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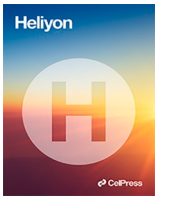

Research article

Protein inhibitor of activated STAT1 (PIAS1) alleviates cerebral infarction and inflammation after cerebral ischemia in rats

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A R T I C L E I N F O

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PIAS1
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A B S T R A C T

Background: Ischemic stroke is a severe disorder with high incidence, disability rate and mortality. Multiple pathogenesis mechanisms are involved in ischemic stroke, such as inflammation and neuronal cell apoptosis. Protein inhibitor of activated signal transducer and activators of transcription 1 (PIAS1) plays a crucial role in various biological processes, including inflammation. PIAS1 is also downregulated in ischemia-reperfusion injury and involved in the disease processes. However, the role of PIAS1 in cerebral ischemia is unclear.

Methods: Sprague-Dawley (SD) rats were induced with middle cerebral artery occlusion (MCAO). The role and mechanisms of PIAS1 in ischemic cerebral infarction were explored by Longa test, 2,3,5-triphenyltetrazolium chloride (TTC) staining, Morris water maze (MWM) test, hematoxylin-eosin (HE) staining, quantification of brain water content, reverse transcription-quantitative polymerase chain reaction (RT-qPCR), enzyme-linked immunosorbent assay (ELISA), terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL), Western blot and immunofluorescence assays.

Results: The expression of PIAS1 in MCAO-induced rat was declined compared to sham rats. Overexpression of PIAS1 reduced the Longa neurological scores, the percent of infarction area, the pathological abnormality, the escape latency of swimming and the percent of brain water content, and increased the number of platform crossings and time in the target quadrant in the MCAO-induced rats. Besides, overexpression of PIAS1 decreased the MCAO-induced the contents of IL-1β, IL-6 and TNF-α, but further elevated the concentrations of IL-10 in both sera and brain tissues. Moreover, overexpression of PIAS1 reversed the MCAO-induced apoptosis rate and the relative protein level of Bax, cleaved caspase3 and Bcl-2. Overexpression of PIAS1 also reversed the level of proteins involved in NF-κB pathway.

Conclusion: PIAS1 reduced inflammation and apoptosis, thereby alleviating ischemic cerebral infarction in MCAO-induced rats through regulation NF-κB pathway.

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1. Introduction

Stroke is the one of the primary causes of disability and also the second leading cause of death around the world [1]. The incidence of stroke has been reported to increase rapidly in the population aged 65 and older, and the incidence is estimated to increase faster year by year due to aging [2]. In addition, the incidence of stroke has been demonstrated to be increasing in young people duo to the unhealthy eating habits [2]. More than 80 % of stroke cases are identified as ischemic stroke, which results in poor life quality of patients and immense public health burden [3,4]. Several approached have been used for the treatment of stroke [5]. Thrombolysis and anticoagulants remain the mainstay of the stroke therapy. However, thrombolysis has a limited treatment time window, and only 10 % or less cases can receive the treatment within a suitable time frame [6]. Besides, nerve stimulation is an auxiliary or alternative strategy for the patient failed with thrombolysis treatment, which can not only protect the brain during acute ischemia, but also repair the nerve function with severe functional damage in the poststroke stage with an exception for enhancing the recovery of neuronal function [7]. Nevertheless, prospective preclinical studies have not been translated into positive outcomes in clinical trials yet, although a large number of candidate agents have exhibited the efficacy and safety in a variety of animal stroke models over the past few decades [8]. Thus, deep understanding of the mechanisms of cerebral ischemia is essential for the identification of new curative targets.

Protein inhibitor of activated signal transducer and activators of transcription 1 (PIAS1) is a small ubiquitin-like modifier (SUMO) E3 ligase, which plays a crucial role in variety of biological processes both *in vitro* and *in vivo*, such as inflammation [9]. It is reported that the expression of PIAS1 is downregulated after traumatic brain injury (TBI) [10]. Inhibition of PIAS1 in CA1 area lead to the damage of memory and spatial learning in rats [11]. PIAS1 relieves diabetic peripheral neuropathy in mice, and PIAS1 effectively inhibits the expression of sciatic Col IV and the sustained increase in fibrotic area [12]. More importantly, the expression of PIAS1 is also decreased in myocardial ischemia-reperfusion injury [13] and hepatic ischemia-reperfusion injury [14]. PIAS1 deficiency after myocardial ischemia exacerbates inflammation and apoptosis of cardiomyocytes through activation of the NF-κB pathway [13]. PIAS1 mitigates hepatic ischemia-reperfusion injury, in which PIAS1 inhibits the apoptosis and inflammatory response of hepatocytes [14]. However, the role and related mechanisms of PIAS1 in cerebral ischemia are unclear.

Therefore, Sprague-Dawley (SD) rats were induced with middle cerebral artery occlusion (MCAO) to establish an ischemic cerebral infarction model *in vivo*. The role and mechanisms of PIAS1 in ischemic cerebral infarction were explored in MCAO-induced rats. Our findings have the potential to identify a potential target for the treatment of ischemic stroke.

2. Materials and methods

2.1. Animal

Sprague Dawley (SD) male rats (180–220 g) were provided by Vital River (Beijing, China) and raised to laboratory room with 12 h/12 h light-dark cycle and 40%–60 % the relative humidity at 22 °C. All the processes were grimly executed in keeping with the Guide for the Care and Use of Laboratory Animals [15] and the Animal Research Ethics Committee of ***Hospital.

2.2. Construction of MCAO model

The MCAO model was constructed in SD rats based on the previous study [16]. Rats were anesthetized with 2 % isoflurane (RWD Life Science, Shenzhen, China), and then a midline cervical incision was generated. The branches of the right external carotid artery were ligated, and the right internal carotid artery was occluded with a thread to induce ischemia. The sham rats were treated the similar operative procedures without the MCAO.

2.3. Animal group and treatment

Rats were stochastically apportioned into four groups (n = 6), namely sham, MCAO, MCAO + AAV-empty and MCAO + AAV-PIAS1. The sequence of PIAS1 and the relevant negative control were constructed and packed into adeno-associated virus 2 (AAV2) virus (GENECHEM, Shanghai, China) to upregulate the expression of PIAS1. AAV particles (2 μl) were stereotactically injected into the cortex at the rate of 0.5 μl/min at 3 sites (AP + 1.2 [site 1], 0.3 [site 2], −0.6 [site 3]; ML + 5.5; DV -3.5 mm from the skull) based on the previous study [17] by an automatic injector. After the administration, the needle was stayed for extra 5 min, and withdrawn a short distance for residence another 2 min. Rats in MCAO, MCAO + AAV-empty and MCAO + AAV-PIAS1 groups were first stereotactically injected with phosphate buffer saline (PBS) (P1020, Solarbio, Beijing, China), AAV particles packed scrambled control and AAV particles packed PIAS1, severally. After 15 d, rats were challenged with MCAO. Rats in sham group were stereotactically injected with PBS, and then conducted the similar operative procedures without the MCAO. After being subjected to neurological scores, blood sampling and Morris water maze (MWM) test, rats were sacrificed by dislocation. The timeline of the experimental procedure was exhibited in Fig. 1.

2.4. Assessment of neurological scores

The neurological deficit was assessed with the Longa test according to the previous report [16]. Briefly, rats were classified into four

grades. Grade 0 was identified when rats were without neurological impairment (normal), grade 1 referred that the left forepaw of rats was inextensible when the rats' tail was lifted (mild), the condition that rats were circling to the left side when walking (moderate) was defined as grade 2, grade 3 was identified when the rat had hard walking or leant to the left (severe), and grade 4 referred that the rats could not spontaneously walk (very severe).

2.5. MWM tests

As described in the previous study [18], MWM experiments were performed in a swimming pool with a diameter of 110 cm filled with a depth of approximately 30 cm water (22 ± 2 °C). The pool was divided into four quadrants, one of which was always placed with a platform in the center except for the experiments executed on the last day. The pool was encompassed with different cues to assist rats to recognize the spatial orientation. Learning ability of rats was assessed for four consecutive days. Each rat was put into the pool facing the wall and trained to discover the platform with four trials per day. Rats were permit to arrive at the platform within 90 s, and the time and tracks for rats to find the platform were documented through a video camera. If the time that rats reach the platform exceeded 90 s, the rat was guided to the platform manually for 10 s to accommodate the environment and the latency was designated as 90 s. One day after the final training, the platform was removed and the rats were administrated to a free exploration experiment for 90 s. The time spent in the target quadrant as well as tracks for rats were monitored and analyzed via a video tracking apparatus (Ethovision XT, Noldus, Netherlands).

2.6. 2,3,5-Triphenyltetrazolium chloride (TTC) staining

Brain tissues were rapidly segregated and sliced into six coronal sections at 2-mm intervals. The slices were hatched with 1 % TTC staining solution (G3005, Solarbio) at 37 °C for 20 min, and then photographed. The infarcted areas were measured through ImageJ software (National Institutes of Health, USA), and then the percentage of infarct volume was determined by the infarct volume/the total contralateral hemispheric volume × 100 %.

2.7. Hematoxylin-eosin (HE) staining

Brain tissues were fixed into 4 % paraformaldehyde (P1110, Solaibio) for overnight. The samples were embedded in paraffin (YA0012, Solaibio) and sliced into sections with a 5 μm thickness. Then, the sections were stained with Hematoxylin and Eosin Staining Kit (C0105S, Beyotime), and the images were captured by a light microscopy (Olympus, Tokyo, Japan).

2.8. Quantification of brain water content

Brain tissues were separated and weighted by an electronic scale (wet weight), and then dried at 105 °C overnight. The weight of dried brains was measured (dry weight). The brain water content was determined as the following formula [(wet weight - dry weight)/wet weight] × 100 %.

2.9. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA from brain tissues was isolated using TRIzol reagent (15596026, TaKaRa Biotechnology Co., Ltd., Dalian, China) and RT was performed by Bio-Rad ScripTM cDNA Synthesis Kit (1708890, Bio-Rad Laboratories, Inc., Hercules, CA, USA) based on the operating instruction. RT-qPCR was conducted using the Bio-Rad CFX Manager software (Bio-Rad Laboratories, Inc.). The primer sequences of PIAS1 (Forward primer: 5′-TCCTGCTGTAGATACAAGCTAC-3′, Reverse primer: 5′-TGCCAAAGTGGACGCTGTGTC-3′) were synthesized in Sangon Biotech (Shanghai, China). The RT-qPCR conditions were as follows: 5 min at 94 °C, followed by 40 cycles between 94 °C for 15 s and 60 °C for 30 s, and 72 °C for 30 s. The relative expression level of FGF13 was calculated using the $2^{-\Delta\Delta CT}$ method and normalized to *GAPDH*.

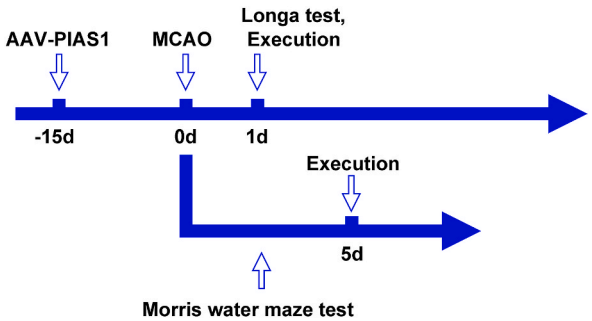

Fig. 1. The timeline of the experimental procedure.

2.10. Enzyme-linked immunosorbent assay (ELISA)

Rat's blood was collected from the orbital artery 24 h post ischemic-reperfusion. The blood samples were stood at room temperature for 2 h, and then at 4 °C for overnight. Subsequently, blood samples were centrifuged with 4000 g at 4 °C for 10 min to isolate the serum samples. The concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-10 in sera and brain tissues were examined by using Rat TNF-α ELISA Kit (PT516, Beyotime, Shanghai, China), Rat IL-1β ELISA Kit (PI303, Beyotime), Rat IL-6 ELISA Kit (PI328, Beyotime) and Rat IL-10 ELISA Kit (PI525, Beyotime) based on the working instructions. The absorbance was examined at 450 nm with a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

2.11. Terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) assay

The brain tissues were isolated and soaked into 4 % paraformaldehyde. Subsequently, the tissues were dehydrated with gradient alcohol and inset into paraffin. Paraffin-embedded tissues were sliced into 5 μm slices, and treated with a TUNEL Apoptosis Assay Kit (T2190, Solarbio) following the operation instruction. The images were acquired by a fluorescence microscopy (IX71, Olympus, Tokyo, Japan). Ten random non-overlapping fields (× 400) were selected to calculate the apoptosis rate through the formula of TUNEL stained cells/DAPI stained cells × 100 % by ImageJ software (National Institutes of Health, USA). Three slices from each rat (a total of three rats) were used for the analysis.

2.12. Western blot

According to the previous studies [19,20], total protein samples were harvested from brain tissues by a Total Protein Extraction Kit (BC3711, Solarbio), and measured with the BCA Protein Assay Kit (PC0020, Solarbio). Then, protein samples were separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto polyvinylidene fluoride (PVDF) membranes (IPVH00010, EMD Millipore, Billerica, MA, USA). The membranes were sealed with western blocking buffer (SW3010, Solarbio) and hatched with primary antibodies at 4 °C overnight. Bounds were determined with goat anti-rabbit IgG H&L (HRP) (ab6721, 1:10000, Abcam) and ECL Western Blotting Detection Kit (Goat IgG) (SW2030, Solarbio). The band intensity was examined via QUANTITY ONE software (Bio-Rad, Hercules, CA, USA). The expression level of each protein was shown after being normalized with GAPDH. The primary antibodies contained anti-PIAS1 (1:10000, ab109388, Abcam, Cambridge, UK), anti-Bax (1:5000, ab32503, Abcam), anti-Bcl-2 (1:2000, ab196495, Abcam), anti-cleaved caspase3 (1:1000, 9661, Cell Signaling Technology, Inc., Danvers, MA, USA), anti-p65 (1:1000, 4764, Cell Signaling Technology), anti-phosphorylated p65 (p-p65) (1:1000, 3031, CST), anti-IκBα (1:1000, 9242, CST), anti-p-IκBα (1:1000, 2859, CST) and anti-GAPDH (1:10000, ab181602, Abcam).

2.13. Immunofluorescence (IF) assay

Rats were transcardially perfused with ice-cold 0.1 M PBS (P1020, pH 7.4, Solarbio) followed by ice-cold 4 % buffered paraformaldehyde. Then, rats were sacrificed, and the brains were quickly removed and immersed in 4 % buffered paraformaldehyde overnight. The brain tissues were cut into 5 μm-thick coronal sections consecutively after embedded with OCT (SAKURA, CA, USA) with Leica CM 1950 Frozen slicer (Leica microsystems, Wetzlar, Germany). After air-dried for 30 min at room temperature, brain sections were washed with 0.1 M PBS to wipe out OCT compound. Subsequently, sections were hatched with blocking buffer (PBS including 3 % bovine serum albumin (BSA, SW3015, Solarbio) and 0.2 % Triton X-100 (T8200, Solarbio)) followed by incubation with rabbit monoclonal to Iba1 (1:200, ab178846, Abcam) or rabbit monoclonal to p-p65 (1:800, 3033, Cell Signaling Technology) at 4 °C overnight. After three times of wash with 0.1 M PBS for 10 min, sections were hatched with Goat anti-rabbit IgG-AlexaFluor 488 (1:500, ab150077, Abcam) or Goat anti-rabbit IgG-AlexaFluor 647 (1:500, ab150079, Abcam) for 1 h at room temperature. Sections were mounted using Mounting Medium, antifading (with DAPI) (S2110, Solarbio) after rinsed three times in 0.1 M PBS. Sections were analyzed by a fluorescence microscopy.

2.14. Statistical analysis

All results were exhibited as mean ± standard deviation (SD). Data analysis was conducted by SPSS 20.0 software (IBM, Armonk, New York, USA) with one-way analysis of variance (ANOVA) followed by *Post Hoc* Bonferroni test. $P < 0.05$ was defined as significant difference.

3. Results

3.1. PIAS1 reduced cerebral infarction area in MCAO-induced rats

To explore the role of PIAS1 in ischemic cerebral infarction, rats were challenged with MCAO to simulate ischemic cerebral infarction *in vivo*. The Longa neurological scores of MCAO-induced rats were notably increased compared with sham rats (Fig. 2A). The relative mRNA and protein levels of PIAS1 in brain tissues from MCAO-induced rats were markedly decreased compared with that from sham rats (Fig. 2B and C). Treatment with PIAS1-packaged AAV significantly upregulated the relative mRNA and protein levels of PIAS1 (Fig. 2B and C). Overexpression of PIAS1 in MCAO-induced rats significantly decreased the Longa neurological scores (Fig. 2A).

Moreover, overexpression of PIAS1 notably reduced the MCAO-induced the percentage of infarction area in rats (Fig. 2C). Thus, these results indicated that PIAS1 reduced cerebral infarction area in MCAO-treated rats.

3.2. PIAS1 improved MCAO-induced brain tissue damage

Next, pathological staining of cortex tissues showed that the cells of the rat cerebral cortex were neatly arranged and morphologically normal in sham rats, while the cerebral cortex cells were loosely arranged, swollen, and nucleus contracted after MCAO surgery (Fig. 3A). The abnormal pathological results in MCAO-induced rats were obviously improved with the overexpression of PIAS1 (Fig. 3A). The pathological changes of hippocampus tissues were assessed by HE staining. The results showed that the pyramidal neurons in the CA1 region of the hippocampus in sham rats were neatly arranged, clearly stained, moderately sized and had normal microstructure, while obvious pathological changes, including loose neuron arrangement, shallow staining, and obvious atrophy and loss of neurons, were observed in MCAO-induced rats (Fig. 3B). The abnormal pathological results in hippocampus tissues from MCAO-induced rats were observably improved with the overexpression of PIAS1 (Fig. 3B). In addition, the percentage of brain water content in MCAO-challenged rats was observably increased compared to that in sham rats, which was markedly counteracted with the overexpression of PIAS1 (Fig. 3C). MWM tests showed that MCAO induced a prominent increase in the escape latency of swimming, and a remarkable reduction in the number of platform crossings and time in the target quadrant were observed the overexpression of

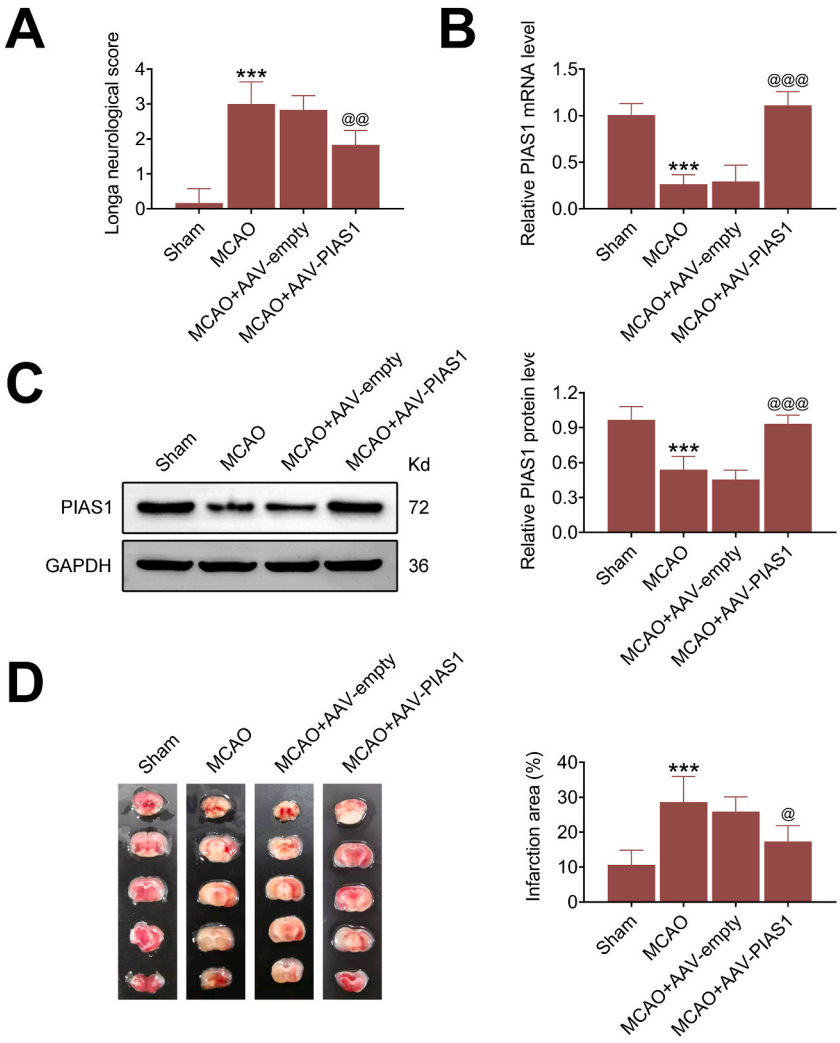

Fig. 2. PIAS1 decreased cerebral infarction area in MCAO-induced rats. (A) The neurological deficit was assessed with the Longa test. (B) The relative mRNA expression of PIAS1 was examined by RT-qPCR. The results were expressed after being normalized with *GAPDH*. (C) The relative protein expression of PIAS1 was examined by Western blot. The results were expressed after being normalized with GAPDH. The full and non-adjusted images have been provided as supplementary Fig. 1 (D) The cerebral infarction area was determined by TTC staining. ***$P < 0.001$ *vs.* sham; @$P < 0.05$, @@$P < 0.01$ and @@@$P < 0.001$ *vs.* MCAO + AAV-empty.

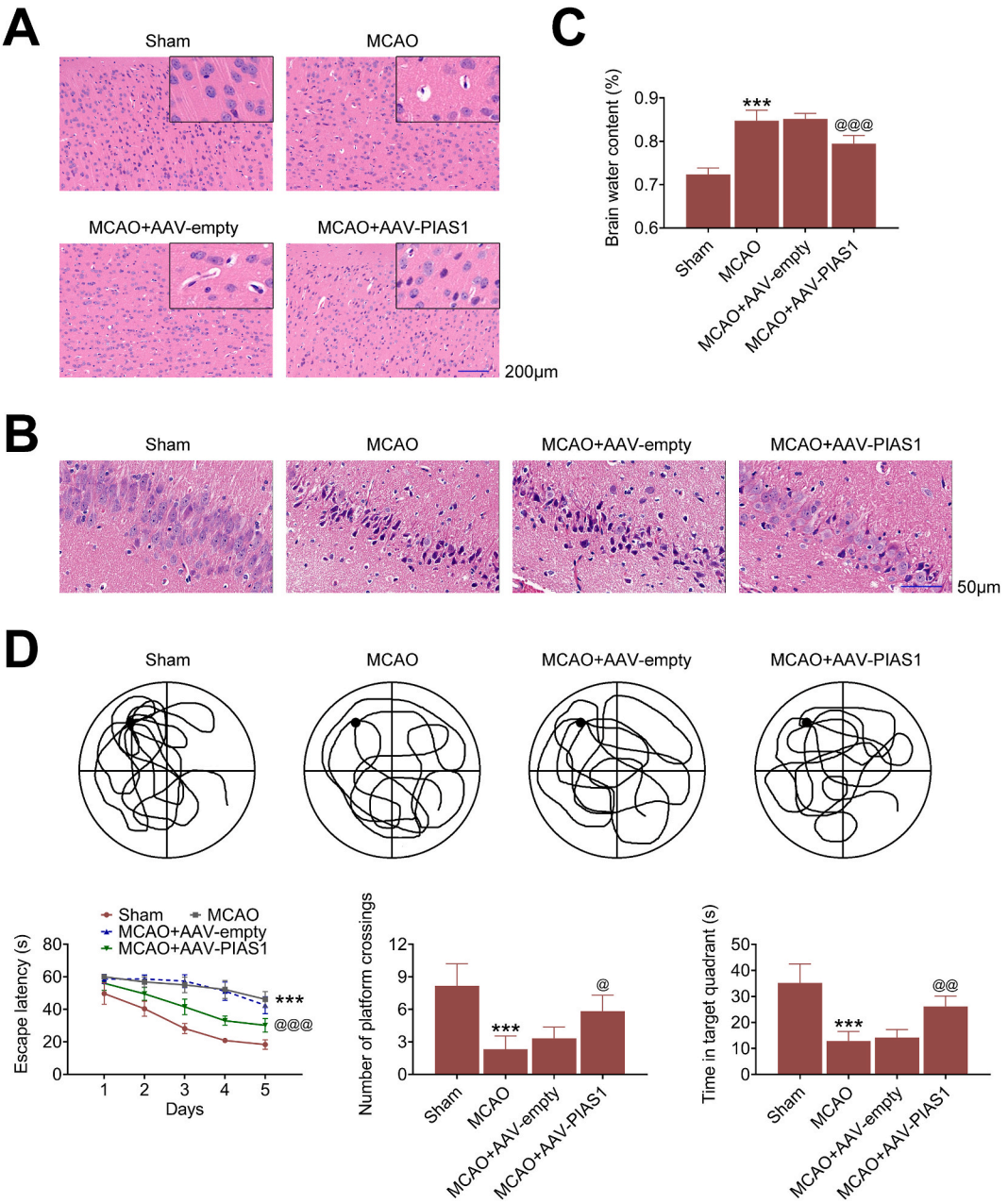

Fig. 3. PIAS1 improved MCAO-induced brain tissue damage. (A) The pathological changes in cortexes were determined by HE staining. Scale bar = 200 μm. (B) The pathological changes in hippocampus were assessed by HE staining. Scale bar = 50 μm. (B) The percent of brain water content was measured and calculated by [(wet weight - dry weight)/wet weight] × 100 %. (D) The escape latency, number of platform crossings and the time of target quadrant of rats were evaluated by MWM tests. ***$P < 0.001$ *vs.* sham; @$P < 0.05$, @@$P < 0.01$ and @@@$P < 0.001$ *vs.* MCAO + AAV-empty.

PIAS1 (Fig. 3D), indicating that overexpression of PIAS1 could improve A MCAO-induced memory defect. Therefore, these results suggested that PIAS1 ameliorated MCAO-induced brain tissue damage and memory defect.

3.3. PIAS1 decreased the inflammatory response in MCAO-induced rats

To address the role of PIAS1 in inflammatory response in ischemic cerebral infarction, the contents of several inflammatory factors, including TNF-α, IL-1β, IL-6 and IL-10 were examined by ELISA assay. The serum levels of TNF-α, IL-1β and IL-6 were prominently enhanced in MCAO-induced rats, which were significantly attenuated with the overexpression of PIAS1 (Fig. 4A). However, the serum

levels of IL-10 were notably increased in MCAO-induced rats, which were significantly further enhanced with the overexpression of PIAS1 (Fig. 4A). Besides, similar results were observed in the concentrations of TNF-α, IL-1β, IL-6 and IL-10 in brain tissues (Fig. 4B). Moreover, the effect of PIAS1 on microglia was examined by IF experiments, and the results showed that the level of microglia from MCAO-induced rats was obviously enhanced compared with that in sham rats, which was distinctly attenuated with the overexpression of PIAS1 (Fig. 4C). Hence, these results indicated that PIAS1 inhibited the release of inflammatory factors in MCAO-induced rats.

3.4. PIAS1 improved MCAO-elicited apoptosis of brain tissue

To determine the role of PIAS1 in apoptosis in ischemic cerebral infarction, the apoptosis rate and the expression level of apoptosis-related proteins were examined. As shown in Fig. 5A, the apoptosis rate was markedly increased in MCAO-induced rats compared with sham rats, which was observably reversed with the overexpression of PIAS1 (Fig. 5A). In addition, the treatment of MCAO evoked a prominent enhancement in the relative protein level of Bax and cleaved-caspase3, but a remarkable decrease in the relative protein level of Bcl-2 (Fig. 5B). However, overexpression of PIAS1 significantly reduced the relative protein level of Bax, cleaved-caspase3 and enhanced Bcl-2 in MCAO-induced rats (Fig. 5B). Moreover, the ratio of Bax/Bcl-2 was markedly enhanced in MCAO-induced rats, which was significantly counteracted with the overexpression of PIAS1 (Fig. 5B). Thus, these results suggested that PIAS1 suppressed MCAO-induced apoptosis of brain tissue.

3.5. PIAS1 inhibited NF-κB pathway in MCAO-induced rats

To explore the mechanism of PIAS1 in ischemic cerebral infarction, the expression of proteins-involved in NF-κB pathway was examined via Western blot. The relative protein level of p-p65/p65 was markedly increased in MCAO-induced rats compared with sham rats, which was notably neutralized with the overexpression of PIAS1 (Fig. 6A). Similar results were also found in the relative protein level of p-IκBα (Fig. 6A). However, the relative protein level of IκBα was observably decreased in MCAO-induced rats relative to

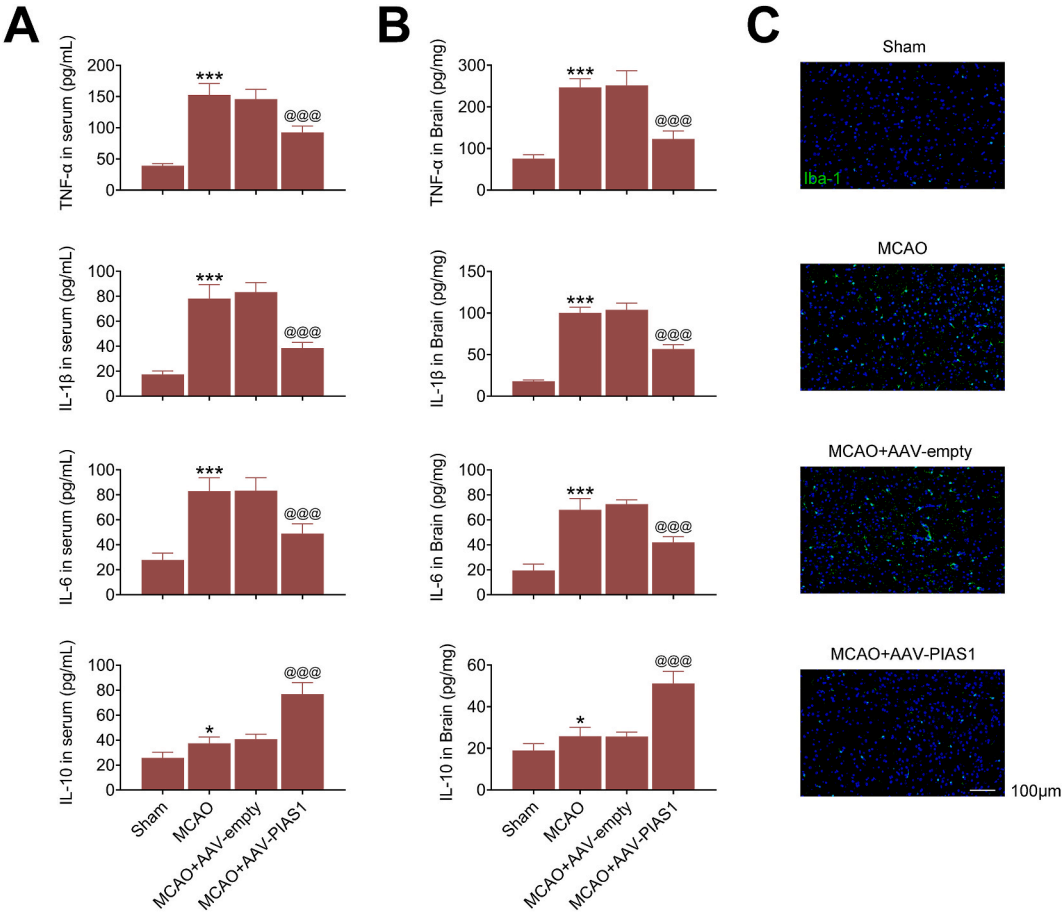

Fig. 4. PIAS1 suppressed the release of inflammatory factors in MCAO-treated rats. (A) The serum levels of TNF-α, IL-1β, IL-6 and IL-10 were examined by ELISA. (B) The concentrations of TNF-α, IL-1β, IL-6 and IL-10 in brain tissues were measured by ELISA. (C) The microglial cells were stained by Iba1 antibody. Scale bar = 100 μm $^{*}P < 0.05$ and $^{***}P < 0.001$ *vs.* sham; @@@$P < 0.001$ *vs.* MCAO + AAV-empty.

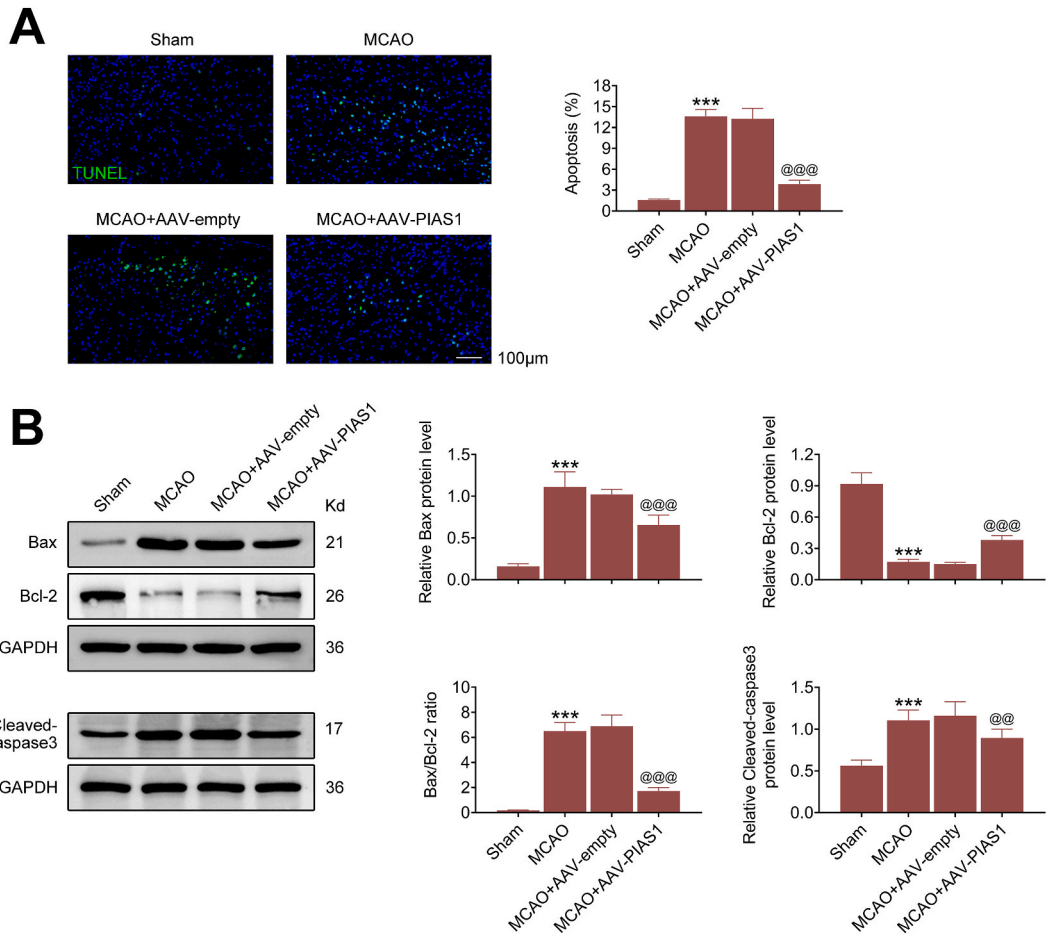

Fig. 5. PIAS1 repressed MCAO-induced apoptosis of brain tissue. (A) The apoptosis rate was measured by TUNEL staining. (B) The relative protein expression of Bax, Bcl-2 and cleaved caspase 3 was examined by Western blot. The results were expressed after being normalized with GAPDH. The full and non-adjusted images have been provided as Supplementary Fig. 2. ***$P < 0.001$ *vs.* sham; @@$P < 0.01$ and @@@$P < 0.001$ *vs.* MCAO + AAV-empty.

that in sham rats, which was prominently restored with the overexpression of PIAS1 (Fig. 6A). In addition, the effect of PIAS1 overexpression on NF-κB nuclear translocation in ischemia was addressed, and the results showed that MCAO induced an obvious decrease in the nuclear expression of p-p65 in rats compared with the sham rats, which was visibly restored with the overexpression of PIAS1 (Fig. 6B). Collectively, PIAS1 repressed NF-κB pathway in MCAO-induced rats.

4. Discussion

In the current study, rats were treated with middle cerebral artery occlusion (MCAO) to simulate ischemic cerebral infarction *in vivo*. The expression of PIAS1 in MCAO-challenged rats was declined compared with sham rats. Overexpression of PIAS1 reduced MCAO-induced cerebral infarction area and improved MCAO-induced brain tissue damage and memory defect. At the molecular level, overexpression of PIAS1 inhibited the release of inflammatory factors and apoptosis in MCAO-induced rats. Mechanically, overexpression of PIAS1 reversed the level of proteins involved in NF-κB pathway induced by MCAO. Taken together, PIAS1 alleviated ischemic cerebral infarction in MCAO-induced rats, which was closely related to NF-κB pathway (Fig. 7).

MCAO is commonly used to establish animal model to simulate the ischemic cerebrovascular disorder [17,21,22]. In this study, rats were treated with MCAO to cause a middle cerebral artery ischemia, thereby inducing a model of ischemic cerebral infarction *in vivo*, in accordance with the previous studies [16,17,21,22]. MCAO treatment prominently increased the Longa neurological scores, the percent of infarction area, the pathological abnormality, memory defect and the percent of brain water content in rats, consistant with the previous findings [16], which indicated thatthe model of ischemic cerebral infarction was successfully constructed in rats. Our results showed that the expression of PIAS1 was decreased in MCAO-induced rats. Overexpression of PIAS1 recovered the above-mentioned indicators in MCAO-induced rats. Thus, PIAS1 improved ischemic cerebral infarction *in vivo*.

Numerous studies have demonstrated that inflammation serves an important role in the progression of ischemic brain injury

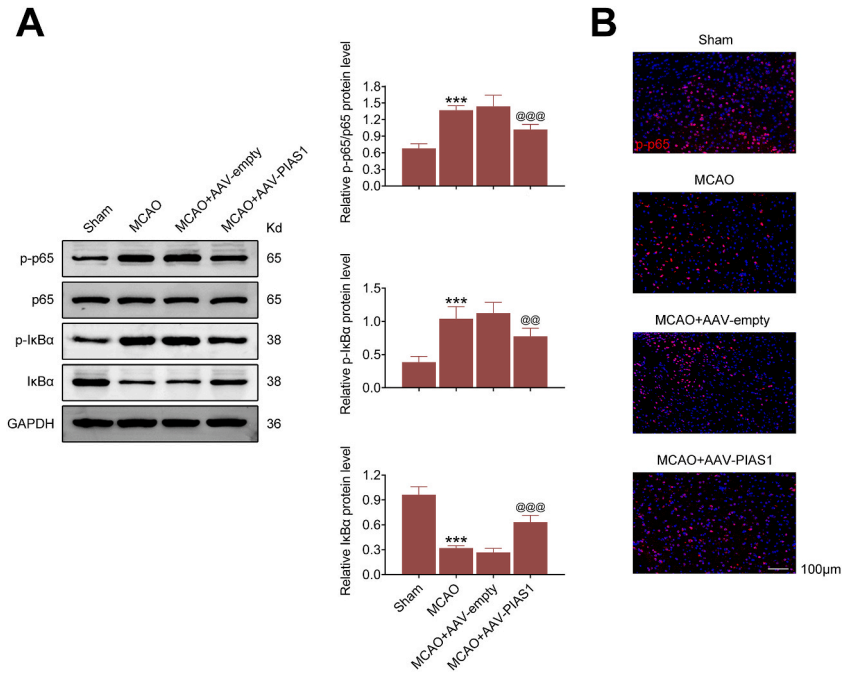

Fig. 6. PIAS1 inhibited NF-κB pathway in MCAO-treated rats. (A) The relative protein expression of p65, p-p65, IκBα and p-IκBα was determined by Western blot. The results were expressed after being normalized with GAPDH. The full and non-adjusted images have been provided as supplementary Fig. 3 (B) The expression of p-p65 was assessed by IF assays. ***$P < 0.001$ *vs.* sham; @@$P < 0.01$ and @@@$P < 0.001$ *vs.* MCAO + AAV-empty.

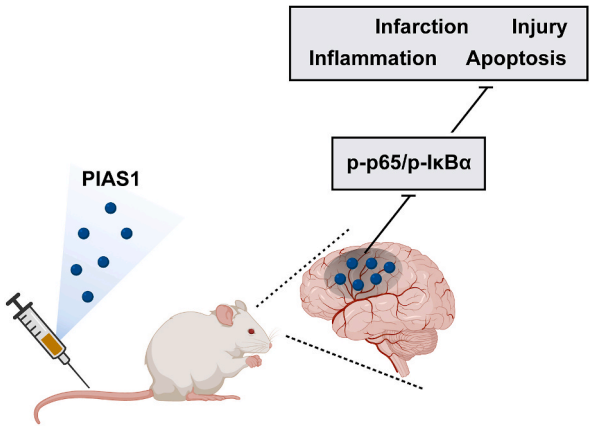

Fig. 7. The schematic diagram of this study. Upregulation of PIAS1 in MCAO-induced rats reduced cerebral infarction area and improved MCAO-induced brain tissue damage and memory defect. At the molecular level, overexpression of PIAS1 inhibited the release of inflammatory factors and apoptosis in MCAO-induced rats. Mechanically, overexpression of PIAS1 reversed the level of proteins involved in NF-κB pathway. Taken together, PIAS1 alleviated ischemic cerebral infarction in MCAO-induced rats, which was strongly involved in NF-κB pathway.

[23–26]. Inflammation is closely associated with poor clinical outcomes in patients with stroke [27]. Pre-clinical studies have shown that inflammatory cells are recruited and activated in focal cerebral ischemic strokes in a time-dependent fashion, while inhibition of inflammatory response ameliorates the neurological deficit and reduces the infarct volume [28]. The levels of inflammatory factors, such as TNF-α, IL-1β and IL-6 were enhanced 24 h after ischemia in a transient MCAO model, and the decrease in these factors benefits the treatment of ischemic strokes [29]. Consistently, the levels of TNF-α, IL-1β, IL-6 and IL-10 were found to be upregulated in both sera and brain tissues from MCAO-induced rats, suggesting an activated inflammatory response in MCAO-induced rats. However, over-expression of PIAS1 decreased the MCAO-induced the contents of TNF-α, IL-1β and IL-6, but further elevated the concentrations of IL-10 in both sera and brain tissues. Activated PIAS1 has been revealed to recruit inflammatory gene promoters, and therefore therapeutical strategies targeting the PIAS1 SUMO ligase pathway is alternative approaches for the treatment of inflammatory diseases

[30]. PIAS1 regulates inflammation of endothelial cell [31]. Enhancement of PIAS1 prevents sodium taurocholate-evoked severe acute pancreatitis related to acute lung injury [32]. PIAS1 suppresses inflammatory cascades in adipose tissue to prevent obesity-elicited insulin resistance [33]. Knockdown of PIAS1 aggravates inflammation of cardiomyocytes in myocardial ischemia-reperfusion injury [13]. Collectively, PIAS1 inhibited the release of inflammatory factors in ischemic cerebral infarction.

Acute ischemic stroke can result in neurons damage, and apoptosis is a critical pathogenesis of ischemic brain damage [34]. Tang et al. [35] reported that delayed recanalization after MCAO represses apoptosis to relieve ischemic stroke. Restoration of long OPA1 mitochondrial dynamin like GTPase (L-OPA1) mitigates acute ischemic stroke injury by suppressing apoptosis [36]. In the present study, a prominent upregulation in the apoptosis rate and the relative protein expression of Bax, and a remarkable reduction in the relative protein level of Bcl-2 were discovered in MCAO-induced rats, which were reversed with the overexpression of PIAS1. PIAS1 has been revealed to modulate the sumoylation of p53 to control stress-evoked apoptosis of lens epithelial cells via the proapoptotic modulator Bax [37]. PIAS1 can attenuate the apoptosis of Schwann cells in mice with diabetic peripheral neuropathy [12]. Down-regulation of PIAS1 promotes the cardiomyocytes apoptosis in myocardial ischemia-reperfusion injury [13]. Upregulation of PIAS1 inhibits apoptosis of hepatocytes in hepatic ischemia-reperfusion injury [14]. Collectively, PIAS1 suppresses apoptosis in ischemic cerebral infarction.

NF-κB is a transcription factor that specifically binds to the promoters and enhancers of various genes to participate in different cellular functions, and therefore NF-κB pathway is involved in the progression of many diseases [38,39]. NF-κB pathway is also demonstrated to be strongly associated with the inflammatory responses [40], and can cause neuroinflammatory responses and damage following ischemic stroke [41]. Suppression of NF-κB pathway reduces the level of inflammatory response genes, ameliorates neurological deficits and decreases infarct size after acute cerebral infarction [42]. In the current study, the relative protein level of p-p65/p65 was increased in MCAO-induced rats. In addition, the relative protein level of p-IκBα was enhanced but the relative protein level of IκBα was downregulated in MCAO-induced rats, indicating that the activity of IκBα was enhanced in spite of the reduced expression of total protein. However, overexpression of PIAS1 reversed the level of proteins related to NF-κB pathway. PIAS1 has been identified as a negative modulator of NF-κB [43]. PIAS1 interacts with PRRS virus nucleocapsid protein to mediate the activation of NF-κB and evoke proinflammatory factors during viral infection [44]. Inhibition of PIAS1 enhances apoptosis and inflammation of cardiomyocytes by activating the NF-κB pathway after myocardial ischemia-reperfusion injury [13]. Therefore, PIAS1 represses NF-κB pathway in ischemic cerebral infarction.

In conclusion, PIAS1 reduced inflammation and apoptosis to alleviate ischemic cerebral infarction in MCAO-induced rats, which was related to NF-κB pathway. However, the direct role of NF-κB pathway in ischemic cerebral infarction should be addressed in the following study. In addition, the role of PIAS1 can be studied through *in vitro* models, such as lipopolysaccharide (LPS) + interferon-γ (IFN-γ) and oxygen-glucose deprivation (OGD) induced neuroinflammatory microglia model, which can deeply dissolve the participation of the PIAS1 in the survival and death of cells in brain ischemia. Moreover, more pre-clinical and clinical trials need to be conducted in the future. In summary, our results demonstrate that PIAS1 may be a potential target for the treatment of cerebral ischemic injury, and even other related cerebrovascular diseases.

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Data availability statement

No data was used for the research described in the article. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics approval

Ethical approval was obtained from the Ethics Committee of Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University (Approval No. 2019-014).

All animal experiments should comply with the ARRIVE guidelines and conducted in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

CRediT authorship contribution statement

Mingyang Wang: Conceptualization. **Pingzhi Wang:** Formal analysis. **Bo Li:** Conceptualization, Visualization. **Guohu Zhao:** Supervision. **Nan Zhang:** Supervision, Writing – original draft. **Ruifeng Cao:** Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not application.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24743.

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