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Original Article

Semaglutide alleviates early brain injury following subarachnoid hemorrhage by suppressing ferroptosis and neuroinflammation via SIRT1 pathway

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Abstract: Objectives: Subarachnoid hemorrhage (SAH) is a major cause of incapacity and death, imposing a significant economic burden globally. Additionally, SAH is the third most prevalent form of stroke. Semaglutide affects oxidative stress, inflammation, and mitochondrial biogenesis. Specifically, the potential neuroprotective effect of semaglutide in SAH and its underlying mechanism is unclear. Accordingly, the present research intended to explore the neuroprotective effect of semaglutide in SAH and its potential molecular mechanisms. Methods: We constructed a C57BL/6 mouse model of SAH. The parameters assessed were neuronal ferroptosis, neuroinflammatory cytokine levels, reactive oxygen species (ROS) levels, glutathione (GSH) and malondialdehyde (MDA) levels, brain water content, and neurological score. Results: The results showed that the activation of semaglutide significantly increased neurological scores, relieved cerebral edema, decreased the levels of inflammatory cytokine nuclear factor kappa B, interleukin (IL)-1β, IL-6, tumor necrosis factor-alpha, MDA, and ROS, and increased the levels of GSH. Suppression of SIRT1 reversed these effects, indicating that semaglutide activated SIRT1 to reduce neuroinflammation, ferroptosis, and neuronal cell death after SAH. Thus, the activation of the Nrf2/HO-1 signaling pathway contributes to the neuroprotective properties of semaglutide. Conclusions: Semaglutide can improve murine neurological outcomes and reduce neuronal damage against neuroinflammation and ferroptosis.

Keywords: Ferroptosis, neuroinflammation, early brain injury, SIRT1, SAH

Introduction

Subarachnoid hemorrhage (SAH) is a prevalent type of hemorrhagic stroke and a major contributor to disability, thus it remains a significant public health concern. SAH is associated with significant financial burden and mortality worldwide [1]. Specifically, SAH exhibits a remarkably increased prevalence in Asia and Iceland, with Japan experiencing a predominance of cases resulting from intracranial aneurysms (22.7 cases per 100,000 individuals), which contributes to a high disability and death rate [2]. Many individuals who survive develop permanent cognitive decline, including sadness, emotional disorders, hearing impairment, and diminished quality of life, due to the effects of surgery [3]. Thus, it is imperative to explore novel and effective pharmacological treatments for SAH and gain a deeper understanding of the pathophysiological mechanisms underlying the disease.

The pathologic mechanisms after SAH is quite complex, including primary and secondary brain injuries, which contribute significantly to neuronal death, and neurological impairments [2, 4, 5]. Efforts to prevent such injuries are often ineffective, and reversing the damage is an impossible task. In SAH, aneurysm rupture is linked to a range of intricate pathophysiology and mechanisms, including neuroinflammation, oxidative stress, apoptosis, necrosis, pyroptosis, autophagy, ferroptosis, and disorders in cell electrophysiology [2]. However, the pathological progression can be broadly divided into two phases: early brain damage (EBI) and subsequent delayed brain damage (DBI) [2]. EBI, in

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particular, has been found to play a significant role in mediating the unfavorable outcomes of SAH [6-10]. The neuroprotective effect of inhibiting necroptosis and neuroinflammation is still controversial.

The interaction between ferroptosis and neuroinflammation in central nervous system diseases is intricate and still unclear. The activation of the Fas ligand and/or tumor necrosis factor-alpha (TNF-α) induces necroptosis, a process that is different from apoptosis and relies on caspases [11]. Recent studies have demonstrated that blocking the ferroptosis pathway can effectively inhibit neuroinflammation [12]. Moreover, in animals, the suppression of ferroptosis and neuroinflammation can relieve cerebral edema and enhance neurological impairments following cerebral ischemia/reperfusion injury [7, 10, 13]. Nevertheless, the correlation between neuroinflammation and ferroptosis in SAH has not been thoroughly investigated. Consequently, therapeutic strategies targeting the suppression of ferroptosis, neuroinflammation, and reactive oxygen species (ROS) production are of utmost importance in the clinical treatment of EBI after SAH. It is well known that sirtuin 1 (SIRT1) modulates gene transcription as well as affects oxidative stress, inflammation, and mitochondrial biogenesis. Sirtuins function as transcriptional regulators in cellular metabolism causing the deacetylation of enzymes and transcriptional regulators via nicotinamide adenine dinucleotide (NAD). Numerous studies have shown that *SIRT1* can significantly induce antioxidation, thus playing a crucial role in safeguarding the nervous system following SAH [14, 15]. Semaglutide drugs that activate the glucagon-like peptide 1 receptor (GLP-1R) are highly significant in the pharmaceutical industry. Specifically, semaglutide is a novel GLP-1R agonist with extended duration of action that alleviates inflammation and endoplasmic reticulum stress [16-18]. A study by Wang *et al*. demonstrated that semaglutide can improve cognitive function and regulate glucose metabolism in patients with Alzheimer's disease via the GLP-1R/SIRT1/GLUT4 pathway [16]. Furthermore, early studies have indicated that semaglutide can reduce blood brain barrier (BBB) permeability and brain damage following an ischemic stroke [19-21]. Nevertheless, the correlation between neuroinflammation and ferroptosis in SAH has not been thoroughly investigated. Although semaglutide's effect on neuroinflammation has been acknowledged, a comprehensive assessment of its effects on EBI after SAH is missing.

Accordingly, this study established a mouse SAH model to evaluate the effect of semaglutide on EBI as well as investigate the relationship between neuronal inflammation and ferroptosis.

Material and methods

Animal models

All animal experiments were approved by the Ethics Committee of Joint Logistics Support Unit No. 904 Hospital (YXLL-2022-058), guaranteeing compliance with the National Institutes of Health's guidelines for the treatment of laboratory animals. C57BL/6J adult mice (weight: 22-25 g; Nantong University, Nantong, China) were used in this study. The mice were provided unlimited access to food and water and were housed under a 12-h light/dark cycle.

Mouse SAH model

As previously reported, endovascular perforation was used to establish the SAH mouse model [7, 8, 10]. Briefly, all mice were anesthetized by intraperitoneally administering 50 mg/kg pentobarbital sodium. Incisions were made between the clavicles to reveal both external and internal carotid arteries. The left external carotid artery was ligated and cut to subsequently uncover a 3-mm external carotid stump. A sharp thread of single-strand nylon was inserted through the stump of the external carotid artery to pierce the point where the anterior and middle cerebral arteries divided. Upon encountering resistance, the suture was inserted 2 mm further to penetrate the division of the middle and anterior cerebral arteries. Reperfusion of the internal carotid artery resulted in SAH. Mice in the sham group did not undergo arterial perforation. All mice were euthanized by intraperitoneally administering 150 mg/kg pentobarbital sodium.

Treatment with siRNA and semaglutide

After the mice received pentobarbital sodium (50 mg/kg), they were positioned using the Narishige stereotaxic apparatus (Tokyo, Japan). To expose the bregma point, a burr hole was

created in the left hemisphere, specifically at 0.2 mm posterior, 1 mm lateral, and 2.2 mm below the horizontal plane of the bregma. A specially designed syringe (Hamilton Company, Reno, USA) was used to administer the siRNA or scramble siRNA (1 g/L) into the lateral ventricle. These injections were administered 12 and 24 hours before SAH for enhancing the suppressive effect. Subcutaneous semaglutide (SEMA, Novo Nordisk) was administered at a concentration of 60 μg/kg/day for 2 days.

Evaluation of neurobehavioral function

Brain damage severity was assessed at 48 h post-SAH induction by evaluating neurological function using a predetermined grading system [7, 8]. The parameters assessed were motor abilities, sensory functions, reflexes, and balance. The overall neurological score ranged from 0 to 18 and was calculated by summing the individual scores of the assessed parameters. For all mice, higher scores indicated better neurological functioning. An uninformed observer who was blinded to the experimental conditions documented the behavior scores of mice.

Quantification of brain water content

To evaluate the extent of brain edema, the traditional wet-dry method, as previously explained, was employed to measure the cerebral water content in mice [7, 8, 10]. At 48 h post-SAH, mice were euthanized and their brains were collected. The brain tissue was then dissected into the cerebellum, basal ganglia on the same side and opposite side, and cortices on the same side and opposite side (measured by wet weight). Subsequently, the brain specimens from each group were dehydrated at 105°C for 24 h to obtain their dry mass. The brain water content (percentage) was calculated as follows: Percentage of brain water content = (Wet weight - dry weight) × 100/wet weight.

Analysis of lipid peroxidation

MDA and GSH levels were measured using the lipid peroxidation assay kit (excitation/emission wavelengths: 532/553 nm, Ab118970; Abcam, UK) as per the manufacturer's instructions (7).

Cytokine levels in the ipsilateral cortical tissue

The ELISA kit was used to determine the levels of interleukin (IL)-1β (ab197742; Abcam), IL-6 (ab222503; Abcam), TNF-α (ab208348; Abcam), and Nuclear factor kappa B (NF-κB) (ab176663; Abcam), as per the manufacturer's instructions.

TUNEL staining

The severity of hippocampal neuronal damage was evaluated by TUNEL assay, and using a commercial kit, as per the manufacturer's instructions. TUNEL reaction solution was added to each slide, and the slides were then incubated at 37°C for 1 hour. In the next step, the slides were treated with DAPI for 5 minutes at room temperature without being exposed to light, then analyzed by fluorescence microscopy. The negative control did not contain the TUNEL reaction solution.

Western blot analysis

As described previously [7], samples of the cerebral cortex were collected, crushed, and then subjected to electrophoresis using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The separated proteins were quantified using the BCA Protein Assay Kit (Beyotime). Subsequently, the separated protein samples were placed onto immobilon nitrocellulose membranes, which were prepared by incubating them with 5% nonfat milk at ambient temperature for 1 h. They were then incubated overnight at 4°C with their respective primary antibodies: mouse monoclonal anti-SIRT1 (1:1000; Abcam, ab189494), GPX4 (1:1000; ab125066, Abcam), FSP1 (1:1000; ab197896, Abcam), Nrf2 (1:1000; A11159, Abclonal); anti-heme oxygenase 1 (HO-1) (1:1000; ab13248, Abcam), and rabbit anti-GAPDH (1:2500; ab9485, Abcam). After washing three times with TBST, the membranes were incubated at room temperature with HRP-conjugated secondary antibodies (goat anti-rabbit IgG or goat anti-mouse IgG; 1:5000) for 1.5 hours. The protein bands obtained were detected using Bio-Rad imaging equipment (Bio-Rad, Hercules, CA, USA). Quantification of the bands was performed using the ImageJ software.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Briefly, qRT-PCR was performed as described previously [7, 8]. According to the manufacturer's instructions, total RNA was extracted from cell cultures or hippocampal brain tissue sam-

Table 1. Primers used in the manuscript

Primer	Forward (5'-3')	Reverse (5'-3')
SIRT1	TGCCGGAAACAATACCTCCA	AGACACCCCAGCTCCAGTTA
Nrf2	ACAGTGCTCCTATGCGTGAA	GAGCCTCTAAGCGGCTTGAA
HO-1	TGCTAGCCTGGTGCAAGATA	GCCAACAGGAAGCTGAGAGT
GAPDH	ATGGGTGTGAACCACGAGA	CAGGGATGATGTTCTGGGCA

ples using the TRIzol reagent (Gibco; Thermo Fisher Scientific, Inc., USA). Next, in order to synthesize complementary DNA (cDNA), RNA was converted using the RevertAid First Strand cDNA Synthesis Kit (K1622; Thermo Fisher Scientific Inc., IL). Subsequently, the levels of total TLR4 and NF-κB mRNA in the samples were determined by conducting PCR using the SYBR Green Master Mix (Toyobo Co., Ltd., Japan). Internal control was performed using GAPDH. For qPCR, the thermocycling conditions included an initial step of 45°C for 2 min and 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min. To guarantee precision, every specimen was assessed thrice. The specific primers used in the present study can be seen in **Table 1**.

Detection of ROS

For ROS detection, the cells were placed in six-well plates at a density of 1×10^5 cells/mL and were treated with different concentrations of 1,5-anhydroglucitol. After 24 h of incubation, the cells were washed twice and were then suspended in a serum-free solution containing 10 μM of the fluorescent dye DCFH-DA (S0033S, Beyotime). Afterward, the cells were incubated in a dark environment at 37°C for 20 min. Flow cytometry was used to analyze the cells after being washed in phosphate-buffered saline.

SAH patients sample collection

In order to determine whether serum SIRT1 levels are associated with the clinical outcomes of SAH patients. The SAH Patients serum samples were collected at 48 hours after SAH. Hunt-Hess levels data, six months mRS scores (good outcome defined as 0-2 scores, poor outcome defined as 3-6 scores) and clinical data also were collected and analyzed. The Inclusion Criteria as: (1) Radiological clear diagnosis of SAH; (2) Hunt -Hess scale was 1 to 5; (3) Age from 18 to 80 years. The Exclusion Criteria as: (1) Patients unlikely to survive on admission; (2) Traumatic SAH; (3) History of mental illness or epilepsy; (4) Severe lung disease or multiple organ dysfunction; (5) Oral administration of drugs that may affect SIRT1 expression. The study protocol was approved by the Joint Logistics Support Unit No. 904 Hospital Clinical Research Ethics Committee (YXLL-2022-062). We obtained written informed consent from the family members of SAH patients whose competence was established by accurate orientation to time, place, and person and understanding of the recruiter's description of the trial.

Statistical analysis

All data are presented as means and standard errors of the mean. The statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA) and SPSS 14.0 software (SPSS, Chicago, IL, USA). A one-way analysis of variance was used to make comparisons between multiple groups based on Tukey's post hoc test. A statistically significant result was defined as one with a p value of 0.05.

Results

SIRT1 expression following SAH

SIRT1 expression in brain tissues after SAH was evaluated using western blot analysis and qRT-PCR. We obtained the relevant literature and accessed FerrDb V2 (http://www.zhounan.org/ferrdb/current/), which led to the discovery of a correlation between ferroptosis and SIRT1 (**Figure 1A**). As shown in **Figure 1B**, qRT-PCR revealed that SIRT1 mRNA levels decreased significantly following SAH. The findings of western blot analysis validated these results, providing further support to the previous observations (**Figure 1C**, **1D**). To date, the time point of this experiment was 48 hours after SAH.

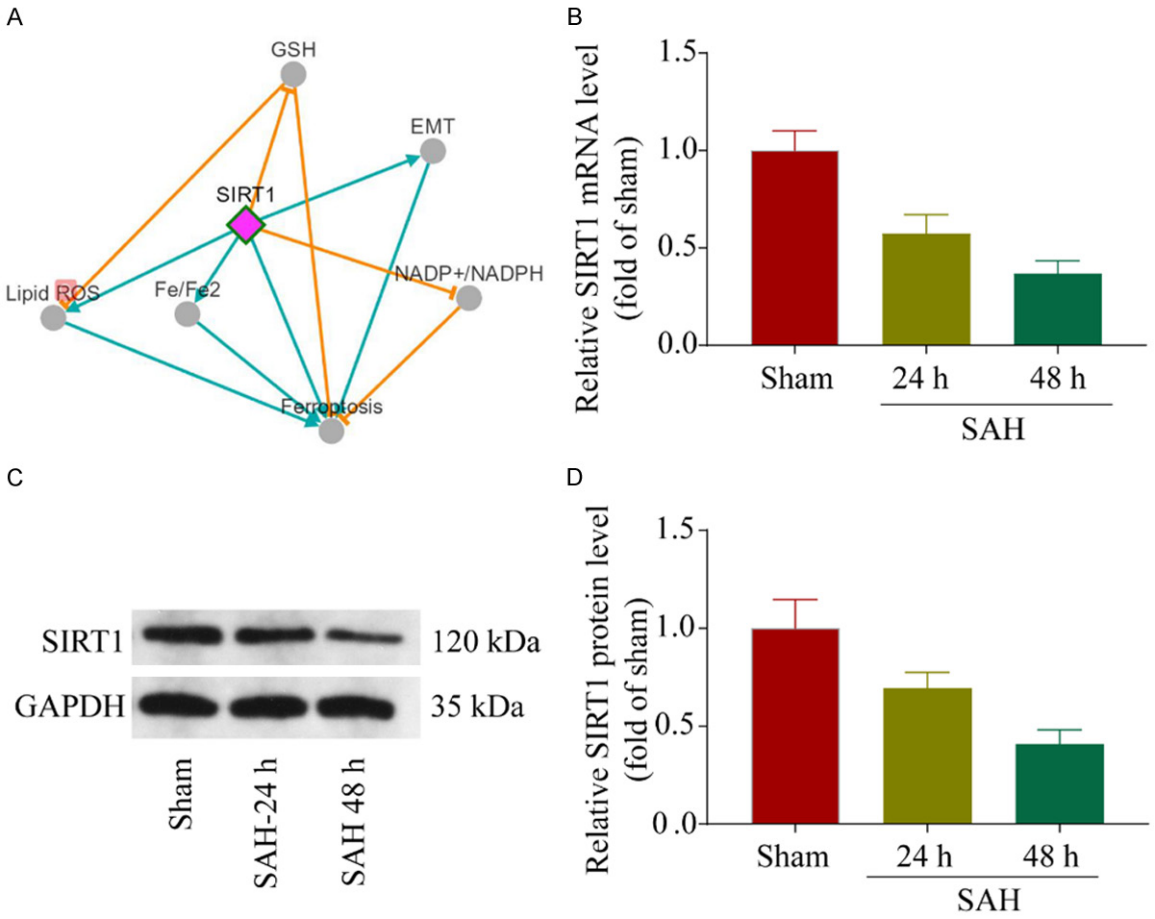

Figure 1. SIRT1 expression after SAH induction. A. The correlation between ferroptosis and SIRT1 was demonstrated by FerrDb V2 (http://www.zhounan.org/ferrdb/current/). B. The levels of SIRT1 mRNA at various time points in mouse brains were determined using real-time polymerase chain reaction. C, D. Western blot analysis was performed to assess SIRT1 expression in mouse cerebral cortex at different time intervals after SAH. GAPDH was used as the loading control.

Semaglutide alleviates neuronal damage in SAH mice

To investigate the neuroprotective effects of semaglutide following SAH, the water levels in mouse brains were assessed. The findings showed that SAH caused brain swelling, whereas semaglutide relieved this swelling (**Figure 2A**). In addition, a significant decrease in neurological scores was noted following SAH; however, semaglutide significantly enhanced neurological function (**Figure 2B**). Neuronal death is the main factor contributing to EBI after SAH. Accordingly, the extent of cell death in mice after 24 h of SAH induction was determined using TUNEL assay. The results revealed increased neuronal death in the hippocampal area, which was mitigated by the administration of semaglutide (**Figure 2C**).

Semaglutide alleviates ferroptosis

Studies have indicated that ferroptosis plays a role in the occurrence of EBI following SAH, and uncontrolled ferroptosis can worsen EBI (7). In line with this, the present study demonstrated a significant increase in MDA levels in SAH mice, which were decreased following semaglutide treatment (**Figure 3A**). In addition, semaglutide treatment initially reduced the GSH levels significantly but then increased the levels (**Figure 3B**). Furthermore, semaglutide significantly reduced ROS levels, as demonstrated by flow cytometry analysis (**Figure 3C**). Western blotting to evaluate the levels of proteins associated with ferroptosis (**Figure 3D**) revealed that semaglutide increased the expression of FSP1 and GPX4. These results highlighted that semaglutide could alleviate

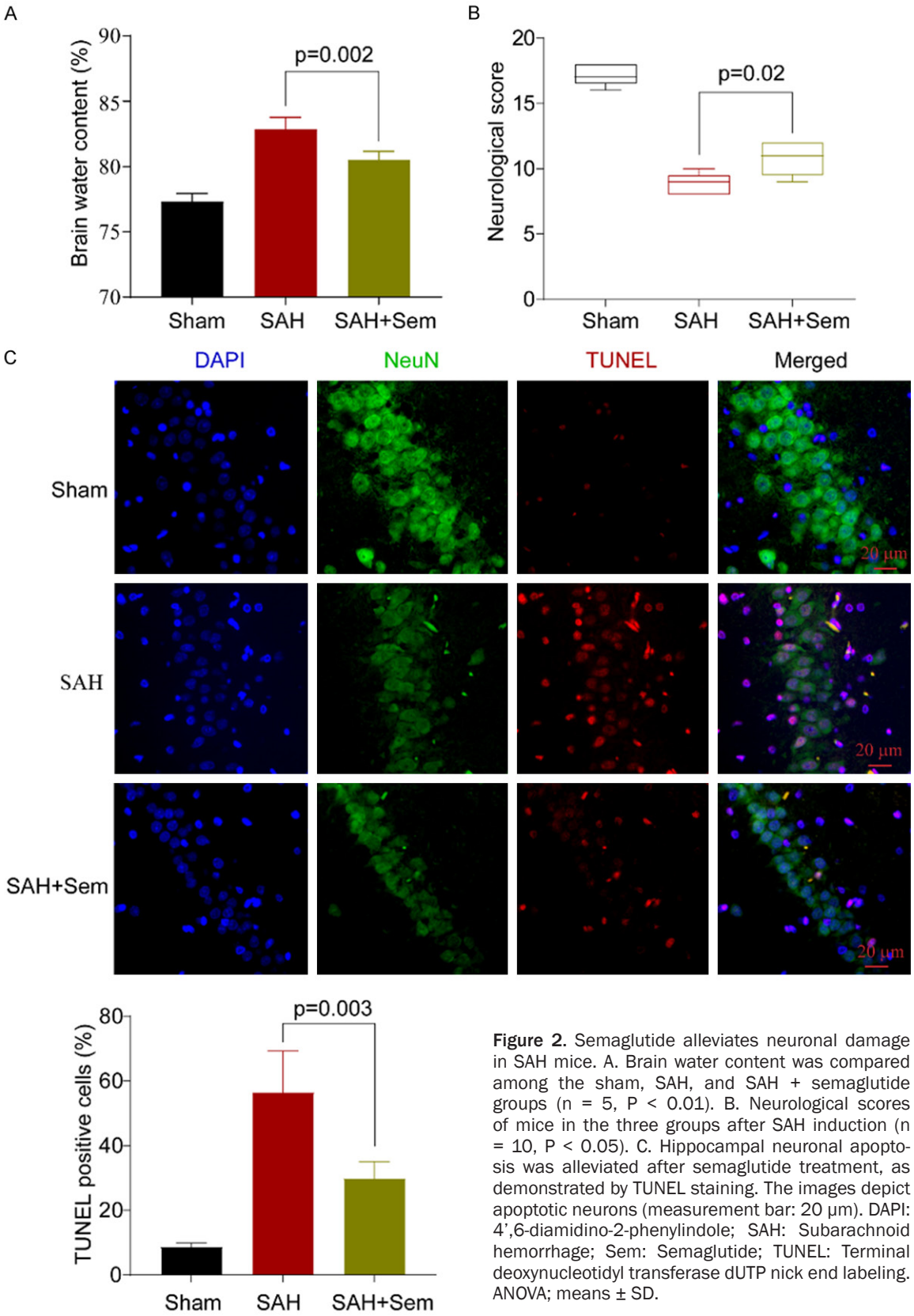

Figure 2. Semaglutide alleviates neuronal damage in SAH mice. A. Brain water content was compared among the sham, SAH, and SAH + semaglutide groups (n = 5, P < 0.01). B. Neurological scores of mice in the three groups after SAH induction (n = 10, P < 0.05). C. Hippocampal neuronal apoptosis was alleviated after semaglutide treatment, as demonstrated by TUNEL staining. The images depict apoptotic neurons (measurement bar: 20 μm). DAPI: 4',6-diamidino-2-phenylindole; SAH: Subarachnoid hemorrhage; Sem: Semaglutide; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling. ANOVA; means ± SD.

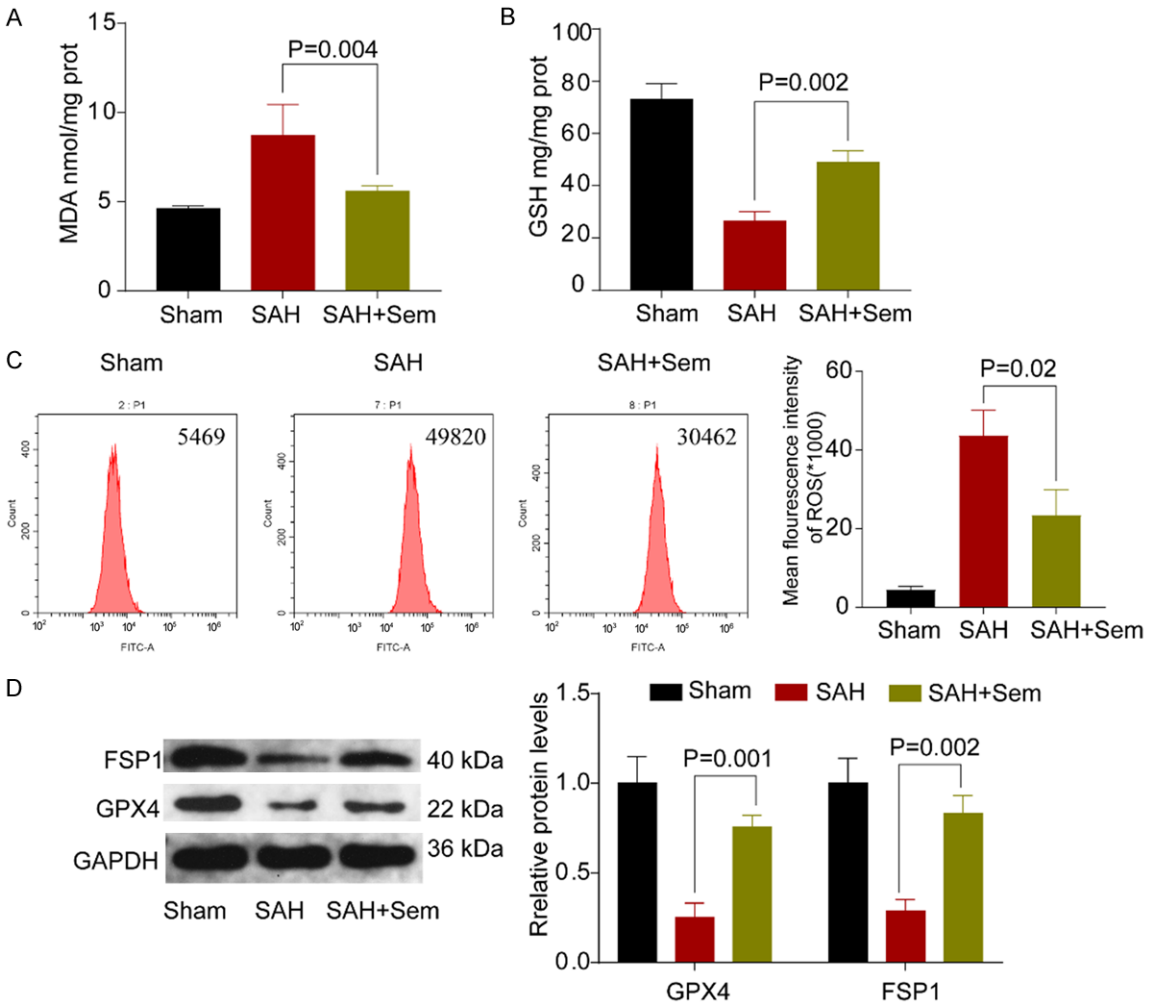

Figure 3. Semaglutide alleviates ferroptosis in SAH mice. A. MDA levels in mouse brain tissue following semaglutide treatment for 48 h after SAH induction. B. GSH levels in mouse brain tissue following semaglutide treatment for 48 h after SAH induction. C. Flow cytometry analysis of ROS levels in mouse brain tissue. D. FSP1 and GPX4 levels in the brain cortex of mice with SAH were analyzed using western blotting. GAPDH was used as the loading control. ROS: reactive oxygen species; GSH: glutathione; MDA: malondialdehyde.

cell lipid peroxidation and protect against neuronal ferroptosis after SAH.

Semaglutide activates SIRT1 and exerts neuroprotective effects in SAH mice

To determine if semaglutide alleviates SAH-induced neuronal damage by activating the SIRT1 pathway, the levels of sirt1 were assessed. Semaglutide treatment substantially increased sirt1 levels, as determined using RT-PCR (**Figure 4A**) and western blotting (**Figure 4B**). Furthermore, the knockdown of SIRT1 expression using SIRT1-specific siRNA was conducted to assess if semaglutide mediates its neuroprotective effects via SIRT1 activation. The results of water content in the mouse brain (**Figure 4C**) and neurological scores (**Figure 4D**) suggest that the neuroprotective effects of semaglutide are diminished after SIRT1 knockdown. Furthermore, the ability of semaglutide to inhibit ferroptosis was diminished when SIRT1 expression was suppressed by both MDA (**Figure 4E**) and GSH (**Figure 4F**).

Semaglutide modulates neuronal ferroptosis by regulating the Nrf2/HO-1 signaling pathway

There are a number of signaling pathways involved in ferroptosis, including Nrf2/HO-1. The present study assessed if semaglutide modulates the Nrf2/HO-1 signaling pathway to

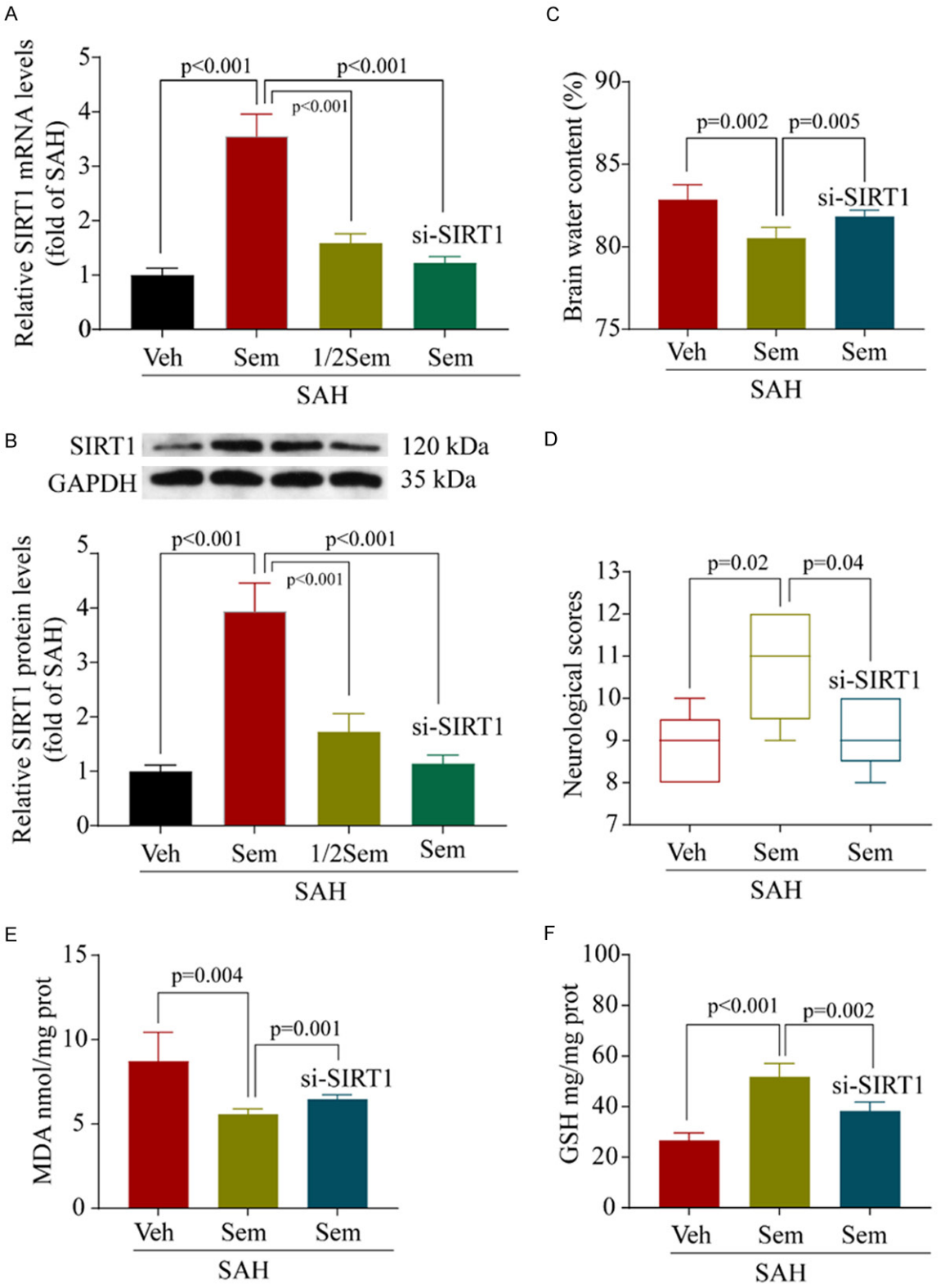

Figure 4. Semaglutide activates SIRT1 and induces a neuroprotective role in SAH mice. A. Levels of SIRT1 mRNA in the brains after Semaglutide treatment were measured by real-time PCR. B. Levels of SIRT1 protein after Semaglutide treatment were measured by Western blotting. C. Comparison of the brain water content across the three groups. D. Neurological scores of mice in the three groups after SAH. E. MDA levels in brain tissue. F. GSH levels in brain tissue in the three groups.

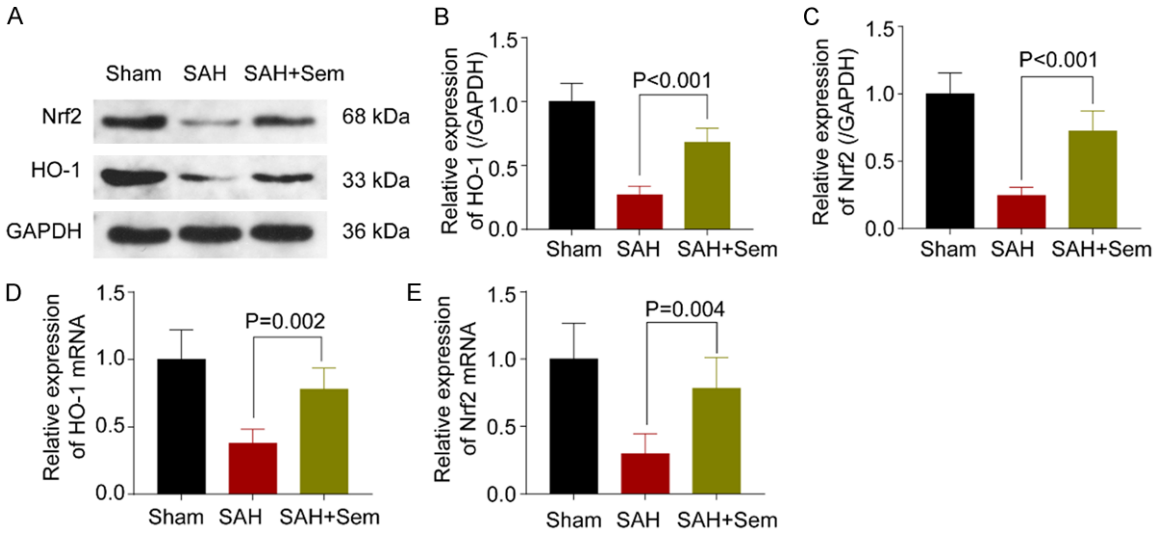

Figure 5. Semaglutide modulates neuronal ferroptosis in SAH via the regulation of the Nrf2/HO-1 signaling pathway. (A-C) Western blotting was performed to assess Nrf2 and HO-1 expression in mouse cerebral cortex (loading control: GAPDH). The mRNA levels of HO-1 (D) and Nrf2 (E) in the brain tissues of SAH mice (n = 5) were measured using real-time PCR. The data are presented as the average ± standard deviation.

control ferroptosis in mice with SAH. For this, Nrf2/HO-1 protein levels were assessed using western blotting (**Figure 5A**), and the results revealed that Nrf2 and HO-1 levels significantly decreased in the SAH group but were increased in the semaglutide group (**Figure 5B**, **5C**). The results of the RT-PCR experiment were comparable (**Figure 5D**, **5E**). In general, these results confirmed the potential of semaglutide to modulate the neuroprotective function by controlling the Nrf2/HO-1 signaling pathway.

Semaglutide alleviates neuroinflammation in SAH mice

Research has revealed that neuroinflammation plays a crucial role in the initiation and progression of EBI following SAH [8]. The inflammatory mediators induce the secretion of proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β, leading to the activation of proinflammatory signaling through NF-κB. Therefore, TNF-α, NF-κB, IL-6, and IL-1β expression in the hippocampus was assessed using ELISA. Following SAH induction, the levels of proinflammatory cytokines were significantly increased; however, treatment with semaglutide significantly reduced proinflammatory cytokine expression (**Figure 6A-D**). These findings indicate the significant anti-inflammatory effect of semaglutide on the neuroinflammatory response triggered by SAH.

Relationship between serum SIRT1 level, SAH severity, and clinical outcomes

In order to determine whether serum SIRT1 levels are associated with the clinical outcomes of 50 patients with SAH, Hunt-Hess levels data, six month mRS scores and clinical data were collected and analyzed. A significant difference in baseline characteristics could not be found between patients with SAH and controls. The results indicated a significant decrease in serum SIRT1 levels in patients with SAH than in healthy controls (5.456 ± 0.267 vs. 3.002 ± 0.233, $P < 0.001$) (**Figure 7A**). The low-grade SAH group had significantly higher serum SIRT1 levels (5.886 ± 0.328) than the poor-grade SAH group (2.37 ± 0.144, $P < 0.001$) (**Figure 7B**). Serum SIRT1 levels were positively associated with better SAH outcomes (2.354 ± 22.050.158 vs. 5.057 ± 0.488, $P < 0.001$) (**Figure 7C**). We also found that the area under the curve (AUC) was 0.905 by SIRT1, while the area under the curve (AUC) was 0.855 by the Hunt-Hess (**Figure 7D**). Even though, the predictive value of SIRT1 and Hunt-Hess had no significant difference in SAH outcomes ($P = 0.377$), while the AUC of SIRT1 was higher than that of Hunt-Hess. A notable association was found between SIRT1 levels in the blood and the severity of SAH as well as the clinical outcomes. These results indicated that SIRT1 might function as a predictive factor for patients with SAH.

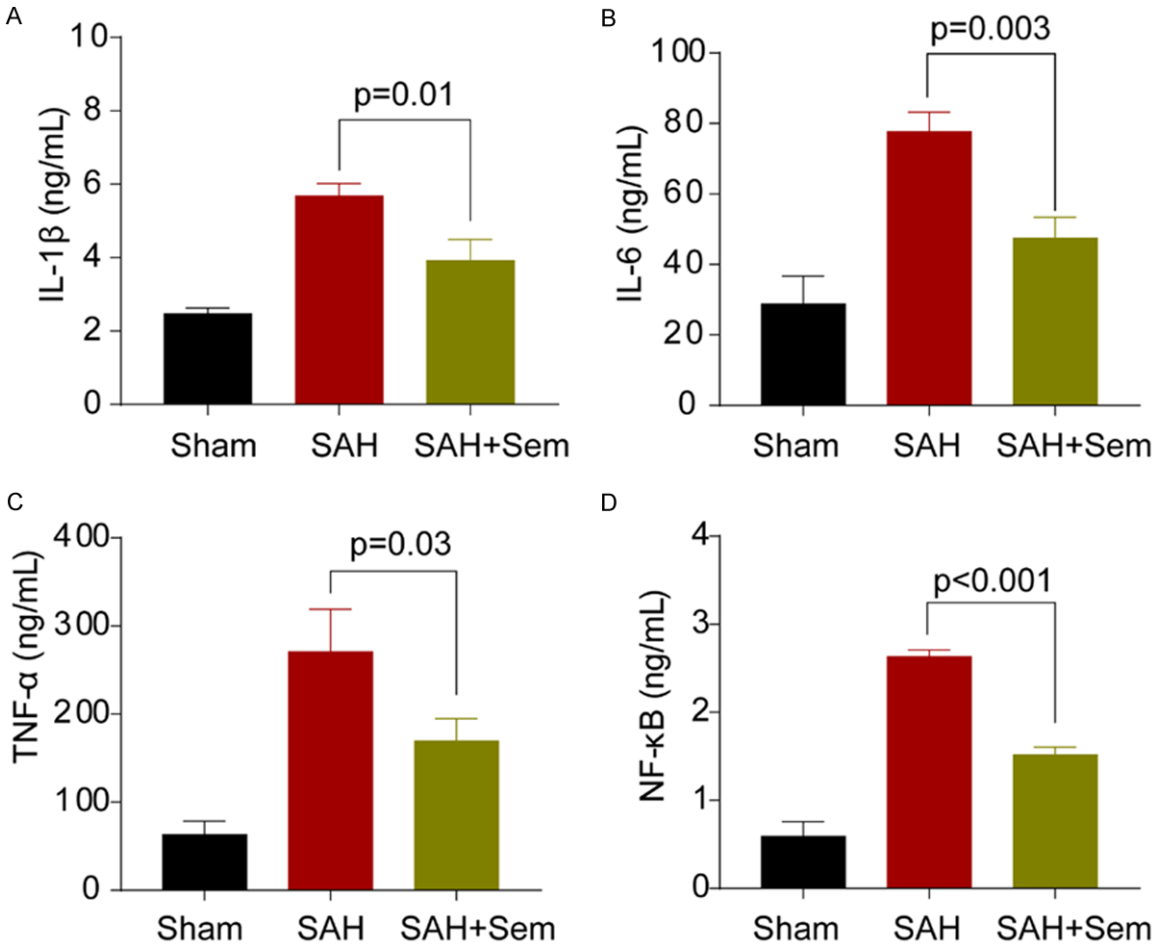

Figure 6. Semaglutide alleviates neuroinflammation in SAH mice. In mice with SAH, semaglutide treatment for 48 h significantly reduced the levels of hippocampal IL-1β (A), IL-6 (B), TNF-α (C), and NF-κB (D). The data are expressed as the average ± standard deviation.

Discussion

The present study aimed to evaluate the therapeutic importance of semaglutide in improving EBI in an SAH mouse model. The findings demonstrated that semaglutide exerted a neuroprotective effect by decreasing EBI after SAH. In addition, semaglutide mitigated neurological deficits and brain edema resulting from traumatic brain injury (TBI) after SAH. Additionally, it reduced neuronal death and improved ferroptosis in mice with SAH. Furthermore, it alleviated neuroinflammation caused by SAH, consequently reducing the extent of inflammation-induced brain damage. The anti-ferroptosis and anti-neuroinflammatory properties of semaglutide may be mediated by the SIRT1/Nrf2/HO-1 signaling pathway (**Figure 8**).

Semaglutide, a novel, long-acting agonist of the GLP-1R, is a widely used hypoglycemic agent owing to its extended half-life of 7 days *in vivo*. In April 2021, semaglutide can reduce weight in addition to its hypoglycemic effect, with studies indicating its safety and efficacy as a weight loss drug [22]. GLP-1 can modulate various pathophysiological mechanisms, such as brain glucose levels [23], BBB [24], and heart rate. In a mouse model of ischemic stroke, semaglutide was found to reduce BBB disruption, decrease infarction volume and atrophy, and enhance neurobehavioral outcomes by inhibiting the formation of C3d/GFAP astrocytes [19]. Additional research has demonstrated that semaglutide can reduce infarct size, suppress inflammation by regulating the p38 MAPK-MKK-c-Jun- NF-κB signaling pathway, and induce apoptosis via the ERK2/Bcl-2/BAX and Caspase-3 pathway [21]. Hence, Semaglutide plays an important role in brain injury via modulation of neuroinflammation, apoptosis, and BBB disruption.

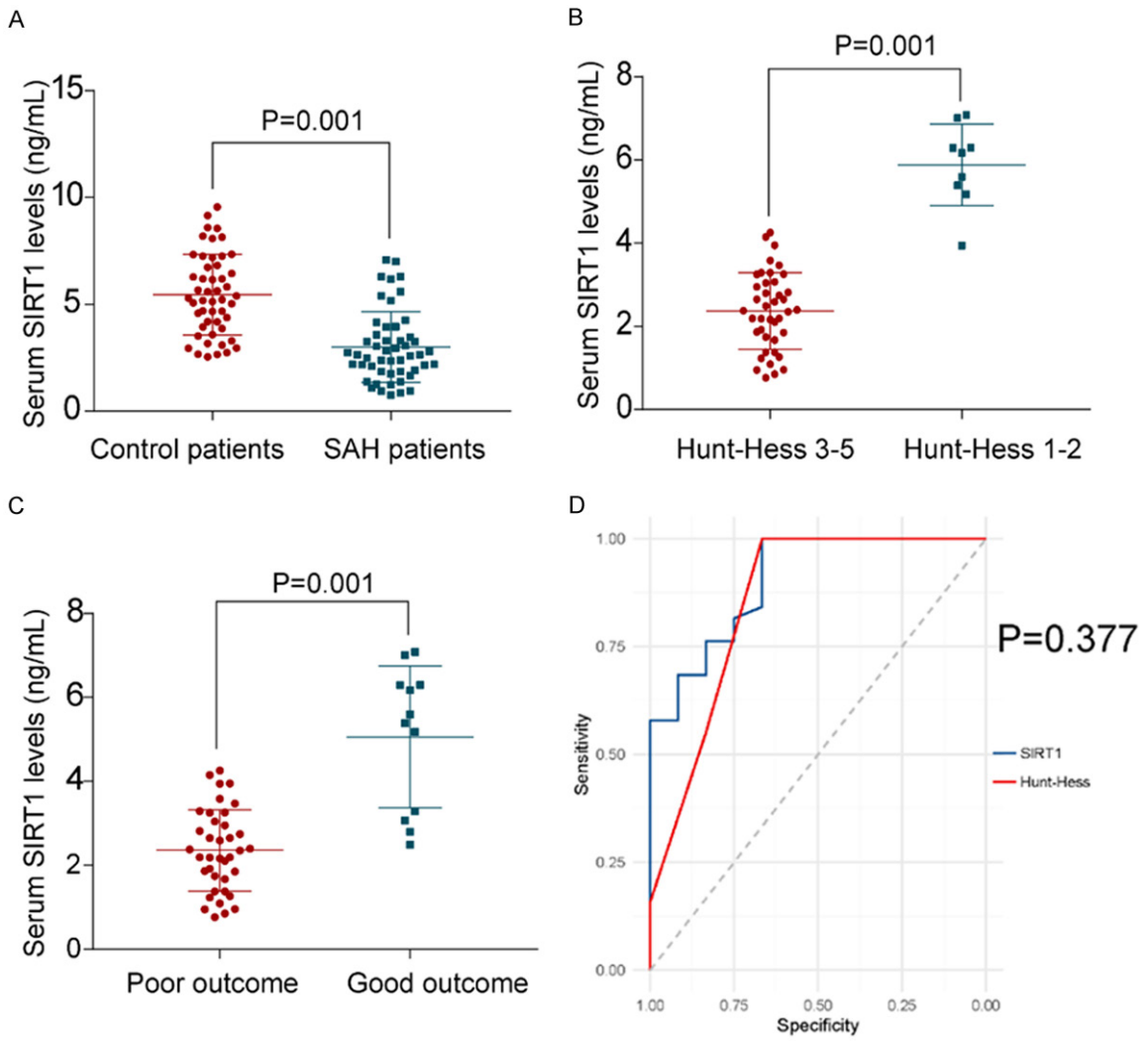

Figure 7. The correlation between serum SIRT1 levels, SAH severity, and clinical results. A. The serum SIRT1 levels were compared between patients with SAH and healthy controls ($P < 0.01$; *t*-test; mean ± standard deviation). B. The levels differed significantly ($P < 0.01$; *t*-test; mean ± standard deviation) between patients with low-grade and poor-grade SAH. C. The SIRT1 levels differed significantly ($P < 0.01$; *t*-test; mean ± standard deviation) between patients with positive and negative outcomes. D. The predictive value of serum SIRT1 levels and Hunt-Hess scores for the long-term prognosis of SAH patients.

Ferroptosis is a distinct, iron-dependent, conventional cell death mechanism that is unrelated to autophagy or apoptosis [25]. Ferroptosis is characterized by the disappearance of mitochondrial cristae, considerable constriction of the mitochondrial membrane, increased thickness of the lipid bilayer membrane, and decreased cell connectivity, leading to cell detachment [2]. The findings of this research emphasize that Nrf2 is triggered and offers defense after SAH, and the proof endorsing the increase of Nrf2 was assessed for its possible application as a treatment approach post-SAH [26]. According to a recent investigation, ferroptosis is a vital mediator of neuronal death in SAH. In line with this, the present study demonstrated that SIRT1 overexpression can alleviate neuronal ferroptosis in SAH mice. Furthermore, Yuan *et al*. [15] have found that SAH-induced ferroptosis can be suppressed by activating SIRT1 through the upregulation of GPX4 and FSP1. According to Yao *et al*. [27], activating the SIRT1/PGC-1α signaling pathway can reduce melatonin-induced suppression of both mitochondrial dynamics and the Nrf2 antioxidant system.

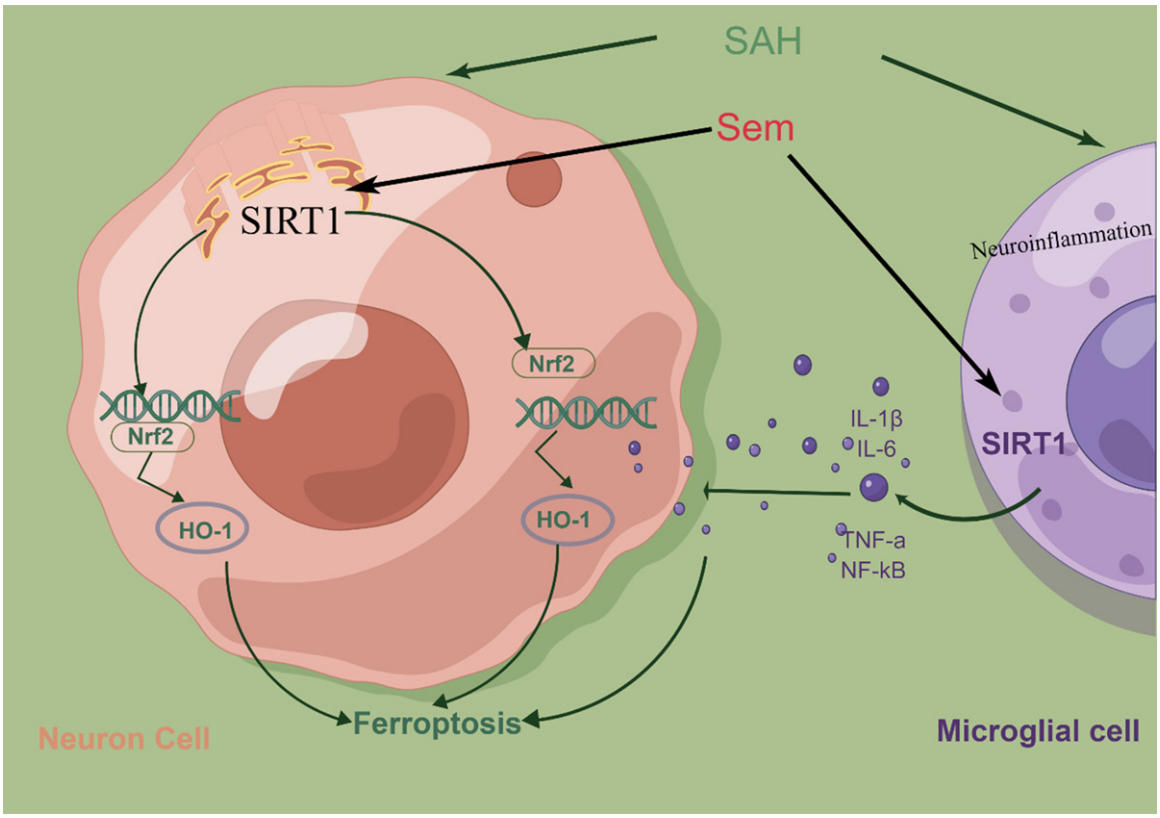

Figure 8. The proposed model of the observed sirt1/Nrf2/HO-1-mediated regulation of neuroinflammation and ferroptosis after SAH as well as potential mechanisms underlying the effects of semaglutide in SAH.

Under non-stress conditions, Nrf2 is primarily regulated via the proteasomal degradation that is mediated by Keap1, a protein associated with Kelch-like ECH. However, under oxidative stress, Keap1 dissociates from Nrf2, following which Nrf2 is transported to the nucleus to trigger the transcription of genes containing antioxidant response elements [28, 29]. According to Fan *et al*. [30], the activation of Nrf2-Keap1 signaling enhances the release of glutamate and regulates xCT, thus influencing the tumor microenvironment. In aged mice, inhibiting hippocampal ferroptosis could enhance cognitive function via the activation of the SIRT1/Nrf2/HO-1 signaling pathway in mesenchymal stem cell-derived exosomes dNCR [31]. Zhu *et al*. [32] conducted *in vitro* and *in vivo* experiments and demonstrated that DHA decreases the production of NOX in TBI by regulating Nrf2 signaling. Administering a prompt acute emergency intervention with a solitary intense dose of DHA after TBI can substantially enhance neurological outcomes in the long run. Furthermore, the levels of DHA-derived mediators, including resolvins and protectins, are also increased and offer supplementary neuroprotection [33]. Additionally, it has been observed that DHA administration can substantially enhance the levels of NAD(P)H quinone oxidoreductase and HO-1 [34]. In the present study, semaglutide was found to activate SIRT1, thus demonstrating the potential to mitigate ferroptosis to some extent by activating the Nrf2/HO-1 pathway. Nevertheless, further investigation is required to understand the precise mechanism and identify possible additional molecular pathways involved.

The NAD-dependent sirtuin proteins are enzymes or transcriptional regulators that undergo deacetylation by sirtuins. Seven sirtuins have been identified in mammals, and these function independently to adapt to environmental and metabolic stressors [35]. SIRT1, the most extensively researched member of the sirtuin family, is a class III histone deacetylase that plays a crucial role in controlling various cellular processes such as oxidative stress, inflamma-

tion, energy metabolism, DNA repair, and apoptosis [35]. Recent research suggests that SIRT1 is involved in deacetylating lysine residues in various substrates, such as p53, PGC-1α, HIF-1α, and STAT3. Moreover, SIRT1 can control various biological processes, such as cell death, iron-induced cell death, genetic material restoration, immune response, energy conversion, and pressure [15, 36]. Earlier studies had identified that SIRT1 expression is upregulated in the brain [37, 38], with the expression being widespread in the hippocampus, prefrontal cortex, and basal ganglia within the human nervous system [39]. Growing evidence suggests that SIRT1 plays a role in various central nervous system disorders, such as cerebral ischemia [40], traumatic brain injury [41], Alzheimer's disease [42], and Parkinson's disease [43]. Reportedly, pharmacological agents that activate or inhibit SIRT1 can control both neuroinflammation and neurodegenerative disorders. In line with this, the present study identified that SIRT1 activation could mitigate EBI following SAH, while SIRT1 suppression could also alleviate EBI. The potential mechanisms underlying these effects include ferroptosis. In mice with aneurysms and low estrogen levels, the absence of ERα and Sirt1 might trigger the NLRP3/IL-1β/MMP-9 pathway, leading to the rupture of intracranial aneurysms under estrogen-deficient conditions [44]. According to Liu *et al*. [45], isoliquiritigenin may protect against oxidative damage caused by SAH by influencing the Nrf2-mediated antioxidant signaling pathway, with SIRT1 playing a key role in this modulation. In particular, hypoxic conditioning and resveratrol treatment both protect against functional connectivity deficits due to SIRT1 signaling in SAH [46].

There are a few limitations in the current study. Firstly, this study only examines the effects of a single dose semaglutide in alleviating EBI after SAH; Secondly, this study has not been able to identify the long-term neuroprotective of semaglutide, such as 3 days, 4 days or more; Thirdly, the mechanisms of anti-ferroptosis has not been thoroughly explored, and the specific mechanism of Sirt1 regulating iron death is not clear. Furthermore, the SIRT1 pathway serves as the foundation for the molecular mechanisms of numerous neuroprotective medications following SAH therapy [47]. As a result, the SIRT1 pathway plays a critical role in the initiation and advancement of EBI following SAH, and the associated mechanisms are highly intricate.

Conclusions

The study indicated that the Nrf2/HO-1 pathway plays a vital role in regulating cellular processes and in the development of EBI following SAH through its involvement in ferroptosis and neuroinflammatory responses. Furthermore, semaglutide treatment upregulated SIRT1-induced ferroptosis via the Nrf2/HO-1 pathway. These findings provide a novel understanding of the effects and mechanisms underlying the anti-inflammatory, anti-ferroptosis, and neuroprotective properties of semaglutide.

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Written informed consent was obtained from the family members of SAH patients.

Disclosure of conflict of interest

None.

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