Research Article

Electroacupuncture Therapy Effectively Protects the Rat Brain after Intracerebral Hemorrhage

Li Huang,1 Xuehui Fan,2 Yao Chen,3 Heng Lin,4 Xiaoqian Jiang,4 and Chaoxian Yang2,4,5

1Department of Anesthesiology, Second Affiliated Hospital of Army Medical University, Chongqing 400037, China
2Ministry of Education and Medical Electrophysiological Key Laboratory of Sichuan Province, Southwest Medical University, Luzhou 646000, China
3Department of Pathology, People’s Hospital of Chongqing Banan District, Chongqing 401320, China
4Department of Anatomy, College of Basic Medicine, Southwest Medical University, Luzhou 646000, China
5Preclinical Medicine Research Center, Southwest Medical University, Luzhou 646000, China

Correspondence should be addressed to Chaoxian Yang; lyycx@foxmail.com

Received 2 July 2023; Revised 17 December 2023; Accepted 31 January 2024; Published 15 February 2024

Academic Editor: Gabriele Sansevero

Copyright © 2024 Li Huang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. Electroacupuncture (Ea) is a useful complementary and alternative therapy for intracerebral hemorrhage (ICH). However, the neurobiological basis for the Ea treatment of ICH is still unclear. The primary aim of the present study was to explore whether Ea prevents brain edema, apoptosis, excitotoxicity, and neuroinflammation in rats after hemorrhagic stroke.

Methods. Rats were randomly divided into Sham, Control, and Ea groups. We used modified neurological severity score (mNSS) and gait analysis to estimate neurological function in rats, and PET/CT to assess glucose uptake and the hemorrhagic focus volume. Measurement of the brain water content and TUNEL staining were used to evaluate brain edema and cell apoptosis, respectively. The serum myelin basic protein (MBP), neuron-specific enolase (NSE), calcium-binding protein B (S100B), and tumor necrosis factor-α (TNF-α) concentrations were examined with ELISA. The expression levels of the Glu, NR2A, AQP4, AQP9, Bax, and Glu proteins around the hematoma were detected via immunohistochemistry staining. Western blot was used to analyze the levels of the AQP4, AQP9, Bax, Bcl-2, INOS, and Arg-1 proteins.

Results. Ea treatment improved neurological function and reduced the hemorrhagic area and brain water content in rats after ICH. The serum concentrations of MBP, NSE, S100B, and TNF-α all decreased significantly in the Ea group compared with the Control group. Expression levels of the Glu, NR2A, AQP4, AQP9, Bax, GFAP, iNOS, and CD68 proteins in brain tissue surrounding the hematoma were obviously suppressed in ICH rats following Ea treatment. Moreover, Ea stimulation increased the levels of the MAP2, GALC, Glu2R, Arg-1, and Bcl-2 proteins, but reduced the number of TUNEL-positive cells in rats after ICH.

Conclusion. The results of this study suggest that Ea may exert neuroprotective effects by suppressing brain edema, apoptosis, excitotoxicity, and neuroinflammation.

1. Introduction

Intracerebral hemorrhage (ICH), a globally significant cause of death and disability, is one of the most common lethal diseases of the central nervous system (CNS) [1]. The incidence of ICH is increasing year by year, and the average age at morbidity is declining. ICH not only has high mortality and disability rates but also seriously invokes a financial burden on families and society as a whole [2]. Despite notable progress in clinical treatments for ICH, current therapies are still unsatisfactory.

Electroacupuncture (Ea), a combination of traditional acupuncture and modern electrostimulation, is a complementary, alternative therapy for ICH whose use and understanding have developed rapidly in recent decades [3, 4]. Ea is effective for treating a number of conditions, including hyperalgesia, postaccident ICH through the acceleration of angiogenesis, and inflammation following spinal cord injury in rats [4–8]. Our previous research demonstrated that Ea stimulation of the Baihui (GV20) and Dazhui (GV14) acupoints can improve neurological outcomes [9]. Although there is significant evidence that Ea could play a crucial role in ICH
treatment, its mechanism of action is not yet fully understood.
Thus, we investigated the effect of Ea on hemorrhagic brain
injury and sought to identify the possible mechanisms involved.

2. Materials and Methods

2.1. Animals. Eight to ten week-old adult male specific pathogen
free (SPF) Sprague–Dawley (SD) rats weighing 250–300 g were
used in this study. All these animals were housed under con-
trolled conditions at 23 ± 2 °C under a 12 hr light–dark cycle
with free access to food and water. The experimental protocol
was approved by the Ethical Committee for Animal Experimen-
tal Center of the Southwest Medical University and was in accor-
dance with the guidelines of the People’s Republic of China for
experimental animals.

2.2. ICH Model. Rats were randomly divided into the follow-
ing groups: Sham, Control, and Ea. The ICH rats were estab-
lished, as previously described [10]. Briefly, after anesthetizing
by intraperitoneal injection of 2% pentobarbital sodium
(2 µL/g), the rats were placed in a stereotaxic frame (Stoelting
Co., USA) in the prone position, keeping the anterior and poste-
rerior fontanelles at the same level. Following scalp disinfection and
longitudinal incision, a burr hole was drilled on the right cranial
bone (location: 0.2 mm anterior to the bregma, 3 mm right lateral
to the midline), and a Hamilton syringe needle was inserted at a
point 6 mm vertical and ventral to the hole was used to inject 2 µL
collagenase I (0.125 U/µL) and 1 µL heparin (2 U/µL) at a rate of
0.3 µL/min for 10 min (Figure 1(a)). After 5 min, the needle was
slowly removed at a rate of 1 mm/min. After infusion, the bore-
hole was sealed with bone wax, and the scalp was sutured. Rats in
the Sham group underwent the same surgical procedure but with-
out the collagenase I and heparin injection.

2.3. Ea Treatment. Two days after ICH, the Ea group began
daily Ea stimulation according to our previous methods at the
GV20 and GV14 acupoints until sacrifice [9] (Figure 1(b)).
One acupuncture needle was obliquely inserted 2 mm into the
GV20 acupoint and another was vertically inserted 5 mm into

![Diagram of ICH model with Ea stimulation](image)
2.4. Modified Neurological Severity Score (mNSS). The mNSS test was used to assess neurobehavioral deficits through a series of motor, sensory, balance, and reflex measurements [10]. The score of 0–18 indicated the degree of injury (normal score, 0; slight injury, 1–6; moderate injury, 7–12; serious injury, 13–18). ICH rats with final scores of higher than 8 were included in subsequent experiments. Researchers were blinded to the experimental conditions and treatment groups.

2.5. Gait Analysis. The rat limb motor functions were tested with the TreadScan™ Gait System (Clever System Inc., USA) 7 days after treatment using a previously described procedure [9]. The rats ran on a treadmill at 8 cm/s for 20 s, the footprints were recorded at 100 frames/s. The videos were analyzed using TreadScan™ software to assess the gait performance. The parameters assessed in this study were the run speed, regularity index of normal step sequence, print area, stance, and swing time.

2.6. Positron-Emission Tomography. Positron-emission tomography (PET) imaging of rat brains was conducted using microPET/computed tomography (CT) (Siemens, Germany). The rats were not provided with food and water for the overnight period prior to PET scanning. Rats were then anesthetized with 2% pentobarbital sodium (2 μL/g) and injected with 18F-fluoro-deoxyglucose (18F-FDG) synthesized by the Department of Nuclear Medicine, Affiliated Hospital of Southwest Medical University. Thirty minutes later, a PET scan of the head was performed, and the PET images of the rat brain were analyzed to measure the hemorrhagic foci volume and glucose uptake.

2.7. Brain Water Content. The brain water content, a surrogate measure for brain edema, was measured as previously reported [11]. Briefly, animals were anesthetized and decapitated. The brains were quickly removed, and right hemispheres were weighed (wet weight). After drying for 24 hr at 100°C, the hemispheres were weighed again (dry weight). The brain water content (%) was calculated using the following formula: (wet weight − dry weight)/wet weight × 100%.

2.8. Enzyme-Linked Immunosorbent Assay (ELISA). After anesthesia, blood was collected for serum preparation. ELISA kits were used to detect serum myelin basic protein (MBP) (Novus Biologicals), tumor necrosis factor-α (TNF-α) (Boster), neuron-specific enolase (NSE), and S100 calcium-binding protein b (S100B) concentrations (Nanjing Jiancheng Corp.) according to the manufacturer’s instructions.

2.9. Pathologic Examination. Rats were perfused transcardially with 0.9% ice-cold normal saline (NS) followed by 4% paraformaldehyde (PFA) under deep anesthetization with an overdose anesthetic drug. The rat brains were removed and subjected to postfixation, concentration gradient dehydration, and snap freezing. The brains were then cut into 7 μm thick coronal sections using a frozen slicer (Leica, Germany). After staining with hematoxylin and eosin (H&E), samples were observed under an optical microscope (Olympus, Japan).

2.10. Immunohistochemistry Staining. The frozen sections were treated with 0.3% tritonX-100 in 0.01 M phosphate-buffered saline (PBS) and 3% hydrogen peroxide (H2O2) for permeabilization and to block endogenous peroxidase activity, respectively. They were then incubated with 10% goat serum for 20 min at room temperature to block any nonspecific staining. The sections were incubated with rabbit anti-galactocerebrosidase (GALC) (1:100, Invitrogen), clusters of differentiation 68 (CD68) (1:200, CST), arginase-1 (Arg-1) (1:100, CST), inducible nitric oxide synthase (iNOS) (1:00, CST), N-methyl D-aspartate acid receptor subunit 2A (NR2A) (1:200, CST), glutamate receptor-2 (Glut2R) (1:100, Boster), aquaporin-4 (AQP4) (1:100, Bioworld), mouse antimicrotubule-associated protein 2 (MAP2) (1:200, Santa Cruz), glial fibrillary acidic protein (GFAP) (1:200, Santa Cruz), aquaporin-9 (AQP9) (1:100, Santa Cruz), B-cell lymphoma-2 (Bcl-2) (1:200, Santa Cruz), Bcl-2-associated x (Bax) (1:200, Santa Cruz), and glutamine (Glu) (1:200, Abcam) at 4°C overnight. Sections were then rinsed before further incubation with corresponding biotinylated secondary antibodies (Invitrogen) at 37°C for 30 min. Then, following treatment with streptavidin–biotin complex solution (SABC) at 37°C for 30 min, the sections were colored with diaminobenzidine (DAB), and target proteins were labeled. Finally, sections were stained with hematoxylin at room temperature for 3 min to detect the nuclei, and images were obtained using a microscope (Olympus, Japan).

2.11. Terminal Deoxynucleotidyl Transferase-Mediated (dUTP) Nick End Labeling (TUNEL) Assay. According to the standard procedure for TUNEL (Roche), frozen brain sections were washed three times in PBS and then incubated with a solution of proteinase k at 37°C for 15 min. After washing twice with PBS, the sections were treated with TUNEL reaction mixture, a red fluorescein-labeled TUTP solution containing 10% terminal deoxynucleotidyl transferase (TdT) at 37°C for 1 hr in the dark. The nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI) at room temperature for 5 min. The number of TUNEL-positive cells was quantified by selecting five microscopic fields from each section.

2.12. Western Blot. The total protein content was extracted from the right striatum and was estimated with the BCA protein assay kit (Beyotime). Equal amounts of sample proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a polyvinylidene difluoride membrane. After blocking with 5% nonfat milk, the membrane was incubated overnight with the primary antibodies (AQP4, AQP9, Bax, Bcl-2, iNOS, Arg-1, and β-actin) at 4°C, with β-actin acting as a control. The next day, further incubation with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (Invitrogen) at room temperature for 2 hr. Blots were then read by a chemiluminescence system.
3.1. Ea Reduced Neurological Symptoms. After ICH, the rats showed obvious neurobehavioral dysfunction. The mNSS scores in the Control and Ea groups were similar before treatment, but significantly higher than those in the Sham group (Figure 2(a)). After Ea treatment for 1 week, the Ea group showed significantly lower scores than the Control group ($P < 0.05$, Figure 2(b)).

The footprints and strides of rats also changed significantly after ICH (Figures 3(a) and 3(b)). The gait analysis revealed that the run speed, regularity index of normal step sequence, and print area (rear left paw (RL)/rear right paw (RR)) decreased, while the stance time (RL) and swing time (RR) increased after ICH ($P < 0.05$, Figure 3(c)–3(p)). However, Ea treatment was associated with significant increases in the run speed, regularity index of normal step sequence, print area (RL/RR), and stance time (RR) and decreases in the stance time (RL) and swing time (RL) in ICH rats ($P < 0.05$, Figure 3(c)–3(p)).

3.2. Ea Reduced Hemorrhagic Foci. An $^{18}$F-FDG micro-PET scan showed that the hemorrhagic area volumes were significantly smaller in the Ea compared with the Control group ($P < 0.05$, Figures 4(a) and 4(b)). Moreover, there was no significant difference in the level of glucose uptake (ipsilateral/contralateral) between the Control and Ea groups (Figure 4(c)).

3.3. Ea Improved the Brain Tissue Structure. Figure 5(a) shows that the Control and Ea groups developed prominent hemorrhagic foci after collagenase I and heparin injection, while no hemorrhagic focus was found in the Sham group, as expected. The Ea group presented significantly better brain tissue structure and smaller hematomas than the Control group ($P < 0.05$, Figures 5(a) and 5(b)). H&E staining showed significantly larger lesion areas and an increased red blood cell (RBC) number in the Control group compared with the Ea group ($P < 0.05$, Figures 5(c) and 5(d)).

3.4. Ea Regulated AQPs and Alleviated Cerebral Edema. The brain water content was significantly higher in the Control group compared with the Sham group, but lower in the Ea group compared with the Control group ($P < 0.05$, Figure 6(a)). Furthermore, the expression levels of AQP4 and AQP9, which are also closely related to the brain water content, reduced significantly following Ea treatment ($P < 0.05$, Figure 6(b)–6(h)).

3.5. Ea Alleviated Brain Injury. We tested the degree of brain damage by immunohistochemistry staining and ELISA. The data revealed that the MAP2 (a neuronal marker) and GALK (an oligodendrocyte marker) proteins increased significantly, but the number of GFAP (an astrocyte marker) positive cells decreased obviously in the Ea group compared with the Control group ($P < 0.05$, $P < 0.05$, Figure 7(f)). Furthermore, the serum of NSE (a neuronal marker), MBP (an oligodendrocyte marker), and S100B (a marker of astrocyte activation and brain dysfunction) concentrations decreased significantly in the Ea group compared with the Control group ($P < 0.05$, Figure 7(g)–7(i)).

3.6. Ea Reduced Cell Apoptosis and Regulated the Expression of Apatosis-Related Proteins. The number of TUNEL-positive cells surrounding the hematoma significantly increased in the Control and Ea groups compared with the Sham group but decreased in the Ea group compared with the Control group ($P < 0.05$, Figures 8(a) and 8(b)). The immunohistochemistry and western blotting analyses revealed that the Bcl-2 (an antiapoptotic marker) protein decreased; however, the Bax (a proapoptotic marker) protein increased after ICH ($P < 0.05$, Figure 8(c)–8(i)). Bcl-2 expression increased and Bax expression decreased significantly in the Ea group compared with the Control group ($P < 0.05$, Figure 8(c)–8(i)).
FIGURE 3: Continued.
3.7. Ea Attenuated Excitotoxicity. Glu (a neurotransmitter) expression around hematomas was significantly upregulated following ICH-induced injury, but Ea treatment was associated with the downregulation of Glu expression ($P<0.05$, Figures 9(a) and 9(d)). Moreover, the expression of NR2A (a subunit of N-methyl-D-aspartic acid (NMDA) receptor) exhibited the same trend ($P<0.05$, Figures 9(b) and 9(e)). The Glu2R (a subunit of amino-3-hydroxy-5- methylisoxazole- 4-propionic acid (AMPA) receptor) level increased in the Ea group compared with the Control group ($P<0.05$, Figures 9(c) and 9(f)).

3.8. Ea Alleviated the Inflammatory Reaction. The number of cells positive for CD68 (a macrophage antigen marker) around the hematomas increased significantly in the Control and Ea groups compared with the Sham group. However, the number of CD68-positive cells decreased significantly in the Ea group compared with the Control group ($P<0.05$, Figure 10(a) and 10(b)).

The iNOS (M1 marker) and Arg-1 (M2 marker) positive cells significantly increased after ICH ($P<0.05$, Figure 10(c)–10(f)). The number of iNOS-positive cells was significantly lower in the Ea group than in the Control group ($P<0.05$, Figure 10(e)). However, the number of Arg-1 positive cells was higher in the Ea groups than in the Control group ($P<0.05$, Figure 10(f)). Western blot results revealed that Ea treatment upregulated the Arg-1 protein but downregulated the iNOS protein in rats subjected to ICH ($P<0.05$, Figure 10(g)–10(i)).

In comparison with the Sham group, serum TNF-$\alpha$ concentrations increased significantly in the Control and Ea groups ($P<0.05$), while serum TNF-$\alpha$ concentrations decreased obviously in the Ea group compared with the Control group ($P<0.05$, Figure 10(j)).

4. Discussion

In this study, we used mNSS and the TreadScan Gait System to assess the effects of Ea on neurological function in an ICH rat model. We found significant differences between the Control and Ea groups in terms of mNSS scores and some gait parameters such as the run speed and regularity index of normal step sequence. The results reveal that Ea administration could significantly improve neurological function in ICH rats.

By using $^{18}$F-FDG micro-PET scanning to detect the bleeding area [9–11], the results showed that Ea treatment reduced the hemorrhagic focal area. This is significant as the extent of a brain injury is closely associated with brain edema and the duration of edema [12, 13]. Our results showed that the brain water content increased in rats by Day 7 after ICH and that Ea therapy was associated with brain edema alleviation. Aquaporins (AQPs), a family of water-channel proteins, play critical roles in homeostasis and water movement [14, 15]. Some studies have shown that AQP4 and AQP9 are associated with brain edema induced by several types of brain damage, including ICH, brain trauma, and ischemic stroke [16–18]. However, the effect of Ea on AQPs expression in the brain after ICH remains unclear. Our experimental study showed that the expression levels of the AQP4 and AQP9 proteins were lower in the Ea groups than in the
Control group, suggesting that Ea may reduce brain edema by regulating the AQP4 and AQP9 proteins.

ICH can lead to significant cell death through a variety of mechanisms, including those involving neurons and glial cells [19–21]. The death of oligodendrocytes and neurons has been associated with demyelination and neurological dysfunction [19, 22]. Previous studies have shown that Ea improves neurobehavioral function and brain injury in ICH rats [9]. In the present study, Ea stimulation increased MAP2 and GALC expression and decreased GFAP expression, implying that Ea promotes the survival of neurons and oligodendrocytes and inhibits astrocyte hyperplasia in rats subjected to ICH. In addition, serum concentrations of the MBP, NSE, and S100B proteins, common indicators of the severity of hemorrhagic brain injury [23, 24], are often used as diagnostic and prognostic biomarkers for brain injury [25–27]. We found that Ea treatment downregulated these proteins in the serum, indicating that Ea treatment relieved brain injury in rats after ICH.

A growing body of evidence suggests that cell apoptosis plays an important role in the pathogenesis of ICH-induced brain injury [28, 29]. The Bcl-2 and Bax proteins play vital roles in regulating apoptosis [30, 31]. Previously, Ea treatment has been shown to inhibit cell apoptosis, thereby inducing neuroprotection against ischemic injury [32]. In order to investigate whether the regulation of apoptosis by Ea (GV20-GV14) could play a neuroprotective role against ICH, the effects of Ea on cell apoptosis and apoptosis-related...
proteins were evaluated. The results showed that Ea treatment may inhibit ICH-induced apoptosis by upregulating Bcl-2 and decreasing Bax expression.

Glu, the most abundant neurotransmitter in the CNS, is not restricted to synaptic clefts but exists in the extrasynaptic regions as ambient glutamate [33]. Previous studies using ICH models have investigated the relationship between brain injury and Glu levels in perihematomal brain tissues, the impact of the Glu level on outcomes in ICH patients, and the challenges posed by Glu excitotoxicity in the management of hemorrhagic stroke [34–36]. In a rabbit model of ICH, minimally invasive removal of intracerebral hematoma was found to decrease perihematomal glutamate level and neurological deficit score [37]. In addition, a clinical study found that the Glu level was increased in the perihematomal region after ICH and decreased during hematoma drainage [34]. The NMDA receptor (NR) is an ionotropic glutamate receptor composed of NR1, NR2A–D, and NR3 subunits that play critical roles in the physiological and pathological processes of the CNS [38]. The fragmentation of the NR2 subunit into NR2A and NR2B peptides may be caused by stroke or neurotoxicity; thus, the NR peptide is an indicator of Glu excitotoxicity associated with stroke pathophysiology [39]. GluR2 plays an important role in the prevention and cure of brain injury, largely by reducing the permeability of the AMPA receptors (AMPARs) to Ca$^{2+}$ [40]. A decrease in GluR2 subunits may cause an influx of Ca$^{2+}$, which may be involved in cortical neuron neurotoxicity [41]. In this study, we found that ICH increased the expression of Glu, NR2A, and GluR2, but Ea decreased the expression of Glu and NR2A, and further improved the expression of GluR2. We speculate that Ea depresses the Ca$^{2+}$-permeability of AMPARs with GluR2 subunits and alleviates the effect of excitotoxicity mediated by Glu and NR2A in ICH rat brains.

Numerous studies have demonstrated the important role of inflammation characterized by microglia/macrophage activation in hemorrhagic brain injury [42–44]. Microglia in the CNS milieu can be categorized into two opposite types, proinflammatory M1 phenotype and anti-inflammatory M2 phenotype, which produce cytotoxic and neuroprotective effects, respectively. Activation of the microglia after ICH exacerbates the production of proinflammatory factors, such as TNF-α and iNOS. Therefore, anti-inflammatory methods may alleviate brain injury and improve neurological functions after ICH [45]. Several studies have reported that Ea significantly induced anti-inflammatory responses in obese and ischemic stroke patients, as well as in individuals with infectious and inflammatory disorders [46–48]. Our data show that Ea significantly inhibited microglia activation, downregulated TNF-α, CD68, and iNOS expression, and upregulated Arg-1 expression in rats after ICH. Ea also exerted an anti-inflammatory effect on ICH rats.

There are mutual influences and connections between brain edema, inflammation, and excitatory toxicity caused by ICH. After collagenase-induced ICH, the brain water content increased on Day 1, reached a peak on Day 3, and was still higher than that in the sham group on Day 10 [49]; Microglia are activated as early as 1 hr post-ICH. The
FIGURE 6: The effects of Ea on mNSS, brain edema, and expressions of AQP4 and AQP9 proteins in rats after ICH. (a) The brain water content of rats from different groups. Representative images of AQP4 (b) and AQP9 (d) immunohistochemical staining in the rat ipsilateral striatum. Statistical analyses of AQP4 (c) and AQP9 (e) immunopositive staining. Representative results (f) and quantitative analyses (g, h) of western blot showing the relative expression levels of the AQP4 and AQP9 proteins in the rat ipsilateral striatum. *$P<0.05$, **$P<0.01$, ***$P<0.001$, and ****$P<0.0001$. Bar = 100 μm.

FIGURE 7: Continued.
number of activated microglia/macrophages then peaks at 72 hr and gradually returns to normal levels, which takes several weeks [50]. ICH causes the release of many endogenous molecules, including heme, glutamate, TNF-α, and reactive oxygen species. These molecules are involved in mitotic signaling transduction and excitatory toxicity. Mitotic signaling initiates the cell cycle to produce the normal division of neural stem cells and microglia, whereas aberrant mitotic signaling may lead to excitatory toxicity [51]. After ICH, IL-10 defective mice have been found to present more serious brain edema, inflammation, and neurological deficits related to delayed hematoma clearance [52]. On the one hand, insufficient RBC clearance by activated microglia may prolong injury and worsen outcomes. On the other hand, overactive microglia may lead to secondary damage [52, 53]. Microglia respond to ICH by becoming activated and developing into a classic M1 phenotype or an alternative M2 phenotype. Microglial polarization affects both the pathological process and recovery post-ICH, and this may be associated with the production of proinflammatory or anti-inflammatory cytokines and chemokines [54–56]. Moreover, M2 phenotype microglia can promote the phagocytosis of RBCs and tissue debris, which is the main contributor to hematoma clearance [54].

Several limitations of this study need to be mentioned. First, Ea plays a variety of roles in tissue repair and neurological rehabilitation. Although we observed the effects of Ea on the brain water content and aquaporins, the underlying mechanisms need to be further investigated. Second, our immunohistochemistry and western blot experiments show that Ea promoted the transformation of microglia from the M1 type to the M2 type after ICH, but further research on its signal regulation is required. In addition, we only observed the brain water content and microglial polarization during
Figure 8: Continued.
FIGURE 8: The effects of Ea on cell apoptosis and the expression of apoptosis-related proteins. (a) Representative images of TUNEL staining in the rat ipsilateral striatum. (b) Quantitative analysis of TUNEL-positive cells in each group. Representative images of Bax (c) and Bcl-2 (d) immunohistochemical staining in the ipsilateral striatum of the rats. Statistical analyses of Bax (e) and Bcl-2 (f) immunopositive staining. Representative results (g) and quantitative analyses (h, i) of Western blot showing the relative expression of the Bax and Bcl-2 proteins in the rat ipsilateral striatum. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. Bar = 100 μm.

FIGURE 9: Continued.

(a) GluA

(b) NR2A

(c) GluR2
FIGURE 9: The effect of Ea on the levels of Glu, NR2A, and Glu2R proteins in the brain. Representative images of the Glu (a), NR2A (b), and Glu2R (c) immunohistochemical staining in rat ipsilateral striatum. Quantification analyses of Glu (d), NR2A (e), and Glu2R (f) immunohistochemical staining. *P < 0.05 and ****P < 0.0001. Bar = 100 μm.

FIGURE 10: Continued.
the first week of Ea treatment, while research on sustained changes in hematomas and the brain water content after microglial polarization is still lacking.

5. Conclusions

In short, although the present study has some limitations, we demonstrated that Ea treatment could effectively improve the symptoms of neurological deficits and reduce brain edema, apoptosis, and excitotoxicity in rats after ICH. We speculate that Ea plays an important role in inhibiting inflammatory responses. As a consequence of this neuroprotection, brain tissue functions may be restored. Further studies and an optimized experimental design are now warranted to better understand and investigate the mechanisms involved. Nevertheless, the results of the present study suggest that the
administration of Ea may be an important therapeutic strategy to alleviate hemorrhagic brain injury.

**Data Availability**
The data used to support the findings of this study are available from the corresponding authors upon reasonable request.

**Ethical Approval**
This study was carried out in accordance with the guidelines of the People’s Republic of China on experimental animals. The animal experiment was submitted to and approved by the Ethical Committee for Animal Experimental Center of the Southwest Medical University.

**Conflicts of Interest**
The authors declare that they have no conflicts of interest.

**Authors’ Contributions**
CY conceived and designed the experiments and revised the manuscript. LH, XF, YC, HL, and XJ performed the experiments and analyzed the data. LH and XF wrote the manuscript. All authors read and approved the final manuscript. Li Huang and Xuehui Fan contributed equally to this work.

**Acknowledgments**
This study was supported by the Research Project of Medical Electrophysiological Key Laboratory (KeyME-2022-05) and the Natural Science Foundation of Sichuan Province (2022NSFSC0718).

**References**


[20] Y. Kong, S. Li, M. Zhang et al., “Acupuncture ameliorates neuronal cell death, inflammation, and ferroptosis and downregulated miR-23a-3p after intracerebral hemorrhage


