RESEARCH PAPER

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Fasudil alleviates cerebral ischemia-reperfusion injury by inhibiting inflammation and improving neurotrophic factor expression in rats

Min-Fang Guo[1], Hui-Yu Zhang[1], Pei-Jun Zhang[1], Yi-Jin Zhao[1], Jing-Wen Yu[1], Tao Meng[1], Meng-Di Li[2], Na Li[2], Cun-Gen Ma[1,2], Li-Juan Song[2,4]*, Jie-Zhong Yu[1,3]*

[1] Institute of Brain Science, Shanxi Datong University, Datong, China
[2] Research Center of Neurobiology, The Key Research Laboratory of Benefiting Qi for Acting Blood Circulation Method to Treat Multiple Sclerosis of the State Administration of Traditional Chinese Medicine, Shanxi University of Chinese Medicine, Jinzhong, China
[3] Department of Neurology, Datong Forth People's Hospital, Datong, China
[4] Department of Physiology, Shanxi Medical University, Taiyuan, China
** Email: sxdtyjz2020@163.com; slj_0354@126.com*

The Rho kinase inhibitor fasudil exerts neuroprotective effects. We previously showed that fasudil can regulate M1/M2 microglia polarization and inhibit neuroinflammation. Here, the therapeutic effect of fasudil on cerebral ischemia-reperfusion (I/R) injury was investigated using the middle cerebral artery occlusion and reperfusion (MCAO/R) model in Sprague-Dawley rats. The effect of fasudil on the phenotype of microglia and neurotrophic factors in the I/R brain and its potential molecular mechanism was also explored. It was found that fasudil ameliorated neurological deficits, neuronal apoptosis, and inflammatory response in rats with cerebral I/R injury. Fasudil also promoted the polarization of microglia into the M2 phenotype, in turn promoting the secretion of neurotrophic factors. Furthermore, fasudil significantly inhibited the expression of TLR4 and NF-κB. These findings suggest that fasudil could inhibit the neuroinflammatory response and reduce brain injury after I/R injury by regulating the shift of microglia from an inflammatory M1 phenotype to an anti-inflammatory M2 phenotype, which may be related to the regulation of the TLR4/ NF-κB signal pathway.

Key words: neuroinflammatory, cerebral ischemia/reperfusion, microglia polarization, neurotrophic factors, apoptosis, TLR4 signal pathways

INTRODUCTION

Ischemic stroke, characterized by the interruption of blood supply to the brain, is associated with high morbidity and mortality. Globally, ischemic stroke accounts for nearly 70% of total stroke patients, and this figure continues to rise annually. At present, therapy for ischemic stroke is centered on thrombolysis and neuroprotection (Feigin et al., 2017), but these confer only limited benefits, and more treatment options are needed (Fonarow et al., 2011). It is well established that inflammation plays a key role in the pathogenesis and development of ischemic stroke, characteristics of which include activation of resident microglia, infiltration of peripheral immune cells, and accumulation of immune mediators (Jayaraj et al., 2019). Microglia are innate immune cells in the central nervous system (CNS), which play an important role in regulating the immune and inflammatory response after cerebral ischemia. Microglia/macrophages exhibit different phenotypes and functions in the process of ischemic brain injury (M1/ pro-inflammation and M2/anti-inflammation) (Qin et al., 2019; Jiang et al., 2020). The M1 phenotype expresses characteristic markers, including CD16/32 and

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inducible nitric oxide synthase (iNOS), and tends to release pro-inflammatory mediators and neurotoxic compounds such as interleukin (IL)-1β, IL-6, tumor necrosis factor alpha (TNF-α), reactive oxygen species, nitric oxide, and prostaglandin E2; these are important factors for neuronal death in cerebral ischemia. In contrast, the M2 phenotype, characterized by the expression of molecules such as arginase-1 (Arg1) and CD206, produces anti-inflammatory mediators, including IL-4 and IL-10 (Williams et al., 2004; Hu et al., 2012). Therefore, inhibiting microglia from shifting to the M1 phenotype and/or converting M1 microglia/macrophages to the M2 phenotype may represent potential therapeutic options in cerebral ischemia-reperfusion (I/R) injury.

As one of the critical neurotrophic factors, brain-derived neurotrophic factor (BDNF) is involved in neuronal survival and regulates synaptic function and the proliferation and differentiation of neurons (Huang et al., 2001; Sabbaghziarani et al., 2017). BDNF can reduce infarct size and improve neurological prognosis after cerebral ischemia through an exogenous supply (Schäbitz et al., 2000; 2007). Glial cell-derived neurotrophic factor (GDNF) has been shown to confer a neuroprotective effect in an animal model of middle cerebral artery occlusion (MCAO) (Mokhtari et al., 2017). It has been shown that injection of Sendai viral vectors containing the GDNF gene and GDNF protein into the brain of rats after cerebral ischemia can significantly reduce infarct size (Yamashita et al., 2016). In addition, it has been demonstrated that M2 microglia/macrophages produce neurotrophic factors to play a neuroprotective role (Du et al., 2017).

Fasudil, a Rho kinase (ROCK) inhibitor, is used for the treatment of hemorrhagic stroke in Japan and China, especially the cerebral vasospasms that occur after subarachnoid hemorrhage (Lingor et al., 2019). It has also been shown to inhibit inflammation, oxidative stress, neuronal apoptosis, and hemodynamic dysfunction (Nagata et al., 1993; Yamashita et al., 2007; Satoh et al., 2007; Feske et al., 2009). However, the exact mechanisms of fasudil in cerebral I/R injury remain unclear. In this study, we used a rat model of MCAO/reperfusion (MCAO/R) to explore the effect of fasudil on I/R-induced brain injury and investigated its underlying mechanism.

METHODS

Animal

Healthy male Sprague-Dawley rats, weighing 250-280 g, were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All experiments conformed to the guidelines of the International Council for Laboratory Animal Science and were approved by the Ethics Committee of Shanxi Datong University, Datong, China.

Focal cerebral I/R model

After 1 week of adaptive feeding, all rats were randomly divided into sham operation group (Sham group), model group (I/R group), or fasudil-treated group (Fasudil group), with 12 rats in each group. Fasudil was obtained from Tianjin Chasesun Pharmaceutical Company, and the dosage of fasudil (25 mg/kg) was selected based on our previous experiments (Guo et al., 2020). An MCAO model was used for the rats' I/R injury according to the methods described previously (Zhang et al., 2020). Briefly, rats were deeply anesthetized. A 2-cm incision was made in the middle of the neck, and the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were separated in turn. A monofilament coated with a silicon tip was inserted and slowly pushed into the ICA until a slight resistance was felt. After being in place for 2 h, the monofilament was pulled out to restore blood perfusion for 24 h. During and after the operation, until the rats were awake, they were placed on a heating pad. Fasudil was intraperitoneally injected once at the onset of reperfusion in rats belonging to the Fasudil group at a dose of 25 mg/kg. The same volume of normal saline was intraperitoneally injected in the sham group and the I/R group rats.

Twenty four hours after I/R injury, neurological deficits (n=12 in each group) were scored according to the classical Zea Longa method (Longa et al., 1989). 0 = no neurological deficits; 1 = the contralateral forelimb cannot be straightened when the tail is lifted; 2 = turning to the hemiplegic side when walking; 3 = falling to the hemiplegic side when walking; and 4 = no spontaneous walking and loss of response to stimulation.

2,3,5-triphenyltetrazolium chloride (TTC) staining was employed to test the infarct volume (n=6 in each group). The staining results showed that the infarcted area appeared white and the noninfarcted region appeared deep red. The size of infarct regions was expressed as the percentage of infarct volume to total brain volume. Infarct areas were quantified by Image-Pro Plus software. Infarct volumes (%) = infarct area/total area × 100%.

Nissl staining and TUNEL assay

Rats (n=3 in each group) were anesthetized and perfused with saline and 4% paraformaldehyde. Frozen brain sections (10 μm) were prepared. Nissl stainings were made according to the manufacturer's protocol (Beyotime Bio-

technology, China). Finally, the sections were observed with an optical microscope (Olympus, Japan).

Cell apoptosis was detected by TUNEL assay (Beyotime Biotechnology, China). The sections were washed with PBS and permeabilized with 0.3% Triton X-100 for five minutes. Then TUNEL detection solution was added to the sample and incubated in the dark at 37°C for 60 min. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; 1 μg/mL) for 10 minutes. Sections were observed with a confocal laser scanning microscope (CLSM, Olympus, Tokyo, Japan). One section per animal was used, and each section used three fields to calculate the average value.

ELISA assay

RIPA lysis buffer (Beyotime Biotechnology, China) was utilized to extract proteins from the ischemic brain tissue of rats (n=3 in each group). Pro-inflammatory factors (IL-6, TNF-α, IL-1β) and an anti-inflammatory factor (IL-10) in the protein extract from the brain were measured by an ELISA kit (PeproTech) according to the manufacturer's instructions. Briefly, coated antibody working solution was added to a 96-well plate and left overnight at 4°C, then washed and blocked with 1% bovine serum albumin (BSA) at room temperature for 1 h. The plate was washed, and standard diluent or protein extracted from the brain was added, and it was incubated at room temperature for 2 h. Then, the samples were washed, and antibody detection working solution was added, followed by incubation at room temperature for 1 h. Finally, HRP-labeled avidin working solution and termination solution were added. The levels of inflammatory factors were determined by measuring the optical density (OD) at 450 nm using a quantified microplate reader.

Histology and immunohistochemistry

The prepared frozen sections were taken out and dried at room temperature for 1 h, washed with PBS for 5 min, and blocked with phosphate buffer saline (PBS) containing 0.3% Triton X-100 and 1% BSA. Then the following primary antibodies were added: anti-ionized calcium binding adaptor molecule 1 (Iba1)/CD16/32 (Abcam/eBioscience), anti-Iba1/iNOS (Abcam), anti-Iba1/Arg1 (Abcam/Cell Signaling Technology), anti-Iba1/toll-like receptor 4 (TLR4) (Abcam/Cell Signaling Technology), anti-microtubule association protein-2 (MAP2) (Cell Signaling Technology), anti-neuron-specific nuclear protein (NeuN) (Abcam), anti-GDNF (Cell Signaling Technology) and anti-BDNF (Cell Signaling Technology). After incubating overnight at 4°C, the slices were washed with PBS 3 times for 5 min each wash. Sections were then incubated with Alexa Fluor 488-conjugated secondary antibodies and/or Alexa Fluor 594-conjugated secondary antibodies (Cell Signaling Technology) at room temperature for 2 h. Sections were observed with a confocal laser scanning microscope. The fluorescent area (polygon) of positive cells was analyzed with Image-Pro Plus software. One section per animal was used, and each section used three fields to calculate the average value.

Western blot analysis

Equal amounts of protein (50 μg) extracted from brain in each group of rats (n=3 in each group) were separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) gels. Protein samples were transferred onto a 0.22-μm polyvinylidene fluoride (PVDF) membrane (Immun-Blot, BD) after electrophoresis. Membranes were blocked with 5% nonfat milk at room temperature for two hours. Then the following primary antibodies were added and left overnight at 4°C: anti-iNOS (Abcam), anti-Arg1 (Cell Signaling Technology), anti-Bcl2 (Abcam), anti-Bax (Abcam), anti-TLR4 (Cell Signaling Technology), anti-nuclear factor kappa-B (NF-κB) (Cell Signaling Technology) and anti-GAPDH (Cell Signaling Technology). The membranes were washed three times with PBST for 5 min each wash. The secondary antibody, labeled with horseradish peroxidase, was added and the membrane was incubated at room temperature for 2 h. The membranes were washed three times with PBST, and then chemiluminescence (ECL) solution was added and visualized under the ECL system (Bio-rad, Hercules, CA, USA). Image Lab Software (Bio-rad, Hercules, CA, USA) was used to analyze the bands. The amount of the protein of interest was determined by normalizing with GPADH and was calculated as the densitometric density.

Statistical analysis

All statistical analyses employed GraphPad Prism 5.0 (Graph-Pad Software, San Diego, CA). Data are presented as mean ± SEM. One-way analysis of variance, followed by Dunnett's *post hoc* test, was used to compare data between the groups. The confidence level of the Type I error was defined as 95 percent. Statistical significance was assumed at $P<0.05$.

RESULTS

Fasudil attenuated neurological deficits and decreased infarction volume

In order to investigate the effect of fasudil on I/R brain injury, neurological deficits were assessed after 24 h of reperfusion. As displayed in Fig. 1A ($F_{(2,35)}$=207.6, P<0.0001), compared with the sham group, neurological scores of the I/R group were significantly increased. Fasudil treatment markedly attenuated neurological deficits, decreasing neurological scores compared with those of the I/R group. TTC staining results showed that there was no infarcted tissue in the sham group, while the infarct volume of the I/R group was significantly larger. Compared with the I/R group, the infarct volume of the fasudil group was significantly reduced ($F_{(2,17)}$=117.5, P<0.0001) (Fig. 1B). These results suggest that fasudil can improve cerebral I/R injury.

Fasudil reduced the neuronal loss in MCAO rats

Nissl staining indicated that the morphology of neurons in the sham group was normal. In the I/R group, the number of neurons in the ischemic region of the cerebral cortex was significantly reduced. Neurons appeared loose and disordered, and Nissl bodies were significantly reduced with nuclear pyknosis. Fasudil treatment significantly increased the number of surviving neurons in the ischemic region of the cerebral cortex, cell morphology was significantly improved, and Nissl bodies were increased (Fig. 2A).

In TUNEL analysis, the rate of neuronal apoptosis was increased significantly in the I/R model group compared with the sham group, while fasudil treatment reduced the apoptosis rate ($F_{(2,8)}$=88.40, P<0.0001), indicating that fasudil treatment significantly inhibited neuronal apoptosis in MCAO rats (Fig. 2B).

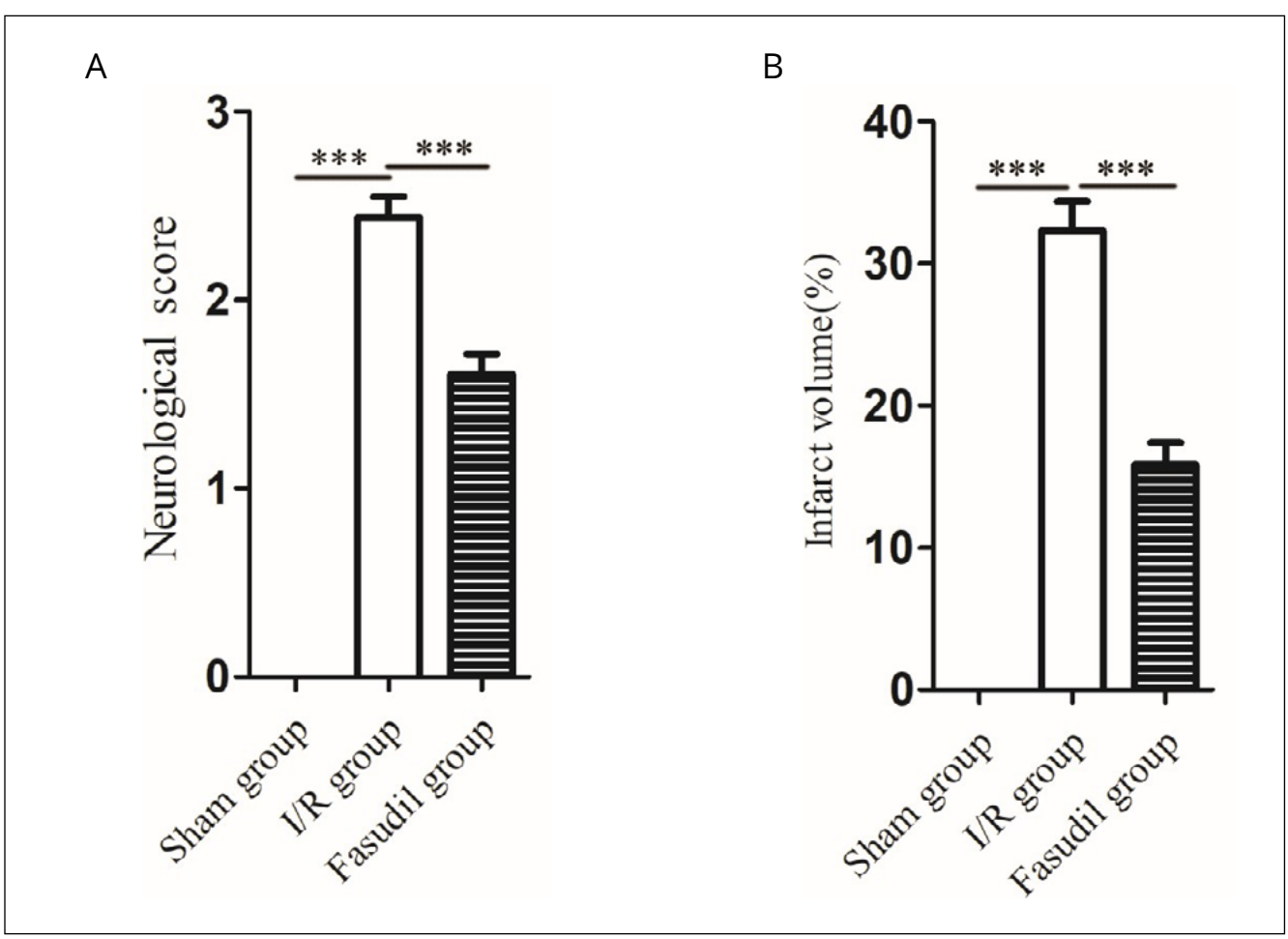
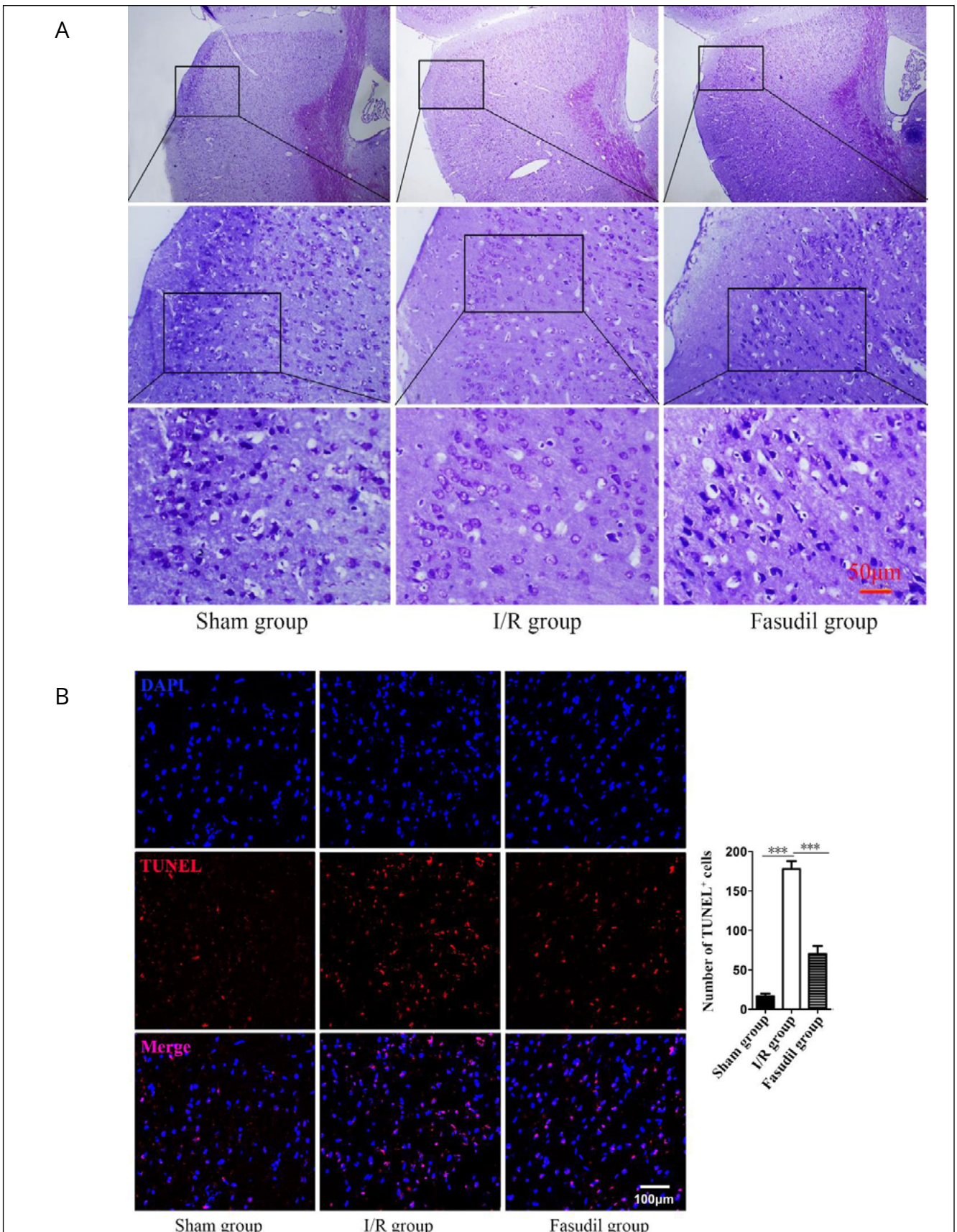


Fig. 1. Fasudil protects rats against brain damage after I/R. (A) Neurological function scores, n=12. (B) Quantification of infarct volume, n=6. The graphed data are expressed as the mean ± SEM. ***P<0.001.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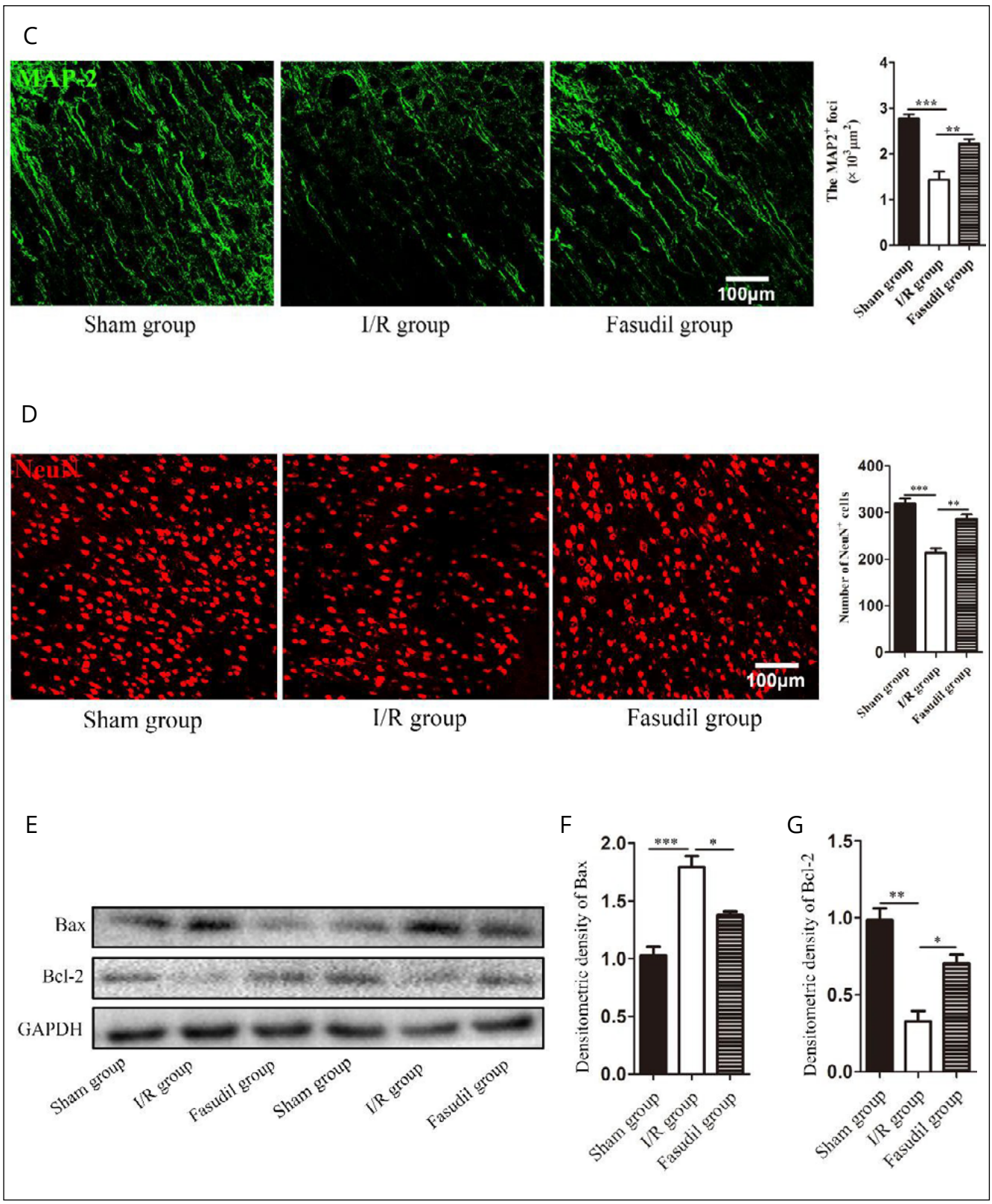


Fig. 2. Effect of fasudil on neuronal injury in rat cerebral cortex. (A) Representative photomicrographs of Nissl staining. (B) Representative confocal images of TUNEL staining. (C) Representative confocal images of MAP2 immunohistochemistry. (D) Representative confocal images of NeuN immunohistochemistry. (E) Western blot to detect the expression of Bax and Bcl-2. (F) Quantification analysis of Bax protein. (G) Quantification analysis of Bcl-2 protein. Data are expressed as the mean ± SEM, n=3. *P<0.05, **P<0.01, ***P<0.001.

MAP-2 is used as a marker for dendrites and axons. NeuN is one of the markers of mature neurons. The result of immunofluorescence staining revealed obvious dendritic and axonal damage and neuronal loss in the I/R group relative to the sham group, with the expression of MAP-2 and the number of NeuN-positive cells decreased significantly. Fasudil increased MAP-2 expression ($F_{(2,8)}$=70.59, P<0.0001) and the number of NeuN positive cells ($F_{(2,8)}$=32.50, P=0.0006) relative to the I/R group (Fig. 2C, D).

We further detected the expression of apoptosis-related proteins, such as Bax and Bcl-2. Western blot analysis showed an increase in Bax and a decrease in Bcl-2 in the I/R group compared with the sham group. Fasudil treatment reduced the expression of Bax ($F_{(2,8)}$=32.50, P=0.0008) and increased the expression of Bcl-2 ($F_{(2,8)}$=32.50, P=0.0017) compared with the I/R group (Fig. 2E). These results indicate that fasudil may reduce cell death in the ischemic region of the cerebral cortex and reduce brain injury.

Fasudil inhibited the inflammatory response in MCAO rats

We explored the effect of fasudil on the inflammatory response with an ELISA assay. As shown in Fig. 3, the levels of pro-inflammatory factors (IL-6, TNF-α, IL-1β) were significantly increased in the I/R group compared to the sham group. Fasudil treatment inhibited levels of IL-6 ($F_{(2,8)}$=62.54, P<0.0001), TNF-α ($F_{(2,8)}$=51.05, P<0.0001) and IL-1β ($F_{(2,8)}$=111.30, P<0.0001), while increasing the levels of IL-10 ($F_{(2,8)}$=127.9, P<0.0001).

Fasudil inhibited the activation of microglia in the brain of MCAO rats

We performed histology and immunohistochemical analysis to examine the expressions of the microglia/macrophage-specific marker Iba1. Expression of Iba1 was significantly increased in MCAO rats compared to the sham group. Fasudil treatment significantly decreased the expression of Iba1 compared with the I/R group ($F_{(2,8)}$=28.72, P=0.0008) (Fig. 4). This suggests that fasudil can inhibit the activation of microglia, which may be one mechanism by which it inhibits neuroinflammation.

Fasudil promoted M2 microglial polarization after MCAO

To confirm whether fasudil affects microglial polarization, double immunofluorescence labeling was used to detect the expression levels of M1 phenotype markers (CD16/32, iNOS) and M2 phenotype markers (Arg1). Western blot was used to detect the expression levels of iNOS and Arg1. In comparison with the sham group, the I/R group showed higher expression of CD16/32 ($F_{(2,8)}$=64.96, P<0.0001) and iNOS ($F_{(2,8)}$=50.11, P<=0.0002; $F_{(2,8)}$=130.9, P<0.0001) (Fig. 5A, B, D). Fasudil-treated rats showed lower expression of M1 markers relative to the I/R group and higher expression of M2 markers ($F_{(2,8)}$=50.38, P=0.0002; $F_{(2,8)}$=50.53, P=0.0002) (Fig. 5A-D). These results indicated that fasudil could exert its protective effects by modulating microglial polarization toward an M2 type.

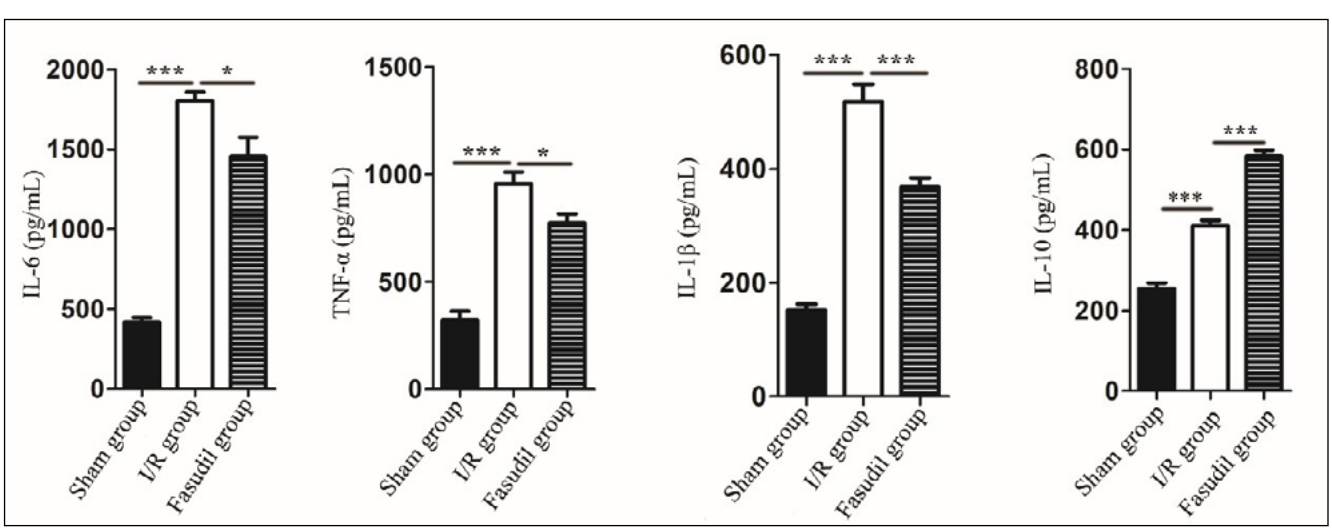


Fig. 3. Fasudil inhibited the inflammatory response in rat cerebral cortex. The level of inflammatory factors was measured with ELISA kits. Data are expressed as the mean ± SEM, n=3. *P<0.05, ***P<0.001.

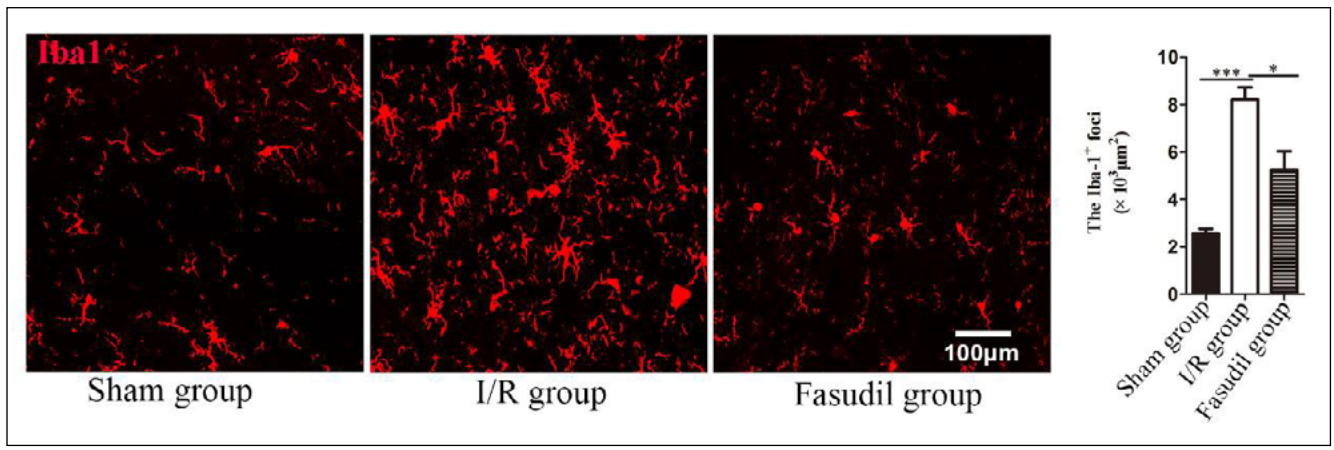


Fig. 4. Fasudil inhibited the activation of microglia in rat cerebral cortex. Representative confocal images of Iba-1 immunohistochemistry. Data are expressed as the mean ± SEM, n=3. *P<0.05, ***P<0.001.

Fasudil promoted the secretion of neurotrophic factors

We used immunohistochemistry to detect whether fasudil affected the expression of BDNF and GDNF. Compared with the sham group, the expression of BDNF and GDNF was significantly decreased in the I/R group; however, fasudil treatment successfully increased BDNF ($F_{(2,8)}$=21.423, P=0.0019) and GDNF ($F_{(2,8)}$=136.2, P<0.0001) expression relative to the I/R group (Fig. 6A, B).

Fasudil inhibited the activation of TLR4/NF-κB signaling in the brain of MCAO rats

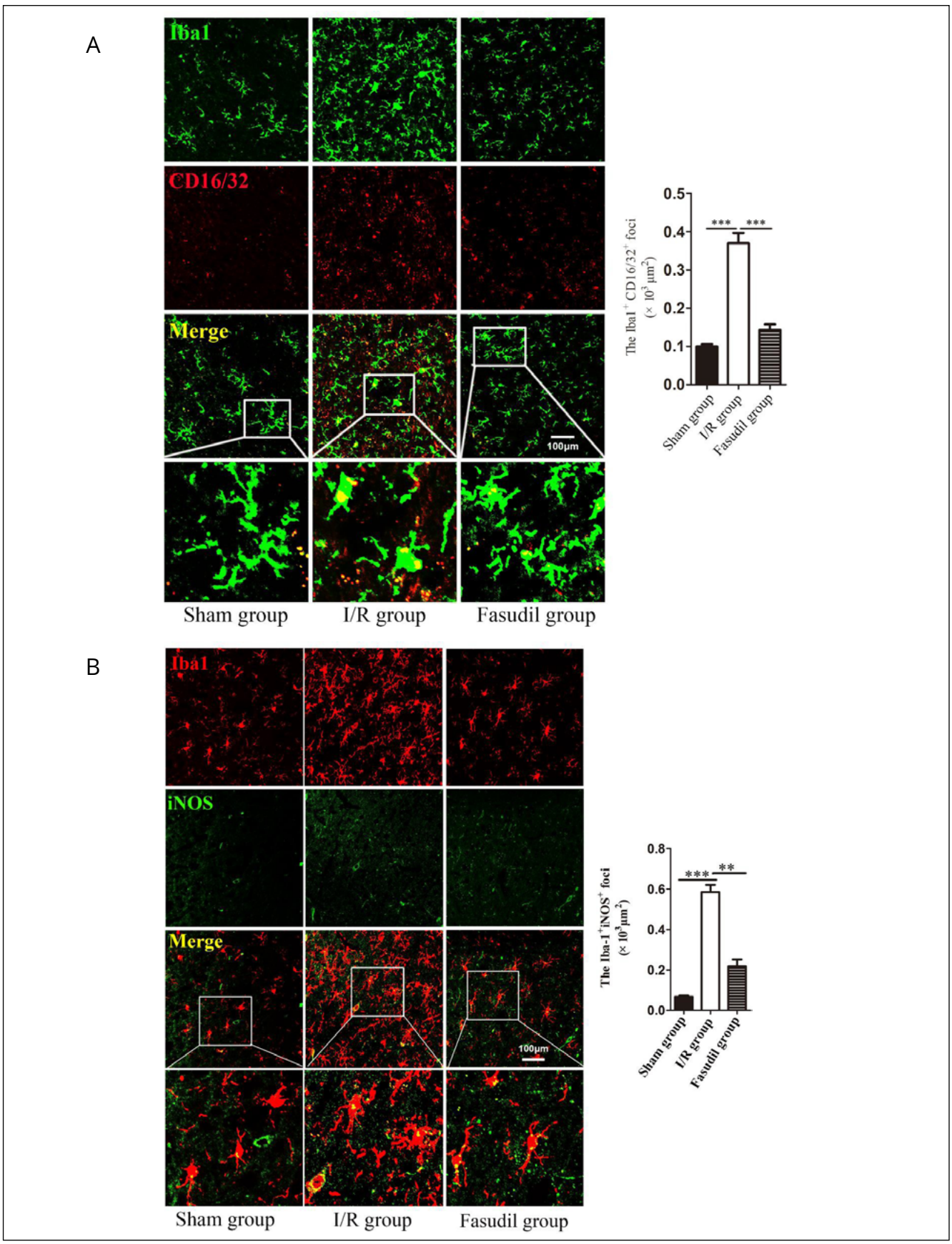
TLR4 is involved in the activation of microglia and is a key regulator involved in the inflammatory process (Rodríguez-Gómez et al., 2020). To clarify the molecular mechanisms of M1/M2 microglia transformation in MCAO after fasudil treatment, we detected the expression of TLR4 and NF-κB by immunohistochemistry and Western blot. The results showed that the expression of TLR4 ($F_{(2,8)}$=13.86, P=0.0056; $F_{(2,8)}$=84.71, P<0.0001) and NF-κB ($F_{(2,8)}$=61.93, P<0.0001) were upregulated in the I/R group, and fasudil treatment attenuated the expression of these molecules (Fig. 7). These results highlight that fasudil suppressed the inflammatory response by inhibiting the activation of microglia through the inhibition of TLR4/NF-κB activation.

DISCUSSION

Previous studies have demonstrated the potential neuroprotective effects of fasudil by mechanisms mainly relating to the reduction of inflammation, oxidation, apoptosis, and blood-brain barrier permeability, as well as the promotion of neurotrophic factor secretion (Yan et al., 2019). Our study provides further evidence that fasudil was able to improve neurological deficits of cerebral infarction in rats with I/R, and as well as demonstrates that fasudil could promote the transformation of microglia from an M1 to M2 phenotype after cerebral I/R, to inhibit the neuroinflammatory response.

Under physiological conditions, microglia carry out a surveillance role, monitoring the microenvironment in order to maintain brain homeostasis. Upon sensing endogenous injury or exogenous stimulation, microglia are rapidly activated and migrate to the injured area (Jiménez Fernández et al., 2015). Microglia/macrophages present two different phenotypes and have different functions in the process of cerebral ischemic injury. M2 microglia promote the survival of cortical neurons by clearing cell debris and releasing nutritional factors. Although M1 microglia are involved in the clearance of cell debris in the early stage of stroke, they may also produce neurotoxic substances and may exacerbate brain injury under conditions of unregulated or chronic activation (Hu et al., 2015). During the pathological process of cerebral ischemia, the phenotype of microglia/macrophages changes dynamically. In the early stage of cerebral ischemia, microglia/macrophages present as an M2 phenotype, but are gradually replaced by the M1 phenotype. In the late stage, the M1 phenotype predominates (Hu et al., 2012). The fate of microglial/macrophage differentiation into M1 or M2 depends largely on signals in the microenvironment. However, the mechanisms regulating M1 or M2 microglial polarization have not been clarified.

Studies have shown that inhibiting M1 and/or inducing M2 microglial activation in the ischemic pen-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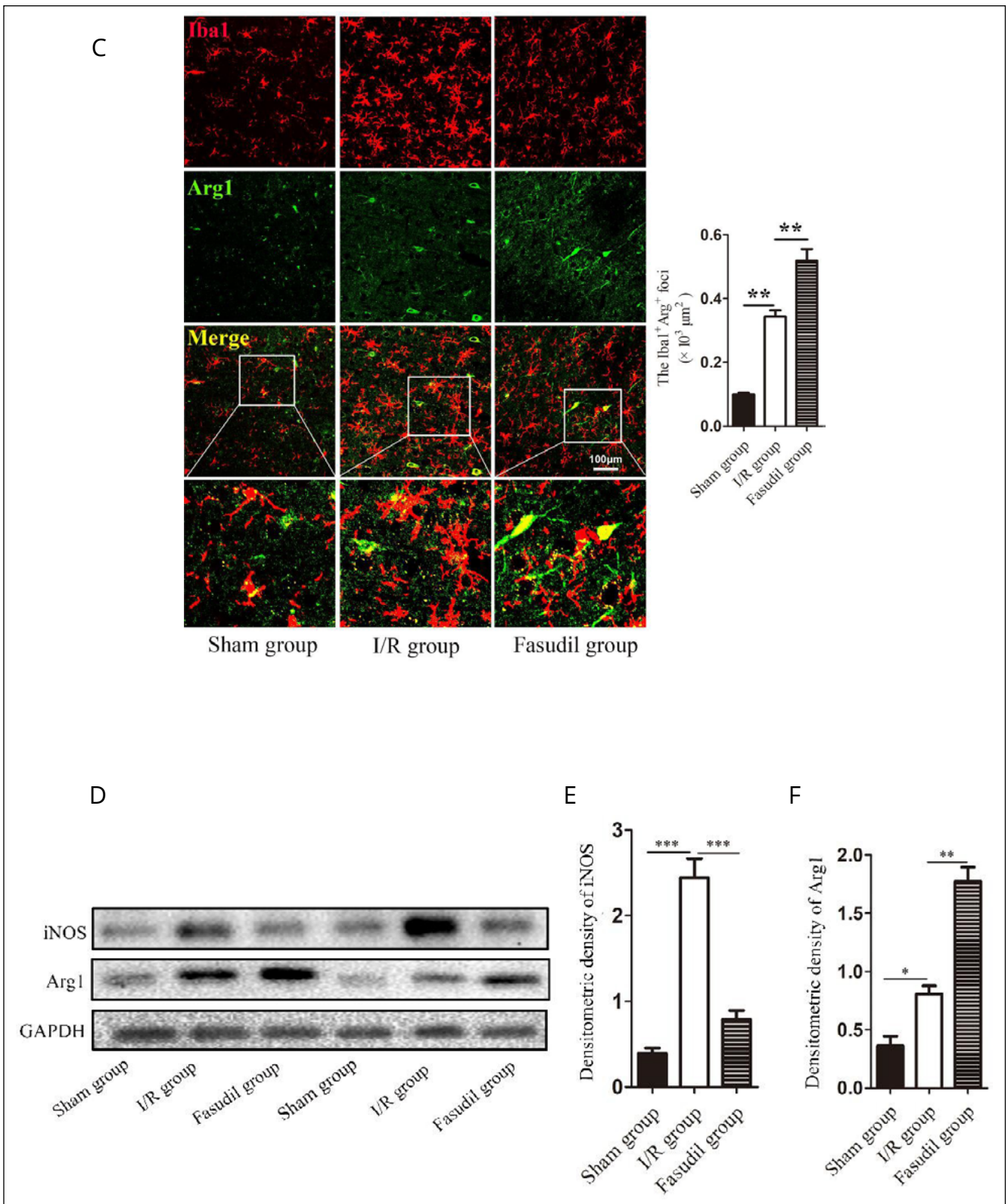

Fig. 5. Fasudil promoted M2 microglial polarization in rat cerebral cortex. (A) Immunofluorescence image of microglia (Iba1, green) and CD16/32 (red). (B) Immunofluorescence image of microglia (Iba1, red) and iNOS (green). (C) Immunofluorescence image of microglia (Iba1, red) and Arg1 (green). (D) Western blot to detect the expression of iNOS and Arg1. (E) Quantification analysis of iNOS protein. (F) Quantification analysis of Arg1 protein. Data are expressed as the mean ± SEM, n=3. *P<0.05, **P<0.01, ***P<0.001.

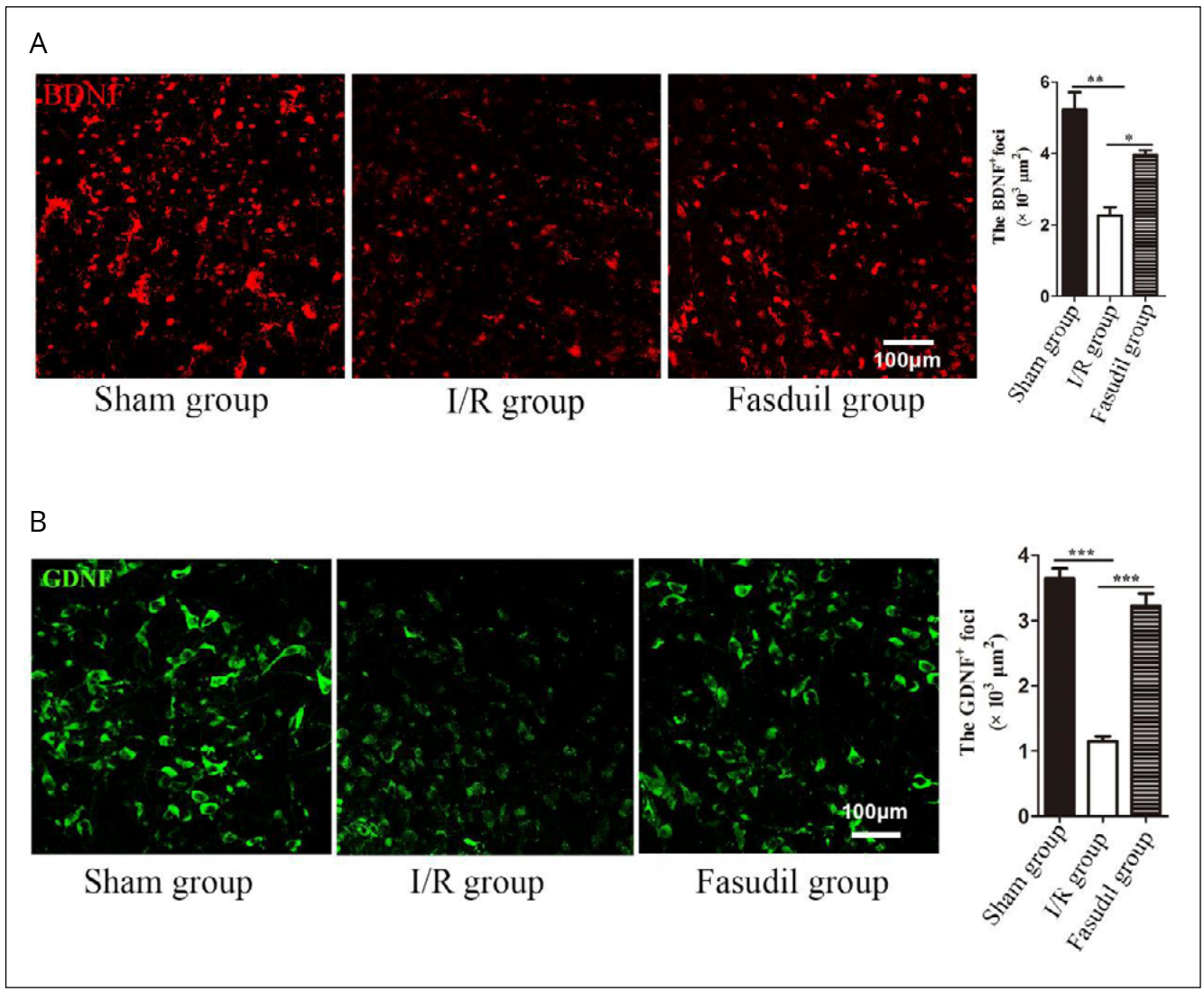

Fig. 6. Fasudil promoted the secretion of neurotrophic factors. (A) Representative confocal images of BDNF immunohistochemistry. (B) Representative confocal images of GDNF immunohistochemistry. Data are expressed as the mean ± SEM, n=3. **P*<0.05, ***P*<0.01, ****P*<0.001.

umbra is beneficial in the early stages of brain I/R injury. Our previous studies showed that fasudil could reverse microglia/macrophages from the M1 to the M2 phenotype, suggesting a possible mechanism of action in neurodegenerative diseases such as Alzheimer's disease and multiple sclerosis (Liu et al., 2013; Guo et al., 2020), However, whether fasudil has a beneficial effect on cerebral ischemia by shifting microglia/macrophage M2 polarization has not been studied. The results of the present study showed that fasudil could significantly improve neurological function and neuronal injury and reduce the volume of cerebral infarction after I/R injury. Moreover, we showed that fasudil treatment inhibited the expression of the characteristic M1 markers iNOS and CD16/32 and promoted the expression of the M2 marker Arg 1. The ELISA results further confirmed that levels of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, secreted by M1 microglia/macrophages, were decreased after fasudil treatment, while the level of the anti-inflammatory cytokine IL-10, secreted by M2 microglia/macrophages, was increased. These data suggest that fasudil treatment could promote the transformation of microglia/macrophages from M1 to M2 phenotype in the ischemic brain.

Apoptosis and neuroregeneration have risen to prominence in I/R injury research. Following cerebral ischemia, cells within the central area of cerebral infarction die, while those at the ischemic penumbra, still metabolically active, are in a state of reversible

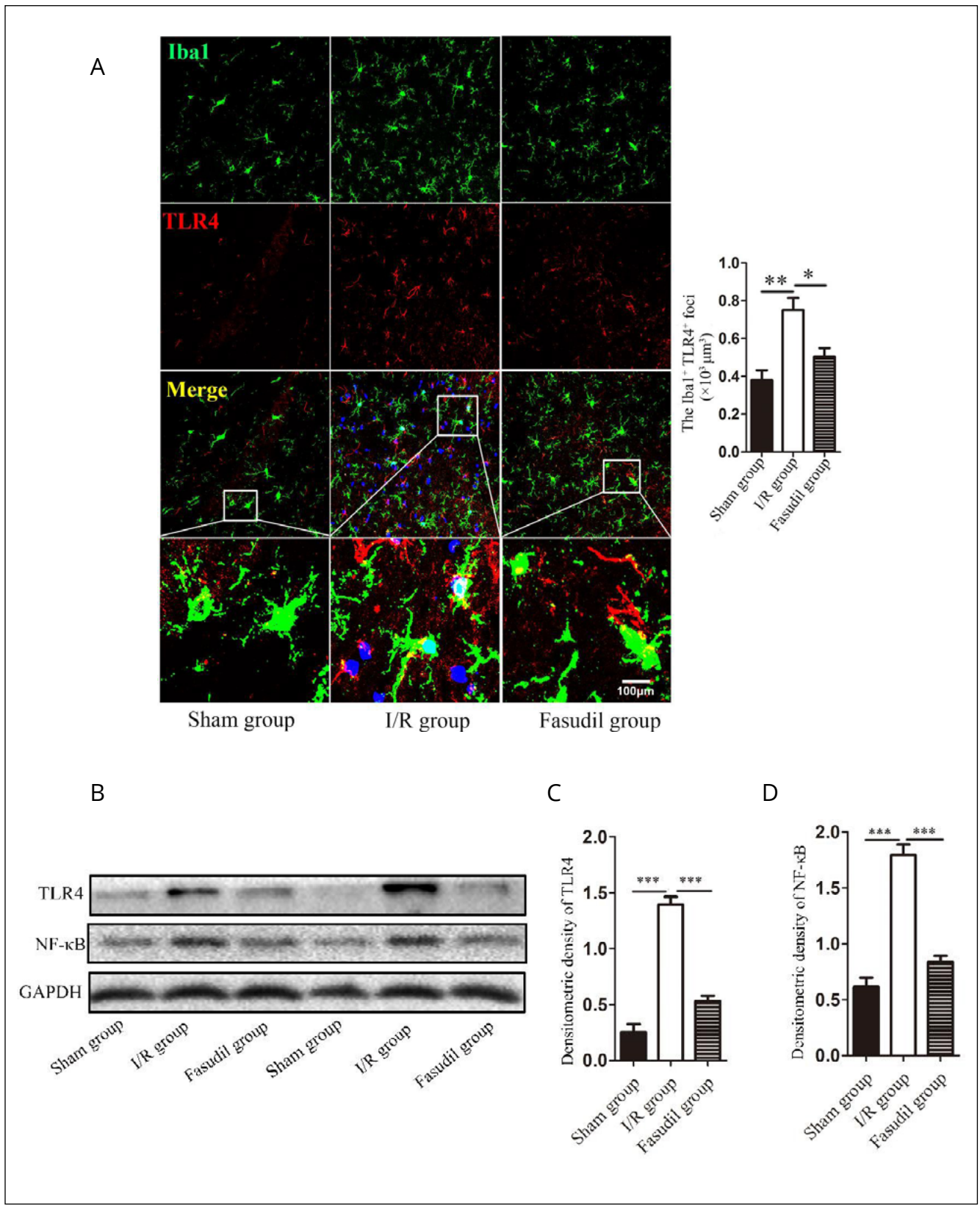

Fig. 7. Fasudil inhibited the activation of TLR4/NF-κB signaling. (A) The expression of TLR4 (red) positive microglia (green) by double immunofluorescence. (B) Western blot to detect the expression of TLR4 and NF-κB. (C) Quantification analysis of TLR4 protein. (D) Quantification analysis of NF-κB protein. Data are expressed as the mean ± SEM, n=3. **P*<0.05, ***P*<0.01, ****P*<0.001.

apoptosis (Radak et al., 2017). Therefore, preventing these apoptotic neurons from reaching a state of irreversible apoptosis by the promotion of regeneration is of particular interest in clinical neuroscience. In addition to releasing anti-inflammatory cytokines, M2 microglia/macrophages can also alleviate cell apoptosis and promote tissue repair by releasing neurotrophic factors such as GDNF, BDNF, NGF, and NT3 (Du et al., 2017). In this study, it was found that fasudil led to a significant decrease in the rate of apoptosis, as well as a significant decrease in the expression of the pro-apoptotic protein Bax and a significant increase in the expression of the anti-apoptotic protein Bcl-2. Furthermore, expression of BDNF and GDNF were increased after fasudil treatment. These results suggest that fasudil may have a protective effect on cerebral I/R injury, which may be conferred through the inhibition of apoptosis by promoting the secretion of neurotrophic factors.

Toll-like receptors (TLRs) are important recognition receptors of the innate immune system. TLR4, one of the most important members of the TLR family, is activated by many internal and external danger signals, leading to the activation of the myeloid differentiation factor 88 (MyD88) signaling pathway, leading to the activation of NF-κB, and giving rise to an inflammatory response (Aravalli et al., 2007). It has been found that TLR4 plays an important role in the inflammatory response of ischemic brain injury (Pascual et al., 2021). In the CNS, TLR4 is widely expressed in microglia, astrocytes, neurons, and endothelial cells, and the TLR4/NF-κB signal pathway is involved in the inflammatory activation of microglia (Rodríguez-Gómez et al., 2020). Our previous studies have shown that fasudil can inhibit the neuroinflammatory response by inhibiting the TLR4/NF-κB signaling pathway (Guo et al., 2020). The results of the present study showed that the expression of TLR4 and NF-κB in the brain of cerebral I/R rats was decreased after fasudil treatment, which further confirms that the mechanism by which fasudil inhibits the cerebral ischemic inflammatory response may be through regulation of the TLR4/NF-κB signaling pathway.

CONCLUSION

The present study provides further evidence of a treatment that can alleviate inflammation and confer a neuroprotective effect in cerebral I/R injury. Fasudil appears to inhibit the activation of the M1 microglial/macrophage phenotype and/or the switch from the M1 to the M2 phenotype, which may be achieved through the modulation of TLR4/NF-κB signaling.

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