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Artesunate Regulates the NLRP3/ASC/Caspase-1 Signaling Pathway to Attenuate Inflammation and Protect Neurological Function in a Mouse Model of Intracerebral Hemorrhage: A Postprint Study

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Abstract

Background Inflammatory response is a major factor in the pathological progression of intracerebral hemorrhage (ICH). Artesunate (ART) possesses antibacterial and anti-inflammatory pharmacological activities, and ART achieves high concentrations in the brain, but its neuroprotective effects against ICH injury remain unclear. **Objective** To investigate the effects of ART on inflammatory response after ICH and explore its underlying mechanism. **Methods** From March 2022 to February 2023, 108 male C57BL/6 mice aged 8-10 weeks were selected and randomly divided into sham operation group (Sham group, n=36), ICH control group (ICH+Vehicle group, n=36), and ART treatment group (ICH+ART group, n=36). The ICH model was established. The ICH+ART group received intraperitoneal injection of ART solution 150 $mg \cdot kg^{-1} \cdot d^{-1}$ at 2 h after modeling, while the ICH+Vehicle group received intraperitoneal injection of 5% sodium bicarbonate solution for 3 consecutive days. Behavioral indicators of mice were observed, hematoxylin-eosin (HE) staining was used to observe brain tissue damage, immunohistochemical staining was performed to detect the number of positive cells per unit area for interleukin (IL)-6, IL-1β, and myeloperoxidase (MPO), IBA1 immunofluorescence staining was used to observe microglia/macrophage activation, TUNEL/NeuN double immunofluorescence staining was performed to observe neuronal death, and Western blotting was used to compare the levels of MPO, IL-1β, tumor necrosis factor-α (TNF-α), NOD-like receptor protein 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), and cysteine-aspartic acid protease-1 (Caspase-1) in mice. **Results** There were statistically significant differences in modified neurological severity scores and right-turn percentage among the three groups of mice ($P<0.05$), with the ICH+Vehicle group being higher than the Sham group,

and the ICH+ART group being lower than the ICH+Vehicle group (P<0.05). HE staining results showed that the Sham group had minimal blood around the striatum and negligible edema, the ICH+Vehicle group showed severe brain tissue damage with increased intercellular spaces, perivascular hematoma, and increased inflammatory cell infiltration; these pathological changes were significantly ameliorated in the ICH+ART group. There were statistically significant differences in the number of positive cells per unit area for IL-6, IL-1β, and MPO among the three groups (P<0.05), with the ICH+Vehicle group having higher numbers of IL-6, IL-1β, and MPO positive cells than the Sham group, and the ICH+ART group having lower numbers than the ICH+Vehicle group (P<0.05). There were statistically significant differences in microglia/macrophage activation numbers among the three groups (P<0.05), with the ICH+Vehicle group being higher than the Sham group, and the ICH+ART group being lower than the ICH+Vehicle group (P<0.05). There were statistically significant differences in neuronal death numbers among the three groups (P<0.05), with the ICH+Vehicle group being higher than the Sham group, and the ICH+ART group being lower than the ICH+Vehicle group (P<0.05). There were statistically significant differences in the levels of MPO, IL-1β, TNF-α, NLRP3, ASC, and Caspase-1 among the three groups (P<0.05), with the ICH+Vehicle group having higher levels than the Sham group, and the ICH+ART group having lower levels than the ICH+Vehicle group (P<0.05). **Conclusion** ART treatment after ICH can alleviate striatal inflammatory response in mice by targeting the NLRP3/ASC/Caspase-1 signaling pathway, reduce microglial activation, and ultimately attenuate striatal neuronal apoptosis and improve cerebral edema.

Full Text

Mechanism of Artesunate Regulating NLRP3/ASC/Caspase-1 Signaling Pathway to Reduce Inflammation and Protect Neurological Function in Mice With Intracerebral Hemorrhage

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Abstract

Background: Inflammatory response is a major factor in the progression of intracerebral hemorrhage (ICH). Artesunate (ART) exhibits antibacterial and anti-inflammatory pharmacological activity and achieves high concentrations in the brain, yet its neuroprotective effect on cerebral hemorrhage injury remains

unclear. **Objective:** To observe the effect of ART on inflammatory response after ICH and explore its underlying mechanism. **Methods:** From March 2022 to February 2023, 108 male C57BL/6 mice aged 8–10 weeks were randomly divided into three groups: sham-operated group (Sham, n=36), ICH control group (ICH+Vehicle, n=36), and ART treatment group (ICH+ART, n=36). The ICH model was established by collagenase injection. The ICH+ART group received intraperitoneal injection of ART solution ($150\ mg \cdot kg^{-1} \cdot d^{-1}$) 2 hours after modeling, while the ICH+Vehicle group received 5% sodium bicarbonate solution for 3 consecutive days. Behavioral indicators were observed, brain tissue damage was assessed by HE staining, and immunohistochemical staining was used to detect the number of positive cells per unit area for interleukin (IL)-6, IL-1β, and myeloperoxidase (MPO). IBA1 immunofluorescence staining was performed to observe microglial/macrophage activation, TUNEL/NeuN double staining was used to assess neuronal death, and Western blotting was employed to compare protein levels of MPO, IL-1β, tumor necrosis factor α (TNF-α), NOD-like receptor protein 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), and cysteine-aspartic protease 1 (Caspase-1). **Results:** Significant differences were observed among the three groups in modified neurological severity scores and right-turn percentages ($P<0.05$). The ICH+Vehicle group showed higher values than the Sham group, while the ICH+ART group demonstrated lower values than the ICH+Vehicle group ($P<0.05$). HE staining revealed minimal blood around the striatum with negligible edema in the Sham group, severe brain tissue damage with increased intercellular space, perivascular hematoma, and inflammatory cell infiltration in the ICH+Vehicle group, and significant improvement of these pathological changes in the ICH+ART group. The numbers of IL-6, IL-1β, and MPO positive cells per unit area differed significantly among groups ($P<0.05$), with the ICH+Vehicle group showing higher counts than the Sham group and the ICH+ART group showing lower counts than the ICH+Vehicle group ($P<0.05$). Microglial/macrophage activation numbers also differed significantly ($P<0.05$), following the same pattern of being highest in the ICH+Vehicle group and reduced in the ICH+ART group ($P<0.05$). Neuronal death counts showed significant intergroup differences ($P<0.05$), with the ICH+Vehicle group exhibiting higher numbers than the Sham group and the ICH+ART group showing lower numbers than the ICH+Vehicle group ($P<0.05$). Protein levels of MPO, IL-1β, TNF-α, NLRP3, ASC, and Caspase-1 differed significantly among groups ($P<0.05$), with the ICH+Vehicle group showing elevated levels compared to the Sham group and the ICH+ART group showing reduced levels compared to the ICH+Vehicle group ($P<0.05$). **Conclusion:** ART treatment after ICH attenuates striatal inflammatory response and reduces microglial activation in mice by targeting the NLRP3/ASC/Caspase-1 signaling pathway, ultimately reducing striatal neuronal apoptosis and improving cerebral edema.

Keywords: Cerebral hemorrhage; Inflammation; Artesunate; NOD-like receptor protein 3; Caspase-1; Mouse

Introduction

Intracerebral hemorrhage (ICH) is a high-risk cerebrovascular disease accounting for 15% of all strokes, characterized by high morbidity, disability, and mortality rates. The global incidence of ICH continues to rise annually, with approximately 5 million new diagnoses each year and about 3 million deaths. Only 12–39% of patients achieve favorable neurological outcomes. The increasing incidence is associated with hypertension, and the pathophysiology involves both primary injury from vascular rupture and multiple secondary inflammatory cascades, including oxidative stress, various forms of cell apoptosis, and neuroinflammation. In clinical practice, the primary injury in most ICH patients is irreversible, so treatment focuses on preventing secondary neurological damage. The inflammatory cascade after ICH ultimately leads to extensive neuronal apoptosis and becomes the main factor affecting patient prognosis. However, clinical translation of pharmacological interventions for ICH remains inadequate.

NOD-like receptor protein 3 (NLRP3) inflammasome activation plays a crucial role in macrophage activation during ICH. Inhibition of NLRP3 inflammasome-related proteins can attenuate injury in various organs, including lung, liver, myocardium, and kidney. Artemisinin and its derivatives possess broad pharmacological activities, including anti-parasitic, anti-viral, anti-cancer, anti-inflammatory, and antioxidant effects. Artesunate (ART), a water-soluble derivative of artemisinin, can cross the blood-brain barrier and maintain high concentrations in brain tissue. ART has demonstrated protective effects in various central nervous system injuries, including traumatic brain injury, subarachnoid hemorrhage, and ischemia-reperfusion injury. Previous studies have shown that ART can directly protect neurons in malaria-infected mice by reducing apoptosis, and it can protect neurons and promote neurotrophic factor release after traumatic brain injury in rats, leading to improved neurological function scores. These findings suggest that ART may play an important role in neuroprotection after ICH.

The NLRP3 inflammasome comprises NLRP3 receptor protein, ASC adaptor protein, and Caspase-1 effector protein, forming an essential component of the innate immune system. Host-derived pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns bind to pattern recognition receptors, activating NLRP3. NLRP3 then recruits ASC to activate pro-Caspase-1, which undergoes hydrolysis and simultaneously cleaves pro-IL-1β and pro-IL-18, ultimately releasing IL-1β and IL-18 and exacerbating the inflammatory response. Additionally, activated Caspase-1 can mediate pyroptosis through gasdermin D (GSDMD). Studies have shown that Caspase-1 knockout significantly improves survival rates in ICH mice. While ART has been reported to effectively protect the nervous system after lipopolysaccharide-induced central nervous system inflammation, previous research did not examine NLRP3/ASC/Caspase-1 expression. Therefore, this study hypothesized that ART participates in the NLRP3 signaling pathway to

protect neurological function after ICH.

Given that local inflammatory cascades around the hematoma after ICH cause irreversible primary damage to neurological function, this study aimed to investigate ART's effect on reducing secondary inflammatory damage. Using a mouse model of collagenase injection to simulate ICH pathogenesis, we examined the relationship between ART and NLRP3/ASC/Caspase-1 signaling pathway expression and its impact on neurological recovery, exploring the underlying mechanisms.

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1. Materials and Methods

1.1 Experimental Materials

1.1.1 Experimental Animals

One hundred eight male C57BL/6 mice aged 8–10 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (License No. SCXK (Beijing) 2021-0006), with body weights of 19–22 g. All mice were housed in a specific pathogen-free (SPF) animal facility at Henan Academy of Medical Sciences under standard conditions: 25°C ambient temperature, appropriate humidity, 12-hour light/dark cycle, with free access to food and water. The study was approved by the Ethics Committee of Zhengzhou University (Approval No. 2023042).

1.1.2 Main Reagents and Instruments

The following reagents were used: 4% paraformaldehyde fixative (Beijing Solarbio Science & Technology Co., Ltd., Cat. No. P1110), Type VII collagenase (Sigma-Aldrich, Cat. No. C0773), artesunate (Shanghai Macklin Biochemical Co., Ltd., Cat. No. 182824-33-5), rabbit anti-tumor necrosis factor α (TNF-α) polyclonal antibody (Abcam, Cat. No. ab183218), rabbit anti-interleukin (IL)-1β polyclonal antibody (Abcam, Cat. No. ab283818), rabbit anti-myeloperoxidase (MPO) polyclonal antibody (Abcam, Cat. No. ab208670), rabbit anti-IL-6 polyclonal antibody (Abcam, Cat. No. ab208113), rabbit anti-ASC polyclonal antibody (Abcam, Cat. No. ab283684), rabbit anti-NLRP3 polyclonal antibody (Abcam, Cat. No. ab263899), rabbit anti-Caspase-1 polyclonal antibody (Abcam, Cat. No. ab207802), rabbit anti-actin polyclonal antibody (Abcam, Cat. No. ab8226), rabbit anti-ionized calcium-binding adaptor molecule 1 (IBA1) antibody (Wako, Cat. No. 019-19741), goat anti-rabbit IgG (Cy3) pre-adsorbed secondary antibody (Abcam, Cat. No. ab6939), goat anti-mouse IgG (Beyotime Biotechnology, Cat. No. A0568), and HRP-conjugated goat anti-rabbit IgG (Sangon Biotech, Cat. No. D110058).

Instruments included: digital stereotaxic apparatus for mice (Anhui Zhenghua Biological Instrument Equipment Co., Ltd., Model: ZH-Blue Star B/S), 1 L microsyringe (Hamilton, Model: 7001KH), upright optical microscope (Zeiss, Model: LSM880), and laser confocal microscope (Olympus, Model: FV1000).

1.2 Experimental Methods

1.2.1 Animal Grouping

Mice were randomly divided into three groups: sham-operated group (Sham, n=36), ICH control group (ICH+Vehicle, n=36), and ART treatment group (ICH+ART, n=36).

1.2.2 ICH Model Preparation

Male SPF-grade C57BL/6 mice aged 8–10 weeks were anesthetized with 10% chloral hydrate (4 mL/kg) and placed in a stereotaxic frame. A small burr hole (0.6 mm diameter) was drilled 0.2 mm posterior to the bregma and 2 mm lateral to the midline. The ICH control and ART treatment groups received 0.75 L of 0.1 U/ L collagenase Type VII injected into the basal ganglia at a rate of 0.1 L/min, with the syringe left in place for 10 minutes to prevent backflow. The sham group received 0.75 L of 0.9% saline. Postoperative mice were kept warm until full recovery. Artesunate (60 mg) was dissolved in 6 mL of 5% sodium bicarbonate solution. The ICH+ART group received intraperitoneal injection of ART solution ($150\ \text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) 2 hours after modeling, while the ICH+Vehicle group received 5% sodium bicarbonate solution, with injections continuing for 3 consecutive days.

1.2.3 Behavioral Observation and Assessment

At 3 days post-surgery, 6 mice from each group were assessed using the modified neurological severity score (mNSS) to evaluate motor, sensory, reflex, and balance functions (total score 18, with higher scores indicating more severe neurological deficits). Additionally, 6 mice per group underwent corner turn testing to assess sensorimotor and postural asymmetry. Normal mice turn equally to the right or left, while ICH mice tend to turn toward the lesion side. Two 20-cm glass plates formed a 30-degree corner; when mice entered the deep corner, vibrissae stimulation on both sides induced a turning behavior. Left and right turns were recorded for 20 trials per mouse with at least 30 seconds between trials. The right-turn percentage was calculated as (right turns / total turns) × 100%.

1.2.4 Brain Tissue Specimen Preparation

At 3 days post-surgery, 6 mice from each group were anesthetized with chloral hydrate and transcardially perfused with 20–40 mL PBS followed by 4% paraformaldehyde. Brains were rapidly removed and immersed in 4% paraformaldehyde, then routinely embedded in paraffin and sectioned at 5 m thickness for HE staining, IBA1 immunofluorescence, IL-6/IL-1β/MPO immunohistochemistry, and NeuN/TUNEL staining.

1.2.5 Hematoxylin-Eosin (HE) Staining

Paraffin sections were deparaffinized and stained with HE at room temperature. Brain tissue damage was observed under an optical microscope and photographed.

1.2.6 Immunohistochemical Staining

Before blocking, paraffin sections were incubated with oxidase solution (3% H_2O_2, 10% methanol, diluted in PBS) for 15 minutes. Sections were then incubated overnight at 4°C with rabbit anti-IL-6 antibody (1:300), rabbit anti-IL-1β antibody (1:500), or rabbit anti-MPO antibody (1:700). After washing three times in PBS, sections were incubated with HRP-conjugated goat anti-rabbit Cy3 secondary antibody (1:3,000) for 1 hour. All sections were developed with diaminobenzidine (DAB) reagent, counterstained with hematoxylin, dried, and examined under a microscope. Four microscopic fields were examined per brain slice, with three slices analyzed per mouse, to calculate the number of positive cells per unit area.

1.2.7 Immunofluorescence Staining

Paraffin sections were deparaffinized, subjected to antigen retrieval, and blocked before incubation with rabbit anti-IBA1 monoclonal antibody (1:2,000) overnight at 4°C. After washing three times with PBS, sections were incubated with goat anti-rabbit Cy3 secondary antibody (1:3,000) for 1 hour at room temperature. Microglial/macrophage activation was observed under a fluorescence microscope, and IBA1-positive cells were counted using ImageJ/Fiji software.

1.2.8 TUNEL/NeuN Double Immunofluorescence Staining

Paraffin sections were deparaffinized and incubated with anti-NeuN antibody (1:600) overnight at 4°C. According to the TUNEL apoptosis kit protocol, sections were incubated with goat anti-mouse IgG (1:3,000) for 2 hours at room temperature, then washed and mounted. Under a fluorescence microscope, four high-power fields were randomly selected per slice for analysis. TUNEL and NeuN double-positive cells were counted as dead neurons.

1.2.9 Western Blotting

Five mice from each group were sacrificed at 3 days post-surgery, and brains were removed. Brain tissue from the 1-mm area surrounding the hematoma was dissected on ice, weighed, and homogenized in RIPA lysis buffer. After centrifugation, supernatants were collected and quantified using Nanodrop. Samples (30 g) were prepared and boiled. Proteins were separated by electrophoresis, transferred to membranes, and blocked before overnight incubation at 4°C with the following primary antibodies: rabbit anti-IL-1β (1:1,000), rabbit anti-MPO (1:1,000), rabbit anti-TNF-α (1:2,000), rabbit anti-NLRP3 (1:1,000), rabbit anti-ASC (1:1,000), rabbit anti-Caspase-1 (1:1,000), and rabbit anti-actin (1:3,000). After washing, membranes were incubated with HRP-conjugated goat anti-rabbit IgG (1:1,000) for 2 hours at room temperature. Chemiluminescence imaging was performed using a Bio-Rad system, and optical density (OD) signals were analyzed using ImageJ/Fiji software.

1.3 Statistical Analysis

Statistical analysis was performed using Prism 8 software. Normally distributed data are expressed as mean ± standard deviation ($\bar{x} \pm s$) and analyzed using one-way ANOVA with Tukey's multiple comparisons test for pairwise comparisons. Non-normally distributed data are expressed as median (interquartile range) [$M(P_{25}, P_{75})$] and analyzed using Kruskal-Wallis H test with Mann-Whitney U

test for pairwise comparisons. $P<0.05$ was considered statistically significant.

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2. Results

2.1 Behavioral Assessment

Significant differences were observed among the three groups in mNSS scores ($P<0.05$). The ICH+Vehicle group exhibited higher scores than the Sham group, while the ICH+ART group showed lower scores than the ICH+Vehicle group ($P<0.05$). Similarly, the percentage of right turns differed significantly among groups ($P<0.05$), with the ICH+Vehicle group showing higher values than the Sham group and the ICH+ART group demonstrating lower values than the ICH+Vehicle group ($P<0.05$) .

2.2 HE Staining Results

HE staining revealed minimal blood around the striatum with negligible edema in the Sham group. The ICH+Vehicle group showed severe brain tissue damage, increased intercellular space, perivascular hematoma, and enhanced inflammatory cell infiltration. These pathological changes were significantly ameliorated in the ICH+ART group [Figure 1: see original paper].

2.3 Immunohistochemical Staining Results

Positive reactions appeared as brown-yellow granules, with IL-6, IL-1β, and MPO positivity representing local inflammatory cell infiltration in the striatum and MPO positivity indicating neutrophil activation. Significant differences were observed among the three groups in the numbers of IL-6, IL-1β, and MPO positive cells per unit area ($P<0.05$). The ICH+Vehicle group showed higher counts than the Sham group, while the ICH+ART group exhibited lower counts than the ICH+Vehicle group ($P<0.05$) [Figure 2: see original paper] .

2.4 IBA1 Immunofluorescence Staining Results

Significant differences were observed among the three groups in microglial/macrophage activation numbers ($P<0.05$). The ICH+Vehicle group showed higher activation than the Sham group, while the ICH+ART group demonstrated lower activation than the ICH+Vehicle group ($P<0.05$) [Figure 3: see original paper] .

2.5 TUNEL/NeuN Double Staining Results

Dead cells stained green with TUNEL, neurons stained red with NeuN, and dead neurons appeared as red-blue merged signals. Significant differences were observed among the three groups in neuronal death counts ($P<0.05$). The ICH+Vehicle group exhibited higher neuronal death than the Sham group, while

the ICH+ART group showed lower neuronal death than the ICH+Vehicle group ($P<0.05$) [Figure 4: see original paper] .

2.6 Protein Expression Levels

Significant differences were observed among the three groups in protein levels of MPO, IL-1β, TNF-α, NLRP3, ASC, and Caspase-1 ($P<0.05$). The ICH+Vehicle group showed higher levels than the Sham group, while the ICH+ART group exhibited lower levels than the ICH+Vehicle group ($P<0.05$) [Figure 5: see original paper] .

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3. Discussion

ICH is a disease with high morbidity and mortality that often leads to severe neurological deficits and death, imposing heavy burdens on individuals, families, and society. The inflammatory response after ICH is a major factor driving disease progression, involving microglial recruitment and release of pro-inflammatory cytokines. Additionally, peripheral inflammatory cells such as leukocytes can enter the central nervous system through the damaged blood-brain barrier, further amplifying inflammatory injury and worsening the condition. Therefore, reducing inflammatory infiltration and suppressing inflammatory cascades after ICH represents a promising therapeutic strategy with important clinical and social value.

ART is a water-soluble derivative of artemisinin with high efficacy, low toxicity, and good tolerability. Beyond its anti-parasitic effects, ART possesses anti-inflammatory, anti-tumor, and anti-microbial activities through multiple mechanisms, including inhibiting inflammatory factor production, inducing iron-dependent apoptosis, and suppressing angiogenesis. As a multi-potential drug, ART exerts neuroprotective effects in central nervous system diseases. Studies have shown that ART can directly protect neurons and reduce apoptosis in malaria-infected mice, and it can protect neurons while promoting neurotrophic factor release after traumatic brain injury in rats, ultimately improving neurological function scores. These findings suggest ART may play an important role in neuroprotection after ICH.

Previous studies have demonstrated that ART protects the blood-brain barrier via the sphingosine-1-phosphate receptor 1/phosphatidylinositol 3-kinase (S1PR1/PI3K) signaling pathway in subarachnoid hemorrhage mice, and in vitro studies have shown that ART promotes neural stem cell proliferation through the PI3K/protein kinase B (Akt) signaling pathway. Other research has reported ART's protective effects after ICH, such as activating nuclear factor erythroid 2-related factor 2 (Nrf2) and reactive oxygen species (ROS)-dependent p38 mitogen-activated protein kinase to inhibit oxidative and inflammatory processes and prevent ICH-reperfusion injury. ART has also been reported to exert neuroprotective effects against cerebral ischemia-reperfusion injury in rats

through the Toll-like receptor 4 (TLR4)/nuclear factor- B (NF- B) pathway. However, ART's involvement in the NLRP3 inflammasome has not been previously reported.

The NLRP3 inflammasome, comprising NLRP3 receptor protein, ASC adaptor protein, and Caspase-1 effector protein, is an essential component of the innate immune system. Host-derived pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns bind to pattern recognition receptors, activating NLRP3. NLRP3 then recruits ASC to activate pro-Caspase-1, which undergoes hydrolysis and simultaneously cleaves pro-IL-1β and pro-IL-18, ultimately releasing IL-1β and IL-18 and exacerbating inflammatory responses. Additionally, activated Caspase-1 can mediate pyroptosis through gasdermin D (GSDMD). Studies have shown that Caspase-1 knockout significantly improves survival rates in ICH mice. While ART has been reported to effectively protect the nervous system after lipopolysaccharide-induced central nervous system inflammation, these studies did not examine NLRP3/ASC/Caspase-1 expression. Therefore, this study hypothesized that ART participates in the NLRP3 signaling pathway to protect neurological function after ICH.

Given that local inflammatory cascades around the hematoma after ICH cause irreversible damage to neurological function, this study investigated ART's effect on reducing secondary inflammatory damage. The results demonstrated that ART significantly improved cerebral edema and neurological outcomes after ICH in experimental animals, as evidenced by improved corner turn test and mNSS-R scores. We examined the expression of TNF-α, IL-1β, and MPO after ART treatment and used immunofluorescence to assess microglial infiltration, finding that ART significantly ameliorated neuroinflammation at 3 days post-ICH. To investigate the underlying mechanism, we examined NLRP3/ASC/Caspase-1 signaling axis expression levels and found that ART at 150 mg/kg significantly reduced NLRP3, ASC, and Caspase-1 expression levels after ICH, explaining the reduced local inflammatory infiltration in the ICH+ART group.

The significance of inflammation-mediated neuronal apoptosis after ICH is profound. Neurons influence the neurovascular unit through nutritional support effects that regulate blood-brain barrier tight junctions. Damage to the neurovascular unit after ICH leads to local protein leakage, fibrotic scar formation, astrocyte activation, and glial scar formation, ultimately impairing neurological function. Studies have shown that early neuronal apoptosis after ICH correlates with poor outcomes, including reduced cortical thickness, loss of white matter integrity, and hippocampal atrophy. Therefore, early inhibition of inflammatory infiltration after ICH has long-lasting and far-reaching implications for prognosis. In summary, this study demonstrates that ART protects neurological function in experimental animals after ICH by blocking the NLRP3/ASC/Caspase-1 signaling pathway, thereby reducing local inflammatory cell activation in the early stage, ultimately decreasing neuronal apoptosis and cerebral edema.

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