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Original Article

Schisandrin B Inhibits NLRP3 Inflammasome Pathway and Attenuates Early Brain Injury in Rats of Subarachnoid Hemorrhage*

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ABSTRACT **Objective:** To determine whether Schisandrin B (Sch B) attenuates early brain injury (EBI) in rats with subarachnoid hemorrhage (SAH). **Methods:** Sprague-Dawley rats were divided into sham (sham operation), SAH, SAH+vehicle, and SAH+Sch B groups using a random number table. Rats underwent SAH by endovascular perforation and received Sch B (100 mg/kg) or normal saline after 2 and 12 h of SAH. SAH grading, neurological scores, brain water content, Evan's blue extravasation, and terminal transferase-mediated dUTP nick end-labeling (TUNEL) staining were carried out 24 h after SAH. Immunofluorescent staining was performed to detect the expressions of ionized calcium binding adapter molecule 1 (Iba-1) and myeloperoxidase (MPO) in the rat brain, while the expressions of B-cell lymphoma 2 (Bcl-2), Bax, Caspase-3, nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3), apoptosis-associated speck-like protein containing the caspase-1 activator domain (ASC), Caspase-1, interleukin (IL)-1β, and IL-18 in the rat brains were detected by Western blot. **Results:** Compared with the SAH group, Sch B significantly improved the neurological function, reduced brain water content, Evan's blue content, and apoptotic cells number in the brain of rats ($P<0.05$ or $P<0.01$). Moreover, Sch B decreased SAH-induced expressions of Iba-1 and MPO ($P<0.01$). SAH caused the elevated expressions of Bax, Caspase-3, NLRP3, ASC, Caspase-1, IL-1β, and IL-18 in the rat brain ($P<0.01$), all of which were inhibited by Sch B ($P<0.01$). In addition, Sch B increased the Bcl-2 expression ($P<0.01$). **Conclusion:** Sch B attenuated SAH-induced EBI, which might be associated with the inhibition of neuroinflammation, neuronal apoptosis, and the NLRP3 inflammatory signaling pathway.

KEYWORDS schisandrin B, subarachnoid hemorrhage, early brain injury, inflammation, neuronal apoptosis, nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3, Chinese medicine

Subarachnoid hemorrhage (SAH) is a serious stroke event which has high levels of morbidity and mortality worldwide. Previous study on SAH has been focused primarily on cerebral vasospasm and delayed cerebral ischemia; however, anti-vasospastic drugs fail to improve patient outcomes in clinical trials.[1] Recent study has shown that early brain injury (EBI) is one of most common causes of disability and death in patients suffering from SAH.[2] The potential mechanisms underlying EBI after SAH include the increase of intracranial pressure, the reduction of cerebral blood flow, inflammatory reactions, and neuronal apoptosis.[3] Anti-inflammatory[4] and anti-apoptotic[5] treatments have been reported to reduce EBI caused by SAH. Therefore, developing new agents which target inflammation and apoptosis have been considered as promising therapeutic strategies to improve the clinical outcomes of SAH patients.

The nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome contains the NOD-like receptor, NLRP3, apoptosis-associated speck-like protein containing the caspase-1 activator domain (ASC), and caspase-1.[6] Recently, several reports have shown that the NLRP3 inflammasome mediates

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the inflammatory response and contributes to neuroinflammation in various neurological diseases, including intracranial hemorrhage,[7] ischemic stroke,[8] traumatic brain injury,[9] spinal cord injury,[10] Alzheimer's disease,[11] and SAH.[4]

SAH causes the activation of NLRP3 inflammasome, recruiting and activating ASC and pro-caspase-1, and subsequently promoting the cleaving of pro-interleukin (IL)-1β and pro-IL-18 into their active forms (IL-1β and IL-18). Both can promote neuroinflammation and EBI after SAH.[12] The specific NLRP3 inhibitor MCC950 has been demonstrated to ameliorate neurological dysfunction and severe brain edema after SAH. Neuroprotection by MCC950 in SAH was attributed to the decreased expression of NLRP3 inflammasome and proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), IL-1β, and IL-6.[13]

Schisandrin B (Sch B), the main active constituent in Chinese medicine, is derived from *Schisandra chinensis* and has been shown to reduce oxidative stress and inflammatory reactions in various inflammatory diseases.[14,15] Several reports have shown that Sch B attenuates inflammatory reactions via the inhibition of toll-like receptor-4 (TLR4), nuclear factor kappa-B (NF-κB), and the mitogen-activated protein kinase (MAPK) inflammatory signaling pathway.[16-18] Lee, et al[19] demonstrated that Sch B protects against ischemic brain damage in rats with cerebral ischemia. The neuroprotection associated with Sch B in ischemic stroke was considered to be associated with the inhibition of TNF-α and IL-1β and the degradation of matrix metalloprotein-2 (MMP-2) and MMP-9 in ischemic hemispheres. However, it remains unknown whether Sch B protects against EBI after SAH. Therefore, this study was performed to confirm the neuroprotection of Sch B in SAH and to further explore the underlying mechanisms.

METHODS

Animals and Modeling

Adult male Sprague-Dawley rats (clean grade, weighing 250–300 g) were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Sciences [certificate No. SCXK (Hu) 2017-0012]. Animals were housed in a colony room under controlled temperature (22 ℃), humidity (50%), and a 12 h/12 h light-dark cycle, with food and water available. All animal experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Fujian Medical University (approval No. 2021-8CAARM098).

The SAH rat model was induced by endovascular perforation described previously.[20] Briefly, animals were transorally intubated after induced anesthesia with 5% isoflurane in 70%/30% medical air/oxygen and a small rodent respirator was used to maintain adequate respiration. Anesthesia was maintained with 3% isoflurane in 70%/30% medical air/oxygen. The right common carotid artery (CCA), external carotid artery, and internal carotid artery (ICA) were carefully exposed through a ventral midline neck incision. A blunted 4–0 monofilament nylon suture was inserted into the ICA until resistance was felt (18–20 mm from the CCA bifurcation). The suture was carefully pushed approximately 3 mm further to perforate the artery wall to create a SAH. Sham-operation rats were manipulated in the same way without perforation. SAH success was determined by observing the base of brain after postmortem removal. Animals that died or where SAH was not confirmed were excluded from the experiment.

Grouping and Administration

Rats were divided into 4 groups using a random number table: (1) sham group (*n*=24), which underwent sham operation; (2) SAH group (*n*=29), which was subjected to SAH; (3) SAH+vehicle group (*n*=30), which was subjected to SAH and received vehicle; and (4) SAH+Sch B group (*n*=28), which was subjected to SAH and treated with Sch B (100 mg/kg). Sch B (lot No. 61281-37-6, Chengdu Herbpurify Co. Ltd., China) was dispersed in an aqueous solution of Tween 20 (5% w/v) and injected intraperitoneally twice, at 2 and 12 h after the onset of SAH. Rats were treated with normal saline as a vehicle control at the same volume as Sch B.

Neurological Scores

Neurological scores were evaluated 24 h after SAH according to the scoring system of Garcia, et al[21] with minor modifications. Briefly, the neurobehavioral study consisted of 6 tests, including spontaneous activity, symmetry in the movement of the 4 limbs, forepaw outstretching, climbing, body proprioception, and response to vibrissae touch. A lower score represents a more serious deficiency in neurological function. Neurological scores were carried out by a blinded assessor.

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SAH Grading

The severity of SAH was quantified as reported previously.[22] Rats were deeply anesthetized with 5% isoflurane and sacrificed with decapitation 24 h after SAH, and brains were removed. The scale evaluation was based on the amount of subarachnoid blood in 6 segments of the basal cistern: grade 0, no subarachnoid blood (0 score); grade 1, minimal subarachnoid blood (1 score); grade 2, moderate blood clot with recognizable arteries (2 scores); grade 3: blood clot obliterating all arteries within the segment (3 scores). A total score ranging from 0 to 18 was obtained by the addition of the scores from each of the 6 segments.

Assessment of Brain Edema

Brain edema was determined by assessing the cerebral water content according to a previous report.[23] The rat brains were removed and gently blotted with filter-paper and weighed on an electronic balance, as the wet weights (WWs), and then dried for 24 h in a 100 ℃ vacuum oven to obtain the dry weights (DWs). Cerebral water content was calculated according to the following formula: H_2O (%)=(WW–DW)/WW × 100%.

Measurement of Blood Brain-Barrier Permeability

Evan's blue dye (2% in saline) was injected into the left jugular vein 23 h after SAH. Rats were then transcardially perfused with phosphate buffered saline (PBS) to remove the intravascular dye. Brains were homogenized in a tenfold volume of 50% trichloroacetic acid solution to precipitate protein and centrifuged for 10 min at 3,000 r/min. The supernatant was diluted with ethanol (1:3), and its fluorescence was measured at 610 nm for the absorbance of Evan's blue. The blood brain-barrier (BBB) permeability were expressed as μg/g tissue.

Terminal Transferase-Mediated dUTP Nick End-Labeling Analysis

Terminal transferase-mediated dUTP nick end-labeling (TUNEL) staining was carried out using the *in situ* cell death detection kit (lot No. 11684795910, Roche, Switzerland) according to the manufacturer's instructions. Briefly, paraffin-embedded sections (4 μm thick) were deparaffinized with xylene and rehydrated with graded alcohol. After deparaffinizing, the protein in brain sections was digested using proteinase K. Slides were placed in the equilibration buffer and then incubated in the TdT enzyme at 37 ℃ for 60 min, followed by 2 × SSC to stop the reaction. Endogenous peroxidase activity was then blocked with 0.3% H_2O_2 and the slices were incubated in streptavidin horseradish-peroxidase (HRP) solution for 30 min at room temperature. Diaminobenzidine was used as chromogen, and the sections were counterstained with hematoxylin. Both the numbers of total cells and TUNEL-positive cells in the ischemic hemisphere at 400 × magnification were manually counted. The number of TUNEL-positive cells was expressed as a percentage of the total counted cells.

Immunofluorescent Staining

After 24 h of cerebral ischemia, rats were perfused with 4% paraformaldehyde in PBS (pH 7.4). Rat brains were removed, fixed, and embedded in paraffin. The paraffin-embedded sections (4 μm thick) were deparaffinized with xylene and rehydrated with graded alcohol. Then, brain sections were incubated in 3% H_2O_2 for 15 min to block endogenous peroxidase activity. The primary antibodies for ionized calcium binding adapter molecule 1 (Iba-1, 1:200, lot No. 019-19741, Wako, Japan), myeloperoxidase (MPO, 1:100, lot No. ab208670, Abcam, UK), and NLRP3 (1:100, lot No. ab214185, Abcam) were used. Afterward, the sections were washed with PBS and incubated with fluorescein isothiocyanate-labeled goat anti-rabbit antibody or goat anti-mouse antibody (1:1000, lot No. A32731, Invitrogen, USA) for 2 h at room temperature in the dark. The sections were rinsed and stained with DAPI and mounted with glycerol. Sections were observed using a fluorescence microscope (Leica DMi8, Germany).

Western Blot Analysis

Total protein from brain samples was extracted using a protein extraction kit (Beyotime Biotech. Co., China). Protein samples (50 μg) were separated on 8%–12% sodium dodecyl sulfate (SDS) polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were incubated at 4 ℃ for 2 h with primary antibodies against B-cell lymphoma 2 (Bcl-2, 1:1000, lot No. ab194583), Bax (1:1000, lot No. ab32503), Caspase-3 (1:1000, lot No. ab184787), NLRP3 (1:500, lot No. ab214185), ASC (1:500, lot No. ab47092), Caspase-1 (1:500, lot No. ab1872), IL-1β (1:500, lot No. ab9722), or IL-18 (1:500, lot No. ab52914), all of which were purchased from Abcam. The nitrocellulose

membranes were incubated with HRP conjugated secondary antibodies (1:2000, lot No. ZB-2313, Zhongshan Biotechnology Co. Ltd., China) for 2 h at 25 ℃ and developed with an enhanced chemiluminescence detection system. β-Actin was used as a loading control. The optical densities of protein bands were analyzed using the Image J software (version 1.8.0, NIH, USA).

Statistical Analysis

SPSS 13.0 software (USA) was used for statistical analysis. Data were presented as mean ± standard deviation ($\bar{x} \pm s$). Statistical analysis was performed using the analysis of variance (ANOVA) test followed by the least significant difference test for individual comparisons between groups. A value of $P<0.05$ was considered statistically significant.

RESULTS

Mortality of Rats

No rats died in the sham group, while 17.24% (5/29) rats died in the SAH group, 20.00% (6/30) died in the SAH+vehicle group, and 14.29% (4/28) died in the SAH+Sch B group. No significant difference was observed for mortality among the SAH, SAH+vehicle, and SAH+Sch B groups ($P>0.05$).

Sch B Improves SAH Grading, Neurological Scores, Brain Edema, and BBB Permeability of Rats

After 24 h of SAH, subarachnoid blood clots were found on the ipsilateral side of rat brains, around the circle of Willis, and within the ventral surface of the brain stem (Figure 1A). No significant difference was observed for SAH grading scores among the SAH, SAH+vehicle, and SAH+Sch B groups ($P>0.05$, Figure 1B). The neurological score in the SAH group was significantly lower than the sham group ($P<0.01$); however, Sch B improved the neurological function in rats with SAH ($P<0.05$, Figure 1C). The brain water content and Evan's blue content were significantly increased in rat brains after 24 h of SAH compared to the sham group ($P<0.01$), both of which were significantly reduced by Sch B ($P<0.01$, Figures 1D and 1E).

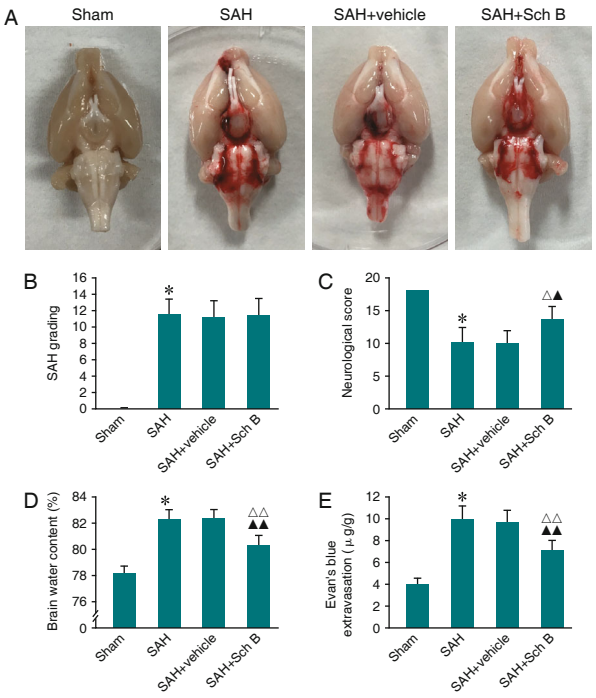

Figure 1. Effect of Sch B on SAH-Induced SAH Grading, Neurological Score, Cerebral Water Content, and BBB Permeability of Rats ($\bar{x} \pm s$, n=6)

Notes: A: representative macroscopic images of rat brain base; B: SAH grading scores; C: neurological scores; D: cerebral water content; E: Evan's blue content of rat brain. SAH: subarachnoid hemorrhage; Sch B: schisandrin B; and the same below. $^{*}P<0.01$ *vs*. sham group, $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$ *vs*. SAH group, $^{\blacktriangle}P<0.05$, $^{\blacktriangle\blacktriangle}P<0.01$ *vs*. SAH+vehicle group

Sch B Reduces SAH-Induced Neuronal Apoptosis of Rats

TUNEL staining showed that compared with the sham group, SAH caused enormous neuronal apoptosis in the brain cortex ($P<0.01$). Sch B significantly reduced the number of apoptotic cells in the brain after 24 h of SAH compared with the SAH and SAH+vehicle groups ($P<0.01$, Figure 2).

Sch B Attenuates Microglial Activation and Neutrophil Infiltration of Rats

Immunofluorescent staining demonstrated that compared with the sham group, the expressions of Iba-1 and MPO were increased in rat brains 24 h after SAH ($P<0.01$), which were significantly attenuated by Sch B ($P<0.01$, Figure 3).

Sch B Increases Bcl-2 Expression and Reduces Expressions of Bax and Caspase-3 of Rats

The Bcl-2 expression in rat brains was down-regulated while the Bax and Caspase-3 expressions were increased in the SAH group after 24 h of SAH compared with the sham group ($P<0.01$), all of which were significantly reversed by Sch B ($P<0.01$, Figure 4).

Sch B Inhibits Expression of NLRP3 Inflammasome Pathway of Rats

Double-immunofluorescent staining showed that

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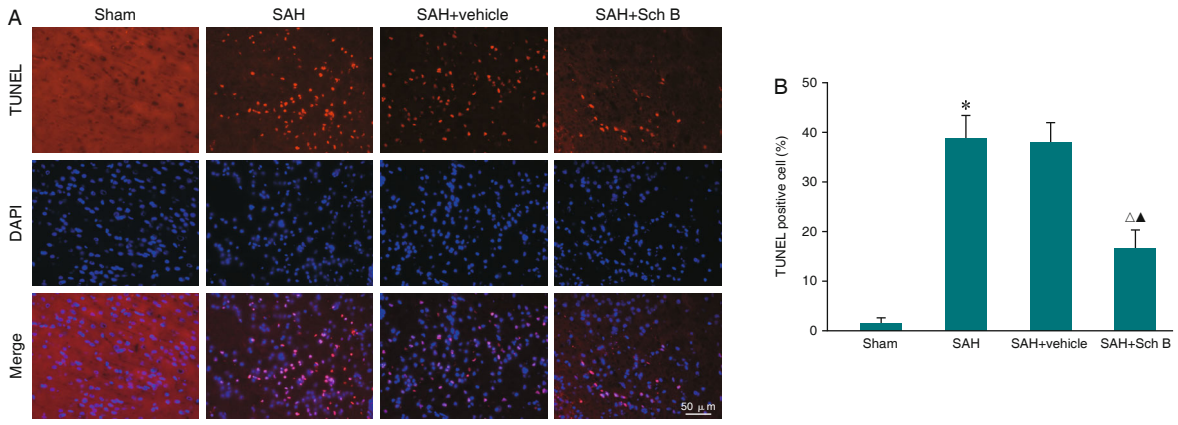

Figure 2. Effect of Sch B on SAH-Induced Neuronal Apoptosis of Rats by TUNEL Staining ($\bar{x} \pm s$, n=6)

Notes: $^{*}P$<0.01 *vs*. sham group, $^{\triangle}P$<0.01 *vs*. SAH group, $^{\blacktriangle}P$<0.01 *vs*. SAH+vehicle group

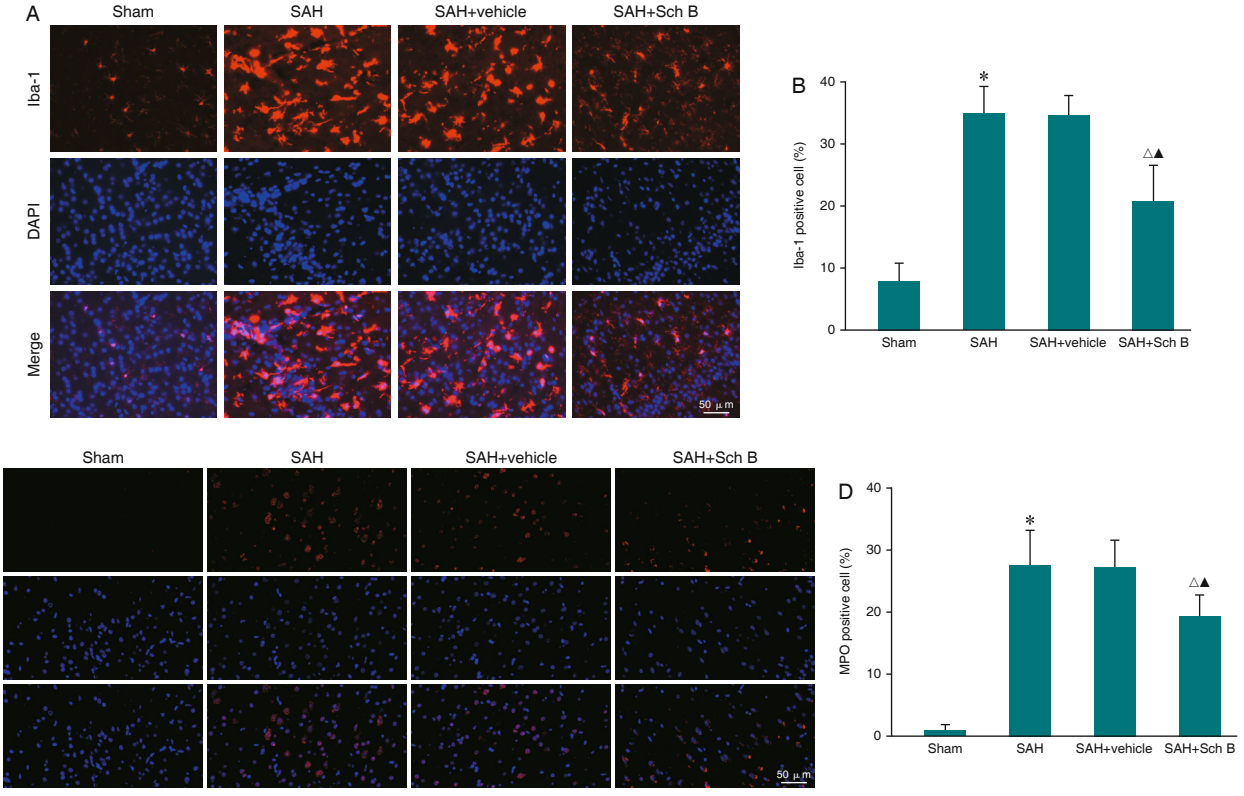

Figure 3. Effect of Sch B on SAH-Induced Microglial Activation and Neutrophil Infiltration of Rats by Immunofluorescent Staining ($\bar{x} \pm s$, n=6)

Notes: Iba-1: ionized calcium binding adapter molecule 1; MPO: myeloperoxidase; and the same below. $^{*}P$<0.01 *vs*. sham group, $^{\triangle}P$<0.01 *vs*. SAH group, $^{\blacktriangle}P$<0.01 *vs*. SAH+vehicle group

NLRP3 was localized in activated microglial cells and infiltrated neutrophils (Figure 5A). SAH caused up-regulated expressions of NLRP3, ASC, Caspase-1, IL-1 β , and IL-18 in rat brains (P<0.01), all of which were significantly inhibited by Sch B (P<0.01, Figure 5B).

DISCUSSION

Recent study has reported that Sch B provides a neuroprotective effect on ischemic brain injury in rats with focal cerebral ischemia. Sch B's neuroprotective, anti-oxidative, and anti-inflammatory properties have been demonstrated as potential mechanisms for the treatment of ischemic stroke.[19] However, it remains unknown whether Sch B can protect from EBI after SAH. In the present study, we found that Sch B reduced SAH-induced neurological dysfunction, attenuated brain edema, BBB disruption, and neuronal apoptosis after SAH, reduced SAH-induced microglial

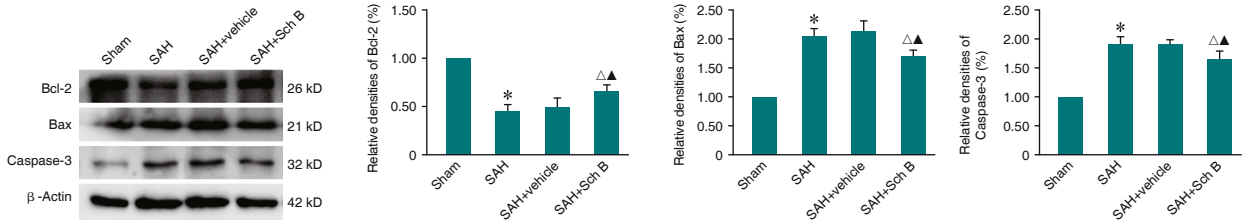

Figure 4. Effect of Sch B on Expressions of Bcl-2, Bax and Caspase-3 of Rats Detected by Western Blot ($\bar{x} \pm s$, n=6)

Notes: $^{*}P$<0.01 *vs*. sham group, $^{\triangle}P$<0.01 *vs*. SAH group, $^{\blacktriangle}P$<0.01 *vs*. SAH+vehicle group

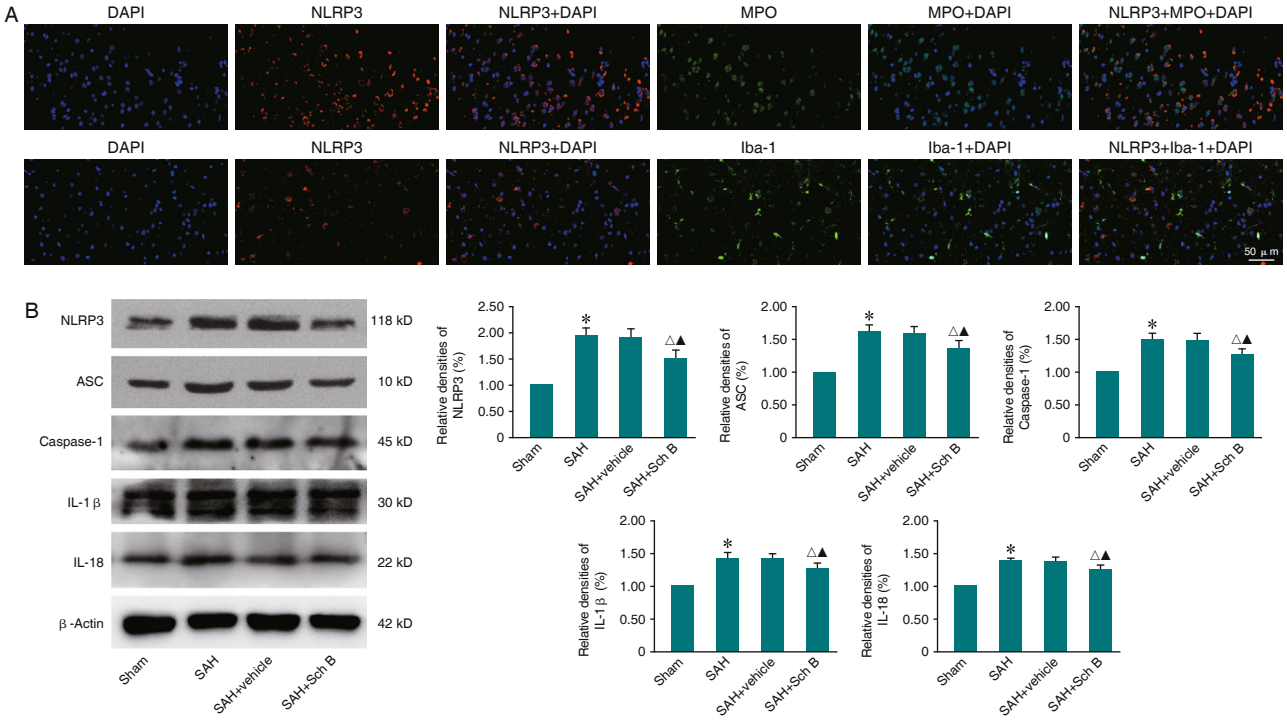

Figure 5. Effect of Sch B on Expression of NLRP3 Inflammasome Pathway in Rats by Double-Immunofluorescent Staining ($\bar{x} \pm s$, n=6)

Notes: NLRP3: nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; ASC: apoptosis-associated speck-like protein containing the caspase-1 activator domain; IL: interleukin. $^{*}P$<0.01 *vs*. sham group, $^{\triangle}P$<0.01 *vs*. SAH group, $^{\blacktriangle}P$<0.01 *vs*. SAH+vehicle group

activation and neutrophil infiltration, up-regulated Bcl-2 expression, down-regulated Bax and Caspase-3 expressions, and inhibited the expression of NLRP3 inflammasome pathway. These findings demonstrate that Sch B attenuates EBI in rats with SAH, which might be partially attributed to the attenuation of neuroinflammation and neuronal apoptosis via inhibition of the NLRP3 inflammasome pathway.

Our results showed that Sch B reduced SAH-induced neurological dysfunction and cerebral water content but did not improve SAH grading. This may be because Sch B reduced SAH-induced neurological dysfunction and cerebral water content via inhibition of neuroinflammation and neuronal apoptosis but did not reduce the amount of subarachnoid blood.

Neuroinflammation and neuronal apoptosis have been popularly considered to play key roles in the pathophysiological processes of SAH-induced EBI.[24-26] The pathological features of EBI caused by SAH include high intracranial pressure, BBB disruption, brain edema, and neuronal death.[3] In the early stage of SAH, neuroinflammation may lead to acute neuronal death and increase BBB permeability and brain edema, ultimately causing an increase of intracranial pressure and aggravation of neurological dysfunction.[2,12] SAH can trigger a series of inflammatory cascades, involving many key inflammatory mediators such as NF-κB, MPO, TNF-α, and IL-1β.[27,28] Apoptosis is referred

to programmed cell death and the launch of apoptosis depends on the activation of a series of cysteine-aspartic proteases called caspases.[29] The increase of intracranial pressure brought about by SAH, the neurotoxicity of blood decomposition components, and the acute blood vessel lead to the activation of endogenous and exogenous apoptotic pathways.[3]

Members of the Bcl-2 gene family play a central role in regulating programmed cell death by controlling the intracellular signals of pro-apoptosis and anti-apoptosis.[30] The activation of the Bax/Bcl-2 signaling pathway is key to neuronal apoptosis in EBI caused by SAH. Bax is a biomarker for pro-apoptosis while Bcl-2 is a biomarker for anti-apoptosis, both of which are key molecules for apoptosis.[31] Pro-apoptotic proteins can undergo post-translational modifications, including dephosphorylation and cleavage, causing activation and translocation to the mitochondria, where they can initiate the apoptosis.[32] Activated Caspase-9 in the mitochondria further activates the downstream caspase cascade, resulting in the induction of apoptosis.[33] We analyzed the expressions of Bcl-2, Bax, and Caspase-3 by Western blot, and found that the expression of Bcl-2 in the rat brain was decreased after SAH while was increased by Sch B treatment. The expressions of Bax and Caspase-3 were increased after SAH but were reduced by Sch B. These results demonstrate that Sch B reduces EBI and neuronal apoptosis caused by SAH through the modulation of Bcl-2/Bax and Caspase-3 expressions.

Activation of microglia and neutrophil infiltration play key roles in the inflammatory reaction after SAH.[34] Our results show that Sch B reduces SAH-induced microglial activation and neutrophil infiltration, indicating that Sch B inhibits SAH-induced inflammation. Infiltrated leukocytes and activated microglia produce and release many pro-inflammatory cytokines that aggravate brain damage after SAH.[3] NLRP3 interacts with its adaptor protein ASC which recruits pro-capase-1 and activates this to capase-1, cleaving IL-1β and IL-18 precursors, which produce corresponding mature cytokines, participating in the body's response to pathogens. The activation of NLRP3 can facilitate the secretion of IL-1β and further induce pyroptosis.[35] Pyroptosis is a type of programmed cell death, which plays a key role in maintaining homeostasis and eliminating unnecessary cells. The activation of pyrolysis can further induce the secretion of inflammatory mediators, thereby promoting the inflammatory response.[36]

NLRP3 is closely related to the pathogenesis and progression of central nervous system diseases. A recent report has shown that the NLRP3 inflammatory pathway plays an important role in cerebral ischemic injury.[8] Danger-associated molecular pattern released from cerebral ischemic injury can activate the NLRP3 inflammatory signal pathway and aggravate ischemic brain injury by inducing the production of downstream inflammatory mediators. In a murine model of middle cerebral artery occlusion, the cerebral infarction volume, brain edema, BBB destruction, and neurological dysfunction in NLRP3 knockout mice were significantly reduced compared with those of wild-type mice, indicating that the expression of NLRP3 promotes injury in cerebral ischemia.[8]

Several reports have shown that Sch B can inhibit the inflammatory response and the expression of inflammatory mediators. Lin, et al[37] showed that Sch B inhibits the LPS-induced inflammatory response in human umbilical vein endothelial cells by activating Nrf2. Mou, et al[38] demonstrated that Sch B alleviates diabetic nephropathy in mice through suppressing excessive inflammation and oxidative stress. Qin, et al[39] reported that Sch B improves renal function in IgA nephropathy rats through inhibition of NF-κB signaling. Sch B was also demonstrated to have anti-inflammatory property in brain inflammation. In addition, Sch B has been demonstrated to effectively suppress IL-1β secretion and pyroptosis by inhibiting NLRP3 inflammasome activation in *Propionibacterium* acnes-infected THP-1 cells.[40] However, it is unknown whether Sch B reduces EBI after SAH by inhibiting inflammation and the NLRP3 singling pathway. Our experimental results show that Sch B reduces the microglia activation and neutrophils infiltration after SAH, demonstrating an anti-inflammatory effect. A report has shown that NLRP3 is mainly expressed in microglia but not in other cell types.[34] Our double-immunostaining showed that NLRP3 was expressed in microglia and neutrophils. Furthermore, our study demonstrated that Sch B inhibits the expression of NLRP3 inflammasome pathway including the expression of NLRP3, ASC, Caspase-1, IL-1β, and IL-18 in rat brains after SAH, which may be the molecular mechanisms to explain

the anti-inflammatory effect of Sch B.

In summary, Sch B reduces EBI following SAH by attenuating inflammation and neuronal apoptosis through inhibiting NLRP3 inflammasome and modulating apoptotic signaling pathways (Bcl-2/Bax and cleaved caspase-3). Sch B, a Chinese medicine ingredient, may be a promising therapeutic drug for SAH in the future.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

Tu XK designed the experiments and drafted the manuscript. Shi SS helped perform the analysis with constructive discussions and revised the manuscript. Chen S and Ding YH carried out the experiments and the statistical analysis. All authors read and approved the final manuscript.

REFERENCES

1. Caner B, Hou J, Altay O, Fujii M, Zhang JH. Transition of research focus from vasospasm to early brain injury after subarachnoid hemorrhage. J Neurochem 2012;123(Suppl 2):12-21.
2. Sehba FA, Hou J, Pluta RM, Zhang JH. The importance of early brain injury after subarachnoid hemorrhage. Prog Neurobiol 2012;97:14-37.
3. Chen S, Feng H, Sherchan P, Klebe D, Zhao G, Sun X, et al. Controversies and evolving new mechanisms in subarachnoid hemorrhage. Prog Neurobiol 2014;115:64-91.
4. Shao A, Wu H, Hong Y, Tu S, Sun X, Wu Q, et al. Hydrogen-rich saline attenuated subarachnoid hemorrhage-induced early brain injury in rats by suppressing inflammatory response: possible involvement of NF-kappaB pathway and NLRP3 inflammasome. Mol Neurobiol 2016;53:3462-3476.
5. Zhang XS, Wu Q, Wu LY, Ye ZN, Jiang TW, Li W, et al. Sirtuin 1 activation protects against early brain injury after experimental subarachnoid hemorrhage in rats. Cell Death Dis 2016;7:e2416.
6. Lamkanfi M, Dixit VM. Modulation of inflammasome pathways by bacterial and viral pathogens. J Immunol 2011;187:597-602.
7. Ma Q, Chen S, Hu Q, Feng H, Zhang JH, Tang J. NLRP3 inflammasome contributes to inflammation after intracerebral hemorrhage. Ann Neurol 2014;75:209-219.
8. Yang F, Wang Z, Wei X, Han H, Meng X, Zhang Y, et al. NLRP3 deficiency ameliorates neurovascular damage in experimental ischemic stroke. J Cereb Blood Flow Metab 2014;34:660-667.
9. Liu HD, Li W, Chen ZR, Hu YC, Zhang DD, Shen W, et al. Expression of the NLRP3 inflammasome in cerebral cortex after traumatic brain injury in a rat model. Neurochem Res 2013;38:2072-2083.
10. Jiang W, Huang Y, He F, Liu J, Li M, Sun T, et al. Dopamine D1 receptor agonist A-68930 inhibits NLRP3 inflammasome activation, controls inflammation, and alleviates histopathology in a rat model of spinal cord injury. Spine 2016;41:E330-E334.
11. Yin J, Zhao F, Chojnacki JE, Fulp J, Klein WL, Zhang S, et al. NLRP3 inflammasome inhibitor ameliorates amyloid pathology in a mouse model of Alzheimer's disease. Mol Neurobiol 2018;55:1977-1987.
12. Liu H, Zhao L, Yue L, Wang B, Li X, Guo H, et al. Pterostilbene attenuates early brain injury following subarachnoid hemorrhage via inhibition of the NLRP3 inflammasome and Nox2-related oxidative stress. Mol Neurobiol 2017;54:5928-5940.
13. Luo Y, Lu J, Ruan W, Guo X, Chen S. MCC950 attenuated early brain injury by suppressing NLRP3 inflammasome after experimental SAH in rats. Brain Res Bull 2019;146:320-326.
14. Leong PK, Ko KM. Schisandrin B induces an Nrf2-mediated thioredoxin expression and suppresses the activation of inflammasome *in vitro* and *in vivo*. Biofactors 2015;41:314-323.
15. Thandavarayan RA, Giridharan VV, Arumugam S, Suzuki K, Ko KM, Krishnamurthy P, et al. Schisandrin B prevents doxorubicin induced cardiac dysfunction by modulation of DNA damage, oxidative stress and inflammation through inhibition of MAPK/p53 signaling. PLoS One 2015;10:e0119214.
16. Ran J, Ma C, Xu K, Xu L, He Y, Moqbel SAA, et al. Schisandrin B ameliorated chondrocytes inflammation and osteoarthritis via suppression of NF-kappaB and MAPK signal pathways. Drug Des Devel Ther 2018;12:1195-1204.
17. Xu J, Lu C, Liu Z, Zhang P, Guo H, Wang T. Schizandrin B protects LPS-induced sepsis via TLR4/NF-kappaB/MyD88 signaling pathway. Am J Transl Res 2018;10:1155-1163.
18. You S, Qian J, Wu G, Qian Y, Wang Z, Chen T, et al. Schizandrin B attenuates angiotensin Ⅱ induced endothelial to mesenchymal transition in vascular endothelium by suppressing NF-kappaB activation. Phytomedicine 2019;62:152955.
19. Lee TH, Jung CH, Lee DH. Neuroprotective effects of schisandrin B against transient focal cerebral ischemia in Sprague-Dawley rats. Food Chem Toxicol 2012;50:4239-4245.
20. Shi SS, Yang WZ, Chen Y, Chen JP, Tu XK. Propofol reduces inflammatory reaction and ischemic brain damage in cerebral ischemia in rats. Neurochem Res 2014;39:793-799.

21. Garcia JH, Wagner S, Liu KF, Hu XJ. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke 1995;26:627-634,635.
22. Sugawara T, Ayer R, Jadhav V, Zhang JH. A new grading system evaluating bleeding scale in filament perforation subarachnoid hemorrhage rat model. J Neurosci Methods 2008;167:327-334.
23. Xing Y, Zhang M, Wang MM, Feng YS, Dong F, Zhang F. The anti-apoptosis effect of single electroacupuncture treatment via suppressing neuronal autophagy in the acute stage of ischemic stroke without infarct alleviation. Front Cell Neurosci 2021;15:633280.
24. Wu Q, Qi L, Li H, Mao L, Yang M, Xie R, et al. Roflumilast reduces cerebral inflammation in a rat model of experimental subarachnoid hemorrhage. Inflammation 2017;40:1245-1253.
25. Zhang T, Su J, Guo B, Wang K, Li X, Liang G. Apigenin protects blood-brain barrier and ameliorates early brain injury by inhibiting TLR4-mediated inflammatory pathway in subarachnoid hemorrhage rats. Int Immunopharmacol 2015;28:79-87.
26. Mo J, Enkhjargal B, Travis ZD, Zhou K, Wu P, Zhang G, et al. AVE 0991 attenuates oxidative stress and neuronal apoptosis via Mas/PKA/CREB/UCP-2 pathway after subarachnoid hemorrhage in rats. Redox Biol 2019;20:75-86.
27. Zhang HB, Tu XK, Song SW, Liang RS, Shi SS. Baicalin reduces early brain injury after subarachnoid hemorrhage in rats. Chin J Integr Med 2020;26:510-518.
28. Xu W, Li T, Gao L, Zheng J, Yan J, Zhang J, et al. Apelin-13/APJ system attenuates early brain injury via suppression of endoplasmic reticulum stress-associated TXNIP/NLRP3 inflammasome activation and oxidative stress in a AMPK-dependent manner after subarachnoid hemorrhage in rats. J Neuroinflammation 2019;16:247.
29. D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Int 2019;43:582-592.
30. Ashkenazi A, Fairbrother WJ, Leverson JD, Souers AJ. From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. Nat Rev Drug Discov 2017;16:273-284.
31. Song Y, Zhong M, Cai FC. Oxcarbazepine causes neurocyte apoptosis and developing brain damage by triggering Bax/Bcl-2 signaling pathway mediated caspase 3 activation in neonatal rats. Eur Rev Med Pharmacol Sci 2018;22:250-261.
32. Shamas-Din A, Brahmbhatt H, Leber B, Andrews DW. BH3-only proteins: orchestrators of apoptosis. Biochim Biophys Acta 2011;1813:508-520.
33. Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B, et al. Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. Cell Prolif 2012;45:487-498.
34. Zhang X, Wu Q, Zhang Q, Lu Y, Liu J, Li W, et al. Resveratrol attenuates early brain injury after experimental subarachnoid hemorrhage via inhibition of NLRP3 inflammasome activation. Front Neurosci 2017;11:611.
35. Sutterwala FS, Haasken S, Cassel SL. Mechanism of NLRP3 inflammasome activation. Ann N Y Acad Sci 2014;1319:82-95.
36. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. Nat Rev Microbiol 2009;7:99-109.
37. Lin Q, Qin X, Shi M, Qin Z, Meng Y, Qin Z, et al. Schisandrin B inhibits LPS-induced inflammatory response in human umbilical vein endothelial cells by activating Nrf2. Int Immunopharmacol 2017;49:142-147.
38. Mou Z, Feng Z, Xu Z, Zhuang F, Zheng X, Li X, et al. Schisandrin B alleviates diabetic nephropathy through suppressing excessive inflammation and oxidative stress. Biochem Biophys Res Commun 2019;508:243-249.
39. Qin JH, Lin JR, Ding WF, Wu WH. Schisandrin B improves the renal function of IgA nephropathy rats through inhibition of the NF-kappaB signalling pathway. Inflammation 2019;42:884-894.
40. Guo M, An F, Yu H, Wei X, Hong M, Lu Y. Comparative effects of schisandrin A, B, and C on *Propionibacterium* acnes-induced, NLRP3 inflammasome activation-mediated IL-1beta secretion and pyroptosis. Biomed Pharmacother 2017;96:129-136.

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