Research Article

Cycloastragenol Confers Cerebral Protection after Subarachnoid Hemorrhage by Suppressing Oxidative Insults and Neuroinflammation via the SIRT1 Signaling Pathway

Weibin Lin, Hao Yao, Jinqing Lai, Yile Zeng, Xieli Guo, Shu Lin, Weipeng Hu, Junyan Chen, and Xiangrong Chen

1Department of Neurosurgery, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China
2Department of Neurosurgery, The Jinjiang Municipal Hospital, Quanzhou, Fujian Province, China
3Centre of Neurological and Metabolic Research, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China

Correspondence should be addressed to Junyan Chen; 13905980777@139.com and Xiangrong Chen; xiangrong_chen281@126.com

Received 20 February 2022; Revised 21 April 2022; Accepted 3 May 2022; Published 2 June 2022

Academic Editor: Xiangsheng Zhang

Copyright © 2022 Weibin Lin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Subarachnoid hemorrhage (SAH) is an acute cerebral vascular disease featured by oxidative insults and neuroinflammation. Cycloastragenol (CAG), the major active component of Astragalus radix, has a wide range of biological functions. However, the potential beneficial effects and the underlying molecular mechanisms of CAG on SAH remain obscure. In the current study, the cerebroprotective effects and mechanism of CAG on SAH were evaluated both in vivo and in vitro. Our results indicated that CAG significantly suppressed SAH-triggered oxidative insults, inflammatory mediators production, microglia activation, and the neutrophil infiltration in the brain. In addition, CAG improved neurological function and ameliorated neuronal apoptosis and degeneration after SAH. In vitro results also revealed the therapeutic effects of CAG on neurons and microglia co-culture system. Mechanistically, CAG treatment upregulated sirtuin 1 (SIRT1) expression, inhibited the levels of FoxO1, nuclear factor-kappa B, and p53 acetylation, and suppressed the subsequent oxidative, inflammatory, and apoptotic pathways. In contrast, inhibiting SIRT1 by pretreatment with Ex527 abrogated the protective actions of CAG both in vivo and in vitro models of SAH. Collectively, our findings indicated that CAG could be a promising and effective drug candidate for SAH.

1. Introduction

Subarachnoid hemorrhage (SAH) is a subtype of lethal stroke featured by high fatality and poor functional recovery. Although significant progress has been achieved to improve SAH outcomes, effective therapies for SAH are still lacking [1]. Recently, mounting evidence has indicated that early brain injury (EBI), characterized by inflammatory damage and robust oxidative insults, is the main cause of poor neurological outcome in SAH [2–6]. Theoretically, new therapies aimed at diminishing inflammation and oxidative stress would improve neurological outcome after SAH.

Sirtuin 1 (SIRT1), a member of the sirtuins family, exhibits a critical role in many biological processes, including oxidative stress, inflammatory response, aging, and energy homeostasis [7–9]. Emerging evidence has demonstrated that SIRT1 could protect against oxidative insults and inflammation in different research areas, such as ischemia reperfusion-induced renal, liver, brain, and heart injury [10–13]. In addition, the neuroprotective function of SIRT1 has emerged as a critical mechanism in a variety of central nervous system (CNS) diseases. In experimental SAH models, several studies have showed that SIRT1 is able to inhibit reactive oxygen species (ROS) generation, modulate
microglia polarization, and ameliorate cerebral vasospasm [14–16]. All these suggest that SIRT1 could be a feasible target for treating SAH.

Astragalus radix, a traditional Chinese herbal medicine, has great potential for cardiovascular diseases, cerebrovascular diseases, diabetes, and cancers [17]. Cycloastragenol (CAG, Figure 1(a)) is the major active component of Astragalus radix [18]. Recent studies have demonstrated that CAG exhibits different biological actions including free-radical elimination, anti-inflammatory, and neurovascular protection activities [19–21]. More importantly, CAG could pass the blood-brain barrier and induce SIRT1 upregulation to ameliorate acute ischemic brain injury [18]. However, whether CAG is beneficial in SAH and the possible molecular mechanisms remain unknown. Therefore, we investigated the beneficial actions of CAG both in vivo and in vitro models of SAH and the underlying molecular mechanisms.

2. Materials and Methods

2.1. Experimental Animals. A total of 154 adult male Sprague-Dawley rats (230-260 g) were housed in standard conditions and were allowed to food and water freely. All experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of Fujian Medical University. Randomization of animals was conducted by using the website http://Randomization.com/.

2.2. In Vivo SAH Model. Rats were intraperitoneal injection with 1% sodium pentobarbital (50 mg/kg) before surgery. After anesthesia, a 4–0 monofilament nylon suture was inserted into the left internal carotid artery. After reaching the bifurcation of the anterior and middle cerebral arteries, the filament was advanced to puncture the artery [22]. Sham animals were treated via the same way except that vessel perforation. SAH hemorrhage severity score was evaluated by using an 18-point scoring system [23]. The sample taken for investigation is shown in Figure 1(b).

2.3. In Vivo Experimental Groups. In the first experiment, 91 rats were used. We divided animals into five groups: the vehicle-treated sham group (n=14), the vehicle-treated SAH group (n=21, 5 rats died), the 5 mg/kg CAG-treated SAH group (n=20, 4 rats died), the 10 mg/kg CAG-treated SAH group (n=18, 2 rats died), and the 20 mg/kg CAG-treated SAH group (n=18, 2 rats died). Postassessments included neurological functions, Nissl staining, and western blotting.

In the second experiment, 59 rats were used. We divided animals into four groups: the vehicle-treated sham group (n=12), the vehicle-treated SAH group (n=16, 4 rats died), the 10 mg/kg CAG-treated SAH group (n=15, 3 rats died), and the 10 mg/kg CAG plus Ex527-treated SAH group (n=16, 4 rats died). Postassessments included biochemical estimation, enzyme-linked immunosorbent assay (ELISA) assay, histopathological study, western blotting, and neurological functions.

2.4. In Vitro SAH Model. Haemoglobin- (Hb-) induced in vitro SAH model was performed according to a previous study [5]. For cell culture, the cortex was dissociated from rat pups (postnatal days 0-1). The primary cortical neurons and microglia co-culture system was maintained with Dulbecco’s modified Eagle Medium with 10% fetal bovine serum. For SAH model in vitro, primary cells were incubated with 25 μM Hb. The neuron-microglia co-cultures were assigned into control group, Hb + vehicle group, Hb +5 μM CAG group, Hb +10 μM CAG group, Hb +15 μM CAG group, and Hb +15 μM CAG + Ex527 group. Postassessments included cell viability analysis, biochemical estimation, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence staining.

2.5. Drug Administration. For in vivo experiments, CAG (Yuanye Biotechnology, Shanghai, China) was dissolved in 5% DMSO (in saline). Different doses of CAG or vehicle were intraperitoneally administered at 3 h, 12 h, and then once daily for up to three days [18]. Ex527 (Sigma-Aldrich, St. Louis, MO) (10 mg/kg) or vehicle (1% DMSO) was intraperitoneally administered before SAH construction once daily for 3 days. For in vitro experiments, CAG was dissolved in culture medium. Ex527 (20 μM in 1% DMSO) was applied for 24 h before Hb stimulation.

2.6. Cell Viability Evaluation. The Cytotoxicity Detection Kit (Roche) was performed in accordance with the manufacturer’s protocols. Cell viability of each sample was determined at the wavelength of 490 nm using a spectrophotometer.

2.7. Intracellular ROS Generation. ROS Assay Kit (S0033, Beyotime, China) was employed to investigate ROS production. According to the manufacturer’s protocols, the ROS content was measured by recording the mean relative fluorescence intensity.

2.8. Neurological Evaluation. As previously described, neurological deficit was calculated using a modified neurological severity score system. The low score indicated severe neurological deficits [23]. To further evaluate the motor impairments after SAH, the rotarod test was conducted. According to a previous report [24], rats were trained for 3 days before the formal testing. During the procedure, the latency to fall was recorded by investigators blinded to group information.

2.9. Western Blotting. Protein levels of ac-NF-κB, ac-FoxO1, SIRT1, and ac-p53 in brain samples were evaluated by western blotting according to standard protocols. In brief, the protein samples were blotted onto the PVDF membrane. Then, the membrane was incubated with primary antibodies against SIRT1 (Ab12193, 1:1000, Abcam), ac-FoxO1 (sc-49437, 1:200, Santa Cruz), ac-NF-κB (12629S, 1:500, Cell Signaling), ac-p53 (2570, 1:500, Cell Signaling), and appropriate secondary antibodies. β-Actin (BM0627, 1:4000, Boster Biotech) was the internal loading control for the proteins. Membranes were observed with enhanced chemiluminescence solution.
Figure 1: Continued.
2.10. Immunoﬂuorescence Staining. The brain sections and coverslips were ﬁxed with 4% paraformaldehyde. Then, they were incubated in 0.1% Triton X-100 for 5 min. The slides were then incubated with primary antibodies including Iba-1 (SC-98468, 1:50, Santa Cruz), myeloperoxidase (MPO, SC-16128, 1:50, Santa Cruz), 8-hydroxydeoxyguanosine (8-OHdG, ab62623, 1:100, Abcam), SIRT1 (Ab12193, 1:100, Abcam), NeuN (MAB377, 1:200, EMD Millipore). After several times washes, they were incubated with appropriate secondary antibodies. The images were visualized by using an epiﬂuorescence microscope.

2.11. TUNEL Staining. TUNEL staining was conducted by using a commercial kit (Roche Diagnostics, Indianapolis). The brain sections and coverslips were incubated with the reaction solution according to the instructions. The images were visualized by using an epiﬂuorescence microscope.

2.12. Nissl Staining. In brief, brain sections were stained with Nissl solution for 10 min. After three times washing with PBS, they were sealed with neutral balsam. The damaged neurons had shrunk or contained vacuoles. The normal neurons had a relatively big and full soma, with round nuclei.

2.13. ELISA. After 24h after SAH, animals were over-anesthetized. The supernatant samples from brain tissues were obtained. The levels of tumor necrosis factor-a (TNF-a), IL-6, IL-1β, and intercellular cell adhesion molecule-1 (ICAM-1) in tissue supernatant or culture medium were evaluated by using commercial ELISA assays.

2.14. Measurements of MDA, SOD, and CAT. After 24h after SAH, animals were over-anesthetized. The supernatant samples from brain tissues were obtained. Commercial malondialdehyde (MDA) assay kit, superoxide dismutase (SOD) assay kit, and catalase (CAT) assay kit were used and the samples were evaluated as per the instructions.

2.15. Statistical Analysis. Results were expressed as the mean ± SD. One-way analysis of variance (ANOVA) test with Tukey’s multiple comparison test was conducted. A value of $P < 0.05$ was considered signiﬁcant. GraphPad Prism 8.02 was used for statistical analysis.

3. Results

3.1. Mortality. A total of 154 rats were used in our study, 4 rats with SAH grading score less than 8 were excluded. The mortality rate was 24.3% (9/37 rats) in the SAH + vehicle group; 15.5% (11/71 rats) in the CAG-treated SAH group; 25.0% (4/16 rats) in the SAH + CAG + Ex527 group. No rats died in the vehicle-treated sham group.

3.2. CAG Exerts a Beneﬁcial Effect against SAH. As shown, western blotting results showed that CAG treatment at 10 and 20 mg/kg, but not 5 mg/kg, could signiﬁcantly increase SIRT1 expression after SAH (Figures 1(c) and 1(d)). In addition, Nissl staining indicated that SAH insults induced overt morphologic changes and cellular degeneration, which could be ameliorated after different doses of CAG administration (Figure 1(e)). We further evaluated the behavior functions after SAH. It revealed that the functional deﬁcits and motor function were severely impaired after SAH. In contrast, CAG treatment at 10 and 20 mg/kg could signiﬁcantly reduce the neurological deﬁcit scores and improve the time spent on the rotarod (Figures 1(f) and 1(g)). But CAG treatment at 5 mg/kg did not markedly make better functional behavior after SAH. No signiﬁcant differences between 10 mg/kg and 20 mg/kg CAG treatment on neurological outcomes and SIRT1 expression were detected. These results revealed that CAG treatment at 10 mg/kg had the maximal protective...
Figure 2: Continued.
Figure 2: Effects of CAG on SIRT1 signaling pathway. (a) Western blot analysis for SIRT1, ac-FoxO1, ac-NF-κB, and ac-p53. Quantification of protein expressions of SIRT1 (b), ac-FoxO1 (c), ac-NF-κB (d), and ac-p53 (e) after CAG treatment (n = 6 per group). (f) Quantification of SIRT1 immunofluorescence staining after CAG treatment (n = 6 per group). (g) Representative photomicrographs of immunofluorescence staining for SIRT1. Scale bar = 50 μm. *P < 0.05.
Figure 3: Continued.
effects after SAH. Thus, we used this dose in subsequent studies.

3.3. CAG Upregulated SIRT1 Expression and Reduced FoxO1, NF-κB, and p53 Acetylation after SAH. In the brain, SIRT1 is highly expressed in neurons and exerts an endogenous neuroprotection in many CNS diseases. As shown, western blotting data revealed that the expression of SIRT1 was further increased after CAG administration. Meanwhile, the acetylation levels of FoxO1, NF-κB, and p53 were effectively decreased in the SAH + CAG group as compared with SAH + vehicle group (Figures 2(a)–2(e)). Double immunofluorescent staining further confirmed that SIRT1 is mainly expressed in neurons post-SAHA. In contrast, CAG further enhanced the expression of SIRT1 in neurons following SAH (Figures 2(f) and 2(g)). However, pretreatment with Ex527 abated the effects of CAG on SIRT1 expression as well as the subsequent SIRT1-mediated signaling pathway.

Figure 3: Influence of CAG on neuroinflammation after SAH. The levels of IL-1β (a), IL-6 (b), TNF-α (c), and ICAM-1 (d) were determined by ELISA assay (n = 6 per group). (e) Immunostaining of Iba1 in brain sections. (f) Quantification of Iba1+ staining after CAG treatment (n = 6 per group). (g) Immunostaining of MPO in brain sections. (h) Quantification of MPO+ staining after CAG treatment (n = 6 per group). Scale bar = 50 μm. *P < 0.05.
3.4. CAG Suppressed Inflammatory Response after SAH.
Microglia activation, neutrophil infiltration, and the subsequent proinflammatory cytokines release play important roles in the neuroinflammation after SAH. We next evaluated the effects of CAG on neuroinflammation after SAH. As expected, SAH insults markedly raised the levels of IL-1β, TNF-α, IL-6, and ICAM-1 (Figures 3(a)–3(d)). In addition, SAH significantly induced microglia activation and neutrophil infiltration (Figures 3(e)–3(h)). In contrast, CAG supplementation effectively suppressed SAH-triggered microglia activation, neutrophil infiltration, and the proinflammatory cytokines production. But the anti-inflammatory effects of CAG on SAH were abated by Ex527 treatment (Figures 3(a)–3(h)).

3.5. CAG Inhibited Oxidative Insults after SAH.
Next, we measured the antioxidant effects of CAG after SAH. 8-OHdG immunofluorescence staining confirmed that SAH aggravated oxidative insults (Figures 4(a) and 4(b)). Moreover, our data showed that SAH induced a significant
increase in lipid peroxidation. In addition, the endogenous antioxidant enzymes including SOD and CAT were significantly suppressed after SAH insults (Figures 4(c)–4(e)). Treatment with CAG markedly reduced 8-OHdG immunoreactivity, decreased the content of MDA, and restored the impairment antioxidant systems. However, all these changes were counteracted by Ex527 (Figures 4(a)–4(e)).

3.6. CAG Inhibited Neuronal Apoptosis and Improved Neurological Function after SAH. Emerging evidence has shown that neuronal apoptosis is a key mechanism involved in neurological impairment after SAH. It revealed that SAH insults significantly aggravated behavior impairment. Moreover, TUNEL staining indicated that SAH insults induced a marked increase in neuronal apoptosis (Figures 5(a)–5(d)). In contrast, rats in the CAG-treated SAH group showed reduced percentage of apoptotic neurons and better neurological functions than rats in the SAH group. However, these changes were abated by SIRT1 inhibition with Ex527 (Figures 5(a)–5(d)).

![Figure 5: Influence of CAG on behavior impairment and neuronal apoptosis following SAH. Changes of neurological deficits score (a) and motor performance (b) (n = 12 per group). (c) Quantification of TUNEL + staining after CAG treatment (n = 6 per group). (d) Representative TUNEL images. Scale bar = 50 μm. * P < 0.05.](attachment://figure5.png)
Figure 6: Continued.
3.7. CAG Ameliorated Hb-Stimulated Neuronal Damage. We further evaluated the effects of CAG on Hb-stimulated neurons and microglia co-culture system. A significant reduction in cell viability was observed in Hb-treated group. CAG treatment markedly improved cell viability after Hb stimulation in a dose-dependent manner (Figure 6(a)). In addition, Hb incubation triggered upregulations of inflammatory mediators (IL-1β and TNF-α) production, lipid peroxidation (MDA), and ROS production, all of which were diminished by CAG supplementation (Figures 6(b)–6(f)). In addition, CAG treatment markedly attenuated neuronal apoptosis after Hb stimulation. However, all the beneficial effects of CAG were abrogated by Ex527 treatment (Figures 6(a)–6(h)).

3.8. CAG Enhanced SIRT1 Expression In Vitro. We then measured the effects of CAG on SIRT1 expression in primary neurons. As shown, western blotting data revealed that the expression of SIRT1 was further increased after CAG administration (Figures 7(a) and 7(b)). In addition, compared to the Hb + vehicle group, SIRT1 immunoreactivity in primary neurons was further enhanced in the CAG-treated Hb group. These suggested that CAG could also upregulate SIRT1 expression in vitro (Figures 7(c) and 7(d)). In contrast, Ex527 abated the CAG-induced SIRT1 upregulation in primary neurons.

4. Discussion

This study verified that CAG protected against EBI after SAH both in vivo and in vitro. In vivo, CAG reduced SAH-triggered oxidative insults, inflammatory mediators production, microglia activation, and the neutrophil infiltration in the brain. In addition, CAG improved neurological function and ameliorated neuronal apoptosis and degeneration after SAH. In vitro, CAG also attenuated oxidative damage, inflammation, and neuronal cytotoxicity. All these actions of CAG were related with the upregulation of SIRT1 and the reduced acetylation of FoxO1-, NF-κB-, and p53-mediated oxidative, inflammatory, and apoptotic pathways. In contrast, Ex527 inhibited SIRT1 expression and abated the neuroprotective effects of CAG after SAH. Thus, our data suggested that CAG attenuated SAH-induced EBI by activating of SIRT1-dependent pathway, and CAG might be a novel therapeutic drug for SAH treatment.

Accumulating evidence has revealed that oxidative damage and inflammatory response play critical roles in the pathogenesis of SAH [25–29]. Diminishing oxidative damage and inflammatory response could significantly improve neurological outcome after SAH [30–33]. CAG is the active form of Astragalus Radix, which exhibits a variety of pharmacological effects [18]. Previous studies have demonstrated that CAG could inhibit ROS production,
ameliorate oxidative insults, and suppress inflammatory response in different disease models [17, 18]. However, the effect of CAG against SAH has yet to be investigated. In the current study, our data showed that SAH triggered a significant oxidative damage as evidenced by increased ROS production and decreased endogenous antioxidant activity. In addition, SAH markedly induced microglia activation, neutrophil infiltration, and proinflammatory cytokines release. Moreover, SAH insults resulted in evident neuronal death and neurological impairment. On the contrary, CAG treatment could significantly suppress oxidative insults and neuroinflammation after SAH. Meanwhile, CAG decreased neuronal apoptosis and improved functional outcomes in a SAH rat model. In vitro, CAG also dose-dependently ameliorated oxidative insults, inflammation, and neuronal damage. These suggest that CAG could protect against EBI after SAH by diminishing oxidative damage and neuroinflammation.

SIRT1, an NAD⁺-dependent class III histone deacetylase, exhibits a wide range of biological processes [34]. In the brain, SIRT1 is highly expressed in neurons and exerts an endogenous neuroprotection in a variety of neurological disorders [34–36]. Macarena et al. reported that SIRT1 protected the brain against cerebral ischemic damage by inhibition of neuroinflammation and apoptosis [35]. Zhou et al. suggested that activation of SIRT1 promoted mitochondrial biogenesis and reduced apoptosis after intracerebral hemorrhage [37]. In SAH, several studies have showed that activation of SIRT1 by pharmacological therapies could ameliorate neuroinflammation and oxidative insults, and improve
motor functions [38–40]. In addition, SIRT1 has the potential to ameliorate cerebral vasospasm after SAH [14]. Whether CAG affects SIRT1 activation to exhibit neuroprotection after SAH remains obscure.

A previous study by Li et al. reported that CAG upregulated SIRT1-mediated signaling pathway and ameliorated brain injury in a middle cerebral artery occlusion mice model [18]. In agreement with this study, our data also showed that CAG significantly increased the expression of SIRT1 both in vivo and in vitro models of SAH. But how SIRT1 activation by CAG affects EBI after SAH remains unclear. As a histone deacetylase, SIRT1 could deacetylate a wide range of substrates [34]. In addition to histones, SIRT1 could deacetylate nonhistone protein substrates. For example, Sirt1 can target FoxO1 to protect cells from oxidative damage [41]. FoxO1 plays a key role in the maintenance of redox homeostasis. Overexpression of FoxO1 can induce endogenous antioxidant enzymes expression and enhance hydrogen peroxide scavenging [10]. Additionally, SIRT1 can deacetylate lys310 residue of RelA/p65 to downregulate NF-κB activity and inhibit proinflammatory mediators release [42]. Moreover, SIRT1 is able to deacetylate p53 to prevent the subsequent apoptosis [43]. Interestingly, our data revealed a significant increase in FoxO1, NF-κB, and p53 acetylation in the brain after SAH insults. In contrast, CAG upregulated SIRT1 expression and decreased FoxO1, NF-κB, and p53 acetylation after SAH. As a result, the oxidative stress, neuroinflammation, and neuronal apoptosis were all ameliorated by CAG treatment. To further confirm that SIRT1 has a beneficial role in the protective actions of CAG against SAH, we employed a selective SIRT1 inhibitor Ex527. As expected, Ex527 pretreatment inhibited SIRT1 expression, increased the acetylation of FoxO1, NF-κB, and p53, and abated the cerebroprotective effects of CAG against SAH. Therefore, our results strongly suggested that CAG could ameliorate EBI after SAH by upregulation of SIRT1-mediated neuroprotection.

Our study has several potential limitations. Firstly, some other targets of SIRT could be neuroprotective, for example, peroxisome proliferator-activated receptor-γ coactivator-1α and heat shock factor. Whether CAG could regulate these molecular targets after SAH remains unknown. Secondly, it is not convinced enough that activation of microglia is assessed only with iba1 staining. Other sensitive markers for microglia activation will be employed in our ongoing research. Thirdly, we did not evaluate the long-term effects of CAG after SAH. Moreover, although no side effects of CAG were reported, the pharmacokinetic studies are still required to validate the translational potential of CAG for SAH treatment.

5. Conclusion

Taken together, this study has identified that CAG notably ameliorated EBI after SAH by suppressing oxidative insults and neuroinflammation. The overall beneficial effects of CAG might involve in the SIRT1-dependent pathway. Our experimental results indicate that CAG may be a promising drug candidate for treating SAH.

Abbreviations

- 8-OHdG: 8-Hydroxydeoxyguanosine
- CAG: Cycloastragenol
- CAT: Catalase
- CNS: Central nervous system
- EBI: Early brain injury
- ELISA: Enzyme-linked immunosorbent assay
- Hb: Haemoglobin
- ICAM-1: Intercellular cell adhesion molecule-1
- MDA: Malondialdehyde
- MPO: Myeloperoxidase
- ROS: Reactive oxygen species
- SAH: Subarachnoid hemorrhage
- SIRT1: Sirtuin 1
- SOD: Superoxide dismutase
- TNF-a: Tumor necrosis factor-a.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no competing interests.

Authors’ Contributions

Conceptualization was performed by Weibin Lin and Xiangrong Chen; in vivo experiments were conducted by Weibin Lin, Jinqing Lai, Yile Zeng, and Xieli Guo; in vitro experiments were completed by Shu Lin and Weipeng Hu; Xiangrong Chen and Junyan Chen reviewed and revised the manuscript. Weibin Lin and Hao Yao contributed equally to this work.

Acknowledgments

This work was supported by grants from the funds for China Postdoctoral Science Foundation (2020M682069) and Fujian Province Scientific Foundation (2019J01168) from Dr. Xiangrong Chen.

References

[1] J. J. Liu, J. S. Raskin, R. McFarlane, R. Samatham, and J. S. Cetas, “Subarachnoid hemorrhage pattern predicts acute cerebral blood flow response in the rat,” Acta Neurochirurgica. Supplement, vol. 127, pp. 83–89, 2020.
[2] G. J. Liu, T. Tao, X. S. Zhang et al., “Resolv in d1 attenuates innate immune reactions in experimental subarachnoid hemorrhage rat model,” Molecular Neurobiology, vol. 58, no. 5, pp. 1963–1977, 2021.
[3] X. Hu, J. Yan, L. Huang et al., “Int-777 attenuates nlrp3-asc inflammasome-mediated neuroinflammation via tgr5/camp/pka signaling pathway after subarachnoid hemorrhage in rats,” Brain, Behavior, and Immunity, vol. 91, pp. 587–600, 2021.
[4] R. Heinz, S. Brandenburg, M. Nieminen-Kelha et al., “Microglia as target for anti-inflammatory approaches to prevent...
secondary brain injury after subarachnoid hemorrhage,” *Journal of Neuroinflammation*, vol. 18, no. 1, pp. 1–13, 2021.

[5] Z. H. Zhang, J. Q. Liu, C. D. Hu et al., “Luteolin confers cerebroprotection after subarachnoid hemorrhage by suppression of nlrp3 inflammasome activation through nfr2-dependent pathway,” *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 5838101, 18 pages, 2021.

[6] Q. Liu, C. Hou, H. Zhang et al., “Impaired meningeal lymphatic vessels exacerbate early brain injury after experimental subarachnoid hemorrhage,” *Brain Research*, vol. 1769, p. 147584, 2021.

[7] A. Z. Herskovits and L. Guarente, “Sirt1 in neurodevelopment and brain senescence,” *Neuron*, vol. 81, no. 3, pp. 471–483, 2014.

[8] J. Xu, C. W. Jackson, N. Khoury, I. Escobar, and M. A. Perez-Pinzon, “Brain sirt1 mediates metabolic homeostasis and neuroprotection,” *Frontiers in Endocrinology*, vol. 9, p. 702, 2018.

[9] J. Zhang, R. Bi, Q. Meng et al., “Catalpol alleviates adriamycin-induced nephropathy by activating the sirt1 signalling pathway in vivo and in vitro,” *British Journal of Pharmacology*, vol. 176, no. 23, pp. 4558–4573, 2019.

[10] H. Mao, L. Wang, Y. Xiong, G. Jiang, and X. Liu, “Fucosaxthrin attenuates oxidative damage by activating the sirt1/nrf2/ho-1 signaling pathway to protect the kidney from ischaemia-reperfusion injury,” *Oxidative Medicine and Cellular Longevity*, vol. 2022, Article ID 7444430, 28 pages, 2022.

[11] L. Liu, Q. Cao, W. Gao et al., “Melatonin ameliorates cerebral ischemia-reperfusion injury in diabetic mice by enhancing autophagy via the sirt1-bmal1 pathway,” *The FASEB Journal*, vol. 35, no. 12, article e22040, 2021.

[12] H. Liu, W. Wang, X. Weng et al., “The h3k9 histone methyltransferase g9a modulates renal ischemia reperfusion injury by targeting sirt1,” *Free Radical Biology & Medicine*, vol. 172, pp. 123–135, 2021.

[13] B. Wu, J. Y. Feng, L. M. Yu et al., “Icariin protects cardiomyocytes against ischaemia/reperfusion injury by attenuating sirtuin 1-dependent mitochondrial oxidative damage,” *British Journal of Pharmacology*, vol. 175, no. 21, pp. 4137–4153, 2018.

[14] A. K. Vellimana, D. J. Aum, D. Diwan et al., “Sirt1 mediates hypoxic preconditioning induced attenuation of neurovascular dysfunction following subarachnoid hemorrhage,” *Experimental Neurology*, vol. 334, p. 113484, 2020.

[15] A. K. Vellimana, D. Diwan, J. Clarke, J. M. Gidday, and G. J. Zipfel, “Sirt1 activation: a potential strategy for harnessing endogenous protection against delayed cerebral ischemia after subarachnoid hemorrhage,” *Neurosurrgy*, vol. 65, CN_suppl_1, pp. 1–5, 2018.

[16] X. S. Zhang, Y. Lu, W. Li et al., “Cerebroprotection by dioxin after experimental subarachnoid haemorrhage via inhibiting nlrp3 inflammasome through sirt1-dependent pathway,” *British Journal of Pharmacology*, vol. 178, no. 18, pp. 3648–3666, 2021.

[17] M. Li, B. Han, H. Zhao et al., “Biological active ingredients of astragali radix and its mechanisms in treating cardiovascular and cerebrovascular diseases,” *Phytomedicine*, vol. 98, p. 153918, 2022.

[18] M. Li, S. C. Li, B. K. Dou et al., “Cycloastragenol upregulates sirt1 expression, attenuates apoptosis and suppresses neuroinflammation after brain ischemia,” *Acta Pharmacologica Sinica*, vol. 41, no. 8, pp. 1025–1032, 2020.

[19] G. Wang, C. Ma, K. Chen et al., “Cycloastragenol attenuates osteooclasisogenesis and bone loss by targeting rankl-induced nfr2/keap1/are, nf-kappab, calcium, and nfatc1 pathways,” *Frontiers in Pharmacology*, vol. 12, p. 810322, 2022.

[20] Y. Wang, C. Chen, Q. Wang, Y. Cao, L. Xu, and R. Qi, “Inhibitory effects of cycloastragenol on abdominal aortic aneurysm and its related mechanisms,” *British Journal of Pharmacology*, vol. 176, no. 2, pp. 282–296, 2019.

[21] M. Ikram, M. H. Jo, K. Choe et al., “Cycloastragenol, a triterpene nolid saponin, regulates oxidative stress, neurotrophic dysfunctions, neuroinflammation and apoptotic cell death in neurodegenerative conditions,” *Cell*, vol. 10, no. 10, p. 2719, 2021.

[22] Y. Huang, Y. Guo, L. Huang et al., “Kisseptin-54 attenuates oxidative stress and neuronal apoptosis in early brain injury after subarachnoid hemorrhage in rats via GPR54/ARRB2/AKT/GSK3β signaling pathway,” *Free Radical Biology & Medicine*, vol. 171, pp. 99–111, 2021.

[23] T. Sugawara, R. Ayer, V. Jadhav, and J. H. Zhang, “A new grading system evaluating bleeding scale in filament perforation subarachnoid hemorrhage rat model,” *Journal of Neuroscience Methods*, vol. 167, no. 2, pp. 327–334, 2008.

[24] B. Liu, Y. Tian, Y. Li et al., “Aca attenuates oxidative stress by promoting mitophagy via cb1r/nrf1/pink1 pathway after subarachnoid hemorrhage in rats,” *Oxidative Medicine and Cellular Longevity*, vol. 2022, Article ID 1024279, 18 pages, 2022.

[25] X. S. Zhang, Y. Lu, T. Tao et al., “Fucosaxthrin mitigates subarachnoid hemorrhage-induced oxidative damage via sirtuin 1-dependent pathway,” * Molecular Neurobiology*, vol. 57, no. 12, pp. 5286–5298, 2020.

[26] B. Yuan, X. M. Zhou, Z. Q. You et al., “Inhibition of aim2 inflammasome activation alleviates gsdmd-induced pyroptosis in early brain injury after subarachnoid haemorrhage,” *Cell Death & Disease*, vol. 11, no. 1, p. 76, 2020.

[27] A. Shao, H. Wu, Y. Hong et al., “Hydrogen-rich saline attenuated subarachnoid hemorrhage-induced early brain injury in rats by suppressing inflammatory response: possible involvement of NF-κB pathway and nlrp3 inflammasome,” *Molecular Neurobiology*, vol. 53, no. 5, pp. 3462–3476, 2016.

[28] J. Li, J. Chen, H. Mo et al., “Minocycline protects against nlrp3 inflammasome-induced inflammation and p53-associated apoptosis in early brain injury after subarachnoid hemorrhage,” *Molecular Neurobiology*, vol. 53, pp. 2660–2678, 2015.

[29] J. Dai, S. Xu, T. Okada et al., “T0901317, an agonist of liver x receptors, attenuates neuronal apoptosis in early brain injury after subarachnoid hemorrhage in rats via liver x receptors/interferon regulatory factor/p53 upregulated modulator of apoptosis/dynamin-1-like protein pathway,” *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 8849131, 16 pages, 2021.

[30] Y. Liu, J. Qiu, Z. Wang et al., “Dimethylfumarate alleviates early brain injury and secondary cognitive deficits after experimental subarachnoid hemorrhage via activation of keap1-nrf2-are system,” *Journal of Neurosurgery*, vol. 123, no. 4, pp. 915–923, 2015.

[31] X. Zhang, Q. Wu, Y. Lu et al., “Cerebroprotection by salvianolic acid b after experimental subarachnoid hemorrhage occurs via nfr2- and sirt1-dependent pathways,” *Free Radical Biology & Medicine*, vol. 124, pp. 504–516, 2018.

[32] Y. Lu, X. S. Zhang, X. M. Zhou et al., “Peroxiredoxin 1/2 protects brain against h2o2-induced apoptosis after...
subarachnoid hemorrhage,” *The FASEB Journal*, vol. 33, no. 2, pp. 3051–3062, 2019.

[33] T. Zhang, P. Wu, E. Budbazar et al., “Mitophagy reduces oxidative stress via keap1 (kelch-like epichlorohydrin-associated protein 1)/nrf2 (nuclear factor-c2-related factor 2)/phb2 (prohibitin 2) pathway after subarachnoid hemorrhage in rats,” *Stroke*, vol. 50, no. 4, pp. 978–988, 2019.

[34] X. S. Zhang, Q. Wu, L. Y. Wu et al., “Sirtuin 1 activation protects against early brain injury after experimental subarachnoid hemorrhage in rats,” *Cell Death & Disease*, vol. 7, no. 10, p. e2416, 2016.

[35] M. Hernandez-Jimenez, O. Hurtado, M. I. Cuartero et al., “Silent information regulator 1 protects the brain against cerebral ischemic damage,” *Stroke*, vol. 44, no. 8, pp. 2333–2337, 2013.

[36] X. Chen, C. Chen, S. Fan et al., “Omega-3 polyunsaturated fatty acid attenuates the inflammatory response by modulating microglia polarization through sirt1-mediated deacetylation of the HMGB1/NF-κB pathway following experimental traumatic brain injury,” *Journal of Neuroinflammation*, vol. 15, no. 1, p. 116, 2018.

[37] Y. Zhou, S. Wang, Y. Li, S. Yu, and Y. Zhao, “SIRT1/PGC-1α signaling promotes mitochondrial functional recovery and reduces apoptosis after intracerebral hemorrhage in rats,” *Frontiers in Molecular Neuroscience*, vol. 10, p. 443, 2018.

[38] D. Y. Xia, J. L. Yuan, X. C. Jiang et al., “Sirt1 promotes m2 microglia polarization via reducing ros-mediated nlrp3 inflammasome signaling after subarachnoid hemorrhage,” *Frontiers in Immunology*, vol. 12, article 770744, 2021.

[39] M. Liu, K. Jayaraman, T. Giri, G. J. Zipfel, and U. Athiraman, “Role of sirt1 in isoflurane conditioning-induced neurovascular protection against delayed cerebral ischemia secondary to subarachnoid hemorrhage,” *International Journal of Molecular Sciences*, vol. 22, no. 8, p. 4291, 2021.

[40] W. Xu, J. Yan, U. Ocak et al., “Melanocortin 1 receptor attenuates early brain injury following subarachnoid hemorrhage by controlling mitochondrial metabolism via AMPK/SIRT1/PGC-1α pathway in rats,” *Theranostics*, vol. 11, no. 2, pp. 522–539, 2021.

[41] L. Zhao, R. An, Y. Yang et al., “Melatonin alleviates brain injury in mice subjected to cecal ligation and puncture via attenuating inflammation, apoptosis, and oxidative stress: the role of sirt1 signaling,” *Journal of Pineal Research*, vol. 59, no. 2, pp. 230–239, 2015.

[42] F. Zhang, S. Wang, L. Gan et al., “Protective effects and mechanisms of sirtuins in the nervous system,” *Progress in Neurobiology*, vol. 95, no. 3, pp. 373–395, 2011.

[43] L. Raz, Q. G. Zhang, D. Han, Y. Dong, L. De Sevilla, and D. W. Brann, “Acetylation of the pro-apoptotic factor, p53 in the hippocampus following cerebral ischemia and modulation by estrogen,” *PLoS One*, vol. 6, no. 10, p. e27039, 2011.