Pulsed Electromagnetic Field Protects Against Brain Injury After Intracerebral Hemorrhage: Involvement of Anti-Inflammatory Processes and Hematoma Clearance via CD36

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Abstract

Intracerebral hemorrhage (ICH) causes high mortality and morbidity, but its therapy methods are limited. In the present study, pulsed electromagnetic field (PEMF) was demonstrated to have beneficial effects on an intracerebral hemorrhage (ICH) model. This study explored the effects and underlying mechanisms of PEMF in a mouse model of ICH and cultured BV2 cells. PEMF was applied 4 hours after collagenase-induced ICH at day 0 and 4 hours per day for seven consecutive days. The expression levels of proinflammatory factors were assessed by ELISA kits and western blotting. Hematoma volume was measured by histological analysis. The effects of PEMF on phagocytosis of the erythrocytes were observed in cultured BV2 cells and ICH mouse models. Seven days after ICH, the hematoma volume was significantly reduced in PEMF-treated animals compared to nontreated mice. We found that PEMF decreased the hematoma volume and the expression levels of proinflammatory factors after ICH. Moreover, PEMF enhanced the erythrophagocytosis of microglia via CD36. Furthermore, we found that downregulation CD36 with Genistein blocked the effects of PEMF-induced hematoma clearance and anti-inflammations effects. Thus, the PEMF-mediated promotion of neurological functions may at least partly involve anti-inflammatory processes and hematoma clearance. These results suggest that PEMF treatment promoted the hematoma clearance and alleviated the inflammation after ICH.

Keywords ICH · PEMF · Inflammation · Hematoma clearance · CD36

Introduction

Intracerebral hemorrhage (ICH) is a devastating nervous system disease with high mortality and morbidity rates (Krishnamurthi et al. 2013; Ekker et al. 2019). Accumulating evidence suggests that neuroinflammation, including activation of microglia and...
the release of inflammatory cytokines, plays an essential role in ICH pathogenesis, which begins immediately after hematoma formation and is a critical host defense response to secondary brain injury after ICH (Xue and Yong 2020; Wang et al. 2018). Hematoma absorption following ICH was accomplished by microglia, resident macrophages of the central nervous system (CNS), which are considered to be associated with inflammation and are generally involved in the phagocytosis process (Liu et al. 2021; Zhao et al. 2015). Different types of microglia, including M1 and M2 microglia, have distinct effects during ICH-related injury (Fu et al. 2021; Bai et al. 2020). The accumulation of M1 microglia increases the secretion of inflammatory cytokines, chemokines, and other neurotoxic mediators, leading to widespread cellular damage. M2 microglia can release anti-inflammatory cytokines, resulting in hematoma removal, neurogenesis, and neuroprotection, which are associated with neurologic improvement (Chen et al. 2020; Chang et al. 2020). It has been pointed out that stimulating microglia/macrophage to promote endogenous hematoma phagocytosis could be a promising strategy for ICH (Zhao et al. 2009).

Pulsed electromagnetic field (PEMF) has been regarded as a potential alternative method to traditional pharmacological treatments in mass brain injury studies: PEMF exposure has been shown to attenuate tissue damage with decreased infarct size in transient focal ischemia rabbits (Grant et al. 1994), and to exert anti-inflammatory effects after traumatic brain injury in rats (Rasouli et al. 2012). Our previous study also proved that PEMF exposure leads to neuroprotective effects against excitotoxicity in HT22 cells (Matsumoto et al. 2017). However, there are relatively few studies that explore the effects of PEMF against ICH-induced neuroinflammation. Whether the PEMF could influence the cleanup of the hematoma following ICH has never been investigated in previous studies. Besides, the role of microglia in the neuroprotective effects of PEMF is still not clear. An in vitro study showed that PEMF can inhibit inflammation induced by lipopolysaccharide in N9 microglial cells (Varani et al. 2017). However, the in vivo regulatory effect of PEMF on microglia after ICH remains unclear.

As a scavenger receptor, CD36 was involved in the inflammation, phagocytosis, and metabolism, angiogenesis. CD36-mediated hematoma absorption occurs in ICH patients and mice, and hematoma absorption was slower in CD36-deficient ICH patients and CD36−/− mice with aggravated neurologic deficits (Fang et al. 2014). Peroxisome proliferator-activated receptors gamma (PPARγ), a member of the nuclear hormone receptor superfamily of ligand-activated nuclear transcription factors, plays an important role in the upregulating CD36 expression (Flores et al. 2016). It has been shown that activating PPARγ could increase CD36 expression on microglia/macrophages to boost hematoma resolution in adult ICH experimental models (Zhao et al. 2007). We found that PEMF increased the expression of CD36 around hematoma, indicating that CD36 upregulation may participate in PEMF-induced hematoma clearance; thus, further investigation was needed to elucidate the underlying mechanisms.

In this study, PEMF therapy using parameters reported in a previous study (Kenny et al. 2019) was performed to illustrate the regulatory effects of PEMF after ICH. We showed that PEMF therapy decreased the release of inflammatory cytokines, promoted hematoma dissolution, ameliorated brain injury, and improved functional recovery in ICH mice. This study aims to provide direct evidence that PEMF could be a potential therapeutic approach for ICH-related injury and improve our understanding of the efficacy and underlying mechanisms of PEMF treatment against hematoma clearance.

Materials and Methods

Animals

The experimental protocol was approved by the Animal Care and Use Committee of Air Force Medical University. C57BL/6 J mice from 8 to 10 weeks were purchased from the Animal Center of Air Force Medical University. The mice were group-housed in individually ventilated cages on a 12-h light–dark cycle with ad libitum access to standard mouse feed and water. After 1-week acclimatization, mice were randomly divided into three groups, namely Sham group, ICH group, and ICH + PEMF group.

ICH Model

The collagenase injection ICH model was constructed as described previously (Xu et al. 2020). After the mice were anesthetized, 1 μl 0.03 U type IV-S collagenase (Sigma-Aldrich) was injected into the right striatum (coordinates: −0.2 mm anterior, 2.5 mm lateral, 3.5 mm deep; relative to bregma). A heating pad was applied to maintain a rectal temperature of 37.0 °C. The animals were returned to their cages after recovery from anesthesia. PEMF treatment was applied 4 h after ICH and for 4 h per day for 7 days. On day 1, day 3, and day 7, all mice were subjected to the corner turn test and forelimb use asymmetry test. Baseline test was performed before the ICH surgery. Genistin (AMQUAR, China) was injected intraperitoneally at 10 mg/kg after ICH, and continuously given one time per day till sacrificed. Control group was given the same volume of saline.
Cell Culture

BV2 cells were seeded at a density of $1 \times 10^6$ cells/ml in DMEM supplemented with 1% antibiotic (penicillin–streptomycin) and 10% heat-inactivated FBS. The cells were cultured at 37°C in a humidified 5% CO₂ with 95% air atmosphere.

PEMF Application

PEMF was generated using a system composed of a pulsed signal generator and two-array, as shown in Fig. 1b (GHY-III, FMMU, Xi’an, China; Chinese patent no. ZL02224739.4). The waveforms generated by the PEMF system consisted of a pulse burst (burst width, 5 ms; pulse width, 0.2 ms; pulse wait, 0.02 ms; burst wait, 60 ms; pulse rise, 0.3 μs; pulse fall, 2.0 μs) repeated at 15 Hz as described in our previous studies (Kenny et al. 2019). The distance between the two-array coils (20 cm diameter) was 10 cm, the turn number of the enamel-coated copper wire was 80, and the diameter of the copper wire was 1.0 mm. An oscilloscope was connected to the two sides of a 2-Ω resistor that was connected in series with the coils to display the voltage-current waveform. The current waveform could be obtained by dividing the voltage waveform by the 2-Ω resistor value. The current flowing through the resistor and the Helmholtz coils was the same. The waveform of the output magnetic fields was obtained on the basis of the current waveform. The determined peak intensity of the PEMF of the coils was 5 Gauss (0.5 mT).

For the animal study, the mice that underwent ICH surgery were placed in the center of the coils 4 h after surgery and for 4 h per day (Zhang et al. 2022). The untreated ICH group mice were placed in a different chamber with inactivated Helmholtz coils. Measurement accuracy of the electromagnetic field output was confirmed using a gaussmeter (Model 455 DSP gaussmeter, Lake Shore Cryotronics).

For cellular study, the bottom of the culture plates containing BV2 cells was aligned with the center of the coils. For ELISA assay, the PEMF treatment group received PEMF treatment immediately after red blood cell (RBC)- or hemoglobin (Hb)-induced injury for 4 h, and the cells were collected after 24 h. The untreated group was placed in a different chamber under the same conditions with inactivated Helmholtz coils. For immunofluorescent staining, the BV2 cells were treated with PEMF for 4 h after Hb-induced injury, and the cells were collected after 24 h. For erythrophagocytosis assay, the BV2 cells received PEMF treatment immediately after PKH-26 labels RBCs for 3 h. Then, the cells were fixed immediately for immunofluorescent staining.
Corner Turn Test

Each mouse was placed in a 30° corner, and the direction that the mouse exited from the corner was recorded 10 times. The percentage of right turns was calculated. The behavioral tests were conducted by a blinded investigator.

Forelimb Use Asymmetry

A forelimb use asymmetry test was performed as previously described (Yang et al. 2006). The mouse was placed in a cylinder (15 cm × 9 cm), and forelimb usage during at least 15 exploratory movements (less than 20) within a period of up to 10 min was recorded. The forelimb use asymmetry score was calculated as \[\frac{I-C}{I+C+B} \times 100\], where I is the number of times the ipsilateral forelimb was used, C is the number of times the contralateral forelimb was used, and B is the number of times the bilateral forelimbs were used.

Hematoma Volume Measurement

On day 1, day 3, and day 7 after ICH, the mice were perfused with 0.01 M PBS 50 ml after being anesthetized. Hematoma volume at day 1 was applied as the baseline level of hematoma volume compared with day 3 or day 7 hematoma volume. Their brains were collected and sectioned with 1-mm coronal mouse brain matrices according to the standard procedure developed by a previous study (Chang et al. 2015). The brain slices were digitalized by camera, and the cubic hematoma volume was measured and calculated as the total area × thickness by using ImageJ software (NIH, Bethesda, USA) in a blinded manner.

Western Blot Analysis

The protocol was the same as that described in previous articles (Yang et al. 2017). Briefly, the protein samples were quantified by the BCA method. Then, equal amounts of the samples (50 μg) were loaded and subjected to electrophoresis on an SDS-PAGE gel. After the proteins were transferred to a membrane, the PVDF membrane was blocked with 5% fat-free milk and then incubated with primary antibody overnight at 4 °C. The following primary antibodies were used: IL-1β (1:1000, Abcam, ab9722), TNF-α (1:1000, Cell Signaling Technology, CST#11948S), CD36 (1:1000, Abcam, ab222931). The images were analyzed with ImageJ software.

ELISA

The cells were suspended at a density of 1 × 10^6 cells/ml and seeded in 24-well plates. The cells were incubated for 24 h in the absence or in the presence of Hb (20 μM) (H7379, Sigma-Aldrich). Whole blood (murine) was washed and centrifuged twice with PBS and then RBCs were isolated for experiment. Mouse brain was collected at the end of treatment, and suspended cells were collected and centrifuged at 1000 x g for 10 min. The levels of the proinflammatory cytokines TNF-α and IL-1β were determined with a specific quantitative sandwich ELISA kit according to the manufacturer’s instructions. The reaction was developed with streptavidin–horseradish peroxidase, and the optical density was read at a wavelength of 450 nm.

TUNEL Staining

For quantification of neuronal apoptosis, TUNEL (green) staining was performed using the In Situ Apoptosis Detection Kit (Roche, Indianapolis, IN, USA) according to the manufacturer’s instructions at day 3 after ICH. Mice who underwent ICH surgery were sacrificed on day 3. Their brains were fixed with 4% paraformaldehyde for 4 h and then dehydrated with 30% sucrose solution. The specimens were cut into 30 μm sections for staining. The number of TUNEL-positive neurons in the perihematomal area in six sections per brain at 20 × magnification was counted manually using ImageJ software (ImageJ 1.4, NIH). The data are expressed as the number of TUNEL-positive cells.

Erythrophagocytosis Assay

BV2 cell erythrophagocytosis was evaluated as previously described (Chang et al. 2020). For the microglia with CD36 or IgG group, CD36 neutralized antibody (endotoxin, Azide free, ab222931) was pretreated on each cell (2 μg/ml) or IgG (ab172730) were pretreated on each cell (2 μg/ml) 4 h before erythrophagocytosis assay. Briefly, RBCs (2 × 10^7) were isolated from the whole blood of donor mice. Then, they were washed with 0.01 M PBS, centrifuged at 750 rpm/min for 5 min, resuspended in diluent, and stained with PKH-26 (PKHGL, Sigma-Aldrich) for 4 min. Serum was applied for 1 min to stop the staining process. Then, the PKH-26-labeled RBCs were washed three times to remove the residual dye. The cells were counted for further experiments and added to BV2 cells, and erythrophagocytosis was assessed.

Immunofluorescence Staining

After anesthesia, mice were perfused with ice-cold 0.01 M phosphate-buffered saline (PBS) and then with 4% paraformaldehyde. The brains were collected and postfixed for another 4 h and then dehydrated with 30% sucrose. Frozen coronal slices (30 mm thick) were obtained with a freezing
The sections were washed with 0.01 M PBS and blocked with 5% normal goat serum for 30 min. Then, they were incubated with primary antibody CD36 (ab252923, Abcam) and Iba1(ab283319, Abcam) at 4 °C overnight and washed three times with 0.01 M PBS. We applied appropriate secondary antibodies at room temperature for 2 h. Antifade solution with or without DAPI was applied, and the sections were observed with an Olympus microscope after they were completely dried.

**Statistical Analysis**

The data are expressed as the mean ± SD of at least three independent experiments. When relative values were compared between the sham, ICH, and ICH with PEMF groups, statistical analysis was performed by one-way ANOVA. In all cases, P values of < 0.05 and < 0.01 were considered significant and are indicated as * and #, respectively. When values between two groups were compared, statistical significance was assessed using paired two-way repeated-measures ANOVA. Statistical analysis was performed using Prism 6.0.

**Results**

**PEMF Promoted Neurofunctional Recovery After ICH**

C57BL/6 J mice were divided into three groups: Sham group, ICH group, and ICH + PEMF treatment group. As shown in Fig. 1a, ICH model was established and then mice in the ICH + PEMF group were treated with 4 h/day PEMF stimulation for 3 days or 7 days respectively. As shown in Fig. 1c, animals were then subjected to behavior tests on day 0, day 1, day 3, and day 7. At day 3 and day 7, the average hematoma volumes were measured and compared.

To assess neurofunctional deficits, we subjected the three groups of mice to the corner turn test and forelimb use asymmetry test at different timepoints before and after the ICH surgery. The results of the two behavioral test showed similar baseline in the three groups at day 0 before the ICH surgery (Fig. 2a, b). For the corner turn test, the average number of corner turn score showed no significant difference in ICH group and ICH + PEMF group at day 1 and day 3, indicating that the ICH mice exhibited similar neurological impairments with or without PEMF exposure for 1 day and 3 days. At day 7, the ICH + PEMF mice exhibited significantly

![Fig. 2](image.png)

Fig. 2 PEMF promoted the hematoma resolution and neurological recovery after ICH. a and b Neurological functions were assessed by the corner turn test and forelimb use asymmetry test. n = 6 for each group. *p < 0.05, #p < 0.01 by two-way repeated measures ANOVA.

The asterisks on the top indicate group differences. c Coronal brain sections near the hematoma center showing the red-hued hematoma in ICH or ICH with PEMF treated mice at day 3 and day 7 after ICH. d Quantification of hematoma volumes on images. n = 7 per group
better performance in the corner turn test after ICH with a significant decrease in the corner turn score compared with ICH group \((p < 0.05, \text{Fig. 2a})\). Similarly, the increased forelimb use asymmetry score of ICH mice and ICH + PEMF mice showed no obvious difference at day 1 and day 3 (Fig. 2b). By day 7 after ICH, there was significant difference between the ICH group and ICH + PEMF group \((p < 0.01, \text{Fig. 2b})\).

Fig. 3 PEMF attenuated inflammation both in vivo and in vitro. \(a\) and \(b\) Brain tissues were collected at day 3 after ICH for TNF-α or IL-1β ELISA test, \(n=5\) for each group. \(c\) and \(d\) BV2 cells were collected at 24 h after hemoglobin or erythrocyte treated for IL-1β or TNF-α ELISA test, \(n=5\) for each group. \(*p<0.05, \#p<0.01\) by one-way ANOVA test. 

Fig. 4 PEMF treatment mitigate neuronal death at day 3. \(a\) Representative image of TUNEL in the perihematomal area at day 3 after ICH. \(b\) and \(c\) Quantitative analyses of TUNEL-positive cells and NEUN-positive cells in the basal ganglia area at day 3 after ICH. Error bars are represented as mean±SD. \(n=6\) per group. Scale bar=100 μm. \(\ast p<0.01\) by one-way ANOVA test. \(d\) DAPI image represent as the field of interest and representative images of ICH mouse brain. Scale bar=500 μm.
PEMF Alleviated Hematoma Volume and Tissue Damage After ICH

To evaluate the influence of PEMF stimulation on ICH mice at different timepoints, hematoma volume of each mouse was measured and compared in the ICH group and ICH + PEMF group at day 1, day 3, and day 7 (Fig. 2c, d). According to the statistical result, the average hematoma volumes were comparable in both groups at day 1. At day 3, there was no obvious difference of the hematoma volumes in two groups although a reducing tendency was observed in ICH + PEMF group when compared with ICH group. As PEMF treatment progressed to day 7, the results showed that a significantly difference between the two groups and the hemorrhage size was reduced in ICH + PEMF group (p < 0.05), indicating the beneficial effects of PEMF regarding hematoma dissolution.

To identify the tissue damage of ICH mice, we assessed the number of TUNEL-positive cells in the perihematomal region after 3-day PEMF treatment. Our results showed that the number of TUNEL-positive cells in the striatal region inside the hematoma was obviously decreased and the NEUN positive cells was increased in the ICH + PEMF group compared with the ICH group (Fig. 3a–c). These results indicate that cell apoptosis in the basal ganglia region was alleviated in ICH mice by exposed to PEMF stimulation.

PEMF Alleviated Neuroinflammation by Decreasing the Release of Inflammatory Cytokines

To verify the anti-inflammatory effect of PEMF treatment, we assessed the in vivo levels of related proteins and inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). After PEMF treatment or placebo for 3 days, the mice brain specimens were collected for further examination. Firstly, we used ELISA to assess the release of two inflammatory factors from brain specimens in Sham, ICH, and ICH + PEMF mice. As shown in Fig. 4a, b, TNF-α (p < 0.01) and IL-1β
(\(p < 0.05\)) expression markedly decreased in ICH + PEMF group when compared with the ICH group. In vitro study, we also detected the level of TNF-\(\alpha\) and IL-1\(\beta\) of BV2 cells after exposure to hemoglobin or RBC. The ELISA results showed the PEMF alleviated the level of TNF-\(\alpha\) (\(p < 0.01\)), but not IL-1\(\beta\) (\(p < 0.05\)) (Fig. 4c, d). Secondly, the western
**PEMF Enhanced the Erythrophagocytosis of Microglia via CD36 in BV2 Cells**

To clarify the mechanism of PEMF on hematoma clearance, Hb-treated BV2 cells were exposed to PEMF stimulation. Representative confocal images showed the immunofluorescence co-stained of DAPI, Iba1, and CD36. The results of immunostaining and western blot demonstrated that the expression level of CD36 were elevated in BV2 cells of Hb + PEMF group when compared with Hb group.

BV2 cells were firstly incubated with PKH26-labeled erythrocytes to investigate the kinetics of erythrophagocytosis. Figure 5c illustrated that the erythrocytes engulfed by BV2 cells at 0.5 h had intact cell structures and that had been converted into debris within the BV2 plasma at 3 h. To further explore the effect of PEMF on erythrophagocytosis, RBC-treated BV2 cells were exposed to PEMF stimulation for 3 h in the RBC + PEMF group. The results demonstrated that phagocytosis index was higher in the PEMF treatment group, indicating exposure of BV2 cells to PEMF enhanced microglial phagocytosis (Fig. 5d, e). Moreover, we administered a CD36 neutralizing antibody to verify the core roles of CD36 in the phagocytosis process. And the group treated with the CD36 neutralizing antibody exhibited a lower phagocytosis index than that in PEMF treatment group (Fig. 5d, e). These results further demonstrated the critical role of CD36 in the promotion effect of PEMF stimulation on erythrophagocytosis.

**Genistein Blocked the Effect of CD36-Mediated PEMF Treatment on ICH Mice**

We detected the mechanism of PEMF treatment effects in murine model of ICH. PEMF treatment was performed at 4 h after collagenase was injected for 4 h/day, 3 days. Both the western blot and immune-fluorescence staining showed that CD36 expression enhanced at ICH + PEMF group compared with ICH group. CD36 positive cells were co-localized with Iba1. Previous study has been proved that Genistein attenuated CD36 signaling in mice hepatitis model, thus we applied genistein to determine the role of CD36 in the process of PEMF function (Xu et al. 2018). Our data revealed that the important role of CD36 in PEMF-induced hematoma clearance and anti-inflammatory effects. Western blot analysis showed that Genistein decreased CD36 protein levels after PEMF was applied (Fig. 6a, b). Immunofluorescent staining also showed that Genistein blocked the CD36 expression after PEMF was applied (Fig. 6c, d). The hematoma volume was also aggravated in the group applied genistein (Fig. 6e). Besides, the decrease of CD36 expression via genistein could reverse the anti-inflammation or the erythrocytosis effects of PEMF (Fig. 6f). These results demonstrated that CD36 plays an important role in the PEMF treatment process.

**Discussion**

The present study showed that PEMF alleviated neurological function deficits in the corner turn test and forelimb use asymmetry test and attenuated brain injury by reducing the hematoma area and cell death in ICH mice. We found that PEMF treatment not only mitigated ICH-induced neuroinflammation in vivo but also suppressed the release of inflammatory factors from microglia. In vitro study showed that PEMF decreased hemoglobin (Hb)- and erythrocyte-induced inflammatory effects, further indicating that PEMF exerts anti-inflammatory effects and protective effects against ICH. Our findings also revealed that CD36 plays important role in the promotion effect of PEMF on hematoma resolution in ICH mice and erythrophagocytosis in RBC-treated BV2 cells. The CD36 expression level was positively altered after PEMF was applied, which downregulating the CD36 via pharmacology methods could reverse its therapeutic effects. In sum, this study represents the first effort identifying that PEMF stimulation enhanced CD36-mediated hematoma clearance and reduced proinflammatory responses in ICH mice, providing experimental data that may contribute to the development of clinical therapeutic effects in patients with ICH.

Clinically, transcranial magnetic stimulation (TMS) involves the targeted application of an external magnetic field to the brain, inducing an electric current within a selected region of the brain. Repetitive TMS (rTMS) and PEMF have been demonstrated as efficient magnetic field therapy in treating several animal models of injury and disease, revealing its many beneficial behavioral and neuroprotective effects. Our study is the first...
study that explored the effects of PEMF in experimental ICH model so far, and the behavioral test results clearly proved the promotion of neurofunctional recovery of ICH mice. Our study contributes to expanding the applicability of PEMF therapy, and the following results of other experiments aim to clarify the underlying mechanisms.

Increasing evidence suggests that neuroinflammation plays a critical role in the progression and prognosis of ICH, which is considered a key therapeutic target for ICH (Wang 2010; Zhang et al. 2017). Recently, rTMS treatment was proven to alleviate the effects of ischemic stroke by inhibiting microgliosis, inducing a shift in M1/M2 polarization, and suppressing proinflammatory cytokine production after photothrombotic stroke (Zong et al. 2020). Previous studies have demonstrated that PEMF exerts an anti-inflammatory effect on neuron-like cells after ischemic injury and hypoxic damage (Sherrard et al. 2018; Vinhas et al. 2020). In N9 microglia, PEMFs exposure significantly reduced ROS production and pro-inflammatory cytokine release (Vincenzi et al. 2017). In addition, PEMF exposure inhibited the release of acute inflammatory cytokines (IL-6, IL-1β, and TNF-α) 7 days after needle stick injury in rat tail discs (Chan et al. 2019). Meanwhile, the effect of PEMF stimulation may not be limited to downregulation of proinflammatory cytokines. Another study found that PEMF exposure promoted the expression of the anti-inflammatory cytokine IL-10 (Gomez-Ochoa et al. 2011). These results indicate that PEMF stimulation has dual effects on upregulation of anti-inflammatory factors and downregulation of pro-inflammatory factors.

However, it is still unknown whether PEMF can protect against the inflammation occurred after ICH. Our in vivo and in vitro study showed clear evidence that the expression levels of pro-inflammatory cytokines TNF-α and IL-1β elevated post-ICH. These results are in consistent with other collagenase-induced and autologous blood-induced models of ICH in the previous studies, which are currently thought to be produced by activated M1 microglia (Lan et al. 2017). Our results showed that PEMF stimulation sharply reduced the expression of M1-sapirnate cytokines at day 3 post-ICH, indicating the microglial function might be changed by PEMF stimulation. Additionally, our findings demonstrated that PEMF therapy reduced apoptotic cells in basal ganglia at day 3. The results are consistent with those of our earlier investigation, which revealed that PEMF exposure combined with 5G dramatically reduced cell apoptosis in MLO-Y4 cells (Wang et al. 2019). On the one hand, PEMF markedly reduced the expression of genes linked to apoptosis. On the other hand, the prevention of inflammatory harm following ICH may contribute to this protective effect.

In addition, several studies have documented that PEMF can affect the phagocytic ability of macrophages (Ouyang et al. 2020). Nevertheless, little is known about the function of PEMF in microglia and their effects on hematoma clearance. CD36, also known as the scavenger receptor B2, is a vital hematoma clearance receptor expressed in microglia (Li et al. 2021). In our study, PEMF stimulation significantly promoted hematoma resolution by improving CD36-mediated microglial erythrophagocytosis, and thus cell apoptosis and tissue damage in ICH mice with alleviated neurological function deficits.

Taken together, these data suggest that PEMF treatment may be a potential noninvasive therapy for ICH. We also observed that PEMF stimulated microglial CD36 activation in vivo and in vitro after PEMF treatment. We assumed that PEMF affects the CD36 expression during the process of therapy, which may be the underline mechanisms of PEMF influence the hematoma clearance or inflammation process. In order to determine the CD36 role, we used genistein to blockage the CD36 during the PEMF treatment. The results showed that PEMF treatments was reversed after genistein administrated compared with the saline group. These results at least partly illustrated the vital role of CD36 in the PEMF treatments process.

Collectively, present study demonstrates that PEMF treatment leads to stronger suppression of neuroinflammation and hematoma dissolution after ICH than no treatment. PEMF alleviated inflammation and promoted phagocytosis in BV2 cells and an ICH model. The CD36 expression level was positively altered after PEMF was applied and downregulating the CD36 via pharmacology methods could reverse its therapy effects. We conclude that PEMF treatment is a possible adjuvant therapy for ICH patients. Further studies elucidating the CD36-related pathway mechanisms underlying the effect of PEMF on the brain recovery process after ICH will be of great interest.

**Author Contribution** Yuefan Yang designed, performed, and analyzed most figures. Pan Wang and Anlai Liu performed the behavior test. Shuhui Dai, Jialiang Wei, and Zhuoyuan Zhang provided studying materials and technical support. Zedong Yan and Xiuquan Wu performed the statistics work. Xin Li, Peng Luo, and Erping Luo conceived and coordinated the study and wrote the paper. The authors read and approved the final manuscript.

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**Declarations**

**Ethics Approval** All procedures were approved by the Animal Care and Use Committee of Air Force Medical University.

**Consent for Publication** All authors listed agree to publish this article.

**Conflict of Interest** The authors declare no competing interests.
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