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ORIGINAL RESEARCH

Synergistic Effect of Acupuncture and Traditional Chinese Medicine on Cerebral Infarction in Rats: Roles of Short-Chain Fatty Acids and Interleukin-17

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Purpose: To investigate the synergistic effect of Acupuncture combined with Chinese Herbal Medicine (CHM) in treating cerebral infarction (CI) rats, focusing on its impact on gut short-chain fatty acids (SCFAs) and serum interleukin-17 (IL-17) expression.

Methods: 36 male SD rats were divided into 6 groups (n=6): Sham, Model, Acupuncture, CHM, Combined Therapy, and Western Medicine (positive control). The CI model was established by middle cerebral artery occlusion (MCAO). The Combined group received both acupuncture (at bilateral "Hegu" (LI4), "Taichong" (LR3), "Zusanli" (ST36), and "Fenglong" (ST40)) and CHM (oral Banxia Baizhu Tianma Decoction combined with Taoren Honghua Decoction). Treatment lasted 14 days. Neurological deficit scores (Longa and horizontal wooden stick tests) were assessed. SCFA content in colonic contents was analyzed by gas chromatography, and serum IL-17 levels by ELISA. Subsequently, the correlation between SCFAs and IL-17 levels was analyzed.

Results: The combined therapy group showed significantly better improvements in neurological function compared to all single-therapy groups ($P < 0.05$). Compared to the model group, the total content of SCFAs (including acetic acid, propionic acid, and butyric acid) was significantly lower in the model group, while IL-17 levels were significantly elevated. All treatment groups showed increased SCFA content and decreased IL-17 levels, with the combined group demonstrating superior effects compared to single therapies ($P < 0.05$). A significant negative correlation was found between total SCFAs, acetic acid, propionic acid, and butyric acid, and serum IL-17 ($R^2 = 0.601–0.711$, $P < 0.05$).

Conclusion: The combination of acupuncture and CHM significantly improved neurological deficits in CI rats. This synergistic effect is likely associated with the regulation of gut microbiota-derived SCFAs and the suppression of IL-17-mediated neuroinflammation.

Keywords: cerebral infarction, acupuncture plus Chinese herbs, short-chain fatty acids, gut microbiota, interleukin-17

Introduction

Cerebral Infarction (CI) is a significant global health concern, characterized by focal cerebral ischemia, which results in neurological deficits.[1] This phenomenon is particularly evident in China. A recent report indicated that the incidence rate of ischemic stroke among residents aged 40 and above in the country was 538.1 per 100,000, with an age-standardized incidence rate of 413.3 per 100,000.[2] In addition to the direct neurological impairment, mounting evidence underscores a pivotal bidirectional communication between the gastrointestinal tract and the brain, formally designated as the microbiota-gut-brain axis.[3] This axis plays a significant role in the pathophysiology of CI through neural, immune, and endocrine pathways.

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Short-chain fatty acids (SCFAs), including acetic acid, propionic acid, and butyric acid, are pivotal metabolites produced by the fermentation of dietary fiber by gut microbiota.[4] These cells play a crucial role in maintaining intestinal homeostasis and exerting broad anti-inflammatory effects.[5] SCFAs have been demonstrated to exert a positive influence on neurological recovery following stroke by modulating pro-inflammatory cytokine expression and enhancing neuronal plasticity.[6] Conversely, inflammatory factors have been demonstrated to play a detrimental role in CI progression.[7] Interleukin-17 (IL-17), predominantly secreted by Th17 cells, is a highly pro-inflammatory cytokine.[8] Within the context of CI, effector T cells migrate from the gut to the brain, where they secrete IL-17, leading to increased chemokine levels and aggravating ischemic neuroinflammation.[9] Research has confirmed that CI leads to alterations in the gut microbiota, promoting the migration of T cells from the gut to the pia mater, and IL-17 secretion exacerbates the neuroinflammatory response.[10] Recent studies indicate that SCFAs in the gut can suppress the activation of peripheral T cells and attenuate the activation of γδ T cells in the meninges.[11] This leads to a reduction in IL-17 concentrations within the brain. However, a critical gap remains in understanding how therapeutic interventions like acupuncture and traditional Chinese medicine, which are known to influence gut health, specifically modulate this SCFA-17 signaling axis to produce neuroprotective effects in cerebral infarction.

Acupuncture and traditional Chinese medicine (TCM) have been extensively utilized in the rehabilitation of cerebral infarction (CI), with their efficacy supported by both traditional theories and modern clinical evidence.[12,13] From a TCM perspective, CI is often attributed to wind-phlegm and blood stasis obstructing the brain's meridians. Acupuncture, particularly at acupoints such as Hegu (LI4) and Taichong (LR3) (collectively known as "Siguan"), is believed to soothe the liver, extinguish wind, and resolve phlegm, while Zusanli (ST36) and Fenglong (ST40) help strengthen the spleen and resolve phlegm.[14,15] Similarly, TCM herbal formulas like Banxia Baizhu Tianma Decoction (BBTD) and Taoren Honghua Decoction (THD) are clinically applied to address the wind-phlegm and blood stasis pattern, and have been shown to improve cerebral blood flow, promote circulation, facilitate nerve function recovery, and prevent thrombosis.[16,17]

In recent years, a growing number of clinical and experimental studies have begun to explore the combined application of acupuncture and herbal medicine. For instance, clinical evidence suggests that the combination of acupuncture with TCM formulations such as Buyang Huanwu Decoction may improve clinical outcomes and reduce recurrence rates in patients with ischemic stroke.[18] However, a recent meta-analysis has indicated that the current evidence grade for such combined therapy remains relatively low, underscoring the need for further investigation.[19] Modern pharmacological research has further revealed that bioactive components in these herbs, such as gastrodin from Gastrodia elata and quercetin from Carthamus tinctorius, exert anti-inflammatory, anti-thrombotic, and antioxidant effects.[20,21] Beyond localized actions, acupuncture may also modulate systemic pathways, including the gut–brain axis, by regulating autonomic nervous system activity (particularly the vagus nerve), thereby influencing gut permeability and systemic inflammation.[22]

Notably, both acupuncture and TCM have been shown to influence gut microbiota composition and metabolic activity. Acupuncture can enhance the diversity and metabolite profile of intestinal flora,[21] while certain TCM prescriptions promote the production of beneficial metabolites such as short-chain fatty acids (SCFAs).[23] Despite these promising findings, previous studies on combined therapy have predominantly focused on functional improvements or examined each modality in isolation. However, it remains unclear whether acupuncture and TCM herbs can act synergistically to modulate specific inflammatory pathways, which includes the SCFA-IL-17 axis. Therefore, this study aims to investigate their synergistic potential, focusing on the role of the SCFA-IL-17 pathway in the recovery process after CI.

Materials and Methods

Experimental Animals and Grouping

Thirty-six healthy 3-month-old male Sprague-Dawley rats (240–270 g) were obtained from Hunan Slaike Jingda Laboratory Animal Co., LTD. and housed at the Animal Experimental Center of Hunan University of Traditional Chinese Medicine under controlled temperature and humidity. After a 1-week acclimation period, the rats were randomly divided into six groups (n = 6 per group): sham operation, model, acupuncture, Chinese medicine, combined acupuncture and Chinese medicine, and Western medicine. A computer-generated random number sequence was used for group allocation to minimize bias and ensure that all groups were comparable at baseline.

To minimize assessment bias, the study was conducted with blinding of outcome assessors. The researchers responsible for the neurological function evaluations (Longa score and horizontal stick test), laboratory assays (SCFAs and IL-17 measurements), and data analysis were kept unaware of the group assignments throughout the experiment.

This study received approval from the Animal Experiment Ethics Review Committee of Hunan University of Traditional Chinese Medicine (approval no. LLBH202211280002). All animal procedures complied with the Guiding Opinions on the Good Treatment of Laboratory Animals issued by the Ministry of Science and Technology, PRC, 2006, the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978), and the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (2020).

Experimental Reagents and Instruments

The reagents used herein included isoflurane (provided by the Animal Laboratory of Hunan University of Traditional Chinese Medicine), penicillin sodium for injection (North China Pharmaceutical Co., Ltd.; Sinopharm approval no. H13020657;800000units), Traditional Chinese Medicine granules of BBTD and THD (provided by the Second Affiliated Hospital of Hunan University of Traditional Chinese Medicine), Bifidobacterium Lactobacillus triple viable tablets (provided by the Second Affiliated Hospital of Hunan University of Traditional Chinese Medicine), a Rat IL-17 ELISA Kit (E-EL-R0566c, 48T; Elabscience), sodium chloride (analytical grade; Sinopharm Chemical Reagent Co., Ltd)., concentrated sulfuric acid (analytical grade; Kaifeng Dongda Chemical Co., Ltd)., anhydrous ether (analytical grade; Sinophasic Chemical Reagent Co., Ltd)., 2-ethyl butyric acid (Sigma Corporation, USA), heptylic acid (Sigma Corporation), hexanoic acid (Dr. Ehrenstorfer, Germany), N-valeric acid (Sigma Corporation), isovaleric acid (Sigma Corporation), N-butyric acid (Sigma Corporation), isobutyric acid (Sigma Corporation), propionic acid (Sigma Corporation), acetic acid (Sigma Corporation), N-hexane (Chemical Pure; TEDIA Inc., USA), methanol (Chemical Pure; Fisher Scientific Inc., USA), and high-purity helium (99.9999%; Wuhan NewRED Special Gas Co. LTD).

Instruments used herein included a respiratory anesthesia machine (provided by the Animal Laboratory of Hunan University of Traditional Chinese Medicine), round stick (radius, 5 cm; length, 50 cm), disposable acupuncture needles (0.30 mm × 25 mm; Suzhou Acupuncture Supplies Co., LTD)., a micro-high-speed centrifuge (C2500-R-230V; Labnet, USA), electric thermostatic incubator (ICV-450; ASONE, Japan), automatic microplate reader (Multiskan MK3; Thermo Scientific, USA), gas chromatograph hydrogen flame detector (GC-FI-D; Agilent, USA), 7890A GC-FID (Agilent), capillary column (Agilent DB-FFAP; 30 m × 0.25 mm × 0.25 μm), centrifuge (5415R; Eppendorf), vortex (XW-80A; Shanghai Huxi Analytical Instrument Co., LTD)., and analytical balance (XP205; Metteler, Germany).

Model Preparation

The stroke models were established in the model, acupuncture, Chinese medicine, combined, and Western medicine groups. In the sham operation group, only the right neck muscles of the rats were isolated; upon exposure of the carotid arteries, the surgical incisions were sutured.

For all surgical procedures, rats were anesthetized using isoflurane (3–3.5% for induction, 2–2.5% for maintenance) delivered via a precision vaporizer in a mixture of oxygen. The depth of anesthesia was monitored by the absence of pedal and corneal reflexes. Body temperature was maintained at 37°C using a heating pad throughout the procedures to prevent hypothermia.

The cerebral ischemia model of middle cerebral artery occlusion was created using the modified method of Longa's isoline occlusion.[24] Each rat was placed in the induction box of a respiratory anesthesia machine under an induced concentration of isoflurane of 3–3.5%. Once the rat's eyes narrowed slightly and the body shook slightly, the rat was fixed on the operating table in a supine position, the nose and mouth were placed in the anesthesia mask, and the isoflurane was maintained at a concentration of 2–2.5%. Upon neck exposure and skin preparation and disinfection, a 2–3-cm incision was made at the right of the midline of the rat's neck, the neck muscles were separated, and the common carotid, external carotid, and internal carotid artery and its branches were exposed, and the occipital, superior thyroid, and Hebei palatal arteries were ligated. The internal carotid artery was clipped with a microvascular clip in the region near the heart of the Hebei palatine artery; simultaneously, the common carotid artery near the heart was clamped with a microvascular clip, the external carotid artery ligated, and the vessel away from the heart cut. An oblique incision was made about 0.4 cm from the bifurcation of the common carotid artery, the thread was inserted, the common carotid artery near the heart was ligated, the thread plug was fixed, the microvascular clip was released, and the incision was sterilized using a layer-by-layer suture. Penicillin sodium40000U/d was injected intraperitoneally for 3 days post-operative to prevent infection. On postoperative day 1, the Longa 5 score was 2–3 points, and the horizontal stick test duration was <3 min, indicating successful model creation.[25]

After the 14-day intervention, rats were euthanized for sample collection. Euthanasia was performed by an overdose of isoflurane (5%) followed by cervical dislocation.

Interventional Methods

In the acupuncture group, acupuncture was administered on the first day after successful modeling to the Hegu (LI4), Taichong (LR3), Zusanli (ST36), and Fenglong (ST40) acupoint locations of the affected limb according to Experimental Acupuncture and Moxibustion,[26] the planning textbook for colleges and universities of traditional Chinese Medicine in the new century, and the rat acupoint map of Huaxingbang.[27] The rats were restrained on the operating table in a supine position, and acupuncture was applied vertically at the LI4, LR3, ST36, and ST40 acupoints for approximately 3 mm, and then to the LR3 acupoint at a slanting angle for approximately 3 mm. The LI4, LR3, and ST40 acupoints were stimulated using the lifting and thrusting reduction method, while the ST36 acupoint was stimulated using the lifting and thrusting enhancement method. The treatment duration was 30 min. The rats were also treated with distilled water (0.01 L·kg-1·d-1) by gavage twice daily for 2 weeks.

In the Chinese medicine group, on the first day after successful modeling, the rats were subjected to the same restraint as those in the acupuncture group and administered BBTD and THD at concentrations of 0.194 g/mL (0.5 mL·100 g-1·d-1) twice a day for 2 weeks.

In the combined group, the interventional methods and treatment course were the same as those of the acupuncture and Chinese medicine groups.

In the Western medicine group, on the first day after successful modeling, the rats were treated with the same restraints as in the acupuncture group and gavaged with 63 g/L Bifidobacterium and Lactobacillus triple viable tablets solution at a dose of 0.63 g·L-1·d-1 twice a day for 2 weeks.

The rats in the six groups were fed routinely. The rats in the sham operation and model groups were treated with the same restraints and given distilled water by gavage as those in the other groups.

Observation Indices and Detection Methods

Neurological Deficit Score

The Longa 5 score and horizontal stick test were performed on all rats preoperatively and at 1 and 14 days post-operatively. For the Longa 5 score, the rats were placed in the open field, their activity state, crawling posture, and direction were observed, and they were scored according to their performance. The Longa 5-point scale was scored as follows: 0, no neurological deficit (normal); 1, failure to fully extend the contralateral forepaw (mild deficit); 2, circling to the contralateral side (moderate deficit); 3, falling to the contralateral side (severe deficit); 4, no spontaneous walking with a depressed level of consciousness.[28]

For the horizontal stick test,[25] a round wooden stick (50 cm in length, 5 cm in radius) was fixed horizontally 50 cm above a soft sponge pad to prevent injury from falling. Each rat was placed in the middle of the stick, and the duration (in seconds) it maintained its balance without falling was recorded. The maximum observation time was set at 300 seconds. A shorter duration indicates more severe motor coordination impairment. All behavioral assessments were performed by investigators who were blinded to the group assignments.

SCFAs Detected by Gas Chromatography

After the 14-day intervention, the rats in each group were anesthetized and their colonic contents collected in Eppendorf tubes, sealed, and quickly placed at −80°C for storage. An aliquot (0.1 g) of cryopreserved colonic content was precisely weighed, 500 μL of ultrapure water was added, and the mixture was vortexed for 1 min, sonicated in an ice water bath for 15 min, and left to rest for 15 min. The samples were centrifuged at13000rpm for 5 min at 4°C, and the supernatant was removed for use. Approximately 0.1 g of sodium chloride was weighed in a 1.5-mL centrifuge tube, 200 μL of sample supernatant was added and vortexed for 30s, and 20 μL of 50% concentrated sulfuric acid and 200 μL of anhydrous diethyl ether were added. The sample was vortexed with 8 μL of internal standard for 30s and shaken up and down for 1 min. The sample was centrifuged at13000rpm for 5 min at 4°C, and 80 μL of the supernatant was taken to the machine for testing.

IL-17 Detected by Enzyme-Linked Immunosorbent Assay

After the 14-day intervention, the rats in each group were anesthetized, whole blood was collected from the abdominal aorta and left at room temperature for 1 h or 2–8°C overnight, the samples were centrifuged at 1000×g for 20 min at 2–8°C, and the supernatant was removed for detection.

Statistical Methods

The data were analyzed using SPSS 26.0, and diagrams were charted using GraphPad Prism 9.5. The data were tested for homogeneity of variances and normality. Normally distributed variables are expressed as mean ± standard deviation ($\bar{x} \pm s$). Homogeneity of variance was satisfied, one-way analysis of variance was used for multiple-group comparisons, and least significant difference was used for intergroup pairwise comparisons. The Kruskal–Wallis test was used when the assumptions of data normality were not met, and the results are expressed as median (first quartile, third quartile) [M (P25, P75)]. The same group was compared before and after treatment; the paired *t*-test was used to determine if normality was satisfied, and the Wilcoxon signed-rank sum test was used to determine if normality was not satisfied. The dependent variables satisfied normality, and the relationship between the independent and dependent variables is represented by a linear regression equation. The test level was α=0.05, with values of P < 0.05 considered statistically significant.

Results

Neurological Deficit Scores

Longa 5 Score

There were no significant differences in the preoperative Longa 5 scores among all experimental groups (P > 0.05). On the first day following the establishment of the CI model, the Longa 5 scores of the model, acupuncture, Chinese medicine, combined, and Western medicine groups were significantly increased compared to the sham operation group (P < 0.05), indicating successful establishment of the cerebral ischemic model and the presence of significant neurological deficits. Although no significant differences were observed among the treatment groups and the model group on this day (P > 0.05), a significant improvement was observed after 14 days of intervention. The Longa 5 scores in the acupuncture, Chinese medicine, combined, and Western medicine groups were all significantly decreased compared to postoperative day 1 (P < 0.05). Notably, the combined group exhibited a median Longa 5 score of 0.00, demonstrating a significantly superior therapeutic effect compared to the individual acupuncture, Chinese medicine, and Western medicine groups (P < 0.05), highlighting a clear synergistic effect. Detailed data are presented in Table 1 and Supplementary Table 1.

Horizontal Stick Test

Preoperatively, there was no significant difference in the duration that rats remained on the horizontal stick among all groups (P > 0.05). On postoperative day 1, the duration for all groups except the sham operation group was significantly shortened (P < 0.05), indicating impairment of motor coordination due to cerebral infarction. Following the 14-day intervention, the duration for the acupuncture, Chinese medicine, combined, and Western medicine groups was significantly prolonged compared to postoperative day 1 (P < 0.05), suggesting a recovery of neuromotor function. The duration for the combined group (161.50 ± 28.22s) was significantly longer than that of the acupuncture, Chinese medicine, and Western medicine groups (P < 0.05), further providing strong evidence for the pronounced advantage of

Table 1 Longa 5 Scores by Study Group

Group	n	Preoperative	Postoperative Day 1	After 14-Day Treatment
Sham operation	6	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
Model	6	0.00 (0.00, 0.00)	2.00 (2.00, 3.00)[a]	2.00 (1.00, 3.00)
Acupuncture	6	0.00 (0.00, 0.00)	2.00 (2.00, 2.25)[a]	1.00 (1.00, 1.25)[b,c]
Chinese medicine	6	0.00 (0.00, 0.00)	2.50 (2.00, 3.00)[a]	1.50 (0.75, 3.00)[b,c]
Combined group	6	0.00 (0.00, 0.00)	2.00 (2.00, 3.00)[a]	0.00 (0.00, 1.00)[b]
Western medicine	6	0.00 (0.00, 0.00)	2.00 (2.00, 3.00)[a]	1.00 (1.00, 2.00)[b,c]

Notes: Values are shown as median (P_{25}, P_{75}). [a]Compared with sham operation group, $P < 0.05$; [b]Compared with postoperative day 1, $P < 0.05$; [c]Compared with the combined (acupuncture plus Chinese medicine) group, $P < 0.05$.

the combined treatment in improving neurological function. Detailed data are presented in Table 2 and Supplementary Table 2.

SCFA Content by Study Group

Contents of Total SCFAs, Acetic Acid, Propionic Acid, and Butyric Acid

TCompared to the sham-operated group, the levels of total SCFAs, acetic acid, propionic acid, and butyric acid in the colonic contents of the model group were significantly lower ($P < 0.05$; Figure 1 and Supplementary Table 3), which directly confirms that cerebral infarction induces severe dysbiosis of gut microbial metabolism. After 14 days of treatment, the concentrations of these four SCFAs in all treatment groups were significantly increased compared to the model group ($P < 0.05$; Figure 1 and Supplementary Table 3). Crucially, the combined acupuncture and Chinese medicine group exhibited a remarkable synergistic effect, with significantly higher levels of total SCFAs, acetic acid, propionic acid, and butyric acid compared to any of the monotherapy groups ($P < 0.05$; Figure 1 and Supplementary Table 3). This is the first experimental evidence demonstrating that the combined therapy has a more powerful regulatory effect on gut microbial metabolites.

Contents of Isobutyric, Isovaleric, Valeric, Hexanoic, and Heptanoic Acids

In contrast to the primary SCFAs, the concentrations of isobutyric, isovaleric, valeric, hexanoic, and heptanoic acids showed no significant changes across all experimental groups ($P > 0.05$; Figure 2 and Supplementary Table 3). This finding suggests that the interventions specifically modulated a distinct subset of SCFAs primarily associated with gut bacterial fermentation of dietary fiber.

IL-17 Contents

Compared to the sham operation group, the serum IL-17 levels in the model group were significantly increased ($P < 0.05$; Figure 3 and Supplementary Table 4), consistent with the severe neuroinflammatory response that occurs after cerebral infarction. Following the 14-day treatment intervention, the IL-17 content in the acupuncture, Chinese medicine, combined, and Western medicine groups was all significantly reduced compared to the model group ($P < 0.05$; Figure 3 and Supplementary Table 4). Notably, the combined group exhibited the most significant reduction, with its

Table 2 Horizontal Stick Durations by Study Group

Group	n	Preoperative	Postoperative day 1	After 14-day treatment
Sham operation	6	193.00±14.03	190.33±19.67	212.33±20.44
Model	6	192.00±11.03	94.83±61.27[a]	113.67±41.54
Acupuncture	6	187.17±13.34	80.50±42.17[a]	106.67±42.25[b,c]
Chinese medicine	6	188.00±23.46	93.50±43.65[a]	122.50±36.41[b,c]
Combined group	6	192.17±11.50	97.00±42.46[a]	161.50±28.22[b]
Western medicine	6	190.33±12.01	73.00±39.65[a]	104.33±49.93[b,c]

Notes: Values are shown as s, $\bar{x} \pm s$. [a]Compared with the sham operation group, $P < 0.05$; [b]Compared with postoperative day 1, $P < 0.05$; [c]Compared with the combined (acupuncture plus Chinese medicine) group, $P < 0.05$.

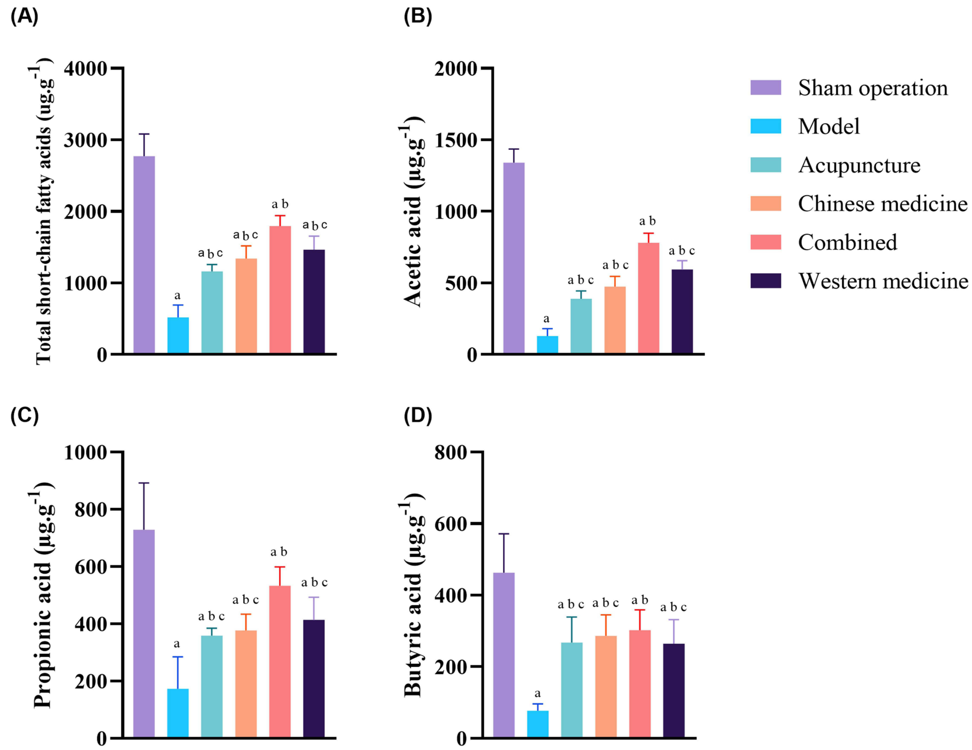

Figure 1 Contents of total short-chain fatty acids, acetic acid, propionic acid, and butyric acid by study groups. [a]Compared with sham operation group, P < 0.05; [b]Compared with model group, P < 0.05; [c]Compared with combined group, P < 0.05. (**A**) shows total fatty acids, (**B**) shows acetic acid, (**C**) shows propionic acid, and (**D**) shows butyric acid.

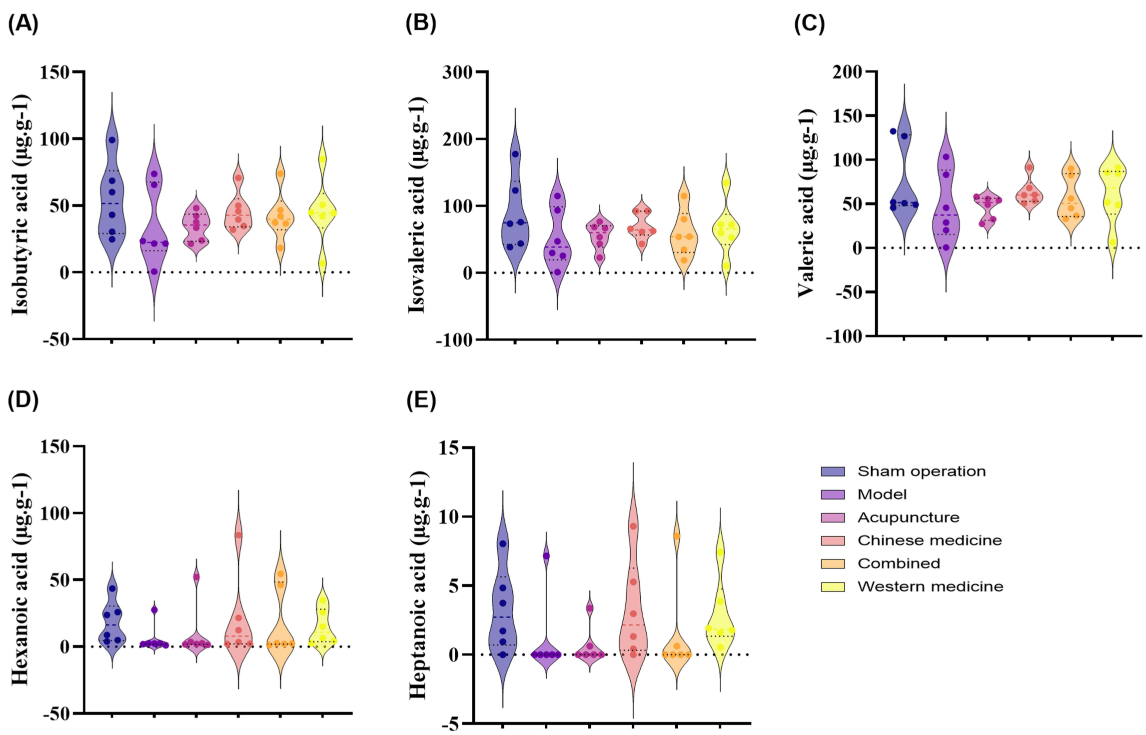

Figure 2 Contents of isobutyric, isovaleric, valeric, hexanoic, and heptanoic acids by study group. (**A**) shows isobutyric acids, (**B**) shows isovaleric acid, (**C**) shows valeric acid, (**D**) shows hexanoic acid, and (**E**) shows heptanoic acid.

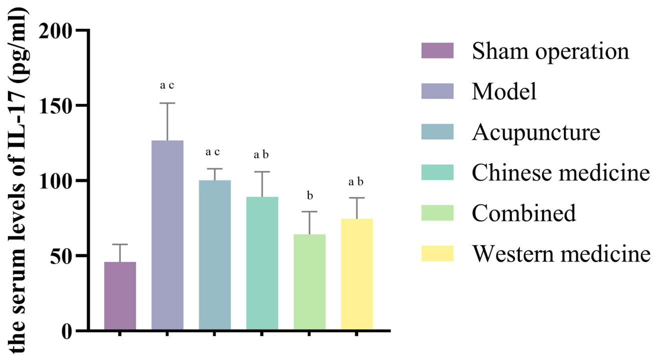

Figure 3 Interleukin-17 contents in rats by study group. [a]Compared with sham operation group, $P < 0.05$; [b]Compared with model group, $P < 0.05$; [c]Compared with combined group, $P < 0.05$.

IL-17 content being significantly lower than that of the individual acupuncture and Chinese medicine groups ($P < 0.05$; Figure 3 and Supplementary Table 4). This result indicates that the combined therapy is more effective in inhibiting the inflammatory cytokine IL-17.

Regression Analysis of IL-17 and SCFAs Contents

To investigate the relationship between SCFAs and IL-17, a regression analysis was performed. The results revealed a significant negative correlation between the levels of total SCFAs, acetic acid, propionic acid, and butyric acid and the serum IL-17 levels ($P < 0.05$; Figure 4). The regression equations and coefficients of determination (R^2) were as follows: Total SCFAs: $y = -0.036x + 137.375$, $R^2 = 0.711$; Acetic acid: $y = -0.063x + 122.754$, $R^2 = 0.663$; Propionic acid: $y = -0.122x + 135.985$, $R^2 = 0.601$; Butyric acid: $y = -0.183x + 134.113$, $R^2 = 0.614$. The highest R^2 value for total SCFAs with IL-17 indicates that changes in total SCFA levels can explain 71.1% of the variation in IL-17 levels, providing strong evidence for a causal relationship between the two. Furthermore, the contents of minor SCFAs such as isobutyric acid, isovaleric acid, valeric acid, caproic acid, and heptanoic acid showed no significant correlation with the IL-17 level ($P > 0.05$; Figure 5).

Discussion

The results of this study provide compelling evidence that the combined intervention of acupuncture and the Chinese herbal formula, BBTD plus THD, not only improves neurological outcomes but also addresses the underlying pathological mechanisms through the modulation of the microbiota-gut-brain axis. The therapeutic effect is primarily mediated by increasing beneficial gut microbiota-derived SCFAs and concurrently reducing the pro-inflammatory cytokine IL-17. This modulation of the SCFA-IL-17 axis represents a central mechanism underlying the neuroprotective effect of this combined therapy.

The selection of acupoints and the herbal formula in this study was grounded in classical TCM theory and contemporary clinical practice for the wind-phlegm and blood stasis pattern, the most common syndrome type in CI. The acupoints LI4, LR3, ST36, ST40 are frequently used in clinical CI treatment.[15,29] The pair of LI4 and LR3, collectively referred to as the "Siguan", plays a pivotal role in regulating qi and blood, calming liver wind, and unblocking the meridians.[30] Contemporary research indicates that the concurrent utilization of these acupoints may exert a synergistic influence on the autonomic nervous system, particularly the vagus nerve, which is a pivotal component of the gut-brain axis.[14] The activation of this pathway has been demonstrated to modulate intestinal permeability and anti-inflammatory responses.[31] ST36 and ST40, both located on the Stomach Meridian, work synergistically to strengthen the spleen, resolve phlegm, and regulate stomach qi.[32] This combination of acupoints is intended to address the fundamental TCM pathophysiology of CI holistically. In a similar manner, the herbal formula BBTD, in combination with THD, is recommended by the nationally planned textbook Chinese Internal Medicine for the treatment of CI characterized by wind-phlegm and blood stasis.[33] The former is chiefly responsible for the elimination of dampness and

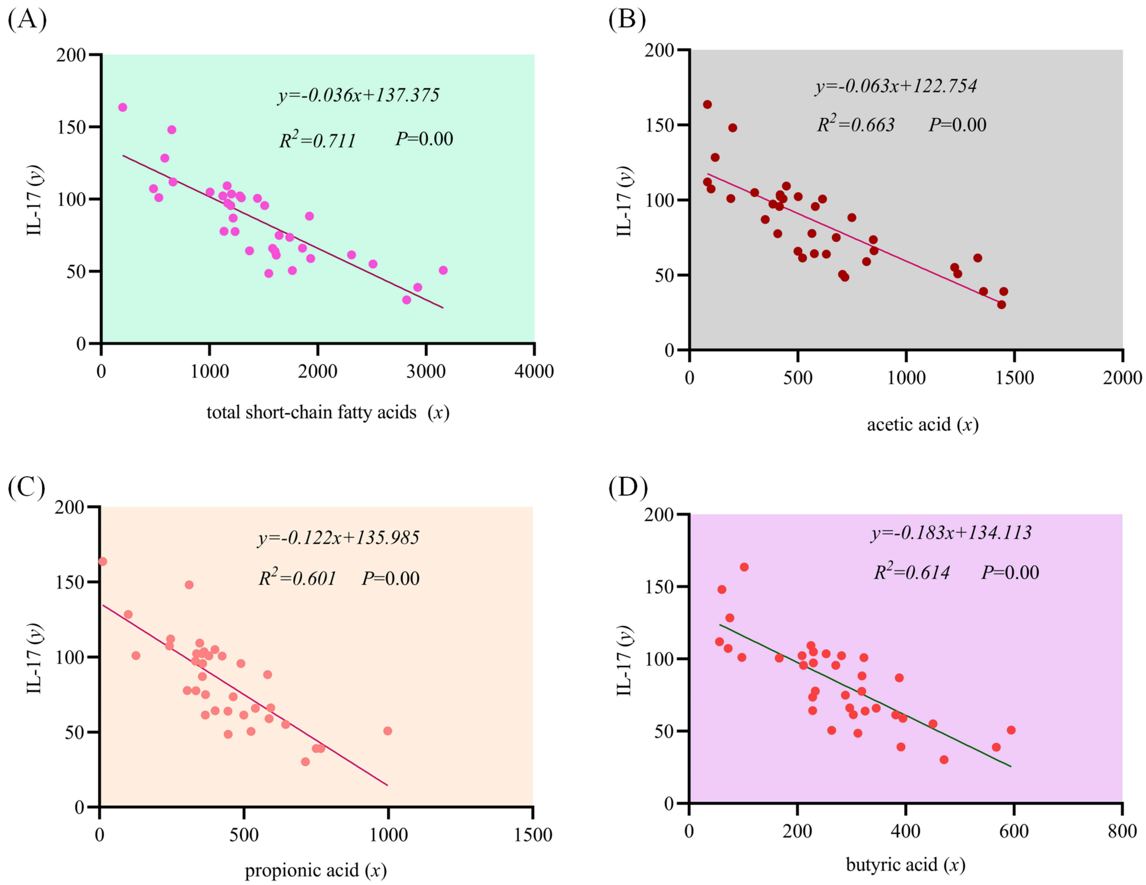

Figure 4 Effects of total short-chain fatty acids, acetic acid, propionic acid, and butyric acid on interleukin-17 (IL-17) contents. (**A**) shows effect of total short-chain fatty acids on IL-17, (**B**) shows effect of acetic acid on IL-17, (**C**) shows effect of propionic acid on IL-17, (**D**) shows effects of butyric acid on IL-17.

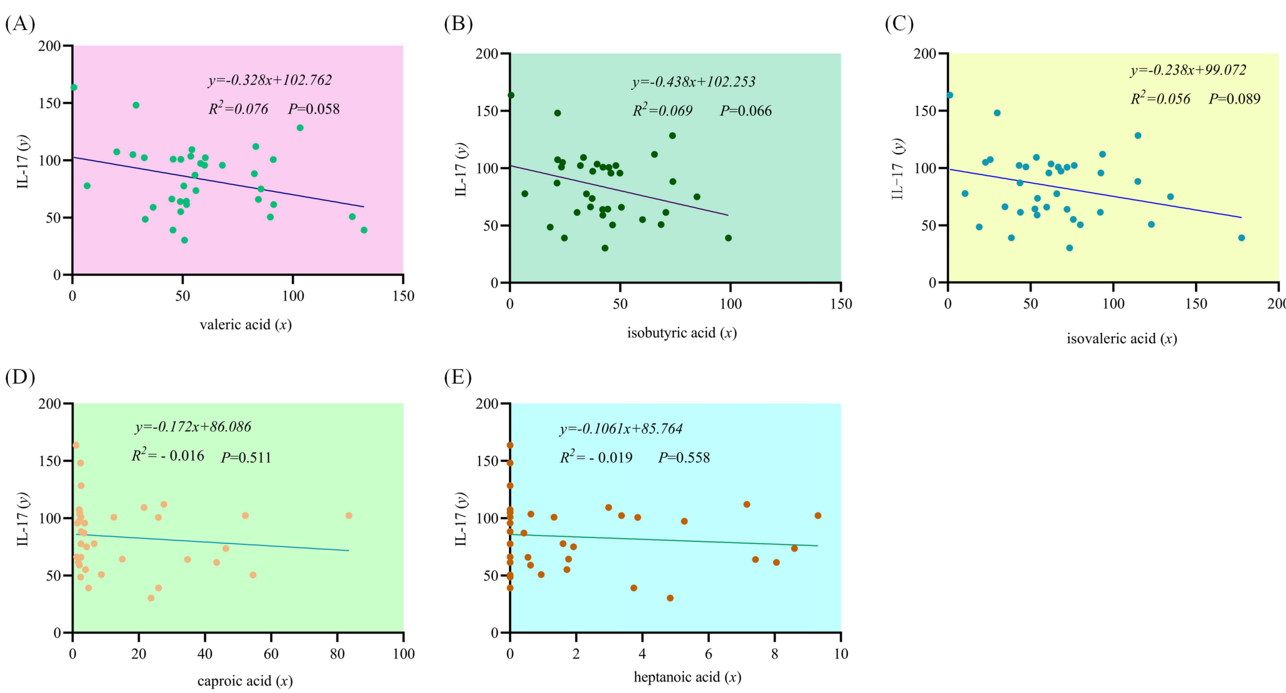

Figure 5 Effects of valeric, isobutyric, isovaleric, caproic, and heptanoic acids on interleukin-17 (IL-17) contents. (**A**) shows effects of valeric acid on IL-17, (**B**) shows effect of isobutyric acid on IL-17, (**C**) shows effect of isovaleric acid on IL-17, (**D**) shows effect of caproic acid on IL-17, (**E**) shows effect of heptanoic acid on IL-17.

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the transformation of phlegm, while the latter is known for its ability to stimulate blood circulation and resolve stasis. The combination of these elements is designed to target both the manifestations and the underlying causes of the disease. Modern pharmacological studies have confirmed that their bioactive components, including gastrodin, liquiritin, quercetin possess anti-inflammatory, anti-thrombotic, and gut-modulating properties.[34,35] These compounds likely contribute to the observed increase in SCFAs by promoting the growth of specific beneficial bacteria or directly acting on host cells to enhance SCFA production.

The pathogenesis of CI is increasingly linked to gut microbiota dysbiosis, characterized by a reduction in beneficial bacteria and an increase in pathogenic species.[36] This imbalance leads to decreased production of SCFAs and an exacerbated inflammatory response.[37,38] The results obtained in this study are consistent with those studies, as they demonstrate that rats exposed to the CI model exhibited significantly lower levels of total SCFAs, acetate, propionate, and butyrate, along with a marked increase in IL-17 levels compared to the sham group. This provides direct experimental support for the disruption of the gut-brain axis following CI.

After the 14-day intervention, neurological function, as measured by Longa scores and horizontal stick duration, was significantly improved in all treatment groups compared to the model group. Most importantly, the combination therapy group demonstrated superior recovery compared to either acupuncture or herbal medicine alone, indicating a clear synergistic effect. This clinical improvement was mirrored at the molecular level. The combined treatment group showed significantly higher levels of total SCFAs, acetate, propionate, and butyrate than the monotherapy groups, coupled with a more substantial reduction in IL-17. The regression analysis further solidified this inverse relationship, suggesting that the enhanced efficacy of the combined protocol is likely mediated through a more robust restoration of gut microbiota homeostasis, leading to increased SCFA production and subsequent suppression of IL-17-mediated neuroinflammation. The enhanced SCFA production may exert its anti-inflammatory effects by activating G protein-coupled receptors (GPCRs), such as GPR43 and GPR41, on immune cells and enteroendocrine cells.[39] This activation pathway is known to suppress the differentiation of Th17 cells and subsequently reduce IL-17 production, thereby mitigating the neuroinflammatory cascade in the brain.[40] This finding suggests a novel synergistic mechanism where acupuncture and Chinese medicine may work via complementary pathways to restore gut health and mitigate stroke-related inflammation.

The findings from this preclinical study provide a rationale for translating the combined acupuncture and CHM protocol into clinical practice. The specific acupoints (LI4, LR3, ST36, ST40) and herbal formulas (BBTD and THD) used in this study, which target the wind-phlegm and blood stasis pattern, can be directly adopted in clinical rehabilitation programs for ischemic stroke patients. Furthermore, the mechanistic insights gained here suggest that monitoring gut microbiota metabolites (eg, SCFAs) and inflammatory markers (eg, IL-17) could serve as valuable biomarkers for assessing treatment response and guiding personalized therapy. For instance, patients with evident gut dysbiosis or high inflammatory burden might benefit most from this integrative regimen. Future clinical trials should validate this combined protocol in human subjects and explore the feasibility of using SCFA profiles to predict and optimize therapeutic outcomes, thereby paving the way for a more precise, mechanism-driven application of TCM in stroke recovery.

The study presents substantial evidence in support of the synergistic effects of acupuncture and traditional TCM on cognitive improvement outcomes via the gut-brain axis. However, it is imperative to acknowledge the inherent limitations of this approach. First, while the study's sample size and observation period were sufficient to observe significant changes, they may not have fully accounted for individual variability or long-term therapeutic effects. Secondly, although the study measured systemic changes in SCFAs and IL-17, it was limited by funding constraints and did not assess local alterations in the brain or gut environment. Instead, it focused on the end products of gut-brain axis activity. Consequently, the scope of research on the gut-brain axis remains incomplete. A more comprehensive approach would entail the application of techniques such as 16S rRNA gene sequencing or metagenomics to fully characterize changes in gut microbiota composition. Moreover, the study did not delve into the molecular mechanisms through which SCFAs exert their inhibitory effect on IL-17 expression. This includes the interactions between SCFAs and specific GPCRs, as well as their role in regulating T-cell differentiation. Elucidation of the precise receptor–ligand relationships and downstream signaling pathways represents a critical direction for future research.

Conclusion

In conclusion, the study provides compelling preclinical evidence that the combination of acupuncture and the classical herbal formula exerts a synergistic effect in the treatment of cerebral infarction. This effect is likely mediated through the modulation of the gut-brain axis, specifically by increasing beneficial SCFAs and inhibiting the pro-inflammatory cytokine IL-17. These findings provide a scientific rationale for an integrative TCM approach and contribute to the growing understanding of the role of the gut microbiota in post-stroke recovery.

Data Sharing Statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The author(s) report no conflicts of interest in this work.

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