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Ac-YVAD-cmk improves neurological function by inhibiting caspase-1-mediated inflammatory response in the intracerebral hemorrhage of rats

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ABSTRACT

Objective: Intracerebral hemorrhage (ICH) is acknowledged as a serious clinical problem lacking effective treatments. And caspase-1-mediated inflammatory response happened during the progression of ICH. Therefore, we aimed to investigate the effects of caspase-1 inhibitor Ac-YVAD-cmk on ICH.

Materials and methods: Microglia cells were isolated and activated by thrombin for 24 h. Then the transcript and protein expressions of NLRP3 and inflammatory factors were assessed by RT-PCR and western blotting. Moreover, Ac-YVAD-cmk was injected into the ICH model. The mNSS and brain water content were tested at 24 h post-ICH. Finally, the pathological changes of microglia activation following ICH were discovered by the immunohistochemical and HE staining ways.

Results: Ac-YVAD-cmk inhibited the activation of pro-caspase-1 and decreased brain edema, in association with decreasing activated microglia and the expression of inflammation-related factors at 24 h post-ICH. Consequently, Ac-YVAD-cmk reduced the release of mature IL-1β/IL-18 in perihematoma, improved the behavioral performance, and alleviated microglia in perihematoma region in ICH rats.

Conclusions: These results indicate that caspase-1 could amplify the plural inflammatory responses in the ICH. Administration of Ac-YVAD-cmk has the potential to be a novel therapeutic strategy for ICH.

1. Introduction

Intracerebral hemorrhage (ICH) accounts for 10%–15% of all strokes in Europe and the United States, and in Asia the percentage can be up to 20%–30%, with high mortality and morbidity which affect > 2 million people worldwide per year. Moreover, the survivors usually suffer from chronic disabilities [1,2]. Unfortunately, there is no effective pharmacologic treatment in the clinical setting, mainly due to the fact that the mechanisms of post-ICH brain damage are unclear. In previous publications, ICH has been shown to not only cause primary brain injury via its biochemical and mechanical effects, but also induces secondary brain injury, including local inflammatory responses to ICH and the toxic effects of blood breakdown products including hemoglobin, iron, and thrombin. Secondary brain injury proceeds over hours to days, and thus it is probable that something could intervene in this process therapeutically [3]. However, there is also emerging evidence suggesting that inflammation contributes to brain injury during the acute phase of ICH, including activation of microglia or breakdown of the blood-brain barrier [4,5]. Therefore, the suppression of inflammatory responses after ICH might be a novel strategy for reducing secondary brain injury.

The resident microglia are innate immune cells of the central

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nervous system (CNS). Numerous studies have confirmed that microglia are activated in the early stages within 1 h after collagenase-induced ICH, whereas infiltration of blood-derived neutrophils into the perihematomal takes 4–5 h [6]. Although microglia are mainly responsible for the removal of tissue debris after ICH, activated microglia also release a series of harmful substances such as chemokines, inflammatory cytokines (such as IL-1β), reactive oxygen species, nitric oxide, heme oxygenase-1 protease and other toxic immunoreactive molecules [7–9], which exacerbate inflammatory cells infiltration and lead to neuron death [10]. It seems that activated-microglia initiate inflammatory response and aggravate brain damage after ICH. The inhibition of microglia activation or these inflammatory molecules can suppress further development of the inflammatory process, thereby reducing early brain injury following ICH. However, the specific mechanisms of microglia-mediated inflammatory responses are still unclear.

Recent studies have shown that ICH-induced inflammatory responses are primarily initiated through the innate immune system, in which inflammasomes play a critical role in the processing of innate immune immunity initiated inflammatory responses [11]. Inflammasomes are multiprotein complexes which contain the intracellular Nod-like receptors and the effector protein pro-caspase-1. While inflammasomes are activated, pro-caspase-1 is cleaved to caspase-1, after which it can not only promote the maturity of IL-1β/IL-18, but also leads to pyroptosis. Finally, a variety of cytoplasmic contents, such as cytokines and chemokines, are transferred to the extracellular region via the pores on the cytomembrane exacerbating the caspase-1 active inflammatory responses consequently [12]. Researchers have demonstrated that caspase-1 plays an important role in the development of many central nervous system diseases, such as spinal cord injury [13], traumatic brain injury [14] and cerebral ischemia-reperfusion injury [15], but the effects of caspase-1 on ICH remain unclear, and whether caspase-1 is related to microglia-mediated inflammatory response or not also requires discussion.

Therefore, in the present study, we demonstrate that Ac-YVAD-cmk (A selective irreversible inhibitor of caspase-1 which can prevent the proinflammatory cytokine IL-1β activation.) remarkably ameliorated ICH injury induced by local injection of collagenase IV in the striatum of rats, and this effect was associated with a decrease in activated microglia and suppression of the expression of inflammation-related factors. In addition, treatment with Ac-YVAD-cmk improved neurological function, which may provide a new approach to potentially reduce ongoing edema and improve the neurological outcome after ICH.

2. Materials and methods

2.1. Animals

Neonatal (day 0–2) and adult (male, 280–300 g) SD rats were obtained from the Animal Center of the Harbin Medical University (Harbin, China). Animals were housed in cages with free access to water and food at a controlled temperature and humidity in a 12-h light/dark cycle. All experiments for this study were conducted according to animal care guidelines approved by Animal Ethics Committee of the First Affiliated Hospital of Harbin Medical University (201512) and the National Institutes of Health.

2.2. Primary cell cultures

For microglia cells culture, cerebral hemispheres of neonatal (day 0–2) SD rats were dissected, the meninges and the blood vessels were removed, cortical tissue was dissected and digested with 0.25% trypsin for 10 min at 37 °C, then growth medium was added and centrifuged at 1500RPM for 5 min. The cells were cultured with saturated humidity at 37 °C in 5% CO_2 atmosphere. Growth medium were refreshed once every three days. These mixed cells were cultured for about 10 days, microglia were detached by shaking flasks on a gyratory shaker. The culture medium was centrifuged, the cells in the supernatant were placed on a poly-L-lysine coated 6-well culture plate and incubated at 37 °C for 30 min. These cells were washed with phosphate-buffered saline (PBS); the supernatant, which contained oligodendrocytes, was discarded and the pelleted microglia were grown in the growth medium. The purity of microglia was examined by immunohistochemical staining for the microglia-specific antibody CD11b. The purity of microglia > 95%.

2.3. Cell treatment and experimental groups in vitro

Microglia cells (1×10^5) were stimulated with the caspase-1 inhibitor Ac-YVAD-cmk (Sigma-Aldrich, USA) (dissolved by DMSO) for 1 h. Thrombin (Sigma-Aldrich, USA) was added at a final concentration of 10 U/mL to activate the microglia cells for 24 h. The experiments were divided into three groups: (i) Control group, microglia cells were cultured alone. (ii) Thrombin-activated group, microglia cells cultured with thrombin for 24 h. (iii) Inhibitor treatment group, microglia cells were stimulated with Ac-YVAD-cmk dissolved by DMSO for 1 h, then activated with thrombin for 24 h.

2.4. ICH model

Briefly, rats were anesthetized with an intraperitoneal injection of 3.6% chloral hydrate (1 mL/100 g) and fixed on a rat stereotaxic frame. A 1-mm-diameter burr hole was made with dentist's micro-drill and a 5-μL needle was inserted through the burr hole into the left striatum (coordinates: 1.0 mm posterior, 5.5 mm ventral and 3.0 mm lateral to the bregma) under aseptic conditions. ICH was induced by the administration of 0.5 U collagenase type VII (Sigma-Aldrich, USA) in 2-μL sterile normal saline during a period of 5 min. After injection the needle was held in place for 10 min, then the needle was gently removed. The burr hole was sealed with bone wax, and the scalp was sutured. Throughout the procedure, body temperature was maintained at 37 °C, the rats were given free access to food and water after they woke up. No rat deaths occurred after the procedure.

2.5. Drug administration and intracerebral ventricular injection

The caspase-1 inhibitor Ac-YVAD-cmk was dissolved in DMSO and further diluted in sterile normal saline in order to achieve the final concentration of DMSO < 0.2%. The drug Ac-YVAD-cmk was then injected into the left lateral ventricle (coordinates: 1.0 mm posterior, 1.5 mm lateral, and 4.5 mm ventral to the bregma) using a 5-μL needle 30 min before induction of ICH. Vehicle group was treated with the same volume of DMSO diluted in sterile normal saline. The experiments were divided into four groups: (i) Normal group. (ii) Sham group, the sham-operated rats were administered of 2-μL sterile normal saline in left striatum. (iii) ICH group. (iiii) Inhibitor treatment group, the ICH rats were administered of Ac-YVAD-cmk in left lateral ventricle at 1 μg/rats and the concentration of Ac-YVAD-cmk is 1 μg/μL.

2.6. Evaluation of neurological scores

Neurological outcomes were assessed with the modified Neurological Severity Scores (mNSS) by an investigator who was blinded to the experimental groups at 24 h and 72 h after ICH. The mNSS, a composite of motor, sensory, reflex and balance tests; the higher the score, the more severe the injury [16].

2.7. Brain water content measurement

Brain water content was measured by a common wet/dry method. Briefly, at 24 h after ICH, rats were decapitated under deep anesthesia and brain immediately removed and separated into three parts: contralateral hemisphere, ipsilateral hemisphere and the cerebellum and

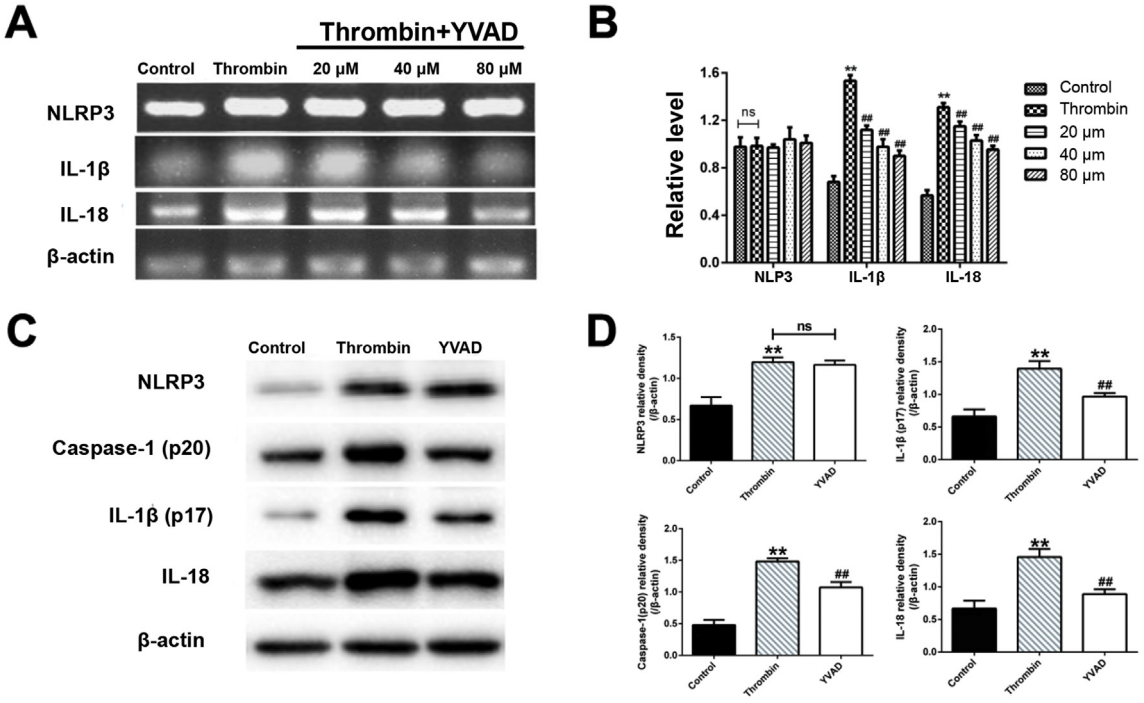

Fig. 1. Ac-YVAD-cmk inhibited caspase-1 mediated microglia inflammation in vitro.
(a) The representative mRNA images of NLRP3 and IL-1β/IL-18 in microglia treated with thrombin for 24 h in the thrombin-activated microglia group. In the treatment group, microglia were cocultured with different concentrations of caspase-1 inhibitor Ac-YVAD-cmk (20 μmol/L, 40 μmol/L and 80 μmol/L) for 1 h, then cells were treated with thrombin for 24 h ($n = 3$ per group). (b) The related RNA quantification of NLRP3, IL-1β and IL-18. (c) The protein levels of NLRP3, caspase-1 (p20), and mature IL-1β/IL-18 were assayed by western blot (n = 3 per group). In the treatment group, microglia were cocultured with caspase-1 inhibitor Ac-YVAD-cmk (40 μmol/L) for 1 h, cells were then treated with thrombin for 24 h. (d) Quantification of NLRP3, caspase-1 (p20), and mature IL-1β/IL-18 is presented. $^{**}P < 0.01$ vs control group, $^{\#\#}P < 0.01$ vs thrombin-activated group.

wet weighed (WW). The cerebellum was used as an internal control for brain water content. Then brain specimens were dried at 100 °C for 24 h to obtain the dry weight (DW). The brain water content was calculated as a percentage of the wet weight: (WW-DW)/WW × 100%.

2.8. RNA isolation and RT-PCR

The total RNA was extracted from the microglial cells using 1 mL Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA (1 μg) was reverse-transcribed to cDNA using the oligo (dT) primer (Promega, Madison, WI, USA). The PCR products were electrophoresed on 1% agarose gel containing ethidium bromide and visualized by ultraviolet transillumination. β-actin was used as internal control. The relative intensity of mRNAs means relative to the internal control (β-actin). The following primers of NLRP3, IL-1β, IL-18 and β-actin were used in the study:

NLRP3: Forward, 5′-GTGGAGATCCTAGGTTTCTCTG-3′
Reverse, 5′-CAGGATCTCATTCTCTTGGATC-3′
IL-1β: Forward, 5′-CCCTGCAGCTGGAGAGTGTGG-3′
Reverse, 5′-TGTGCTCTGCTTGAGAGGTGCT-3′
IL-18: Forward, 5′-ACAACCGCAGTAATACGGAGCA-3′
Reverse, 5′-TGTGCTCTGCTTGAGAGGTGCT-3′
β-actin: Forward, 5′-AGACCTTCAACACCCCAG-3 ′
Reverse, 5′-CACGATTTCCCTCTCAGC-3′

2.9. Western blot

Protein extraction and cell lysis (Beyotime, China –be specific about kit) was followed by centrifugation at 12,500 × *g* for 15 min at 4 °C. The protein concentration was then measured with a BCA protein assay kit (Beyotime, China). Equivalent protein samples (cells, 50 μg; tissue, 100 μg) were loaded on a 10% SDS polyacrylamide gel and transferred onto a nitrocellulose filter membrane, membranes were incubated overnight at 4 °C, subsequently blocked by 5% non-fat milk dissolved in PBS at 22 °C for 1 h. The following primary antibodies were used: rabbit polyclonal anti-NLRP3, rabbit polyclonal anti-caspase-1 (p20), rabbit polyclonal anti-IL-1β (p17) and rabbit polyclonal anti-IL-18 (Boster, China). β-Actin (Boster, China) was used as internal control. The membranes were processed with secondary antibodies (Boster, China) for 1 h room temperature. The relative intensity of protein means relative to the β-actin. The bands were quantified using the Odyssey Infrared Imaging System (Bio-Rad, USA).

2.10. Immunohistochemical and HE staining

The brain tissue was fixed in 4% paraformaldehyde, then embedded in paraffin, subsequently cut into 5-μm thick sections and stained with immunohistochemical and H&E for histological analysis. The immunohistochemistry steps are as follows: Paraffin sections were deparaffinized taken, hydrated. Antigen repair, cooling to room temperature. PBS washed 2 to 3 times, each time 5 min; 3% H_2O_2 at room temperature was allowed to stand 10 min; PBS washed 2 to 3 times, each time 5 min. Goat serum was added dropwise, at room temperature for 20 min; dropwise a primary antibody overnight at 4 °C; rewarming 30 min, PBS washed three times, each time 5 min; dropping secondary antibody, 37 °C 1 h; PBS washed three times, each time 5 min. DAB color was observed under the microscope; PBS rinsed 10 min; hematoxylin, differentiation; graded alcohol dehydration, xylene, were mounted and photographed under light microscope. The HE staining main steps are as follows: The main processes of dyeing are as follows: (1) Both were subjected to dewaxing treatment with xylene. (2) The sections were washed, and the liquid substances used were absolute ethanol, different concentrations of ethanol, and distilled water. (3) After about 4 min, the Sumu purple is dyed and washed. (4) After the

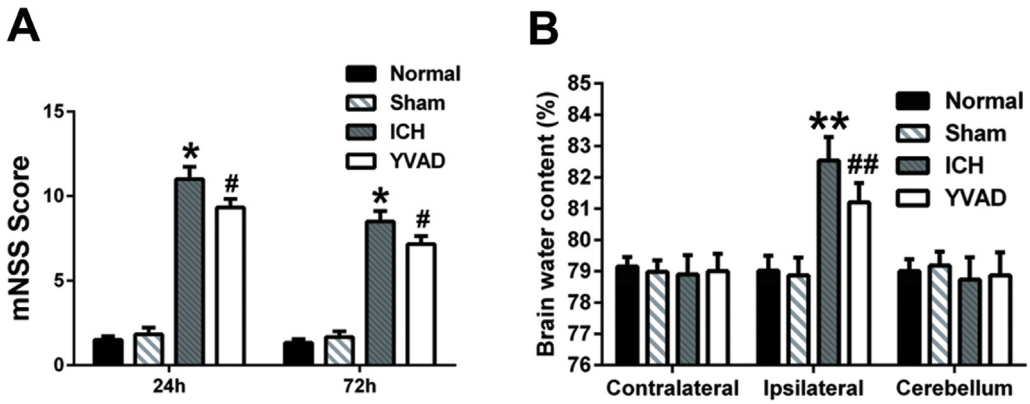

Fig. 2. Ac-YVAD-cmk improved neurological function and reduced brain edema in ICH rats.
(a) At 24 h and 72 h after ICH on rats, the neurological impairment tests were determined by modified Neurological Severity Scores (mNSS) ($n = 6$ per group). (b) The changes in percentage of water content was measured for brain edema assay at 24 h after ICH on rats (n = 6 per group). $^{*}P < 0.05$ vs normal group or sham group, $^{\#}P < 0.05$ vs ICH group. $^{**}P < 0.01$ vs normal group or sham group, $^{\#\#}P < 0.01$ vs ICH group.

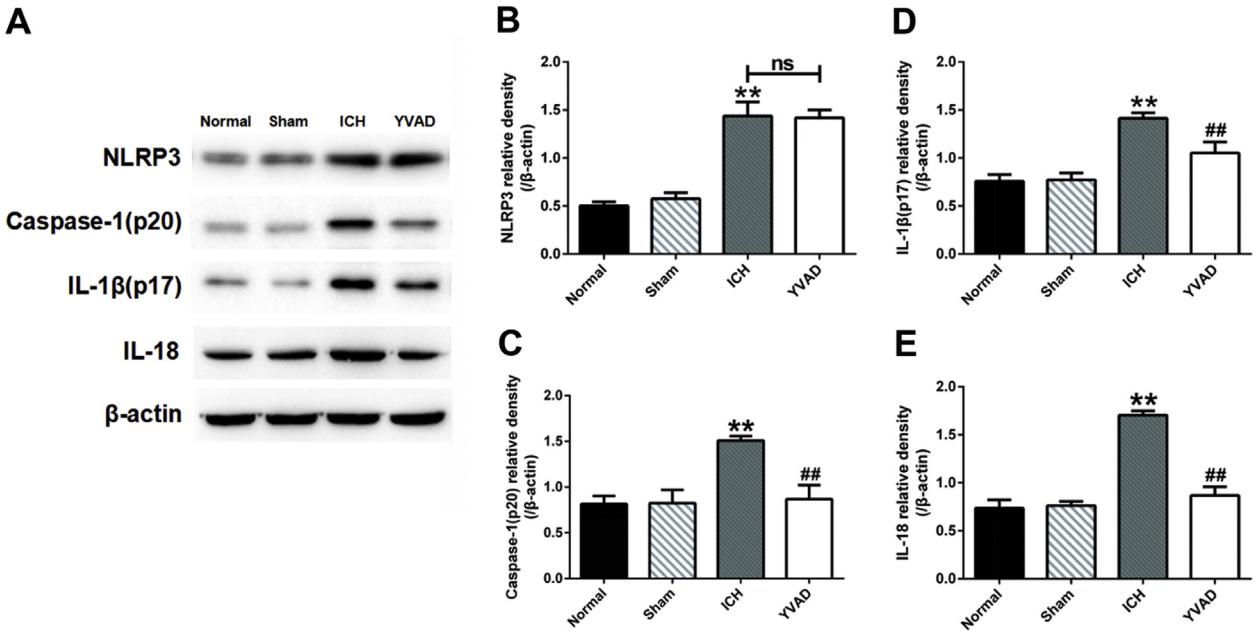

Fig. 3. Ac-YVAD-cmk inhibited caspase-1-mediated inflammation in perihematoma at 24 h after ICH on rats.
(a) The protein levels of NLRP3, caspase-1 (p20), and mature IL-1β/IL-18 were assayed by western blot (n = 3 per group). (b–e) Quantification of protein NLRP3, caspase-1 (p20), maturation protein IL-1β and maturation protein IL-18 in all groups. $^{**}P < 0.01$ vs normal group or sham group, $^{\#\#}P < 0.01$ vs ICH group.

cell nuclei are stained blue, the washing process is stopped. (5) Each of the following three liquids was used for two stainings, including eosin staining, 95% ethanol, and absolute ethanol. (6) Final treatment with phenol xylene and xylene. We used anti-iba-1 (Abcam, UK) to mark microglia.

2.11. Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (IBM, USA). Data are presented as mean ± SD. The statistical significance of findings between differential groups were determined by variance analysis or χ^2 test and considered statistically significant if $P < 0.05$.

3. Results

3.1. Ac-YVAD-cmk reduced the expression of IL-1β and IL-18 in activated microglia in vitro

The microglia cells were purified by shaking of the cell culture flask leading to differential cell adherence, after which thrombin could effectively activate the resting microglia cells by treatment for 24 h [17]. The mRNA expression of NLRP3 and IL-1β/IL-18 increased in thrombin-activated microglia (Fig. 1a). High level Ac-YVAD-cmk treatment (40 μmol/L or 80 μmol/L) significantly decreased the mRNA levels of IL-1β/IL-18. Furthermore, the protein levels of NLRP3, caspase-1 (p20), mature IL-1β/IL-18 were higher in thrombin-activated microglia group than in control group as seen by western blot. Meantime, the 40 μmol/L dose of Ac-YVAD-cmk significantly reduced the protein levels of caspase-1 (p20) and mature IL-1β/IL-18 compared with the thrombin-activated microglia group (Fig. 1b–f).

3.2. Ac-YVAD-cmk improved neurological function and reduced brain edema after ICH

In order to assess the effects of Ac-YVAD-cmk after ICH, the mNSS was performed at 24 h post-ICH (Fig. 2a). The results of the mNSS test showed that the degree of neurological injury was significantly reduced in the inhibitor treatment group (Ac-YVAD-cmk) compared with the ICH group at 24 h after ICH (9.33 ± 1.21 vs ICH group, 11.00 ± 1.79; $P < 0.05$), similar results were obtained at 72 h after ICH (7.17 ± 1.17 vs ICH group, 8.50 ± 1.52; $P < 0.05$). Meanwhile, to investigate whether Ac-YVAD-cmk could attenuate brain edema, we monitored brain water content in the experimental groups 24 h post-ICH. We found that the brain edema alleviated in inhibitor treatment

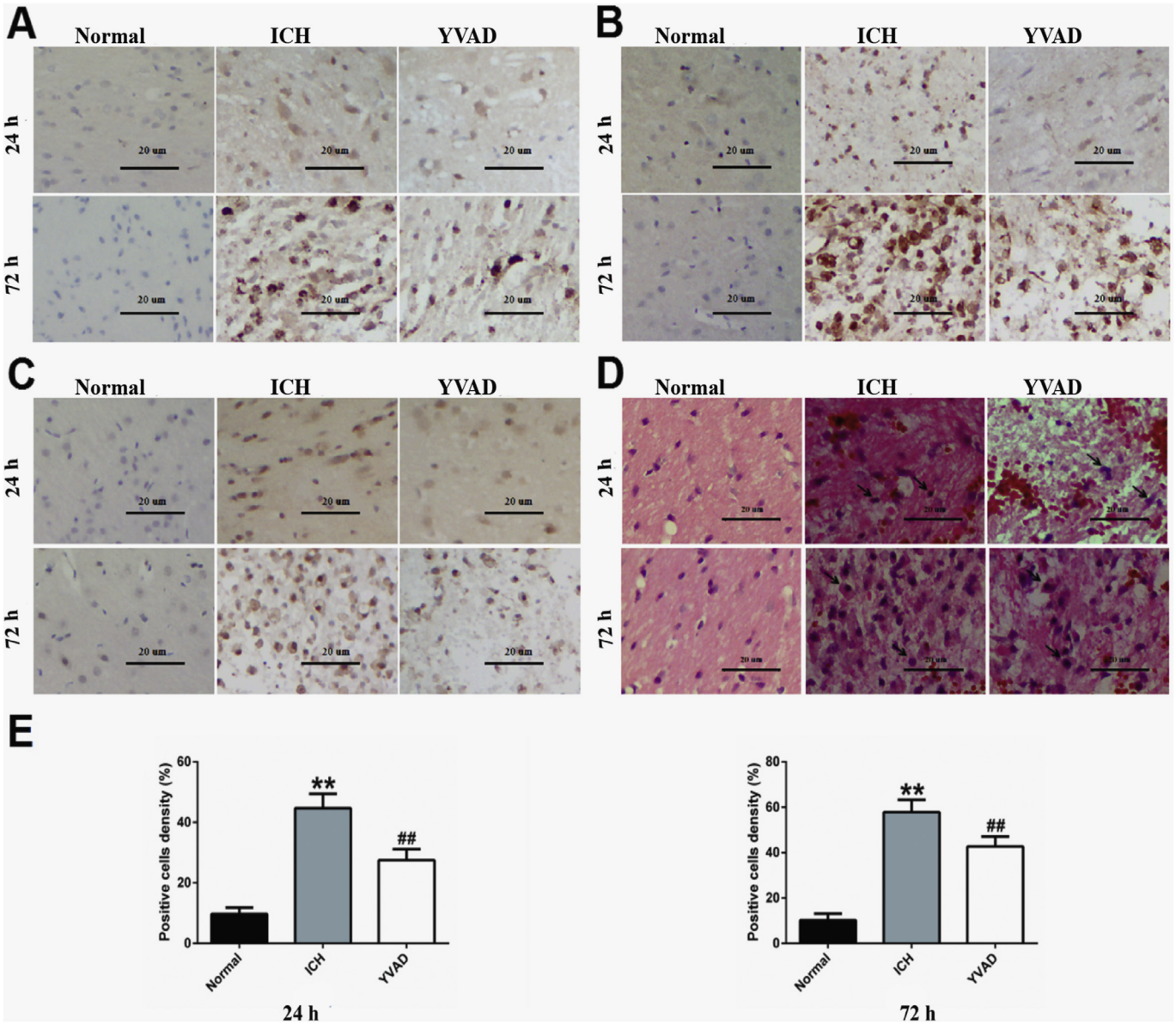

Fig. 4. Ac-YVAD-cmk inhibited caspase-1-mediated inflammation in perihematoma at 24 h and 72 h after ICH on rats.
The protein expression of caspase-1(p20), and mature IL-1β/IL-18 were assayed by immunohistochemistry. Ac-YVAD-cmk treatment decreased the protein levels of caspase-1(p20), IL-1β/IL-18 maturation and inhibited the infiltration of inflammatory cells in the perihematoma at 24 h and 72 h after ICH on rats (n = 6 per group). (a) The protein level of caspase-1 (p20) (b) The protein level of mature IL-1β. (c) The protein level of mature IL-18. (d) H&E staining was used to observe inflammatory cells infiltration. (e) Quantification of positive cells density (inflammatory cell) at 24 h and 72 h after ICH. Scale bar: 20 μm.

group compared with the ICH group at 24 h following ICH in different positions (81.20 ± 0.61% vs ICH group, 82.53 ± 0.75%; $P < 0.01$) (Fig. 2b).

3.3. Ac-YVAD-cmk mitigated the caspase-1 mediated inflammatory response after ICH

To investigate the anti-inflammatory effects of Ac-YVAD-cmk, we measured the protein levels of NLRP3, caspase-1(p20), pro-inflammatory factors (IL-1β and IL-18) in the injured hemisphere at 24 h post-ICH by western blot. The results showed that the protein levels of NLRP3, caspase-1 (p20), mature IL-1β/IL-18 were significantly increased in perihematoma compared with the normal group or the sham group, whereas Ac-YVAD-cmk treatment (Ac-YVAD-cmk dose: 1 μg/rats) significantly decreased the protein levels of caspase-1 (p20), mature IL-1β/IL-18 compared with the ICH group (Fig. 3). Then we detected the protein levels of caspase-1 (p20) and mature IL-1β/IL-18 by immunohistochemistry in perihematoma at 24 h and 72 h after ICH to confirm the above results and make long-term observation (Fig. 4a–c). Furthermore, we found that Ac-YVAD-cmk could reduce the infiltration of inflammatory cells in the perihematoma by H&E staining at 24 h and 72 h after ICH as well (Fig. 4d–e).

3.4. Ac-YVAD-cmk alleviated microglia infiltration after ICH

Microglia activation is an important feature of the pathological changes following ICH. As shown in Fig. 5, we found that Ac-YVAD-cmk could significantly alleviate microglia infiltration in perihematoma by immunohistochemistry compared with ICH group at 24 h (28.17 ± 3.82% vs ICH group, 45.50 ± 4.39%; $P < 0.05$) or 72 h after ICH. (38.83 ± 5.42% vs ICH group, 75.00 ± 8.27%; $P < 0.01$) And both groups were compared with normal group at each time point

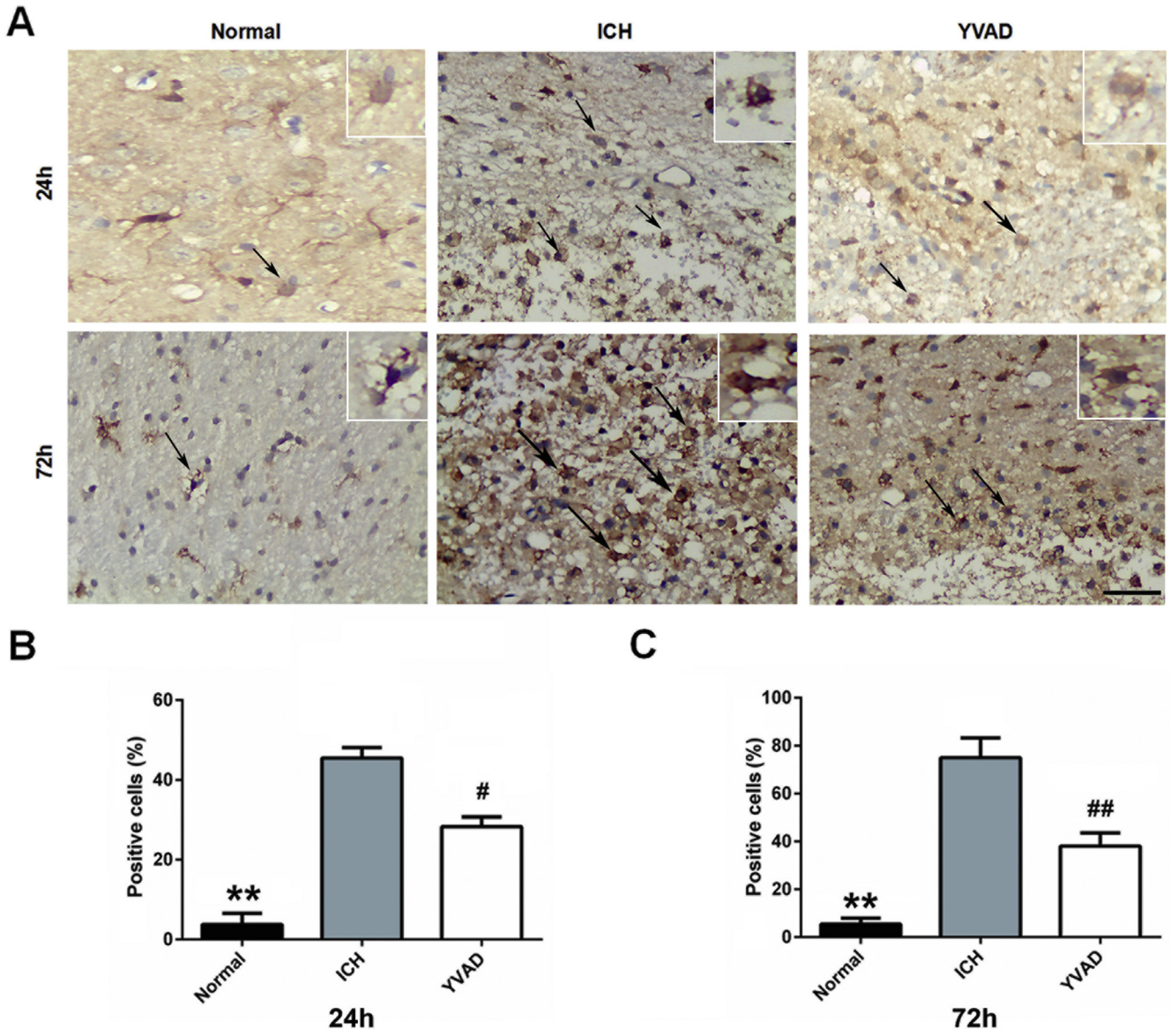

Fig. 5. Ac-YVAD-cmk inhibited microglia infiltration in perihematoma at 24 h and 72 h after ICH on rats.
(a) The mount of microglia was assayed by immunohistochemistry with the special anti-iba-1. Ac-YVAD-cmk treatment decreased the mount of microglia in the perihematoma at 24 h and 72 h after ICH on rats. (n = 6 per group). (b) Quantification of positive cells density (microglia) at 24 h after ICH. (c) Quantification of positive cells density (microglia) at 72 h after ICH. ICH or Ac-YVAD-cmk group vs normal group, $^{**}P < 0.01$; Ac-YVAD-cmk group vs ICH group, $^{\#}P < 0.05$ (24 h), $^{\#\#}P < 0.01$ (72 h). Arrows show microglia. The enlarged photos are located at the top right corner. Scale bar: 20 μm.

($P < 0.01$).

4. Discussion

In the present study, we unraveled three important novel findings of caspase-1 in ICH. First, caspase-1 is activated in activated-microglia both in vitro and in perihematomal after ICH. Second, inhibition of caspase-1 by Ac-YVAD-CMK can inhibit caspase-1-mediated microglia inflammation in vitro and reduce brain edema and neurological deficits via blocking the activation/processing of mature IL-1β/IL-18 and alleviated microglia infiltration on ICH rats (Fig. 6).

Growing studies have demonstrated that pro-caspase-1 is cleaved to activated caspase-1 which is dependent on activated inflammasomes to release the IL-1β/IL-18 [18]. In the present study we considered microglia as the target cells in vitro because the resident microglia are the earliest activation immune cells and play an important role in brain injure after ICH [6–9]. Our research shows that caspase-1 is highly expressed in thrombin-activated microglia in vitro and the perihematomal region of ICH rats, as well as an upregulation in mature IL-1β/IL-18 expression. These results suggest that activated microglia trigger inflammatory response via activated caspase-1 and processing the activation of IL-1β/IL-18 in vitro, also suggesting that the activated microglia are one of the important sources of caspase-1/IL-1β/IL-18 in ICH rats.

The synthesis and activation of inflammasomes depend on the activation of related receptors. The NLRs (nucleotide-binding domain and leucine-rich repeat containing), belong to a class of PRRs (pattern recognition receptors), and are one of the important cytosolic pattern recognition receptors in the innate immune system [19]. After NLRs detect exogenous or endogenous threatening signals, NLRs subsequently generate the inflammasomes containing pro-caspase-1, after which pro-caspase-1 is cleaved to caspase-1 which further promotes proinflammatory cytokine maturation and causes cells pyroptosis, finally inducing cell death. Among all inflammasomes, the NLRP3-

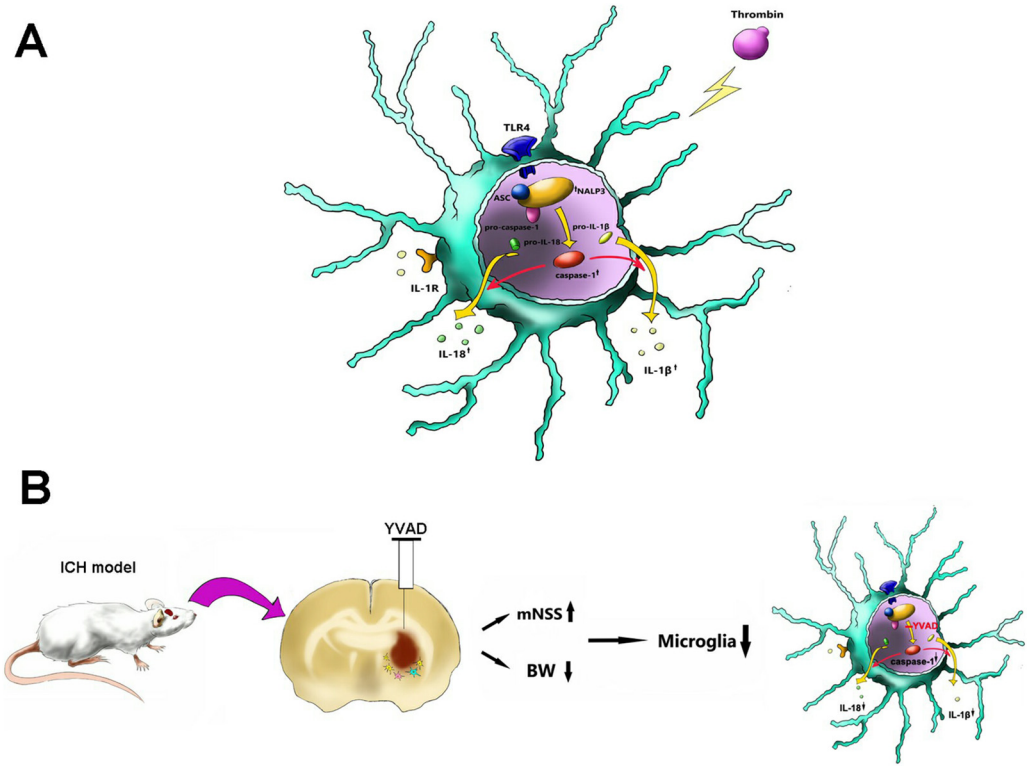

Fig. 6. Schematic illustration indicates that caspase-1 is activated in activated-microglia by thrombin in vitro (a) and in perihematomal after ICH (b). Inhibition of caspase-1 by Ac-YVAD-CMK can reduce brain edema and neurological deficits via blocking the activation/processing of mature IL-1β/IL-18 and alleviated microglia infiltration on ICH rats. mNSS: modified Neurological Severity Scores, BW: brain water.

inflammasome is the most familiar. As a receptor of the best-studied NLRP3-inflammsome, NLRP3 should be activated after receiving stimulation signals, and it contributes to the immunity to pathogen infection and sterile inflammation, what's more, it can be activated by a multitude of substances such as LPS, pore-forming toxins, nucleic acid, endogenous ligands and crystalline substances including ATP, silica, asbestos and alum [20]. In our study, we found that the increased expression of NLRP3 in activated microglia in vitro and in perihematoma on ICH rats is consistent with the previous findings [21]. We considered that activated microglia are one of the sources of NLRP3-inflammsome on ICH rats. Meanwhile, the NLRP3 activation could promote NLRP3-inflammasome formation and further activate caspase-1 [12]. However, treatment with Ac-YVAD-cmk does not affect the expression of NLRP3. For the reason that caspase-1 is a kind of downstream protein of NLRP3, and has no feedback effect on NLRP3. Moreover, several different mechanisms have been proposed to explain NLRP3 activation, including potassium (K^+) efflux, intracellular calcium alteration, ubiquitination and reactive oxygen species (ROS) generation. Previous studies found that ROS plays an important role in NLRP3 activation in ICH [22,23], but the specific mechanisms of its activation need to be elucidated.

In this study, we focused on the effect of caspase-1 that acts as an effector protein consisting of many inflammasomes and the best-described inflammatory caspase in innate immune response. As we all know, caspase-1 is one of the key proteins for releasing IL-1β and IL-18 [11]. We found that to inhibit caspase-1 not only inhibited the maturation of IL-1β and IL-18, but also reduced the mRNA expression of IL-1β and IL-18 in activated microglia in vitro. We infer the reason may be Ac-YVAD-cmk could block the positive feedback synthesis pathways of IL-1β and IL-18. IL-1β plays a crucial role in the development of inflammation in CNS. First, it can induce chemokines and adhesion molecules from astrocytes and endothelial cells leading to release of inflammatory factors and infiltration of leukocytes and T-cells to the injury site during the early stage [24]. Second, it can also regulate the expression of inflammatory cytokines through the activation of NF-κB by ROS [25]. Third, IL-1β regulates glutamate metabolism and enhances the uptake of excitotoxicity and finally adversely affects neuronal structure which leads to neuron death [26]. IL-18 induces IFN-γ production and contributes to the activation of T cells, macrophages and other cell types [27]. Both IL-1β and IL-18 play crucial parts in inflammation. Rothwell et al. [28] demonstrated that IL-1 inhibitor have protective effects in ischemic brain injury models. We found that downregulation of IL-1β expression by transplanting human amniotic epithelial stem cells (HAESCs) on ICH rats was significantly reduced brain edema and neurobehavioral deficits in the previous study. In addition, Masada et al. [29] have shown that overexpression of IL-1ra can reduce the inflammatory response and brain edema in an autologous blood induced models of ICH. Moreover, decreasing the expression of IL-1β can reduce the blood-brain barrier degradation in ICH mice [5]. Our results demonstrate that inhibition of inflammation could play the neuroprotective role by using Ac-YVAD-cmk, which can reduce the expression of activated caspase-1, further inhibiting the process of maturation and secretion of IL-1β and IL-18 in activated microglia in vitro and in perihematoma on ICH rats.

Finally, in our study we found that the microglia infiltration also alleviated while the activated caspase-1/IL-1β/IL-18 reduced at 24 h or 72 h in the Ac-YVAD-cmk treatment group compared with ICH group on ICH rats. We speculate that the reasons may be as following: I. mature IL-1β can stimulates innate immunocytes migration and expression of many kinds of cytokines/chemokines and formed a positive feedback [26], meanwhile, Ac-YVAD-cmk reduced the expression of IL-1β directly or indirectly inhibited microglia infiltration; II. mature IL-18 contributes to the activation of many kinds of immune cells [27], Ac-YVAD-cmk reduced the expression of IL-18 which has an impact on microglia activation and proliferation. Further study needs carrying out to verify our hypothesis. The reduction of microglia infiltration can further reduce brain injure following ICH. However, whether Ac-YVAD-cmk plays a neuroprotective role by affecting other immune cells besides microglia is not clear. Moreover, the evidence that Ac-YVAD-cmk inhibited the expression of IL-1β/IL-18 is convinced, but there might be other mechanisms to inhibit the inflammatory response after ICH. To excessive suppress inflammation after ICH whether has influence on the nerve repair remains unknown, long-term studies are needed.

In conclusion, our research shows that inhibition of caspase-1 can reduce the expression of mature IL-1β/IL-18 in activated microglia in vitro and mitigate inflammatory cells or microglia infiltration in perihematoma, further reduce brain edema and improve neurological function on ICH rats. Meanwhile, we certified for the first time that to inhibit caspase-1 could alleviate microglia infiltration. We demonstrated that inhibition of caspase-1 plays an important role in reducing brain injury on ICH rats by blocking microglia-induced inflammation. Overall, it is possible that the inhibition of caspase-1 constitutes a new important step in the therapy of ICH.

Availability of data and material

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Declaration of Competing Interest

All authors announce no competing interests.

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