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ORIGINAL ARTICLE

Effect of Gastrodin on Early Brain Injury and Neurological Outcome After Subarachnoid Hemorrhage in Rats

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Abstract Gastrodin is a phenolic glycoside that has been demonstrated to provide neuroprotection in preclinical models of central nervous system disease, but its effect in subarachnoid hemorrhage (SAH) remains unclear. In this study, we showed that intraperitoneal administration of gastrodin (100 mg/kg per day) significantly attenuated the SAH-induced neurological deficit, brain edema, and increased blood-brain barrier permeability in rats. Meanwhile, gastrodin treatment significantly reduced the SAH-induced elevation of glutamate concentration in the cerebrospinal fluid and the intracellular Ca^{2+} overload. Moreover, gastrodin suppressed the SAH-induced microglial activation, astrocyte activation, and neuronal apoptosis. Mechanistically, gastrodin significantly reduced the oxidative stress and inflammatory response, up-regulated the expression of nuclear factor erythroid 2–related factor 2, heme oxygenase-1, phospho-Akt and B-cell lymphoma 2, and down-regulated the expression of BCL2-associated X protein and cleaved caspase-3. Our results suggested that the administration of gastrodin provides neuroprotection against early brain injury after experimental SAH.

Keywords Subarachnoid hemorrhage · Gastrodin · Early brain injury · Neuroprotection · Neuronal apoptosis

Introduction

Subarachnoid hemorrhage (SAH) is a subtype of stroke that has a high morbidity and mortality due to ruptured aneurysms and cerebrovascular emergencies. Early brain injury, which occurs within the first 72 h after SAH, plays a primary role in determining the prognosis. After early brain injury occurs, the intracranial pressure, cerebral blood flow, and cerebral perfusion pressure decrease, cellular Ca^{2+} homeostasis is rapidly impaired, and microglia and astrocytes are activated, all leading to the initiation of oxidative stress and cerebral inflammation, which result in increased blood-brain barrier (BBB) permeability, the development of brain edema, and neuronal death [1, 2].

Gastrodin (4-hydroxybenzyl alcohol-4-O-β-D-glucopyranoside), a phenolic glycoside from the rhizome of the plant *Gastrodia elata*, has a wide variety of effects including anti-oxidation, anti-inflammation, modulating neurotransmitters, suppressing microglial activation, and regulating mitochondrial cascade activity in preclinical models of CNS disorders, such as epilepsy, Parkinson's disease, Alzheimer's disease, cerebral ischemia/reperfusion, cognitive impairment, and affective disorders [3]. Previous studies have demonstrated that, after systemic administration, gastrodin can pass through the blood-brain barrier and is quickly distributed throughout the brain [4, 5]. Gastrodin has been reported to improve neurological deficits in ischemic brain damage [6–8], reduce malondialdehyde (MDA) content and the pro-inflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α

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[8], and suppress the expression of Bax and cleaved caspase-3 [8, 9]. However, it is not yet known whether gastrodin has neuroprotective effects against SAH-induced early brain injury.

This study was designed to study the effects of gastrodin on intracellular Ca^{2+} overload, neuro-inflammation, oxidative stress, and neuronal apoptosis in early brain injury after SAH in a rat model.

Materials and Methods

Experimental Animals

Male Sprague-Dawley rats (12 weeks old, weighing 330–360 g) were purchased from the Animal Center of Shandong University (Jinan, China) and housed under a reversed 12-h/12-h light/dark cycle at constant temperature and stable humidity. All procedures were approved by the Committee on Animal Care of the Second People's Hospital of Liaocheng and were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Rat SAH Model and SAH Grading

The rat SAH model was produced by endovascular perforation as described previously [10]. Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). The femoral artery was catheterized with a PE-50 catheter to allow measurement of blood pH, $PaCO_2$, PaO_2, and mean arterial blood pressure. A sharpened 3-0 monofilament nylon suture was introduced into the right external carotid artery and advanced into the internal carotid artery until resistance was felt ($\sim$18 mm), and then pushed 3 mm further to penetrate the internal carotid artery near the bifurcation with the middle cerebral artery. The suture was withdrawn after 10 s to allow reperfusion of the internal carotid artery and producing SAH.

SAH was graded according to a published grading scale [10]. Briefly, photographs of the brain were taken 72 h after SAH. The score was based on the amount of blood in six segments of the basal cistern. No subarachnoid blood, grade 0; minimal subarachnoid blood, grade 1; moderate blood clot, grade 2; blood clot covering arteries, grade 3. The total score (0–18) was the sum of six segments.

Drug Administration

Saline or gastrodin (100 mg/kg per day, purity >99.2%, Kunming Pharmaceutical Corp., China) was intraperitoneally injected at 2, 24, and 48 h after SAH. This dose was equivalent to that used for the treatment of experimental cerebral ischemia [6, 8].

Mortality and Neurological Scores

Mortality was calculated at 72 h after SAH. Neurological function was evaluated at 72 h after SAH on the basis of the modified Garcia score system as previously described [10]. In brief, the system consisted of six tests (tests for spontaneous activity, forepaw outstretching, and symmetrical movement of 4 limbs, scored as 0–3; and tests for proprioception, climbing, and response to whisker stimulation, scored as 1–3).

Brain Water Content and Evans Blue Extravasation

The brain was quickly separated into left and right hemispheres at 72 h after SAH. The percentage water content in each hemisphere was calculated as (wet weight − dry weight)/wet weight × 100%.

The permeability of the BBB was evaluated using extravasation of Evans blue dye (E2129, Sigma-Aldrich; Exλ = 620 nm, Emλ = 680 nm) as previously described [11]. In brief, 30 min after femoral intravenous injection of 2% Evans blue dye, the rat was perfused transcardially with saline to remove intravascular dye. The brain was quickly separated into left and right hemispheres, homogenized in saline, and then centrifuged. Then 1 mL of supernatant was mixed with 1 mL trichloroacetic acid with ethanol (1:3), incubated for 6 h, and the supernatant of sample and standards were measured using a microplate reader. The Evans blue content was calculated from the standard curve, and presented as μg/g.

Biochemical Analysis

At 72 h after SAH, the cerebrospinal fluid (CSF) and brain were collected. The glutamate in CSF was measured with a Glutamate Assay Kit (MAK004, Sigma-Aldrich, St. Louis, MO). The intracellular Ca^{2+} level in basal cortex was measured with a tissue Ca^{2+} concentration quantitative determination kit (GMS10157.2, Genmed Scientifics, Shanghai, China). In addition, the basal cortex was homogenized in saline, followed by centrifugation. The total protein concentration was determined by a BCA protein assay kit (P0010S). Levels of IL-1β, TNF-α, MDA, superoxide dismutase (SOD), 3-nitrotyrosine (3-NT), and 8-hydroxy-2-desoxyguanosine (8-OHDG) were determined with a rat IL-1β ELISA kit (P1303, Beyotime), rat TNF-α ELISA kit (PT516, Beyotime), lipid peroxidation MDA assay kit (S0131, Beyotime), total SOD kit (S0101, Beyotime), rat 3-NT ELISA kit (E02A0670, BlueGene), and rat 8-OHDG ELISA kit (E02H0007, BlueGene),

respectively, according to the instructions of the manufacturers.

Western Blot

The basal cortex was harvested for western blotting at 72 h post-SAH, following the standard protocol. In brief, the basal cortex was lysed in RIPA buffer and mixed with a protease inhibitor cocktail (P8340, Sigma-Aldrich), followed by centrifugation. A total of 30 μg tissue lysate (as quantified by the BCA protein assay kit) was resolved by SDS-PAGE electrophoresis and transferred to nitrocellulose membranes. After blocking with 5% nonfat milk for 2 h, the membranes were incubated with primary antibodies [anti-CD68 (ED-1, 1:1000, ab31630), anti-GFAP (1:1000, ab7260), anti-Nrf2 (1:1000, ab62352), anti-HO-1 (1:1000, ab13248), anti-IL-1β (1:1000, ab2105), anti-TNF-α (1:1000, ab6671), Abcam (Cambridge, UK); anti-Akt (1:1000, #9272), anti-p-Akt (1:1000, #4060), anti-Bcl-2 (1:1000, #3498), anti-Bax (1:1000, #2772), anti-cleaved caspase-3 (1:1000, #9661), and anti-GAPDH (1:1000, #5174), Cell Signaling Technology, Beverly, MA] for 14 h at 4 °C. Then the membranes were washed with Western Wash Buffer (P0023C6, Beyotime) and incubated with HRP-linked anti-mouse or anti-rabbit IgG for 2 h. Next, the membranes were washed with Western Wash Buffer and measured using an enhanced chemiluminescence substrate kit (#15159, Thermo Scientific, Waltham, MA) on a ChemiDoc^{TM} MP Imaging System (Bio-Rad, Richmond, CA). The relative density of protein bands was quantified with ImageJ software.

Immunofluorescent Staining

At 72 h after SAH, rats from each group were perfused with 4% paraformaldehyde/PBS solution under anesthesia. Then 10-μm coronal sections of the basal cortex were fixed for 30 min in 4% paraformaldehyde and washed in PBS, blocked in 5% normal donkey serum, and incubated with primary antibodies [anti-ED-1 (1:200, ab31630); anti-GFAP (1:200, ab7260); Abcam; anti-cleaved caspase-3 (1:200, #9661, Cell Signaling Technology); and NeuN (1:200, MABN140, Merck Millipore, Darmstadt, Germany)] overnight at 4 °C. Then the sections were washed with PBS and incubated with anti-rabbit IgG-FITC (F9887, Sigma) or anti-mouse IgG-TRITC (T5393, Sigma) for 2 h. For TUNEL staining, the sections were used for TdT-mediated dUTP Nick-End Labeling according to the manufacturer's instruction (QIA39, Merck, Germany). The sections were washed with PBS and coverslipped. Images were obtained from three fields in basal cortex using a fluorescence microscope. The numbers of cells positive for ED-1, GFAP, cleaved caspase-3/NeuN, and TUNEL/NeuN staining were quantified (six random images).

Statistical Analysis

All results are expressed as mean ± SEM. SPSS (version 17.0) was used to analyze the data. Comparisons among three groups were assessed by one-way analysis of variance. $P < 0.05$ was considered statistically significant.

Results

SAH Grade, Mortality, Physiological Parameters, Body Weight, and Neurological Score

There was no statistical difference in SAH grade between the SAH + vehicle and SAH + gastrodin groups (Fig. 1A). The mortality at 72 h after SAH was 25.0% (8 of 32 rats) in the SAH + vehicle group and 21.8% (7/32) in the SAH + gastrodin group. No rats died in the sham group. There was no significant difference in pH, $PaCO_2$, PaO_2, or body temperature among the sham, SAH + vehicle, and SAH + gastrodin groups (Table 1). There was a statistical increase in the mean arterial blood pressure immediately after SAH (Table 1). Compared with the sham group, the SAH + vehicle group had a lower body weight and neurological score at 72 h after SAH, while gastrodin treatment reduced the SAH-induced loss of body weight and neurological deficit (Fig. 1B, C).

Gastrodin Alleviated SAH-Induced Brain Edema and Increased BBB Permeability

Compared with the sham group, there was an increase of brain water content in both hemispheres at 72 h in the SAH + vehicle group, while gastrodin significantly reduced it (Fig. 2A). Consistently, the SAH + vehicle group showed marked extravasation of Evans blue dye into both hemispheres at 72 h after SAH, while gastrodin treatment reduced the SAH-mediated increase of Evans blue dye extravasation (Fig. 2B).

Gastrodin Reduced the Level of Glutamate and Intracellular Ca^{2+} After SAH

The glutamate concentration in CSF and the intracellular Ca^{2+} level in basal cortex were significantly higher at 72 h in the SAH + vehicle group than in the sham group. However, this increase was reversed in the SAH + gastrodin group (Fig. 3A, B).

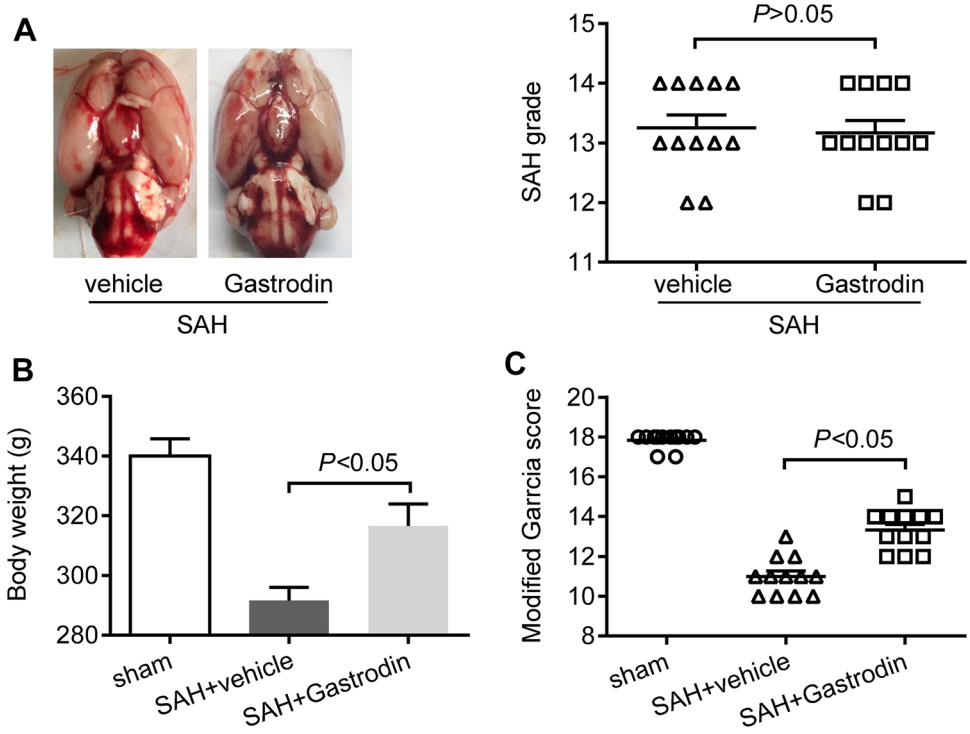

Fig. 1 Effect of gastrodin on neurological function and body weight in SAH rats. **A** Representative image of brains and SAH grades in the SAH + vehicle and SAH + gastrodin groups. **B**, **C** Body weight (**B**) and neurological score (**C**) with vehicle or gastrodin treatment 72 h after SAH (n =12/group).

Table 1 Physiological parameters.

Parameter	Sham	SAH + vehicle	SAH + gastrodin
pH	7.36 ± 0.06	7.39 ± 0.04	7.40 ± 0.05
$paCO_2$ (mmHg)	37.30 ± 2.26	36.59 ± 2.57	36.15 ± 2.70
paO_2 (mmHg)	81.58 ± 4.71	84.59 ± 5.29	83.05 ± 4.96
Body temperature	36.8 ± 0.3	37.3 ± 0.5	37.4 ± 0.4
MABP (pre-SAH)	84.50 ± 2.77	85.57 ± 2.60	86.92 ± 2.85
MABP (0 min post-SAH)	83.75 ± 2.94	106.51 ± 2.67	103.75 ± 2.80
MABP (30 min post-SAH)	82.80 ± 2.59	86.78 ± 2.93*	88.32 ± 2.72*

MABP, mean arterial blood pressure. *$P < 0.05$ *vs* 0 min post-SAH.

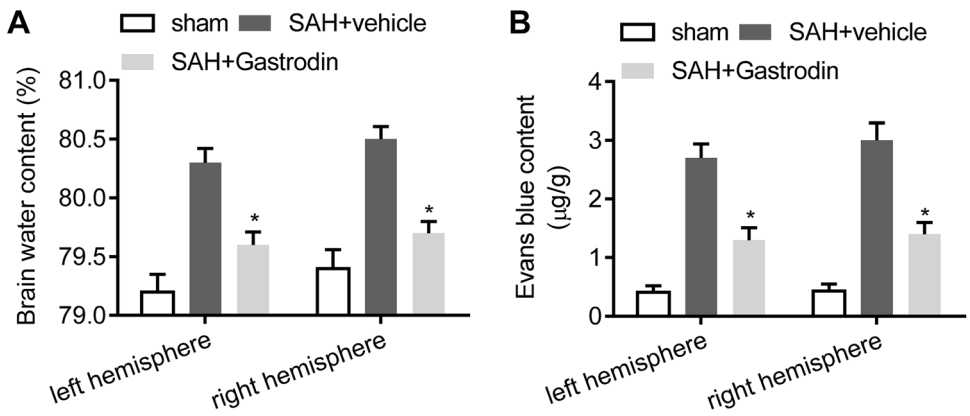

Fig. 2 Effect of gastrodin on brain water content and Evans blue extravasation in SAH rats. The water content (**A**) and Evans blue extravasation (**B**) in the left and right hemispheres were quantified in the sham, SAH + vehicle, and SAH+ gastrodin groups at 72 h after SAH. *$P < 0.05$, compared with the SAH + vehicle group, n = 6/group.

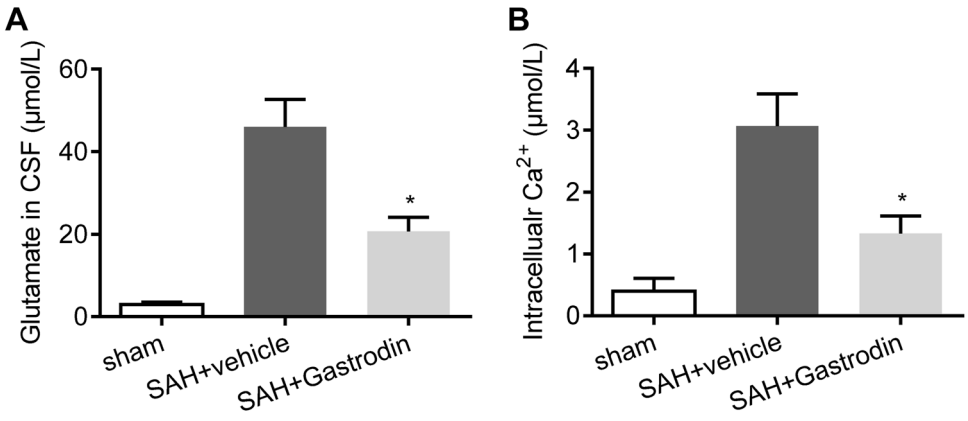

Fig. 3 Effect of gastrodin on glutamate concentration in cerebrospinal fluid (CSF) and intracellular Ca^{2+} level in SAH rats. The glutamate in CSF (**A**) and intracellular Ca^{2+} level (**B**) of basal cortex were measured in the sham, SAH + vehicle, and SAH + gastrodin groups at 72 h after SAH. $^*P < 0.05$ compared with the SAH + vehicle group, $n = 6$/group.

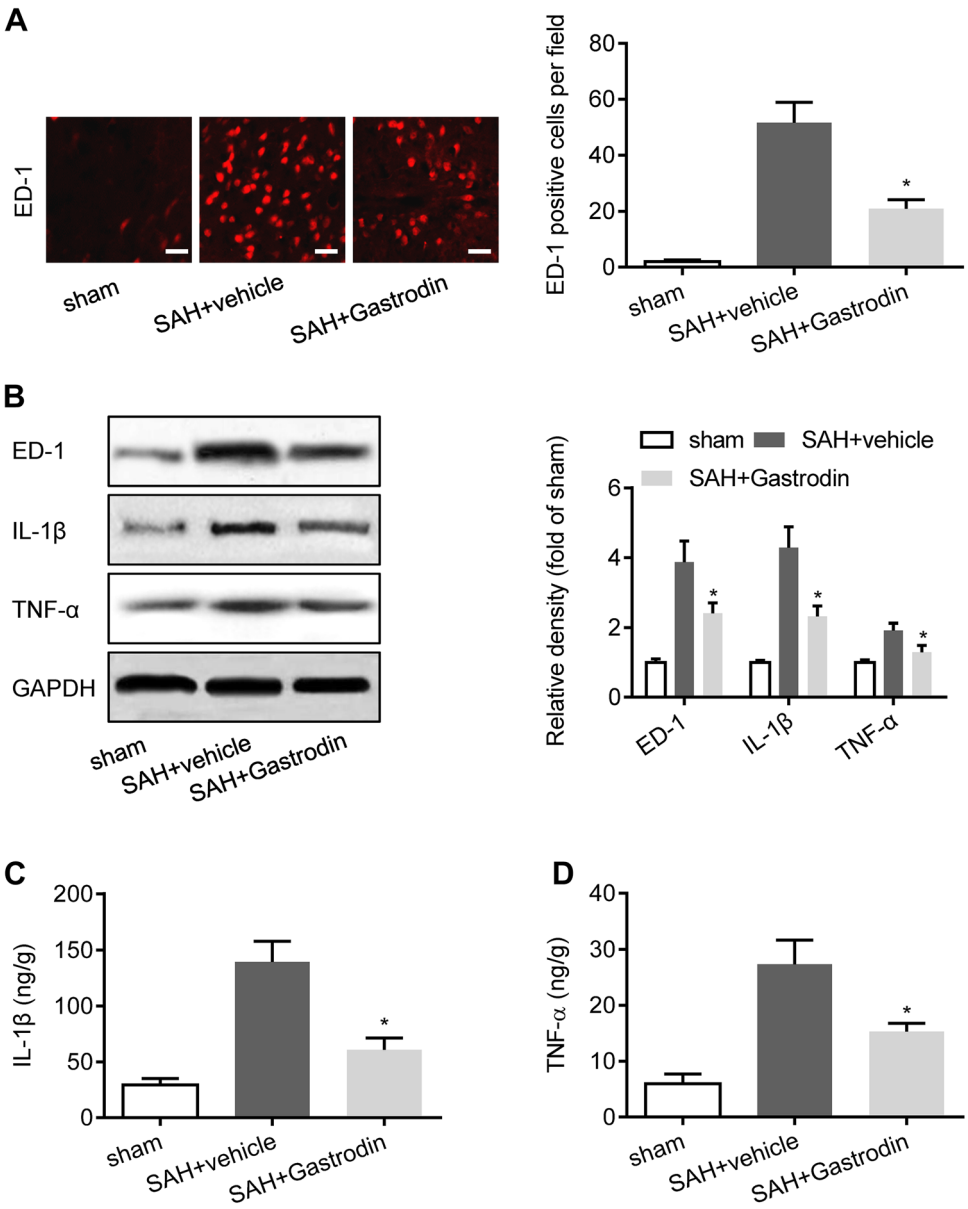

Fig. 4 Effect of gastrodin on microglial activation and levels of IL-1β and TNF-α in sham, SAH + vehicle, and SAH + gastrodin groups 72 h after SAH. **A** Representative images of ED-1 staining (left) and cell counts (right) in basal cortex. **B** Representative Western blots (left) and statistics (right) of the expression of ED-1, IL-1β, and TNF-α. **C**, **D** IL-1β (**C**), and TNF-α concentrations (**D**). $^*P < 0.05$ compared with SAH + vehicle group. Scale bars, 20 μm; $n = 6$/group.

Effects of Gastrodin on Microglial and Astrocyte Activation After SAH

SAH significantly increased the numbers of cells positive for ED-1 (anti-CD68, a marker of activated microglia/macrophages) and the protein expression of ED-1 in basal cortex compared to the sham group, while gastrodin treatment reduced the SAH-mediated increases of ED-1-positive cells and ED-1 expression at 72 h following SAH (Fig. 4A, B). Furthermore, the levels of IL-1β and TNF-α in the basal cortex were significantly higher in the SAH + vehicle group than in the sham group at 72 h. However, gastrodin treatment significantly attenuated the SAH-mediated increases of IL-1β and TNF-α (Fig. 4B–D). Next, to investigate the potential role of gastrodin on SAH-induced astrocyte activation, the level of GFAP (glial fibrillary acidic protein, a marker of activated astrocytes) was examined at 72 h after SAH. The GFAP expression following SAH as determined by immunostaining and Western blot in basal cortex was higher than in sham-operated rats. However, gastrodin treatment significantly reduced the SAH-elevated GFAP expression (Fig. 5A, B).

Effects of Gastrodin on Oxidative Stress After SAH

To assess the effect of gastrodin on SAH-induced oxidative stress injury, the oxidative stress markers of lipid, protein, and DNA damage (MDA, 3-NT, and 8-OHDG, respectively) were evaluated at 72 h after SAH. We found significantly higher MDA, 3-NT, and 8-OHDG (Fig. 6A–C) and lower total SOD (Fig. 6D) in the basal cortex in the SAH + vehicle group than in the sham group. However, gastrodin treatment induced a significant decrease in MDA, 3-NT, and 8-OHDG and a significant increase in the total SOD level compared with the SAH + vehicle group (Fig. 6A–D). To support these results, we evaluated the effect of gastrodin on the activation of the Nrf2/HO-1 antioxidant pathway. Compared with the SAH + vehicle group, gastrodin treatment significantly upregulated the Nrf2/HO-1 signaling cascade in basal cortex 72 h after SAH (Fig. 6E).

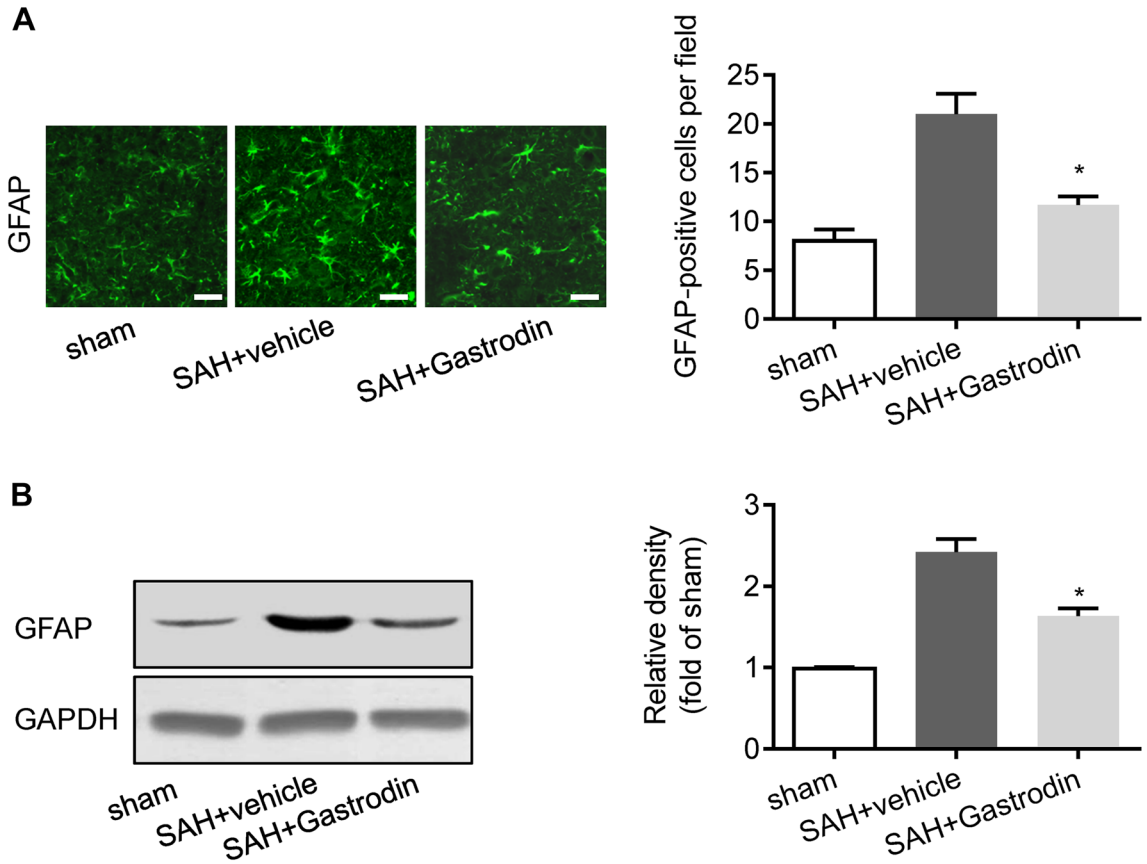

Fig. 5 Effect of gastrodin on astrocyte activation in sham, SAH + vehicle, and SAH + gastrodin groups 72 h after SAH. **A** Representative images of GFAP staining in the basal cortex (left) and quantification (right). **B** Representative Western blots (left) and analysis of the expression of GFAP (right). *$P < 0.05$ compared with SAH + vehicle group; scale bars, 20 μm; $n = 6$/group.

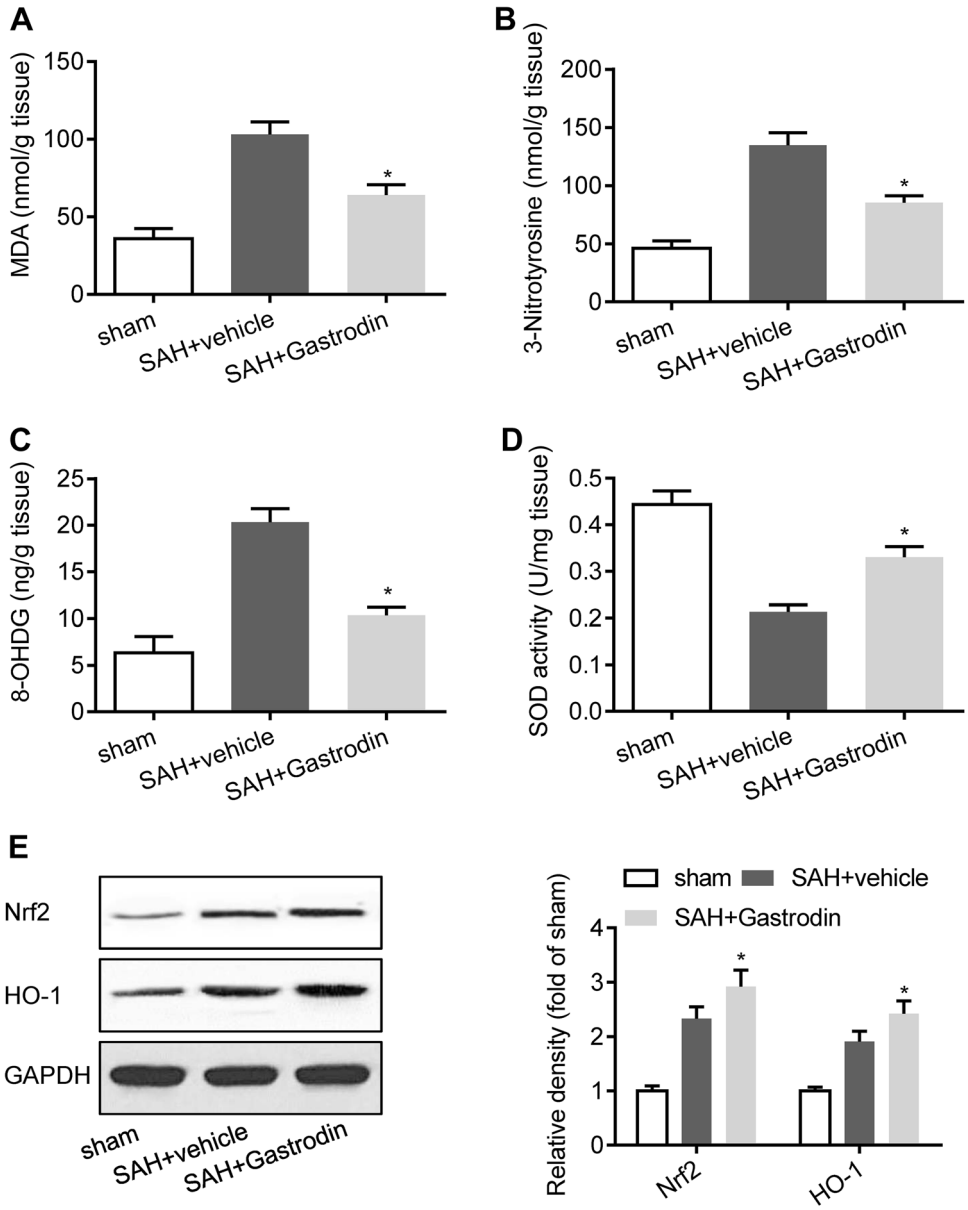

Fig. 6 Effect of gastrodin on oxidative stress in SAH rats. MDA concentration (**A**), 3-nitrotyrosine content (**B**), 8-OHDG content (**C**), SOD activity (**D**), and Western blots and statistics (**E**) of the expression of Nrf2 and HO-1 in sham, SAH + vehicle, and SAH + gastrodin groups 72 h after SAH. $*P < 0.05$, compared with the SAH + vehicle group, $n = 6$/group.

Effect of Gastrodin on Neuronal Apoptosis After SAH

The cleaved caspase-3/NeuN and TUNEL/NeuN staining directly reflect the occurrence of neural cell apoptosis. In this study, we found more apoptotic neurons in the basal cortex after SAH than in the sham group, while gastrodin treatment significantly reduced this apoptosis (Fig. 7A, B). We further focused on the apoptotic signaling pathways to understand the possible mechanisms of neuronal apoptosis after gastrodin treatment. Compared with the SAH group, gastrodin treatment significantly increased the p-Akt and Bcl-2 levels, and decreased the Bax and cleaved caspase-3 levels (Fig. 7C, D).

Discussion

In the present study, we showed that intraperitoneal administration of gastrodin attenuated the neurological deficit and brain edema, and the increased BBB permeability and levels of glutamate and intracellular Ca^{2+}, microglial and astrocyte activation, oxidative stress, and neuronal apoptosis at 72 h in a rat model of SAH. These results showed for the first time that gastrodin is neuroprotective during early brain injury after SAH through its anti-inflammatory, anti-oxidative, and anti-apoptotic activity.

Early brain injury following SAH is often associated with BBB disruption and brain edema, which is an independent risk factor for neurological deficits and a poor

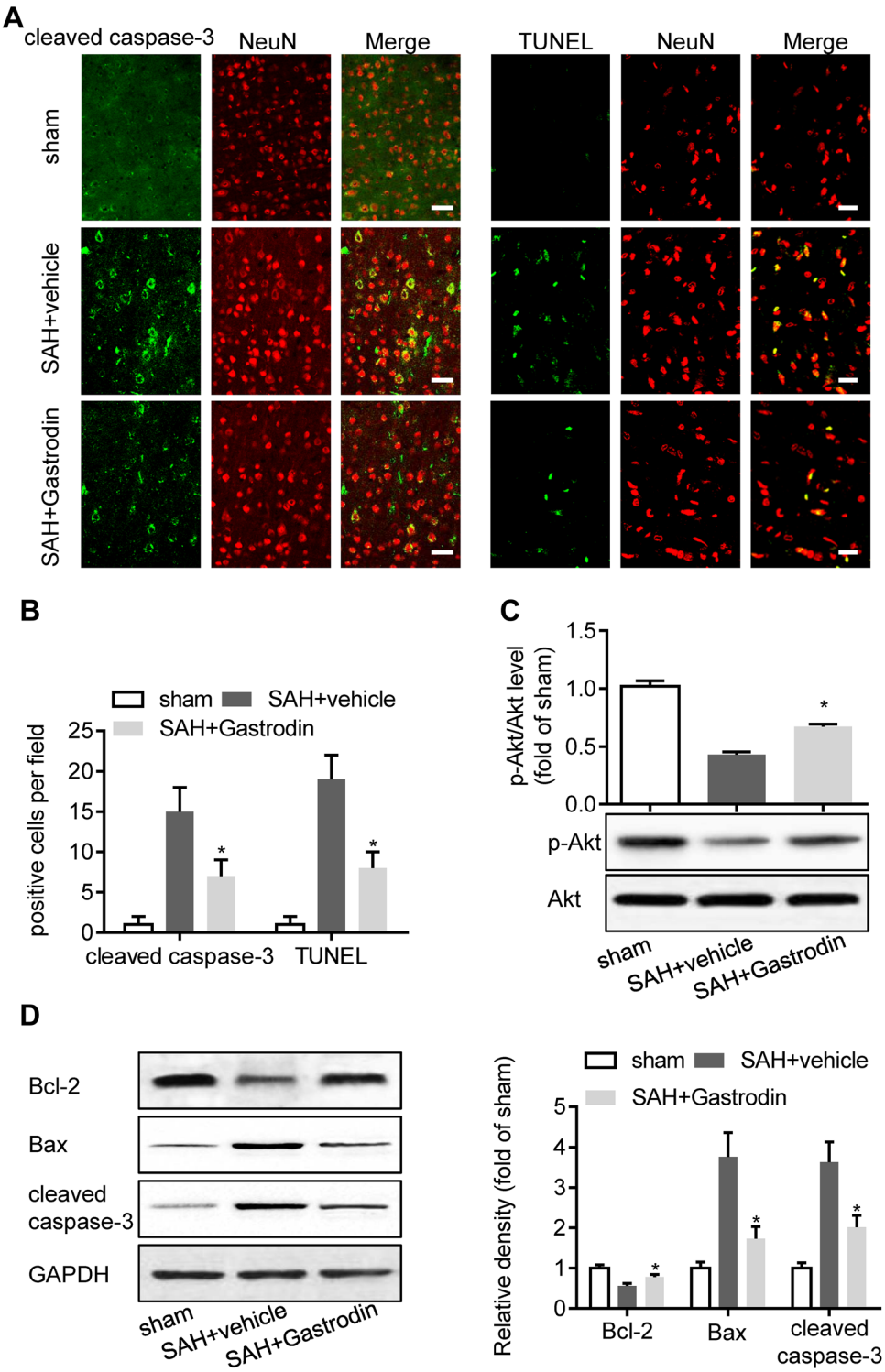

Fig. 7 Effect of gastrodin on neuronal apoptosis in SAH rats. Representative images of cleaved caspase-3/NeuN and TUNEL/NeuN staining in the basal cortex (**A**), positive cells per field (**B**), Western blots and statistics of the expression of p-Akt (**C**), and Bcl-2, Bax, and cleaved caspase-3 (**D**) in sham, SAH + vehicle, and SAH + gastrodin groups 72 h after SAH. *$P < 0.05$ compared with the SAH + vehicle group, n = 6/group. Scale bars, 20 μm.

outcome [12]. Our results showed that gastrodin treatment attenuated the neurological deficit as assessed by a modified Garcia score system; this finding is consistent with previous reports that gastrodin improves neurological function in a rat MCAO model of ischemic stroke [6, 9, 13]. In our study, gastrodin treatment attenuated the SAH-induced brain edema as determined by the wet/dry weight method, and reduced the SAH-mediated increase of BBB permeability as measured by Evans blue extravasation, a reliable means of assessing BBB permeability in animal models [14]. Glutamate is a major excitatory transmitter that is elevated in the CSF after SAH

[15–17], and the excessive glutamate induces excitotoxicity and subsequently causes the rise of intracellular Ca^{2+}, which plays an important role in neuronal death [18]. Our results showed that gastrodin treatment significantly reduced the SAH-induced increase of glutamate and intracellular Ca^{2+} level; this result is consistent with previous studies showing that gastrodin attenuates the glutamate concentration and Ca^{2+} overload in an experimental MCAO model of ischemic stroke [6, 7, 13]. In brain, glutamate is not acquired from the circulation and not synthesized in neurons [19], but is synthesized and released by activated microglia and astrocytes [1, 20]. In the early stage following SAH, activated microglia and astrocytes have beneficial effects. However, excessive activation of microglia and astrocytes aggravates SAH-induced brain injury by secreting inflammatory factors [1, 21, 22], yet inhibition of microglial and astrocyte activation attenuates brain injury after SAH. As anticipated, our results showed that gastrodin treatment significantly attenuated the SAH-induced microglial and astrocyte activation, and reduced the pro-inflammatory cytokines IL-1β and TNF-α, suggesting that gastrodin acts against neuro-inflammation *via* blocking microglial and astrocyte activation. It is possible that gastrodin reduces the SAH-elevated glutamate concentration and intracellular Ca^{2+} level by inhibiting microglial and astrocyte activation. Further study is warranted to explore the possible mechanism of action of gastrodin on glutamate-mediated excitotoxicity after SAH.

Mounting reports show that SAH induces the early generation of reactive oxygen species and oxidative stress. Lipid peroxidation, protein breakdown, and DNA damage are found in many types of cell damage. Our results showed that gastrodin treatment significantly attenuated the SAH-induced MDA, 3-NT, and 8-OHDG elevation, and restored the SAH-induced decrease of SOD, an essential anti-oxidant enzyme. This finding is consistent with previous reports that gastrodin significantly reduces oxidative stress in a mouse MCAO model of ischemic stroke [8]. Moreover, the up-regulation of Nrf2 and HO-1 expression in SAH rats after gastrodin treatment indicated involvement of the Nrf2/HO-1 signaling pathway in the anti-oxidant activity of gastrodin as reported in other studies [23, 24]. Apoptosis plays an essential role in SAH pathology, and neuronal apoptosis occurs following SAH [25]. In the endovascular perforation SAH model, apoptosis occurs in most regions of brain, especially in the basal cortex, which is exposed to bloody CSF [26]. Our results showed that gastrodin significantly reduced the number of neurons positive for cleaved caspase-3/NeuN in basal cortex that was elevated by SAH, preserved expression of the anti-apoptotic protein Bcl-2, and suppressed the expression of pro-apoptotic Bax and cleaved caspase-3. In addition, Akt phosphorylation was increased by gastrodin, suggesting that the activation of Akt plays an essential role in the pharmacological action of gastrodin. Even with the limitation of this study, our findings showed that gastrodin is protective against SAH-induced early brain injury by preventing microglial and astrocyte activation, oxidative stress, and neuronal apoptosis.

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Conflict of interest The authors declare that there are no conflicts of interest.

References

1. 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.
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. Liu Y, Gao J, Peng M, Meng H, Ma H, Cai P, *et al.* A review on central nervous system effects of gastrodin. Front Pharmacol 2018, 9: 24.
4. Lin LC, Chen YF, Tsai TR, Tsai TH. Analysis of brain distribution and biliary excretion of a nutrient supplement, gastrodin, in rat. Anal Chim Acta 2007, 590: 173–179.
5. Lin LC, Chen YF, Lee WC, Wu YT, Tsai TH. Pharmacokinetics of gastrodin and its metabolite p-hydroxybenzyl alcohol in rat blood, brain and bile by microdialysis coupled to LC-MS/MS. J Pharm Biomed Anal 2008, 48: 909–917.
6. Zeng X, Zhang S, Zhang L, Zhang K, Zheng X. A study of the neuroprotective effect of the phenolic glucoside gastrodin during cerebral ischemia *in vivo* and *in vitro*. Planta Med 2006, 72: 1359–1365.
7. Bie X, Chen Y, Han J, Dai H, Wan H, Zhao T. Effects of gastrodin on amino acids after cerebral ischemia-reperfusion injury in rat striatum. Asia Pac J Clin Nutr 2007, 16 Suppl 1: 305–308.
8. Peng Z, Wang S, Chen G, Cai M, Liu R, Deng J, *et al.* Gastrodin alleviates cerebral ischemic damage in mice by improving anti-oxidant and anti-inflammation activities and inhibiting apoptosis pathway. Neurochem Res 2015, 40: 661–673.
9. Liu B, Li F, Shi J, Yang D, Deng Y, Gong Q. Gastrodin ameliorates subacute phase cerebral ischemiareperfusion injury by inhibiting inflammation and apoptosis in rats. Mol Med Rep 2016, 14: 4144–4152.
10. 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.
11. Zhang ZY, Jiang M, Fang J, Yang MF, Zhang S, Yin YX, *et al.* Enhanced therapeutic potential of nano-curcumin against subarachnoid hemorrhage-induced blood-brain barrier disruption through inhibition of inflammatory response and oxidative stress. Mol Neurobiol 2017, 54: 1–14.
12. Claassen J, Carhuapoma JR, Kreiter KT, Du EY, Connolly ES, Mayer SA. Global cerebral edema after subarachnoid hemorrhage: frequency, predictors, and impact on outcome. Stroke 2002, 33: 1225–1232.

13. Zeng X, Zhang Y, Zhang S, Zheng X. A microdialysis study of effects of gastrodin on neurochemical changes in the ischemic/reperfused rat cerebral hippocampus. Biol Pharm Bull 2007, 30: 801–804.
14. Manaenko A, Chen H, Kammer J, Zhang JH, Tang J. Comparison Evans Blue injection routes: Intravenous *versus* intraperitoneal, for measurement of blood-brain barrier in a mice hemorrhage model. J Neurosci Methods 2011, 195: 206–210.
15. Zhang Z, Liu J, Fan C, Mao L, Xie R, Wang S, *et al.* The GluN1/GluN2B NMDA receptor and metabotropic glutamate receptor 1 negative allosteric modulator has enhanced neuroprotection in a rat subarachnoid hemorrhage model. Exp Neurol 2018, 301: 13–25.
16. Wu CT, Wen LL, Wong CS, Tsai SY, Chan SM, Yeh CC, *et al.* Temporal changes in glutamate, glutamate transporters, basilar arteries wall thickness, and neuronal variability in an experimental rat model of subarachnoid hemorrhage. Anesth Analg 2011, 112: 666–673.
17. Boyko M, Melamed I, Gruenbaum BF, Gruenbaum SE, Ohayon S, Leibowitz A, *et al.* The effect of blood glutamate scavengers oxaloacetate and pyruvate on neurological outcome in a rat model of subarachnoid hemorrhage. Neurotherapeutics 2012, 9: 649–657.
18. Kostandy BB. The role of glutamate in neuronal ischemic injury: the role of spark in fire. Neurol Sci 2012, 33: 223–237.
19. Hertz L, Peng L, Dienel GA. Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. J Cereb Blood Flow Metab 2007, 27: 219–249.
20. Takeuchi H, Jin S, Wang J, Zhang G, Kawanokuchi J, Kuno R, *et al.* Tumor necrosis factor-alpha induces neurotoxicity *via* glutamate release from hemichannels of activated microglia in an autocrine manner. J Biol Chem 2006, 281: 21362–21368.
21. Murakami K, Koide M, Dumont TM, Russell SR, Tranmer BI, Wellman GC. Subarachnoid hemorrhage induces gliosis and increased expression of the pro-inflammatory cytokine high mobility group box 1 protein. Transl Stroke Res 2011, 2: 72–79.
22. Hanafy KA. The role of microglia and the TLR4 pathway in neuronal apoptosis and vasospasm after subarachnoid hemorrhage. J Neuroinflammation 2013, 10: 83.
23. Wang XL, Xing GH, Hong B, Li XM, Zou Y, Zhang XJ, *et al.* Gastrodin prevents motor deficits and oxidative stress in the MPTP mouse model of Parkinson's disease: involvement of ERK1/2-Nrf2 signaling pathway. Life Sci 2014, 114: 77–85.
24. Zhao X, Zou Y, Xu H, Fan L, Guo H, Li X, *et al.* Gastrodin protect primary cultured rat hippocampal neurons against amyloid-beta peptide-induced neurotoxicity *via* ERK1/2-Nrf2 pathway. Brain Res 2012, 1482: 13–21.
25. Hasegawa Y, Suzuki H, Sozen T, Altay O, Zhang JH. Apoptotic mechanisms for neuronal cells in early brain injury after subarachnoid hemorrhage. Acta Neurochir Suppl 2011, 110: 43–48.
26. Park S, Yamaguchi M, Zhou C, Calvert JW, Tang J, Zhang JH. Neurovascular protection reduces early brain injury after subarachnoid hemorrhage. Stroke 2004, 35: 2412–2417.