Electroacupuncture reduces corpus callosum injury in rats with permanent cerebral ischemia by inhibiting the activation of high-mobility group box 1 protein and the receptor for advanced glycation end products

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Previous studies have shown that cerebral ischemia can cause white matter injury in the brain. This study aimed to investigate the potential mechanism of electroacupuncture (EA) at the Baihui (GV20) and Zusanli (ST36) acupoints in protecting white matter. Sprague–Dawley rats were used to establish permanent middle cerebral artery occlusion (pMCAO) rat models. Comprehensive motor functions were assessed using the mesh experiment. Morphological changes in the myelin sheath were assessed with Luxol fast blue staining. Morphological changes in oligodendrocytes and myelinated axons were evaluated using Nissl staining. The expressions of high-mobility group box 1 protein (HMGB1) and the receptor for advanced glycation end products (RAGE) in the corpus callosum were detected by immunohistochemical staining and Western blot analysis. pMCAO caused severe injury to the corpus callosum, evidenced by significant loss of white matter fibers and myelinated axons, and induced overexpression of HMGB1 and RAGE in the corpus callosum. EA treatment significantly improved comprehensive motor function alleviated white matter damage, and downregulated the expression of HMGB1 and RAGE. Its effects were comparable to those of FPS-ZM1, a RAGE receptor inhibitor. In conclusion, EA effectively improves comprehensive motor function in rats with cerebral infarction and alleviates corpus callosum injury. This effect may be related to the inhibition of HMGB1 and RAGE overexpression. *NeuroReport* **35: 963–971**

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Keywords: cerebral ischemia, corpus callosum, electroacupuncture, high-mobility group box 1 protein, the receptor of advanced glycation end products

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Introduction

Ischemic stroke has a high disability and mortality rate and is often accompanied by white matter lesions (WMLs), including corpus callosum injury caused by acute ischemia [1]. The main pathological changes in corpus callosum injury are the death of oligodendrocytes and loss of myelin sheaths, which may lead to neurological deficits such as motor disorders, sensory, and cognitive changes [2]. Previous studies have suggested that the activation of high-mobility group box protein B 1 protein (HMGB1) and receptor for advanced glycation end products (RAGE) may be involved in neuronal damage induced by cerebral ischemia [3,4]. HMGB1 is a late proinflammatory factor that induces neuroinflammation and cognitive impairments by directly binding to RAGE [5]. It is, however, currently obscure whether HMGB1 and its receptor RAGE are involved in white matter damage induced by cerebral ischemia.

The treatment of ischemic brain injury remains a challenge, and traditional thrombolytic therapy is limited by the time window and difficult to improve the prognosis. Although FPS-ZM1 is an efficient RAGE inhibitor that can provide neuroprotection in middle cerebral artery occlusion (MCAO) rats [6], this potent RAGE antagonist shows great potential in treating various inflammatory conditions but has not been reported in clinical trials [7]. Electroacupuncture (EA) has been proven to be an effective and well-established method in the treatment of cerebral ischemia, with potential mechanisms related to anti-inflammatory responses [8,9]. In addition, acupoint EA, namely, needling at Baihui (GV20) and Zusanli (ST36) acupoints, also has a certain therapeutic effect on secondary hypothalamic injury after permanent middle cerebral artery occlusion (pMCAO). Preliminary studies by our group have shown that EA at GV20 and ST36 acupoints can inhibit the overexpression of HMGB1 and RAGE in the cortex M1 area and striatum of pMCAO rats, alleviate neuronal damage, and improve neurological dysfunction [3,4]. Yet it is unclear whether EA can also regulate the abnormal expression of HMGB1 and RAGE in

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the brain white matter and exert protective effects on ischemic white matter injury.

The purpose of this study is to explore the potential mechanism of EA's neuroprotective effect on the corpus callosum of pMCAO rats. This study focuses on exploring the role of HMGB1 and RAGE, aiming to provide experimental evidence for EA treatment of ischemic white matter injury.

Materials and methods

Animals

A total of 72 male adult Sprague–Dawley rats (6–8 weeks old; 230 ± 20 g) were purchased from Nanjing Animal Center of Qinglongshan [permit no. SCXK (Zhejiang, China) 2019-0002] and kept in a specific pathogen-free animal laboratory with a temperature of 25 ± 1 °C, humidity of 70%, and a 12-h light-dark cycle. The rats had free access to food and water. After 1 week, the rats were randomly divided into the normal group (N group), sham surgery group (S group), model group (M group, received pMCAO surgery), electroacupuncture group (EA group, received pMCAO surgery and electroacupuncture treatment), FPS-ZM1 inhibitor group (MF group, received pMCA surgery and inhibitor treatment), and sham electroacupuncture group (SEA group, received pMCAO surgery, connected to the electroacupuncture device but without electric stimulation), with 12 rats in each group. Rats that died during the experiment or did not meet the inclusion criteria were excluded, and the number of rats was replenished through the same steps. This experiment was conducted under the guidance of the Animal Care and Use Committee of Wannan Medical College (permission no. LLSC-2021–025). Our utmost effort was made to minimize the pain of the rats throughout the experiment.

Establishment of the permanent middle cerebral artery occlusion model

The pMCAO rat model used in this study was established based on the study by Ma *et al*. [10]. Briefly, the rats were fixed on an animal surgery table in a supine position. Prior to the surgery, anesthesia was induced by intraperitoneal injection of 30 mg/kg pentobarbital sodium. A midline incision was made on the neck skin to expose the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA). The CCA and ECA were ligated, and the ICA was clamped near its proximal end. Then, a nylon filament for MCAO was gently inserted into the ICA and advanced along the ICA toward the intracranial region to block the blood flow in the right middle cerebral artery. The filament was inserted to a length of approximately 18 mm until a slight resistance was felt. Afterward, the ICA was ligated and the filament was fixed. The incision was disinfected with iodophor cotton balls and sutured routinely. The rats were screened using the modified Longa scoring method, and only rats with successful modeling were used in the experiment. In group S, rats were anesthetized with an equal dose of pentobarbital sodium. The surgery involved only the separation of the same artery, followed by a suture of the incision and the same disinfection treatment. While N group rats did not perform any operations.

Intervention methods

The methodology of EA treatment is based on the study by Ma *et al*. [10]. In brief, the rats in the EA group started receiving acupuncture stimulation 24 h after successful modeling. The Baihui acupoint (GV20, 2 mm obliquely upward from the midpoint of the line connecting the two ears) and the Zusanli acupoint (ST36, located 5 mm below the head of the fibula, directly inserted to a depth of 7 mm) on the left side of the rats were selected as the treatment points. EA was delivered with a frequency of 2 Hz/10 Hz alternating waves, an intensity of 1 mA, for a duration of 30 min, once daily for 14 consecutive days. In the SEA group, the rats received shallow acupuncture at GV20 and ST36 without electrical stimulation. In the MF group, the rats received intraperitoneal injections of FPS-ZM1 (dose: 1 mg/kg) once daily for 14 consecutive days. The remaining groups did not receive the above interventions.

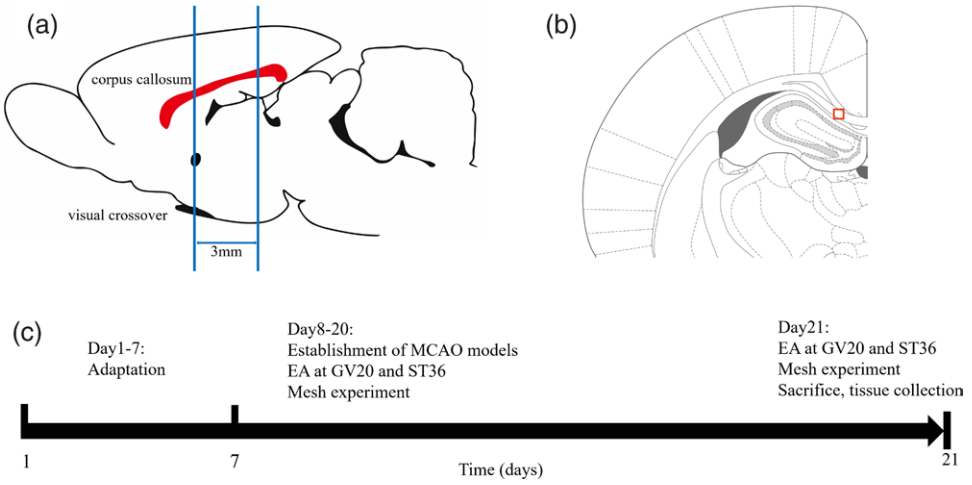
Mesh experiment test

A grid test was performed on the first and 14th day after modeling to evaluate the comprehensive motor ability of rats, including muscle strength, muscle tension, and balance [11]. The grid test apparatus consisted of a 50 cm × 40 cm grid divided into 1 cm × 1 cm squares by wooden strips. The apparatus was placed 80 cm above the ground and a sponge cushion was placed on the ground underneath. The rats were placed on the horizontal grid and within 2 s, the grid was slowly rotated 90 degrees to a vertical position and held for 5 s. The duration of the rats staying on the grid was observed and scored. The scoring criteria were as follows: five points for rats that could firmly grip the grid and climb upwards; four points for rats that could grip the grid with their limbs and stay on the grid for 5 s; three points for rats that could temporarily grip the grid but would slide down a certain distance; two points for rats that fell off the grid within 5 s; one point for rats that immediately fell off when the grid became vertical.

Specimen collection

After EA treatment (Fig. 1a), six rats from each group were euthanized by intraperitoneal injection of 45 mg/kg sodium pentobarbital, followed by rapid intravenous infusion of 150 ml of 0.9% saline and perfusion fixation with 300 ml of ice-cold fixative stored in a refrigerator at 4 °C through the left ventricle. The brains were then harvested. The brain tissue from the desired section

Fig. 1



A schematic diagram of the experimental design. (c) Experimental design timeline. (a) Localization of the corpus callosum for sampling. (b) Observation and photography area of the corpus callosum histopathology and immunohistochemistry results marked by the red box.

(between 8.28 mm and 5.28 mm interaural for sampling position, visually confirmed as 3 mm behind the midpoint of the optic chiasm) was dissected according to the stereotaxic rat brain atlas. The dissected tissue was then embedded in paraffin. Coronal sections of 5 μm thickness were cut from the paraffin-embedded brain tissue. The sections were divided into four sets for Luxol fast blue (LFB) staining, Nissl staining, and immunohistochemistry staining (HMGB1 and RAGE). Additionally, six rats from each group were euthanized by intraperitoneal injection of the same dose of sodium pentobarbital, and their brains were immediately harvested. The brain tissue from the desired section was dissected from the paraffin-embedded blocks and the corpus callosum tissue was isolated and stored at –80 °C for the Western blot experiment.

Luxol fast blue staining

According to the instructions of the LFB staining kit (DK0009, Leagene, Beijing, China), staining was performed on the sliced sections following the steps, and the slides were sealed with neutral gum. Representative images of the right corpus callosum LFB staining were obtained using an Olympus microscope. According to a previous study [12], the grading criteria for the severity of WMLs were as follows: grade 0, normal; grade 1, neurofiber disorder; grade 2, formation of labeled vesicles; grade 3, disappearance of myelinated fibers.

Nissl staining

The paraffin sections containing the corpus callosum tissue were dewaxed in xylene, dehydrated in graded ethanol, and then stained following the steps of the Nissl staining kit (DK0022, Leagene, China). After coverslipping with neutral gum, the sections were observed under a microscope and photographed to examine the changes in oligodendrocytes in the corpus callosum region. Three coronal sections of the corpus callosum were taken from each rat. The image J software (Media Cybernetics, Rockville, Maryland, USA) was used to count the Nissl-positive cells and the average count of the three sections from each rat was used for statistical analysis.

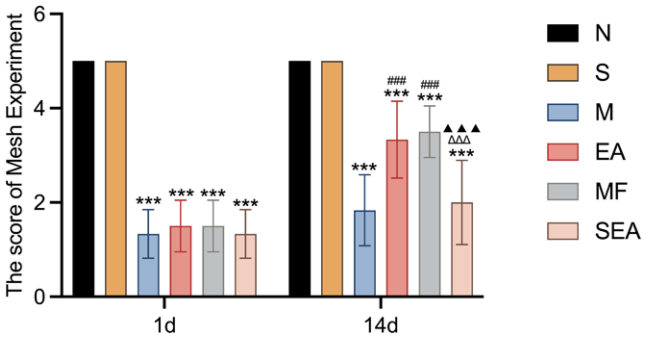
Immunohistochemistry staining

The paraffin sections containing the corpus callosum tissue were dewaxed in xylene and dehydrated in graded ethanol. The sections were treated with Triton-100 for 30 min and then incubated in 3% H_2O_2 to block endogenous peroxidase activity. The sections were then incubated in a citrate buffer solution and blocked with 5% BSA (AR0004, Boster, Hubei, China) for 30 min. Subsequently, the sections were incubated overnight at 4 °C with rabbit anti-HMGB1 antibody (1 : 200, ab79823, Abcam, UK) or anti-RAGE antibody (1 : 20, ab216329, Abcam, UK). After washing with PBS three times, the sections were incubated with goat antirabbit secondary antibody for 30 min. Finally, the sections were stained with DAB chromogen, sealed with neutral gum, and observed and photographed under a microscope to examine the right corpus callosum region. Three sections of the corpus callosum were taken from the same part of each rat (Fig. 1a,b). The average optical density values of three slices from each rat were used for statistical analysis.

Western blot test

The corpus callosum tissue was taken from the –80 °C freezer, homogenized in protein lysis buffer, and centrifuged, and the supernatant was collected and stored at –80 °C. Equal amounts of protein were loaded onto SDS-PAGE gels for electrophoresis. After electrophoresis, the proteins were transferred to p olyvinylidene fluoride (FFP32; Beyotime, Shanghai, China) membranes,

Fig. 2



Beneficial effect of EA on the comprehensive motor ability of pMCAO rats. The neurological function scores of the rats in each group ($n = 12$) on first day and 14th day after modeling were compared and analyzed. N represents the normal group. S represents the sham group. M represents the pMCAO group. EA represents the pMCAO + electroacupuncture group. MF represents the pMCAO + FPS-ZM1 group. SEA represents the pMCAO + sham electroacupuncture group. ***$P < 0.0001$ indicates significant differences compared to the N group, ###$P < 0.0001$ vs the M group; ΔΔΔ$P < 0.0001$ vs the EA group, and ▲▲▲$P < 0.0001$ vs the MF group. EA, electroacupuncture; pMCAO, permanent middle cerebral artery occlusion.

placed in blocking solution for 2 h, and then were incubated overnight (12–15 h) in the icebox on the rocker. The primary antibodies used were anti-HMGB1 (1 : 1000, ab79823, Abcam, UK), RAGE (1 : 1000 and ab216329, Abcam, UK), and β-tubulin (1 : 1000, AF7011, Affinity, China). After incubation with the primary antibodies, the strips were placed in goat antirabbit secondary antibodies and incubated at room temperature for 1 h. Then, chemiluminescent detection was used to visualize the protein expression on the strips and the pictures were taken by GE Amersham Imager600 and the grayscale values of the target protein bands were analyzed using Image J software. The relative expression levels of the protein HMGB1 and RAGE were calculated using β-tubulin as an internal reference.

Statistical analysis

All data were analyzed using SPSS 26.0 software (IBM SPSS Inc.,Chicago, Illinois, USA). The figures were mapped with GraphPad Prism 9.0 software (GraphPad, Boston, Massachusetts, USA). The mean ± SD was employed for the measurement data conforming to a normal distribution. One-way analysis of variance (one-way ANOVA) was used to compare the data between multiple groups and Tukey's multiple comparisons test was used for two-by-two comparisons between groups. Least significant difference test was used to compare data between two groups. A P value <0.05 was considered statistically significant.

Results

Electroacupuncture alleviates comprehensive motor ability of permanent middle cerebral artery occlusion rats

The grid-walking test was used to evaluate the comprehensive motor ability of rats. On the first day of the experiment, the Mesh scores of rats in the N group and S group were five, while the Mesh scores of the other four groups were significantly lower than the N group. Furthermore, on the 14th day, the scores of the other four groups were still lower than the N group, but the EA group and MF group had significantly higher scores than the M group and SEA group (Fig. 2). This indicates that EA treatment for 2 weeks significantly improves the comprehensive motor ability of rats, and its effect is comparable to the inhibitor FPS-ZM1, while SEA does not.

Electroacupuncture alleviates corpus callosum damage caused by permanent middle cerebral artery occlusion

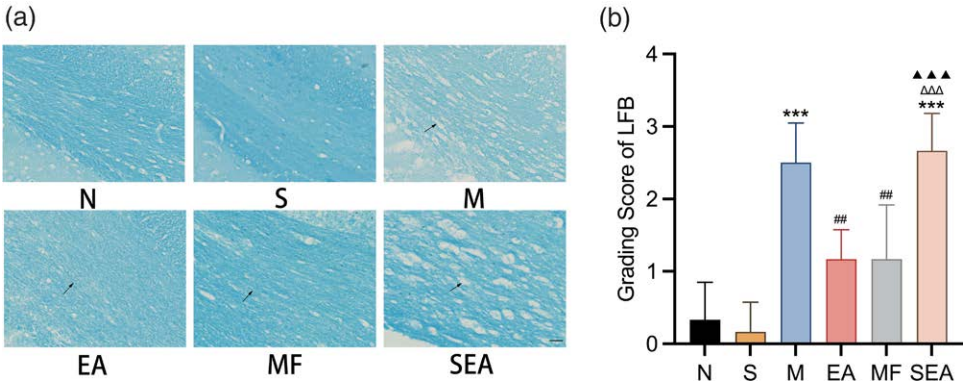
LFB staining was used to observe the morphological changes of myelin in the corpus callosum. Myelin phospholipids are abundant in the brain white matter. Damage to the myelin sheath may lead to structural and functional impairment of the brain white matter. In Fig. 3, the results showed that the myelin fibers in the corpus callosum of rats in the N group and S group were arranged neatly and densely. In contrast, the structure of the myelin sheath in the corpus callosum of rats in the M group was very loose, with vacuoles appearing. The LFB scores of the M group and SEA group were significantly higher than those of the N group. Compared with the M group, the myelin sheath structure in the corpus callosum of rats in the EA group was more compact, consistent with the performance of the MF group, and the LFB scores were significantly decreased.

Nissl staining was displayed to observe the changes in oligodendrocytes in the corpus callosum. The normal number and structure of oligodendrocytes are fundamental for normal neuronal function. As shown in Fig. 4a, the distribution of oligodendrocytes in the N group was dense and uniform, with a clear and intact structure. There was no statistically significant difference in the Nissl-positive cell count between the N group and the S group. Nevertheless, the number of Nissl-positive cells in the M group and SEA group was obviously lower than that in the N group, and the cells were dispersed and showed many vacuoles. It is worth noting that after EA or treatment with the inhibitor FPS-ZM1, the number of oligodendrocytes in the brain tissue increased and the vacuoles decreased enormously (Fig. 4).

Electroacupuncture inhibits the activation of high-mobility group box 1 protein and the receptor for advanced glycation end products in corpus callosum induced by permanent middle cerebral artery occlusion

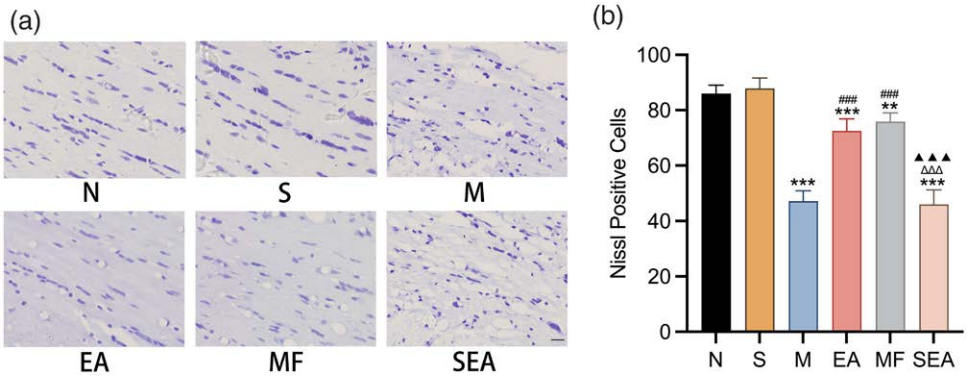
Immunohistochemical staining was used to detect the localization and expression of HMGB1 and RAGE protein in the corpus callosum. Under the microscope, the positive reaction products of HMGB1 and RAGE protein appeared brownish-yellow. As shown in Fig. 5,

Fig. 3



Effect of EA on the myelin sheath in the corpus callosum of pMCAO rats. (a) Representative microscopic photos (magnification: ×400, scale bar: 50 μm) of LFB staining in the corpus callosum of the rats in each group. The arrow points to the myelin sheath. (b) Comparison of LFB scores among the groups ($n = 6$). ***$P < 0.0001$ vs the N group, ##$P = 0.0021$ vs the M group, ΔΔΔ$P < 0.0001$ vs the EA group, and ▲▲▲$P < 0.0001$ vs the MF group. EA, electroacupuncture; pMCAO, permanent middle cerebral artery occlusion.

Fig. 4



Effect of EA on myelin in pMCAO rats. (a) Representative microphotographs of LFB staining in the corpus callosum of rats in each group (magnification: ×400, scale bar: 50μm). (b) Comparison of LFB scores among groups ($n = 6$). **$P < 0.0015$, ***$P < 0.0001$ vs the N group, ###$P = 0.0021$ vs the M group, ΔΔΔ$P < 0.0001$ vs the EA group, ▲▲▲$P < 0.0001$ vs the MF group. EA, electroacupuncture; LFB, Luxol fast blue; pMCAO, permanent middle cerebral artery occlusion.

there was no significant difference in the optical density values of HMGB1 and RAGE between N group and S group. Compared to N group, however, the expression of HMGB1 and RAGE was extremely increased in M group and SEA group. Conversely, in rats from EA group and MF group, the expression of HMGB1 and RAGE was dramatically decreased.

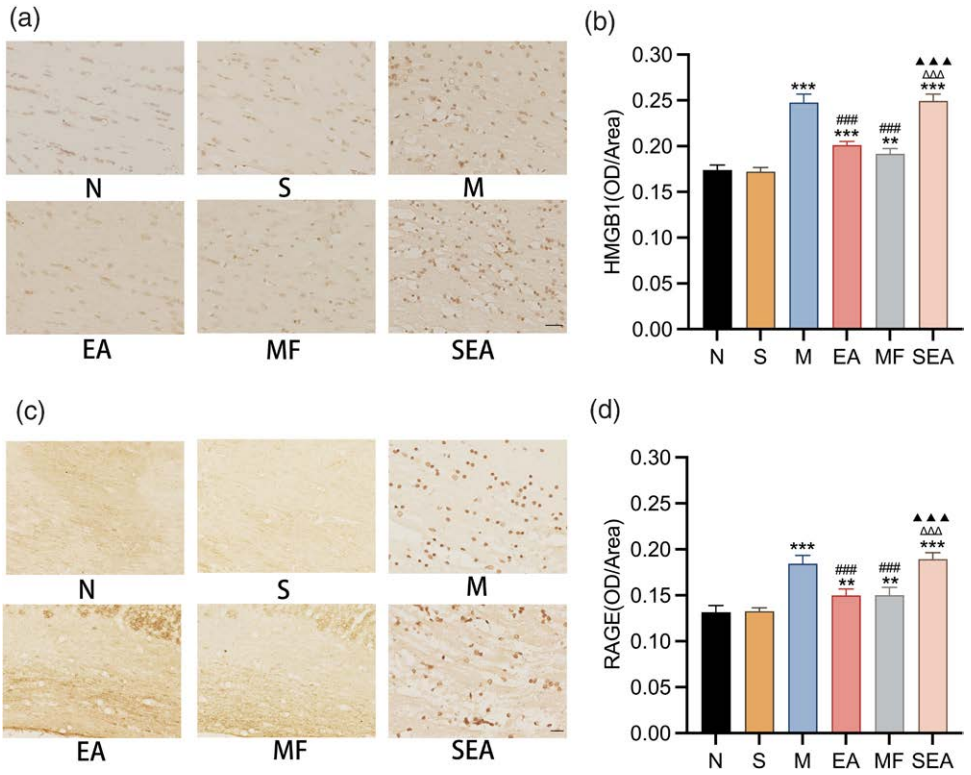
Similarly, Western blot results showed that the expression levels of HMGB1 and RAGE in the corpus callosum of N group and S group were relatively low, with no significant difference between the two groups. Nevertheless, compared to N group, the expression levels of HMGB1 and RAGE were significantly increased in M group and SEA group, and this abnormal increase was inhibited by EA and the inhibitor FPS-ZM1 (Fig. 6).

In summary, the above experimental results suggest that EA treatment can inhibit the activation of HMGB1 and RAGE in the corpus callosum induced by pMCAO, similar to the effect of FPS-ZM1, whereas SEA does not.

Discussion

This study investigated the effects and possible mechanisms of EA treatment on ischemic brain white matter injury using a pMCAO rat model. The researchers also compared the effects of EA with RAGE inhibitor and SEA. The study employed various experimental methods including behavioral tests, histochemical and immunohistochemical staining, and protein immunoblotting to investigate the effects and potential mechanisms of electroacupuncture on ischemic white matter injury. Compared to previous studies, this research made several new findings. First, pMCAO induced the activation of HMGB1 and RAGE in the corpus callosum, which may be associated with the damage to this region. Second,

Fig. 5

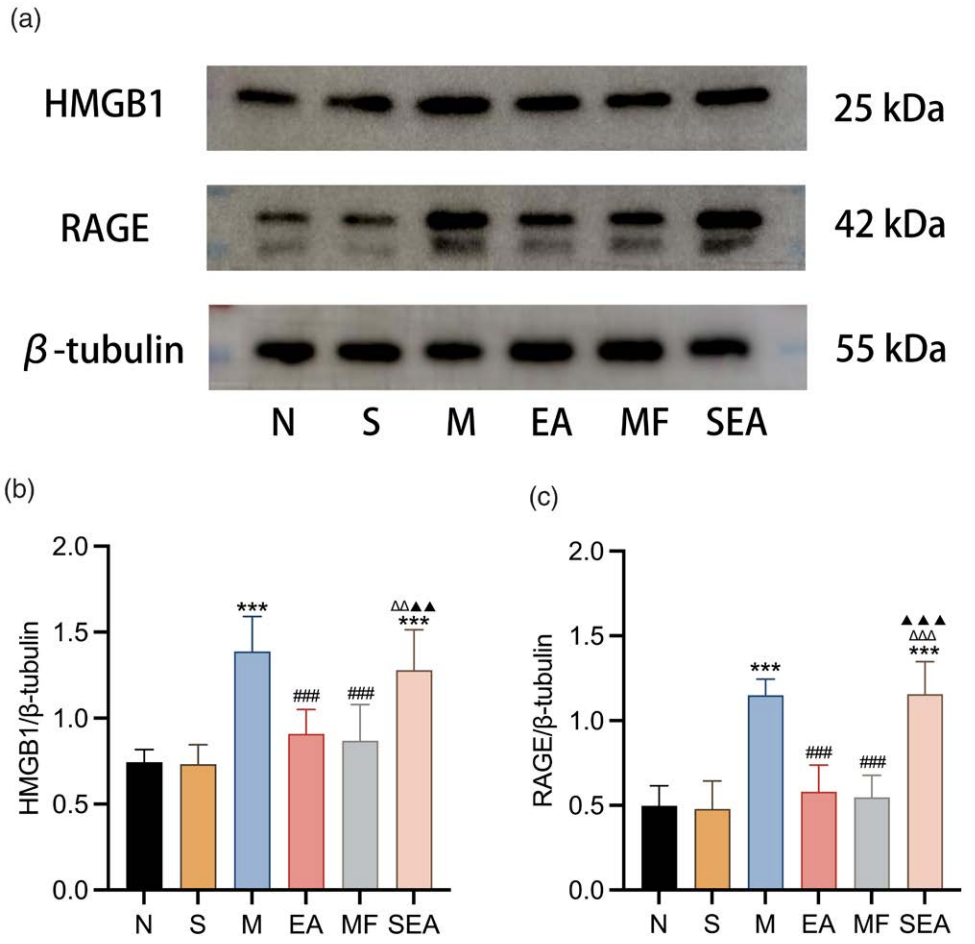


Effect of EA on the expression of HMGB1 and RAGE in the corpus callosum of pMCAO rats. (a) Representative microscopic photos (magnification: ×400) of HMGB1 staining in each group. The scale bar represents 50 μm. (b) Quantitative analysis of HMGB1 immunostaining intensity based on optical density (OD) ($n = 6$). Image J software was used to measure the optical density. ***$P < 0.0001$ vs the N group, **$P = 0.0004$ vs the N group, ###$P < 0.0001$ vs the M group, ΔΔΔ$P < 0.00001$ vs the EA group, and ▲▲▲$P < 0.0001$ vs the MF group. (c) Representative microscopic photos (magnification, ×400) of RAGE staining in each group. The scale bar represents 50μm. (d) Quantitative analysis of RAGE immunostaining intensity based on optical density (OD) ($n = 6$). Image J was used to measure the optical density. ***$P < 0.0001$ vs the N group, **$P = 0.0029$ and **$P = 0.0021$ vs the N group, ####$P < 0.0001$ vs the M group, ΔΔΔ$P < 0.00001$ vs the EA group, ▲▲▲$P < 0.0001$ vs the MF group. EA, electroacupuncture; HMGB1, high-mobility group box 1 protein; pMCAO, permanent middle cerebral artery occlusion; RAGE, the receptor for advanced glycation end products.

EA at GV20 and ST36 acupoints significantly reduced corpus callosum injury and improved motor ability in the rats, which may be closely related to its inhibition of pMCAO-induced activation of HMGB1 and RAGE in the corpus callosum. Third, the therapeutic effect of EA at acupoints was found to be comparable to that of the RAGE inhibitor FPS-ZM1, while SEA had no such effect.

According to the global stroke statistics from the World Stroke Organization and the WHO, stroke is the second leading cause of death worldwide, accounting for approximately 12% of all deaths [13]. Stroke can be classified into two types: ischemic stroke and hemorrhagic stroke. Ischemic stroke accounts for over 85% of stroke cases and is caused by the obstruction of blood circulation in the brain, leading to neuronal death and neurological impairments with high disability and mortality rates [14]. With the development of neuroimaging, a growing number of acute ischemic stroke patients have been found to have comorbid corpus callosum injury, including acute ischemia-induced damage to the corpus callosum. The corpus callosum is the major white matter fiber bundle connecting the left and right cerebral hemispheres, playing a crucial role in interconnecting widespread brain regions, including the frontal, parietal, and temporal cortices, and being closely associated with cognitive, motor, and language ability in the human body. Corpus callosum injury often correlates with corresponding symptoms of neurological deficits, such as motor impairment, sensory, and cognitive changes. Previous studies have indicated that corpus callosum injury after cerebral ischemia is closely associated with the time course of the ischemic insult [15]. Nevertheless, only a few thrombolytic drugs have been developed for ischemic stroke treatment, and the therapeutic time window is very short [16,17]. Therefore, EA, a treatment method that combines traditional Chinese medicine therapy with weak electrical stimulation, has gradually attracted attention.

Fig. 6



Evaluation of the effects of EA on the expression levels of HMGB1 and RAGE in the corpus callosum of rats subjected to pMCAO by protein immunoblotting. (a) Representative protein immunoblotting bands in each group. (b) Comparison of HMGB1 protein quantification among groups ($n = 6$). ***$P < 0.0001$ indicates a significant difference compared to group N, ###$P < 0.00001$ vs the M group, ΔΔ$P = 0.0096$ vs the EA group, ▲▲P = 0.0034 vs the MF group. (c) Comparison of RAGE protein quantification among groups ($n = 6$). ***$P < 0.0001$ vs the N group, ###$P < 0.0001$ vs the M group, ΔΔΔ$P < 0.00001$ vs the EA group, ▲▲▲$P < 0.0001$ vs the MF group. EA, electroacupuncture; HMGB1, high-mobility group box 1 protein; pMCAO, permanent middle cerebral artery occlusion; RAGE, the receptor for advanced glycation end products.

Some studies have suggested that EA can reduce neuronal and brain tissue damage caused by cerebral ischemia [18,19]. Interestingly, in traditional Chinese medicine, there is a theory called 'Brain-Gut Theory'. According to this theory, the brain, located at the top of the body, gathers human consciousness, while the intestines, located in the lower part of the abdomen, gather food residues. If the intestines are clear, 'breath power' and blood circulation will be active, which may contribute to the treatment of brain disorders. GV20 is the meeting point of all the meridians on the head and needling this acupoint has the effect of clearing the mind. ST36 is an acupoint on the stomach meridian of the foot and needling this acupoint has the effect of promoting gastric 'breath power', invigorating Yang, and nourishing vitality. Therefore, in our study, GV20 and ST36 were chosen as the acupoints for EA treatment.

Based on Nissl staining results, it was observed that EA could alleviate the damage to oligodendrocytes caused by cerebral ischemia. Oligodendrocytes play an important role in the nervous system and are essential for the formation of central nervous system myelin sheaths [20,21]. The normal number and structure of oligodendrocytes determine the integrity of myelin sheath structure, which was further verified by LFB staining. Cerebral ischemia leads to the loosening of myelin sheath structures and the loss of many myelinated axons. EA, however, can reverse these changes. The results of LFB and Nissl staining collectively showed that EA treatment significantly reduced the damage to ischemic brain tissue and inhibited demyelination induced by cerebral ischemia. Extensive loss of myelin sheaths can lead to a series of neuronal injuries and symptoms of neurological deficits [22]. The current experimental results indicate that the M group had a

reduction in oligodendrocytes and severe demyelination. Additionally, the rats in the M group had difficulty grasping a grid in the grid-walking test, indicating impaired motor ability, which is one of the symptoms of neurological impairment. Surprisingly, EA treatment significantly improved the overall motor ability of rats. Our current experimental results suggest that EA can improve poststroke neurological damage by increasing the number of oligodendrocytes and protecting the myelin sheath, thus exerting a protective effect on the brain's white matter.

Yet, there is no consensus on mechanism of EA treatment for cerebral ischemia. Recent studies have shown that ischemic stroke is often accompanied by the occurrence of cerebral inflammation [23]. In recent years, HMGB1 in neurological inflammation has gradually received attention. HMGB1 is widely distributed in the nuclei of mammalian cells and is a highly conserved nuclear protein. Studies have indicated that once HMGB1 is secreted outside of cells, it can cause inflammation [24]. Once cerebral ischemia occurs, the brain parenchyma is extensively damaged, leading to neuronal inflammation and necrosis, and various inflammatory mediators such as HMGB1 are released into the blood, exacerbating the inflammatory response. Thus, HMGB1 can serve as a biochemical marker of neuronal damage after cerebral ischemia. Other studies have revealed that HMGB1 can bind to cell surface receptors such as RAGE and disrupt the blood–brain barrier, promoting neuronal necrosis and apoptosis, thereby promoting the production and secretion of inflammatory factors [25]. RAGE, a multiligand receptor, is mainly expressed in neurons and microglia. In this study, the expression of HMGB1 and RAGE in the corpus callosum of the M group was significantly higher than that of the N group, indicating a greater inflammatory response in the corpus callosum during ischemic stroke. Conversely, after EA treatment, the expression of HMGB1 and RAGE decreased distinctly, similar to the results of the MF group. FPS-ZM1 is a high-affinity RAGE receptor inhibitor that inhibits microglial activation and inflammatory response when bound to RAGE. Furthermore, studies have shown that FPS-ZM1 has no toxic effects on normal neurons and has great potential for the treatment of neuroinflammation [6], but it has not yet entered clinical trials. Our experiment compared the EA group with the MF group, and the overall trend of the results in both groups was consistent, suggesting that EA may reduce the inflammatory response in the corpus callosum by inhibiting the activation of HMGB1 and RAGE, protecting the integrity of myelinated fiber structures, and improving comprehensive motor ability in pMCAO rats, thereby exerting a protective effect on ischemic cerebral white matter damage.

Of course, this study also has some limitations. First, the experimental period was 2 weeks, and no longer-term testing, such as over 1 month, was conducted. Additionally, the focus of this experiment was narrow, with the location mainly concentrated in the corpus callosum, and the detection indicators did not focus on other pathways. These limitations will be addressed with further expansion of research in future experiments.

In summary, our current research results at least reveal that pMCAO rats exhibit severe behavioral deficits and cause significant damage to the corpus callosum. We found, however, that EA can improve cerebral white matter damage and reduce the inflammatory response in pMCAO rats, exerting a protective effect on the brain's white matter. This therapeutic effect of EA may be related to its inhibition of HMGB1 and RAGE activation, thereby reducing the negative impact of pMCAO on the corpus callosum. This provides insights and strategies for further research on the mechanisms of acupuncture in promoting neuroinjury repair.

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C.L.: data curation, formal analysis, investigation, methodology, software, writing – original draft, writing – review and editing. Z.N.: data curation, investigation, methodology. H.M.: formal analysis, investigation, software. F.W.: conceptualization, formal analysis, funding acquisition, project administration, supervision, writing – review and editing. X.W.: formal analysis, funding acquisition, project administration, writing – review and editing.

The authors declare that all experiments of this study performed under agreement of ethical committee of Wannan Medical College (permission no. LLSC-2021–025).

Data will be made available on request.

Conflicts of interest

There are no conflicts of interest.

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