

Research Article

Transcutaneous Electrical Nerve Stimulation (TENS) Alleviates Brain Ischemic Injury by Regulating Neuronal Oxidative Stress, Pyroptosis, and Mitophagy

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Background. As a noninvasive treatment, transcutaneous electrical nerve stimulation (TENS) has been utilized to treat various diseases in clinic. However, whether TENS can be an effective intervention in the acute stage of ischemic stroke still remains unclear. In the present study, we aimed to explore whether TENS could alleviate brain infarct volume, reduce oxidative stress and neuronal pyroptosis, and activate mitophagy following ischemic stroke. **Methods.** TENS was performed at 24 h after middle cerebral artery occlusion/reperfusion (MCAO/R) in rats for 3 consecutive days. Neurological scores, the volume of infarction, and the activity of SOD, MDA, GSH, and GSH-px were measured. Moreover, western blot was performed to detect the related protein expression, including Bcl-2, Bax, TXNIP, GSDMD, caspase-1, NLRP3, BRCC3, HIF-1 α , BNIP3, LC3, and P62. Real-time PCR was performed to detect NLRP3 expression. Immunofluorescence was performed to detect the levels of LC3. **Results.** There was no significant difference of neurological deficit scores between the MCAO group and the TENS group at 2 h after MCAO/R operation ($P > 0.05$), while the neurological deficit scores of TENS group significantly decreased in comparison with MCAO group at 72 h following MCAO/R injury ($P < 0.05$). Similarly, TENS treatment significantly reduced the brain infarct volume compared with the MCAO group ($P < 0.05$). Moreover, TENS decreased the expression of Bax, TXNIP, GSDMD, caspase-1, BRCC3, NLRP3, and P62 and the activity of MDA as well as increasing the level of Bcl-2, HIF-1 α , BNIP3, and LC3 and the activity of SOD, GSH, and GSH-px ($P < 0.05$). **Conclusions.** In conclusion, our results indicated that TENS alleviated brain damage following ischemic stroke via inhibiting neuronal oxidative stress and pyroptosis and activating mitophagy, possibly via the regulation of TXNIP, BRCC3/NLRP3, and HIF-1 α /BNIP3 pathways.

1. Introduction

As one of the major causes of high mortality and morbidity rates worldwide, stroke is a multifactorial and devastating disease, which can be broadly classified into two forms: ischemic stroke and hemorrhagic stroke [1]. To sum up, more than 70% of stroke cases are ischemic stroke in the world, and this percentage is reaching nearly 86% in the United States of America [2–5]. Ischemic stroke is usually

occurred due to the embolic or thrombotic occlusion in cerebral arteries, which leads to permanent physical and neurological disabilities, contributing to high expenditure on health care annually. Currently, two major timely revascularization treatments, including endovascular thrombectomy and intravenous thrombolysis, are recommended to apply in the acute phase of ischemic stroke according to the present clinical guidelines [6–8]. However, they have certain limitations, such as the narrow therapeutic window, multiple contraindications,

and strict eligibility standard [9, 10]. Despite progress in the investigation of the pathogenesis of cerebral ischemia, the safe and reliable therapeutic methods are still very limited [11–13]. Thus, a safe and beneficial therapeutic method for stroke is urgently needed in clinical settings.

Electroacupuncture (EA) combines electrical stimulation with traditional acupuncture, which is an extension technique based on acupuncture [14]. EA is widely applied in both clinical settings and experimental research for stroke treatment [15]. Our previous studies and related researches had shown that EA intervention effectively reduced the cerebral damage following ischemic stroke via modulating various pathophysiological processes, including apoptosis, inflammation, autophagy, and analgesia [15–17]. However, EA treatment is invasive with the risks of bleeding and infection, and some stroke patients cannot bear the pains of puncturing. Transcutaneous electrical nerve stimulation (TENS) is a technique of electrical stimulation, which can produce perceptible sensations by electrodes in contact with skin [18]. TENS is used widely in clinical practice in various types of hospitals and medical institutions because of its simplicity, good safety, and noninvasion [19]. Currently, the analgesic effects of TENS on multiple neuropathic pains are extensively studied, such as cancerous pain and musculoskeletal pain [19, 20]. However, TENS has only been proved to exert beneficial effects on patients with ischemic stroke in late stages in clinical trials. For instance, Chen et al. demonstrated that bilateral TENS combined with task-oriented training effectively improved motor recovery of upper limb following stroke [21]. Moreover, a systematic review and meta-analysis demonstrated that TENS was related to the improvement of spasticity and promoted walking speed and static balance in stroke patients [22]. Actually, increasing evidence supports early rehabilitation interventions after stroke are of important significance, which can rebuild brain function, accelerate the average cerebral blood flow, and promote collateral circulation formation, thus improving the neurological function of patients [23–27]. Therefore, clarifying whether and how TENS exerts beneficial effects at the early stage of ischemic stroke will provide theoretical basis and promote the extensive application of TENS in clinic.

Following the obstruction of brain arteries by an in situ thrombosis or embolus, cerebral ischemia occurs and causes irreversible neuronal dysfunction and a cascade of pathological responses, comprising apoptosis, oxidative stress, neuroinflammation, and autophagy [28]. Oxidative stress plays a key role in the fundamental pathological progression of brain injury following ischemic stroke. Oxidative stress occurs when the inherent antioxidant capacity to counteract reactive oxygen species (ROS) is insufficient and cannot maintain the endogenous redox balance [29, 30]. Oxidative stress can result in cytotoxicity due to oxidative damage of nucleic acids, protein, and lipids with detrimental effects on the function and structure of brain tissues [31]. As a primary antioxidant system, thioredoxin (TRX) system is essential to keep the intracellular redox homeostasis, and thioredoxin-interacting protein (TXNIP) is an endogenous suppressor of TRX [32, 33]. Tian et al. reported that TXNIP increased the levels of oxidative stress in ischemic stroke,

and inhibition of TXNIP conferred the neuroprotection against ischemic brain damage. On the other hand, neuroinflammation is a vital event contributing to brain injury after stroke [34]. In the innate immune system, inflammasomes are the key regulators of neuroinflammatory response in ischemic stroke, such as the nod-like receptor pyrin domain-containing 3 (NLRP3) inflammasome [35]. The increase of NLRP3 inflammasomes stimulates pro-caspase-1, which causes the aggravation of pyroptosis, a form of inflammatory programmed cell death [36, 37]. The suppression of NLRP3 obviously reduces cerebral ischemic injury, protecting against neuronal cell death in both in vivo and in vitro settings, which may be an effective strategy for treating stroke [38]. Moreover, BRCA1eBRCA2-containing complex subunit 3 (BRCC3) is a critical modulator of NLRP3 activity through increasing its deubiquitination and making NLRP3 a characterized substrate for the cytosolic BRCC3-containing BRISC complex [39]. However, the precise alteration in BRCC3 levels and whether TENS exerts counteracting effects on oxidative stress and inflammasomes by TXNIP and BRCC3/NLRP3 signaling have barely been investigated in ischemic stroke.

Autophagy is referred to a regulatory mechanism for recycling and degrading cellular constituents and managing protein quality control and organelle renewal, which can provoke a response to a series of stress conditions, such as starvation, ischemia, and many pathological stresses [40, 41]. Mitophagy, one of the selective forms of autophagy, can particularly remove dysfunctional mitochondria to keep mitochondrial homeostasis and cellular survivals [42]. During this procedure, mitochondrial dysfunction is identified by the mitophagy receptors, consisting of BNIP3 (BCL2/adenovirus E1B 19kDa interacting protein 3), NIX (BNIP3L), PINK1/PINK1 (phosphatase and tensin homolog- (PTEN-) induced putative protein kinase 1), and Parkin and other related mediators [43]. Various studies have demonstrated that activating mitophagy to eliminate damaged and accumulated mitochondria can alleviate neuronal damage induced by cerebral ischemia/reperfusion injury [44–46]. Moreover, Chen et al. showed that a sphingosine kinase 2-mimicking TAT-peptide prevented neurons against ischemic injury by upregulating BNIP3-induced mitophagy [47]. Hypoxia inducible factor-1 α (HIF-1 α), a transcription factor, acts as the modulator of oxygen homeostasis, which is the upstream factor of BNIP3 [48]. It has been proved that BNIP3 overexpression can be mediated by HIF-1 α in cerebral ischemia [49, 50]. Moreover, Xu et al. demonstrated that hypoxia-induced HIF-1 α overexpression reduced the inhibition of dexamethasone on mitophagy via BNIP3 signaling pathway, thus suppressing apoptosis of bone cells [51]. Therefore, we hypothesize that HIF-1 α plays a protective role by regulating BNIP3-mediated mitophagy, and TENS might regulate mitophagy through HIF-1 α /BNIP3 pathway in ischemic stroke.

As for the selection of stimulation points, Baihui (GV20) and Hegu (LI4) are proved to be effective for the treatment of ischemic stroke [52]. Several studies have proved the neuroprotective roles of EA in MCAO models via stimulating at Baihui (GV20) and Hegu (LI4) points [53, 54]. Zhan et al.

showed that EA induced anti-inflammatory effects by upregulating the zinc finger protein A20 expression via stimulating Baihui (GV20) and Hegu (L14) acupoints in MCAO rats [55]. In addition, Xie et al. reported that the EA stimulation in Baihui (GV20) and Hegu (L14) acupoints could improve the recovery of neurological function and mediate angiogenesis following ischemic stroke [56]. In clinical practice, these two acupoints were adopted to relieve post-stroke symptoms, including depression, anxiety, and insomnia [57–59]. Therefore, we selected the acupoints of Baihui (GV20) and Hegu (L14) as the stimulating areas of TENS protocol.

Overall, in the present study, we aim to investigate the protective mechanisms of TENS intervention in the process of oxidative stress, neuroinflammation, and mitophagy in the acute stage of ischemic stroke.

2. Materials and Methods

2.1. Animals and Groups. All rats in this experiment were offered by Hebei Province Laboratory Animal Center. All adult male Sprague–Dawley (SD) rats, weighed 250–280 g, were raised in appropriate temperature ($22 \pm 2^\circ\text{C}$) with sufficient water and food in a 12 h light/dark cycle. The whole procedures of the present study were approved by the Animal Care and Use Committee of Hebei Medical University (ethical approval ID: 2021020). All protocols were abided by laboratory animal—guide for ethical review of animal welfare (GB/T 35892-2018) released in 2018 in China. The 75 SD rats were randomly divided into 3 groups ($n = 25/\text{group}$): (i) middle cerebral artery occlusion/reperfusion group (MCAO group), (ii) MCAO+TENS group (TENS group), and (iii) sham-operated group (sham group).

2.2. Middle Cerebral Artery Occlusion (MCAO) Model Establishment. The model of cerebral ischemia was established through blocking the middle cerebral artery (MCA). Before surgery, rats were fasted for 10 h but allowed to drink. The operation details are illustrated in our previous publications [17, 60].

2.3. Transcutaneous Electrical Nerve Intervention. The rats received TENS treatment with self-adhesive surface electrodes via a TENS apparatus (Model G6805-2A; Shanghai Huayi Co., Shanghai, China) after 3% sodium pentobarbital anesthesia. Remove the hair at the midpoint of the parietal bone (the position of Baihui acupoint, GV20) and the midpoint of radial border of the second metacarpal bone (the position of Hegu acupoint, LI4) and sterilize the skin with 75% ethyl alcohol. Then apply electrical stimulation to these two points for 30 min per day with a disperse-dense wave of 4 or 20 Hz, a current of 3–4 mA, and the intensity below the level of causing obvious muscle contraction. TENS was initiated at 24 h after MCAO/R surgery. After the treatment for three times (at 72 h following MCAO/R), the rats were sacrificed. Both sham and MCAO group only received the injection of 3% sodium pentobarbital without TENS intervention.

2.4. Assessment of Neurological Behaviors. The neurological deficit assessment was performed in all rats at 2 h or 72 h after MCAO/R in a blind manner. The details are reported in our published articles [16, 60].

2.5. TTC (2,3,5-Triphenyltetrazolium Chloride) Staining. This staining method was used to the detection of cerebral infarct volume. After sacrifice, the brain tissues from rats in all groups were obtained and frozen at -20°C for 15 min. The whole brain was then dissected coronally into 6 sections (2 mm every section) for TTC staining. The sections were soaked and stained in 2% TTC (TTC, Servicebio, China) at 37°C for 30 minutes. After 24 h to 48 h fixation in 4% paraformaldehyde buffer, photograph all slices. The region of infarction was calculated by Image J.

2.6. Measurement of Oxidative Stress Index. The brain tissues from cerebral cortex of the affected side in the rats ($n = 5/\text{group}$) were homogenized by RIPA buffer. Then collect the proteins. Levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), and glutathione peroxidase (GSH-px) were evaluated by commercially available kits (A001-3/A003-1/A005-1, Nanjing Jiancheng, Nanjing, China) in line with the manufacturer's protocol.

2.6.1. ELISA. Based on the manufacturer's instruction, brain tissues were collected and lysed in lysis buffer. After homogenized ultrasonic treatment, the samples were centrifugated at 4°C , and the supernatant was obtained for ELISA. Read values of different groups at 450 nm with a microplate reader, and the concentrations of IL-6 and TNF- α were measured following the standard curve generated in the meantime.

2.7. Immunofluorescence. Immunofluorescence (IF) was conducted to analyze the expression difference of LC3 protein in the affected hippocampus of rats in three groups. Following anesthesia, five rats in each group were sacrificed. Brain tissues were removed and fixed in formaldehyde. After embedded in paraffin, the brains were sectioned into slices of $4\ \mu\text{m}$ thickness. The slices were blocked with the normal sheep serum at 37°C for 90 min and then immunostained with primary antibodies against LC3 (from rabbit; 1:300; 18725-1-AP, Proteintech Group, USA) at 4°C overnight. Next, the slices were incubated with Rhodamine- (TRITC-) conjugated goat antirabbit IgG (H + L) secondary antibodies (1:400, SA00007-2, Proteintech Group, USA) for 2 h in the darks. After washing 3 times with PBS, use 40, 6-diamidino-2-phenylindole (DAPI) to counterstain nuclei. Capture the images of hippocampus by a fluorescence microscope (OLYMPUS 905). Results were quantified as optical density (OD) by the Image J 1.8.0 program.

2.8. Western Blot. Western blotting was conducted to detect the expression of proteins related to apoptosis, oxidative stress, mitophagy, and neuroinflammation, including Bcl-2, Bax, TXNIP, GSDMD, caspase-1, NLRP3, BRCC3, HIF-1 α , P62, and BNIP3. At 72 h after MCAO/R, rats ($n = 5$ each group) were euthanatized, and hippocampus in injured hemisphere was collected and lysed as protein samples. The samples were subjected to SDS-PAGE to separate

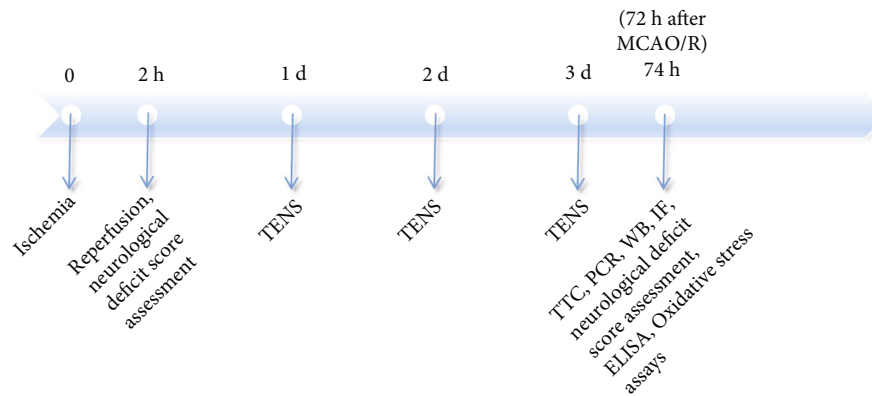


FIGURE 1: The experimental protocol at different time points.

proteins and subsequently transferred onto polyvinylidene difluoride membrane (3010040001, Roche, Mannheim, Germany). After being blocked for 90 minutes with 5% bovine serum albumin, the membranes were put on the rotating mixer and incubated at 4°C for 16 h with the primary antibodies against BRCC3 (1:1000, Cat. #18215, Cell Signaling Technology, USA), TXNIP (1:1000, #14715, Cell Signaling Technology, USA), caspase-1 (1:2000, #ab184787, Abcam), NLRP3 (1:1000, #19771-1-Ig, Proteintech), GSDMD (1:500, #20770-1-AP, Proteintech Group, USA), HIF-1 α (1:1000, ab179483, Abcam, UK), BNIP3 (1:300, ab109362, Abcam, UK), LC3 (1:1000; 18725-1-AP, Proteintech Group, USA), P62 (1:300, 66184-1-Ig, Proteintech Group, USA), Bcl-2(1:1000, #60178-1-Ig, Proteintech Group, Inc.), Bax (1:1000, #60267-1-Ig; Proteintech Group, USA), and GAPDH (1:5000, #60004-1-AP, Proteintech Group, USA). Following washing in 0.1% Tween 20 in Tris-buffered saline (TBST) for 3 times, membranes were then incubated with HRP-conjugated secondary antibodies (1:5000, SA00001-1/SA00001-2, Proteintech Group, USA) for another 1.5 h at room temperature. Use the imaging analysis system of Amersham Imager 600 to make the protein bands visible with an ECL detection kit. At last, the optical density of these protein bands was calculated by Image J 1.8.0 (Scion Corporation).

2.9. Quantitative Real-Time PCR. Extract total RNA from brain tissues by TRIzol reagent (Life Technologies Corporation, Carlsbad, CA, USA). The concentrations and the quality of RNA were detected by a UV Spectrophotometer (N50 Touch; Implen, Germany). Quantitative Real-time PCR was applied using One-Step SYBR PrimeScript RT-PCR. The relative expression of mRNA was evaluated in line with $2^{-\Delta\Delta C_t}$ method normalized to GAPDH. The synthesis of primers was shown as follows: hsa-GAPDH: forward 5'-TGCACCACCAACTGCTTAGC-3' and reverse 5'-GGCATGCAC TGTGGTCATGAG-3', has-NLRP3: forward.

2.10. Statistical Analyses. Make use of the statistic software, SPSS 21.0, to analyze all data. Differences among the multiple groups were conducted by one-way analysis of variance (ANOVA), followed by a Bonferroni test. The data from RT-qPCR and neurological deficits scores were performed by a nonparametric test. Quantitative values were expressed

as mean \pm standard deviation. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. The Effect of TENS on Neuronal Pathological Changes after MCAO/R. The neurological deficits were assessed at 2 h and 72 h after I/R surgery, as demonstrated in Figure 1. As shown in Figure 2(a), the sham group did not show any neurological symptoms, while the TENS group and MCAO group all showed distinct manifestations of neurological deficits. The TENS group exhibited no significant difference in neurological scores (Figure 2(a)) compared with the MCAO group at 2 h after MCAO/R operation ($P > 0.05$). However, in TENS group, neurological deficit scores were evidently reduced at 72 h compared with the MCAO group ($P < 0.05$, Figure 2(b)). Then, the infarction volume evaluation was implemented at 72 h following MCAO/R injury. The sham group showed no infarction. However, there was significant difference of infarct ratio between the TENS group and MCAO group ($P < 0.05$, Figures 2(c) and 2(d)). The infarcted volume of the TENS group was lower than MCAO group ($P < 0.01$). To further explore the mechanism of anti-apoptosis effects of TENS, as shown in Figures 2(e)–2(g), TENS treatment reversed the decrease of Bcl-2 ($P < 0.05$) and increase of Bax ($P < 0.05$) mediated by ischemic stroke.

3.2. Modulation of Oxidative Stress-Associated Proteins by TENS. As shown in Figure 3(b), ischemic stroke obviously increased MDA levels in the cortex ($P < 0.01$), and TENS significantly decreased MDA levels in the cortex ($P < 0.01$). Antioxidant enzymes were also measured. In Figures 3(a), 3(c), and 3(d), MCAO/R decreased the levels of SOD, GSH, and GSH-px compared with the sham group ($P < 0.01$); however, TENS restored the level of SOD, GSH, and GSH-px to some extent ($P < 0.01$). TXNIP level was low in sham group. TENS significantly suppressed the expression level of TXNIP ($P < 0.05$, Figures 3(e) and 3(f)).

3.3. Modulation of Neuroinflammation-Associated Proteins by TENS. As shown in Figure 4, MCAO/R induced the elevation of NLRP3 ($P < 0.01$), GSDMD ($P < 0.05$), and caspase-1 ($P < 0.01$), and TENS reversed the elevation in the affected

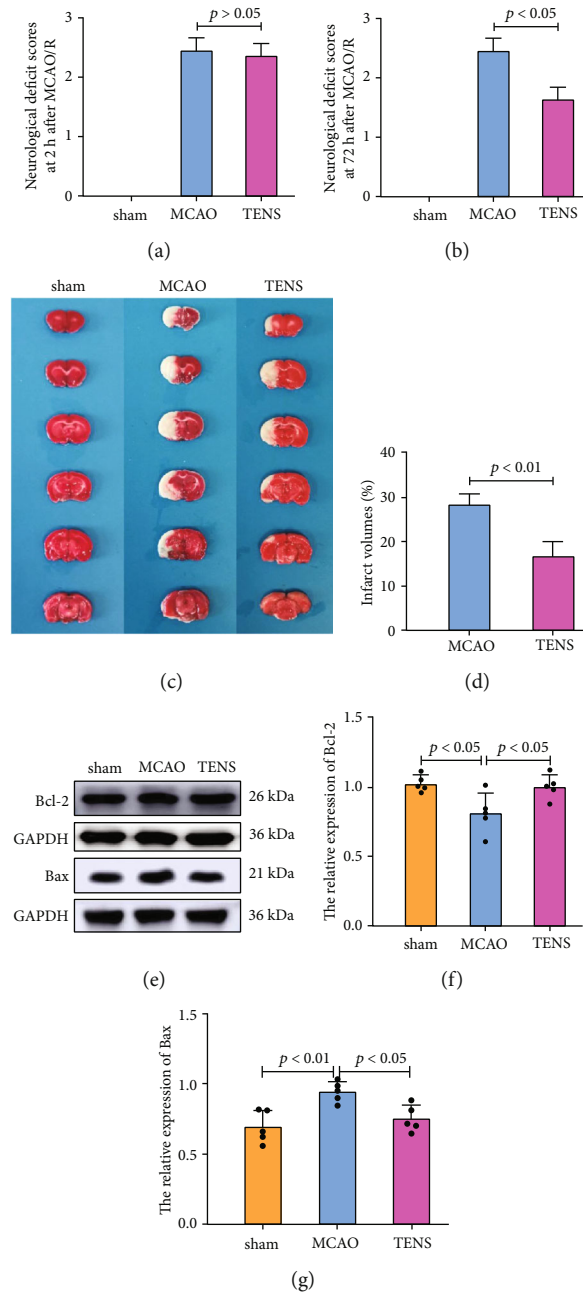


FIGURE 2: The pathological damage after cerebral ischemia/reperfusion (I/R) within 3 days. (a) Statistical analysis of neurological deficit score assessment at 2 h after MCAO/R injury. (b) Statistical analysis of neurological deficit score assessment at 72 h after MCAO/R injury. (c) The 2,3,5-triphenyltetrazolium chloride (TTC) staining demonstrating infarct volumes in MCAO rats with or without TENS. (d) Statistical analysis showing TENS significantly reduced neural damage when compared with the MCAO group. (e) Western blot analysis of the expression levels of Bcl-2 and Bax (GAPDH was used for the loading controls). (f, g) Statistical analysis showing the Bcl-2 and Bax expression in the injured hippocampus among three groups.

hippocampus ($P < 0.05$), suggesting its anti-inflammatory effects (Figures 4(b), 4(c), and 5(a)–5(c)). Furthermore, BRCC3 expression was significantly raised in MCAO group ($P < 0.01$). TENS significantly inhibited the levels of BRCC3 ($P < 0.05$, Figures 5(d) and 5(e)). As shown in Figure 4(a), NLRP3 expression detected by RT-qPCR was increased in the MCAO group ($P < 0.01$). Furthermore, we verified that this elevation could be counteracted by TENS treatment ($P < 0.01$). In Figures 5(f) and 5(g), MCAO/R decreased

the levels of IL-6 and TNF- α compared with the sham group ($P < 0.01$); however, TENS restored the increased level of IL-6 and TNF- α ($P < 0.01$).

3.4. Modulation of Mitophagy-Associated Proteins by TENS.

To explore the modulating mechanism of TENS in mitophagy after MCAO/R injury, the expressions of LC-3II/LC3-I, P62, BNIP3, and HIF-1 α were evaluated by western blot. According to the Figures 6(a), 6(b), and 7(a)–7(d), the

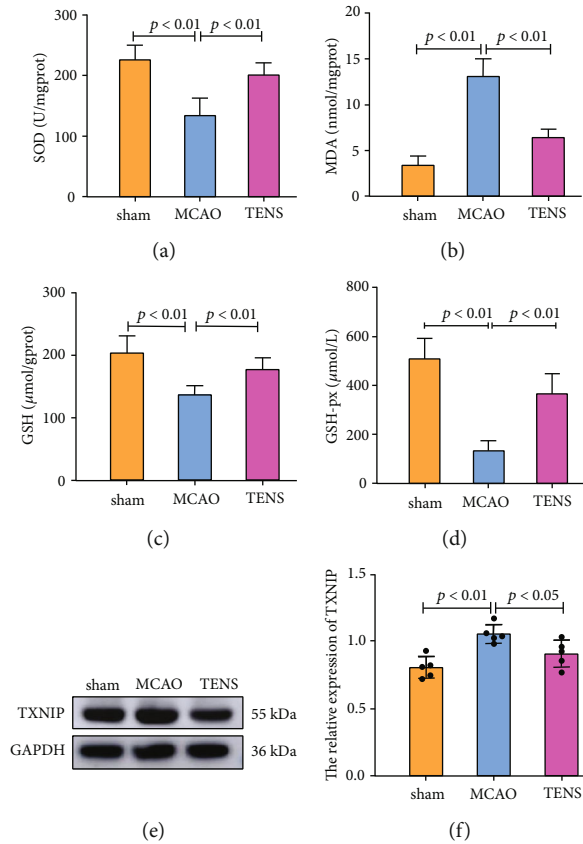


FIGURE 3: The effect of TENS treatment on oxidative stress-related proteins. (a–d) Statistical analysis of the SOD, MDA, GSH, and GSH-px activities assessed by kits. (e) TXNIP expression was detected by western blot. (f) Statistical analysis showing the TXNIP expression in the ipsilateral hippocampus among three groups.

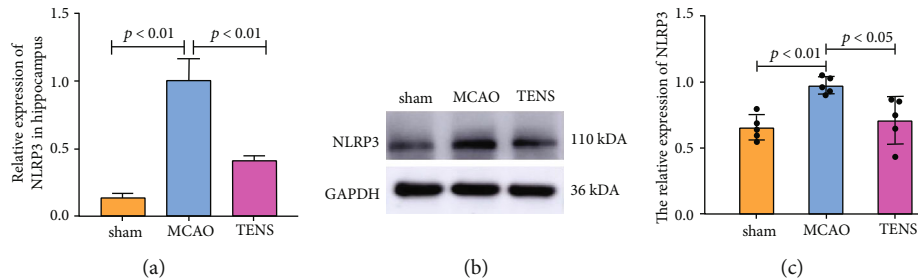


FIGURE 4: The effect of TENS treatment on NLRP3 expression. (a) NLRP3 expression in the injured hippocampus was detected by real-time PCR, in which GAPDH was used for the loading controls. (b) The NLRP3 expression was detected by western blot. (c) Statistical analysis showing the expression difference of NLRP3 and BRCC3 in the injured hippocampus among three groups.

elevated expressions of LC-3 II/LC3-I ($P < 0.01$) and BNIP3 ($P < 0.01$) were detected in the MCAO group compared with the sham group, and their expressions were further increased in the TENS group (LC3-II/LC3-I, $P < 0.05$; BNIP3, $P < 0.01$). Also, the P62 levels were decreased in the MCAO group compared with the sham group ($P < 0.05$) and were further decreased in the TENS group than the MCAO group ($P < 0.01$). Moreover, HIF-1 α was enhanced in the MCAO group compared with the sham group ($P < 0.05$), and its levels in the TENS group were even higher than the MCAO group ($P < 0.05$). As shown in Figures 6(c) and

6(d), the levels of LC3 as analyzed by immunofluorescence were also increased in the TENS group than the MCAO group ($P < 0.05$). These results indicate that TENS intervention can activate the cerebral ischemia/reperfusion-induced mitophagy potentially via the HIF-1 α /BNIP3-dependent pathway.

4. Discussion

Globally, more than 16 million people are afflicted with stroke each year, leading to approximately one-third dying and

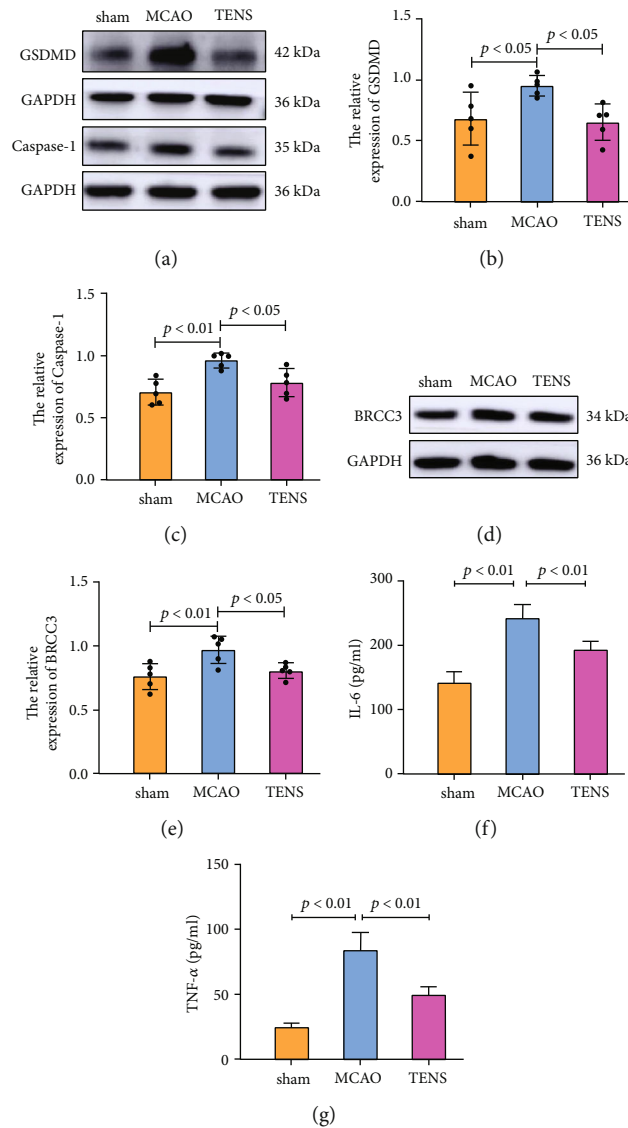


FIGURE 5: The effect of TENS treatment on neuroinflammation-related proteins. (a, d) The GSDMD, caspase-1, and BRCC3 expressions were detected by western blot. (b, c, e) Statistical analysis showing the expression difference of GSDMD, caspase-1, and BRCC3 in the injured hippocampus among three groups. (f, g) Statistical analysis of the levels of IL-6 and TNF- α assessed by ELISA kits.

another third becoming permanent disability, which causes the profound socioeconomic influence [61]. Moreover, previous studies confirmed that the prevalence and mortality rates of stroke were obviously higher in rural population compared with the urban population in China [62]. The rural population had the slower reduction of mortality in stroke than the urban population. These findings suggest that rural areas lag behind urban districts in socioeconomic and healthcare improvement [63]. Nowadays, despite great efforts and development in translational research, the available treatment strategies for stroke, including ischemic stroke, still remain limited, and numerous promising drugs in traditional preclinical evaluation are unable to enter clinical trials [63]. Therefore, a cost-effective and convenient treatment method is urgently needed, which could be important for rural and undeveloped areas.

Up to now, there are a growing number of interests toward the nonpharmacological approaches for improving stroke recovery due to the advantages of easy operation, availability, and economy, such as EA and electrical stimulation. The effects of EA on stroke have been widely studied in both clinical and experimental research [15, 64]. However, EA treatment is invasive with the risks of bleeding and infection, and some stroke patients cannot bear the pains of puncturing. In addition, the method of puncture relies heavily on the doctor's experience. In comparison, TENS is used widely in clinical practice because of its simplicity, good safety, and noninvasion [19]. Several clinical studies have reported the beneficial effect of TENS for poststroke patients, such as the improvement of motor, sensory dysfunction and dysphagia, and the symptomatic relief of shoulder hand syndrome, urinary incontinence, and constipation [65–71]. Currently,

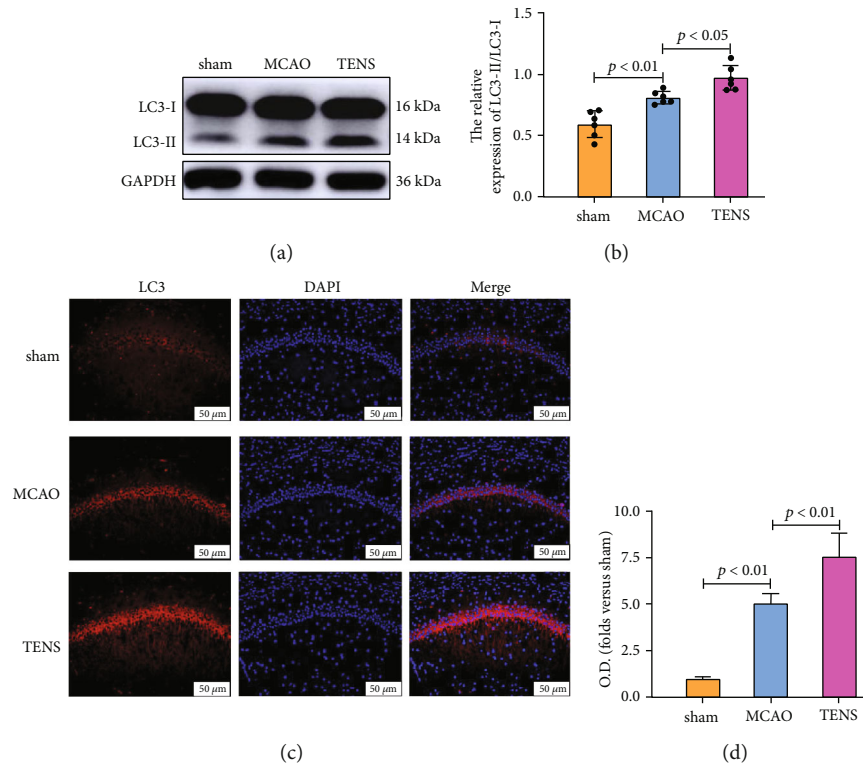


FIGURE 6: The effect of TENS treatment on LC3 expression. (a) The expression of LC3-II/LC3-I was measured by western blot, in which GAPDH was used for the loading controls. (c) IF analysis exhibiting the expression difference of LC3 protein in the ipsilateral hippocampus among three groups. (b, d) Statistical analysis of the expression of LC3 as assessed by IF.

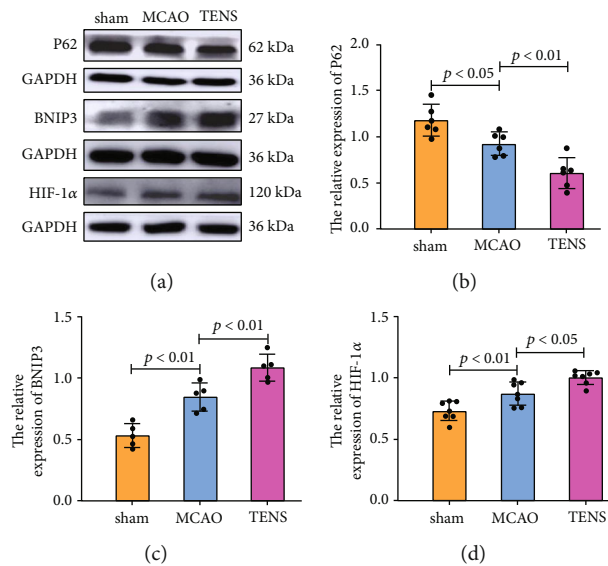


FIGURE 7: The effect of TENS treatment on mitophagy-related proteins. (a) The expressions of P62, BNIP3, and HIF-1 α in the ipsilateral hippocampus were measured by western blot, in which GAPDH was used for the loading controls. (b, c, d) Statistical analysis of the expression difference of P62, BNIP3, and HIF-1 α among three groups.

only the analgesic mechanism of TENS on multiple neuropathic pains is studied, including the electrical transmission modulation, the endogenous opioid peptides generation, and the blood vessel expansion [20, 72, 73]. However, the

exact molecular mechanisms of TENS to treat stroke have not been investigated until now. Thus, in the present study, for the first time according to our knowledge, we demonstrated that TENS effectively decreased the neurological

deficit scores, the cerebral infarction, and the levels of apoptosis in the acute stage of ischemic stroke, suggesting that TENS can be considered as a promising treatment strategy for ischemic stroke.

Furthermore, we investigated the exact mechanisms of neuroprotective effect of TENS on ischemic stroke. Following ischemic stroke, neuron injury was triggered by a series of pathological mechanisms, including oxidative stress, autophagy, neuroinflammation, and pyroptosis. Due to the disequilibrium between endogenous antioxidative system and detrimental ROS, ROS-triggered malondialdehyde (MDA) is evidently accumulated, and antioxidative enzymes (such as SOD and GSH-px) or antioxidant (including GSH) are reduced accordingly [74]. In this study, TENS treatment exhibited the antioxidant potential via promoting SOD, GSH, and GSH-px as well as inhibiting MDA. Moreover, TXNIP is an essential modulator for ROS signaling, which has been proved to accelerate cerebral ischemic injury [75]. In the current research, our results demonstrated that TXNIP expression was increased in the hippocampus following MCAO/R injury and conversely significantly reduced after TENS intervention, indicating the neuroprotective role of TENS.

Moreover, neuroinflammation plays a key role in the process of ischemic stroke [76, 77]. In innate immune system, NLRP3 inflammasome is a key node for immune sensing, readily responding to chronic inflammation. Moreover, NLRP3 can induce pyroptosis, one of the programmed cell death processes with a feature of plasm membrane damage, ensuing release of proinflammatory factors [35, 78]. The essential executor of pyroptosis is Gasdermin D (GSDMD). After being cleaved by caspase-1, GSDMD can form pores in cellular membrane, which causes cell death [79, 80]. Following ischemic stroke, NLRP3 inflammasome was reported to be activated, thus promoting neuron death [81]. Jiang et al. demonstrated that EA intervention inhibited NLRP3 inflammasome and subsequent inflammatory reaction via inhibiting caspase-1, GSDMD, and IL-1 β , thus exerting neuroprotection against cerebral ischemic injury. Consistent with these findings, our results confirmed that TENS dramatically reduced the increased levels of proteins related to NLRP3 inflammasome-dependent pyroptosis and neuroinflammation after ischemic stroke, consisting of NLRP3, GSDMD, caspases-1, IL-6, and TNF- α . Moreover, as a regulator of the ubiquitination and activity of NLRP3, BRCC3 was activated following cerebral ischemia and TENS treatment reversed the promoted BRCC3 levels induced by ischemic condition. These results suggested that TENS may effectively alleviate brain damage against ischemic stroke via suppressing NLRP3 inflammasome activation by regulating BRCC3.

Mitophagy contributes to the physiology of organelle integrity and cellular energy by maintaining the mitochondria biogenesis and homeostasis, which is often considered as the essential mechanism for cell survival [82]. Rapamycin-activated mitophagy was proved to improve mitochondrial function through decreasing malondialdehyde (MDA) and recovering the levels of ATP and mitochondrial membrane potential in the rat models of cerebral ischemia [83]. More-

over, Zhong et al. demonstrated that EA improved cognitive impairment by inhibiting NLRP3 inflammasome activation by upregulating mitophagy induced by melatonin in stroke models [84]. Consistently, in the present study, the expression of BNIP3 and the ratio of LC3-II/I was obviously enhanced, and P62 expression was significantly reduced after MCAO/R injury. TENS treatment further elevated the expression level of BNIP3 and LC3-II/I and reduced the P62 expression. These results indicated that BNIP3-mediated mitophagy was activated following cerebral ischemia, and TENS could exert neuroprotective effects by activating mitophagy. HIF-1 α plays a key role in adaption to hypoxic and ischemic conditions, which modulates the expression of targeted proteins involved in apoptosis and oxidative stress, particularly mitophagy [85–87]. Liu et al. showed that the expression of HIF-1 α was increased at 72 h following MCAO surgery [88]. Furthermore, the upregulation of mitophagy induced by activated HIF-1 α /BNIP3 signaling pathways was proved to inhibit apoptosis in acute kidney injury [89]. In our present study, we showed that HIF-1 α was activated following I/R injury, and TENS treatment markedly promoted the HIF-1 α expression and increased BNIP3-mediated mitophagy levels, thus indicating that HIF-1 α and BNIP3 might participate in the regulation mechanism of TENS treatment for ischemic stroke.

There are several limitations in this research. First, our study focuses on the neuroprotection of TENS against ischemic brain injury, without identifying the different effects among different stimulation sites of TENS. In the future study, we will set different control groups to explore the difference between the stimulated acupoints of “Baihui and Hegu” and other stimulation acupoints. Second, we did not perform rescue experiments with the inhibitors or inducers of autophagy, inflammation, TXNIP, BRCC3, and HIF-1 α . Further studies are needed to confirm the involvement of autophagy, inflammation, TXNIP, BRCC3, and HIF-1 α in the neuroprotective mechanisms of TENS for alleviating cerebral ischemic injury.

5. Conclusion

Taken together, the current results demonstrate that TENS significantly modulates oxidative stress, neuroinflammation, pyroptosis, and neuronal autophagy following ischemic stroke, possibly via regulating TXNIP, BRCC3/NLRP3, and HIF-1 α /BNIP3 signaling pathway, thus reducing the brain damage caused by cerebral ischemia. However, the specific mechanisms for the neuroprotective effect of TENS on ischemic stroke remain to be further explored.

In summary, TENS treatment might be a rational and cost-effective strategy for improving patient function following ischemic stroke and deserves further exploration, thus benefiting more stroke patients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Zixuan Tan, Fang Dong, Linyu Wu, and Yashuo Feng contributed equally to this article.

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References

- [1] F. Zhou, L. Zhou, T. Guo et al., "Plasma proteomics reveals coagulation, inflammation, and metabolic shifts in H-type hypertension patients with and without acute ischemic stroke," *Oncotarget*, vol. 8, no. 59, pp. 100384–100395, 2017.
- [2] Y. S. Jung, S. W. Lee, J. H. Park, H. B. Seo, B. T. Choi, and H. K. Shin, "Electroacupuncture preconditioning reduces ROS generation with NOX4 down-regulation and ameliorates blood-brain barrier disruption after ischemic stroke," *Journal of Biomedical Science*, vol. 23, no. 1, p. 32, 2016.
- [3] M. A. Moskowitz, E. H. Lo, and C. Iadecola, "The science of stroke: mechanisms in search of treatments," *Neuron*, vol. 67, no. 2, pp. 181–198, 2010.
- [4] K. B. Koronowski and M. A. Perez-Pinzon, "Sirt1 in cerebral ischemia," *Brain Circulation*, vol. 1, no. 1, pp. 69–78, 2015.
- [5] T. Jiang, L. Gao, J. Lu, and Y. D. Zhang, "ACE2-Ang-(1-7)-Mas axis in brain: a potential target for prevention and treatment of ischemic stroke," *Current Neuropharmacology*, vol. 11, no. 2, pp. 209–217, 2013.
- [6] G. Turc, P. Bhogal, U. Fischer et al., "European Stroke Organisation (ESO) - European Society for Minimally Invasive Neurological Therapy (ESMINT) guidelines on mechanical thrombectomy in acute ischemic stroke," *Journal of Neurointerventional Surgery*, vol. 11, no. 6, pp. 535–538, 2019.
- [7] W. Powers, A. Rabinstein, T. Ackerson et al., "2018 guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association," *Stroke*, vol. 49, no. 3, pp. e46–e110, 2018.
- [8] T. Leng and Z. G. Xiong, "Treatment for ischemic stroke: from thrombolysis to thrombectomy and remaining challenges," *Brain Circulation*, vol. 5, no. 1, pp. 8–11, 2019.
- [9] J. Baron, "Protecting the ischaemic penumbra as an adjunct to thrombectomy for acute stroke," *Nature Reviews Neurology*, vol. 14, no. 6, pp. 325–337, 2018.
- [10] B. K. Menon, F. S. Al-Ajlan, M. Najm et al., "Association of clinical, imaging, and thrombus characteristics with recanalization of visible intracranial occlusion in patients with acute ischemic stroke," *Journal of the American Medical Association*, vol. 320, no. 10, pp. 1017–1026, 2018.
- [11] H. Zhou, C. Yang, F. Bai et al., "Electroacupuncture alleviates brain damage through targeting of neuronal calcium sensor 1 by miR-191a-5p after ischemic stroke," *Rejuvenation Research*, vol. 20, no. 6, pp. 492–505, 2017.
- [12] M. Zhao, L. Guan, J. P. Collet, and Y. Wang, "Relationship between ischemic stroke locations, etiology subtypes, neurological outcomes, and autonomic cardiac function," *Neurological Research*, vol. 42, no. 8, pp. 630–639, 2020.
- [13] Q. Wang, N. Liu, Y. S. Ni et al., "TRPM2 in ischemic stroke: structure, molecular mechanisms, and drug intervention," *Channels*, vol. 15, no. 1, pp. 136–154, 2021.
- [14] N. Li, R. Wang, X. Ai et al., "Effect of electroacupuncture treatment on functional constipation in adults: a protocol for systematic review," *Medicine*, vol. 100, no. 10, article e24870, 2021.
- [15] Y. Xing, M. Zhang, W. Li, F. Dong, and F. Zhang, "Mechanisms involved in the neuroprotection of electroacupuncture therapy for ischemic stroke," *Frontiers in Neuroscience*, vol. 12, p. 929, 2018.
- [16] M. Wang, M. Zhang, Y. Feng et al., "Electroacupuncture inhibits neuronal autophagy and apoptosis via the PI3K/AKT pathway following ischemic stroke," *Frontiers in Cellular Neuroscience*, vol. 14, p. 134, 2020.
- [17] Y. Xing, M. Wang, Y. Feng, F. Dong, and F. Zhang, "Possible involvement of PTEN signaling pathway in the anti-apoptotic effect of electroacupuncture following ischemic stroke in rats," *Cellular and Molecular Neurobiology*, vol. 38, no. 8, pp. 1453–1463, 2018.
- [18] K. van Dijk, E. Scherder, P. Scheltens, and J. Sergeant, "Effects of transcutaneous electrical nerve stimulation (TENS) on non-pain related cognitive and behavioural functioning," *Reviews in the Neurosciences*, vol. 13, no. 3, pp. 257–270, 2002.
- [19] A. C. Merlocco, K. L. Redington, T. Disenhouse et al., "Transcutaneous electrical nerve stimulation as a novel method of remote preconditioning: in vitro validation in an animal model and first human observations," *Basic Research in Cardiology*, vol. 109, no. 3, p. 406, 2014.
- [20] K. A. Sluka and D. Walsh, "Transcutaneous electrical nerve stimulation: basic science mechanisms and clinical effectiveness," *The Journal of Pain*, vol. 4, no. 3, pp. 109–121, 2003.
- [21] P. Chen, T. W. Liu, P. W. H. Kwong et al., "Bilateral transcutaneous electrical nerve stimulation improves upper limb motor recovery in stroke: a randomized controlled trial," *Stroke*, vol. 53, no. 4, pp. 1134–1140, 2022.
- [22] S. Lin, Q. Sun, H. Wang, and G. Xie, "Influence of transcutaneous electrical nerve stimulation on spasticity, balance, and walking speed in stroke patients: a systematic review and meta-analysis," *Journal of Rehabilitation Medicine*, vol. 50, no. 1, pp. 3–7, 2018.
- [23] P. Zhang, G. Jiang, Q. Wang et al., "Effects of early acupuncture combined with rehabilitation training on limb function and nerve injury rehabilitation in elderly patients with stroke: based on a retrospective cohort study," *BioMed Research International*, vol. 2022, Article ID 8557936, 9 pages, 2022.
- [24] M. van Bloemendaal, S. Bus, F. Nollet, A. Geurts, and A. Beelen, "Feasibility and preliminary efficacy of gait training assisted by multichannel functional electrical stimulation in early stroke rehabilitation: a pilot randomized controlled trial," *Neurorehabilitation and Neural Repair*, vol. 35, no. 2, pp. 131–144, 2021.
- [25] N. Himi, H. Takahashi, N. Okabe et al., "Exercise in the early stage after stroke enhances hippocampal brain-derived neurotrophic factor expression and memory function recovery,"

- Journal of Stroke and Cerebrovascular Diseases : The Official Journal of National Stroke Association*, vol. 25, no. 12, pp. 2987–2994, 2016.
- [26] P. Zhang, H. Yu, N. Zhou et al., “Early exercise improves cerebral blood flow through increased angiogenesis in experimental stroke rat model,” *Journal of Neuroengineering and Rehabilitation*, vol. 10, no. 1, 2013.
- [27] L. Chen, Z. Han, and J. Gu, “Early path nursing on neurological function recovery of cerebral infarction,” *Translational Neuroscience*, vol. 10, no. 1, pp. 160–163, 2019.
- [28] J. Liao, X. Xia, G. Wang, Y. Shi, and J. Ge, “Naotaifang extract treatment results in increased ferroportin expression in the hippocampus of rats subjected to cerebral ischemia,” *Molecular Medicine Reports*, vol. 11, no. 6, pp. 4047–4052, 2015.
- [29] K. Hou, G. Li, J. Yu, K. Xu, and W. Wu, “Receptors, channel proteins, and enzymes involved in microglia-mediated neuroinflammation and treatments by targeting microglia in ischemic stroke,” *Neuroscience*, vol. 460, pp. 167–180, 2021.
- [30] W. Li and S. Yang, “Targeting oxidative stress for the treatment of ischemic stroke: upstream and downstream therapeutic strategies,” *Brain Circulation*, vol. 2, no. 4, pp. 153–163, 2016.
- [31] X. Su, L. Wang, S. Ma et al., “Mechanisms of acupuncture in the regulation of oxidative stress in treating ischemic stroke,” *Oxidative Medicine and Cellular Longevity*, vol. 2020, Article ID 7875396, 15 pages, 2020.
- [32] S. Nasoohi, S. Ismael, and T. Ishrat, “Thioredoxin-interacting protein (TXNIP) in cerebrovascular and neurodegenerative diseases: regulation and implication,” *Molecular Neurobiology*, vol. 55, no. 10, pp. 7900–7920, 2018.
- [33] C. Li, M. A. Thompson, A. T. Tamayo et al., “Over-expression of thioredoxin-1 mediates growth, survival, and chemoresistance and is a druggable target in diffuse large B-cell lymphoma,” *Oncotarget*, vol. 3, no. 3, pp. 314–326, 2012.
- [34] E. Moon, J. E. Han, S. Jeon, J. H. Ryu, J. W. Choi, and J. Chun, “Exogenous S1P exposure potentiates ischemic stroke damage that is reduced possibly by inhibiting S1P receptor signaling,” *Mediators of Inflammation*, vol. 2015, Article ID 492659, 12 pages, 2015.
- [35] Y. Feng, Z. Tan, M. Wang, Y. Xing, F. Dong, and F. Zhang, “Inhibition of NLRP3 inflammasome: a prospective target for the treatment of ischemic stroke,” *Frontiers in Cellular Neuroscience*, vol. 14, p. 155, 2020.
- [36] N. Kim, H. Kim, J. Lee et al., “Juglone suppresses LPS-induced inflammatory responses and NLRP3 activation in macrophages,” *Molecules*, vol. 25, no. 13, p. 3104, 2020.
- [37] Y. S. Feng, Z. X. Tan, L. Y. Wu, F. Dong, and F. Zhang, “The involvement of NLRP3 inflammasome in the treatment of Alzheimer’s disease,” *Ageing Research Reviews*, vol. 64, article 101192, 2020.
- [38] N. Deroide, X. Li, D. Lerouet et al., “MFGE8 inhibits inflammasome-induced IL-1 β production and limits post-ischemic cerebral injury,” *Journal of Clinical Investigation*, vol. 123, no. 3, pp. 1176–1181, 2013.
- [39] B. F. Py, M. S. Kim, H. Vakifahmetoglu-Norberg, and J. Yuan, “Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity,” *Molecular Cell*, vol. 49, no. 2, pp. 331–338, 2013.
- [40] B. Levine and G. Kroemer, “Autophagy in the pathogenesis of disease,” *Cell*, vol. 132, no. 1, pp. 27–42, 2008.
- [41] F. M. Menzies, A. Fleming, and D. C. Rubinsztein, “Compromised autophagy and neurodegenerative diseases,” *Nature Reviews Neuroscience*, vol. 16, no. 6, pp. 345–357, 2015.
- [42] R. J. Youle and D. P. Narendra, “Mechanisms of mitophagy,” *Nature Reviews Molecular Cell Biology*, vol. 12, no. 1, pp. 9–14, 2011.
- [43] H. Wei, L. Liu, and Q. Chen, “Selective removal of mitochondria via mitophagy: distinct pathways for different mitochondrial stresses,” *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1853, no. 10, pp. 2784–2790, 2015.
- [44] H. Wang, S. Chen, Y. Zhang, H. Xu, and H. Sun, “Electroacupuncture ameliorates neuronal injury by Pink1/Parkin-mediated mitophagy clearance in cerebral ischemia-reperfusion,” *Nitric Oxide*, vol. 91, pp. 23–34, 2019.
- [45] W. Wang and J. Xu, “Curcumin attenuates cerebral ischemia-reperfusion injury through regulating mitophagy and preserving mitochondrial function,” *Current Neurovascular Research*, vol. 17, no. 2, pp. 113–122, 2020.
- [46] Y. Di, Y.-L. He, T. Zhao et al., “Methylene blue reduces acute cerebral ischemic injury via the induction of mitophagy,” *Molecular Medicine*, vol. 21, no. 1, pp. 420–429, 2015.
- [47] J. L. Chen, X. X. Wang, L. Chen et al., “A sphingosine kinase 2-mimicking TAT-peptide protects neurons against ischemia-reperfusion injury by activating BNIP3-mediated mitophagy,” *Neuropharmacology*, vol. 181, article 108326, 2020.
- [48] G. L. Semenza, “HIF-1: mediator of physiological and pathophysiological responses to hypoxia,” *Journal of Applied Physiology*, vol. 88, no. 4, pp. 1474–1480, 2000.
- [49] R. Guan, W. Zou, X. Dai et al., “Mitophagy, a potential therapeutic target for stroke,” *Journal of Biomedical Science*, vol. 2015, no. 1, p. 87, 2018.
- [50] A. H. Chourasia and K. F. Macleod, “Tumor suppressor functions of BNIP3 and mitophagy,” *Autophagy*, vol. 11, no. 10, pp. 1937–1938, 2015.
- [51] K. Xu, C. Lu, X. Ren, J. Wang, P. Xu, and Y. Zhang, “Overexpression of HIF-1 α enhances the protective effect of mitophagy on steroid-induced osteocytes apoptosis,” *Environmental Toxicology*, vol. 36, no. 11, pp. 2123–2137, 2021.
- [52] R. Liu, N. G. Xu, W. Yi, and C. Ji, “Electroacupuncture attenuates inflammation after ischemic stroke by inhibiting NF- κ B-Mediated activation of microglia,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2020, Article ID 8163052, 12 pages, 2020.
- [53] H. Xu, Y. Wang, and Y. Luo, “OTULIN is a new target of EA treatment in the alleviation of brain injury and glial cell activation via suppression of the NF- κ B signalling pathway in acute ischaemic stroke rats,” *Molecular Medicine*, vol. 27, no. 1, p. 37, 2021.
- [54] X. Lin, J. Zhan, J. Jiang, and Y. Ren, “Upregulation of neuronal cyclin D1 expression is essential for electroacupuncture-mediated alleviation of neuroinflammatory injury by regulating microglial polarization in rats subjected to focal cerebral ischemia/reperfusion,” *Journal of Inflammation Research*, vol. 14, pp. 2061–2078, 2021.
- [55] J. Zhan, W. Qin, Y. Zhang et al., “Upregulation of neuronal zinc finger protein A20 expression is required for electroacupuncture to attenuate the cerebral inflammatory injury mediated by the nuclear factor- κ B signaling pathway in cerebral ischemia/reperfusion rats,” *Journal of Neuroinflammation*, vol. 13, no. 1, p. 258, 2016.

- [56] C. Xie, X. Gao, Y. Luo, Y. Pang, and M. Li, "Electroacupuncture modulates stromal cell-derived factor-1 α expression and mobilization of bone marrow endothelial progenitor cells in focal cerebral ischemia/reperfusion model rats," *Brain Research*, vol. 1648, Part A, pp. 119–126, 2016.
- [57] P. Wu and S. Liu, "Clinical observation on post-stroke anxiety neurosis treated by acupuncture," *Journal of Traditional Chinese Medicine*, vol. 28, no. 3, pp. 186–188, 2008.
- [58] H. Huang, S. Yang, Z. Mei et al., "Efficacy and safety of electroacupuncture combined with Suanzaoren decoction for insomnia following stroke: study protocol for a randomized controlled trial," *Trials*, vol. 22, no. 1, p. 485, 2021.
- [59] W. Cai, W. Ma, Y. Li, G. Wang, H. Yang, and W. Shen, "Efficacy and safety of electroacupuncture for post-stroke depression: a randomized controlled trial," *Acupuncture in Medicine*, vol. 40, no. 5, pp. 434–442, 2022.
- [60] Y. Xing, M. Zhang, M. M. Wang, Y. S. Feng, F. Dong, and F. Zhang, "The anti-apoptosis effect of single electroacupuncture treatment via suppressing neuronal autophagy in the acute stage of ischemic stroke without infarct alleviation," *Frontiers in Cellular Neuroscience*, vol. 15, 2021.
- [61] R. Kuts, D. Frank, B. F. Gruenbaum et al., "A novel method for assessing cerebral edema, infarcted zone and blood-brain barrier breakdown in a single post-stroke rodent brain," *Frontiers in Neuroscience*, vol. 13, p. 1105, 2019.
- [62] J. Yuan, G. L. Shan, S. D. Li, C. P. Gao, L. Y. Cui, and B. Peng, "Risk factors influencing seeking emergency medical service in urban and rural China among participants with a previous transient ischemic attack," *Frontiers in Neurology*, vol. 11, article 620157, 2021.
- [63] J. Li, B. Li, F. Zhang, and Y. Sun, "Urban and rural stroke mortality rates in China between 1988 and 2013: an age-period-cohort analysis," *The Journal of International Medical Research*, vol. 45, no. 2, pp. 680–690, 2017.
- [64] L. M. Chavez, S. S. Huang, I. MacDonald, J. G. Lin, Y. C. Lee, and Y. H. Chen, "Mechanisms of acupuncture therapy in ischemic stroke rehabilitation: a literature review of basic studies," *International Journal of Molecular Sciences*, vol. 18, no. 11, p. 2270, 2017.
- [65] Z. Guo, Y. Liu, G. Hu, H. Liu, and Y. Xu, "Transcutaneous electrical nerve stimulation in the treatment of patients with poststroke urinary incontinence," *Clinical Interventions in Aging*, vol. 9, pp. 851–856, 2014.
- [66] P. Kwong, G. Ng, R. Chung, and S. Ng, "Bilateral transcutaneous electrical nerve stimulation improves lower-limb motor function in subjects with chronic stroke: a randomized controlled trial," *Journal of the American Heart Association*, vol. 7, no. 4, 2018.
- [67] A. Mahmood, S. Veluswamy, A. Hombali, A. Mullick, N. Manikandan, and J. M. Solomon, "Effect of transcutaneous electrical nerve stimulation on spasticity in adults with stroke: a systematic review and meta-analysis," *Archives of Physical Medicine and Rehabilitation*, vol. 100, no. 4, pp. 751–768, 2019.
- [68] M. Zhou, F. Li, W. Lu, J. Wu, and S. Pei, "Efficiency of neuromuscular electrical stimulation and transcutaneous nerve stimulation on hemiplegic shoulder pain: a randomized controlled trial," *Archives of Physical Medicine and Rehabilitation*, vol. 99, no. 9, pp. 1730–1739, 2018.
- [69] K. Kita, Y. Otaka, K. Takeda et al., "A pilot study of sensory feedback by transcutaneous electrical nerve stimulation to improve manipulation deficit caused by severe sensory loss after stroke," *Journal of Neuroengineering and Rehabilitation*, vol. 10, no. 1, 2013.
- [70] Z. Liu, Y. Ge, F. Xu et al., "Preventive effects of transcutaneous electrical acustimulation on ischemic stroke-induced constipation mediated via the autonomic pathway," *American Journal of Physiology - Gastrointestinal and Liver Physiology*, vol. 315, no. 2, pp. G293–G301, 2018.
- [71] E. Verin, D. Maltete, Y. Ouahchi et al., "Stimulation électrique trans-cutanée sensitive sous-mentonnière à domicile dans les dysphagies oropharyngées neurologiques : étude pilote," *Annals of Physical and Rehabilitation Medicine*, vol. 54, no. 6, pp. 366–375, 2011.
- [72] D. Jin, Y. Xu, D. Geng, and T. Yan, "Effect of transcutaneous electrical nerve stimulation on symptomatic diabetic peripheral neuropathy: a meta-analysis of randomized controlled trials," *Diabetes Research and Clinical Practice*, vol. 89, no. 1, pp. 10–15, 2010.
- [73] G. Gossrau, M. Wähner, M. Kuschke et al., "Microcurrent transcutaneous electric nerve stimulation in painful diabetic neuropathy: a randomized placebo-controlled study," *Pain Medicine*, vol. 12, no. 6, pp. 953–960, 2011.
- [74] M. Long, Z. Wang, D. Zheng et al., "Electroacupuncture pretreatment elicits neuroprotection against cerebral ischemia-reperfusion injury in rats associated with transient receptor potential vanilloid 1-mediated anti-oxidant stress and anti-inflammation," *Inflammation*, vol. 42, no. 5, pp. 1777–1787, 2019.
- [75] S. Ismael, S. Nasoohi, A. Yoo, H. A. Ahmed, and T. Ishrat, "Tissue plasminogen activator promotes TXNIP-NLRP3 inflammasome activation after hyperglycemic stroke in mice," *Molecular Neurobiology*, vol. 57, no. 6, pp. 2495–2508, 2020.
- [76] S. J. Schimmel, S. Acosta, and D. Lozano, "Neuroinflammation in traumatic brain injury: a chronic response to an acute injury," *Brain Circulation*, vol. 3, no. 3, pp. 135–142, 2017.
- [77] Y. Zhu, E. A. Klomparens, S. Guo, and X. Geng, "Neuroinflammation caused by mental stress: the effect of chronic restraint stress and acute repeated social defeat stress in mice," *Neurological Research*, vol. 41, no. 8, pp. 762–769, 2019.
- [78] H. Wang, J. Zhu, L. Jiang et al., "Mechanism of Heshouwuyin inhibiting the Cyt c/Apaf-1/Caspase-9/Caspase-3 pathway in spermatogenic cell apoptosis," *BMC Complementary Medicine and Therapies*, vol. 20, no. 1, p. 180, 2020.
- [79] C. Jia, J. Zhang, H. Chen et al., "Endothelial cell pyroptosis plays an important role in Kawasaki disease via HMGB1/RAGE/cathepsin B signaling pathway and NLRP3 inflammasome activation," *Cell Death & Disease*, vol. 10, no. 10, p. 778, 2019.
- [80] N. M. de Vasconcelos and M. Lamkanfi, "Recent insights on inflammasomes, gasdermin pores, and pyroptosis," *Cold Spring Harbor Perspectives in Biology*, vol. 12, no. 5, 2020.
- [81] T. Jiang, M. Wu, Z. Zhang et al., "Electroacupuncture attenuated cerebral ischemic injury and neuroinflammation through $\alpha 7$ nAChR-mediated inhibition of NLRP3 inflammasome in stroke rats," *Molecular Medicine*, vol. 25, no. 1, p. 22, 2019.
- [82] K. Palikaras and N. Tavernarakis, "Mitochondrial homeostasis: the interplay between mitophagy and mitochondrial biogenesis," *Experimental Gerontology*, vol. 56, pp. 182–188, 2014.
- [83] Q. Li, T. Zhang, J. Wang et al., "Rapamycin attenuates mitochondrial dysfunction via activation of mitophagy in experimental ischemic stroke," *Biochemical and Biophysical Research Communications*, vol. 444, no. 2, pp. 182–188, 2014.

- [84] X. Zhong, B. Chen, Z. Li et al., "Electroacupuncture ameliorates cognitive impairment through the inhibition of NLRP3 Inflammasome activation by regulating melatonin-mediated mitophagy in stroke rats," *Neurochemical Research*, vol. 47, no. 7, pp. 1917–1930, 2022.
- [85] E. Conde, L. Alegre, I. Blanco-Sánchez et al., "Hypoxia inducible factor 1-alpha (HIF-1 alpha) is induced during reperfusion after renal ischemia and is critical for proximal tubule cell survival," *PLoS One*, vol. 7, no. 3, article e33258, 2012.
- [86] Z. Zhang, B. Haimovich, Y. S. Kwon, T. Lu, B. Fyfe-Kirschner, and E. O. Olweny, "Unilateral partial nephrectomy with warm ischemia results in acute hypoxia inducible factor 1-alpha (HIF-1 α) and toll-like receptor 4 (TLR4) overexpression in a porcine model," *PLoS One*, vol. 11, no. 5, article e0154708, 2016.
- [87] M. Föhling, S. Mathia, A. Paliege et al., "Tubular von Hippel-Lindau knockout protects against rhabdomyolysis-induced AKI," *Journal of the American Society of Nephrology: JASN*, vol. 24, no. 11, pp. 1806–1819, 2013.
- [88] F. J. Liu, P. Kaur, D. S. Karolina et al., "miR-335 regulates Hif-1 α to reduce cell death in both mouse cell line and rat ischemic models," *PLoS One*, vol. 10, no. 6, article e0128432, 2015.
- [89] Q. Lin, S. Li, N. Jiang et al., "Inhibiting NLRP3 inflammasome attenuates apoptosis in contrast-induced acute kidney injury through the upregulation of HIF1A and BNIP3-mediated mitophagy," *Autophagy*, vol. 17, no. 10, pp. 2975–2990, 2021.