IBRO Neuroscience Reports 18 (2025) 443–452

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

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

Acupuncture ameliorates inflammation by regulating gut microbiota in acute ischemic stroke

Haoyue Yan [a,1], Yini Hua [a,1], Jinxia Ni [a,*], Xiaona Wu [b], Jingni Xu [c], Ziniu Zhang [a], Juwei Dong [a], Zhihao Xiong [a], Lei Yang [a], Hongwei Yuan [a]

[a] Department of Acupuncture and Moxibustion, Dongzhimen Hospital of Beijing University of Chinese Medicine, China
[b] Department of Rehabilitation, Beijing Fengtai Hospital, China
[c] Dongcheng District Dongzhimen Community Healthcare Center, China

ARTICLE INFO

Keywords:
Acupuncture
Acute Ischemic Stroke
Immune-inflammatory response
Gut microbiota

ABSTRACT

Background: Acute ischemic stroke(AIS) is a major life-threatening disease.Some studies have found that AIS may be related to gut flora and immune responses. Acupuncture is used widely in the treatment of AIS. However its relevant mechanism is unclear enough. Therefore, in this study, we wanted to confirm that acupuncture was treating AIS through gut flora and immune response.
Methods: We randomly divided 18 rats into equal three groups, including Sham, Middle Cerebral Artery Occlusion (MCAO) and Acupuncture.Rats in the Acupuncture group for a continuous period of three days after surgery. Neurological deficits were assessed using Longa's method, and detection of intestinal flora by 16s rRNA gene sequencing, determination of SCFAs by gas chromatography-mass spectrometry, detection of HDAC and inflammatory cytokines by elisa assay, detection of Th17 and Treg cells by flow cytometry and, observation of pathological and morphological changes in brain and colon tissues by HE staining.
Results: Acupuncture improved the degree of impaired neurological function in MCAO rats and regulated the type and abundance of intestinal bacteria, increased SCFAs of MCAO rats, decreased HDAC1 and HDAC2, modulated the Th17/Treg imbalance, reduced the level of inflammatory factors in the peripheral blood and altered the pathology of the intestine and brain.
Conclusion: Acupuncture repaired neurologic deficits after AIS and may be associated with an immune-inflammatory response mediated by gut microbiota.

Background

Ischemic stroke has been threatening human life and health for a long time,and it is aggravated with the aging of the world population. Due to its high incidence, high disability rate and high mortality rate, it brings great pain to the patients and great burden to the family and society (GBD, 2021). Common treatments for stroke brain lesions include drug thrombolysis and mechanical thrombolysis, but with more stringent time Windows, it is difficult to benefit most patients (Powers, 2020). With the deepening of research, people have found the existence of "brain-gut axis", in which the brain and gut interact in physiological and pathological conditions. In particular, intestinal flora plays a crucial role in this process, affecting the occurrence and development of stroke (Peh et al., 2022).

The intestinal microbiota has been verified from multiple perspectives to be closely related to ischemic stroke, influencing the post-stroke brain-gut environment in terms of metabolism, immunity and inflammation via the brain-gut axis (Chidambaram et al., 2022). Inflammatory response is one of the main factors affecting the occurrence and development of ischemic stroke (Huang and Xia, 2021). After stroke,

Abbreviations: AIS, Acute ischemic stroke; MCAO, Middle Cerebral Artery Occlusion; SCFAs, Short-chain fatty acids; STAT3, signal transducers and transcriptional activator 3; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery.

* Corresponding author.
E-mail addresses: 798225208@qq.com (H. Yan), 1248029141@qq.com (Y. Hua), nijinxia118@126.com (J. Ni), 18810550620@163.com (X. Wu), 18811383811@163.com (J. Xu), ziniuzhang@126.com (Z. Zhang), djuwei@163.com (J. Dong), DrXiongzh@163.com (Z. Xiong), leiyang2009@126.com (L. Yang), doctorhwyuan@yeah.net (H. Yuan).
[1] These two authors contributed to the work equally and should be regarded as co-first authors.

https://doi.org/10.1016/j.ibneur.2025.02.007
Received 15 November 2024; Accepted 15 February 2025
Available online 17 February 2025

intestinal flora regulating immune cell differentiation and inflammatory factor, by peripheral circulation to the brain, brain tissue repair. Among them, inhibiting Th-17 and promoting IL-10 secretion are crucial for nerve repair, and intestinal flora can regulate their rise and fall, promote inflammation repair, and improve prognosis (Singh et al., 2016; Benakis et al., 2016). Short-chain fatty acids (SCFAs) is a metabolite of intestinal flora, known as a bridge between brain and intestine (Fang et al., 2023). Researchers have found that it can reduce neurological damage and cerebral infarction size after stroke (Chen et al., 2019). It is also associated with signal transducers and transcriptional activator 3(STAT3), a protein that ameliorates neurological impairment (Davis et al., 2022; Zhao et al., 2018), becoming a link between SCFAs and the brain-gut axis.

In recent years, acupuncture has been shown to regulate gut microbiota and can restore the balance of bacteria after stroke (Jang et al., 2020; Feng et al., 2024). However, the mechanism of acupuncture on brain and intestine remains to be perfected. Therefore, we aim to observe the changes of immune inflammation, microflora and their metabolites in ischemic stroke rats after acupuncture, and further explore the molecular mechanism of acupuncture treatment of stroke.

Materials and methods

Animal

Male Sprague-Dawley rats were obtained from Beijing HFK Bio-Technology(Beijing, China, license No. SCXK2019-0008). Rats were housed in Molecular Biology of Nerve Cells laboratory at Dongzhimen hospital. Light exposure was maintained on a 12-hour light/dark cycle starting at 8:00 a.m. Ambient temperature was 18–26 °C and the relative humidity was 40–70 %. Cage temperature was 1–2 °C higher than the ambient temperature and cage humidity was 5–10 % higher than the relative humidity. All rats had ad libitum access to food and water. All experimental protocols were approved by the Ethics Committee for Animal Experimentation of Dongzhimen hospital (approval No. 21-01, on December 11, 2020). All rats were acclimatized in the cages for 2 days and then randomly divided into three groups according to a random number table: Sham, MCAO, Acupuncture. (Six rats per group, were sacrificed in 3 day after inducing stroke.)

Induction of focal cerebral ischemia

The rats were fasted without food and water for 24 hours before surgery. At room temperature, the rats were weighed and then anesthetized intraperitoneally with 1 % sodium pentobarbital according to the standard of 6 mL/kg, and after the anesthesia was complete and the muscles of the rats were relaxed, the rats were fixed on the surgical table. The neck fur was cleared to expose the skin, and the surgical area was sterilized using alcohol. The skin was incised in the middle of the neck, and a surgical incision of approximately 1.5 cm was made from the inferior border of the jawbone, and the subcutaneous muscles were bluntly separated, and the right sternocleidomastoid and sternocleidomastoid muscles were held with a homemade hook to completely expose the sheath of the common carotid artery. The right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were isolated using a glass split-needle approach to protect the vagus nerve as much as possible and to reduce surgical irritation. A 4–0 surgical wire was used to ligate the proximal end of the CCA and ECA, and an arterial clip was used to clip the ICA, and a live knot was threaded through the distal end of the CCA for backup. Place the wire bolus into the 6-gauge syringe needle, insert the syringe tip into the CCA, push the wire bolus into the vessel from behind, secure the inserted portion of the wire bolus with forceps, withdraw the syringe needle, insert the wire bolus, tighten the backup wire, and open the arterial clip. Continue to feed the bougie until the bougie entered 17–21 mm (from the bifurcation) and then stopped, at which time the head of the bougie had passed the middle cerebral artery and had blocked the blood flow to the middle cerebral artery, tighten the live knot of the spare thread and ligature, cut off the remnants of the bougie, suture the muscle and skin in layers, and disinfect the surgical incision with alcohol and iodine vapour. Postoperative rats were placed in the supine position in the rat cage, and care was taken to keep the head high and feet low when placing them. The rats were awakened 2–4 h after surgery. In the sham group, only the neck muscles were incised to expose and separate the CCA, ICA, and ECA without ligating the blood vessels or inserting the thread plugs, and the rest of the group was sterilized and sutured after the operation.

Neurological deficits were assessed using Longa's method (Longa et al., 1989). This entailed assigning a score of 0 for normal or no neurological impairment, 1 for mild neurological impairment (inability to fully extend the left forelimb), 2 for moderate neurological impairment (turning left when walking), 3 for severe neurological impairment (falling to the left when walking), and 4 for conscious disturbance (inability to walk spontaneously).

Acupuncture

Acupuncture began at first day after inducing stroke. The rats were fixed with the fixators, and referring to the "Rat Acupuncture Points Atlas". The disposable acupuncture needle(0.25 × 25 mm) was inserted 4 mm into Tianshu(ST25) and Zusanli (ST36), and the disposable acupuncture needle also was used to take Baihui(DU20) and Baihui left and right side to open 4 mm,the needle was rotated for 1 min at a frequency of 160 rpm. The needle was retained for 30 min,once a day, for 3 continuous days. The Sham and MCAO groups both received no treatment, while the MCAO group received fixation as same as the acupuncture group.

Neurological function score

Neurological deficits were assessed using Longa's method (Longa et al., 1989). This entailed assigning a score of 0 for normal or no neurological impairment, 1 for mild neurological impairment (inability to fully extend the left forelimb), 2 for moderate neurological impairment (turning left when walking), 3 for severe neurological impairment (falling to the left when walking), and 4 for conscious disturbance (inability to walk spontaneously). The neurological function was evaluated by an independent researcher who was blinded to the experimental conditions. The neurological functions were performed once after inducing stroke and once before sacrificing.

Assessment of intestinal metabolites

The extracted feces were placed in an EP tube, 500 μL of methanol solution was added to make a fecal suspension, the acidity was adjusted to 2–3 with dilute sulfuric acid, shaking and mixing, and then centrifuged for 20 min, the supernatant was taken, then centrifuged again, the supernatant was taken, and placed in an EP tube, and 1 mL of a mixture of benzene and n-hexane (1:1) was added, vortexing and oscillating to homogeneity, and then potassium hydroxide- methanol solution, shake well and leave for about 30 min, after the solution was clarified, the supernatant was aspirated.

Fecal DNA extraction and 16S rRNA sequencing

According to the experimental grouping, feces were extracted from each group of rats on the third day after inducing stroke. About 100 mg of intestinal contents of rats in each group were taken and the 16 S region was sequenced. The primer region was V3V4 front-end primer: CCTAYGGGRBGCASCAG; back primer:GGACTACNNGGGTATCTAAT. The genomic DNA of the samples was extracted using Omega 's Magbindsoil DNAkit DNA extraction kit, and then the purity and concentration of DNA were detected by 1 % agarose gel electrophoresis. The extracted genomic DNA was used as a template for polymerase chain reaction(PCR) amplification of the 16S rRNA gene. The purified product was subjected to Qubit quantification and then sequenced using the IlluminaNovaSeq 6000 sequencing platform.

Detection of HDAC1 and HDAC2 in small intestinal tissues

Small intestinal tissue samples are carefully collected from the rats under sterile conditions and stored at −80 °C until further use. The samples are thawed and homogenized in a buffer containing a protease inhibitor to ensure the integrity of the HDAC protein. The homogenized samples are centrifuged to separate the cellular debris from the supernatant containing the HDAC protein. The supernatant is then collected and stored at −20 °C. The supernatant was evaluated as follows: enzyme-linked immunosorbent assay(ELISA) kits (Beijing Rigor Bioscience Development LTD.Beijing, China) were used to detect the HDAC1 and HDAC2.

Blood sample collection and flow cytometric analysis

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized peripheral blood by density gradient centrifugation with Ficoll–Paque PREMIUM 1.077 (GE Healthcare Life Sciences, Pittsburgh, PA). The cells were then resuspended in RPMI 1640 medium containing phorbol 12-myristate13-acetate (PMA) (50 ng/mL), GolgiStop (1 μl), and ionomycin (1 μg/mL) for 5 h in an incubator at 37 °C with 5 %CO2. The percentages of Th17 and Treg cells were detected by flow cytometry (Calibur, BD, USA) and analysed by fluorescence-activated cell sorting (FACS) (Canto II, BD Biosciences, San Jose, CA) followed by data analysis using FlowJo 7.6 software (TreeStar, San Carlos, CA).

Assessment of systemic inflammatory response

The blood was placed in a high-speed low-temperature centrifuge at 3000 r/min at 4 °C for 10 min, centrifuged and left to stand, then the supernatant was taken by pipette, dispensed into EP tubes and stored in a −80 °C refrigerator for spare use. The supernatant was evaluated as follows: enzyme-linked immunosorbent assay(ELISA) kits (Beijing Rigor Bioscience Development LTD. Beijing, China) were used to detect the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-6, interleukin (IL)-17,and the anti-inflammatory cytokine IL-10.

H&E staining of colon and brain tissues

Alcohol was used to dehydrate the tissues after removal from the 10 % buffered formalin. Paraffin wax-embedded, dehydrated tissue sections were stained with H&E. Pathomorphologic changes in the tissue of the cerebral ischemic semidarkness zone and the tissue of the colon in MCAO rats at different time points observed with a bio-optical microscope.

Statistical analysis

Data were analyzed using SPSS software (version 17.0; SPSS Corporation, Chicago, IL, USA). Data are presented as means and standard errors of the mean (SEMs). One-way analysis of variance followed by Tukey’s honestly significant difference test was used to compare multiple groups. Significance was set at $P < 0.05$.

Result

Acupuncture improves neurologic impairment in MCAO rats

Neurologic deficits were demonstrated by neurologic function scores. Neurologic function scores were assessed using Longa’s method (Longa et al., 1989). Compared with the rats in the MCAO group, the neurological function scores of the rats in the acupuncture group decreased significantly, suggesting that acupuncture can improve the neurological deficits in MCAO rats($P < 0.05$)(Fig. 1).

Acupuncture modulates the gut microbiota of MCAO rats

PCoA of β-diversity showed that the three groups were separately clustered (Fig. 2A). The ANOSIM test of β-diversity indicated a significant difference in bacterial diversity among the three groups ($R = 0.618$, $P = 0.001$; Fig. 2B). Compared with the MCAO group, at the phylum level, the acupuncture group had decreased abundances of Proteobacteria and increased abundance of Firmicutes (Fig. 2C); at the genus level, the acupuncture group had decrease abundances of Escherichia.Shigella and increased abundance of Akkermansia(Fig. 2D).

Acupuncture changes short-chain fatty acid content and Inhibited HDAC in MCAO rats

Acupuncture significantly elevated total SCFAs content in MCAO rats (Fig. 3A). SCFAs including acetic acid, propionic acid, butyric acid, valeric acid, etc. Acupuncture significantly increases acetic acid (Fig. 3B), propionic acid(Fig. 3C), and butyric acid levels(Fig. 3D) in MCAO rats. Acupuncture significantly decreased HDAC1 and HDAC2 in MCAO rats(Fig. 3EF).

Acupuncture regulates Th17/Treg imbalance in peripheral blood of MCAO rats

A decrease in the number of Treg cells and an increase in the number of Th17 cells in the peripheral blood of MCAO rats, and therefore a rise in Th17/Treg ratio, were detected by flow cytometry. Compared with rats in the MCAO group, rats in the acupuncture group had fewer Th17 cells and a decreased Th17/Treg ratio(Fig. 4).

Acupuncture affects in vivo inflammatory response in MCAO rats

We found that the levels of inflammatory factors, including TNF-α, IL-17 and IL-6,in rats in the MCAO group were significantly higher than those in the Sham group. The levels of inflammatory factor in MCAO rats were significantly decreased after acupuncture (Fig. 5ABC). It was also found that the anti-inflammatory factor, IL-10, was significantly higher in the rats in the acupuncture group than in the rats in the MCAO group

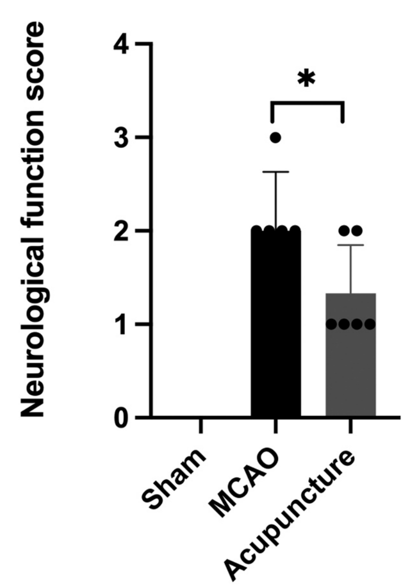

Fig. 1. Acupuncture repairs neurologic deficits in MCAO rats.

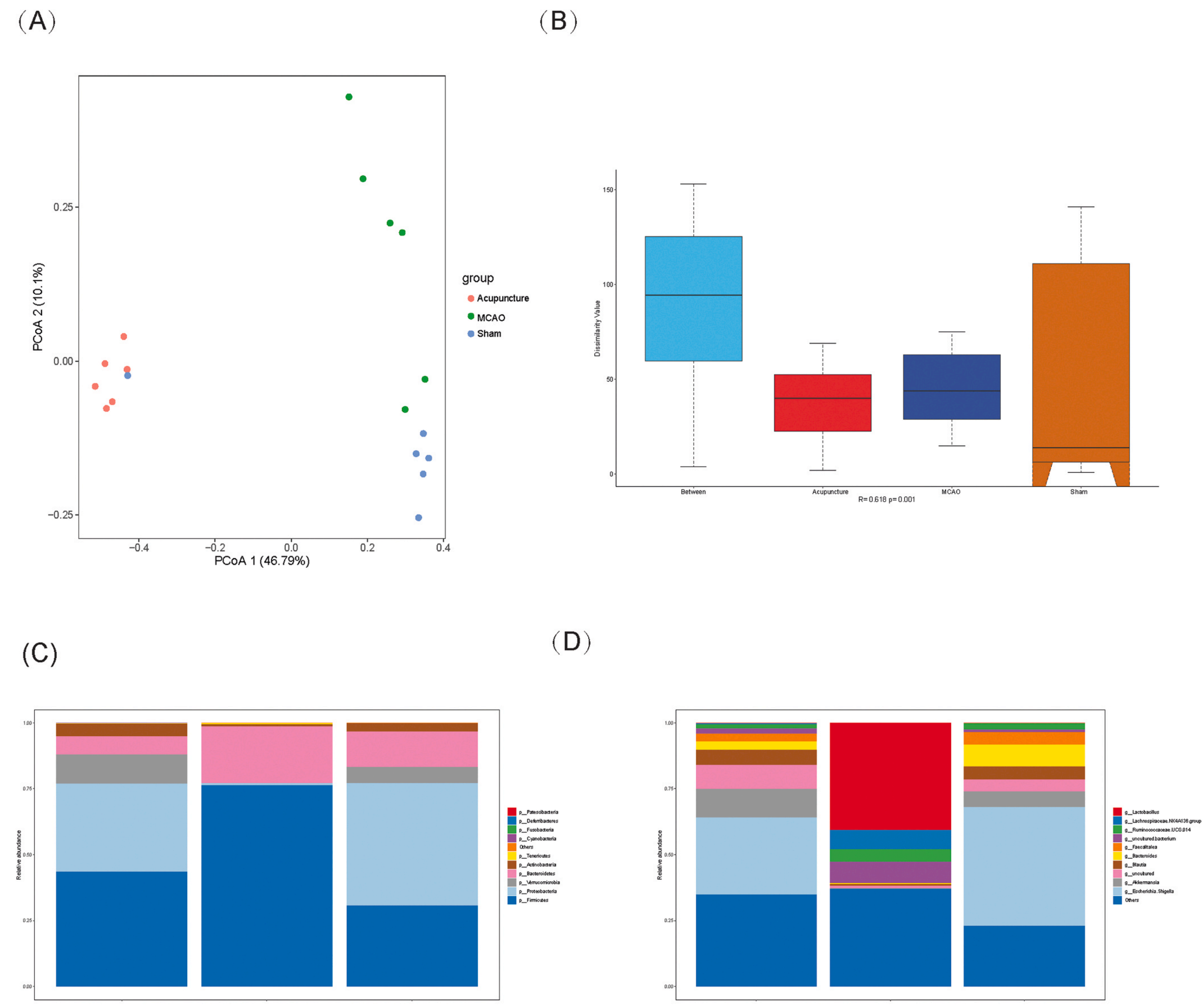

Fig. 2. Effect of acupuncture on intestinal flora of MCAO rats. (A) PCoA plot of β-diversity metrics based on the Bray-Curtis distance matrix of the relative abundances of bacterial taxa. Each point in the PCoA plot represents one sample, and samples from the different groups are shown in different colors.(B)Comparison of β-diversity differences between the sample groups using an ANOSIM test.(C,D) Microbial composition of the three groups at the phylum level and the genus level.

(Fig. 5D). These findings indicated that acupuncture regulates the release of gut inflammatory factors by regulating the abundance of certain gut bacteria.

Acupuncture protects against the intestinal pathology of MCAO rats

H&E staining confirmed that the colonic mucosal epithelium of the Sham group was intact, the intestinal glands were abundant and tightly arranged, goblet cells were abundant, and there were no evident abnormalities in cell morphology. In the MCAO group, H&E staining revealed slight mucosal epithelial cell damage, hyperchromatic nuclear pyknosis, enhanced eosinophilia in the cytoplasm, and occasional slight inflammatory cell infiltration into the lamina propria. By contrast, in the acupuncture group, the mucosal epithelium was intact, the intestinal glands were abundant and tightly arranged, goblet cells were abundant, and cell morphology was normal with no evident abnormalities(Fig. 6).

Acupuncture protects against the brain pathology of MCAO rats

H&E staining confirmed that the brain tissues of the Sham group had clear morphological layers, no necrotic nerve cells, and were neatly structured. In the MCAO group, H&E staining revealed neuronal edema, degenerative changes, atrophic necrosis, and widened cell spacing. In the acupuncture group, neuronal cell alignment disorders, cell loss, tissue vacuolation, and cytosolic consolidation were all improved to varying degrees(Fig. 7).

Discussion

Stroke is the second leading cause of death worldwide and the most common cause of disability. Stroke can be divided into hemorrhagic stroke and ischemic stroke, with ischemic stroke being more prevalent, occurring in about 87 % of stroke patients (Saini et al., 2021). It has been demonstrated that gut microbes can modulate the physiological functions of the brain through the brain-gut axis (Smith, 2015). Gut microbes has emerged as a new target for the treatment of acute ischemic stroke. The gut microbes is a microbial community that primarily inhabits the large intestine and consists of trillions of microorganisms that make up a complex ecosystem (Rajilić-Stojanović and de Vos, 2014). Gut microbes can act against infection in the host. Gut microbes have an anti-infective

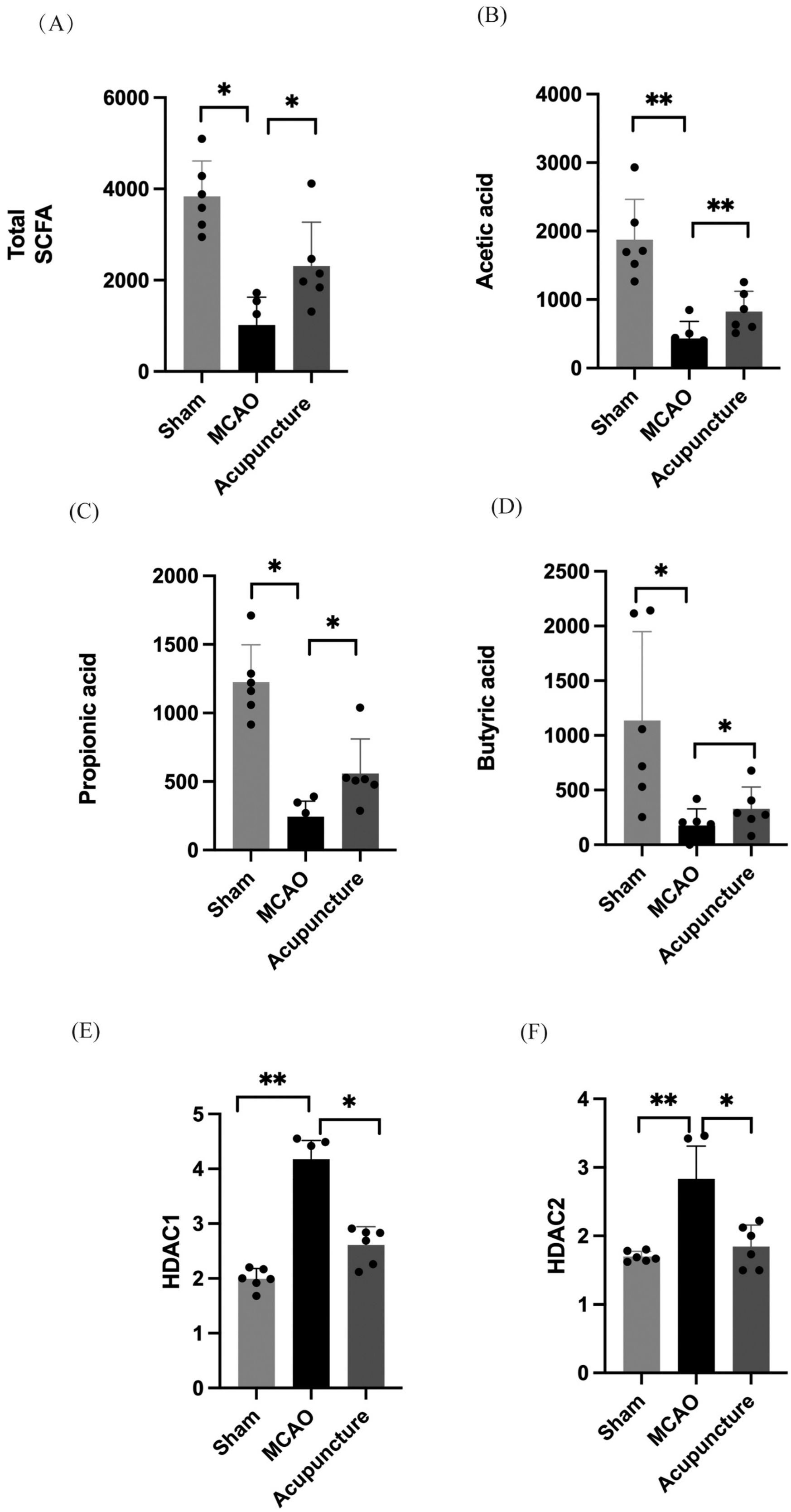

Fig. 3. Effect of acupuncture on SCFA and HDAC in MCAO rats.

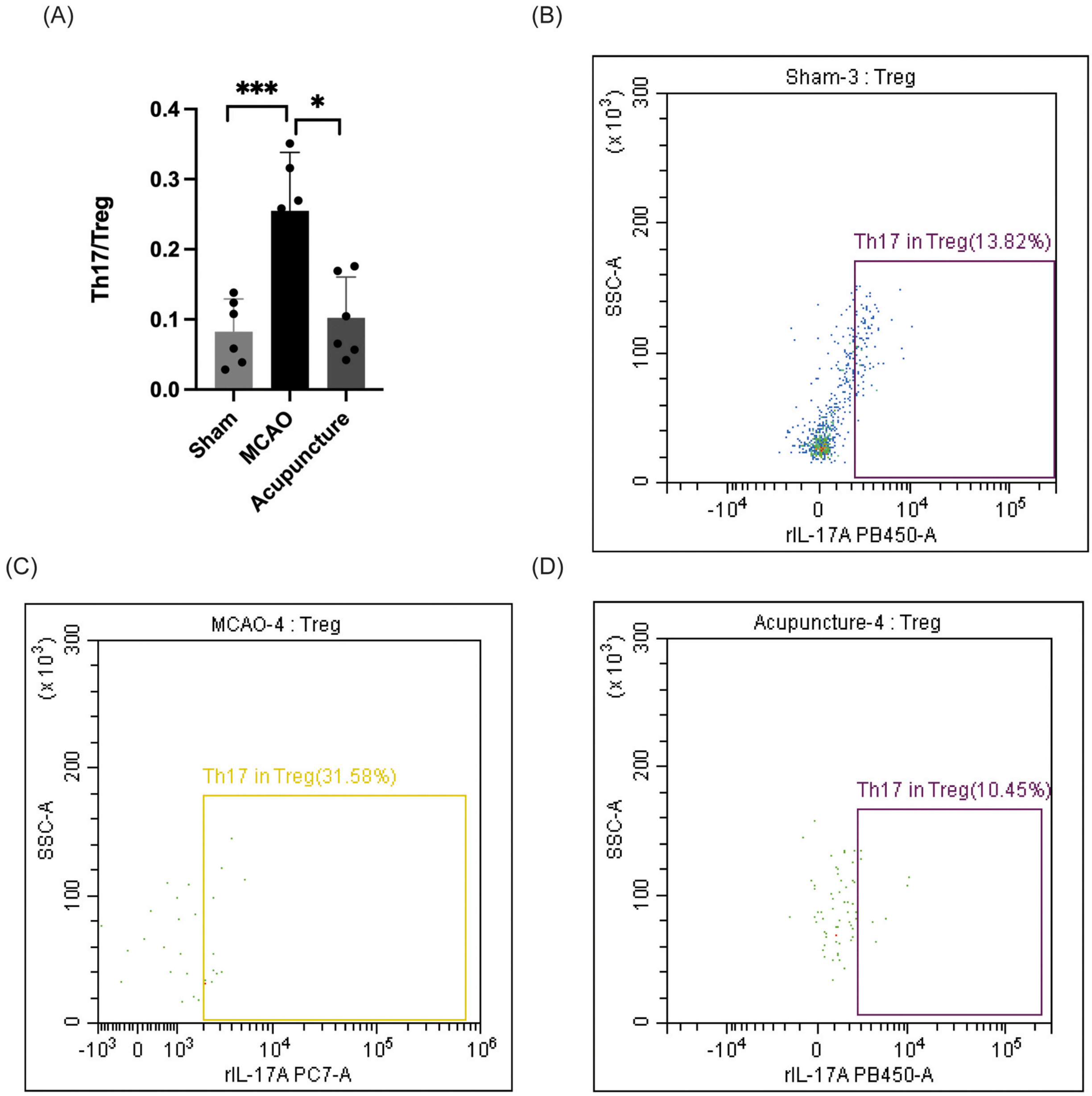

Fig. 4. Acupuncture regulates Th17/Treg balance in MCAO rats.

effect on the host, and the mechanism of action is related to the fact that metabolites of intestinal flora (SCFAs) can regulate the balance between anti-inflammatory Treg or pro-inflammatory Th17 cells (Campbell et al., 2020). Inflammatory response early in acute ischemic stroke found to be major cause of secondary brain injury (Lambertsen et al., 2019). During the hyperacute phase of ischemic stroke, the body's peripheral immune system is rapidly activated (Esposito et al., 2019). Activated immune cells trigger the release of large amounts of pro-inflammatory factors, some of which have been shown to positively correlate with cerebral infarct size (Yang et al., 2019). Treg cells and Th17 cells have a crucial role in the inflammatory response to stroke. Th17 cells are a subpopulation of CD4+ T cells that secrete the cytokine IL-17 that promotes the inflammatory response after acute cerebral ischemia and further aggravates brain injury (Mills, 2023). Treg cells can secrete anti-inflammatory factors,like IL-10,to suppress inflammatory responses after acute ischemic stroke (Duffy et al., 2018). It has been found that the imbalance between Treg cells and Th17 cells that occurs after acute ischemic stroke manifests itself as a decrease in the percentage of peripheral blood Treg cells and an increase in the percentage of Th17 cells (Dolati et al., 2018). Therefore, recent studies have proved that gut flora and immune inflammation are involved in the course of AIS,but whether acupuncture treats AIS by modulating gut flora and immune inflammation has not yet been determined. A rat model of middle cerebral artery occlusion was used in this study to investigate the effect of acupuncture and to explore the influence of intestinal immunity on this process. Our study showed that compared with the Sham group, the neurological function score of the MCAO group was increased and decreased after acupuncture. Moreover, acupuncture repaired intestinal damage in MCAO rats, increased the abundance of intestinal flora in MCAO rats, regulated immune balance, and relieved inflammatory response.

Acupuncture is an external treatment method of Chinese medicine with a long history of good efficacy for many diseases, especially for ischemic strokes. Acupuncture has been proven to be an effective treatment for ischemic stroke through numerous clinical trials (Liu et al., 2021; Li et al., 2022; Song et al., 2022). At the same time, there is a large body of research confirming that acupuncture can regulate intestinal flora disorders (Yan et al., 2023; Yao et al., 2022; Bao et al., 2023). The

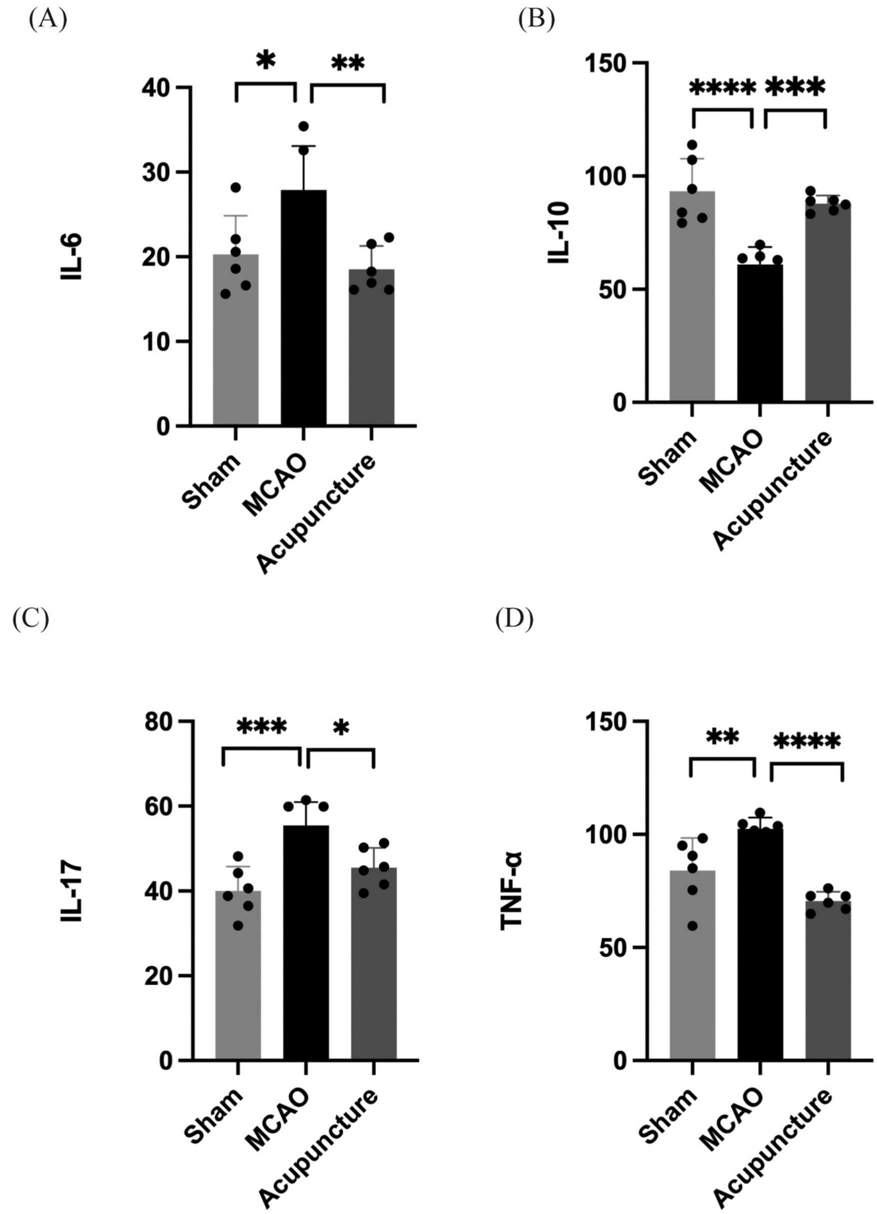

Fig. 5. Effect of acupuncture on inflammatory factors in MCAO rats.

acupuncture treatment protocol adopted in this study was scalp acupuncture with Tianshu(ST25) and Shusanli(ST36). Scalp acupuncture as a special method of acupuncture is more effective for neurological disorders (Wang et al., 2020; Lin et al., 2023) Tianshu(ST25) and Zusanli(ST36) have also been shown to improve gut bacterial dysbiosis (Wang et al., 2024). Our study confirms that such an acupuncture regimen can ameliorate neurological damage after acute ischemic stroke by regulating the gut bacteria.

Our findings suggest that the mechanism of action of acupuncture in the treatment of acute ischemic stroke may be related to the regulation by acupuncture of the disturbed intestinal flora after the onset of ischemic stroke. Our findings revealed that acupuncture could reduce the degree of brain damage in MCAO rats by regulating the intestinal flora and thereby increasing the SCFAs content in MCAO rats to inhabit HDAC further regulate the TH17/Treg ratio imbalance and thereby reducing the inflammatory response in MCAO rats.

Our results showed that at the phylum level, Proteobacteria was significantly decreased in rats in the acupuncture group compared to rats in the MCAO group. Disturbances of the gut bacterial disorders are usually caused by persistent colonization of Proteobacteria, and over proliferation of Proteobacteria is considered to be a biological marker of gut bacterial disorders (Shin et al., 2015). Firmicutes is strongly associated with the development of ischemic stroke (Lee et al., 2021). Abundance of Firmicutes found to be an independent predictor of ischemic stroke risk in clinical studies (Li et al., 2019, 2020) Elevated ratio of Firmicutes/Bacteroidetes exacerbates neurological deficits after ischemic stroke in mice and may increase mortality (Spychala et al., 2018). Our finding suggests at the genus level, the acupuncture group had decrease abundances of Escherichia.Shigella and increased abundance of Akkermansia.Akkermansia has a role in maintaining the integrity of the intestinal barrier and modulating the host immune response. A. muciniphila produces SCFAs that induce regulatory T cells and exert anti-inflammatory effects (Ottman et al., 2017). Escherichia. Shigella has been shown to have pro-inflammatory properties (Baltazar-Díaz et al., 2022). And it was found that a decrease in SCFAs-producing genera was often accompanied by an elevation in Escherichia-Shigella (Hu et al., 2022). Our study found that acupuncture reduced the abundance of opportunistic pathogenic bacteria and

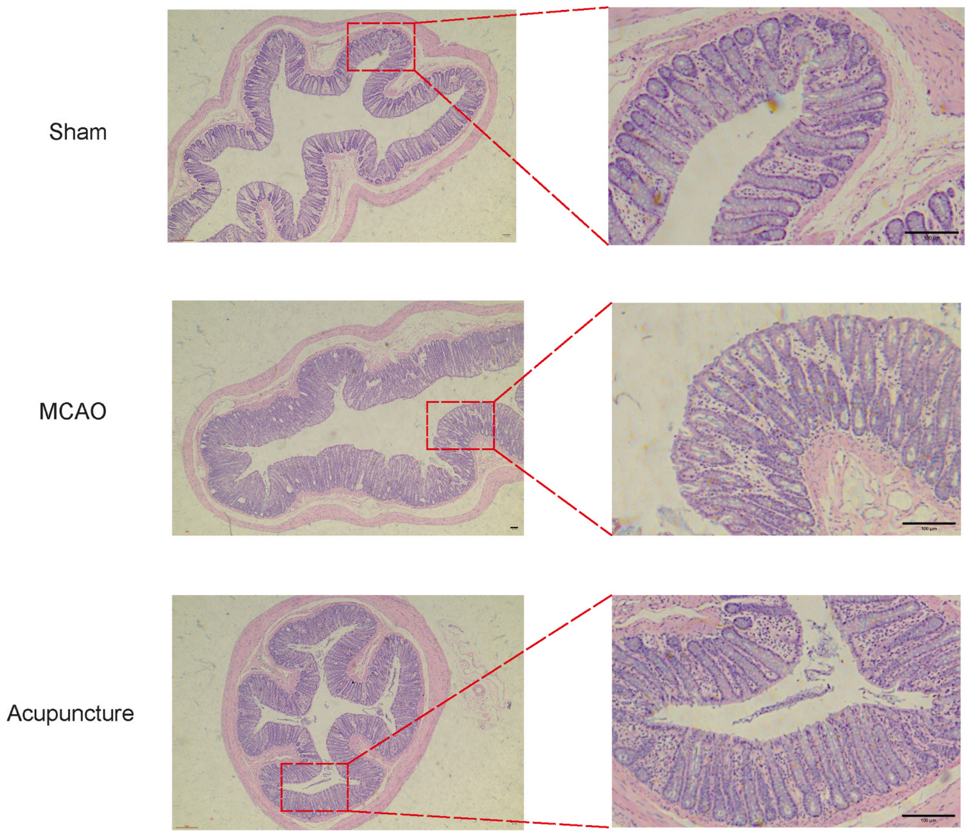

Fig. 6. Acupuncture protection against intestinal pathology of MCAO rats.

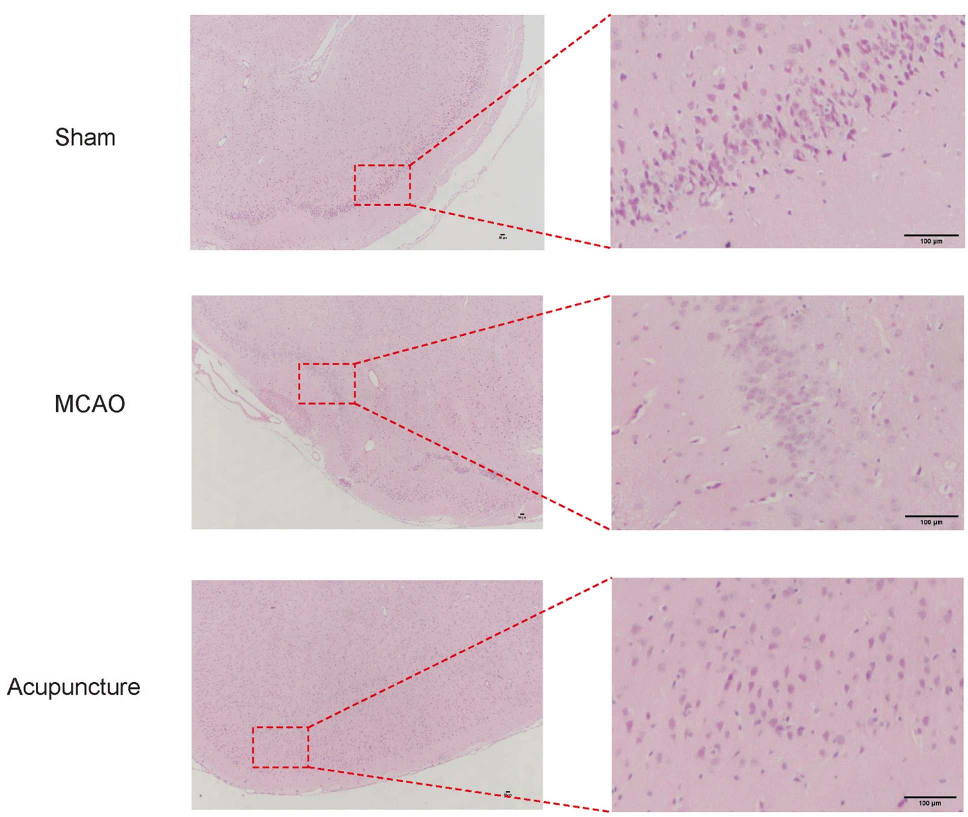

Fig. 7. Acupuncture protection against brain pathology of MCAO rats.

increased the abundance of beneficial bacteria in MCAO rats.

Our results showed that the total short-chain fatty acid content, including propionic acid and butyric acid, was significantly elevated in MCAO rats after acupuncture. Compared with rats in the Sham group, rats in the MCAO group had significantly lower levels of various SCFAs, which is consistent with the findings of other scholars. Butyric acid is a type of short-chain fatty acid, a common product of intestinal flora that can pass from the intestines into the bloodstream or brain (Bergman et al., 1990). Butyric acid is involved in apoptosis, inhibits inflammatory responses, and protects the integrity of the intestinal barrier (Tang et al., 2022). More importantly, studies have found that butyric acid can inhibit the inflammatory response in acute ischemic stroke and reduce neuronal apoptosis (Zhou et al., 2021; Duan et al., 2023). Further research has found that butyric acid improves the prognosis of acute ischemic stroke by regulating the balance of Th17/Treg ratio to achieve inhibition of inflammation, (Chen et al., 2021; Wen et al., 2021) which is also consistent with our findings.SCFAs is a pan-HDAC inhibitor and SCFA exerts immunomodulatory effects by inhibiting HDAC.Our study confirmed that acupuncture indeed significantly elevated SCFAs in MCAO rats and significantly reduced HDAC1 and HDAC2.

There are some limitations in this study.First,this study only set up one observation time point,3 days.The acute phase is often defined in animal studies as 1–3 days after onset (Sicard and Fisher, 2009).The aim of this study was to explore the mechanism of action of acupuncture on AIS.In future experiments, we will set a longer treatment duration to observe the long-term efficacy of acupuncture and explore the mechanism of action of long-term acupuncture.

Second, During the experiment, we observed the changes of stool traits in MCAO rats, but did not record them.

Conclusion

In conclusion, AIS led to a certain degree of neurologic impairment, intestinal flora disturbance and activation of immune inflammatory response. Acupuncture could improve the degree of impaired neurological function in MCAO rats by regulating the type and abundance of intestinal bacteria, increasing the content of SCFAs, inhibiting the HDAC1 and HDAC2, futhur modulating the Th17/Treg imbalance, and down-regulating the secretion of intestinal inflammation-associated factors.

Ethics approval and consent to participate

The present study was performed following the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All sections of this report adhere to the ARRIVE Guidelines for reporting animal research. All experimental protocols were approved by the Ethics Committee for Animal Experimentation of Dongzhimen hospital (approval No. 21–01, on December 11, 2020)

Consent for publication

Not appl icable.

Funding

This work was supported by National Natural Science Foundation of China (Grant Nos. 82074527)

Authors' contributions

Haoyue Yan and Yini Hua had the equal contribution to this research. Jinxia Ni is the corresponding author and she completed the project design.Haoyue Yan and Yini Huaconducted the experiments and drafted the manuscript. Xiaona Wu, Jingni Xu,and Ziniu Zhang contributed to the data analysis.Juwei Dong and Zhihao Xiong prepared experimental reagent materials.Lei Yang and Hongwei Yuan revised the manuscript. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Jingni Xu: Data curation. **Xiaona Wu:** Data curation. **Ziniu Zhang:** Data curation. **Haoyue Yan:** Writing – original draft. **Jinxia Ni:** Writing – review & editing. **Yini Hua:** Writing – original draft. **Zhihao Xiong:** Software. **Juwei Dong:** Software. **Hongwei Yuan:** Writing – review & editing. **Lei Yang:** Writing – review & editing.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgements

None.

Data availability

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

References

Baltazar-Díaz, T.A., González-Hernández, L.A., Aldana-Ledesma, J.M., Peña-Rodríguez, M., Vega-Magaña, A.N., Zepeda-Morales, A.S.M., López-Roa, R.I., Del Toro-Arreola, S., Martínez-López, E., Salazar-Montes, A.M., Bueno-Topete, M.R., 2022. Escherichia/Shigella, SCFAss, and metabolic pathways-the triad that orchestrates intestinal dysbiosis in patients with decompensated alcoholic cirrhosis from Western Mexico. Microorganisms 10 (6), 1231. Jun 16.

Bao, Q., Liu, Y., Zhang, X., Li, Y., Wang, Z., Ye, F., He, X., Xia, M., Chen, Z., Yao, J., Zhong, W., Wu, K., Wang, Z., Sun, M., Chen, J., Hong, X., Zhao, L., Yin, Z., Liang, F., 2023. Clinical observation and mechanism of acupuncture on amnestic mild cognitive impairment based on the gut-brain axis: study protocol for a randomized controlled trial. Front. Med. (Lausanne) 10, 1198579. Jun 21.

Benakis, C., Brea, D., Caballero, S., Faraco, G., Moore, J., Murphy, M., Sita, G., Racchumi, G., Ling, L., Pamer, E.G., Iadecola, C., Anrather, J., 2016. Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. Nat. Med. 22, 516–523.

Bergman, E.N., Zheng, L., Kelly, C.J., Colgan, S.P., Lu, Z., Gui, H., Yao, L., Yan, L., Martens, H., Aschenbach, J.R., et al., 1990. Energycontributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70, 567–590.

Campbell, C., McKenney, P.T., Konstantinovsky, D., Isaeva, O.I., Schizas, M., Verter, J., Mai, C., Jin, W.B., Guo, C.J., Violante, S., Ramos, R.J., Cross, J.R., Kadaveru, K., Hambor, J., Rudensky, A.Y., 2020. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. Nature 581 (7809), 475–479 (May).

Chen, Z., Wang, M., Yang, S., Shi, J., Ji, T., Ding, W., Jiang, L., Fan, Z., Chen, J., Lu, Y., 2021. Butyric acid protects against renal ischemia-reperfusion injury by adjusting the Treg/Th17 balance via HO-1/p-STAT3 signaling. Front. Cell. Dev. Biol. 9, 733308. Nov 2.

Chen, R., Xu, Y., Wu, P., Zhou, H., Lasanajak, Y., Fang, Y., Tang, L., Ye, L., Li, X., Cai, Z., Zhao, J., 2019. Transplantation of fecal microbiota rich in short chain fatty acids and butyric acid treat cerebral ischemic stroke by regulating gut microbiota. Pharmacol. Res. 148, 104403.

Chidambaram, S.B., Rathipriya, A.G., Mahalakshmi, A.M., Sharma, S., Hediyal, T.A., Ray, B., Sunanda, T., Rungratanawanich, W., Kashyap, R.S., Qoronfleh, M.W., Essa, M.M., Song, B.J., Monaghan, T.M., 2022. The influence of gut dysbiosis in the pathogenesis and management of ischemic stroke. Cells 11, 1239.

Davis, C.M., Lyon-Scott, K., Varlamov, E.V., Zhang, W.H., Alkayed, N.J., 2022. Role of endothelial STAT3 in cerebrovascular function and protection from ischemic brain injury. Int. J. Mol. Sci. 23, 12167.

Dolati, S., Ahmadi, M., Khalili, M., Taheraghdam, A.A., Siahmansouri, H., Babaloo, Z., Aghebati-Maleki, L., Jadidi-Niaragh, F., Younesi, V., Yousefi, M., 2018. Peripheral Th17/Treg imbalance in elderly patients with ischemic stroke. Neurol. Sci. 39 (4), 647–654 (Apr).

Duan, H., Hu, J., Deng, Y., Zou, J., Ding, W., Peng, Q., Duan, R., Sun, J., Zhu, J., 2023. Berberine mediates the production of butyrate to ameliorate cerebral ischemia via the gut microbiota in mice. Nutrients 16 (1), 9. Dec 19.

Duffy, S.S., Keating, B.A., Perera, C.J., Moalem-Taylor, G., 2018. The role of regulatory T cells in nervous system pathologies. J. Neurosci. Res. 96 (6), 951–968 (Jun).

Esposito, E., Ahn, B.J., Shi, J., Nakamura, Y., Park, J.H., Mandeville, E.T., Yu, Z., Chan, S. J., Desai, R., Hayakawa, A., Ji, X., Lo, E.H., Hayakawa, K., 2019. Brain-to-cervical lymph node signaling after stroke. Nat. Commun. 10 (1), 5306. Nov 22.

Fang, Z., Chen, M., Qian, J., Wang, C., Zhang, J., 2023. The bridge between ischemic stroke and gut microbes: short-chain fatty acids. Cell. Mol. Neurobiol. 43, 543–559.

Feng, Y.J., Wang, B.Q., Cao, L.L., Dong, L.Y., Zhang, C.Y., Hu, D.J., Zhou, Z., Cao, J.X., 2024. Efficacy of fire-needle therapy in improving neurological function following cerebral infarction and its effect on intestinal flora metabolites. Int. J. Gen. Med. 17, 387–399.

GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Neurol., 2021;20:795-820.

Hu, J., Cheng, S., Yao, J., Lin, X., Li, Y., Wang, W., Weng, J., Zou, Y., Zhu, L., Zhi, M., 2022. Correlation between altered gut microbiota and elevated inflammation markers in patients with Crohn's disease. Front. Immunol. 13, 947313. https://doi.org/10.3389/fimmu.2022.947313. PMID: 36045690; PMCID: PMC9420857.

Huang, Q., Xia, J., 2021. Influence of the gut microbiome on inflammatory and immune response after stroke. Neurol. Sci. 42, 4937–4951.

Jang, J.H., Yeom, M.J., Ahn, S., Oh, J.Y., Ji, S., Kim, T.H., Park, H.J., 2020. Acupuncture inhibits neuroinflammation and gut microbial dysbiosis in a mouse model of Parkinson's disease. Brain Behav. Immun. 89, 641–655.

Lambertsen, K.L., Finsen, B., Clausen, B.H., 2019. Post-stroke inflammation-target or tool for therapy? Acta Neuropathol. 137 (5), 693–714 (May).

Lee, Y.T., Mohd Ismail, N.I., Wei, L.K., 2021. Microbiome and ischemic stroke: a systematic review. PLoS One 16 (1), e0245038. Jan 13.

Li, N., Wang, X., Sun, C., Wu, X., Lu, M., Si, Y., Ye, X., Wang, T., Yu, X., Zhao, X., Wei, N., Wang, X., 2019. Change of intestinal microbiota in cerebral ischemic stroke patients. BMC Microbiol. 19 (1), 191. Aug 19.

Li, H., Zhang, X., Pan, D., Liu, Y., Yan, X., Tang, Y., Tao, M., Gong, L., Zhang, T., Woods, C.R., Du, Y., Gao, R., Qin, H., 2020. Dysbiosis characteristics of gut microbiota in cerebral infarction patients. Transl. Neurosci. 11 (1), 124–133. Jun 8.

Li, L., Zhu, W., Lin, G., Chen, C., Tang, D., Lin, S., Weng, X., Xie, L., Lu, L., Li, W., 2022. Effects of acupuncture in ischemic stroke rehabilitation: a randomized controlled trial. Front. Neurol. 13, 897078. Jun 23.

Lin, D., Gao, J., Lu, M., Han, X., Tan, Z., Zou, Y., Cui, F., 2023. Scalp acupuncture regulates functional connectivity of cerebral hemispheres in patients with hemiplegia after stroke. Front. Neurol. 14, 1083066. May 25.

Liu, H., Jiang, Y., Wang, N., Yan, H., Chen, L., Gao, J., Zhang, J., Qu, S., Liu, S., Liu, G., Huang, Y., Chen, J., 2021. Scalp acupuncture enhances local brain regions functional activities and functional connections between cerebral hemispheres in acute ischemic stroke patients. Anat. Rec. (Hoboken) 304 (11), 2538–2551 (Nov).

Longa, E.Z., Weinstein, P.R., Carlson, S., Cummins, R., 1989. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20 (1), 84–91 (Jan).

Mills, K.H.G., 2023. IL-17 and IL-17-producing cells in protection versus pathology. Nat. Rev. Immunol. 23 (1), 38–54 (Jan).

Ottman, N., Geerlings, S.Y., Aalvink, S., de V os, W.M., Belzer, C., 2017. Action and function of Akkermansia muciniphila in microbiome ecology, health and disease. Best Pract. Res. Clin. Gastroenterol. 31, 637–642.

Peh, A., O'Donnell, J.A., Broughton, B.R.S., Marques, F.Z., 2022. Gut microbiota and their metabolites in stroke: a double-edged sword. Stroke 53, 1788–1801.

Powers, W.J., 2020. Acute ischemic stroke. New Engl. J. Med. 383, 252–260.

Rajilić-Stojanović, M., de Vos, W.M., 2014. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol. Rev. 38 (5), 996–1047 (Sep).

Saini, V., Guada, L., Yavagal, D.R., 2021. Global epidemiology of stroke and access to acute ischemic stroke interventions. Neurology 97 (20 2), S6–S16. Nov 16.

Shin, N.R., Whon, T.W., Bae, J.W., 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33 (9), 496–503 (Sep).

Sicard, K.M., Fisher, M., 2009. Animal models of focal brain ischemia. Exp. Transl. Stroke Med. 1, 7. Nov 13.

Singh, V., Roth, S., Llovera, G., Sadler, R., Garzetti, D., Stecher, B., Dichgans, M., Liesz, A., 2016. Microbiota dysbiosis controls the neuroinflammatory response after stroke. J. Neurosci. 36, 7428–7440.

Smith, P.A., 2015. The tantalizing links between gut microbes and the brain. Nature 526 (7573), 312–314. Oct 15.

Song, Z., Huang, Q., Guo, Y., Song, X., Zhang, X., Xiao, H., 2022. Xingnao kaiqiao acupuncture method combined with temporal three-needle in the treatment of acute ischemic stroke: a randomized controlled trial. Comput. Intell. Neurosci. 2022, 8145374. Jun 29.

Spychala, M.S., Venna, V.R., Jandzinski, M., Doran, S.J., Durgan, D.J., Ganesh, B.P., Ajami, N.J., Putluri, N., Graf, J., Bryan, R.M., McCullough, L.D., 2018. Age-related changes in the gut microbiota influence systemic inflammation and stroke outcome. Ann. Neurol. 84 (1), 23–36. https://doi.org/10.1002/ana.25250. Epub 2018 Jul 18. PMID: 29733457; PMCID: PMC6119509.

Tang, G., Du, Y., Guan, H., Jia, J., Zhu, N., Shi, Y., Rong, S., Yuan, W., 2022. Butyrate ameliorates skeletal muscle atrophy in diabetic nephropathy by enhancing gut barrier function and FFA2-mediated PI3K/Akt/mTOR signals. Br. J. Pharmacol. 179 (1), 159–178 (Jan).

Wang, J., Tian, L., Zhang, Z., Yuan, B., Zhang, T., Li, X., Jiang, H., Du, X., 2020. Scalp-acupuncture for patients with hemiplegic paralysis of acute ischaemic stroke: a randomized controlled clinical trial. J. Tradit. Chin. Med. 40 (5), 845–854 (Oct).

Wang, J., Zhu, H., Song, X., Zhao, J., Zhang, J., Zhang, J., Li, S., Rong, P., 2024. Electroacupuncture regulates gut microbiota to reduce depressive-like behavior in rats. Front. Microbiol. 15, 1327630. Mar 27.

Wen, S., He, L., Zhong, Z., Zhao, R., Weng, S., Mi, H., Liu, F., 2021. Stigmasterol restores the balance of Treg/Th17 cells by activating the butyrate-PPARγ axis in colitis. Front. Immunol. 12, 741934. Oct 6.

Yan, X.Y., Yao, J.P., Li, Y.Q., Xiao, X.J., Yang, W.Q., Chen, S.J., Tang, T.C., Yang, Y.Q., Qu, L., Hou, Y.J., Chen, M., Li, Y., 2023. Effects of acupuncture on gut microbiota and short-chain fatty acids in patients with functional constipation: a randomized placebo-controlled trial. Front. Pharmacol. 14, 1223742. Sep 1.

Yang, C., Hawkins, K.E., Doré, S., Candelario-Jalil, E., 2019. Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. Am. J. Physiol. Cell Physiol. 316 (2), C135–C153. Feb 1.

Yao, J., Yan, X., Chen, L., Li, Y., Zhang, L., Chen, M., Li, Y., 2022. Efficacy and microrna-gut microbiota regulatory mechanisms of acupuncture for severe chronic constipation: study protocol for a randomized controlled trial. Front. Med. (Lausanne) 9, 906403. Jun 28.

Zhao, Y., Chen, F., Wu, W., Sun, M., Bilotta, A.J., Yao, S., Xiao, Y., Huang, X., Eaves-Pyles, T.D., Golovko, G., Fofanov, Y., D'Souza, W., Zhao, Q., Liu, Z., Cong, Y., 2018. GPR43 mediates microbiota metabolite SCFAs regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. Mucosal Immunol. 11 (3), 752–762.

Zhou, Z., Xu, N., Matei, N., McBride, D.W., Ding, Y., Liang, H., Tang, J., Zhang, J.H., 2021. Sodium butyrate attenuated neuronal apoptosis via GPR41/Gβγ/PI3K/Akt pathway after MCAO in rats. J. Cereb. Blood Flow Metab. 41 (2), 267–281 (Feb).