Pulsed Electromagnetic Fields Protect Against Brain Injury After Intracerebral Hemorrhage: Involvement Of Anti-Inflammatory Processes And Hematoma Clearance Via CD36

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Research

Keywords: Intracerebral hemorrhage, PEMFs, Inflammation, Hematoma clearance

Posted Date: July 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-666619/v1>

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Abstract

Background: Intracerebral hemorrhage causes high mortality and morbidity, but its therapy methods are limited. In the present study, pulsed electromagnetic fields (PEMFs) were demonstrated to have beneficial effects on an intracerebral hemorrhage (ICH) model. This study explored the effects and underlying mechanism of PEMFs in a mouse model of ICH and cultured BV2 cells.

Methods: PEMFs were applied 24 hours after collagenase-induced ICH and 4 hours per day for seven consecutive days. The levels of proinflammatory factors were assessed by ELISA kits and western blotting. Hematoma volume was measured by histological analysis. The effects of PEMFs on phagocytosis of the erythrocytes was observed in cultured BV2 cells.

Results: Seven days after ICH, the hematoma volume was significantly reduced in PEMF-treated animals compared to nontreated mice. We found that PEMFs decreased the hematoma volume and the levels of proinflammatory factors after ICH. To evaluate the mechanisms underlying the effects of PEMFs on ICH, we analyzed the transcriptome profile three days after ICH, and we found that PEMFs reversed the changes in inflammation-related pathways and alleviated neurological deficits. Furthermore, PEMFs enhanced the erythrophagocytosis of microglia via CD36. Thus, the PEMF-mediated promotion of neurological functions may at least partly involve anti-inflammatory processes and hematoma clearance.

Conclusions: These results suggest that PEMF treatment is a possible adjuvant therapy for ICH patients.

Background

Intracerebral hemorrhage (ICH) is a devastating nervous system disease with high mortality and morbidity rates^{1–4}. ICH-related injury involves primary and secondary mechanisms. In ICH, primary injury is induced by the mass effect of the intracerebral hematoma, which leads to brain tissue deformation and elevated intracranial pressure^{5,6}. Secondary injury after ICH refers to the subacute and long-term injury caused by neuroinflammation, oxidative stress, and other detrimental factors^{7–9}. Accumulating evidence suggests that neuroinflammation, including activation of microglia and the release of inflammatory cytokines, plays an essential role in ICH pathogenesis⁹. It begins immediately after hematoma formation and is a critical host defense response to secondary brain injury after ICH¹⁰. Although currently available invasive surgical approaches for hematoma removal are effective against primary injury, they do not address secondary brain injury following ICH¹¹.

Electromagnetic field therapy is emerging as a potential alternative to pharmacological treatments for several neurodegenerative diseases¹². Pulsed electromagnetic field (PEMF) treatment, which can be externally and noninvasively applied to injured tissue, increases microvascular perfusion and metabolism after brain injury¹³. Exposure to PEMFs has been shown to attenuate tissue damage following stroke. PEMF stimulation was shown to decrease the infarct size after transient focal ischemia in rabbits¹⁴. PEMF treatment using FDA-approved parameters has been demonstrated to exert inflammatory effects

after traumatic brain injury in rats, as it was shown to decrease the level of the inflammatory cytokine interleukin (IL)-1 β ¹⁵.

Microglia are resident macrophages of the central nervous system (CNS) and account for approximately 5–20% of the total glia in the CNS. Once tissue damage occurs, microglia can be activated within minutes via neurotoxins released into the extracellular space. Microglia are considered to be associated with inflammation and are generally involved in the phagocytosis process¹⁶. Different types of microglia, including M1 and M2 microglia, have distinct effects during ICH-related injury^{17,18}. M1 microglia are strongly associated with worsening of cerebral edema surrounding ICH on radiography¹⁹. 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^{20,21}. In addition, PEMFs have been shown to reduce the production of reactive oxygen species (ROS) induced by lipopolysaccharide (LPS) and alleviate neuron-like cell damage by inhibiting inflammation in N9 microglial cells²². These findings reveal that microglia may be the targets of PEMF. However, the regulatory effect of PEMFs on microglia after ICH in vivo remains unknown.

In this study, PEMF therapy using parameters reported in a previous study²³ was performed to illustrate the regulatory effects of PEMFs after ICH. We showed that in a mouse model of ICH, PEMF therapy decreased the release of inflammatory cytokines, promoted hematoma dissolution, ameliorated brain injury, and improved functional recovery, suggesting that PEMF therapy could represent a potential therapeutic approach for ICH-related injury.

Methods

PEMF application

PEMFs were generated using a system composed of a pulsed signal generator and two-array Helmholtz coils, as shown in Figure 1 (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 μ s; pulse fall, 2.0 μ s) repeated at 15 Hz as described in our previous studies³⁵. 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). The bottom of the culture plates containing BV2 cells was aligned with the center of the coils. The PEMF treatment group received PEMF treatment immediately after red blood cell (RBC)- or Hb-induced injury for

4 hours, and the cells were collected after 24 hours. The untreated group was placed in a different chamber under the same conditions with inactivated Helmholtz coils.

For the animal study, the mice that underwent ICH surgery were placed in the center of the coils 4 hours after surgery and for 4 hours per day. The untreated ICH group mice were placed in a different chamber with inactivated Helmholtz coils.

Animals

The experimental protocol was approved by the Animal Care and Use Committee of Air Force Medical University. A total of 100 8- to 10-week-old C57BL/6J mice were purchased from the Animal Center of Air Force Medical University. The mice were group-housed in individually ventilated cages on a 12-hour light-dark cycle with ad libitum access to standard mouse feed and water.

ICH model

The collagenase injection ICH model was constructed as described previously. After the mice were anesthetized, 1 ml 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 hours after ICH and for 4 hours per day for 7 days. On day 3 and day 7, all mice were subjected to the corner turn test and forelimb use asymmetry test.

Western blot analysis

The protocol was the same as that described in previous articles. Briefly, the protein samples were quantified by the BCA method. Then, equal amount of the samples (50 mg) 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 images were analyzed with ImageJ software.

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 hours and then dehydrated with 30% sucrose. Frozen coronal slices (30 mm thick) were obtained with a freezing microtome. The sections were washed with 0.01 M PBS and blocked with 5% normal goat serum for 30 minutes. Then, they were incubated with primary antibody (CD36, Iba1) at 4 °C overnight and washed three times with 0.01 M PBS. We applied appropriate secondary antibodies at room temperature for 2 hours. Antifade solution with or without DAPI was applied, and the sections were observed with an Olympus microscope after they were completely dried.

Erythrophagocytosis assay

BV2 cell erythrophagocytosis was evaluated as previously described²¹. For the microglia with CD36 or IgG group, CD36 neutralized antibody or IgG were pretreated on each cell (2 mg/ml) 4 hours 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 minutes, resuspended in diluent, and stained with PKH-26 for 4 minutes. Serum was applied for 1 minute 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.

ELISA

The cells were suspended at a density of 10^6 cells/ml and seeded in 24-well plates. The cells were incubated for 24 hours in the absence or in the presence of Hb (20 mM). Mouse brain was collected at the end of treatment, and suspended cells were collected and centrifuged at $1000 \times g$ for 10 minutes. The levels of the proinflammatory cytokines TNF- α and IL-1 β 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.

Hematoma volume measurement

On day 3 and day 7 after ICH, the mice were perfused with 0.01 M PBS 50 ml after being anesthetized. Their brains were collected and sectioned with 1-mm coronal mouse brain matrices. The brain slices were digitalized and analyzed, and the cubic volume of the hematoma was calculated using ImageJ in a blinded manner.

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 72 hours after ICH. The number of TUNEL-positive neurons in the perihematomal area in six sections per brain at 20 $\times$ magnification was counted manually using ImageJ software (ImageJ 1.4, NIH). The data are expressed as the number of TUNEL-positive cells.

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. 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 minutes was recorded. The forelimb use asymmetry score was calculated as $[I-C]/[I+C+B] \times 100$, 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.

Transcriptome resequencing analysis

The mice were divided into four groups: the sham, PEMF, ICH, and ICH with PEMF groups (n = 3). After 3 days, the ipsilateral brain hemispheres of all mice were collected for RNA-seq. Total RNA was extracted using a TRIzol reagent kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. RNA quality was assessed with an Agilent 2100 Bioanalyzer and checked using RNase-free agarose gel electrophoresis. After total RNA was extracted, eukaryotic mRNA was enriched by oligo(dT) beads, while prokaryotic mRNA was enriched by rRNA removal with a Ribo-Zero™ Magnetic Kit. Then, the enriched mRNA was fragmented into short fragments using fragmentation buffer and reverse transcribed into cDNA with random primers. Second-strand cDNA was synthesized by DNA polymerase I, RNase H, dNTPs, and buffer. Then, the cDNA fragments were purified with a QiaQuick PCR extraction kit (Qiagen, Venlo, The Netherlands), end-repaired, poly(A) added, and ligated to Illumina sequencing adapters. The ligation products were size selected by agarose gel electrophoresis and PCR amplified. Finally, the sample library products were sequenced using an Illumina NovaSeq6000 device (Gene Denovo Biotechnology Corporation, Guangzhou, China).

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

PEMFs promoted hematoma dissolution and neurofunctional recovery after ICH

C57BL/6J mice were divided into three groups: the control group, ICH group and ICH with PEMF treatment group. Animals who underwent ICH surgery were subjected to behavior tests on day 3 or day 7. Mice in the ICH with PEMF treatment group were treated with PEMFs 4 hours after ICH and for 3 days or 7 days.

The mice were sacrificed after anesthesia with chloral hydrate (7%), and then their brains were carefully separated with fine forceps. Each brain was sectioned coronally at a thickness of 1 mm and digitally imaged for further analysis. The hematoma volume was calculated as the total area × thickness. The

results showed that the PEMF treatment group had a smaller hemorrhage size on day 7. Thus, these data proved the beneficial effects of PEMFs regarding hematoma dissolution ($p < 0.05$).

To assess neurofunctional deficits, we subjected the three groups of mice to the corner turn test and forelimb use asymmetry test 3 days or 7 days after ICH. The PEMF-treated mice exhibited significantly better performance in the corner turn test ($p < 0.05$) and forelimb use asymmetry test ($p < 0.01$) 7 days after ICH.

PEMFs alleviated tissue damage and cell apoptosis after ICH

Mice who underwent ICH surgery were sacrificed on day 3. Their brains were fixed with 4% paraformaldehyde for 4 hours and then dehydrated with 30% sucrose solution. The specimens were cut into 30 μm sections for staining. Our results showed that the number of TUNEL-positive cells in the striatal region around the hematoma was decreased in the PEMF treated group compared with the ICH group on day 3 ($p < 0.01$).

PEMF reversed the profound alterations in the gene transcription profile of the brain on day 3 after ICH

To gain mechanistic insights into the protective effects of PEMFs against ICH, whole-transcriptome analysis of the brain was performed on day 3 after ICH surgery using RNA-seq. The data were uploaded to the National Center for Biotechnology Information Sequence Read Archive (SRA) under the accession code SRP324536 and are publicly available. A total of 700 genes (566 upregulated genes and 134 downregulated genes) were differentially expressed ($\text{FDR} < 0.05$) in the sham group compared to the ICH group. A total of 500 genes (105 upregulated genes and 395 downregulated genes) were differentially expressed in the PEMF treatment group compared with the ICH group. The differentially expressed genes belonged to multiple categories related to the neuroinflammatory pathway. Taken together, the RNA-seq data revealed that PEMF exposure reverses the profound alterations in the gene transcription profile of brain tissue after ICH. The results of gene set enrichment analysis (GSEA) showed that genes upregulated in the ICH group compared with the sham or ICH with PEMF treatment group were highly enriched in inflammation-related pathways, demonstrating that PEMF treatment has anti-inflammatory effects. However, the expression of was not different between the sham and ICH groups or between the ICH and ICH with PEMF treatment group.

PEMFs alleviated neuroinflammation by decreasing the release of inflammatory cytokines

To verify the anti-inflammatory effect of PEMF treatment, we assessed the levels of related proteins in both in vitro and in vivo models. All mice were randomly divided into three groups: the sham group, ICH group, and PEMF treatment group. After 3 days of treatment or placebo, the mice were sacrificed, and their brains were collected for ELISA analysis and western blot analysis. The western blot results indicated that PEMFs reduced the release of IL-1 β , tumor necrosis factor (TNF)- α , and Nlrp3 in brain tissues. The ELISA results suggested that PEMFs decreased the levels of IL-1 β and TNF- α in vivo.

To further explore the anti-inflammatory effect of PEMFs, we used ELISA to assess the release of inflammatory factors from Hb-injured BV2 cells. The results showed that the TNF- α level was significantly decreased in the PEMF treatment group compared to the untreated group. The level of IL-1 β also declined after PEMF treatment.

PEMFs enhanced the erythrophagocytosis of microglia via CD36

To clarify the effects of PEMF on hematoma clearance, we used BV2 cells to mimic the hemorrhagic environment. The erythrocytes engulfed by BV2 cells at 30 minutes had intact structures and had been converted into debris within the BV2 plasma at 3 hours. The results demonstrated that exposure of BV2 cells to PEMFs enhanced microglial phagocytosis. The fluorescence index was higher in the PEMF treatment group. PEMFs elevated the CD36 protein level after ICH, which promoted hematoma clearance. 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 fluorescence index than the PEMF treatment group. These results further demonstrated the critical effects of CD36.

Discussion

Increasing evidence suggests that neuroinflammation plays a critical role in the progression and prognosis of ICH, inducing oxidative stress, increasing the permeability of the blood-brain barrier, exacerbating brain edema, and ultimately leading to brain injury and neurological deterioration^{24–26}. Therefore, neuroinflammation is considered a key therapeutic target for ICH. A previous study showed that PEMFs can exert anti-inflammatory effects in several human conditions^{27, 28}. Furthermore, PEMF therapy has been clinically applied via remote actuation and minimally invasive approaches to manage pain and delayed wound healing²⁹. Although previous studies have demonstrated that PEMFs exert an anti-inflammatory effect on neuron-like cells after ischemic injury and hypoxic damage, it is unclear whether PEMFs can protect against ICH.

Previous studies have focused on the biological effects of PEMFs, the underlying mechanisms of which are unclear. In the present study, we found that PEMFs reversed the activation of different pathways by ICH through RNA-seq analysis of mouse brain tissues. The transcriptome data provided valuable information regarding the mechanisms of PEMFs in the treatment of ICH-related injury and can be compared with data from previous studies to determine their accuracy.

Importantly, we explored the potential neuroprotective role of PEMF therapy in ICH and found that PEMFs alleviated neurological function deficits, as revealed by the corner turn test and forelimb use asymmetry test, and attenuated brain injury, including by reducing the hematoma area and cell death, in ICH mice. Interestingly, we found that PEMF treatment not only suppressed the release of inflammatory factors from microglia but also mitigated ICH-induced neuroinflammation in vivo. PEMFs decreased hemoglobin

(Hb)- and erythrocyte-induced inflammatory effects, further indicating that PEMFs exert anti-inflammatory effects and protective effects against ICH.

In addition, several studies have documented that PEMFs can affect the phagocytic ability of macrophages³⁰. Nevertheless, little is known about the function of PEMFs in microglia and their effects on hematoma clearance. CD36, also known as the scavenger receptor B2, is a vital hematoma clearance receptor expressed in microglia³¹. We observed that PEMFs stimulated microglial CD36 activation in vivo and in vitro after PEMF treatment. However, the CD36 transcript level was not significantly different between the ICH and ICH with PEMF treatment groups or between the sham and ICH groups, which is inconsistent with the change in CD36 protein expression. The inconsistency between the change in the transcript level and the change in the protein level may have been the result of the CD36-related regulatory pathway. In addition, a previous study reported that Tim3 (Havcr2) can downregulate the expression of CD36, as Tim-3 was shown to inhibit macrophage-mediated phagocytosis of *Listeria monocytogenes* via CD36³². The transcriptome data showed that Tim3 levels were decreased after PEMF treatment, and this decrease at least partly accounted for the promotion of CD36 expression. On the other hand, a previous study proved that TLR4 can negatively regulate CD36-mediated hematoma absorption, and the transcriptome data also revealed that PEMF treatment reversed the TLR level³³. These changes could be the underlying mechanisms by which PEMF regulates CD36 expression levels after PEMF treatment. Taken together, these data suggest that PEMF treatment may be a potential noninvasive therapy for ICH. Collectively, these results demonstrate that PEMF treatment leads to stronger suppression of neuroinflammation and hematoma dissolution after ICH than no treatment. Further studies elucidating the molecular mechanisms underlying the effect of PEMFs on the brain recovery process after ICH will be of great interest.

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) has demonstrated efficacy in treating several animal models of injury and disease, revealing its many beneficial behavioral and neuroprotective effects. Application of rTMS after experimental stroke in an animal model increased the expression of genes involved in neural remodeling, repair, neuroprotection, and the injury response. 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 stroke³⁴. It is commonly known that rTMS and PEMF have similar mechanisms. Thus, our results are consistent with the findings of previous studies of rTMS, providing a better understanding of the effects of rTMS after stroke. Furthermore, our study contributes to expanding the applicability of rTMS and PEMF therapy.

Although our work provides proof that PEMFs attenuate neuroinflammation after ICH in a collagenase injection model, the autologous blood injection model, which is more suitable for evaluating hematoma, was not used in this study. Second, macrophages from the circulatory system also play an essential role in hematoma dissolution, but these macrophages were not assessed in this study. Third, we only

evaluated the effects of PEMF therapy in the early stage after ICH, and its effect on long-term neurological deficits should also be evaluated in further studies.

Conclusions

PEMFs alleviated inflammation and promoted phagocytosis in BV2 cells and an ICH model. The underlying mechanisms of PEMF may be related to reversal of alterations in the transcript levels of genes associated with ICH-related pathways, including the inflammatory pathway and immune pathway. We conclude that PEMF treatment is a possible adjuvant therapy for ICH patients.

Abbreviations

PEMFs: Pulsed electromagnetic fields

ICH: Intracerebral hemorrhage

IL: Interleukin

TNF: Tumor necrosis factor

RBC: Red blood cell

PBS: Phosphate-buffered saline

Declarations

Availability of data and materials

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

Competing interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: the study was supported by the Natural Science Foundation of Shannxi Province, China (grant no. 2021JQ-344 to YF.Y.), and the National Natural Science Foundation of China (grant no. 81771322, 81901186), the Youth talent lifting project (no. 17-JCJQ-QT-037), and Youth Nova Program of Shaanxi Province (2021KJXX-19) and Natural Science Foundation of Shannxi Province, China (grant no. 2020JQ-231)

Authors' contributions

YFY designed, performed and analyzed most figures. WP and ALL performed the behavior test. SHD and JLW provided studying materials and technical support. ZDY and XQW performed the statistics work. XL, PL and EPL conceived and coordinated the study and wrote the paper. The authors read and approved the final manuscript.

Acknowledgments

We thank Xiaoyan Chen for technical support, and the Gene Denovo Biotechnology Co.Ltd (Guangzhou, China) for Sequencing bioinformation analysis.

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Figures

Figure 1

Photograph of the PEMF system used and the experimental design. ICH, intracerebral hemorrhage; PEMF, pulsed electromagnetic field.

Figure 2

PEMF promoted hematoma resolution and neurological recovery after ICH. A. Coronal brain sections from the region near the hematoma center showing red-hued hematomas in mice from the ICH and ICH with PEMF treatment groups at 3 days and 7 days after ICH. B. Quantification of the hematoma volume from images. $n = 7$ per group. C. Neurological functions were assessed by the corner turn test and D. forelimb use asymmetry test. $n=6$ for each group. * $p < 0.05$, # $p < 0.01$ by two-way repeated-measures ANOVA (B, C and D). The asterisks on the top indicate differences groups.

Figure 3

PEMF attenuated inflammation both in vivo and in vitro. A. The protein levels of IL-1 β , TNF- α , and Nlrp3. B, C and D. Western blot analysis of IL-1 β , TNF- α , and Nlrp3 expression. $n = 3$ for each group. $*p < 0.05$, $\#p < 0.01$ by one-way ANOVA test. E. Brain tissue was collected 3 days after ICH to measure IL-1 β and TNF- α levels by ELISA. $n = 5$ for each group. F. BV2 cells were collected 24 hours after Hb or erythrocyte treatment to measure IL-1 β and TNF- α levels by ELISA. $n = 5$ for each group. $*p < 0.05$, $\#p < 0.01$ by one-way ANOVA.

Figure 4

PEMF treatment mitigated neuronal death on day 3. Representative images of TUNEL staining in the perihematoma area on day 3 after ICH. Quantitative analyses of TUNEL-positive cells in the perihematoma area on day 3 after ICH. The error bars are the SD. n = 5 per group. Scale bar = 50 μ m.

Figure 5

PEMF reversed the alterations in gene transcription induced by ICH. A, B. Volcano plots of the sham vs ICH groups and ICH vs ICH with PEMF groups. Down indicates downregulation, up indicates upregulation, and nodiff indicates no difference. C. Heatmap of cluster analysis showing the transcript levels of differentially expressed genes in the ipsilateral hemisphere in the ICH and ICH with PEMF groups on day 3 after ICH or PEMF treatment. Relative expression levels across all samples are illustrated using the color

scale. Red represents an expression level above the mean, and blue represents an expression level lower than the mean. The dendrogram on the left of the heat map shows the clustering of the transcripts. D and F, GSEA of the sham vs ICH groups and ICH vs ICH with PEMF groups. E and G, GSEA of genes associated with the neuroinflammatory response in the sham vs ICH groups and ICH vs ICH with PEMF groups. n = 3 per group.

Figure 6

PEMF alter CD36 levels both in vivo and in vitro. A. RBCs were labeled with PKH-26 to assess the effects of PEMFs on phagocytosis by BV2 cells 0.5 hours, 1 hour, and 3 hours after RBCs were added. B. Immunofluorescent staining of CD36 and Iba1 in ICH model. C. Representative western blot bands and quantitative analyses of CD36 protein levels in brain tissues. The error bars are the SDs. n = 3 per group. D. BV2 cells after incubation with Hb for 24 hours. Immunofluorescent staining of CD36 and Iba1 in BV2 cells. E. Representative western blot bands and quantitative analyses of CD36 protein levels in BV2 cells. The error bars are the SDs. n = 3 per group.

Figure 7

PEMF promoted BV2 cell-mediated erythrocyte phagocytosis via upregulation of CD36 expression A. PEMF treatment promoted BV2 cell-mediated phagocytosis via CD36. Quantitative analyses of PKH-26-positive cells at 3 hours after ICH. The error bars are the SDs. n = 8 per group. Scale bar = 20 μ m. B. Quantitative analyses of PKH26-positive cells.