Upregulation of TIPE2 attenuates macrophage activation and neuroinflammation after intracerebral hemorrhage in mice

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Abstract

Background: Intracerebral hemorrhage (ICH) is a serious disease with high mortality and morbidity, and effective treatment is limited. A large amount of evidence suggests that the inflammatory response contributes to secondary brain damage following ICH. TIPE2 is an essential negative regulator of both innate and adaptive immunity, and depletion of TIPE2 causes inflammatory disease. However, the possible role of TIPE2 following ICH has not been reported.

Methods: In this study, we investigated TIPE2 levels and inflammation in macrophages treated with erythrocyte lysate in vitro, and we observed proinflammatory cytokine production, BBB disruption, cerebral water content and neurological damage in ICH mice in vivo.

Results: We found that TIPE2 levels were significantly decreased in erythrocyte lysate-treated macrophages compared to control macrophages. Upregulation of TIPE2 decreased macrophage activation and cytokine production and accelerated brain damage in ICH mice. In addition, upregulation of TIPE2 attenuated proinflammatory cytokine production, BBB disruption, and severe brain inflammation after ICH.

Conclusion: The results demonstrated that TIPE2 was negatively correlated with the pathogenesis of ICH, which prevented brain injury and attenuated deleterious inflammatory responses following ICH. TIPE2 might serve as a novel target for ICH therapy.

Introduction

Intracerebral hemorrhage (ICH) is a severe neurological disease with high morbidity and mortality [1, 2, 3]. ICH often induces severe neurological impairment because of inflammation-mediated secondary brain damage [4, 5, 6]. Brain inflammation following ICH is characterized by the accumulation of activated inflammatory cells. These activated cells produce inflammatory mediators, including chemokines, cytokines, proteases, and other proinflammatory molecules [7, 8, 9]. Among the factors that contribute to brain inflammation, macrophage activation plays an essential role in the secondary damage after ICH [10, 11, 12].

TIPE2 (tumor necrosis factor (TNF)-induced protein 8-like 2) plays an important role in maintaining immune homeostasis by negatively regulating T cell receptor and Toll-like receptor (TLR) signaling [13, 14, 15]. TIPE2 is preferentially expressed in lymphoid tissues, macrophages and lymphocytes. TIPE2 has been identified to be involved in inflammatory and infectious diseases, such as hepatitis B, systemic lupus erythematosus, and asthma [16, 17, 18].

However, whether TIPE2 plays a role in the regulation of inflammation following ICH has not been reported. Considering TIPE2 is essential for the maintenance of immune homeostasis, we hypothesize that TIPE2 might contribute to inflammation following ICH. In this study, we explore TIPE2 levels and the related effects on inflammation following ICH.

Materials And Methods

Experimental animals

C57BL/6 mice were generated and kept in our laboratory according to a previous study. All mice used were male and 8–10 weeks old and weighed 22–25 g. The mice were maintained in individual cages under controlled conditions (22 ± 2 °C; 55% humidity) with free access to food and fresh water. All animal experiments were approved by the Ethics Committee of Chongqing Medical University.

Primary cell cultures

Cortical neuronal cultures were prepared from the whole cerebral cortices of C57BL/6 mouse embryos (E16). After removal of the meninges, the tissue was digested with 0.005% trypsin/0.002% EDTA (10 min, 37 °C), mechanically dissociated, and centrifuged at 1000 × g for 5 min. The cell pellet was resuspended in neurobasal medium (Gibco) containing B27 serum-free supplement (Gibco) and 500 N m L -glutamine. A total of 2×10^5 cells/well were seeded on sterile poly-L-lysine (Sigma-Aldrich)-coated glass cover slips in a 24-well plate and incubated at 37 °C and 5% CO_2. After 1 h, the culture medium was changed completely. The purity of the neuronal cultures was > 95%, as confirmed by random staining with neuronal and glial markers. Five days after plating, the neurons had developed a dense network of extensions. To isolate primary macrophage cells, the cerebral hemispheres of 1-day-old postnatal mice were digested with 0.1% trypsin. The cells were seeded into a six-well plate coated with poly-L-lysine and cultured with Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA). The culture medium was refreshed twice per week for 2 weeks. Macrophages were detached by gentle shaking and filtered through a nylon mesh to remove astrocytes. After centrifugation at 1000 × g for 10 min, the cells were resuspended in fresh DMEM supplemented with 10% FBS and plated at a final density of 5×10^5 cells/mL on a poly-L-lysine coated 6-well culture plate. The following day, the cells were subjected to experiments. The cell purity was determined by immunohistochemical staining using a macrophage-specific CD11b antibody. The macrophage cultures used were > 95% pure.

Lentivirus encoding TIPE2 construction

A TIPE2 expressing lentivirus gene transfer vector was constructed by Genechem Co., Ltd., Shanghai, China. The identity of the vector was confirmed by PCR and sequencing analysis. The recombinant TIPE2 expressing lentivirus (Lv-TIPE2) and the control lentivirus (Lv-CON) were prepared at a titer of 10^9 TU (transfection unit)/ml.

Lentivirus transduction and detection assay

Macrophage were transduced with lentiviral vectorsat a multiplicity

of infection (MOI) of 50 in complete medium containing polybrene

(5 μg/ml) at 37 °C and 5% CO_2 for 18–24 h. The cells were then

washed and cultured in fresh medium containing 10% FBS. Cells

were harvested 3 days following transduction.

Preparation of erythrocyte lysates

The spleens were removed from C57BL/6 mice. Single-cell suspensions of splenocytes were prepared using stainless steel mesh screens. Then, 1×10^5 splenocytes were incubated with 1 ml of red blood cell lysis solution for 20 min and centrifuged at 2000 rpm for 10 min. The supernatants were utilized as erythrocyte lysates.

Cell treatment

Macrophages (1×10^5) were stimulated with 10 μL of erythrocyte lysate. After 3 days, the supernatants were removed and further analyzed for cytokine production by ELISA. Neurons were cultured in a 96-well plate at a density of 1×10^4 cells per well. For the toxicity experiments, neurons were serum-starved for 4 h and then treated with a mixture of macrophage-conditioned media. For the MTT and apoptosis assays, the cells were treated for 48 h.

ICH model

Briefly, mice were anesthetized by an intraperitoneal injection of 400 mg/kg chloral hydrate and fixed on a mouse stereotaxic frame (Stoelting). A 20-μl volume of autologous nonanticoagulated blood was collected from the tail vein of the mice and then injected into the caudate nucleus at 0.8 mm anterior to bregma, 2 mm lateral to bregma the left, and 3.5 mm deep under stereotactic guidance at a rate of 2 μl/min over a period of 10 min. The needle was held in place for 10 min after injection, and the microsyringe was pulled out after the blood had coagulated. The craniotomy was then sealed with bone wax, and the scalp was closed with sutures. Body temperature was maintained at 37 °C throughout the procedure, and the mice were given free access to food and water after they woke up. The mice that died due to anesthesia were excluded.

Administration of TIPE2 Lentivirus

To study the effects of TIPE2, mice received an intracerebral ventricular injection of TIPE2 Lentivirus or control lentivirus (1×10^9 plaque forming units [Pfu], 2 μl of a 10 mg/ml solution prepared in 0.9% NaCl) 10 min after ICH and sacrificed 48 h after ICH.

RT-qPCR

Total RNA was extracted from mouse brain tissue by using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Reverse transcription was performed with a First Strand

cDNA Synthesis kit (Fermentas, Vilnius, Lithuania). Real-time PCR was performed on an ABI 7300 Sequence Detection System with a SYBR-Green PCR kit (both Applied Biosystems; Thermo Fisher Scientific, Inc.). All primers used in this study were purchased from Sangon Biotech Co., Ltd. (Shanghai, China), and the sequences were as follows: TIPE2, forward 5'-TGA AAC TCA GGT CCG CTT CT-3' and reverse 5'-TCC TAG TGC TGC CTC CAA CT-3'. For qPCR, 1 μl of cDNA, 1 nM primers and 12.5 μl of 2 × SYBR Green were mixed to obtain a final volume of 25 μl. Thermal cycling was performed as follows: 40 cycles at 95 °C for 30 sec, 56 °C for 30 sec and 72 °C for 30 sec. Relative gene expression levels were determined using the comparative Cq method with GAPDH as the control.

Western blot analysis

Total protein was isolated from ipsilateral lesional brain tissues using ice-cold RIPA buffer. The protein concentrations were measured with the BCA Protein Assay Kit (Forevergen Biosciences). The protein samples were separated using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. The proteins were detected by incubation with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies. The immunoblots were visualized using ECL plus Western Blotting Detection Reagents (Pluslight, Forevergen Biosciences, China). GAPDH (1:1000, Cell Signaling Technology, USA) was employed as the loading control. Densitometry analysis was performed with the use of ImageJ software with normalization to GAPDH.

Enzyme-linked immunosorbent assay (ELISA)

Macrophages (1×10^5) were stimulated with 10 μL of erythrocyte lysate. After 3 days, the supernatants were removed and further analyzed for cytokine production by ELISA. ELISA was performed according to the manufacturer's instructions (Dakewe Biotech, Shenzhen, China) to assess the concentrations of TNF-α, IL-1β and IL-6 in the culture supernatant.

MTT assay

The viability of neurons was assessed using a 3-(4,5-dimethyl-2 -thiazolyl) − 2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma-Aldrich) assay. After 48 h, MTT reagent was added to the wells, and the cells were incubated for 4 h at 37 °C and 5% CO_2. After centrifugation, the supernatant was removed from each well. The colored formazan crystal produced from MTT was dissolved with 0.15 mL of DMSO, then the A490 optical density (OD) value was measured with a multiscanner autoreader (Dynatech MR 5000; Dynatech Laboratories, Chantilly, VA, USA). The absorbance was measured at 570 nm. The mean of the readings of triplicate wells was taken as the value. The OD value for the control cultures was considered 100% viability, and the viability of the other samples is expressed as a percentage of the viability of the control cultures.

Flow cytometry analysis of apoptosis

An annexinV-fluorescein isothiocyanate (FITC) apoptosis detection kit (OncogeneResearch Products, Boston, MA) was used to detect apoptosis. The cells were seeded in 1-mL flasks and incubated in DMEM supplemented with 10% fetal bovine serum until they reached approximately 90% confluence. Then, the cells were harvested, washed with ice-cold PBS twice, and resuspended in binding buffer (10 mM Hepes, pH 7.4, 50 mM NaCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 4% bovine serum albumin). Annexin V-fluorescein isothiocyanate (0.5 mg/mL) and propidium iodide (0.6 mg/mL) were then added to a 250 mL aliquot (1×10^6 cells) of this cell suspension according to the manufacturer's protocol. After a 15-minute incubation in the dark at room temperature, the stained cells were immediately analyzed with a flow cytometer (Beckman Coulter, USA). All of the samples were assayed in triplicate, and the cell apoptosis rate was calculated using the following formula: apoptosis rate = (apoptotic cell number / total cell number) × 100%.

Caspase assay

To detect caspase-3-like protease activities, the ApoAlert Caspase-3 Colorimetric Assay kit (Clontech, Palo Alto, USA) was used. Cytosolic lysates were prepared 48 h following transfection and incubated with 50 mm p-nitroanilide (pNA) conjugated to the caspase cleavage site Asp-Glu-Val-Asp (DEVD) for 1 h at 37 °C. Hydrolyzed pNA was detected using a Multiscan MS colorimeter (Thermo Labsystems, Vantaa, Finland) at 405 nm. For control experiments, the lysates were incubated with 10 mM caspase-3 inhibitor DEVD-fmk (Clontech) for 30 min before addition of the substrate.

Histochemical evaluation of macrophage activation

Three days after ICH, the animals were deeply anesthetized with pentobarbital and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PBS, pH 7.4). After the mice were perfused and fixed, the perihematomal regions of the cerebral tissues were collected, fixed in 4% paraformaldehyde for 24 h, dehydrated in 30% sucrose solution for 48 h, embedded, frozen, and cut into 25-μm sections using a Leica CM1900 cryostat. The perihematomal sections were treated with 3% H_2O_2 in 0.01 M phosphate-buffered saline (PBS) and preincubated in 5% normal goat serum. The samples were then incubated in a primary antibody solution containing a rat anti-Iba antibody (Serotec, Fullerton, CA, USA, 1:200) overnight at 4 °C. After washing, the samples were incubated in a secondary IgG antibody (1:200) for 1 h at room temperature (RT). Finally, the sections were incubated in horseradish peroxidase (HRP)-streptavidin (1:200) for 1 h at RT, and the color reaction was conventionally developed with diaminobenzidine (DAB) and H_2O_2. Six representative sections from the brain of each animal were selected. IPP6.0 image processing software (Media Cybernetics, MD, USA) was utilized to count the number of positive cells.

Evans Blue Extravasation

Briefly, under anesthesia, Evans blue dye (2%, 5 mL/kg; Aladdin, Shanghai, China) was injected into the left femoral vein over a period of > 2 minutes and allowed to circulate for 60 minutes. Then, the mice were euthanized by intracardial perfusion of sterile saline. The brain samples were weighed, homogenized in

sterile saline, and centrifuged at 15000 × g for 30 minutes. After that, an equal volume of trichloroacetic acid was added to the resultant supernatant. The samples were then incubated overnight at 4 °C and centrifuged again at 15000 × g for 30 minutes. The resultant supernatant was spectrophotometrically quantified for extravasated Evans blue dye at 620 nm.

Brain water content measurement

Brain water content was measured in mouse cerebral tissues after ICH. Briefly, mice were randomly sampled from each group and anesthetized by intraperitoneal injection of chloral hydrate (n = 5). Next, the cerebral tissues were removed, and the water on the surface of the cerebral tissues was blotted with filter paper. The brain samples were immediately weighed with an electric analytic balance to obtain the wet weight and then dried at 100 °C for 24 h to obtain the dry weight. Brain water content was calculated using the following formula: brain water content (%) = (wet weight – dry weight)/wet weight × 100%.

Evaluation of Neurological Scores

Neurological scores were determined by the Neurological Severity Scale, a composite of motor, sensory, reflex, and balance tests. Neurological function was graded on a scale of 1 to 18; a score of 1 point was awarded for the inability to perform a test or for the lack of a tested reflex. The higher the score, the more severe the injury (normal score 2–3; maximal deficit score 18).

Statistical analysis

All experiments were repeated three times. The significance of differences between groups was determined by two-tailed Student's t test and two-way ANOVA. P values of less than 0.05 were considered statistically significant.

Results

TIPE2 lentivirus improved TIPE2 levels following erythrocyte lysate treatment and ICH.

We analyzed TIPE2 levels in macrophages after erythrocyte lysate treatment by RT-qPCR and western blot analysis. The data demonstrated that TIPE2 levels in macrophages were significantly decreased after erythrocyte lysate treatment. However, TIPE2 lentivirus improved TIPE2 levels compared with control group after erythrocyte lysis treatment (Fig. 1A). Furthermore, we detected the alterations in TIPE2 in the mouse brain 3 ds following ICH. The results demonstrated that TIPE2 levels in the mouse brain were significantly decreased after ICH. However, TIPE2 lentivirus improved TIPE2 levels compared with control group after ICH (Fig. 1B).

TIPE2 lentivirus decreased the macrophage inflammatory response in vitro

To analyze the role of TIPE2 in macrophage inflammation, we utilized erythrocyte lysate to treat macrophages and detected cytokine expression by ELISA. We found that erythrocyte lysate treatment significantly increased TNF-α, IL-1β, and IL-6 levels in macrophages compared with PBS treatment. However, TIPE2 lentivirus decreased TNF-α, IL-1β, and IL-6 levels compared with control group after erythrocyte lysis treatment (Fig. 2). These data suggest that TIPE2 decreases the macrophage- mediated inflammatory response and TIPE2 lentivirus decreased the macrophage inflammatory response in vitro.

TIPE2 lentivirus inhibited macrophage-mediated inflammatory injury in neurons

To assess the effect of TIPE2 on macrophage-mediated inflammatory injury in neurons, we utilized the MTT assay to assess erythrocyte lysate-induced toxicity of macrophages to neurons. The data demonstrated that when neurons were treated with conditioned medium from erythrocyte lysate-treated macrophages, the cell viability significantly decreased. However, when the cells were treated with conditioned medium from TIPE2 lentivirus transduced macrophages, cell viability increased (Fig. 3A). We further detected neuron apoptosis and the specific apoptotic pathways. Neurons were treated with conditioned medium from erythrocyte lysate-treated macrophages for 2 days, and the cell apoptosis ratio and caspase activity were detected by flow cytometry and a colorimetric assay. The results demonstrated that the cell apoptosis rate and caspase-3 activity increased significantly in neurons treated with conditioned medium from erythrocyte lysate-treated macrophages compared with that from TIPE2 lentivirus transduced macrophages (Fig. 3B and C). Therefore, the results suggest that TIPE2 attenuates macrophage-mediated inflammatory injury in neurons in vitro.

TIPE2 lentivirus attenuated macrophage accumulation in the perihematomal region

Macrophage accumulation is involved in brain inflammatory damage. To detect the effect of TIPE2 in macrophage accumulation, we detected Iba-1-positive macrophages in the perihematomal brain tissue 3 days after ICH. Our data indicated that the number of macrophages in the perihematomal brain tissues was increased after 3 days in ICH WT mice compared with sham controls. However, the number of macrophages in TIPE2 lentivirus transduced mice was decreased compared with that in WT mice after ICH (Fig. 4). These results suggest that TIPE2 attenuates macrophage accumulation in the perihematomal region in ICH mice.

TIPE2 lentivirus inhibited inflammation in the perihematomal region

To identify the role of TIPE2 in inflammation following ICH, we analyzed the mRNA and protein levels of inflammatory factors, such as TNF-α, IL-1β and IL-6. The results demonstrated that the mRNA and protein levels of inflammatory factors in the perihematomal brain tissues were significantly increased in ICH WT mice compared with sham controls. However, the mRNA and protein levels of inflammatory factors in TIPE2 lentivirus transduced mice were decreased compared with those in WT mice after ICH (Fig. 5). These data indicate that TIPE2 inhibits inflammation in the perihematomal region.

TIPE2 lentivirus attenuated neurological damage following ICH

To further explore the effect of TIPE2 on neurological damage following ICH, we analyzed BBB integrity, brain water content and the neurological deficit scores of ICH mice. The data indicated that BBB injury, brain water content and neurological deficit scores of ICH mice were much higher than sham controls. However, BBB injury, brain water content and neurological deficit scores in TIPE2 lentivirus transduced ICH mice were much lower than control group (Fig. 6). These results suggest that TIPE2 attenuates neurological damage following ICH.

Discussion

In the current study, we present the following evidence, which has not been reported previously: (1) Erythrocyte lysate treatment and ICH decreased TIPE2 levels. (2) TIPE2 decreased the macrophage-mediated inflammatory response in vitro. (3) TIPE2 inhibited macrophage-mediated inflammatory injury to neurons. (4) TIPE2 inhibited inflammation in the perihematomal region. (5) TIPE2 attenuated neurological damage following ICH.

Hematoma following ICH can directly compress brain tissue, which further induces mechanical damage and secondary brain injury[19, 20, 21]. Inflammation contributes to brain damage following ICH, leading to macrophage activation and blood-brain barrier (BBB) disruption [22, 23, 24]. Therefore, inhibiting inflammatory responses after ICH might be a promising approach for reducing secondary brain injury.

TIPE2 belongs to the TNFAIP8 family and has been proven to be overexpressed in mice with experimental autoimmune encephalomyelitis [25]. Many studies suggest that TIPE2 has the ability to negatively regulate the innate and adaptive immune responses [26, 27, 28]. TIPE2 is preferentially expressed in lymphoid tissues, and TIPE2-deficient cells are sensitive to Toll-like receptor (TLR) and T cell receptor (TCR) activation [29]. TLR4 is activated by many endogenous ligands, such as heme and fibrinogen, which are produced in the brain after ICH. Therefore, TIPE2 might be regulated by erythrocyte lysates. A previous study showed that TIPE2 negatively regulates inflammation by switching arginine metabolism

from nitric oxide synthase to arginase in macrophages [30, 31, 32]. More evidence has suggested that TIPE2 might inhibit M1 macrophage differentiation but induce M2 macrophage polarization [33, 34]. However, the underlying mechanism by which TIPE2 inhibits macrophage inflammation following ICH remains unclear.

To explore the effect of ICH on macrophages, we first utilized erythrocyte lysate to treat macrophages and analyzed macrophage inflammatory responses. We found that erythrocyte lysate significantly increased cytokine levels in macrophages compared with WT macrophages. However, TIPE2 can inhibit the inflammatory response of macrophages induced by erythrocyte lysate. Furthermore, we found that TIPE2 inhibited macrophage-mediated inflammatory injury to neurons in vitro. These data suggest that TIPE2 might directly contribute to the immunosuppressive action of ICH-induced macrophage inflammation. Second, to investigate the role of TIPE2 in the macrophage accumulation and neuroinflammation following ICH, we analyzed Iba-1-positive macrophages and inflammatory factors in the perihematomal brain tissues after ICH. These results suggested that TIPE2 attenuated macrophage accumulation and inflammatory factors in the perihematomal region after ICH. In addition, we observed TIPE2 transduced ICH mice exhibited decreased macrophage accumulation. Finally, to detect the specific role of TIPE2 in ICH-induced brain damage, we assessed BBB integrity, brain water content and the neurological deficit scores of ICH mice. The results indicated that TIPE2 attenuated neurological damage following ICH.

Previous studies have shown that ICH can activate MAPK signaling through phosphorylation of ERK1/2, p38 and JNK proteins, leading to inflammatory cytokine production [35]. In addition, phosphorylation of ERK, p38, and JNK is increased in TIPE2-silenced macrophages stimulated with MP. Furthermore, MAPK inhibitors, including a JNK inhibitor, P38 inhibitor and ERK inhibitor, can partially attenuate the negative role of TIPE2 in MP-triggered cytokine production in THP-1 cells[36]. Therefore, we postulate that the downregulation of TIPE2 expression after ICH might contribute to the elevated levels of inflammatory cytokines, possibly via the APK signaling pathway. However, the relevant molecular mechanisms by which TIPE2 inhibits intracellular signaling pathways remain to be further investigated.

Conclusion:

The data indicated that TIPE2 contributes to the pathogenesis of ICH, which prevents brain injury and attenuates inflammation following ICH. In addition, TIPE2 might represent a promising target for ICH therapy.

Abbreviations

None

Declarations

Availability of data and materials

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

Contributions

ZY conceptualized and designed the study. SDL, WYL and HS performed the experiments and analyzed data. GT designed and performed all cytokine analysis experiments. JWZ performed immunohistochemistry, cell quantification, and data analysis, and prepared the figures. All authors critically reviewed the manuscript, approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Competing interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

All animals received care in compliance with the Principles of Laboratory Animal Care and National Standards.

Availability of data and materials

Please contact author for data requests.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

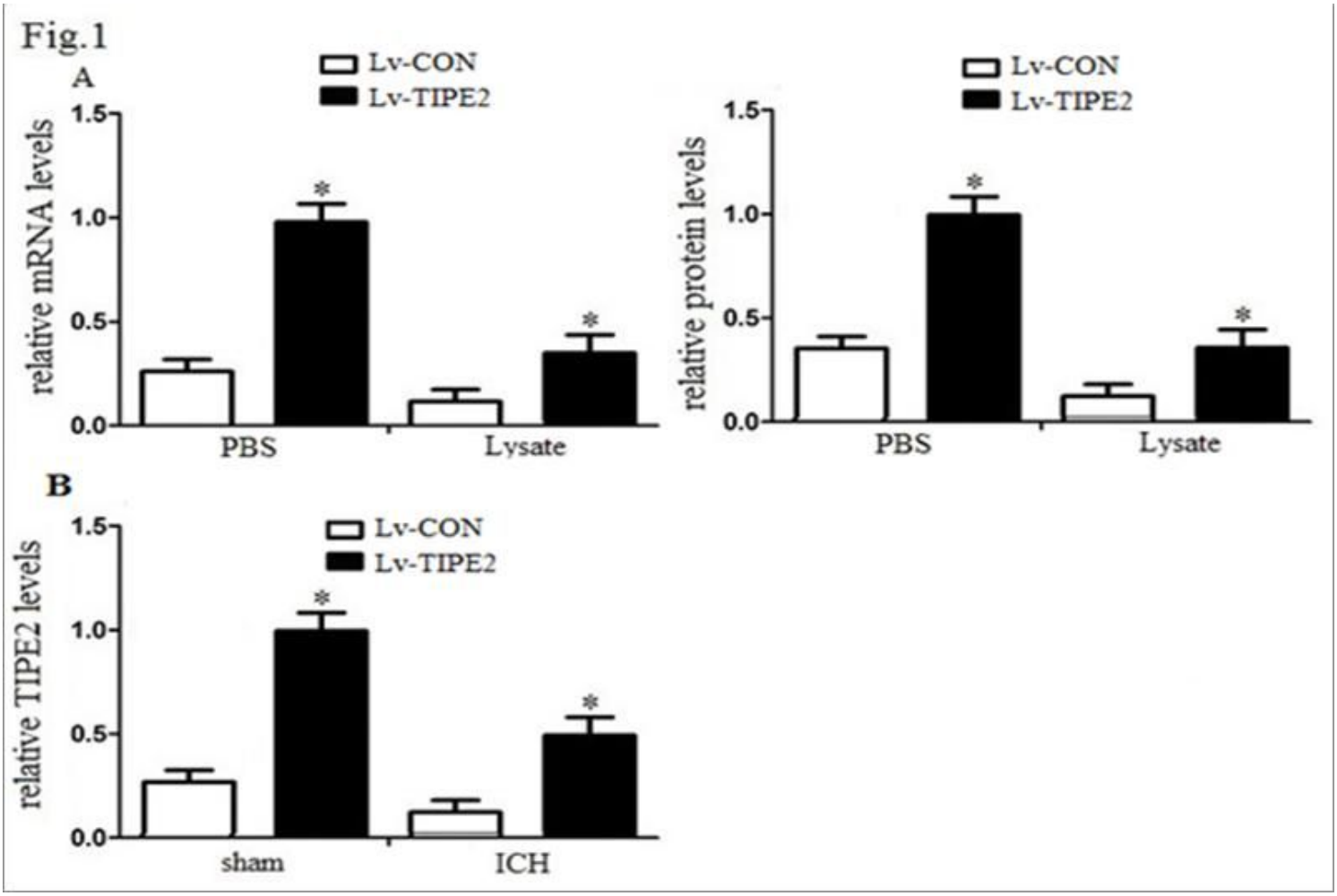

Figure 1

TIPE2 lentivirus improved TIPE2 levels following erythrocyte lysate treatment and ICH. (A) TIPE2 lentivirus transduced or control macrophages (1×105) were stimulated with 10 μL of erythrocyte lysate or

PBS. After 3 days, the supernatants were removed, and macrophages were collected for mRNA and protein analysis by RT-qPCR and western blotting. (D) The mice (n = 10 each group) were equally divided into the sham group and ICH group. After 3 ds, TIPE2 protein levels in the hippocampi of the mice were examined by western blot analysis. Experiments performed in triplicate showed consistent results. The data are presented as the mean ± standard error of the mean (SEM) of three independent experiments. *P < 0.05.

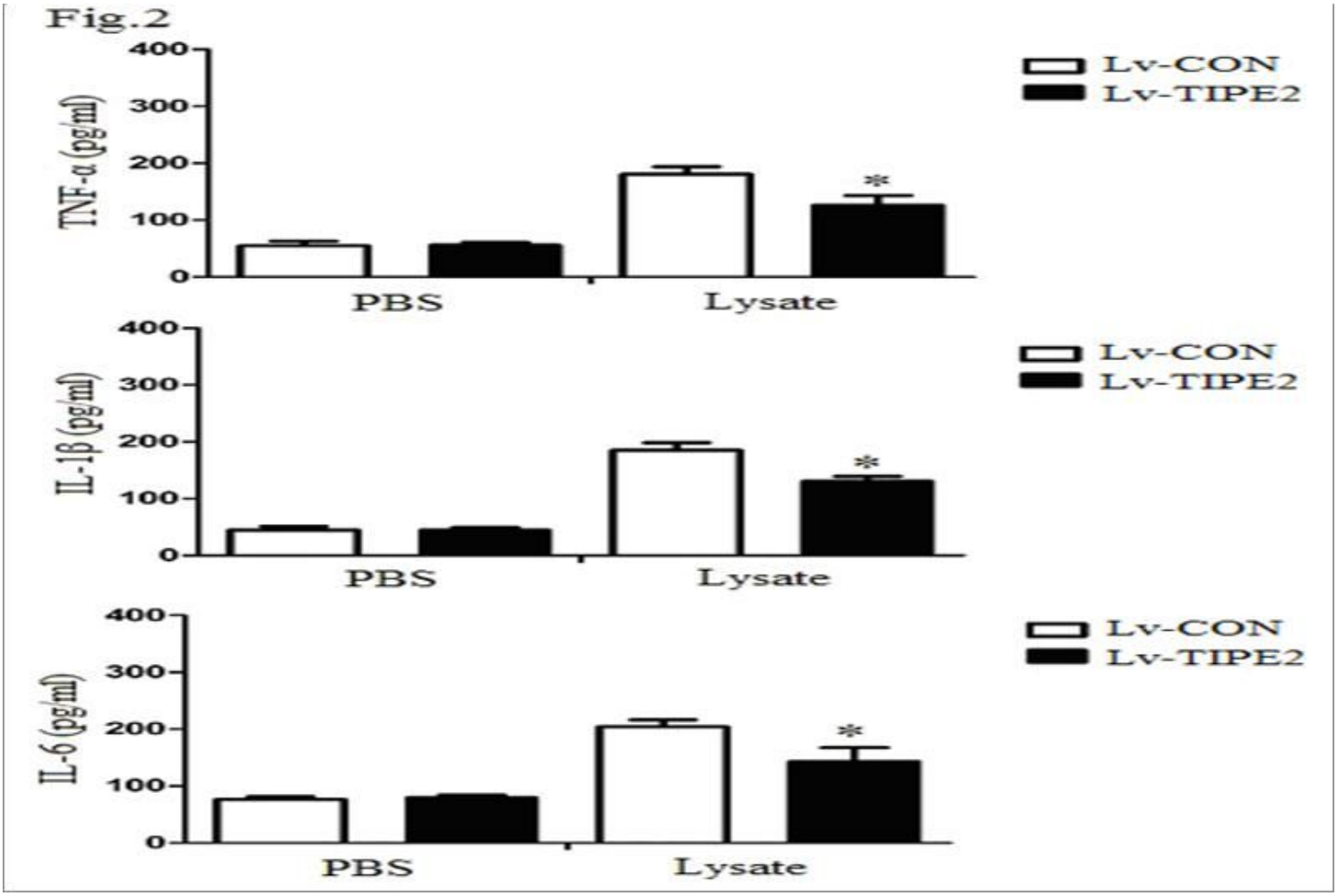

Figure 2

TIPE2 lentivirus decreased the macrophage inflammatory response in vitro. TIPE2 lentivirus transduced or control macrophages (1× 10 5) were stimulated with 10 μL of erythrocyte lysate or PBS. After 3 days, the supernatants were removed, and TNF-α, IL-1β and IL-6 cytokine production was further analyzed by ELISA. Experiments performed in triplicate showed consistent results. The data are presented as the mean ± SD of three independent experiments. *P < 0.05.

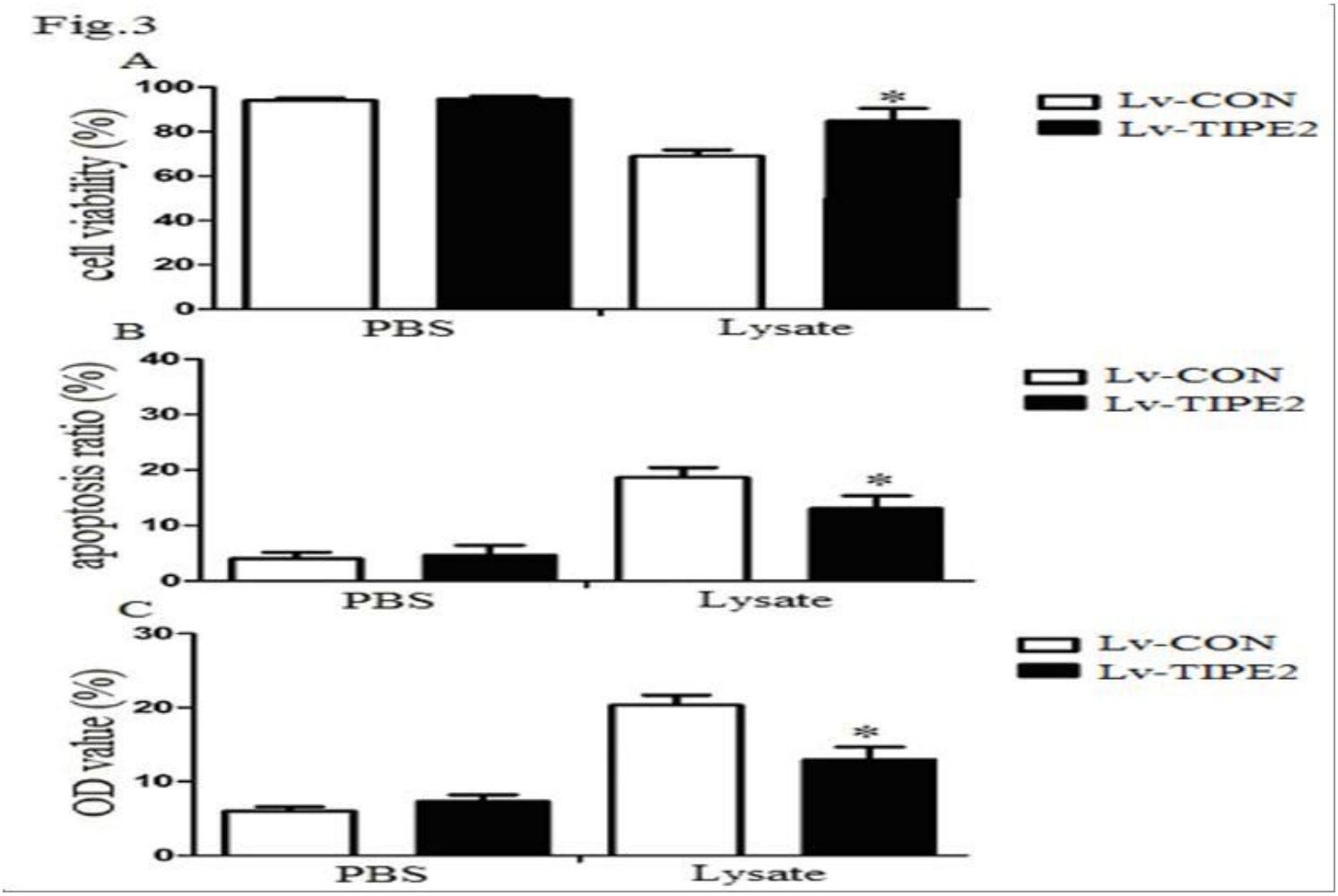

Figure 3

TIPE2 lentivirus inhibited macrophage-mediated inflammatory injury in neurons (A) Neurons were treated with conditioned medium from erythrocyte lysate-treated TIPE2 lentivirus transduced or control macrophages. After 48 h, MTT reagent was added, and cell viability was assessed. (B) After 48 h, neurons were treated with conditioned medium from erythrocyte lysate-treated TIPE2 lentivirus transduced or control macrophages, and the cell apoptosis ratio was detected by flow cytometry. Apoptotic cells were identified as annexin V-positive and propidium iodide (PI)-negative cells. (C) After 48 h, neurons were treated with conditioned medium from erythrocyte lysate-treated TIPE2 lentivirus transduced or control macrophages, and the ApoAlertcaspase-3 Colorimetric Assay Kit (Clontech, Palo Alto, USA) was used. Hydrolyzed pNA was detected using a Multiscan MS colorimeter (Thermo Labsystems, Vantaa, Finland) at 405 nm. Experiments performed in triplicate showed consistent results. The data are presented as the mean ± SD of three independent experiments. *P < 0.05.

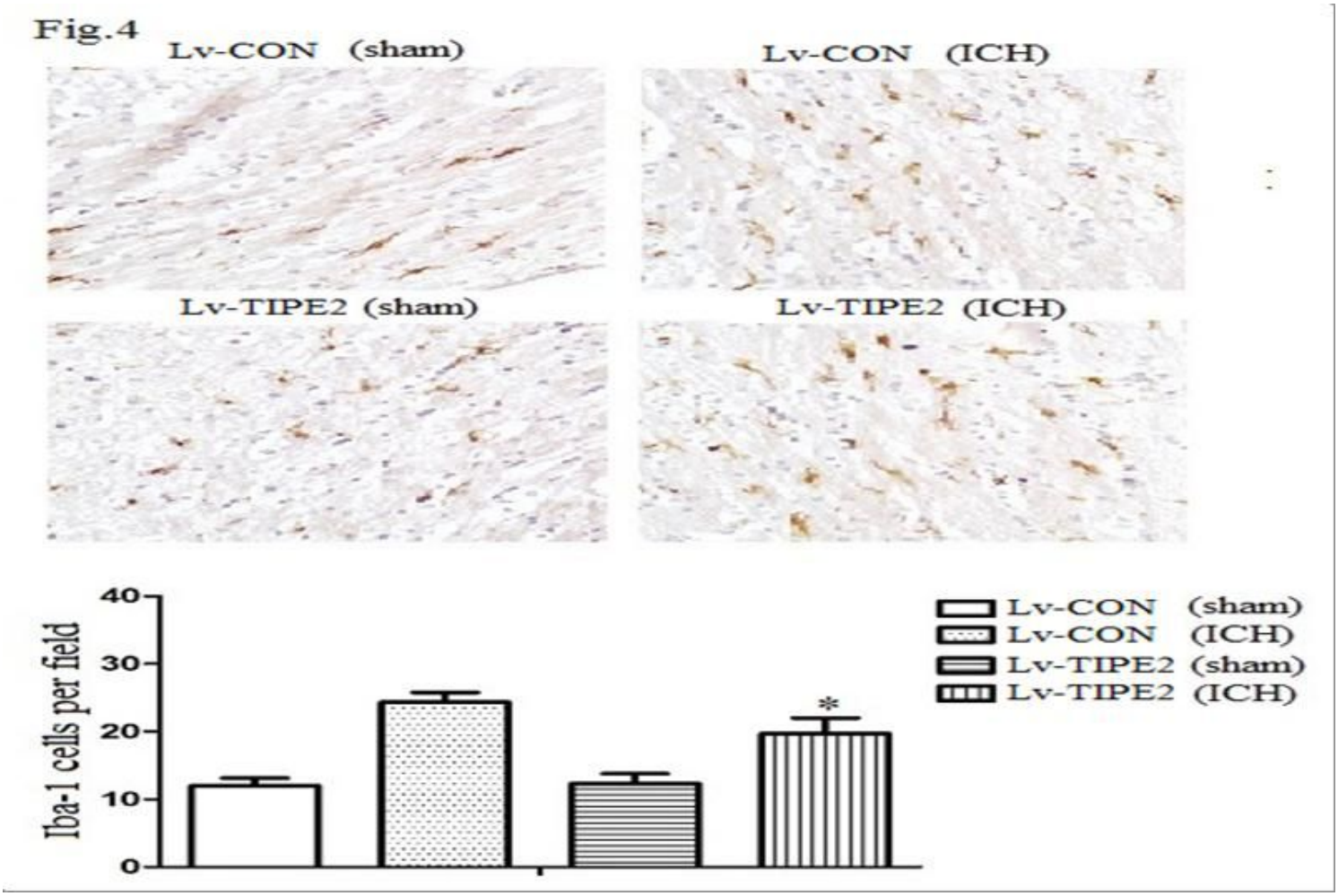

Figure 4

TIPE2 lentivirus attenuated macrophage accumulation in the perihematomal region Three days after ICH, mice (n = 10 per group) were deeply anesthetized transcardially. The brains were removed and postfixed. The perihematomal region of cerebral tissue was collected, and macrophages were analyzed with an anti-Iba-1 antibody (×400 magnification). Experiments performed in triplicate showed consistent results. The data are presented as the mean ± standard error of mean (SEM) of three independent experiments. *P < 0.05.

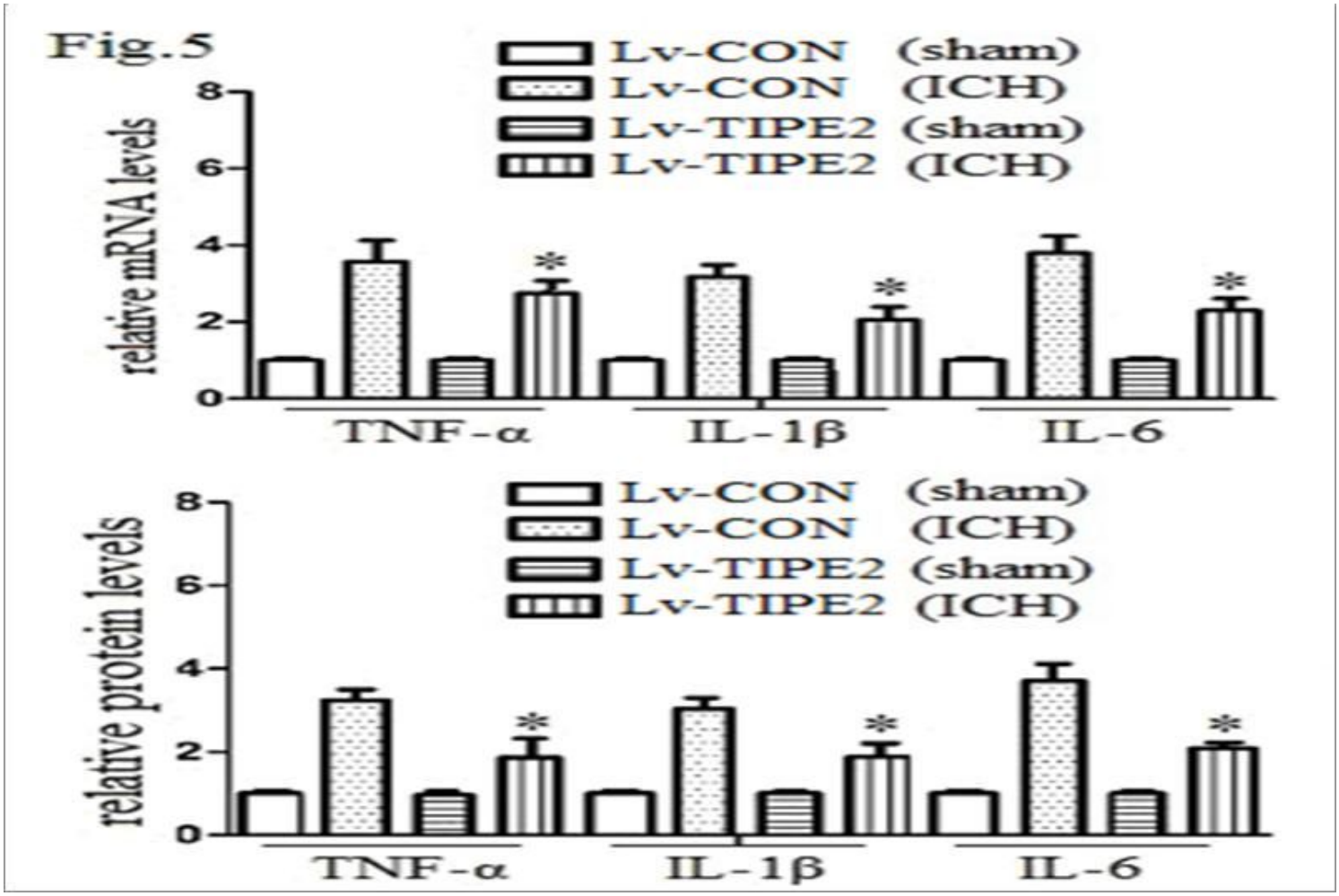

Figure 5

TIPE2 lentivirus inhibited inflammation in the perihematomal region. Three days after ICH, mice (n = 10 per group) were deeply anesthetized transcardially. The brains were removed and postfixed. The perihematomal region of cerebral tissue was collected, and the tissue lysates were further analyzed for cytokine production by qRT-PCR and western blotting. Experiments performed in triplicate showed consistent results. The data are presented as the mean ± standard error of mean (SEM) of three independent experiments. *P < 0.05.

Fig.6A

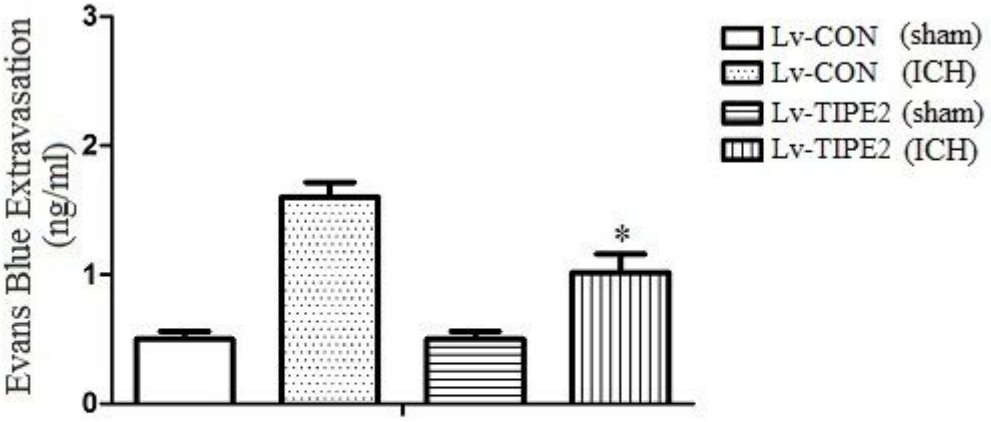

Fig.6B

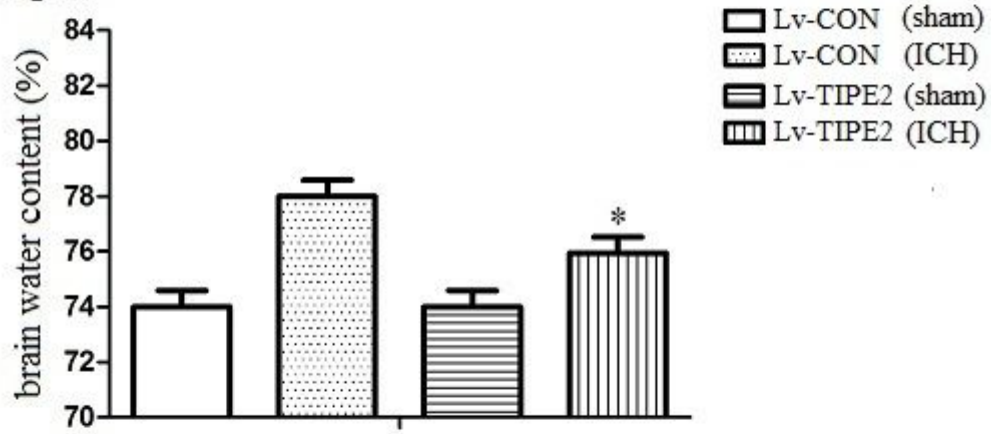

Fig.6C

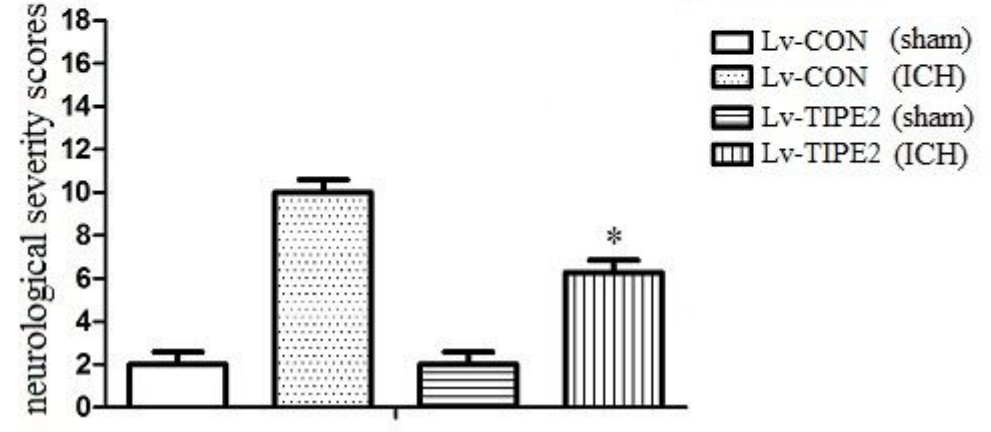

Figure 6

TIPE2 lentivirus attenuated neurological damage following ICH. (A) Three days after ICH, BBB disruption in the mice (n=10 per group) was analyzed. (B) Three days after ICH, the cerebral water content of mice (n=10 per group) was also analyzed. (C) Three days after ICH, the mice (n = 10 per group) were in stable condition, and neurological deficit tests were performed by measuring behaviors, including a composite of motor, sensory, reflex, and balance behaviors. Experiments performed in triplicate showed consistent

results. The data are presented as the mean ± standard error of mean (SEM) of three independent experiments. *P < 0.05.