RESEARCH ARTICLE

Acupuncture through *Baihui* (DU20) to *Qubin* (GB7) mitigates neurological impairment after intracerebral hemorrhage

Xiao-Ying Liu[1,#], Xiao-Hong Dai[2,#], Wei Zou[2,3,*], Xue-Ping Yu[2,*], Wei Teng[2], Ying Wang[4], Wei-Wei Yu[2], Hui-Hui Ma[2], Qiu-Xin Chen[2], Peng Liu[1], Rui-Qiao Guan[1], Shan-Shan Dong[1]

1 Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang Province, China
2 The First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang Province, China
3 Clinical Key Laboratory of Integrated Traditional Chinese and Western Medicine of Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang Province, China
4 Department of Pharmacology of Dali University, Dali, Yunnan Province, China

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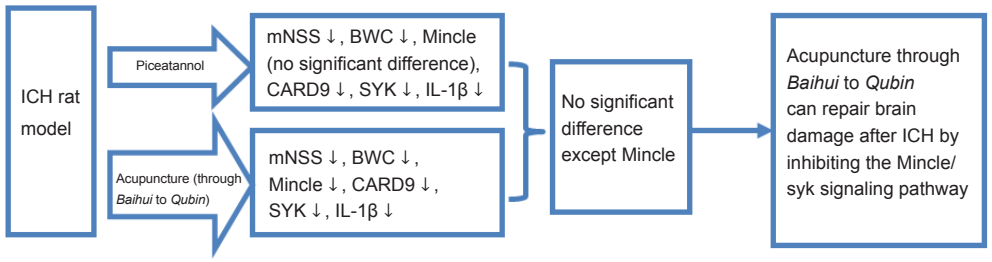
Graphical Abstract

Acupuncture through *Baihui* (DU20) to *Qubin* (GB7) is an effective treatment for intracerebral hemorrhage (ICH) through Mincle/Syk signaling pathway



Correspondence to:
Wei Zou, Ph.D. or Xue-Ping Yu, Ph.D., kuangzou1965@163.com or hzyyxp82@163.com.

#These authors contributed equally to this study.

orcid:
0000-0003-4560-1582 (Wei Zou)
0000-0002-9929-4883 (Xue-Ping Yu)

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Abstract

Inflammation plays an important role in nerve defects caused by intracerebral hemorrhage. Repairing brain damage by inhibiting the macrophage-inducible C-type lectin/spleen tyrosine kinase (Mincle/Syk) signaling pathway is a potential new target for treating cerebral hemorrhage. In this study, we aimed to determine whether acupuncture through *Baihui* (DU20) to *Qubin* (GB7) is an effective treatment for intracerebral hemorrhage through the Mincle/Syk signaling pathway. An intracerebral hemorrhage rat model was established by autologous blood infusion into the caudate nucleus. Acupuncture through *Baihui* to *Qubin* was performed for 30 minutes, once every 12 hours, for a total of three times. Piceatannol (34.62 mg/kg), a Syk inhibitor, was intraperitoneally injected as a control. Modified neurological severity score was used to assess neurological function. Brain water content was measured. Immunohistochemistry and western blot assay were used to detect immunoreactivity and protein expression levels of Mincle, Syk, and CARD9. Real-time polymerase chain reaction was used to determine interleukin-1β mRNA levels. Hematoxylin-eosin staining was performed to observe histopathological changes. Our results showed that acupuncture through *Baihui* to *Qubin* remarkably improved neurological function and brain water content, and inhibited immunoreactivity and expression of Mincle, Syk, CARD9, and interkeukin-1β. Moreover, this effect was similar to piceatannol. These findings suggest that acupuncture through *Baihui* to *Qubin* can improve neurological impairment after cerebral hemorrhage by inhibiting the Mincle/Syk signaling pathway.

Key Words: *nerve regeneration; intracerebral hemorrhage; acupuncture; Baihui (DU20); Qubin (GB7); inflammation; Mincle/Syk signaling pathway; nerve protection; neural regeneration*

Introduction

Approximately 2 million individuals worldwide are diagnosed with intracerebral hemorrhage (ICH) each year (Sudlow and Warlow, 1997; Broderick et al., 2007; Lloyd-Jones et al., 2009; Qureshi et al., 2009). Furthermore, ICH is one of the most fatal types of stroke, and results in severe mental dysfunction generated by secondary nerve damage in patients who survive the primary stroke (Aronowski and Zhao, 2011). Current therapies for ICH have a poor impact on prognosis (Mayer and Rincon, 2005; Keep et al., 2012). Cytotoxicity, oxidative stress, and especially, inflammation play key roles in secondary injury after ICH (Keep et al., 2012; Zhou et al., 2014). Consequently, regulation of inflammation after ICH may provide insight for future ICH therapies (Zhao et al., 2007).

Acupuncture is one of the most important components of traditional Chinese medicine and widely used in the

treatment of various diseases (Lou et al., 2016). Moreover, its curative effect has been widely recognized and accepted worldwide (Meng et al., 2011; Liu et al., 2016, 2017) by the international medical community. Our previous study revealed that acupuncture through *Baihui* (DU20) to *Qubin* (GB7) has a "reparative" function by inducing expression of endogenous glial cell line-derived neurotrophic factor during acute cerebral hemorrhage (Zhang et al., 2012). More specifically, acupuncture can improve recovery of neural stem cells by suppressing expression of both Notch1 and Hes1 (Zou et al., 2015). Acupuncture can also antagonize inflammatory brain injury generated by cerebral hemorrhage by suppressing the classical nuclear factor-κB pathway (Liu et al., 2017). Thus, acupuncture is an effective means of lowering expression of inflammatory mediators in the nervous system. Furthermore, according to current reports, acupuncture has greatly contributed to reducing the rate of stroke-induced disability and improving recovery of neural function. Studies investigating acupuncture have found that it is a highly potent therapy for reducing neural inflammation (Liu et al., 2016), suppressing cell apoptosis, and alleviating nerve dysfunction (Ma et al., 2016) after stroke. Altogether, these studies show that acupuncture has great potential for treating cerebral hemorrhage by inhibiting inflammation.

The immune system plays an important role in the inflammatory response. Compared with the adaptive immune system (which is highly pathogen specific), innate immune receptors recognize a variety of pathogens with similar structures (Park et al., 2006; Tang et al., 2007; Shichita et al., 2012). Toll-like receptors are a classical example of innate immune receptors, and are a "hot topic" of research because of their participation in inflammation induced by neural system disease, including ischemic stroke and cerebral hemorrhage (Fadakar et al., 2014; Lan et al., 2017). Macrophage-inducible C-type lectin (Mincle) is a recently discovered innate immune receptor that was originally recognized as a macrophage target in the peritoneum (Matsumoto et al., 1999). Toll-like receptors recognize ligands expressed on necrotic cells, and interact with downstream spleen tyrosine kinase (Syk) to active a pathway that can induce generation of several inflammatory cytokines, including interleukin (IL)-1β (Brown, 2008). The Mincle/Syk pathway plays a role in traumatic brain injury (de Rivero Vaccari et al., 2015), subarachnoid hemorrhage, and ischemic stroke (Suzuki et al., 2013; He et al., 2015; Xie et al., 2017). However, the regulatory mechanism of the Mincle/Syk pathway in cerebral hemorrhage remains unclear. Furthermore, it is also not known whether acupuncture can treat cerebral hemorrhage by regulating the Mincle/Syk pathway.

In this study, we first used an autohemic blood infusion method to establish a rat model of cerebral hemorrhage for studying the effectiveness of acupuncture through *Baihui* to *Qubin*. We then determined the therapeutic mechanism of acupuncture through the Mincle/Syk pathway.

Materials and Methods

Animals

A total of 312 male specific pathogen-free Sprague-Dawley rats, aged 8 weeks and weighing 280–320 g, were purchased from the Laboratory Animal Center, Heilongjiang University of Chinese Medicine, China (license No. SYXK (Hei) 2016-015). All experiments were approved by the Animal Care and Use Committee of Heilongjiang University of Chinese Medicine of China (approval No. 2016-09-02-01). Animal care and experimental procedures were performed in accordance with the United State National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication No. 85–23, revised 1985). All surgeries were performed under pentobarbital anesthesia, and all efforts were made to minimize suffering. Rats were housed under a 12-hour light/dark cycle at 21 ± 2°C and 50 ± 5% relative humidity, with unlimited access to standard food and water. Rats were randomly divided into four groups: sham (n = 72), intracerebral hemorrhage (ICH) (n = 72), piceatannol (a Syk inhibitor) (n = 72), and acupuncture (n = 72). All groups were evenly divided into four subgroups (n = 18 each), which were assessed at 6, 12, 24, and 72 hours after treatment.

Establishment of a cerebral hemorrhage rat model

Rats were anesthetized with pentobarbital (60 mg/kg; intraperitoneally) (Royalton, Dalian, China), and fixed on a stereotactic frame (STW-3X; Chengdu Instrument Factory, Chengdu, China). Bregma and the coronal sulcus were exposed by cutting the scalp midline. A dental drill was used at the interchange of 0.2 mm behind and 3.5 mm right from bregma. Drilling was performed to a diameter of 1 mm until the meninges was reached. Autologous tail tip blood was injected into the caudate nucleus (20 μL/min) (anteroposterior: −0.24 mm, lateral: 3.5 mm, dorsal: 6 mm) (right side is the injury side) (Paxinos and Watson, 2007). The microsyringe was exited from the caudate–putamen after 5 minutes. The wound was filled using dental cement, and subsequently stitched, bandaged, and sterilized (Liu et al., 2017). According to Berderson's scale (Bederson et al., 1986), the model was successful if the score was 1 to 3 at 2 hours after modeling.

Operations for sham models were identical except for blood injection.

Treatment

After successful model establishment, in the acupuncture group, *Baihui* (DU20) (specific location: head between the middle ears) and *Qubin* (GB7) (specific location: leading edge of ear root) points were identified (Li, 2007). Hair was cut around the *Baihui* (DU20) point, and a 0.30 × 25 mm acupuncture needle (Hua Tuo Brand; Suzhou Medical Appliance, Suzhou, China) used to pierce from the epicranial aponeurosis to the *Qubin* (GB7) point in the bottom right direction. The needle pierced to a depth of 15 mm, and was then rotated at 200 r/min for 5 minutes. For each 30-minute session, needling at 200 r/min was performed for three session, each lasting for 5 minutes.

In the sham and ICH groups, rats were fixed on the frame for 30 minutes without any operation.

In the piceatannol group, rats were intraperitoneally injected with piceatannol (34.62 mg/kg) (license No. 10083-

24-6; Selleck, Shanghai, China) at 1 hour after model establishment.

Modified neurological severity score (mNSS)
The mNSS was analyzed at 6, 12, 24, 72, and 168 hours after model establishment (Lei et al., 2015). Higher scores indicate poorer neurological function. More detailed information is provided in **Additional file 1**.

Brain water content (BWC)
Brains were obtained by decapitation at 6, 12, 24, and 72 hours after treatment in each subgroup. Left and right hemispheres were separated. Wet weight was weighed. Dry weight was weighed after a 72-hour incubation in a drying cabinet (Binder, Germany) at 105°C. BWC was calculated by: wet weight – dry weight) / wet weight × 100%.

Histopathological assay
Brain tissue of rats in each group was collected at 72 hours after surgery, perfused with 4% paraformaldehyde, and cut into 4-μm slices for routine hematoxylin and eosin staining to observe organizational structure. Images were captured using an optical microscope (Leica, Germany) at 200× magnification.

Immunohistochemistry
Rats were treated with pentobarbital (60 mg/kg) by intraperitoneal injection, followed by perfusion fixation with 4% paraformaldehyde. Brain tissue was obtained after decapitation, and fixed in paraformaldehyde following dehydration. After paraffin embedding and histological sectioning (5 μm), sections were incubated with primary antibodies, namely rabbit anti-Mincle (1:500; Bioss, Beijing, China), rabbit anti-Syk (1:500; Bioss), and rabbit anti-caspase recruitment domain family member 9 (CARD9) (1:500; Abcam, Cambridge, UK) overnight at 4°C. Sections were then incubated with goat anti-rabbit IgG (1:2000; Abcam) as a secondary antibody at 37°C for 30 minutes. Images were captured using a microscope imaging system (Moticam 3000; Motic, Hong Kong Special Administrative Region, China). Each sample was examined in captured images from five non-overlapping visual fields under a 400× magnification. Average number of positive cells was calculated (Xue et al., 2014).

Western blot assay
The right hemisphere of rats at 6, 12, 24, and 72 hours after ICH was lysed, with protein samples isolated and collected. Fifty micrograms (50 μg) of sample was loaded onto a sodium dodecyl polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. The membrane was then blocked using 5% non-fat dry milk, and subsequently incubated with primary antibody overnight at 4°C, followed by secondary antibody at room temperature for 2 hours. Rabbit anti-Mincle (1:1000; Bioss), rabbit anti-Syk (1:1000; Bioss), rabbit anti-CARD9 (1:1000; Abcam), and rabbit anti-β-actin (1:1000; Bioss) were used as primary antibodies. Goat anti-rabbit IgG (1:2000; Abcam) was used as the secondary antibody. Protein signal was marked using a commercially available 3, 3′-diaminobenzidine (DAB) electrochemiluminescence system (Beyotime, Jiangsu, China), and images captured (Duris et al., 2011). Optical density values for specific bands were measured using ImageJ software (NIH, Bethesda, MD, USA).

Real-time polymerase chain reaction (PCR)
RNA was extracted from the brain using a commercially available kit (RNAiso Plus; Takara Bio Inc., Dalian, China), according to the manufacturer's instructions. Complementary DNA (cDNA) was reverse transcribed using the Prime Script II 1st Strand cDNA Synthesis Kit (Takara Bio Inc.). The reverse transcription conditions were: 42°C for 60 minutes, 95°C for 5 minutes, and then storage on ice. The primers used were: β-actin-forward, 5′-AAC ACC CCA GCC ATG TAC GTA-3′; β-actin-reverse, 5′-TGG CCA TCT CTT GCT CGA A-3′; IL-1β-forward, 5′-AAG GGG ACA TTA GGC AGC AC-3′; and IL-1β-reverse, 5′-ATG AAA GAC CTC AGT GCG GG-3′. Real-time PCR was performed using the SYBR Premix Ex Taq kit (Takara Bio Inc.). The PCR conditions were: 95°C for 30 seconds, 95°C for 5 seconds, and 60°C for 34 seconds for 40 cycles, with reactions held at 4°C. IL-1β mRNA was calculated by the $2^{-\Delta\Delta Ct}$ method (Xie et al., 2017).

Statistical analysis
Data are represented as mean ± SD for mNSS, western blotting, and as median (interquartile range) for BWC, immunohistochemistry, and real-time PCR. All analyses were performed using Graph Pad Prism 6 software (GraphPad, San Diego, CA, USA). *P* values for mNSS and western blotting were determined by one-way analysis of variance followed by Tukey's *post hoc* test. BWC, immunohistochemistry and real-time PCR were examined using the Kruskal-Wallis test followed by Mann-Whitney *U* test for pairwise comparisons. A value of $P < 0.05$ was considered statistically significant.

Results

Effect of acupuncture on neural function after ICH
No significant differences in mNSS were detectable among subgroups (6, 12, 24, 72, and 168 hours) in the sham group ($P > 0.05$). Compared with the sham group, mNSS score was significantly increased in the ICH group ($P < 0.01$). mNSS was significantly lower in the acupuncture and piceatannol groups compared with the ICH group ($P < 0.01$). No significant differences were observed in mNSS score between the acupuncture and piceatannol groups ($P > 0.05$; **Figure 1**).

Effect of acupuncture on BWC after ICH
BWC was relatively minor in the sham group (**Figure 2**). However, compared with the sham group, BWC was significantly increased in the ICH group ($P < 0.05$). BWC was significantly lower in the acupuncture and piceatannol groups compared with the ICH group ($P < 0.05$). No significant difference in BWC was observed between the acupuncture and piceatannol groups ($P > 0.05$; **Figure 2**).

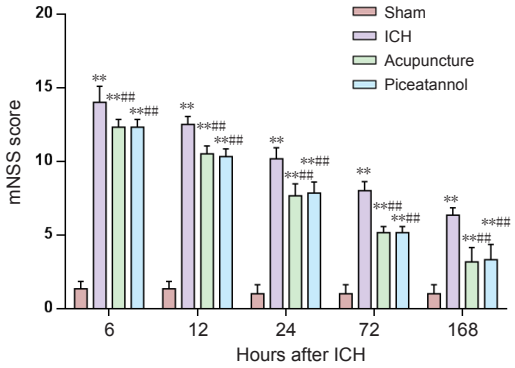

Figure 1 Effect of acupuncture through *Baihui* (DU20) to *Qubin* (GB7) on mNSS in a rat model of intracerebral hemorrhage.
Data are expressed as the mean ± SD (n = 6; one-way analysis of variance followed by Tukey's *post hoc* test). $^{**}P < 0.01$, *vs.* sham group; $^{\#\#}P < 0.01$, *vs.* ICH group. mNSS: Modified neurological severity score; ICH: intracerebral hemorrhage.

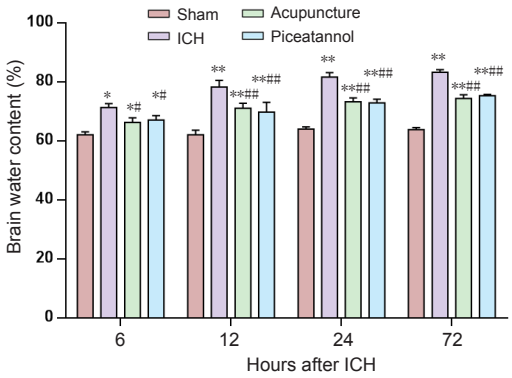

Figure 2 Effect of acupuncture through *Baihui* (DU20) to *Qubin* (GB7) on brain water content in a rat model of intracerebral hemorrhage.
Brain water content = wet weight – dry weight / wet weight × 100%. Data are expressed as the mean ± SD (n = 6; Kruskal-Wallis test followed by Mann-Whitney U test). $^{*}P < 0.05$, $^{**}P < 0.01$, *vs.* sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, *vs.* ICH group. ICH: Intracerebral hemorrhage. The primary data of Figure 2 are expressed in Additional file 2.

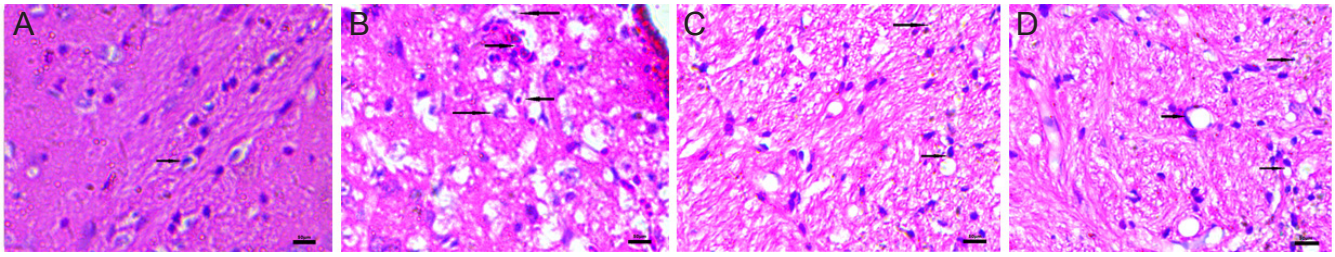

Figure 3 Effect of acupuncture through *Baihui* (DU20) to *Qubin* (GB7) on brain histopathology of rats after intracerebral hemorrhage (hematoxylin and eosin staining, original magnification, 200×).
(A) Sham group; (B) ICH group; (C) piceatannol group; (D) acupuncture group. Rats in the sham group had no blood injection into the brain, and showed no inflammatory infiltration (arrows) from the semi-dark area and normal microscopic features. Pathological features (arrows) in the ICH group were intracranial blood injection, tissue edema, partial nerve cell necrosis, glial cell swelling, intracellular vacuoles, and inflammatory cell infiltration. Pathological features (arrows) were less severe in rats receiving acupuncture or piceatannol treatment after blood injection. ICH: Intracerebral hemorrhage.

Effect of acupuncture on brain histopathology after ICH

Hematoxylin and eosin staining was performed to examine histological changes 72 hours after ICH (**Figure 3**). Rats receiving sham surgery had no blood injection into the brain and showed normal microscopic features, with no inflammatory infiltration from the semi-dark area. However, the ICH group showed intracranial blood injection, tissue edema, partial nerve cell necrosis, glial cell swelling, intracellular vacuoles, and inflammatory cell infiltration. Furthermore, in rats receiving acupuncture or piceatannol treatment after blood injection, the pathological features were less severe.

Effect of acupuncture on the Mincle/Syk pathway in rats with ICH

Immunopositivity of Mincle, Syk, and CARD9 in the brain as detected by immunohistochemistry

Immunopositivity of Syk and CARD9 were significantly reduced in the acupuncture and piceatannol groups compared with the ICH group ($P < 0.05$). Immunopositivity of Syk and CARD9 showed no statistical differences between the acupuncture and piceatannol groups ($P > 0.05$). Compared with the ICH group, Mincle immunopositivity was decreased in the acupuncture group ($P < 0.05$). However, Mincle immunopositivity was not significantly different between the ICH and piceatannol groups ($P > 0.05$; **Figure 4**).

Protein expression of Mincle, Syk, and CARD9 in the brain as detected by western blot assay

Expression levels of Syk and CARD9 were significantly reduced in the acupuncture and piceatannol groups compared with the ICH group ($P < 0.01$). There were no statistically significant differences in Syk and CARD9 expression levels between the acupuncture and piceatannol groups ($P > 0.05$). Compared with the ICH group, Mincle expression levels were significantly decreased in the acupuncture group ($P < 0.01$). However, Mincle expression levels were not significantly different between the ICH and piceatannol groups ($P > 0.05$; **Figure 5**.

IL-1β mRNA expression in the brain as detected by real-time PCR

IL-1β mRNA levels were significantly increased in the ICH group compared with the sham group ($P < 0.01$). Compared with the ICH group, expression levels of IL-1β mRNA were significantly decreased in the acupuncture and piceatannol groups ($P < 0.01$). However, expression levels of IL-1β mRNA were not different between the acupuncture and piceatannol groups ($P > 0.05$; **Figure 6**).

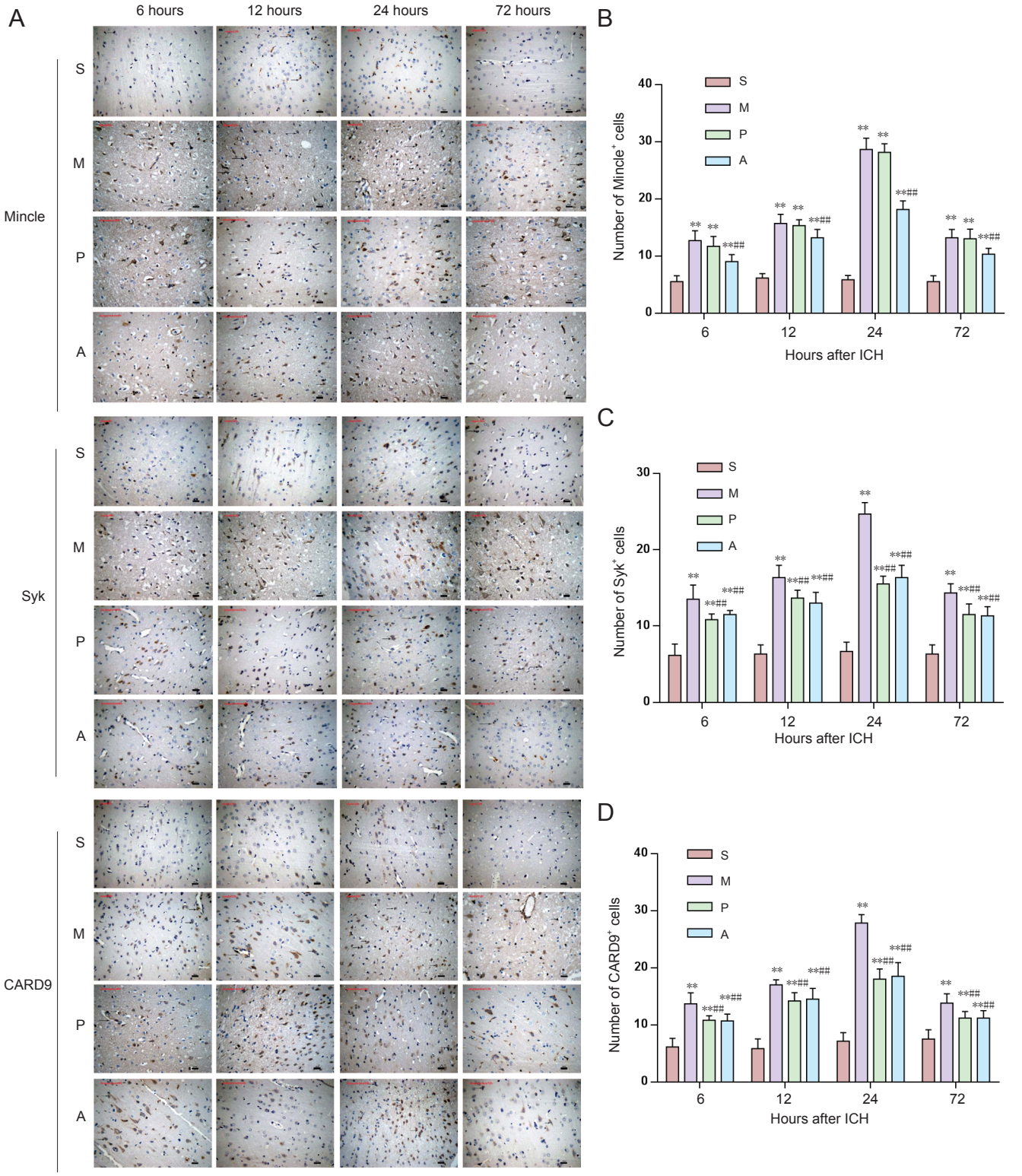

Figure 4 Effect of acupuncture through *Baihui* (DU20) to *Qubin* (GB7) on immunoreactivity of Mincle, Syk, and CARD9 in the brain of rats at 6, 12, 24, and 72 hours after intracerebral hemorrhage (immunohistochemistry).

(A) Immunohistochemical staining of brain tissue (original magnification, 400×). Positive cells are indicated by arrows. Scale bars: 50 μm. (B–D) Number of Mincle$^+$, Syk$^+$, and CARD9$^+$ cells in 400-fold fields, respectively. Data are presented as the mean ± SD (n = 6; Kruskal-Wallis test followed by Mann-Whitney U test). ∗P < 0.05, *vs.* sham group; #P < 0.05, *vs.* ICH group. S: Sham group; M: model group; P: piceatannol group; A: acupuncture group; ICH: intracerebral hemorrhage.

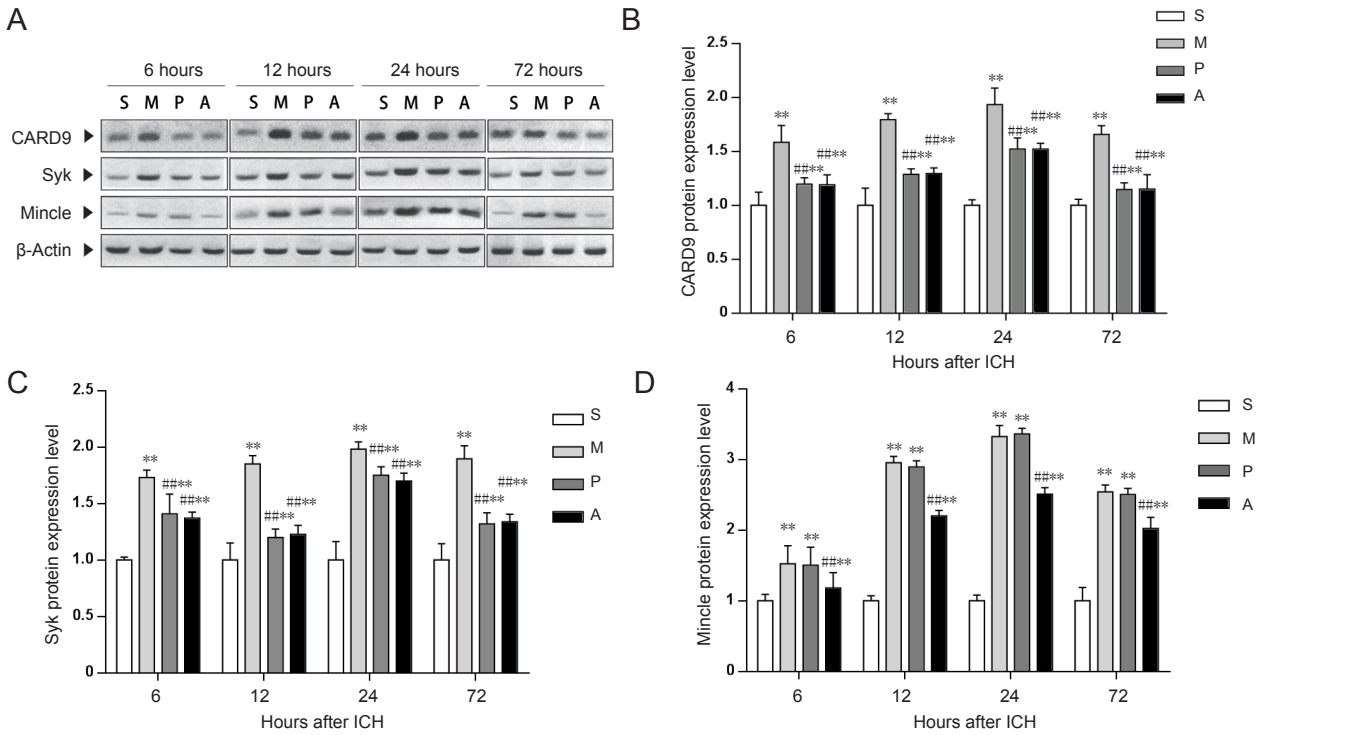

Figure 5 Effect of acupuncture through *Baihui* (DU20) to *Qubin* (GB7) on protein expression of CARD9, Syk, and Mincle in the brain of rats at 6, 12, 24, and 72 hours after intracerebral hemorrhage (western blot assay).

(A) Western immunoblotting analysis of proteins relevant to intracerebral hemorrhage in rats. Protein levels were normalized to β-actin as a loading control. (B–D) Quantitative analysis of protein expression levels of CARD9, Syk, and Mincle, respectively. Data are presented as the mean ± SD ($n = 6$; one-way analysis of variance followed by Tukey's *post hoc* test); $^{**}P < 0.01$, *vs.* sham group; $^{\#\#}P < 0.01$, *vs.* ICH group. S: Sham group; M: model group; P: piceatannol group; A: acupuncture group; ICH: intracerebral hemorrhage; Mincle: macrophage-inducible C-type lectin; Syk: spleen tyrosine kinase.

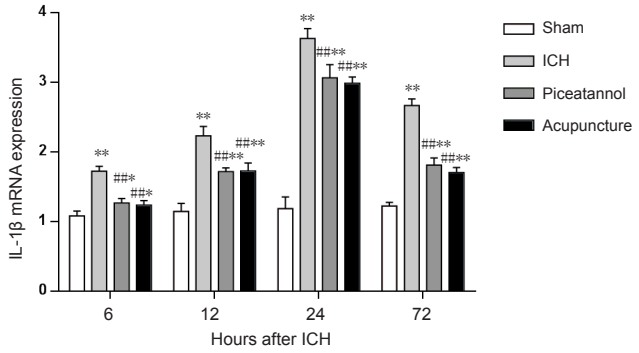

Figure 6 Effect of acupuncture through *Baihui* (DU20) to *Qubin* (GB7) on mRNA expression of IL-1β at 6, 12, 24, and 72 hours in the brain of rats after intracerebral hemorrhage.

Relative mRNA expression of IL-1β was determined by real-time polymerase chain reaction. Data are presented as the mean ± SD ($n = 6$; Kruskal-Wallis test followed by Mann-Whitney *U* test). $^{*}P < 0.05$, $^{**}P < 0.01$, *vs.* sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, *vs.* ICH group. ICH: Intracerebral hemorrhage; IL: interleukin.

Discussion

Peripheral tissues are exposed to a massive amount of blood after ICH, which in turn provokes a variety of cellular and molecular effects. Activation of cells and inflammation can trigger infiltration of polymorphonuclear leukocytes and monocytes, activation of microglia, damage to the blood-brain barrier, and development of brain edema, which contributes to secondary brain injury (Aronowski and Hall, 2005; Wang and Dore, 2007). Meanwhile, previous studies have shown that acupuncture can reduce neural inflammation (Liu et al., 2016), suppress cell apoptosis, and alleviate nerve dysfunction (Ma et al., 2016) after stroke, but it remains unclear whether acupuncture is effective for treating cerebral hemorrhage. Consequently, in this study, we first examined the protective effects of acupuncture through *Baihui* (DU20) to *Qubin* (GB7) in a cerebral hemorrhage animal model. We then further investigated the effect of acupuncture on inflammation and its protective mechanism. Our results indicate that acupuncture could be an excellent tool for ICH therapy.

As already described, inflammation plays an important role in ICH (Tang et al., 2017). Inflammation leads to cell swelling and damage, which in turn induces brain edema. Inflammation, clotting, and erythrocyte lysis caused by primary injury can result in brain edema, which is associated with poor recovery from injury and may lead to more severe and prolonged brain injury (Zheng et al., 2016). Thus, decreasing brain edema is critical for protecting neurovascular structures. Acupuncture is one of the most important components of traditional Chinese medicine, and has greatly contributed to reducing the rate of stroke-induced disability and improving recovery of neural function. Our results show that acupuncture through *Baihui* (DU20) to *Qubin* (GB7) can decrease mNSS and reduce BWC in a cerebral hemorrhage animal model. Moreover, hematoxylin-eosin staining showed that acupuncture through *Baihui* (DU20) to *Qubin* (GB7) also decreased capillary permeability and leakage. Our data are consistent with other similar studies (Liu et al., 2017).

The innate immune system is critical in development of neurovascular injury and a major reason for excessive parenchymatous inflammation. Fang et al., (2013) found that both innate immunity and inflammation participate in the pathological process of ICH. Necrosis of leukocytes can induce microglial cells and macrophages to release proinflammatory cytokines, thereby leading to further brain injury (Wang and Dore, 2007). Animal experiments have shown that factors inhibiting microglia/macrophages can remarkably reduce brain injury and improve neural function in rodents (Zhao et al., 2011).

Mincle is a pattern-recognition receptor primarily expressed in myeloid cells, especially antigen-presenting cells. It is also expressed on the surface of B cells, microglia, neurons, and mast cells (Flornes et al., 2004; McKimmie et al., 2006; Ribbing et al., 2011; Kawata et al., 2012; He et al., 2015). Mincle expression is low under normal circumstances. During infection and tissue damage, Mincle is upregulated and binds to endogenous antigens, leading to recruitment and activation of Syk. Syk can activate protein kinase C-δ, which phosphorylates downstream CARD9. This activates the nuclear factor-κB pathway via CARD9/B-cell lymphoma/leukemia 10 (BCL 10), which ultimately generates biologically active IL-1β (Takizawa et al., 2001; Strasser et al., 2012; Yasukawa et al., 2014). As a mediator of the immune response and activation point of inflammation, IL-1β expression levels directly reflect grades of inflammation after cerebral hemorrhage (Gross et al., 2009). Previous studies have shown that the Mincle/Syk signaling pathway is involved in many innate immune responses including ischemic stroke, traumatic brain injury, and subarachnoid hemorrhage (Suzuki et al., 2013; de Rivero Vaccari et al., 2015; He et al., 2015; Arumugam et al., 2017; Xie et al., 2017). Our study found that acupuncture markedly suppressed protein expression in the Mincle/Syk pathway and decreased IL-1β expression in rat brain tissue following hemorrhage. These results suggest that acupuncture through *Baihui* (DU20) to *Qubin* (GB7) may mitigate cerebral hemorrhage injury neuritis and improve neural function by suppressing the Mincle/Syk pathway, which further downregulates expression levels of downstream inflammatory cytokines.

Piceatannol, a Syk inhibitor, suppresses Syk/CARD9 expression during ischemic stroke and subarachnoid hemorrhage, reduces IL-1β levels, improves neural function, and facilitates brain edema therapy (Suzuki et al., 2013; He et al., 2015; Xie et al., 2017). Our study found no significant differences in the effects of therapy in rats treated with acupuncture compared with the piceatannol group. This further suggests that acupuncture through *Baihui* (DU20) to *Qubin* (GB7) can in fact affect the Mincle/Syk pathway, mitigate inflammatory brain injury, improve neural function, and alleviate brain edema.

Our study has a few limitations. First, we have searched relevant literature within and outside China, but reports on the Mincle/Syk signaling pathway are still limited. The mechanism by which blocking Mincle improves ICH outcome needs further investigation. Second, we did not examine changes of Mincle/Syk between different time points after ICH as the purpose of our study was to determine whether the therapeutic effect of acupuncture was significant compared with the other experimental groups at the same time point. Third, connections between Mincle and other signaling pathways after ICH requires further studies.

In summary, acupuncture through *Baihui* (DU20) to *Qubin* (GB7) may suppress the Mincle/Syk pathway and reduce the release of proinflammatory cytokines, thereby mitigating neurological damage after cerebral hemorrhage.

Author contributions: *XYL and XHD participated in study conception and design. XYL, WZ, and XPY wrote this paper. WT, YW, WWY, and HHM were responsible for data analysis. QXC, PL, RQG, and SSD were responsible for tissue processing. All authors approved the final version of the paper.*
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Institutional review board statement: *All experiments were approved by the Animal Care and Use Committee of Heilongjiang University of Chinese Medicine of China (approval No. 2016-09-02-01). Animal care and experimental procedures were performed in accordance with the United State National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication No. 85-23, revised 1985).*

Data sharing statement: *Datasets analyzed during the current study are available from the corresponding author on reasonable request.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

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Additional files:
Additional file 1: *Modified neurological severity score points.*
Additional file 2: *The primary data of Figure 2.*

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