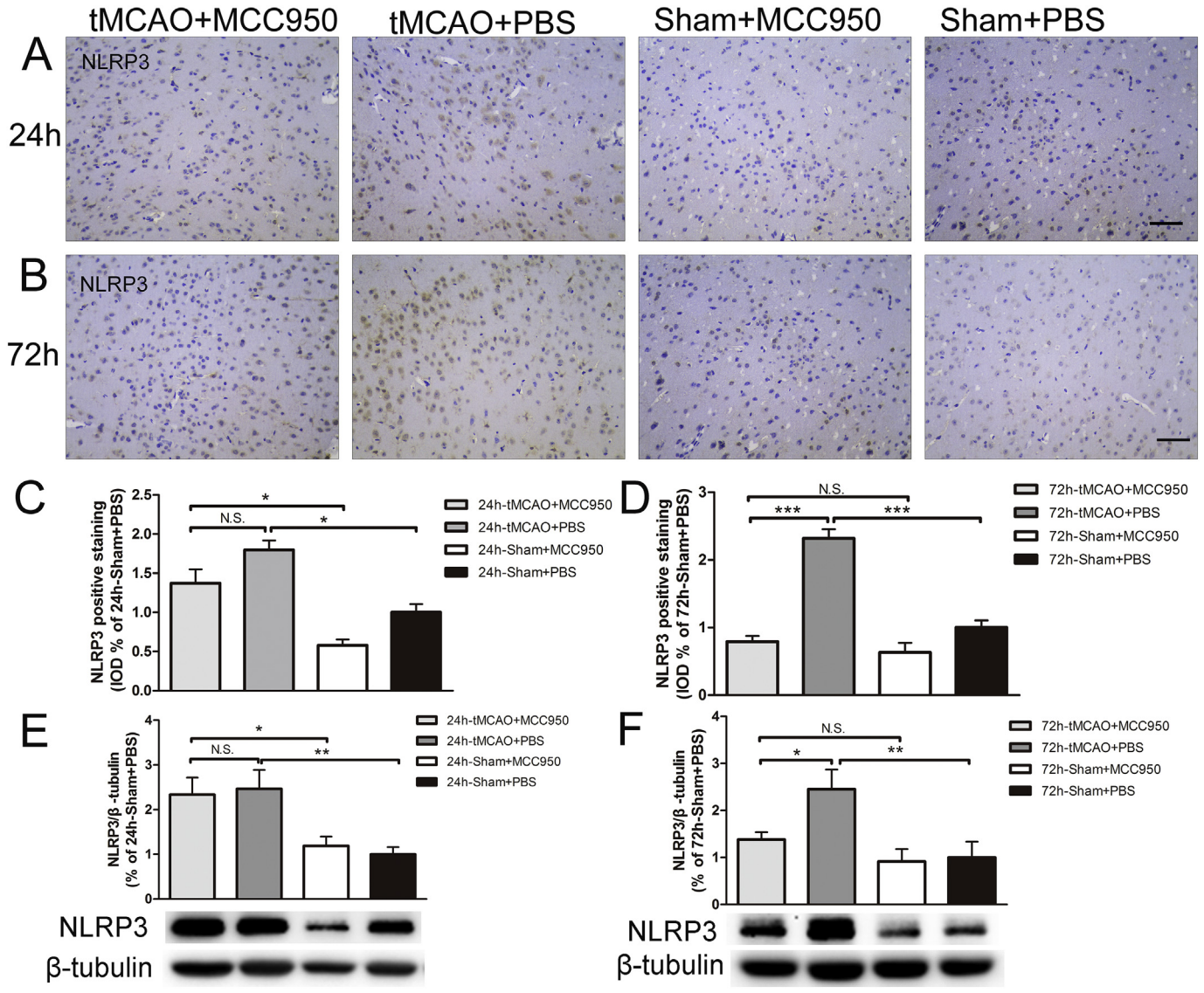

Fig. 4. MCC950 affects the expression of NLRP3 inflammasome in the mouse brain tissues after tMCAO for 24 h and 72 h. (A, B) Immunohistochemical staining of NLPR3 expression in each group (tMCAO + MCC950, tMCAO + PBS, sham + MCC950, sham + PBS) (n = 4) for 24 h and 72 h. (C, D) Comparison of the IOD in NLRP3 positive staining among each group (n = 4). Data are analyzed as the percentage of the sham + PBS group values. (E, F) NLRP3 protein were examined by western blotting among the 24 h and 72 h groups (n = 4). Analysis of the grey value obtained via western blotting, normalized to β-tubulin. Data are expressed as a percentage of the sham + PBS group values. $*p < .05$, $**p < .01$, $***p < .001$ and N.S. not significant. Scale bar = 50 μm.

However, many questions remains, especially the role and mechanism of the NLRP3 inflammasome in ischaemic stroke. To explore the relationship between NLRP3 inflammasome and IL-23/IL-17 axis in ischaemic stroke, we explored the effects of MCC950-mediated inhibition of NLRP3 inflammasome signalling on IL-23/IL-17 axis after tMCAO. In the present study, we confirmed that MCC950 mediated the inhibition of NLRP3 inflammasome and attenuated ischaemia-reperfusion injury in brain tissue through reduction of the activation of IL-23/IL-17 axis after tMCAO in mice. Specifically, we found that the process was also associated with a decreasing in expression of IL-23R.

Cerebral ischaemia induces hypoxia and glucose deprivation, which lead to tissue necrosis and inflammation activation. The brain inflammatory cascade post ischaemia-reperfusion has been shown to be mediated by inflammasome. Inflammasome present in neurons, astrocytes, microglia, and macrophages of ischaemic brain, and mediated the mature process of caspase-1 and IL-1β. IL-1β induces apoptosis of neuron and enhances the expression of chemokine [21]. Expression levels of NLRP3-inflammasome-associated protein were detected at the given time points after tMCAO (Figs. 1D and 2). The results reveals that these protein were significantly increased at approximately 24 h and 72 h after tMCAO, which was consistent with previous research [20,22]. But the peak time points of each protein expression were different and the results achieved significance at a different time point post-reperfusion. We infer that this discrepancy may related to the process of NLRP3 inflammasome activation. The NLRP3 inflammasome is composed of the NLRP3, ASC and caspase-1. Brain tissue expresses NLRP3 inflammasome constitutively which initiate inflammation quickly [23]. The activation of NLRP3 inflammasome converts precursor caspase-1 into cleaved caspase-1 and actives IL-1β and IL-18 [24]. The NLRP3 inflammasome was activated after tMCAO, and we speculated that NLRP3, ASC and caspase-1 were triggered first, while IL-1β and IL-18 were triggered later. The result of western blotting shows that the peak time point of NLRP3 was at 72 h, which is later than that of ASC and caspase-1 after tMCAO. The reason could be that other types of inflammasomes such as NLRP1, NLRC4 and AIM2, were also involved in ischaemic stroke and regulated ASC, caspase-1 and IL-1β directly or indirectly [25,26]. It has been reported that Bruton's tyrosine kinase (BTK) could be a platform for the interacting physically

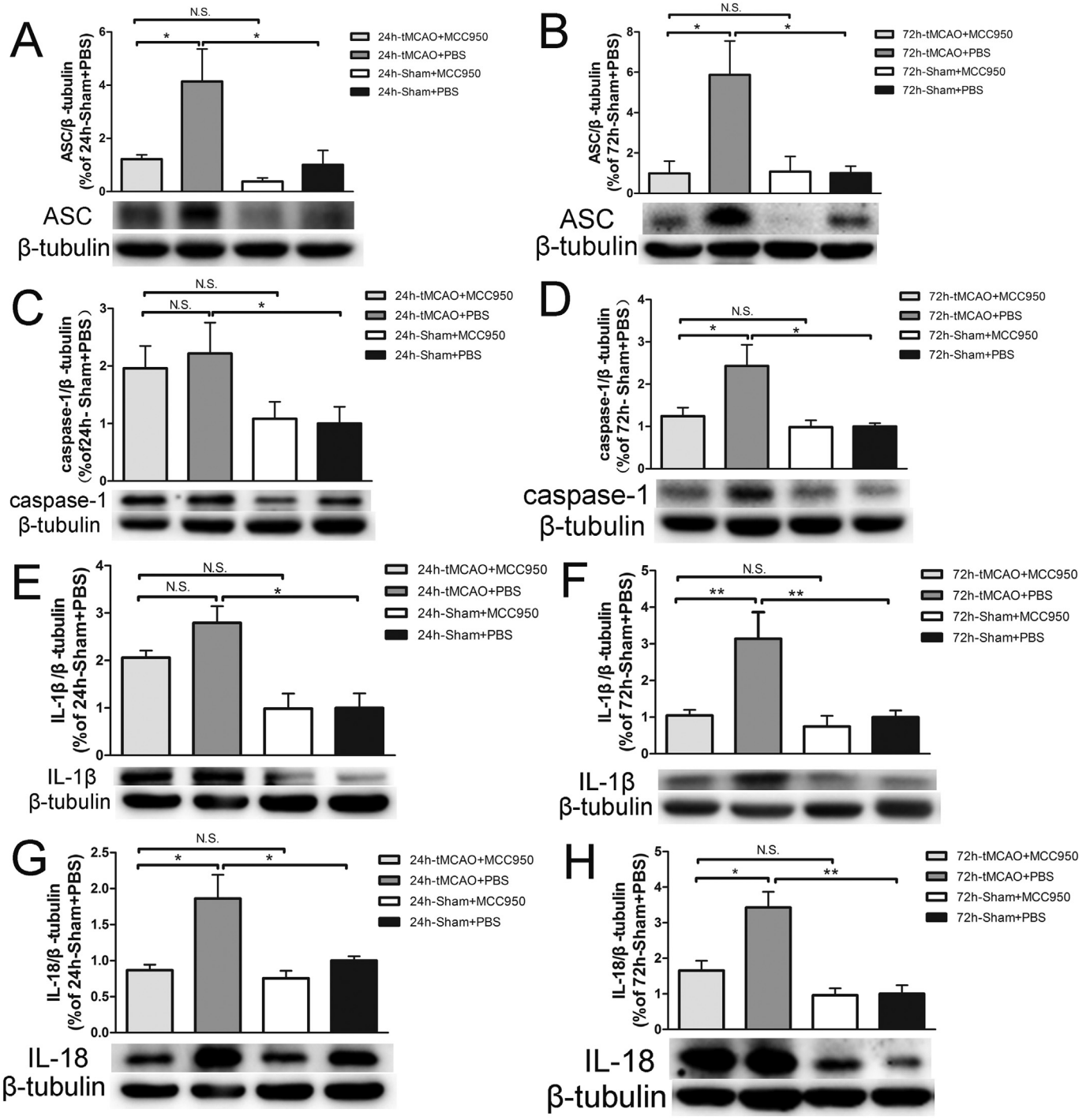

Fig. 5. MCC950 inhibited the NLRP3-inflammasome-associated protein in brain tissues of mice after tMCAO for 24 h and 72 h. (A,B) ASC protein were evaluated by western blotting among the MCC950 and PBS treatments in the 24 h and 72 h groups (n = 4). (C, D) Protein expression levels of caspase-1 were evaluated using western blotting among MCC950 and PBS treatments for 24 h and 72 h groups (n = 4). (E, F) IL-1β protein were evaluated by western blot analysis among groups for 24 h and 72 h groups (n = 4). (G, H) The protein levels of IL-18 were examined by western blot analysis between the MCC950 and PBS treatments for 24 h and 72 h groups (n = 4). Analysis of the grey value obtained via western blotting, normalized to β-tubulin. Data are expressed as a percentage of the values from the sham + PBS group. *$p < .05$, **$p < .01$ and N.S. not significant.

between NLRP3 and ASC [27], which further suggested that the regulatory mechanisms of inflammasome was complex. Our analysis might mix up different inflammasome signalling pathways and give a summation of effects. Furthermore, we confirmed that treatment with MCC950 reduced NLRP3-inflammasome-associated protein for 24 h and 72 h after tMCAO. In the study, the protein of NLRP3, caspase-1 and IL-1β were showed no statistically significant differences in mice treated with MCC950 at 24 h; however, these expression levels were significantly reduced at 72 h after tMCAO. Our data confirmed that the inhibition effect of MCC950 might be associated with the dose and administration time. MCC950 was found to selectively inhibit the formation of the protein complex of NLRP3 inflammasome, reduce IL-1β signalling and decrease the concentration of IL-18 in the blood. Both IL-1β and caspase-1 were reduced in PBMCs of the Muckle-Wells

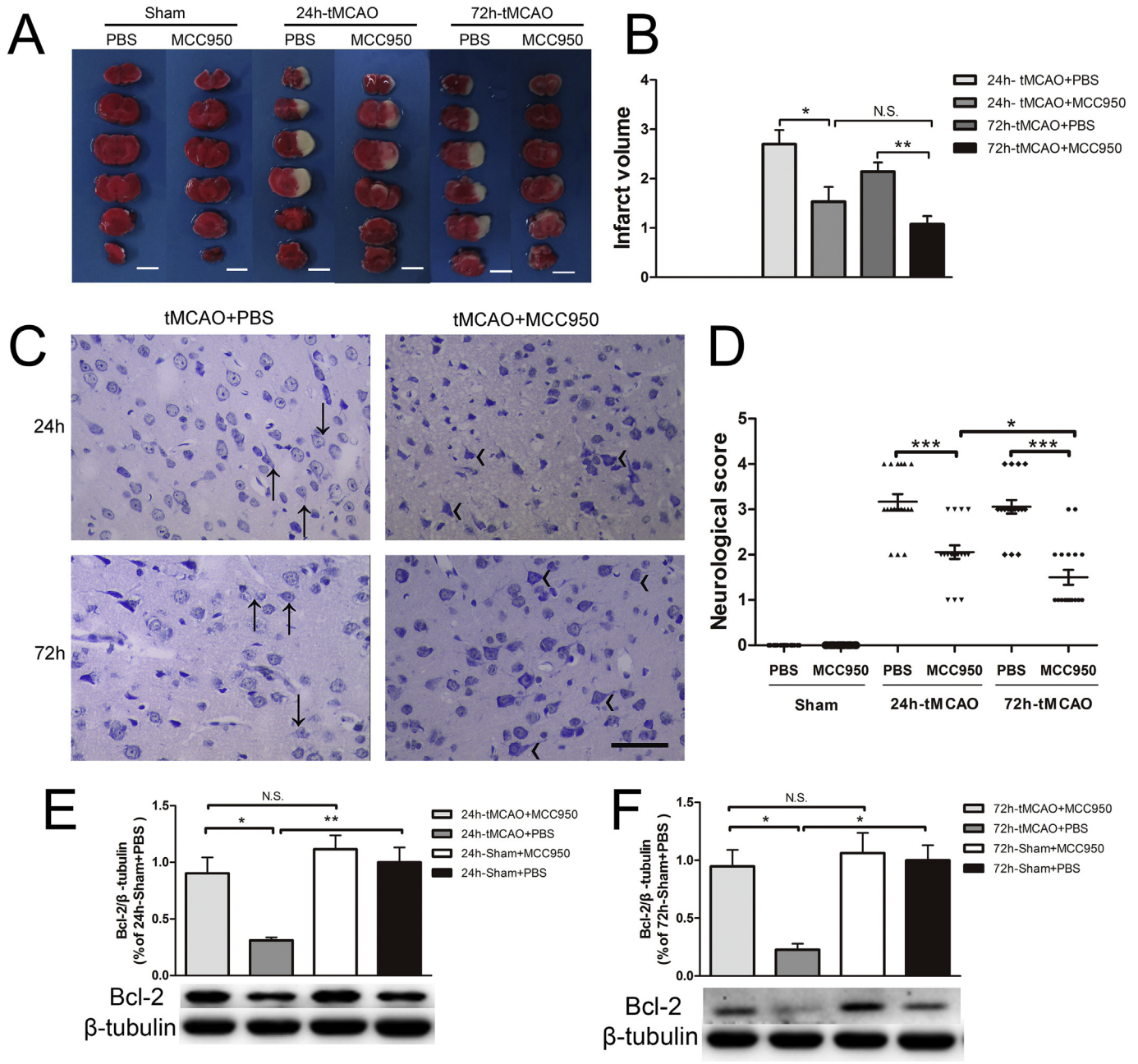

Fig. 6. Effects of MCC950 on ischaemia-reperfusion injury of the brain tissue of mice after tMCAO. (A, B) Representative photographs of the brain sections with TTC-stained and quantitative analysis of the infarct volume in each group (n = 5). Scale bar = 5 mm. (C) Nissl staining of the brain tissue after tMCAO in mice treated with MCC950 or PBS. The Nissl body in neurons was reduced in the tMCAO + PBS groups (↑) and increased in the tMCAO + MCC950 groups (ᐢ) at 24 h and 72 h. Scale bar = 50 μm. (D) Quantitation of the neurological scores in different groups (n = 18). (E, F) The protein of Bcl-2 were evaluated by western blotting in each group (n = 4). *$p < .05$, **$p < .01$, ***$p < .001$ and N.S. not significant.

syndrome (MWS) patients pre-treated with MCC950 [9]. In some studies demonstrated that the treatment with MCC950 reduced IL-1β and caspase-1 in mouse experimental non-alcoholic steatohepatitis (NASH) and in the brain tissue after intracerebral haemorrhage (ICH) at 3 days [19,28].

Our study demonstrated that MCC950 effectively inhibits inflammation in cerebral ischaemic stroke. Inhibition of the NLRP3 inflammasome signalling attenuated cerebral infract volume and functional deficits during ischaemia-reperfusion injury. In NLRP3-knockout mice, NLRP3 deficiency reduced cerebral ischaemic injury, including the reduction of infarction volume, cerebral oedema, and brain barrier permeability [20]. In another study, MCC950 significantly improved the neurological function and the long-term survival rate after ischaemic stroke in diabetic mice; however, it did not reduce the area of cerebral infarction [1]. Together these results including our founding might imply that the negative effect of MCC950 on the NLRP3 inflammasome is not fully equivalent to NLRP3 knockout, which needs further research and discussion. These protective effects might be related to reducing the activation of NF-κB signalling pathway, the decreasing of reactive oxygen species in mitochondria, and the enhancement of autophagy function of cells [29–31]. By treated with MCC950 in mice, Nissl bodies and Bcl-2 protein were increased in the brain tissue after tMCAO. Bcl-2 is a kind of anti-apoptotic protein. It is still unclear the link between inflammasome and neuronal death. The activation of inflammasome in neurons promotes neurodegeneration through a cytokine release mechanism, which induced apoptosis and

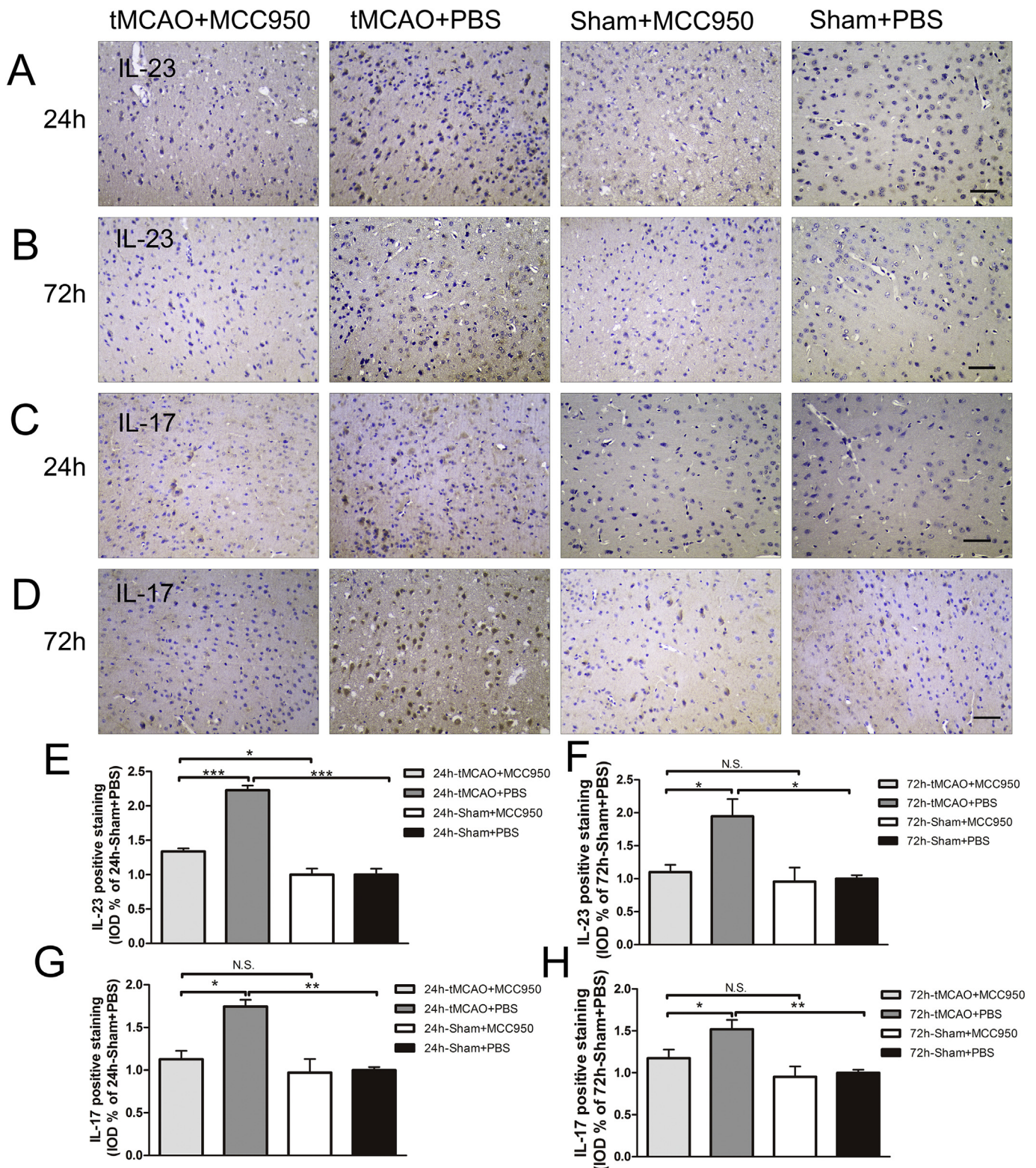

Fig. 7. MCC950 reduced the activation of IL-23/IL-17 axis in mouse brain tissues after tMCAO. (A, B) Immunohistochemical staining of the IL-23 protein expression in brain tissues of mice treated with MCC950 or PBS after tMCAO in the 24 h and 72 h groups (n = 4). (C, D) Immunohistochemical staining of the IL-17 protein expression among groups treated with MCC950 or PBS for 24 h and 72 h (n = 4). (E, F, G, H) The IOD of IL-23- and IL-17-positive staining in each group (n = 4). Data are expressed as percentage of the values from the sham + PBS group. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ and N.S. not significant. Scale bar = 50 μm.

pyroptosis [7]. Caspase-1 is an apical mediator in the processes of neuron death [32]. It demonstrated that NLRP3 inflammasome activation was linked to apoptosis that involved mitochondrial permeabilization [7]. Our results further suggested that there was a good prognosis of ischaemic stroke treated with MCC950. Therefore, it is reasonable to suggest that MCC950 play a key part in improving the neurological outcome via inhibition of NLRP3 inflammasome mediated inflammation in mice after tMCAO.

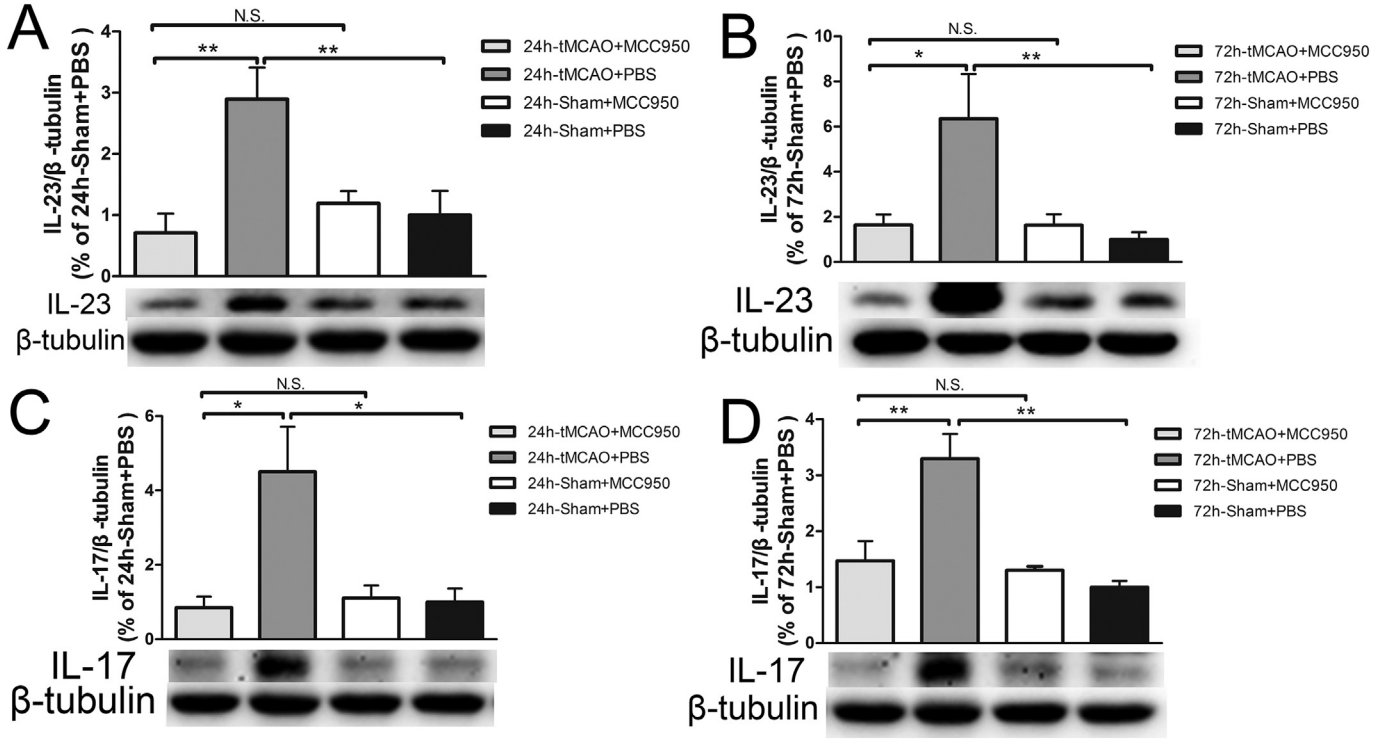

Fig. 8. Western bolt analysis of IL-23 and IL-17 in the brain tissues of mice treated with MCC950 or PBS after tMCAO. (A, B) IL-23 protein were detected in each group (n = 4). (C, D) Western blotting of the IL-17 protein in each group (n = 4). Analysis of the grey value obtained via western blotting, normalized to β-tubulin. Data are expressed as the percentage of values from the sham + PBS group. $*p < .05$, $**p < .01$, $***p < .001$ and N.S. not significant.

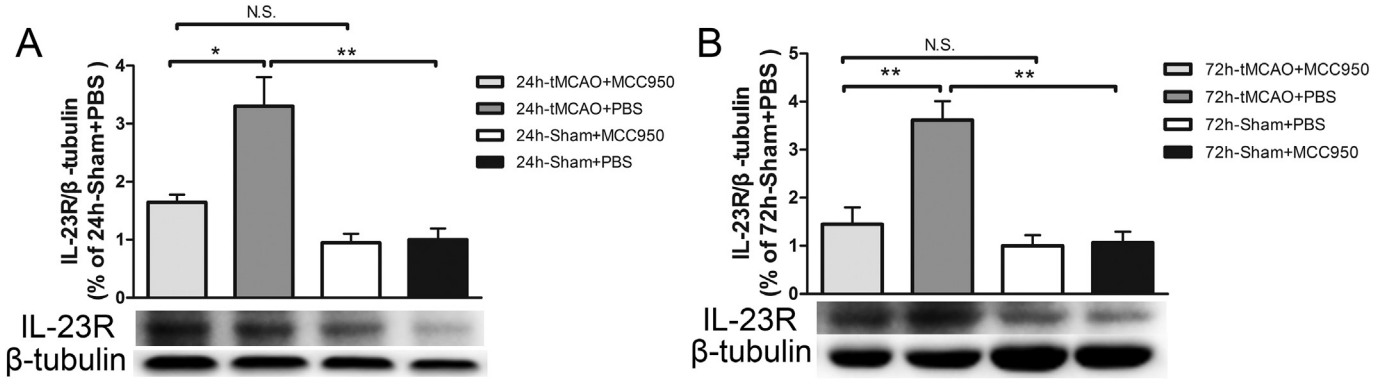

Fig. 9. MCC950 reduced IL-23R in the brain tissues after tMCAO in mice. (A) Western blotting of the protein expression of IL-23R were performed in each group (n = 4) for 24 h. (B) Western blotting of the protein expression of IL-23R were examined in each group (n = 4) for 72 h. Analysis of the grey value obtained via western blotting, normalized to β-tubulin. Data are expressed as the percentage of values from the sham + PBS group. $*p < .05$, $**p < .01$, $***p < .001$ and N.S. not significant.

IL-23 is a growth factor and is required for IL-17-producing cells [33]. IL-23R has an important role in the IL-23/IL-17 axis [34]. It had been demonstrated that IL-23 induced a high mRNA expression of IL-23R, and further more elevated the levels of IL-17, IL-6, and TNF in autoimmune inflammation [35]. In ischaemic stroke, a T-cell-related detrimental mechanism encompassed the production of IL-17 to promote the inflammatory injury. The effects of IL-17 mainly depend on the interaction with the pro-inflammatory chemokines and lead to a rapid neutrophil infiltration. IL-17 is produced within 24 h after the ischaemic attack through the engagement of the cytokine receptors for IL-1β and IL-23 [36]. The IL-23/IL-17 axis as well as IL-23R plays important roles in several autoimmune and inflammatory diseases [35,37–39]. Our previous studies have indicated that IL-23 exerts the toxic effect to neurovascular unit in the condition with oxygen glucose deprivation (OGD) via the IL-23R, and siRNA interference knockdown of IL-23p19 suppressed the gene and protein expression of IL-17 [40,41]. In the present research, we indicated that the IL-23 protein expression was sharply increased at 24 h, while IL-17 reached the peak at 72 h by the analysis of western blotting after tMCAO. This result was in line with a study that indicated that IL-17A from $\gamma\delta^+$ T cells showed peak expression within 3 days and worsened the injury of brain tissue at the acute stage of ischaemic stroke [42]. As shown by the results, there were some differences between the data statistics via immunohistochemical staining and western blot analysis. It is suggested that there are slightly differences among different protein detection methods, which may be related to the experimental principle and process itself.

We found that the IL-23/IL-17 axis was sharply decreased in mice treated with MCC950 for 24 h and 72 h after tMCAO. IL-23 and IL-17 are critical immune regulatory cytokines that as the bridge of the innate

and adaptive immunity in autoimmune and inflammatory pathologies [42,43]. IL-1β has the function in regulating IL-17-producing cells in adaptive immunity, together with IL-23. The production of IL-23 is partially dependent on the NLRP3 inflammasome signalling and IL-1β [44]. Macrophages and dendritic cells are associated with the progress of regulating IL-23 by IL-1β [45]. Inhibitor of caspase-1 suppressed the IL-17 production in EAE; however, the other caspase-1 inhibitor, Ac-YVAD-Cmk, had little effect on inhibiting the production of IL-23 [46]. The study also indicated that IL-18 promoted the $\gamma\delta^+$ T cells and $CD4^+$ T cells to product IL-17 during the development of EAE. We inferred that MCC950 might affect the activation of the IL-17 through its effects on the inhibition of the NLRP3 inflammasome signalling and the reducing of IL-1β, IL-18 and caspase-1, as well as the reducing of IL-23.

It has reported that the inflammasome adaptor molecule ASC plays a critical T cell-intrinsic role in the pathogenesis of Th17-mediated EAE, and the deficiency of ASC in T cells impaired Th17-mediated EAE [16]. Further studies are needed to explore whether ASC is involved in the regulation of IL-23 and IL-17 and its detailed mechanisms.

An important distinction between the prior study and this study is that our results indicated that IL-23R was reduced in mice treated with MCC950. IL-23R is consisting of IL-12R β1 and a specific IL-23R chain, and the gene is located on chromosome-1 [43]. IL-23R is mainly on activated immune cells [34]. IL-23, IL-6, IL-21, TGF -β and activated Th17 cells were the factors increasing IL-23R mRNA expression [43]. We thought that MCC950 affected the expression of IL-23 and inhibited the NLRP3 inflammasome signalling, and the two processes induced the reducing IL-23R expression. Our findings implied that MCC950 may negatively regulate NLRP3 inflammasome activation to reduce IL-17 via modulating IL-23R signalling. At the same time, our further research will focus on the detailed molecular mechanisms of the effect of NLRP3 inflammasome on IL-23R in ischaemia-reperfusion injury after ischaemic stroke.

5. Conclusion

Overall, the results provided an insight into the mechanism by which NLRP3 promotes ischaemia-reperfusion injury after tMCAO. NLRP3 inflammasome signalling activated the IL-23/IL-17 axis to promote cerebral ischaemia-reperfusion damage. The specific NLRP3 inflammasome inhibitor MCC950 efficiently suppressed the NLRP3 inflammasome signalling and the IL-23/IL-17 axis, and decreased the expression of IL-23R. Furthermore, inhibition of the inflammasome might reduce both the innate and adaptive immunity at the same time which are associated with injury after ischaemic stroke. These findings might provide more insights and pharmacological evidence that MCC950 has potential application in the prevention the inflammatory injury and being an effective therapeutic of ischaemic stroke.

Abbreviations

ASC	apoptosis associated speck-like protein containing a CARD
Bcl-2	B-cell lymphoma-2
DAMPs	damage-associated molecular patterns
EAE	experimental autoimmune encephalomyelitis
HE	haematoxylin-eosin
ICH	intracerebral haemorrhage
IL-17	interleukin-17
IL-23	interleukin-23
IL-23R	interleukin-23 receptor
MWS	Muckle-Wells syndrome
NLRP3	NLR family, pyrin domain containing 3
OGD	oxygen glucose deprivation
PAMPs	pathogen-associated molecular patterns
PBS	phosphate- buffered saline
pMCAO	permanent middle cerebral artery occlusion
tMCAO	transient middle cerebral artery occlusion
TTC	2, 3, 5-triphenyltetrazolium chloride

Competing interests

The authors have declared that they have no competing interests.

Authors' contributions

Haining Wang and Di Zhong designed research and wrote manuscript. Guozhong Li designed the research and critically reviewed the manuscript. Haining Wang, Hongping Chen, Jing Jin and Qingqing, Liu performed research. All authors have read and approved the final manuscript.

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References

[1] P. Hong, F.-X. Li, R.-N. Gu, Y.-Y. Fang, L.-Y. Lai, Y.-W. Wang, T. Tao, S.-Y. Xu, Z.-J. You, H.-F. Zhang, Inhibition of NLRP3 inflammasome ameliorates cerebral ischemia-reperfusion injury in diabetic mice, Neural Plast. 2018 (2018) 1–8, https://doi.org/10.1155/2018/9163521.

[2] S. He, C. Wang, H. Dong, F. Xia, H. Zhou, X. Jiang, C. Pei, H. Ren, H. Li, R. Li, H. Xu, Immune-related GTPase M (IRGM1) regulates neuronal autophagy in a mouse model of stroke, Autophagy 8 (2014) 1621–1627, https://doi.org/10.4161/auto.21561.

[3] C. Iadecola, J. Anrather, The immunology of stroke: from mechanisms to translation, Nat. Med. 17 (2011) 796–808, https://doi.org/10.1038/nm.2399.

[4] K. Zhou, L. Shi, Y. Wang, S. Chen, J. Zhang, Recent advances of the NLRP3 inflammasome in central nervous system disorders, J Immunol Res 2016 (2016) 9238290, https://doi.org/10.1155/2016/9238290.

[5] B.K. Davis, H. Wen, J.P. Ting, The inflammasome NLRs in immunity, inflammation, and associated diseases, Annu. Rev. Immunol. 29 (2011) 707–735, https://doi.org/10.1146/annurev-immunol-031210-101405.

[6] L.C. Freeman, J.P. Ting, The pathogenic role of the inflammasome in neurodegenerative diseases, J. Neurochem. 136 (Suppl. 1) (2016) 29–38, https://doi.org/10.1111/jnc.13217.

[7] J.G. Walsh, D.A. Muruve, C. Power, Inflammasomes in the CNS, Nat. Rev. Neurosci. 15 (2014) 84–97, https://doi.org/10.1038/nrn3638.

[8] J. Barrington, E. Lemarchand, S.M. Allan, A brain in flame; do inflammasomes and pyroptosis influence stroke pathology? Brain Pathol. 27 (2017) 205–212, https://doi.org/10.1111/bpa.12476.

[9] Coll RC, Robertson AA, Chae JJ, Higgins SC, Munoz-Planillo R, Inserra MC, Vetter I, Dungan LS, Monks BG, Stutz A, Croker DE, Butler MS, Haneklaus M, Sutton CE, Nunez G, Latz E, Kastner DL, Mills KH, Masters SL, Schroder K, Cooper MA, O'Neill LA, A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases, Nat. Med., 21 (2015) 248–55. doi:https://doi.org/10.1038/nm.3806.

[10] T. Korn, E. Bettelli, M. Oukka, V.K. Kuchroo, IL-17 and Th17 cells, Annu. Rev. Immunol. 27 (2009) 485–517, https://doi.org/10.1146/annurev.immunol.021908.132710.

[11] S.L. Gaffen, R. Jain, A.V. Garg, D.J. Cua, The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing, Neural Plast. 14 (2014) 585–600, https://doi.org/10.1038/nri3707.

[12] T. Shichita, Y. Sugiyama, H. Ooboshi, H. Sugimori, R. Nakagawa, I. Takada, T. Iwaki, Y. Okada, M. Iida, D.J. Cua, Y. Iwakura, A. Yoshimura, Pivotal role of cerebral interleukin-17-producing gammadeltaT cells in the delayed phase of ischemic brain injury, Nat. Med. 15 (2009) 946–950, https://doi.org/10.1038/nm.1999.

[13] V.H. Brait, T.V. Arumugam, G.R. Drummond, C.G. Sobey, Importance of T lymphocytes in brain injury, immunodeficiency, and recovery after cerebral ischemia, J. Cereb. Blood Flow Metab. 32 (2012) 598–611, https://doi.org/10.1038/jcbfm.2010.6.

[14] K.A. Cho, J.W. Suh, K.H. Lee, J.L. Kang, S.Y. Woo, IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1beta by keratinocytes via the ROS-NLRP3-caspase-1 pathway, Int. Immunol. 24 (2012) 147–158, https://doi.org/10.1093/intimm/dxr110.

[15] D. Gris, Z. Ye, H.A. Iocca, H. Wen, R.R. Craven, P. Gris, M. Huang, M. Schneider, S.D. Miller, J.P. Ting, NLRP3 plays a critical role in the development of experimental autoimmune encephalomyelitis by mediating Th1 and Th17 responses, J. Immunol. 185 (2010) 974–981, https://doi.org/10.4049/jimmunol.0904145.

[16] B.N. Martin, C. Wang, C.J. Zhang, Z. Kang, M.F. Gulen, J.A. Zepp, J. Zhao, G. Bian, J.S. Do, B. Min, P.G. Pavicic Jr., C. El-Sanadi, P.L. Fox, A. Akitsu, Y. Iwakura, A. Sarkar, M.D. Wewers, W.J. Kaiser, E.S. Mocarski, M.E. Rothenberg, A.G. Hise, G.R. Dubyak, R.M. Ransohoff, X. Li, T cell-intrinsic ASC critically promotes T(H)17-mediated experimental autoimmune encephalomyelitis, Nat. Immunol. 17 (2016)

583–592, https://doi.org/10.1038/ni.3389.
[17] J.H. Jeong, K.S. Yu, D.H. Bak, J.H. Lee, N.S. Lee, Y.G. Jeong, D.K. Kim, J.J. Kim, S.Y. Han, Intermittent fasting is neuroprotective in focal cerebral ischemia by minimizing autophagic flux dist urbance and inhibiting apoptosis, Exp. Ther. Med. 12 (2016) 3021–3028, https://doi.org/10.3892/etm.2016.3852.
[18] A. Durukan, T. Tatlisumak, Ischemic stroke in mice and rats, Methods Mol. Biol. 573 (2009) 95–114, https://doi.org/10.1007/978-1-60761-247-6 6.
[19] H. Ren, Y. Kong, Z. Liu, D. Zang, X. Yang, K. Wood, M. Li, Q. Liu, Selective NLRP3 (Pyrin domain-containing protein 3) inflammasome inhibitor reduces brain injury after intracerebral hemorrhage, Stroke 49 (2018) 184–192, https://doi.org/10.1161/STROKEAHA.117.018904.
[20] F. Yang, Z. Wang, X. Wei, H. Han, X. Meng, Y. Zhang, W. Shi, F. Li, T. Xin, Q. Pang, F. Yi, NLRP3 deficiency ameliorates neurovascular damage in experimental ischemic stroke, J. Cereb. Blood Flow Metab. 34 (2014) 660–667, https://doi.org/10.1038/jcbfm.2013.242.
[21] T. Shichita, R. Sakaguchi, M. Suzuki, A. Yoshimura, Post-ischemic inflammation in the brain, Front. Immunol. 3 (2012) 132, , https://doi.org/10.3389/fimmu.2012.00132.
[22] D.Y. Fann, S.Y. Lee, S. Manzanero, S.C. Tang, M. Gelderblom, P. Chunduri, C. Bernreuther, M. Glatzel, Y.L. Cheng, J. Thundyil, A. Widiapradja, K.Z. Lok, S.L. Foo, Y.C. Wang, Y.I. Li, G.R. Drummond, M. Basta, T. Magnus, D.G. Jo, M.P. Mattson, C.G. Sobey, T.V. Arumugam, Intravenous immunoglobulin suppresses NLRP1 and NLRP3 inflammasome-mediated neuronal death in ischemic stroke, Cell Death Dis. 4 (2013) e790, https://doi.org/10.1038/cddis.2013.326.
[23] Y. Yin, Y. Yan, X. Jiang, J. Mai, N.C. Chen, H. Wang, X.F. Yang, Inflammasomes are differentially expressed in cardiovascular and other tissues, Int. J. Immunopathol. Pharmacol. 22 (2009) 311–322, https://doi.org/10.1177/039463200902200208.
[24] L. Gao, Q. Dong, Z. Song, F. Shen, J. Shi, Y. Li, NLRP3 inflammasome: a promising target in ischemic stroke, Inflamm. Res. 66 (2017) 17–24 http://10.1007/s00011-016-0981-7.
[25] L. Lammerding, A. Slowik, S. Johann, C. Beyer, A. Zendedel, Poststroke inflammasome expression and regulation in the peri-infarct area by gonadal steroids after transient focal ischemia in the rat brain, Neuroendocrinology 103 (2016) 460–475, https://doi.org/10.1159/000439435.
[26] Denes A, Coutts G, L¨¦n¨¢rt N, Cruickshank SM, Pelegrin P, Skinner J, Rothwell N, Allan SM, Brough D, AIM2 and NLRC4 inflammasomes contribute with ASC to acute brain injury independently of NLRP3, Proc. Natl. Acad. Sci. U. S. A., 112 (2015) 4050–5. doi:https://doi.org/10.1073/pnas.1419090112.
[27] M. Ito, T. Shichita, M. Okada, R. Komine, Y. Noguchi, A. Yoshimura, R. Morita, Bruton's tyrosine kinase is essential for NLRP3 inflammasome activation and contributes to ischaemic brain injury, Nat. Commun. 6 (2015) 7360, https://doi.org/10.1038/ncomms8360.
[28] A.R. Mridha, A. Wree, A.A.B. Robertson, M.M. Yeh, C.D. Johnson, D.M. Van Rooyen, F. Haczeyni, N.C. Teoh, C. Savard, G.N. Ioannou, S.L. Masters, K. Schroder, M.A. Cooper, A.E. Feldstein, G.C. Farrell, NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice, J. Hepatol. 66 (2017) 1037–1046, https://doi.org/10.1016/j.jhep.2017.01.022.
[29] J. Sun, Q. Luan, H. Dong, W. Song, K. Xie, L. Hou, L. Xiong, Inhibition of mitochondrial permeability transition pore opening contributes to the neuroprotective effects of ischemic postconditioning in rats, Brain Res. 1436 (2012) 101–110, https://doi.org/10.1016/j.brainres.2011.11.055.
[30] M. Levy, C.A. Thaiss, E. Elinav, Taming the inflammasome, Nat. Med. 21 (2015) 213–215, https://doi.org/10.1038/nm.3808.
[31] R. Zhou, A.S. Yazdi, P. Menu, J. Tschopp, A role for mitochondria in NLRP3 inflammasome activation, Nature 469 (2011) 221–225, https://doi.org/10.1038/nature09663.
[32] W.H. Zhang, X. Wang, M. Narayanan, Y. Zhang, C. Huo, J.C. Reed, R.M. Friedlander, Fundamental role of the Rip2/caspase-1 pathway in hypoxia and ischemia-induced neuronal cell death, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 16012–16017, https://doi.org/10.1073/pnas.2534856100.
[33] D.J. Cua, J. Sherlock, Y. Chen, C.A. Murphy, B. Joyce, B. Seymour, L. Lucian, W. To, S. Kwan, T. Churakova, S. Zurawski, M. Wiekowski, S.A. Lira, D. Gorman, R.A. Kastelein, J.D. Sedgwick, Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain, Nature 421 (2003) 744–748, https://doi.org/10.1038/nature01355.
[34] C. Parham, M. Chirica, J. Timans, E. Vaisberg, M. Travis, J. Cheung, S. Pflanz, R. Zhang, K.P. Singh, F. Vega, W. To, J. Wagner, A.M. O'Farrell, T. McClanahan, S. Zurawski, C. Hannum, D. Gorman, D.M. Rennick, R.A. Kastelein, R. de Waal Malefyt, K.W. Moore, A receptor for the heterodimeric cytokine IL-23 is composed of IL-12R 1 and a novel cytokine receptor subunit, IL-23R, J. Immunol. 168 (2002) 5699–5708, https://doi.org/10.4049/jimmunol.168.11.5699.
[35] C.L. Langrish, Y. Chen, W.M. Blumenschein, J. Mattson, B. Basham, J.D. Sedgwick, T. McClanahan, R.A. Kastelein, D.J. Cua, IL-23 drives a pathogenic T cell population that induces autoimmune inflammation, J. Exp. Med. 201 (2005) 233–240, https://doi.org/10.1084/jem.20041257.
[36] P. Arunachalam, P. Ludewig, P. Melich, T.V. Arumugam, C. Gerloff, I. Prinz, T. Magnus, M. Gelderblom, CCR6 (CC chemokine receptor 6) is essential for the migration of detrimental natural interleukin-17-producing gammadelta T cells in stroke, Stroke 48 (2017) 1957–1965, https://doi.org/10.1161/STROKEAHA.117.016753.
[37] C. Rong, W. Hu, F.R. Wu, X.J. Cao, F.H. Chen, Interleukin-23 as a potential therapeutic target for rheumatoid arthritis, Mol. Cell. Biochem. 361 (2012) 243–248, https://doi.org/10.1007/s11010-011-1109-6.
[38] L. Song, R. Zhou, S. Huang, F. Zhou, S. Xu, W. Wang, F. Yi, X. Wang, B. Xia, High intestinal and systemic levels of interleukin-23/T-helper 17 pathway in Chinese patients with inflammatory bowel disease, Mediat. Inflamm. (2013) (2013) 425915, https://doi.org/10.1155/2013/425915.
[39] D.W. Wojkowska, P. Szpakowski, D. Ksiazek-Winiarek, M. Leszczynski, A. Glabinski, Interactions between neutrophils, Th17 cells, and chemokines during the initiation of experimental model of multiple sclerosis, Mediat. Inflamm. 2014 (2014) 590409, https://doi.org/10.1155/2014/590409.
[40] Y. Zheng, D. Zhong, H. Chen, S. Ma, Y. Sun, M. Wang, Q. Liu, G. Li, Pivotal role of cerebral interleukin-23 during immunologic injury in delayed cerebral ischemia in mice, Neuroscience 290 (2015) 321–331, https://doi.org/10.1016/j.neuroscience.2015.01.041.
[41] M. Wang, D. Zhong, Y. Zheng, H. Li, H. Chen, S. Ma, Y. Sun, W. Yan, G. Li, Damage effect of interleukin (IL)-23 on oxygen-glucose-deprived cells of the neurovascular unit via IL-23 receptor, Neuroscience 289 (2015) 406–416, https://doi.org/10.1016/j.neuroscience.2015.01.012.
[42] Y. Lin, J.C. Zhang, C.Y. Yao, Y. Wu, A.F. Abdelgawad, S.L. Yao, S.Y. Yuan, Critical role of astrocytic interleukin-17 A in post-stroke survival and neuronal differentiation of neural precursor cells in adult mice, Cell Death Dis. 7 (2016) e2273, https://doi.org/10.1038/cddis.2015.284.
[43] E. Abdollahi, F. Tavasolian, A.A. Momtazi-Borojeni, M. Samadi, H. Rafatpanah, Protective role of R381Q (rs11209026) polymorphism in IL-23R gene in immune-mediated diseases: a comprehensive review, J. Immunotoxicol. 13 (2016) 286–300, https://doi.org/10.3109/1547691X.2015.1115448.
[44] C. Wynick, C. Petes, A. Tigert, K. Gee, Lipopolysaccharide-mediated induction of concurrent IL-1beta and IL-23 expression in THP-1 cells exhibits differential requirements for caspase-1 and cathepsin B activity, J. Interf. Cytokine Res. 36 (2016) 477–487, https://doi.org/10.1089/jir.2015.0134.
[45] C. Peral de Castro, S.A. Jones, C. Ni Cheallaigh, C.A. Hearnden, L. Williams, J. Winter, E.C. Lavelle, K.H. Mills, J. Harris, Autophagy regulates IL-23 secretion and innate T cell responses through effects on IL-1 secretion, J. Immunol. 189 (2012) 4144–4153, https://doi.org/10.4049/jimmunol.1201946.
[46] S.J. Lalor, L.S. Dungan, C.E. Sutton, S.A. Basdeo, J.M. Fletcher, K.H. Mills, Caspase-1-processed cytokines IL-1beta and IL-18 promote IL-17 production by gammadelta and CD4 T cells that mediate autoimmunity, J. Immunol. 186 (2011) 5738–5748, https://doi.org/10.4049/jimmunol.1003597.