IBRO Neuroscience Reports 18 (2025) 370–377

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

Research paper

Acupuncture protects against ischemic stroke by inhibiting the NF-κB pathway

Linlin Wang [a,*], Lanrong Chen [a], Jiayong Wu [a], Chengyan Liu [a], Caixia Chi [a], Shicong Wang [b,*]

[a] Department of Encephalopathy Rehabilitation III, Rehabilitation Hospital Affiliated to Fujian University of Traditional Chinese Medicine, No.13, Hudong Branch Road, Gulou District, Fuzhou, Fujian 350000, China

[b] Department of Outpatient, the Second Affiliated Hospital of Fujian University of Traditional Chinese Medicine, 282 Wusi Road, Fuzhou, Fujian 350000, China

ARTICLE INFO

Keywords:
Acupuncture
Neuroprotection
NF-κB
NLRP3
MCAO

ABSTRACT

Cerebral ischemia is a leading cause of disability and death worldwide. This study aims to investigate the neuroprotective effects of acupuncture on rats with middle cerebral artery occlusion (MCAO) and elucidate the underlying mechanisms involving the nuclear factor kappa-B (NF-κB) pathway and the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome. Acupuncture at specific acupoints, i.e., GV26, GV20, and ST36, was administered to MCAO rats. Infarct volume was measured by TTC (2,3,5-triphenyl-tetrazolium chloride solution) staining. Protein expression levels of NF-κB pathway components and inflammatory markers were determined by western blot and enzyme-linked immunosorbent assay. Terminal deoxynucleotidyl transferase dUTP nick end labeling and hematoxylin-eosin staining were performed to evaluate apoptosis and histopathologic changes. Acupuncture significantly improved neurological function and reduced infarct volume in MCAO rats, as evidenced by decreased TTC staining areas. Meanwhile, acupuncture reduced NF-κB pathway, NLRP3 inflammasome and pro-inflammatory cytokines. Compared with the MCAO group, apoptotic cells were significantly reduced in the acupuncture group, which attenuated the histopathological changes induced by cerebral ischemia, including neuronal cell damage and tissue disorganization. However, application of phorbol 12-myristate 13-acetate partially reversed the beneficial effects of acupuncture, suggesting that the NF-κB pathway plays a key role in mediating the neuroprotective effects of acupuncture. Acupuncture exerts significant neuroprotective effects in MCAO rats, possibly through inhibition of the NF-κB pathway and NLRP3 inflammasome activation. These findings provide insight into the mechanisms of acupuncture in the treatment of ischemic stroke and support its potential therapeutic application.

1. Introduction

Ischemic stroke (IS) is a leading cause of adult mortality and disability worldwide (Zhu et al., 2022). It occurs when a sudden blockage in the cerebral vasculature leads to tissue ischemia and hypoxia, initiating a complex cascade of biochemical and molecular pathological changes including cell apoptosis, inflammatory response, and blood-brain barrier disruption, ultimately resulting in brain tissue damage and neurological dysfunction (Mendelson and Prabhakaran, 2021). Despite reductions in stroke mortality rates due to advances in medical technology, stroke survivors often suffer from functional disabilities that affect mobility, sensation, speech, cognition, and swallowing, significantly reducing the quality of life and placing a substantial burden on healthcare resources and family budgets (Ajoolabady et al., 2021). Currently, rapid thrombolytic therapy to restore blood flow is the mainstay of treatment (Gauberti et al., 2021), but due to the limited therapeutic window and contraindications to thrombolysis, only a minority of patients are eligible. Even reperfusion during IS can sometimes exacerbate brain dysfunction and structural damage, leading to cerebral ischemia-reperfusion injury (Wang et al., 2022). Therefore, identifying effective treatments to mitigate ischemic brain damage and promote neurological recovery remains a focus of medical research.

In recent years, with a deeper understanding of the pathophysiology of stroke, various potential therapeutic strategies have been proposed and investigated, including traditional Chinese medicine treatments (Zhu et al., 2021), neuroprotective agents (Safouris et al., 2021), revascularization procedures (Markus and Michel, 2022), and

* Corresponding authors.
E-mail addresses: Lily033019@163.com (L. Wang), fjwsc@126.com (S. Wang).

https://doi.org/10.1016/j.ibneur.2025.02.014
Received 4 November 2024; Received in revised form 25 February 2025; Accepted 26 February 2025
Available online 26 February 2025

rehabilitative therapies (O'Dell, 2023). Among these strategies, acupuncture, as a traditional Chinese medical approach, has received widespread attention for its unique therapeutic effects and minimal side effects, especially in recent years (Qin et al., 2022; Zhang et al., 2021). Studies such as those by Cao et al. have highlighted the potential synergistic intervention of acupuncture in inflammatory signaling pathways, laying a foundation for exploring the various molecular mechanisms by which acupuncture promotes neurological recovery (Cao et al., 2021); Liu et al. found that scalp acupuncture could alleviate patients' speech processing and motor coordination (Liu et al., 2021a). These studies provide theoretical support for acupuncture's ability to stimulate specific acupoints, regulate the body's qi and blood circulation, improve local blood flow, reduce inflammation, and promote nerve cell repair and regeneration. Thus, acupuncture may serve as an effective adjunct therapy to help stroke patients regain neurological function.

In acupuncture research for cerebral ischemia, acupoints such as GV26 (Renzhong), GV20 (Baihui), and ST36 (Zusanli) are commonly used (Zhang et al., 2022a). These acupoints are selected based on traditional Chinese meridian theory and modern anatomical knowledge, with the aim of treating cerebral ischemia through regulatory effects on the nervous and vascular systems. For example, stimulation of GV26 on the face is believed in Chinese medicine to awaken the spirit and restore consciousness; GV20 on the top of the head can calm the wind, clear the head and eyes, and awaken the brain; ST36 on the leg is believed to benefit qi, strengthen the spleen and stomach, and fortify tendons and bones (Li et al., 2022a; Yang et al., 2022). By applying acupuncture to these points, a comprehensive regulation of the patient's physiological state can be achieved, promoting recovery after cerebral ischemia.

Despite the widespread clinical use of acupuncture in stroke rehabilitation, the exact mechanisms of its effects are not fully understood. With advances in molecular biology and neuroscience, an increasing number of studies have begun to explore the biological basis of acupuncture's therapeutic effects on cerebral ischemia. Research suggests that acupuncture may act through various mechanisms, including inhibiting neuronal apoptosis, reducing the release of inflammatory factors, improving cerebral blood flow, and enhancing antioxidant capacity (Liu et al., 2021b). Among these, modulation of the NF-κB pathway and NLRP3 inflammasome play important roles in acupuncture treatment (Tang et al., 2022; Guo et al., 2021). NF-κB is a critical transcription factor involved in the regulation of inflammation and cell apoptosis, and the NLRP3 inflammasome is a multiprotein complex involved in inflammatory responses and cell death (Li et al., 2021a). By inhibiting the activation of these pathways, acupuncture may alleviate the inflammatory damage and cell death caused by cerebral ischemia, thereby protecting neural tissue and promoting functional recovery.

In conclusion, as a potential therapeutic approach, acupuncture has significant clinical value for the rehabilitation of IS. However, to fully realize its therapeutic potential, further basic and clinical research is needed to elucidate its mechanisms of action, optimize treatment protocols, and evaluate its long-term effects and safety. Through these studies, we can better understand the role of acupuncture in stroke recovery and provide more effective and safer treatment options for patients.

2. Materials and methods

2.1. MCAO model construction

A total of 45 SPF female Sprague-Dawley rats, aged 6–8 weeks old (weighing 120–130 g), were purchased from Sipeifu (Beijing) Biotechnology Co., Ltd. (license number: SCXK (Jing) 2019–0010). After 1 week of acclimatization, rats were housed in polystyrene cages under a 12-hour light-dark cycle at 22°C and 60 % humidity. Standard chow and water were provided ad libitum. The 45 rats were randomly divided into five groups (n = 9 each): Sham, MCAO model, MCAO+acupuncture, MCAO+acupuncture+phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich, MO, USA), and MCAO+acupuncture+dimethyl sulfoxide (DMSO), and PMA acts as an activator of the NF-κB pathway (Kim et al., 2023). Thirty-six randomly selected rats underwent MCAO modeling. After isoflurane gas anesthesia, the rats were placed in the supine position and restrained, the skin was disinfected with iodophor, and a longitudinal incision made in the anterior neck to expose and separate the right common, external, and internal carotid arteries. The external carotid artery was ligated, and the common and internal carotid arteries were temporarily ligated with silk thread. A 45° oblique incision was made at the bifurcation of the external carotid artery, and a filament was inserted from the external carotid artery to the intracranial segment of the internal carotid artery, stopping when the black line of the thread reached the bifurcation. The filament was secured, and the vascular clamp was removed. After layered suturing of the incision, the filament was withdrawn to the external carotid artery after 90 minutes of occlusion for ischemia followed by reperfusion. After modeling, the rats were housed individually for observation. The sham-operated group underwent arterial transection without ligation or filament insertion. After surgery, the animals were placed in a 22°C environment for recovery on normal chow. Beginning on the first day after modeling, acupuncture interventions were performed daily at GV26, GV20, and ST36 for 30 minutes each for six consecutive days (Fig. 1). Sixty minutes before the first acupuncture session, the MCAO+acupuncture+PMA group received an intravenous injection of phorbol 12-myristate 13-acetate (PMA; 200 μg/kg; MeilunBio, Dalian, China) and the MCAO+acupuncture+DMSO group received an intravenous injection of DMSO (MeilunBio; same volume as PMA), and all groups received continuous acupuncture intervention for 6 days. Normal animal care was maintained throughout the intervention period. The study was approved by the Animal Ethics Committee of Fujian Anburui Biotechnology Co., LTD (approval number: IACUC FJABR 2023026007).

2.2. Zea-Longa neurological deficit scoring

Zea-Longa neurological deficit scores were performed on day one and day six after modeling. Scoring criteria: 0 for no neurological deficit; 1 for inability to fully extend the forepaw of the paralyzed side; 2 for circling to the paralyzed side while walking; 3 for falling to the paralyzed side while walking; 4 for inability to walk spontaneously and loss of consciousness; 5 for death. A score of 1 or higher indicated successful modeling; if a score of 5 was achieved, dissection revealed subarachnoid hemorrhage or absence of neurological deficit symptoms (score 0), which was considered MCAO modeling failure.

2.3. Animal tissue collection

Samples were collected from the rats after the procedure (the day of the procedure was counted as day 0). All rats were euthanized with 3 % pentobarbital sodium injection (200 mg/kg) on the sixth day after housing, and brain tissues were collected in a sterile environment. Whole brain tissues (n = 3) were collected for 2,3,5-

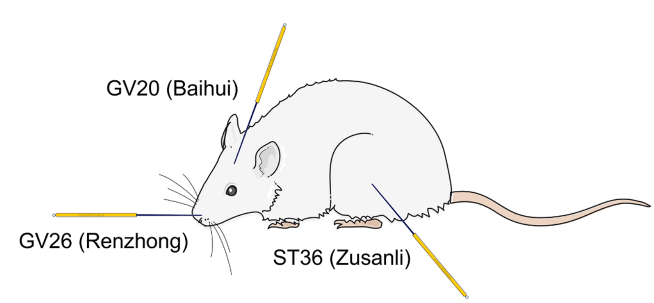

Fig. 1. Photographic documentation of the acupuncture intervention process in MCAO-modeled rats.

triphenyltetrazolium chloride (TTC; Sigma-Aldrich) staining to observe subcortical infarct areas; ischemic side brain tissues (n = 3) were used for subsequent Western blot and enzyme-linked immunosorbent assay (ELISA) analysis; ischemic side brain tissues (n = 3) were fixed in 4 % paraformaldehyde. Dehydrated samples were embedded in paraffin using a KH-BL embedding machine and sectioned (4 μm) for subsequent terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining and hematoxylin-eosin (HE) staining.

2.4. TTC staining

The collected brain tissues were placed in 4°C PBS solution for 5 minutes, then frozen at −20°C for 20 minutes for subsequent sectioning. The tissues were then cut into five 2 mm sections and incubated in 2 % TTC solution (dissolved in phosphate buffered saline (PBS); Gibco, Thermo Fisher Scientific, Waltham, MA, USA) at 37°C in a dark water bath for 30 minutes, washed with PBS solution for 3–5 minutes, and then photographed for observation. After staining, non-infarcted areas of the brain were red, while infarcted tissues were white. Brain infarct areas were analyzed using ImageJ software.

2.5. Western blot analysis

Total protein from ischemic side brain tissues was extracted using RIPA lysis buffer (MeilunBio), and protein concentration was determined using the BCA protein assay kit (MeilunBio). The samples were then electrophoresed on SDS-PAGE and transferred to PVDF membranes (MeilunBio). The membranes were blocked with skim milk powder (BBI CO, LTD, Shanghai, China) and incubated with specific antibodies, including p-p65 (1:2000, AF2006, Affinity Biosciences, Cincinnati, OH, USA), p65 (1:4000, 10745-1-AP, Proteintech, Wuhan, China), p-IKBα (1:2000, AF2002, Affinity Biosciences), IKBα (1:5000, 10268-1-AP, Proteintech), NLRP3 (1:2000, A00034–2, Boster Bio, Pleasanton, CA, USA), ASC (1:2000, bs-6741R, Bioss, Beijing, China), Caspase-1 (1:10000, 22915-1-AP, Proteintech), GAPDH (1:40000, 60004-1-Ig, Proteintech), and goat anti-rabbit HRP-conjugated secondary antibody (1:10000, SA00001–2, Proteintech). Protein signals were visualized using an ECL chromogenic kit (MeilunBio) and quantitated using ImageJ software.

2.6. ELISA analysis

Tissues were washed with precooled PBS, minced, and homogenized on ice in a homogenizer. The process included sonication and repeated freeze-thawing. The homogenate was then centrifuged at 5000 ×g for 5–10 minutes at 4°C, and the supernatant was collected for analysis. ELISA kits for interleukin (IL)-1β, IL-18, IL-6, and TNF-α from Jiangsu Meimian Industrial Co., Ltd (Jiangsu, China) were used for detection. Samples and standards were incubated according to the kit instructions, followed by incubation with detection antibodies and substrates at 37°C in the dark for 15 minutes. Finally, absorbance values were read at a wavelength of 450 nm using an enzyme-linked immunosorbent assay reader (Multiscan MK3, Thermo Fisher Scientific) to calculate concentrations.

2.7. TUNEL staining

TUNEL apoptosis detection kits (Beyotime, Shanghai, China) were used to label and detect DNA fragmentation indicative of apoptosis in brain tissue. Briefly, fixed brain tissue sections were first incubated with proteinase K at 37°C for 30 minutes, followed by three PBS washes. The TUNEL reaction was then performed according to the kit instructions. After staining, the sections were observed under a fluorescence microscope (Olympus Corporation, Tokyo, Japan), and TUNEL-positive cells were counted to assess the degree of apoptosis.

2.8. HE staining

Hematoxylin-eosin solution (Beyotime) were used for HE staining. Fixed, dehydrated, cleared, and paraffin-embedded brain tissue sections were first stained with hematoxylin to stain nuclei, followed by eosin to stain cytoplasm and extracellular matrix. The post-stained sections were observed and photographed under an optical microscope (Leica Microsystems, Wetzlar, Germany) to assess tissue structural changes after ischemic brain injury. Comparison of HE staining results between different groups allows direct assessment of the protective effects of acupuncture intervention on ischemic brain injury.

2.9. Data analysis

Data are expressed as mean ± standard deviation. GraphPad Prism 9.0 software was used for statistical analysis. Differences between groups were determined by one-way ANOVA followed by and Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Acupuncture treatment ameliorates cerebral infarction in MCAO rats

On the first and sixth days after modeling, all rats underwent Zea-Longa neurological deficit scoring to evaluate the effect of acupuncture on MCAO rats. The results showed that Sham rats exhibited normal behavior with a score of 0, while the scores of rats in the MCAO model group increased (all >1) on days 1 and 6, indicating successful establishment of the MCAO model. After 6 consecutive days of acupuncture intervention at GV26, GV20 and ST36 acupoints, there was a significant recovery of neurological function in MCAO rats. However, administration of PMA significantly reversed the ameliorative effect of acupuncture on MCAO rats (Fig. 2A). TTC staining revealed that the infarct size was significantly increased in MCAO model rats (22.97 ± 2.12, $P < 0.001$) compared to Sham rats (2.26 ± 0.93), further confirming the success of MCAO modeling. After 6 days of acupuncture intervention, infarct size was significantly reduced in MCAO rats (7.47 ± 1.94, $P < 0.001$). DMSO did not significantly interfere with the effects of acupuncture (7.59 ± 2.67, $P > 0.05$), but administration of PMA partially counteracted the reduction in infarct size by acupuncture in MCAO rats (20.73 ± 1.98, $P < 0.001$), almost restoring it to MCAO levels (Fig. 2B, C). These results suggest that the ameliorative effects of acupuncture on MCAO rats may involve the NF-κB pathway.

3.2. Acupuncture treatment inhibits NF-κB signaling and inflammasome activation

To confirm the role of NF-κB in the improvement of MCAO rats by acupuncture, we performed Western blot analysis on rat brain tissue. We observed that the phosphorylation levels of the key proteins p65 and IKBα in the NF-κB pathway were significantly increased in MCAO rats compared with the Sham group, indicating that ischemic brain injury activated the NF-κB pathway. Additionally, we observed significant increases in inflammasome-related proteins such as NLRP3, ASC, and Caspase-1 in MCAO rats, further confirming ischemia-induced inflammatory responses. After acupuncture treatment, these protein levels decreased significantly, indicating effective inhibition of the NF-κB pathway and NLRP3 inflammasome activation, thereby attenuating inflammation. However, with the addition of PMA to the acupuncture treatment group, the protective effects of acupuncture were markedly counteracted, with reversed trends in reduced phosphorylation levels of p65, IKBα, and decreased levels of NLRP3, ASC, and Caspase-1 proteins (Fig. 3). This result further confirms the central role of the NF-κB signaling pathway in mediating the anti-inflammatory effects of acupuncture treatment, as well as the activation of inflammasomes by NF-κB signaling.

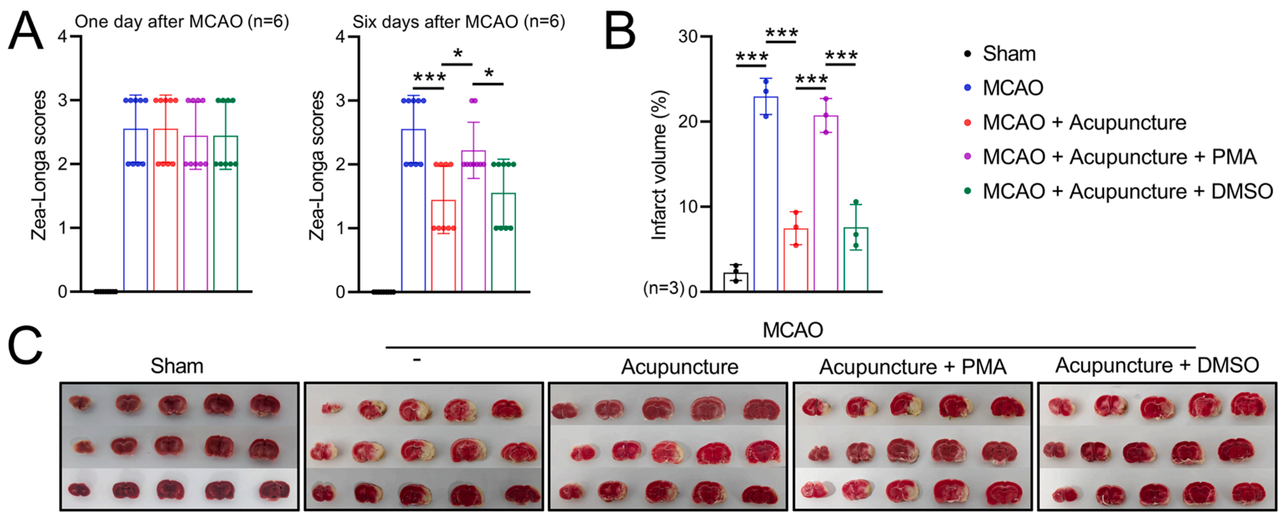

Fig. 2. Acupuncture Treatment Improves Cerebral Infarction in MCAO Rats. **A.** Zea-Longa neurological deficit scoring assessing the clinical features improvement of MCAO rats by acupuncture treatment (n = 6). **B-C.** TTC staining analysis of the ischemic injury in the brain of MCAO rats treated with acupuncture and the effect of PMA on acupuncture treatment (n = 3). All data are shown as means±SD. *$P < 0.05$, * **$P < 0.001$.

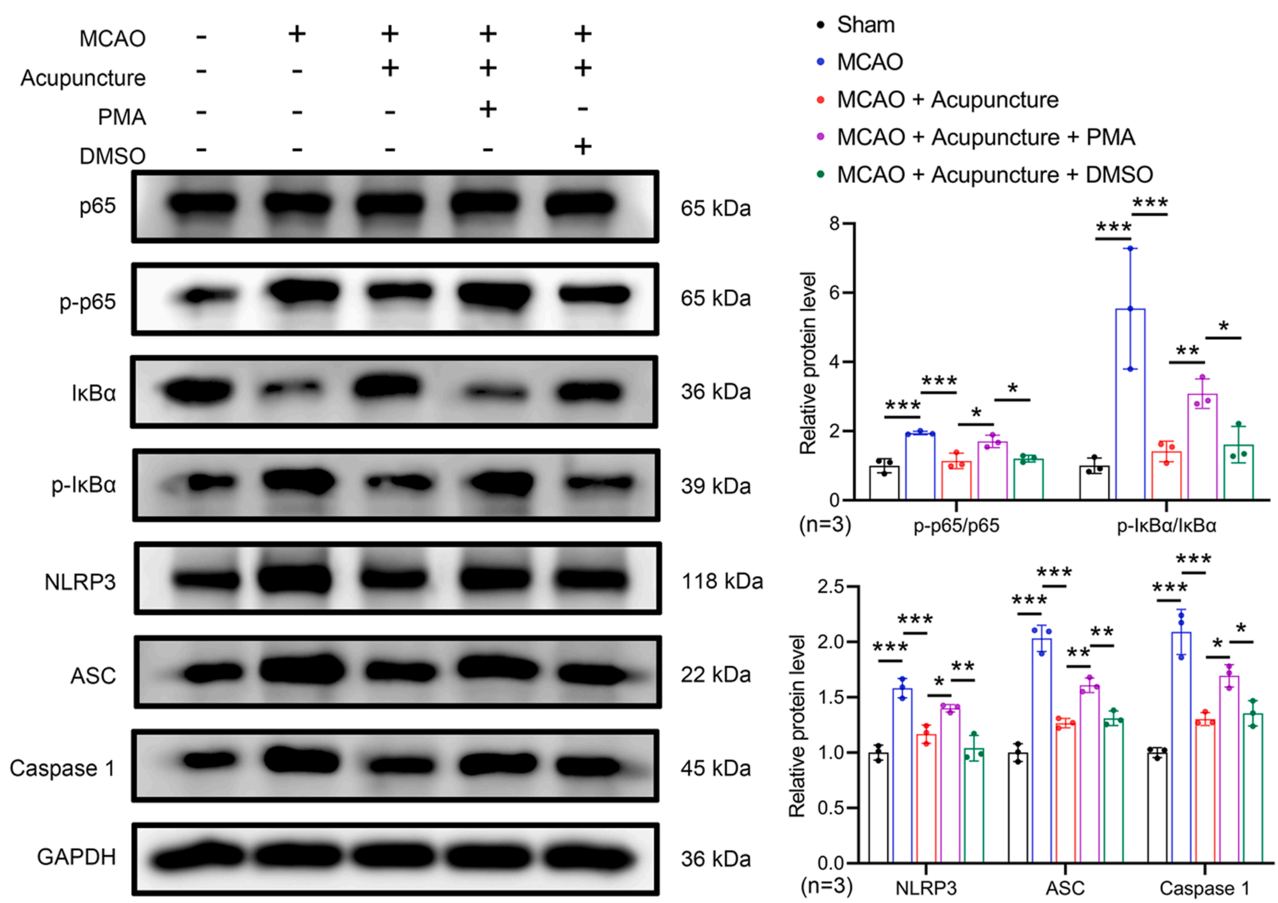

Fig. 3. Acupuncture Treatment Inhibits NF-κB Signaling and Activation of Inflammasomes. Western blot analysis of the effect of acupuncture treatment on the levels of p-p65, p65, p-IKBα, IKBα, NLRP3, ASC, and Caspase-1 proteins in the brain tissue of MCAO rats and the effect of PMA on acupuncture treatment (n = 3). All data are shown as means±SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

3.3. Acupuncture treatment reduces secretion of pro-inflammatory factors

ELISA analysis of brain tissue homogenates showed that compared with Sham rats (5.97 ± 0.40, 9.71 ± 0.32, 14.49 ± 0.79, 20.78 ± 0.40), the levels of inflammatory factors such as IL-1β (24.88 ± 2.14), IL-18 (41.15 ± 0.95), IL-6 (42.39 ± 2.44), and TNF-α (64.45 ± 0.49) were significantly upregulated in MCAO rat brain tissue ($P < 0.0001$), indicating markers of inflammation and neural damage. Activation of inflammasomes further promoted the maturation and secretion of IL-1β and IL-18, exacerbating the inflammatory response. After acupuncture treatment, we observed a significant reduction in these pro-inflammatory factors ($P < 0.0001$), suggesting that acupuncture effectively attenuation the inflammatory response. This mitigation may be due to acupuncture reducting the activation of the NF-κB pathway, thereby inhibiting inflammasomes and reducing the release of pro-inflammatory factors. However, when PMA was added, the reduction effect of acupuncture on pro-inflammatory factors was reversed (Fig. 4), indicating that PMA reactivated the NF-κB pathway and counteracted the anti-inflammatory effect of acupuncture.

3.4. Acupuncture treatment alleviates MCAO-induced apoptosis in brain tissue

Activation of the NLRP3 inflammasome promotes the production of inflammatory cytokines and cell apoptosis and interacts with the NF-κB pathway to drive the inflammatory response. Therefore, we performed TUNEL analysis on rat brain tissue to explore the mechanisms by which acupuncture ameliorates MCAO rats. The results showed that in the sham group, the TUNEL assay revealed few or no apoptotic cells, reflecting a healthy state of brain tissue. However, the MCAO group showed a significant number of TUNEL-positive neurons in the hippocampus and cortex, indicating DNA fragmentation, a direct evidence of cell apoptosis, and reflecting widespread neuronal death due to ischemia. The acupuncture treatment group showed a significant reduction in the number of apoptotic cells, indicating a potential protective role of acupuncture in alleviating cell apoptosis. In the acupuncture+PMA group, due to the activating effect of PMA, the reduction in apoptosis was inhibited or reversed compared with acupuncture treatment alone (Fig. 5), suggesting that activation of the NF-κB pathway may interfere with the protective effect of acupuncture. The results of the acupuncture+DMSO group were similar to those of the acupuncture treatment group.

3.5. Acupuncture treatment protects neuronal cells from MCAO-induced damage

After sectioning and HE staining of rat brain tissue, we observed that in the sham group, HE staining showed normal brain tissue structure with neurons neatly arranged, regular cell morphology, clear nuclei, and uniform staining, indicating of healthy neural tissue characteristics. In contrast, the MCAO group showed typical pathological changes of ischemic brain injury, including disorganized arrangement of neurons, reduced cell volume, concentrated or fragmented nuclei in areas CA3 and CA1, and loose and edematous intercellular spaces with large numbers of inflammatory cells infiltrating the cortex-clear signs of cell damage and death. Compared with MCAO, post-acupuncture treatment showed less neuronal cell damage, more ordered cell arrangement, more normal cell morphology, less nuclear pyknosis, and mild interstitial edema, suggesting that acupuncture may protect neurons from ischemic damage. However, with the addition of PMA, which activates the NF-κB pathway, further deterioration in cell structure was observed compared

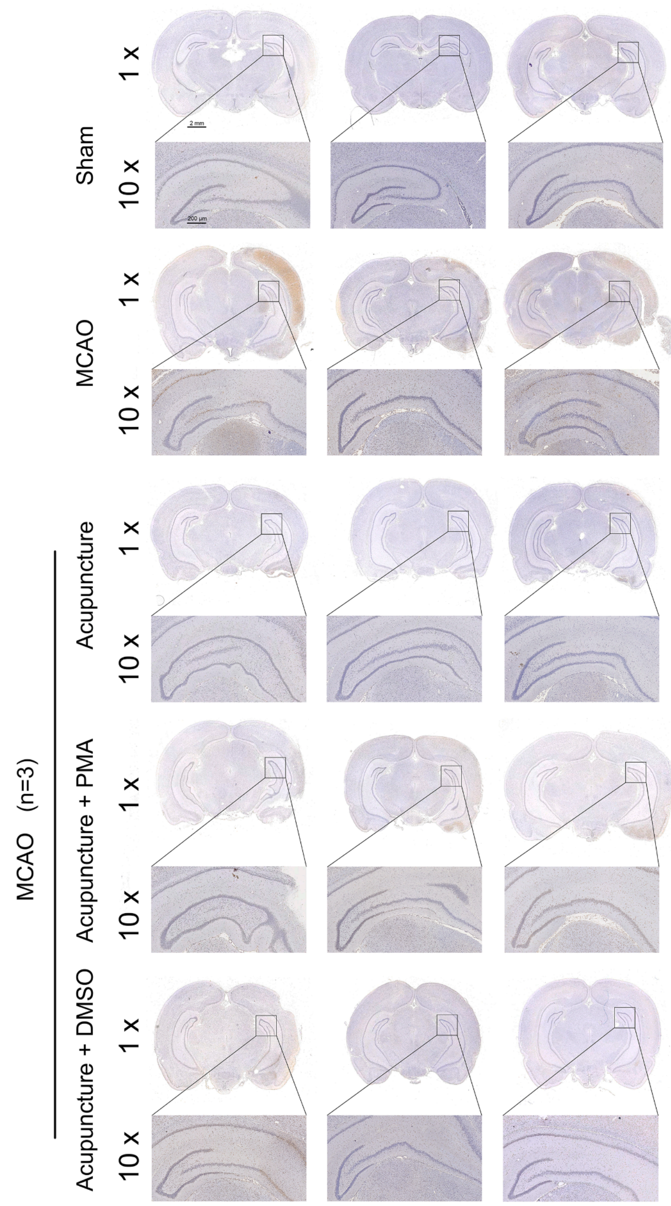

Fig. 5. Acupuncture Treatment Alleviates MCAO-Induced Apoptosis in Brain Tissue. TUNEL analysis of the effect of acupuncture treatment on the apoptosis level in the brain tissue of MCAO rats and the effect of PMA on acupuncture treatment (n = 3).

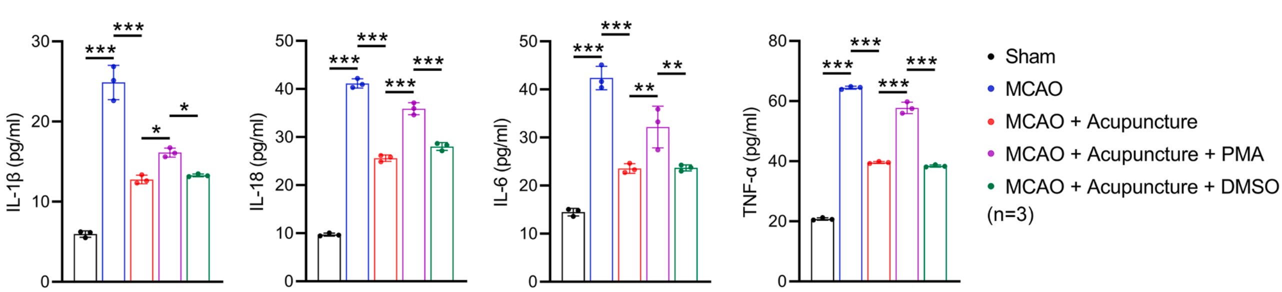

Fig. 4. Acupuncture Treatment Reduces the Secretion of Pro-inflammatory Factors. ELISA analysis of the effect of acupuncture treatment on the levels of pro-inflammatory factors IL-1β, IL-18, IL-6, and TNF-α in the brain tissue of MCAO rats and the effect of PMA on acupuncture treatment (n = 3). All data are shown as means±SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

with acupuncture treatment alone, with some neuronal nuclei pyknosis in area CA3 and a large number of inflammatory cells infiltrating the cortex, confirming increased cell damage and attenuated protective effects of acupuncture. The results of the acupuncture+DMSO group as a drug carrier control were similar to those of the acupuncture treatment group (Fig. 6).

4. Discussion

Current therapeutic approaches such as thrombolysis and mechanical thrombectomy are effective in the acute phase, but still face limitations such as a narrow therapeutic window and limited restricted patient eligibility for IS. Against this background, we believe that traditional acupuncture therapy, as an adjunctive therapy in the prognosis of IS or IS because of its unique mechanism, low cost, and acceptability. Acupuncture has been shown to improve neural functions, promote blood reperfusion, and alleviate neuronal cell damage by stimulating specific acupoints (Hu et al., 2023). Therefore, exploring the application of acupuncture in the recovery of IS, especially its neuroprotective effects, holds great promise for expanding treatment strategies and improving patient prognosis.

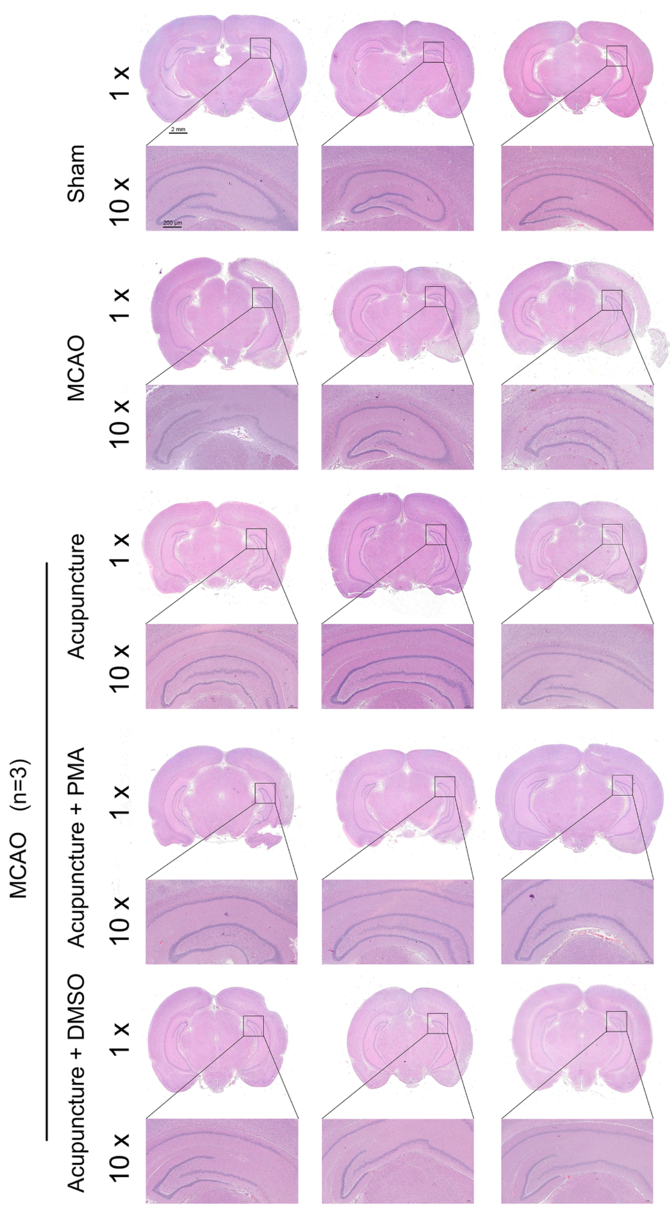

Fig. 6. Acupuncture Treatment Protects Neuronal Cells from MCAO-Induced Damage. HE staining analysis of the effect of acupuncture treatment on neuronal cell damage and inflammatory infiltration in the brain tissue of MCAO rats and the effect of PMA on acupuncture treatment (n = 3).

In our study, acupuncture intervention at Renzhong, Baihui and Zusanli acupoints significantly improved behavioral functions and reduced cerebral infarct volume in MCAO rats. The selection of these points is based on traditional acupuncture theory, which postulates their ability to harmonize cerebral qi and blood, improve local circulation, and facilitate the recovery of neuronal function (Li et al., 2022b; Liu et al., 2022). Specifically, we used Zea-Longa scores to quantify neurological deficits, and the results showed that the scores in the acupuncture-treated rats were lower than the model group, indicating the potential efficacy of acupuncture in improving neurological functions. Concurrently, TTC staining further confirmed the significant reduction in infarct areas, which was consistent with the behavioral improvements. From the perspective of potential mechanisms, modern medical research suggests that acupuncture may exert its effects in several ways: first, by increasing local blood flow and improving blood supply to ischemic areas, thereby mitigating cerebral ischemic injury (Zhang et al., 2022b); secondly, by modulating neurotransmitter and hormone levels in the body, such as increasing cerebral serotonin and endorphins, which are crucial for pain relief and mood improvement and are associated with neuronal cell repair and regeneration (Lee et al., 2019); and finally, functional MRI has shown that acupuncture can alter activity in specific brain areas, regulating neural network functions and thus positively affecting recovery from post-stroke brain dysfunction (Li et al., 2021b). Thus, the potential role of acupuncture may go beyond this, and brain-related disorders may or may not be treated with acupuncture methods, including but not limited to the acupoints used in this study.

Previous research has confirmed that the NF-κB pathway is activated in ischemia and reperfusion injury, promoting the expression of various inflammatory cytokines and adhesion molecules, and exacerbating neuronal injury and death (Dong et al., 2022). Our study found that the phosphorylation levels of the key proteins p65 and IκBα in the NF-κB pathway were significantly decreased after acupuncture, indicating effective inhibition of NF-κB activation. Consistent with previous studies, our research showed that acupuncture reduced the expression of inflammasome-related proteins such as NLRP3, ASC, and Caspase-1, which are central to attenuating inflammasome activation (Cai et al., 2022). These results suggest that acupuncture may alleviate the inflammatory response after stroke and protect neurons from further damage by inhibiting the NF-κB pathway and inflammasome activation. Levels of the pro-inflammatory factors IL-1β, IL-18, IL-6, and TNF-α support the above notion and are key mediators in the stroke-induced inflammatory response (Lyu et al., 2022). We observed a significant increase in these factors in the brain homogenates of MCAO rats and a significant decrease after acupuncture treatment, directly reflecting the attenuation of inflammatory responses, likely due to the regulatory effect of acupuncture on the NF-κB pathway and inflammasome activity. Additionally, activation of the NLRP3 inflammasome can lead to maturation and release of inflammatory factors such as IL-1β and IL-18, exacerbating inflammatory damage (Xu and Nunez, 2023). Therefore, the reduction of NLRP3 inflammasome-related protein expression by acupuncture may also be a mechanism for reducing the secretion of pro-inflammatory factors. Together, these actions reduce the overall level of the inflammatory response, providing a more favorable environment for brain tissue repair after stroke.

In our study, we observed a significant decrease in the number of apoptotic cells in the brain tissue of acupuncture-treated rats compared with MCAO rats. This result suggests that acupuncture may alleviate ischemic brain tissue damage and improve neural function by inhibiting cell apoptosis pathways. In addition, acupuncture reduced the production of inflammatory factors, which may also attenuate apoptosis signaling (Wang et al., 2023). Notably, apoptosis involves various signaling pathways and molecular mechanisms (Li et al., 2022c), suggesting that acupuncture may affect cell apoptosis through multiple pathways (Ji et al., 2018). In addition, acupuncture may improve local blood flow, providing a better survival environment for neurons cells (Chen et al., 2022). These findings provide further scientific evidence for the role of acupuncture in brain protection after stroke or other brain diseases and suggest that acupuncture may exert therapeutic effects through comprehensive regulation of multiple signaling pathways. Similar to previous studies, we found that acupuncture treatment significantly ameliorated MCAO-induced neuronal damage, including reducing the reduction of nuclear condensation, fragmentation, and cell volume shrinkage, associated with apoptosis (Liu et al., 2021c). After acupuncture treatment, the brain tissue structure of rats more closely resembled normal, with more orderly cell arrangement and more typical cell morphology, indicating that acupuncture could improve the overall brain tissue conditions by promoting cell and tissue repair. This effect on pathological changes could be attributed to acupuncture's attenuation of inflammatory responses and reduction of cell damage, thereby alleviating pathological tissue changes. Notably, PMA, as an activator of the NF-κB pathway, reversed the ameliorative effect of acupuncture in MCAO rats, demonstrating that acupuncture may act by inhibiting the NF-κB pathway. However, the role of PMA is not limited to this; it may also affect cellular function and inflammatory regulation by activating other signaling pathways such as protein kinase C (PKC) (Lu et al., 2021). This suggests that the efficacy of acupuncture may involve the interaction of multiple pathways and that the therapeutic response may vary according to individual differences. In summary, the ameliorative effects of acupuncture on the pathological changes in brain tissue of MCAO model rats might result from its comprehensive regulation of various signaling pathways and biological processes, among which inhibition of the NF-κB pathway and inflammasome activity may be key mechanisms. These effects collectively act to reduce cell and tissue damage, promote tissue repair and functional recovery, and thereby improve the pathological condition of brain tissue.

This study still has limitations. First, although the rat MCAO model has some similarities with human IS, there are differences between animal models and human pathophysiological conditions, and the model results may not fully represent the effects in humans. Second, the mechanism of action of acupuncture is complex and diverse, and this study mainly focused on the regulation of the NF-κB pathway and inflammasomes, but acupuncture may also involve other signaling pathways and biological effects, which require further research to comprehensively elucidate its mechanisms. In addition, the optimal parameters for acupuncture treatment (such as acupoint selection, stimulation intensity, frequency, and duration) are not yet clear, and different treatment regimens may significantly affect therapeutic outcomes. Finally, this study focused primarily on the effects of acupuncture on inflammation and cell apoptosis, whereas stroke recovery is a multifaceted process involving neural regeneration, vascular reconstruction, and functional remodeling, among others. Future research should more comprehensively evaluate the effects of acupuncture on these aspects.

5. Conclusion

This study confirmed the potential therapeutic efficacy of acupuncture in improving the outcome of IS through experiments in the MCAO rat model, especially in reducing cerebral infarct volume, suppressing inflammatory response, decreasing cell apoptosis, and ameliorating pathological changes in brain tissue. The effects of acupuncture treatment are closely related to its regulation of the NF-κB pathway and inflammasomes, revealing the potential of acupuncture as a comprehensive treatment modality in stroke recovery. Future work should focus on optimizing acupuncture schedules, delving into mechanisms of action, expanding clinical research, and evaluating long-term effects to better apply this traditional treatment modality in modern medical practice for IS and provide more effective and safer treatment options for patients.

Ethics approval

All procedures involving mice and the corresponding experimental protocols were approved by the Ethics Committee of Fujian Anburui Biotechnology Co., LTD (approval number: IACUC FJABR 2023026007).

Funding

This study was supported by the Young and Middle-aged Backbone Talents Training Project of Fujian Provincial Health Commission (Grant No. 2022GGA048).

CRediT authorship contribution statement

Wang Shicong: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Wu Jiayong:** Visualization, Validation, Supervision, Resources, Formal analysis, Data curation. **Chen Lanrong:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Formal analysis, Data curation, Conceptualization. **Chi Caixia:** Visualization, Validation, Formal analysis, Data curation. **Liu Chengyan:** Writing – review & editing, Validation, Software, Resources, Formal analysis, Data curation. **Wang Linlin:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgements

Not applicable.

Data availability

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

References

Ajoolabady, A., Wang, S., Kroemer, G., Penninger, J.M., Uversky, V.N., Pratico, D., Henninger, N., Reiter, R.J., Bruno, A., Joshipura, K., Aslkhodapasandhokmabad, H., Klionsky, D.J., Ren, J., 2021. Targeting autophagy in ischemic stroke: from molecular mechanisms to clinical therapeutics. Pharm. Ther. 225, 107848.

Cai, L., Yao, Z.Y., Yang, L., Xu, X.H., Luo, M., Dong, M.M., Zhou, G.P., 2022. Mechanism of electroacupuncture against cerebral ischemia-reperfusion injury: reducing inflammatory response and cell pyroptosis by inhibiting NLRP3 and caspase-1. Front. Mol. Neurosci. 15, 822088.

Cao, B.Q., Tan, F., Zhan, J., Lai, P.H., 2021. Mechanism underlying treatment of ischemic stroke using acupuncture: transmission and regulation. Neural Regen. Res. 16, 944–954.

Chen, S., Huang, J., Tang, X., Wang, T., Zeng, Y., 2022. Effects of transient electrical acupuncture stimulation combined with rehabilitation training on hemorheology, neurological function and BDNF in patients with cerebral infarction. Front. Surg. 9, 839523.

Dong, X., Jiang, J., Lin, Z., Wen, R., Zou, L., Luo, T., Guan, Z., Li, X., Wang, L., Lu, L., Li, H., Huang, Y., Yang, Z., Wang, J., Ye, X., Hong, X., Wang, L., Xian, S., Chen, Z., 2022. Nuanxinkang protects against ischemia/reperfusion-induced heart failure through regulating IKKbeta/IkappaBalpha/NF-kappaB-mediated macrophage polarization. Phytomedicine 101, 154093.

Gauberti, M., Martinez de Lizarrondo, S., Vivien, D., 2021. Thrombolytic strategies for ischemic stroke in the thrombectomy era. J. Thromb. Haemost. 19, 1618–1628.

Guo, H., Zhu, L., Tang, P., Chen, D., Li, Y., Li, J., Bao, C., 2021. Carthamin yellow improves cerebral ischemia-reperfusion injury by attenuating inflammation and ferroptosis in rats. Int. J. Mol. Med. 47.

Hu, X., Du, L., Liu, S., Lan, Z., Zang, K., Feng, J., Zhao, Y., Yang, X., Xie, Z., Wang, P.L., Ver Heul, A.M., Chen, L., Samineni, V.K., Wang, Y.Q., Lavine, K.J., Gereau, R.Wt, Wu, G.F., Hu, H., 2023. A TRPV4-dependent neuroimmune axis in the spinal cord promotes neuropathic pain. J. Clin. Invest. 133.
Ji, C., Song, F., Huang, G., Wang, S., Liu, H., Liu, S., Huang, L., Liu, S., Zhao, J., Lu, T.J., Xu, F., 2018. The protective effects of acupoint gel embedding on rats with myocardial ischemia-reperfusion injury. Life Sci. 211, 51–62.
Kim, N.Y., Kim, S., Park, H.M., Lim, C.M., Kim, J., Park, J.Y., Jeon, K.B., Poudel, A., Lee, H.P., Oh, S.R., Ahn, J., Yoon, D.Y., 2023. Cinnamomum verum extract inhibits NOX2/ROS and PKCdelta/JNK/AP-1/NF-kappaB pathway-mediated inflammatory response in PMA-stimulated THP-1 monocytes. Phytomedicine 112, 154685.
Lee, I.S., Cheon, S., Park, J.Y., 2019. Central and peripheral mechanism of acupuncture analgesia on visceral pain: a systematic review. Evid. Based Complement Altern. Med. 2019, 1304152.
Li, X., Cai, L., Jiang, X., Liu, X., Wang, J., Yang, T., Wang, F., 2021b. Resting-state fMRI in studies of acupuncture. Evid. Based Complement Altern. Med. 2021, 6616060.
Li, X., Han, Z., Wang, T., Ma, C., Li, H., Lei, H., Yang, Y., Wang, Y., Pei, Z., Liu, Z., Cheng, L., Chen, G., 2022c. Cerium oxide nanoparticles with antioxidative neurorestoration for ischemic stroke. Biomaterials 291, 121904.
Li, G., Shi, Y., Zhang, L., Yang, C., Wan, T., Lv, H., Jian, W., Li, J., Li, M., 2022b. Efficacy of acupuncture in animal models of vascular dementia: a systematic review and network meta-analysis. Front. Aging Neurosci. 14, 952181.
Li, Y., Xia, Y., Yin, S., Wan, F., Hu, J., Kou, L., Sun, Y., Wu, J., Zhou, Q., Huang, J., Xiong, N., Wang, T., 2021a. Targeting microglial alpha-synuclein/TLRs/NF-kappaB/NLRP3 inflammasome axis in Parkinson's disease. Front. Immunol. 12, 719807.
Li, L., Zhu, W., Lin, G., Chen, C., Tang, D., Lin, S., Weng, X., Xie, L., Lu, L., Li, W., 2022a. Effects of acupuncture in ischemic stroke rehabilitation: a randomized controlled trial. Front. Neurol. 13, 897078.
Liu, H., Jiang, Y., Wang, N., Yan, H., Chen, L., Gao, J., Zhang, J., Qu, S., Liu, S., Liu, G., Huang, Y., Chen, J., 2021a. Scalp acupuncture enhances local brain regions functional activities and functional connections between cerebral hemispheres in acute ischemic stroke patients. Anat. Rec. (Hoboken) 304, 2538–2551.
Liu, J., Ma, W., Zang, C.H., Wang, G.D., Zhang, S.J., Wu, H.J., Zhu, K.W., Xiang, X.L., Li, C.Y., Liu, K.P., Guo, J.H., Li, L.Y., 2021c. Salidroside inhibits NLRP3 inflammasome activation and apoptosis in microglia induced by cerebral ischemia/reperfusion injury by inhibiting the TLR4/NF-kappaB signaling pathway. Ann. Transl. Med. 9, 1694.
Liu, J., Zhao, G., Niu, Y., Gan, T., Yan, Z., Zhang, Y., 2021b. Effect of electro-acupuncture therapy on limb spasm and excitability of motor neurons in stroke rats. Zhejiang Da Xue Xue Bao Yi Xue Ban. 50, 361–368.
Liu, D.N., Zhou, J., Huang, X.R., Sun, G.H., Luo, F., Tan, J.Q., Liao, Y., Peng, T., Feng, W. B., 2022. Effect of electroacupuncture at "Shuigou" (GV26) and "Baihui" (GV20) on autophagy of hippo-campal neurons in rats with cerebral ischemia-reperfusion injury. Zhen Ci Yan Jiu 47, 491–496.
Lu, C.H., Li, K.J., Wu, C.H., Shen, C.Y., Kuo, Y.M., Hsieh, S.C., Yu, C.L., 2021. The FcgammaRIII engagement augments PMA-stimulated neutrophil extracellular traps (NETs) formation by granulocytes partially via cross-talk between Syk-ERK-NF-kappaB and PKC-ROS signaling pathways. Biomedicines 9.
Lyu, H., Ni, H., Huang, J., Yu, G., Zhang, Z., Zhang, Q., 2022. VX-765 prevents intestinal ischemia-reperfusion injury by inhibiting NLRP3 inflammasome. Tissue Cell 75, 101718.
Markus, H.S., Michel, P., 2022. Treatment of posterior circulation stroke: acute management and secondary prevention. Int. J. Stroke 17, 723–732.
Mendelson, S.J., Prabhakaran, S., 2021. Diagnosis and management of transient ischemic attack and acute ischemic stroke: a review. JAMA 325, 1088–1098.
O'Dell, M.W., 2023. Stroke rehabilitation and motor recovery. Continuum (Minne Minn) 29, 605–627.
Qin, S., Zhang, Z., Zhao, Y., Liu, J., Qiu, J., Gong, Y., Fan, W., Guo, Y., Guo, Y., Xu, Z., Guo, Y., 2022. The impact of acupuncture on neuroplasticity after ischemic stroke: a literature review and perspectives. Front. Cell Neurosci. 16, 817732.
Safouris, A., Magoufis, G., Tsivgoulis, G., 2021. Emerging agents for the treatment and prevention of stroke: progress in clinical trials. Expert Opin. Invest. Drugs 30, 1025–1035.
Tang, X.Q., Liao, R.Y., Zheng, L.J., Yang, L.L., Ma, Z.L., Yi, C., Liu, J., Liu, J.C., Kuang, Y. J., Cai, H.A., Huang, L., 2022. Aerobic exercise reverses the NF-kappaB/NLRP3 inflammasome/5-HT pathway by upregulating irisin to alleviate post-stroke depression. Ann. Transl. Med. 10, 1350.
Wang, L., Tu, W., Li, X., Li, C., Lu, J., Dai, P., Chen, Y., Gu, M., Li, M., Jiang, S., Yang, G., Li, S., 2023. Exercise improves cardiac function and attenuates myocardial inflammation and apoptosis by regulating APJ/STAT3 in mice with stroke. Life Sci. 332, 122041.
Wang, L., Zhang, X., Xiong, X., Zhu, H., Chen, R., Zhang, S., Chen, G., Jian, Z., 2022. Nrf2 regulates oxidative stress and its role in cerebral ischemic stroke. Antioxidants (Basel) 11.
Xu, J., Nunez, G., 2023. The NLRP3 inflammasome: activation and regulation. Trends Biochem. Sci. 48, 331–344.
Yang, Y., Deng, P., Si, Y., Xu, H., Zhang, J., Sun, H., 2022. Acupuncture at GV20 and ST36 improves the recovery of behavioral activity in rats subjected to cerebral ischemia/reperfusion injury. Front. Behav. Neurosci. 16, 909512.
Zhang, Y., Ding, N., Hao, X., Zhao, J., Zhao, Y., Li, Y., Li, Z., 2022b. Manual acupuncture benignly regulates blood-brain barrier disruption and reduces lipopolysaccharide loading and systemic inflammation, possibly by adjusting the gut microbiota. Front. Aging Neurosci. 14, 1018371.
Zhang, J., Lu, C., Wu, X., Nie, D., Yu, H., 2021. Neuroplasticity of acupuncture for stroke: an evidence-based review of MRI. Neural Plast. 2021, 2662585.
Zhang, H.Y., Zhao, Y.J., Zhang, P.J., Guo, M.F., Yu, J.W., Chai, Z., Ma, C.G., Song, L.J., Yu, J.Z., 2022a. Acupuncture ameliorates neurological function by suppressing microglia polarization and inflammatory response after cerebral ischemia in rats. Zhen Ci Yan Jiu 47, 941–948.
Zhu, H., Hu, S., Li, Y., Sun, Y., Xiong, X., Hu, X., Chen, J., Qiu, S., 2022. Interleukins and ischemic stroke. Front. Immunol. 13, 828447.
Zhu, T., Wang, L., Feng, Y., Sun, G., Sun, X., 2021. Classical active ingredients and extracts of Chinese herbal medicines: pharmacokinetics, pharmacodynamics, and molecular mechanisms for ischemic stroke. Oxid. Med. Cell Longev. 2021, 8868941.