Acupoint catgut embedding improves learning and memory impairment in vascular dementia rats

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Background: Vascular dementia (VD) is a disease that affects brain function through cerebrovascular disease. Due to its complex pathogenesis, there is no effective drug treatment for VD. The present study aimed to evaluate the role of acupoint catgut embedding in the treatment of rats with VD and its possible molecular mechanism.

Methods: A modified 4 vessel occlusion (4-VO) method was used to establish a VD model rat, and spatial learning and memory ability was assessed using the Morris water maze (MWM) test. The protein expression levels were detected by Western blot. Hematoxylin and eosin (HE) staining was used for histological analysis and enzyme-linked immunosorbent assay (ELISA) was applied for analysis of serum inflammatory factors.

Results: We successfully constructed VD model rats with spatial learning and memory impairment, hippocampus injury, and high inflammatory response. Treatment of VD rats with acupoint catgut embedding significantly reduced escape latency and increased the time in the target quadrant and platform crossing times. VD-mediated hippocampal tissue damage and inflammatory reaction [down-regulating interleukin-1β (IL-1β), interleukin-6 (IL-6)] were significantly alleviated by acupoint catgut embedding treatment. In addition, further mechanism exploration found that acupoint catgut embedding treatment could improve the activity of the toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (MyD88)/nuclear factor-κB (NF-κB) signaling pathway. In summary, acupoint catgut embedding treatment improved spatial learning and memory loss, alleviated pathological damage of the hippocampus, and inhibited inflammation response in VD rats, which was probably related to the inhibition of the TLR4/MyD88/NF-κB signaling pathway.

Conclusions: Acupoint catgut embedding may warrant further study as an adjuvant therapy for the treatment of VD.

Keywords: Vascular dementia (VD); acupoint catgut embedding; learning and memory; inflammatory reaction

Introduction

Vascular dementia (VD), as the second most prevalent cognitive disorder after Alzheimer's disease (AD), is an acquired intelligence disorder syndrome caused by brain dysfunction in various cerebrovascular diseases, which leads to learning and memory disorders (1). In North America and Europe, VD cases account for about 15–20% of dementia cases, whereas in Asia and developing countries,

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the proportion is slightly higher, about 30% (2,3). The incidence rate of VD in Asia continues to rise, which not only increases the economic burden of individuals, but also represents a huge challenge to the medical insurance system of Asian countries. The pathogenesis of VD has not been fully elucidated. Currently, it is known that the imbalance of choline metabolism, synaptic damage, oxidative stress damage, nervous system inflammation, etc., cause changes in the morphological structure of neurons, neurological dysfunction, and ultimately lead to the occurrence and development of VD. Therefore, the research on VD, especially the research on the changes of neural function and morphology caused by it is one of the key points of basic and clinical researchers. Meanwhile, timely prevention and early intervention of VD are of great significance to conserve public medical resources.

At present, the treatment methods of VD in modern medicine mainly include brain metabolism reactivating agents such as cerebral rehabilitation, brain nerve protective agents such as calcium antagonists and combined rehabilitation techniques, while traditional Chinese medicine (TCM) will apply some Chinese medicine or acupuncture treatment according to the specific symptoms of patients and achieve certain clinical effects. Compared with other treatment methods, acupoint catgut embedding has unique advantages, including time saving, low medical costs, simple and convenient operation, no liver or kidney toxicity, low incidence of adverse reactions, mild symptoms and so on. Acupoint catgut embedding therapy refers to the use of sterile tweezers to put a 3-0 catgut (1–1.5 cm) into the needle tip of No. 9 disposable sterile needles, the catgut is parallel with the inner edge of the needle tip, and the needle is followed by a blunt acupuncture needle, inserting the sterile needle into the disinfected acupuncture points. After obtaining the sensation, the acupuncture needle is pushed in while withdrawing the sterile needle, leaving the catgut within the acupuncture point (4). Researches have shown that embedding catgut in specific parts of the skin (acupoint catgut embedding therapy) can continuously generate benign stimulation, affect neurotransmitters and local circulation, repair damaged neural loop networks, restore normal central function, regulate immunity, and inhibit the release of inflammatory factors (5-7). Besides, our early works and others have demonstrated that inflammation plays an important role in the development of VD (8-11). Toll-like receptor 4 (TLR4) is a kind of toll-like receptor (TLR), which is an important pattern recognition receptor expressed in innate immune cells. Numerous studies have shown that nerve inflammation mediated by TLR4 activation plays a key role in stroke, AD, and other neurological diseases at the onset of systemic disease. Due to the complex mechanism involved in the pathogenesis, the treatment is controversial, leading to different roles in the study of the neurological inflammatory disease (12-14). Therefore, we established a VD rat model through 4 vessel occlusion (4-VO) operation. Then, we treated the VD rats with acupoint catgut embedding and explored whether the TLR4/myeloid differentiation factor 88 (MyD88)/nuclear factor-κB (NF-κB) signaling pathway is involved in the role of acupoint catgut embedding in treating VD and its mechanism. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6402/rc).

Highlight box

Key findings
- Acupoint catgut embedding may warrant further study as an adjuvant therapy for the treatment of vascular dementia (VD).

What is known and what is new?
- The study showed that acupoint catgut embedding therapy in specific parts of the skin can continuously generate benign stimulation, affect neurotransmitters and local circulation, repair damaged neural loop networks, restore normal central function, regulate immunity, and inhibit the release of inflammatory factors.
- We further treated VD rats with acupoint catgut embedding and explored whether the TLR4/MyD88/NF-κB signal pathway is involved in the role of acupoint catgut embedding in VD and its mechanism.

What is the implication, and what should change now?
- This study offers a new potential way to treat vascular dementia and may also reduce a heavy burden on the public health system.

Methods

Materials

Rabbit anti-TLR4 antibody (BS-20594R), rabbit anti-MyD88 antibody (BS-1047R), and rabbit Anti-NF-κB p65 antibody (BS-0465R) were obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Rabbit anti-β-actin antibody (AC026) and Goat Anti-Rabbit IgG (ab6721) were purchased from ABclonal (Wuhan, China) and Abcam (Cambridge, UK), respectively. Nimodipine tablets (190309, Yabao Pharmaceutical, Yuncheng, China); Ultra RNA Kit (CW0581M1, CWBIO, Beijing, China); HiScript II One Step qRT-PCR SYBR

Green Kit (Q221-01, Vazyme, Nanjing, China); and Phenol: chloroform: isoamyl alcohol 25:24:1 (P1012, Solarbio, Beijing, China). Hematoxylin dye (BA4041) and eosin dye (BA4022, Zhuhai Beso, Zuhai, China). Nitrocellulose (NC) membrane (Bio-Rad, Hercules, CA, USA). Vertical electrophoresis tank (JY-SCZ4+), electrophoresis instrument (JY200C) (Beijing Junyi, Beijing, China); horizontal decolorization shaker (TY-80A, Jiangsu Keyan, Nanjing, China); chemiluminescence gel imager (5200, Tianneng Technology Co., Ltd., Taiyuan, China); full function microplate reader (MK3, Thermo Waltham, MA, USA); high speed low temperature centrifuge (H2050R, Xiangyi Group, Shanghai, China); real time fluorescence quantizer [QuantStudio 1, Applied Biosystems (AB), Forster City, CA, USA]; TRIzol Reagent: IL-6 ELISA kit (PI328, Beyotime, China); IL-1β ELISA kit (PI303, Beyotime, China).

Animals

This study was approved by the Ethics Committee of Guizhou University of Traditional Chinese Medicine (No. 20221011). All experimental processes were in accordance with the regulations of the Experimental Animal Ethics Committee of Guizhou University of Traditional Chinese Medicine for the care and use of animals. A protocol was prepared before the study without registration. A total of 93 healthy, specific-pathogen-free (SPF) male Sprague-Dawley (SD) rats (10–12 weeks old, 300–320 g) were purchased from Changsha Tianqin Biotechnology Co., Ltd. (Changsha, China), experimental animal production license No. SCXK (Xiang) 2019–0014.The rats were housed under the conditions of 22–26 ℃, 50% humidity, and 12 h/12 h light-dark cycle. They had free access to water and food.

VD rat model establishment and grouping treatment

After a week of acclimatization, the rats were randomly divided into 5 groups: Sham group (n=12), model (Mod) group (n=15), acupoint catgut embedding group (Ace; n=15), non-acupoint catgut embedding group (N-ace; n=15), and nimodipine group (Nmp; n=15). The VD rat model was prepared by modified 4-VO method with reference to a previous study (15). Specifically, rats were anesthetized intraperitoneally with 40 mg/kg pentobarbital sodium and then fixed on the operating platform in a prone position. A longitudinal incision of 1–2 cm was made at the level of the first cervical vertebra in the occipital region to expose the pterygoid foramen of the first cervical vertebra. The incision

was sutured after cauterizing bilateral vertebral arteries 4–5 times with an electric coagulation needle. A longitudinal incision of 1.0–1.5 cm was made in the anterior and middle part of the neck. About 1 cm of bilateral common carotid arteries were separated from both sides of the trachea, and thread was used for standby. The next day, the cervical suture was removed, the common carotid arteries on both sides were clipped with minimally invasive artery clips, and the artery clips were released 15 minutes later to restore blood supply. The incision was sutured, and the rats were fed in clean cages. The Sham group was anesthetized and fixed in the same way to separate the common carotid artery, but the vertebral artery was not burned, the common carotid artery was not clamped, and only the external opening of the pterygoid foramen was exposed. The incision was sutured after operation. Then, the rats were returned to their cages, and fed normally after waking up. The criteria for successful model establishment were as below: loss of consciousness, coma, disappearance of righting and repositioning reflex, gradual pallor of both eyes, bilateral pupil dilation, and disappearance of light reflex after clamping bilateral common carotid arteries. In the experiment, if the above criteria were not met or the rats experienced convulsions and death, the modeling was deemed a failure. Among the total of 93 rats initially included in the experiment, 2 rats died due to modeling, 1 rat did not meet the criteria of modeling, and 90 rats were included in the experiment.

Ace group: on the 8th day after the successful modeling, catgut embedding therapy was carried out at the acupoints of "Baihui" (GV20), "Qihai" (CV6), "Tanzhong" (CV17), "Sanyinjiao" (SP6), and "Geshu" (BL17). Hair removal and skin preparation were performed at the acupoints with electric push scissors. Catgut with a length of about 1.5 cm was taken and buried into the above acupoints with a size 7 disposable catgut embedding needle (Zhenjiang Gaoguan Medical Instrument Co., Ltd., China). The site of needle insertion was pressed with a sterilized dry cotton ball for 1 minute after the embedding. The buried line was replaced once every 15 days, 3 times in total. N-ace group: the catgut embedding treatment was applied at 0.5 mm below the acupoint taken by the acupoint catgut embedding group, once every 15 days, 3 times in total. Nmp group: nimodipine solution was given by gavage for 45 consecutive days at a dose of 20 mg/(kg·d) once a day. The model group, the sham group, the Ace group, and the N-ace group were given 0.9% sodium chloride solution by gavage at the same time, and the drug was administered from the 8th day after the model establishment for 45 consecutive days.

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Spatial learning and memory ability estimated by the Morris water maze (MWM)

The MWM consisted of a circular tank with depth of 35 cm, height of 50 cm, and diameter of 200 cm which was divided into 4 quadrants. The MWM includes 5 days of orientation navigation tests and a 1-day spatial probe test. During the orientation navigation tests, the platform was positioned at the center of a quadrant, 1.5 cm underwater, and the rats were placed in the water from 3 to 4 entry points and allowed to explore the maze and find the platform for 90 seconds. Upon failure to locate the platform, rats were guided to the platform and allowed to stay there for 10 seconds. Records the time spent searching for the escape platform (the escape latency) The training process was repeated for 5 days. During the spatial probe test, the underwater platform was removed, and rats were placed in the water at the R point. Collect and analyze the number of times that the rats entered the platform quadrant and crossed the original platform within 90 seconds (16).

Hippocampal pathomorphology assessed by HE staining

Anesthetized rats were intraperitoneally injected with 0.9% normal saline and 4% paraformaldehyde (PFA) successively. After the perfusion, they were fixed in 4% PFA. The brain of rats was taken for paraffin section. To investigate the pathomorphology in the hippocampus, sections were stained with hematoxylin and eosin (HE) staining solution. After dehydration and paraffin-embedding, rat brain tissue was sectioned to 3 mm slices, and slices were dried and dewaxed, then rinsed with distilled water, stained with hematoxylin, separated with 1% hydrochloric acid alcohol, deionized rinsed, and stained with eosin. Finally, they were processed with 80%, 95%, and 100% ethanol, and xylene I, xylene II in turn. The slices were sealed with neutral gum and the pathological changes were observed under an inverted microscope (BX53 Olympus, Tokyo, Japan).

Immunoblotting

Complete hippocampal tissues of rats were taken and added to radioimmunoprecipitation assay (RIPA) lysate. These were then ground repeatedly in the biological sample homogenizer, placed on ice for 10 minutes, and centrifuged at 4 ℃ at 12,000 r/min for 15 minutes. The supernatant was collected then mixed 120 µL protein supernatant and 30 µL 5× loading buffer. The mixture was boiled in boiling water for 5 minutes. The 10 µL protein samples were separated by 8%/11% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and subjected to electrophoresis at constant voltage of 73 V for 130 minutes; the electrophoresis was discontinued when bromophenol blue electrophoresis reached the bottom of the gel. The protein was electrically transferred to nitrocellulose (NC) membrane and blocked overnight at 4 ℃ with 5% skimmed milk in phosphate-buffered saline with Tween 20 (PBST). Then, the membranes were incubated with the primary antibodies including anti-TLR4 (1:2,000), anti-MyD88 (1:1,000), anti-NF-κB p65 (1:1,000), and anti-β-actin (1:100,000) overnight at 4 ℃. After being washed 3 times (10 minutes each time) with PBST, the membranes were incubated with secondary antibody (1:5,000) for 80 minutes at room temperature. Finally, ChemiDoc XRS (Bio-Rad, USA) was used to scan the membrane added with exposure liquid, and Image Lab software (Bio-Rad) was used to quantify the images.

Detection of messenger RNA expression of TLR4, MyD88, and NF-κB in hippocampus tissues by real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from hippocampal tissue collected from rats using TRIzol reagent. The complementary DNA (cDNA) was obtained by PrimeScript RT reagent Kit (RR047A, Takara, Shiga, Japan) using RNA as a template. The messenger RAN (mRNA) levels of TLR4, MyD88, and NF-κB measured by RT-qPCR according to the TB Green TM Premix Ex TaqTM II [Tli RNaseH Plus (RR820A, Takara, Japan)] instructions and normalized to β-actin. The primer sequences were designed by Primer 5 software and are displayed in *Table 1*. The results were analyzed by the relative quantitative $2^{-\Delta\Delta Ct}$ method.

Detection of serum interleukin-6 (IL-6) and IL-1β levels of rats by enzyme-linked immunosorbent assay (ELISA)

A total of 6 rats were anesthetized by intraperitoneal injection with 40 mg/kg pentobarbital sodium, The cytokines (IL-6, IL-1β, and IL-10), which contribute to inflammation, were assessed using an ELISA kit (IL-1β ELISA KIT:SEKR-0002, Solarbio; IL-6 ELISA KIT:SEKR-0005 Solarbio) based on the manufacturers protocol. A microplate reader (Thermo Fisher, USA) was utilized to detect the absorbance at 450 nm.

Statistical analysis

All values were presented as mean ± standard error of the mean (SEM). Data statistical analysis was performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to compare the differences between groups. A P value <0.05 was considered a significant difference. Results were verified by at least 3 independent experiments.

Results

Acupoint catgut embedding alleviates spatial learning and memory impairment in VD rats

In order to clarify the role of acupoint catgut embedding in VD rats, we established a VD rat model by 4-VO surgery.

Table 1 Nucleotide sequences of the primers used for RT-qPCR

Gene	Primer sequence (5' to 3')
β-actin	Forward: GAAGATCAAGATCATTGCTCC
	Reverse: TACTCCTGCTTGCTATCCA
TLR4	Forward: TTGCTGCCAACATCATCCGGAAG
	Reverse: CAGAGCGGCTACTCAGAAACTGC
MyD88	Forward: ATACGCAACCAGCAGAACAGGAG
	Reverse: GGTGATGCCTCCCATTCCTTTG
NF-κB	Forward: TGGCTACACGGGACCAGAACAGT
	Reverse: GGCTTGCTCCAGTCTCGCTTCTT

RT-qPCR, real-time quantitative polymerase chain reaction; TLR4, toll-like receptor 4; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB.

We foremost investigated spatial learning and memory ability of rats in each group via the escape latency time, time spent in the target quadrant, and times of platform crossing in the MWM experiment. The results showed that the time of the Mod group was significantly longer compared with that of the Sham group in the escape latency during 2–6 days of training (P<0.01), but the mean escape latency of the rats in Ace and Nmp groups was significantly shorter than that of the Mod group (P<0.05), no significant difference in escape latency was observed between N-ace group and Mod group (*Figure 1A*). In addition, the time spent in target quadrant of the Mod group was obviously shorter than that of the Sham group, but those rats in the Ace (P<0.05) and Nmp (P<0.05), not N-ace group, had significantly increased times spent in target quadrant compared to the Mod group rats (*Figure 1B*). Meanwhile, platform crossing times of rats in the Mod group were less than those of rats in the Sham group, and greater than those in the Ace, Nmp (P<0.05) and N-ace (P<0.05) groups (*Figure 1C*). These results indicated that we had successfully established the VD rat model of spatial memory and learning impairment. Acupoint catgut embedding could significantly improve the spatial memory and learning dysfunction of VD rats, whereas non-acupoint catgut embedding had a weak effect on the improvement of related functions of VD rats.

Acupoint catgut embedding reduced the pathological damage of hippocampal neurons in VD rats

We further evaluated the pathological changes of rat hippocampal neurons through HE staining, the staining results are shown in *Figure 2*. Sham group rat hippocampal pyramidal cells were found to have complete form and
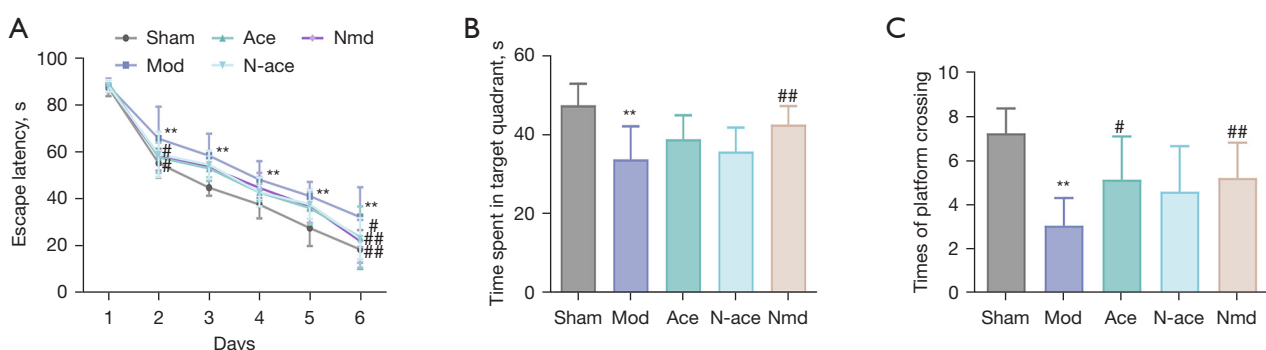
Figure 1 Acupoint catgut embedding alleviates spatial learning and memory impairment in vascular dementia rats. **, P<0.01 compared with Sham group, ##, P<0.01 and #, P<0.05 compared with Mod group.
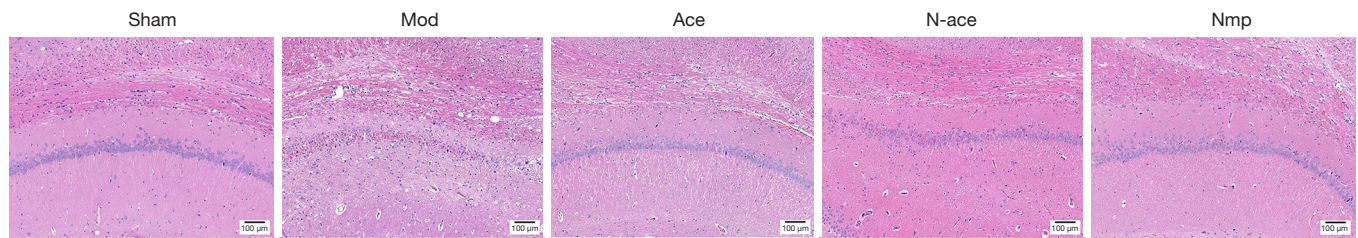
Figure 2 Pathological change of rat hippocampus were detected by HE staining (100×). Scale bar =100 μm. HE, hematoxylin and eosin.
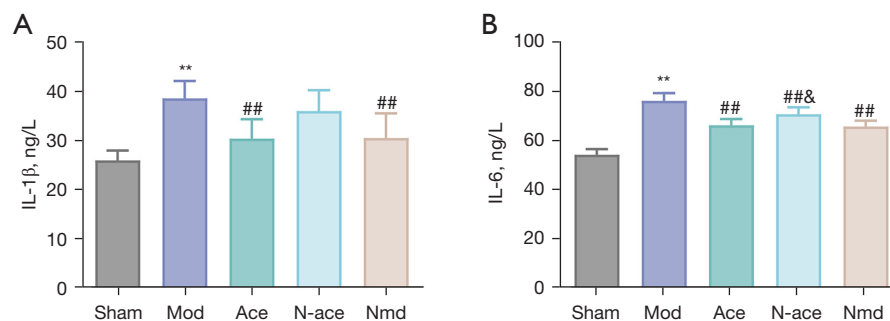
Figure 3 The serum levels of IL-1β (A) and IL-6 (B) in rats were measured using ELISA, **, P<0.01 compared with Sham group; ##, P<0.01 compared with Mod group; &, P<0.05 compared with Ace group. IL-1β, interleukin-1β; IL-6, interleukin-6; ELISA, enzyme-linked immunosorbent assay.

structure, clear edges, neat and close arrangement, uniform dyeing, large nuclei (approximate circle), obvious nucleolus, and abundant cytoplasm. However, the morphology of hippocampal pyramidal cells in the Mod group was irregular and loose, with blurred cell boundaries, and relatively disordered arrangement; a decreased number of normal cells and necrotic neurons were observed, the cell membrane and nuclear envelope were unclear, and the nucleus was irregularly shaped. The color became light, the nucleolus was concentrated, the staining was deep, and the cytoplasm was cloudy. These results suggest that there was obvious histopathological injury and cell necrosis in the hippocampus of VD rats. In addition, we found that hippocampal pyramidal cells in the Ace and Nmp group had regular morphology, clear boundaries, clear laminations, and orderly arrangement. Compared with the VD model, the number of normal cells was significantly increased, the number of necrotic neurons was decreased, the shape of the nucleus was more regular, the nuclear envelope and nucleolus were clearer, and the cytoplasm was more abundant. The HE staining results showed that acupoint catgut implantation alleviated the pathological damage of hippocampus in rats with VD.

Acupoint catgut embedding decreases the levels of serum IL-1β and IL-6 in VD rats

To examine the effects of Acupoint catgut embedding on VD-induced inflammation *in vivo*, the levels of serum IL-1β and IL-6 in rats were determined by ELISA and the results are presented in *Figure 3A,3B*. The levels of hippocampus IL-1β (38.5 *vs.* 25.9 pg/L) and IL-6 (75.9 *vs.* 54 pg/L) in the Mod group were markedly increased than those in rats in the Sham group. The hippocampus IL-1β of rats in the Ace, Nmp, and N-ace group decreased to 30.3, 30.4, and 35.9 pg/L, respectively. Meanwhile, the serum IL-6 of rats in the Ace, Nmp, and N-ace groups decreased to 65.9, 65.5, and 70.5 pg/L, respectively. These results suggested that acupoint catgut embedding could inhibit the serum levels of IL-1β and IL-6 in VD rats.

Acupoint catgut embedding inhibited the TLR4/MyD88/NF-κB signaling pathway in VD rats

To preliminarily explore the molecular mechanism of acupoint catgut embedding's anti-inflammatory role in VD rats, we evaluated whether acupoint catgut embedding exerted effect associated with downregulating the TLR4/MyD88/NF-κB
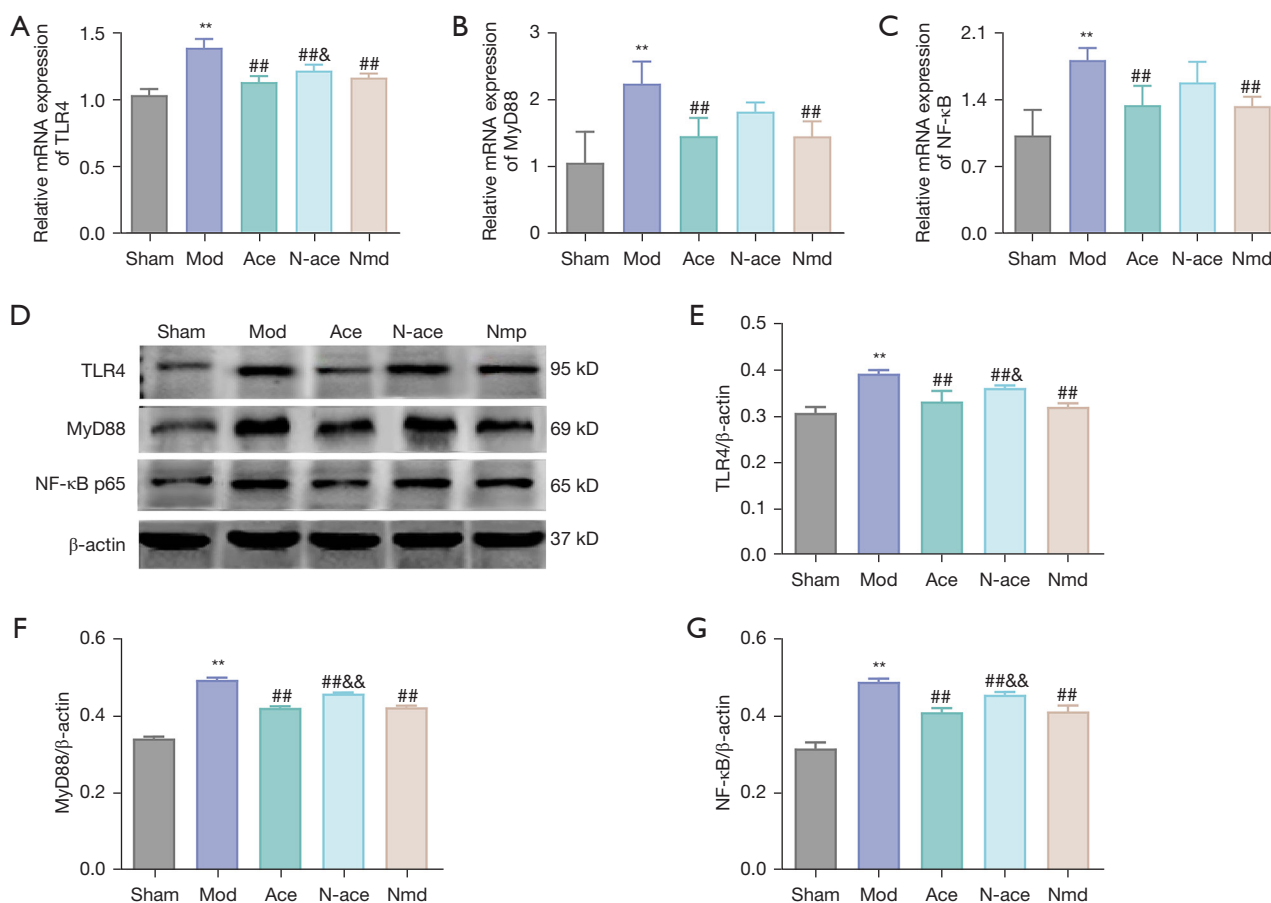
Figure 4 The mRNA (A-C) and protein (D-G) of TLR4, MyD88, and NF-κB in rat hippocampus tissues detected by RT-PCR and western blot, respectively. **, $P<0.01$ compared with Sham group; ##, $P<0.01$ compared with Mod group; &, $P<0.05$ and &&, $P<0.01$ compared with Ace group. mRNA, messenger RNA; TLR4, toll-like receptor 4; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; RT-PCR, real-time polymerase chain reaction.

signaling pathway activity of the hippocampus in VD rats. The results are shown in *Figure 4A-4G*. The mRNA and protein levels of TLR4, MyD88, and NF-κB in hippocampal tissues were significantly increased in the Mod group compared with the Sham group ($P<0.05$). In contrast, rats in the Ace, N-ace, and Nmp groups showed obviously reduced protein and mRNA levels of TLR4, MyD88, and NF-κBp65 in comparison to those in the Mod group ($P<0.05$); no significant difference was found between the Ace and Nmp groups ($P>0.05$). These results suggested that acupoint catgut embedding could inhibit the activity of the TLR4/MyD88/NF-κB signaling pathway activated by 4-VO in rat hippocampal tissues.

Discussion

Dementia has been studied in TCM and modern medicine

for a long time. After years of in-depth research, people have realized that vascular disease plays an important role in the occurrence and development of dementia, which has led to it being defined as VD, of which the clinical manifestations are mainly cognitive impairment and memory loss. The results of the water maze experiment showed that the swimming time in the third quadrant and the times of crossing platform in Ace group were significantly improved compared with the Mod group, indicating that the acupoint catgut embedding can improve the learning and memory ability of VD rats. HE staining of the hippocampal tissue related to learning and memory also showed that the neuronal damage in the hippocampus of VD rats treated with acupoint catgut embedding was less than that in the Mod group. In conclusion, acupoint catgut embedding therapy can improve nerve function injury by

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inhibiting hippocampal neuron damage in VD rats. Besides, inflammation resulting from ischemia is one of 2 major contributors to the pathogenesis of VD (17). Several studies have shown that over stimulation of inflammatory cytokines will lead to cell death, induce arterial occlusion, neuronal damage, and cell apoptosis, thus aggravating VD (18-20). Profiling of plasma cytokines by Zuliani *et al.* revealed that blood samples from VD patients had higher levels of inflammatory cytokines IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) compared with older controls (21). We established VD model rats by modified 4-VO method. Obvious learning and memory impairment, hippocampal injury, and up-regulation of inflammatory factors (IL-1β and IL-6) were observed in VD model rats induced by 4-VO. These results are consistent with previous studies (15,22) and indicated that we had successfully established the VD model rats with this method.

In recent years, acupoint catgut embedding, as a kind of TCM, has been shown to exert obvious effects on the treatment of VD (23,24), but its mechanism remained to be further explored. Chen *et al.* found that acupoint catgut embedding significantly reduced the average escape latency and increased the times of platform crossing of VD rats, in other words, acupoint catgut embedding improved the spatial learning and memory and location memory disorders of VD rats (25). Another study by Tang *et al.* also showed that acupoint catgut embedding improved the learning and memory ability and reduced the serum IL-1β and IL-6 levels in VD rats (26). Our research also showed that acupoint catgut embedding and nimodipine treatment alleviated spatial learning and memory impairment in VD rats, improved the pathological changes of neuron structure (atrophy and necrosis), inhibited inflammation by down-regulating IL-1β and IL-6, and played a neuroprotective role in VD rats.

TLRs are receptors that mainly recognize pathogenic microorganisms in the natural immune system. When cerebral ischemia occurs, TLRs are activated, sending signals to trigger inflammatory reactions, and an abundance proinflammatory cytokines and adhesion factors are expressed, leading to brain tissue damage (27-29). There are many classifications of TLRs, among which TLR4 distributed on the cell surface can be expressed by vascular smooth muscle cells and endothelial cells in the brain, which can stimulate the secretion of inflammatory substances, and participate in and mediate inflammatory injury (30,31). MyD88 is expressed in a variety of tissues and cells and is a key junction molecule in the TLR signal

pathway. When the death domain and intermediate domain in MyD88 are expressed at the same time, it can activate downstream NF-κB, which plays an important role in transmitting upstream information and disease occurrence and development (32,33). Therefore, we made a hypothesis: the TLR4/MyD88/NF-κB-mediated inflammatory signal pathway is involved in the process of acupoint catgut embedding in VD. To test this hypothesis, we detected the mRNA and protein expression of TLR4, MyD88, and NF-κB. The results showed that acupoint catgut embedding and nimodipine treatment could reduce TLR4, MyD88, and NF-κB expression in the hippocampus of VD rats, indicating that acupoint catgut embedding inhibited the activity of the TLR4/MyD88/NF-κB signaling pathway.

In conclusion, these results indicate that acupoint catgut embedding down-regulated the release of inflammatory factors, inhibited the damage of neurons in hippocampus, and then restored the learning and memory function of VD rats. However, this study is only a preliminary exploration of the therapeutic mechanism of acupoint catgut embedding in VD. We did not perform an experiment to verify that acupoint catgut embedding inhibited inflammatory response through the TLR4/MyD88/NF-κB signaling pathway, thereby alleviating learning and memory impairment and pathological damage in VD rats. In addition, it is not clear whether the therapeutic effect of acupoint catgut embedding is different in high-risk groups of VD of different ages and genders, so as to formulate different refined interventions to better recover their learning and memory disorders. Further experiments are needed to explore the exact molecular mechanism and therapeutic effect of acupoint catgut embedding in VD.

Conclusions

The present study demonstrated that acupoint catgut embedding treatment could improve spatial learning and memory loss, alleviate pathological damage of the hippocampus, and inhibit the inflammatory response in VD rats, which is probably related to inhibition of the TLR4/MyD88/NF-κB signaling pathway. This study presents a novel approach to treating VD which may also help to reduce the heavy burden on the public health system.

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