Ginsenoside-MC1 Alleviates Stroke by Modulating AMPK/SIRT1 Pathway in a Rat Model

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Received October 17, 2023
Accepted April 18, 2024

Summary

Stroke and cerebral ischemia/reperfusion (IR) injury are neurodegenerative conditions characterized by impaired blood flow to specific brain regions, resulting in brain tissue infarction and loss of sensorimotor function. Ginsenoside-MC1 (GMC1) has exhibited diverse effects in reducing various cerebrovascular disorders. Thereby, this study aimed to ascertain the neuroprotective effect of GMC1 against cerebral IR injury in a rat model of middle cerebral artery occlusion (MCAO) and examine the involvement of the AMPK/SIRT1 pathway in mediating this effect. Male Wistar rats (n=60, 250–280g, 12 weeks old) were used to induce cerebral IR through MCAO. GMC1 (10 mg/kg) was administered intraperitoneally for 28 days prior to tissue sampling. The assessment included measurements of cerebral infarct volume, neurological scores using the corner test and adhesive removal test, mitochondrial function indices (mitochondrial ROS, membrane potential, and ATP levels), oxidative stress markers (8-isoprostane and GSH), inflammatory markers (IL-6, IL-10, TNF-α, and p65-NF-κB), and the expression of p-AMPK and SIRT1 proteins. Treatment with GMC1 significantly reduced infarct volume, improved neurological scores, and enhanced mitochondrial function. Additionally, GMC1 administration increased enzymatic antioxidant activity, reduced 8-isoprostane levels, suppressed the inflammatory response, and upregulated p-AMPK and SIRT1 proteins. Notably, inhibiting AMPK with compound C, as an AMPK inhibitor, reversed the positive effects of GMC1 in rats with cerebral IR injury. GMC1 exhibited mitoprotective, antioxidative, and anti-inflammatory actions, providing neuroprotection against stroke outcomes in rats. The underlying mechanism involved the modulation of the AMPK/SIRT1 signaling pathway. Thus, GMC1 demonstrates promise as a potential therapeutic approach for improving the quality of life in stroke patients.

Keywords

Cerebral ischemia/reperfusion • Ginsenoside MC1 • Inflammation • Mitochondria • Oxidative stress • Stroke

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Introduction

Stroke stands as one of the primary contributors to global mortality and long-term disability, posing a significant burden on healthcare systems. Ischemic stroke, resulting from the occlusion of cerebral blood vessels, leads to the deprivation of oxygen and nutrients, ultimately triggering neuronal cell death and neurological dysfunction [1]. The restoration of blood flow, termed reperfusion, is a common treatment strategy to salvage the ischemic brain tissue. However, reperfusion itself can exacerbate tissue injury through the induction of oxidative stress, inflammation, and mitochondrial dysfunction [2,3]. Therefore, developing interventions that target these pathological processes is crucial for effective stroke management [4].

Mitochondrial dysfunction plays a critical role in the progression of ischemia/reperfusion (IR) injury [5]. During ischemia, the lack of oxygen and nutrients impairs mitochondrial respiration, leading to the generation of reactive oxygen species (ROS) and a decline in adenosine triphosphate (ATP) production [6]. Subsequent reperfusion exacerbates mitochondrial dysfunction, triggering a cascade of events that contribute to neuronal

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damage and cell death [7]. The impaired mitochondrial function leads to oxidative stress and release of pro-inflammatory mediators, ultimately resulting in infarct formation and loss of neurological function [8].

Ginsenoside-MC1 (GMC1), a bioactive compound derived from Panax ginseng, has garnered attention for its potential therapeutic effects on various cerebrovascular disorders. GMC1 possesses antioxidant, anti-inflammatory, and anti-apoptotic properties, emerging as a highly promising option for neuroprotection [9]. Studies have indicated the advantageous impacts of GMC1 in ischemic stroke models, suggesting its potential as a viable therapeutic agent for stroke treatment [10,11]. However, the precise mechanisms underlying GMC1's neuroprotective effects in the context of IR injury and its impact on mitochondrial function remain to be elucidated.

Understanding the molecular mechanisms underlying GMC1's actions will provide valuable insights into its therapeutic potential and contribute to the advancement of innovative approaches for stroke management. In this study, we aimed to investigate the effects of GMC1 on stroke outcomes in a rat model of cerebral IR injury. Specifically, we focused on evaluating the impact of GMC1 on mitochondrial function and oxidative stress/inflammatory responses and the involvement of the adenosine monophosphate-activated protein kinase (AMPK)/sirtuin 1 (SIRT1) signaling pathway in this scenario.

Methods and materials

Experimental animals

Sixty male Wistar rats weighing between 250 and 280g and aged 12 weeks were used in this study. The rats were housed in standard laboratory conditions (20-24 °C temperature and 55 % humidity) with a 12-hour light-dark cycle and provided with food and water ad libitum. The animal studies conducted in this research adhered to the guidelines set forth by the National Institutes of Health for the ethical use of laboratory animals (8^{th} Edition, revised 2011). All procedures involving animals were conducted in accordance with ethical guidelines and regulations of the National Key Research and Development Program of China (2020YFC2005404).

Animal grouping

The rats were randomly divided into 5 experimental groups to assess the effects of GMC1 on stroke outcomes. The number of rats in each group (n=60) was determined based on statistical power analysis to ensure adequate sample size for reliable data analysis. Randomization was employed to minimize bias, and the researchers conducting the assessments were blinded to the group assignments to avoid subjective influences on the results. The following groups were included:

(1) Control group: Rats underwent the surgical procedure without middle cerebral artery occlusion (MCAO) induction or GMC1 administration. This group served as the control for baseline measurements;

(2) IR group: Rats underwent MCAO surgery to induce cerebral IR injury without GMC1 administration. This group was used to evaluate the effects of IR alone;

(3) IR+GMC group: Rats received intra-peritoneal injection of 10 mg/kg GMC1 (Ambo Institute, Seoul, Korea) for 28 days and then underwent MCAO surgery. This group was used to investigate the therapeutic effects of GMC1 on stroke outcomes [12];

(4) IR+CC group: Rats received intravenous injection of 0.2 mg/kg/72h compound C (CC; Sigma–Aldrich Inc., USA), as an AMPK inhibitor, for 28 days and then underwent MCAO surgery; and

(5) IR+CC+GMC group: Rats received GMC1 as in the IR+GMC1 group plus compound C as in the IR+CC group and then underwent MCAO surgery. This group was incorporated to assess the role of the AMPK pathway in the favorable effects of GMC1.

At 24 h after surgery, 6 rats per group underwent the assessment of cerebral infarct volume. At this time point, 6 rats from each group were subjected to neurological scoring and then were sacrificed under deep anesthesia and their brain tissues were collected for further evaluation of molecular and biochemical parameters.

Construction of MCAO model

The MCAO model was employed to induce cerebral IR injury in the rats, mimicking the pathophysiology of stroke. The surgical procedure was performed under aseptic conditions and with careful attention to minimize potential complications. First, rats were anesthetized using an intraperitoneal injection of ketamine (60 mg kg^{-1}) and xylazine (10 mg kg^{-1}) mixture. Following anesthesia, the rats were positioned in a supine posture on a heating pad to ensure a constant body temperature during the procedure. The neck area was carefully shaved and disinfected using an antiseptic solution. A midline incision was performed in the neck

area to provide visibility and access to the right common carotid artery, external carotid artery, and internal carotid artery. The connective tissue and muscles were gently separated to allow clear visualization of the carotid arteries. The external carotid artery was then ligated, and a nylon monofilament with a silicone-coated tip was inserted through the external carotid artery into the internal carotid artery until resistance was felt. The monofilament remained in place for 30 min to induce cerebral ischemia, after which it was gently withdrawn to allow reperfusion. Rats in the Control group underwent an identical surgical procedure, but without the insertion of the filament. After the surgery, the incision was carefully sutured, and the rats were provided with a warm, calm, and controlled environment for their recovery. The rats were then returned to their housing facilities and closely monitored during the post-operative period.

Assessment of cerebral infarct volume

After 24 h of reperfusion, the rats were deeply anesthetized by injecting a mixture of ketamine (60 mg kg^{-1}) and xylazine (10 mg kg^{-1}) intraperitoneally. Transcardial perfusion was performed using heparinized saline followed by 4 % paraformaldehyde. Brains were carefully removed from the skull and were post-fixed in 4 % paraformaldehyde for 24 h and then transferred to 30 % sucrose solution for cryoprotection. The frozen brains were coronally sectioned on a cryostat, producing slices with a thickness of 20 μm. A series of sections were collected at standardized intervals throughout the infarcted region. Brain sections were subjected to incubation in a 2 % solution of 2,3,5-triphenyltetrazolium chloride (TTC) prepared in phosphate-buffered saline (PBS) at 37 °C for 30 min. Following incubation, brain sections were washed with PBS to remove excess TTC solution. Sections were fixed on glass slides and cover slipped using a mounting medium. Digital images of the stained brain sections were captured using a bright-field microscope equipped with a digital camera. The infarcted area (pale, unstained region) and the contralateral hemisphere were outlined manually using image analysis software. The infarcted area was quantified using planimetry techniques. To calculate the infarct volume, the areas from all sections were summed and multiplied by the section thickness.

Neurological scoring

The corner test and adhesive removal test were employed to assess the neurological function of the rats. The corner test evaluated sensorimotor function, while the adhesive removal test assessed tactile sensation and motor coordination. Scoring was performed by trained observers blinded to the experimental groups.

- *Corner Test.* The purpose of using the corner test was to evaluate sensorimotor and postural asymmetries. In this test, rats were positioned between two plastic boards arranged at a 30° angle towards a corner. When a mouse ventured further into the corner, its vibrissae were stimulated to encourage it to rear up and then turn to one side. In healthy rats, the frequency of turning to either side was equal, whereas rats that underwent MCAO surgery tended to turn more towards the same side as the brain injury. The number of turns was recorded over 10 trials to determine the average percentage of turns towards the ipsilateral side.
- *Adhesive removal test.* The adhesive removal test is a widely employed method to assess tactile sensation and motor coordination in rodents. This test was conducted following 24 h reperfusion for assessing the rats' ability to detect and remove adhesive stimuli from their forelimbs as follows: Small adhesive patches were applied to the rats' forelimbs in a symmetrical manner. The rats were placed in an enclosed testing arena. The rats' behavior and response to the adhesive stimuli were observed and recorded. The time taken by the rats to detect and remove the adhesive stimuli from each forelimb was measured. The adhesive removal time was recorded for each forelimb separately. A maximum cutoff time (120 s) was set to prevent excessive stress or potential tissue damage. Changes in adhesive removal time served as indicators of neurological deficits or improvements following GMC1 treatment in the MCAO model.

Assessment of mitochondrial function

- *Brain mitochondria isolation.* At 24 h after surgery, rats were deeply anesthetized by injecting a mixture of ketamine (60 mg kg−1) and xylazine (10 mg kg−1) intraperitoneally. Transcardial perfusion was performed using ice-cold PBS to remove blood contaminants. The brain was quickly removed and placed in ice-cold isolation buffer to preserve mitochondrial integrity. The ischemic hemisphere was dissected from the brain

using a sterile blade. The dissected tissue was homogenized using a tissue grinder in ice-cold isolation buffer. The homogenate underwent centrifugation at 1,300×g for 3 min to eliminate cell debris and nuclei. The resulting supernatant was collected and subjected to another round of centrifugation at 10,000×g for 10 min to obtain the crude mitochondrial fraction. The resulting mitochondrial pellet underwent washing with isolation buffer and was centrifuged once more to achieve a purified mitochondrial fraction. The pellet containing mitochondria was then resuspended in a storage buffer for further analysis. Protein concentration in the mitochondrial suspension was determined using Bradford assay.

- *Measurement of mitochondrial ROS production.* The mitochondrial ROS production in isolated brain mitochondria was measured using a fluorogenic probe. The mitochondrial fractions were incubated with DCFDA (2',7'-dichlorofluorescin diacetate) for 30 min at 37 °C in the dark, which selectively binds to mitochondrial ROS. The emitted fluorescence from the probe was quantified using a fluorescence microplate reader with excitation at 480 nm and emission at 530 nm. The fluorescence intensity was quantified, and higher fluorescence levels indicated increased mitochondrial ROS production.
- *Assessment of mitochondrial membrane potential.* Mitochondrial membrane potential reflects the integrity and functionality of the mitochondria. Changes in mitochondrial membrane potential were measured using a fluorescent dye 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1). The brain mitochondrial fractions were incubated with the membrane potential-sensitive dye in the dark at room temperature. After a 10-20 min, the fluorescence emitted by the dye was detected using a fluorescence microplate reader. In order to determine the changes in mitochondrial membrane potential, the ratio of red (aggregates) to green (monomers) fluorescence was calculated.
- *Quantification of ATP levels.* ATP levels were measured to assess mitochondrial energy production and function. A commercially available ATP assay kit (Sigma, USA) was employed to quantify ATP content in the brain mitochondrial fractions based on the manufacturer's guidelines. Samples were mixed with the ATP assay reagent, and luminescence intensity was measured using a luminometer. To determine the ATP levels in the samples, a standard curve was created using known ATP concentrations.

Measurement of oxidative stress markers

The levels of 8-isoprostane, a marker of lipid peroxidation, and the activity of enzymatic antioxidant glutathione (GSH) were measured via enzyme-linked immunosorbent assay (ELISA) method using specific assay kits (Randox Laboratories, Ltd). These assays were conducted following the manufacturer's protocols.

Measurement of inflammatory markers

The inflammatory response was evaluated by measuring the levels of pro-inflammatory and anti-inflammatory markers in brain tissue samples. The concentrations of interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), and nuclear factor kappa B (NF-κB) were quantified using commercially available ELISA kits following the guidelines given by the manufacturer's (MyBioSource, San Diego, USA).

Western blot analysis

Protein expression levels of p-AMPK and SIRT1 were analyzed through Western blotting. Protein samples were extracted from the brain tissue using ice-cold lysis buffer (Beyotime Biotechnology, China). The protein concentration was assessed utilizing the bicinchoninic acid method. Equal amounts of protein were separated by gel electrophoresis and transferred to a nitrocellulose membrane. The membrane was blocked and then incubated with primary antibodies specific to p-AMPK (Thr172), AMPK, SIRT1, and β-actin as a loading control (diluted at 1:1000; Sigma, USA). After incubation with secondary antibody (diluted at 1:2500; Sigma, USA), the protein bands were made visible utilizing enhanced chemiluminescence that generated a detectable signal. Quantification was performed using densitometry analysis software to compare protein expression levels between experimental groups.

Statistical analyses

The data obtained in the present work were reported as mean ± standard deviation (SD). Data were

assessed by one-way analysis of variance (ANOVA) and Tukey post hoc test. Statistical significance was determined with a P-value less than 0.05 ($P < 0.05$).

Results

GMC1 alleviated infarct volume in IR-injured brains of rats

The effects of GMC1 on cerebral infarct volumes were assessed to evaluate its neuroprotective potential in the context of MCAO-induced cerebral IR injury. Treatment with GMC1 significantly lessened the infarct volumes in comparison with the IR group ($p<0.001$). The mean infarct volume in the GMC1-treated group was 46±5.9 %, whereas the IR group exhibited a mean infarct volume of 28±2.4 %. Notably, the inhibitory effect of GMC1 on infarct volumes was reversed upon administration of compound C, an inhibitor of AMPK ($p<0.01$). The mean infarct volume in the IR+CC+GMC group was 40±4.9 %, which was not significantly different from the IR group. These results indicate that the neuroprotective effect of GMC1 on cerebral infarct volumes was dependent on AMPK activity (Fig. 1A).

GMC1 improved neurological function following MCAO-induced cerebral IR injury in rats

The corner test and adhesive removal test were conducted to assess the effects of GMC1 on neurological function in rats subjected to MCAO-induced cerebral IR injury (Figs. 1B and 1C). In the corner test, GMC1-treated rats exhibited improved neurological scoring in comparison with the IR group ($p<0.001$). However, co-administration of compound C attenuated the beneficial effects of GMC1 on neurological function ($p<0.01$). Similar results were found in the adhesive removal test, where GMC1-treated rats showed significant improvement in sensory and motor function in comparison with the IR group ($p<0.01$). When compound C was co-administered, it reversed the beneficial effects of GMC1 on sensory and motor function ($p<0.05$).

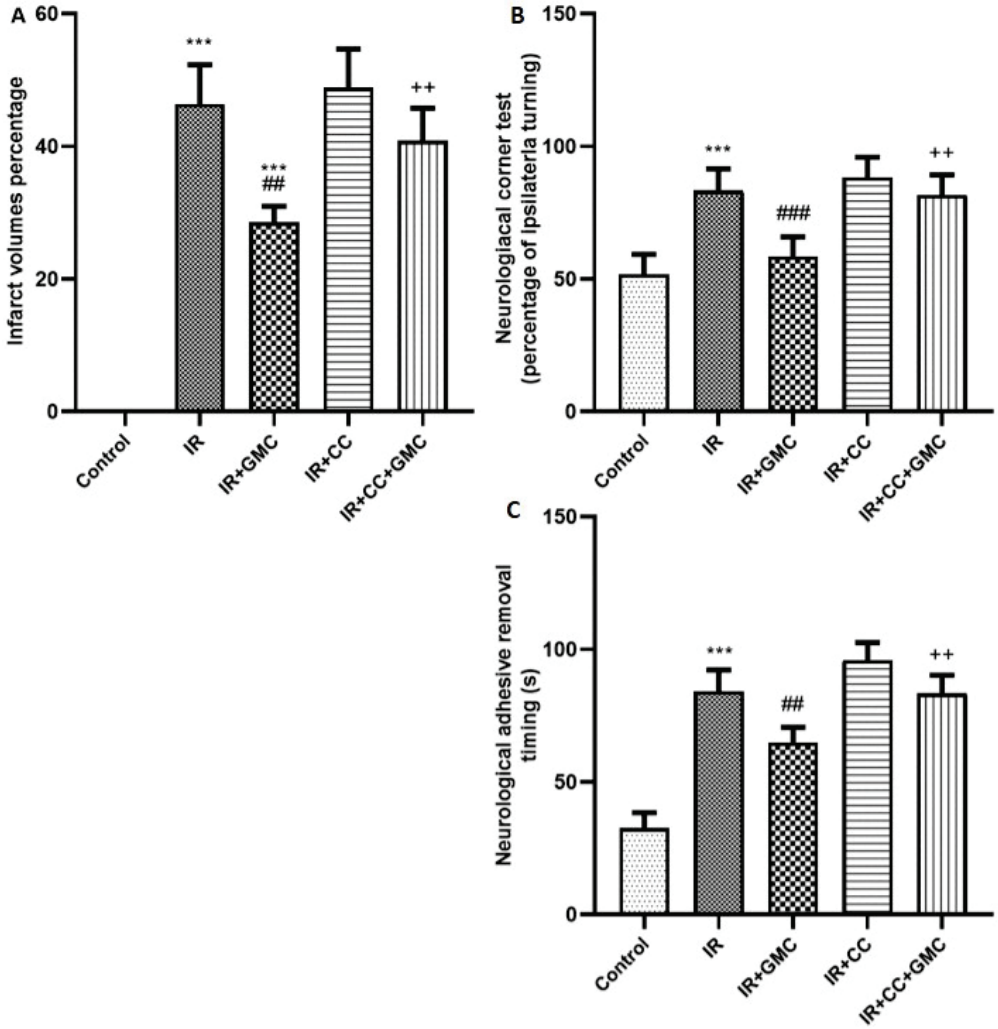

Fig. 1. GMC1 alleviated infarct volume and improved neurological function following MCAO-induced cerebral IR injury in rats. Effect of ginsenoside MC1 (GMC) and compound C (CC), as an AMPK inhibitor, on the cerebral injury **(A)** and neurological function tests **(B** and **C)** in rats with IR injury. The data were expressed as mean ± SD (n = 6 per group). (*** $P < .001$ vs. Control group, ## $P < .01$, and ### $P < .001$ vs. IR group, ++ $P < .01$ vs. IR+GMC group). IR: ischemia-reperfusion.

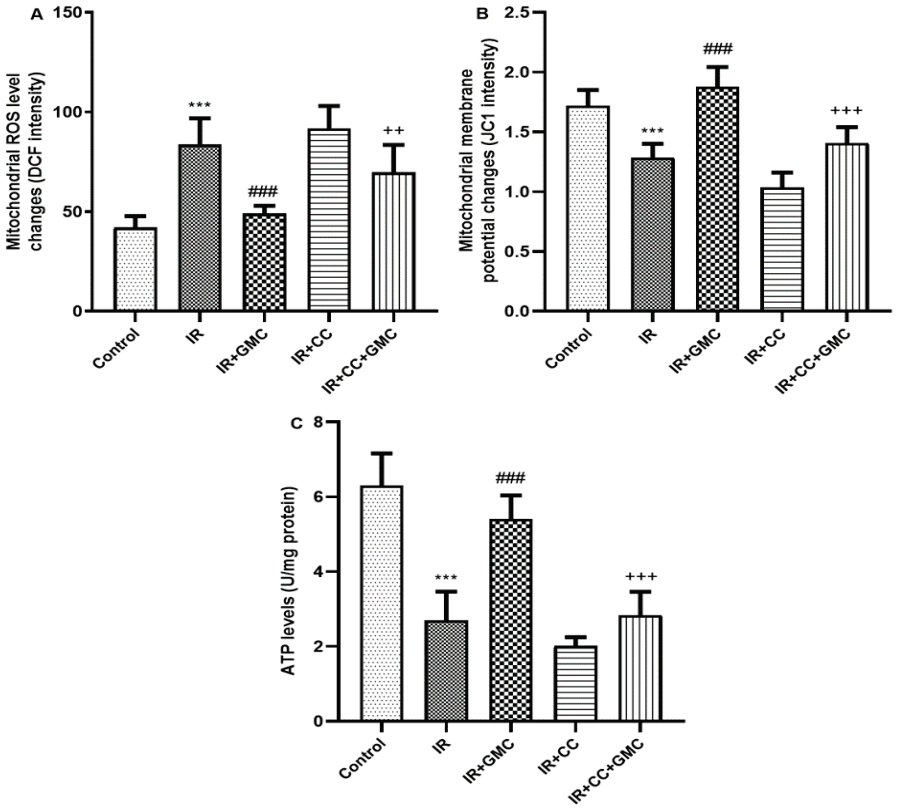

Fig. 2. GMC1 improved mitochondrial function in IR-injured brains of rats. Effect of ginsenoside MC1 (GMC) and compound C (CC), as an AMPK inhibitor, on the mitochondrial ROS **(A)**, mitochondrial membrane potential **(B)**, ATP **(C)** levels in rats with IR injury. The data were expressed as mean ± SD (n = 6 per group). (*** P < .001 vs. Control group, ### P < .001 vs. IR group, ++ P < .01, and +++ P < .001 vs. IR+GMC group). IR: ischemia-reperfusion.

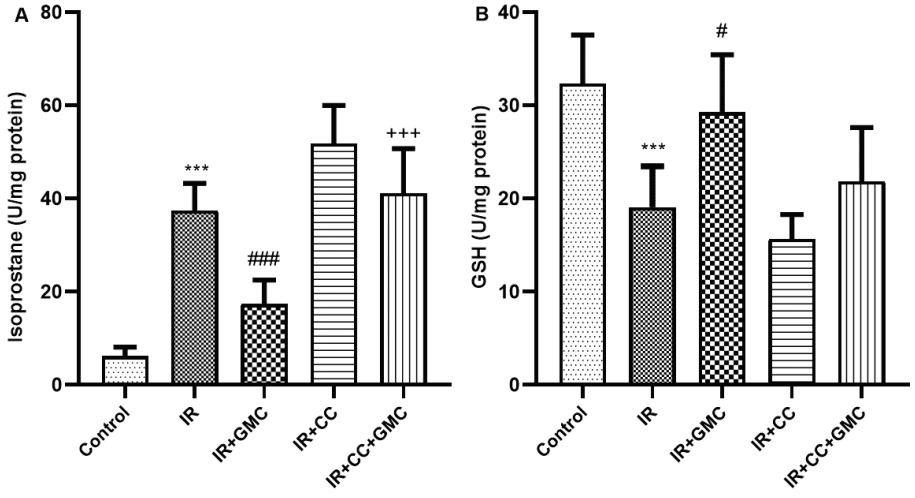

Fig. 3. GMC1 decreased oxidative stress in IR-injured brains of rats. Effect of ginsenoside MC1 (GMC) and compound C (CC), as an AMPK inhibitor, on the cerebral isoprostane **(A)** and GSH **(B)** levels in rats with IR injury. The data were expressed as mean ± SD (n = 6 per group). (*** P < .001 vs. Control group, # P < .05, and ### P < .001 vs. IR group, +++ P < .001 vs. IR+GMC group). IR: ischemia-reperfusion.

GMC1 improved mitochondrial function in IR-injured brains of rats

The mitochondrial ROS levels, membrane potential, and ATP levels were examined to assess the effects of GMC1 on mitochondrial function in rats subjected to MCAO-induced cerebral IR injury. Treatment with GMC1 significantly improved mitochondrial function parameters compared to the IR group (Figs. 2A-C). GMC1-treated rats exhibited decreased mitochondrial ROS levels ($p<0.001$), indicating reduced oxidative stress (Fig. 2A). GMC1 treatment also led to an improvement in mitochondrial membrane potential ($p<0.001$), suggesting enhanced mitochondrial integrity and functionality (Fig. 2B). Nevertheless, co-administration of compound C attenuated the improvement in ROS levels and mitochondrial membrane potential induced by GMC1 ($p<0.01$). Additionally, GMC1 treatment significantly increased ATP levels ($p<0.001$), indicating improved mitochondrial energy production (Fig. 2C). However, the beneficial effect of GMC1 on ATP levels was also abolished upon administration of compound C ($p<0.001$). These findings

demonstrate that GMC1's positive effects on mitochondrial function are dependent on AMPK activation in cerebral IR injury.

GMC1 decreased oxidative stress in IR-injured brains of rats

The levels of 8-isoprostane, a marker of lipid peroxidation, and the activity of enzymatic antioxidant glutathione (GSH) were examined to assess the effects of GMC1 on oxidative stress in rats subjected to MCAO-induced cerebral IR injury. GMC1 treatment resulted in elevated levels of GSH ($p<0.05$), suggesting increased antioxidant capacity. Additionally, GMC1 pretreatment significantly reduced 8-isoprostane levels ($p<0.001$), a marker of lipid peroxidation and intracellular oxidative stress. However, the inhibitory effect of GMC1 on 8-isoprostane levels was abolished upon administration of compound C ($p<0.001$) (Figs. 3A and 3B).

GMC1 alleviated inflammatory response in IR-injured brains of rats

The levels of IL-6, IL-10, TNF-α, and NF-κB were evaluated to assess the effects of GMC1 on inflammatory response in rats subjected to MCAO-induced cerebral IR injury. As shown in Figs. 4A-D, treatment with GMC1 significantly reduced the levels of pro-inflammatory markers, IL-6, TNF-α, and p65-NF-κB ($p<0.001$), and increased the level of anti-inflammatory cytokine, IL-10 ($p<0.001$) demonstrating suppression of the inflammatory response and a shift toward an anti-inflammatory state in cerebral IR injury. Nonetheless, co-administration of compound C resulted in a significant reversal of the anti-inflammatory effects of GMC1 ($p<0.01$).

GMC1 induced neuroprotection by targeting AMPK/SIRT1 signaling pathway in IR-injured brains of rats

Protein expression levels of p-AMPK and SIRT1 were examined to assess the effects of GMC1 on AMPK/SIRT1 signaling pathway in rats subjected to MCAO-induced cerebral IR injury. Western blotting analysis revealed that pretreatment with GMC1 significantly enhanced the phosphorylation levels of

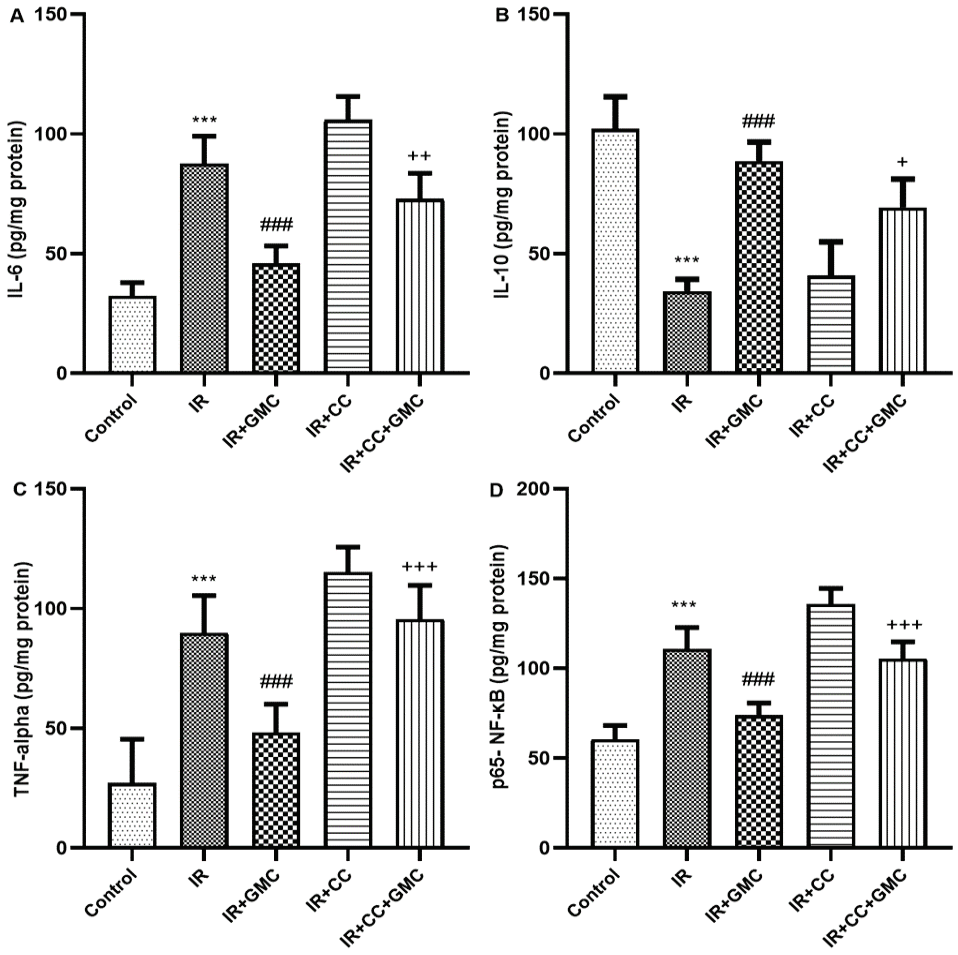

Fig. 4. GMC1 alleviated inflammatory response in IR-injured brains of rats. Effect of ginsenoside MC1 (GMC) and compound C (CC), as an AMPK inhibitor, on the cerebral IL-6 **(A)**, IL-10 **(B)**, TNF-alpha **(C)**, and p65-NF-κB **(D)** levels in rats with IR injury. The data were expressed as mean ± SD (n = 6 per group). (*** P < .001 vs. Control group, ### P < .001 vs. IR group, ++ P < .01, and +++ P < .001 vs. IR+GMC group). IR: ischemia-reperfusion.

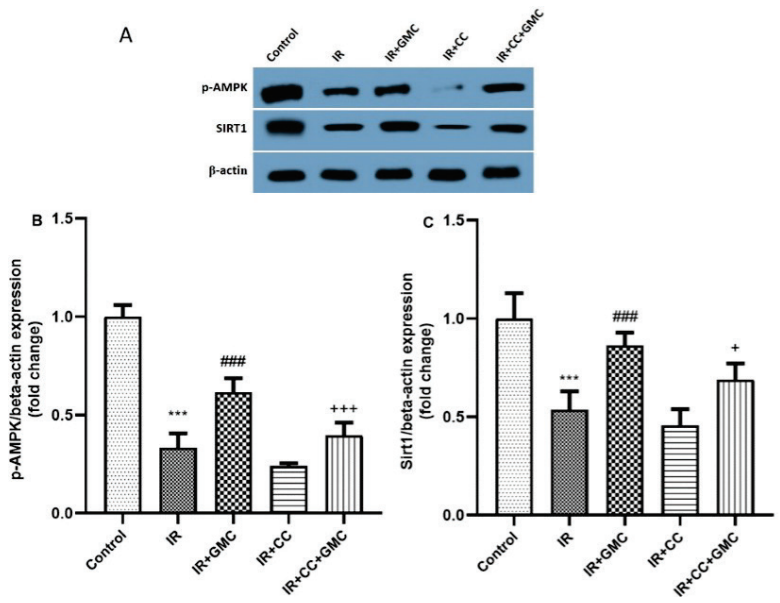

Fig. 5. GMC1 induced neuroprotection by targeting AMPK/SIRT1 signaling pathway in IR-injured brains of rats. Effect of ginsenoside MC1 (GMC) and compound C (CC), as an AMPK inhibitor, on the representative immunoblots **(A)**, pAMPK **(B)**, and Sirt1 **(C)** protein expression levels in rats with IR injury. The data were expressed as mean ± SD (n = 6 per group). (*** P < .001 vs. Control group, ### P < .001 vs. IR group, + P < .01, and +++ P < .001 vs. IR+GMC group). IR: ischemia-reperfusion.

SIRT1 ($p<0.001$). However, the modulation of AMPK and SIRT1 protein expression induced by GMC1 was reversed in the presence of compound C ($p<0.001$ and $p<0.05$). The findings suggest that GMC1 exerts neuroprotective benefits by engaging the AMPK-SIRT1 pathway, and these effects are dependent on AMPK activity (Figs. 5A and 5B).

Discussion

The current investigation was designed for exploring the potential neuroprotective impact of GMC1 pretreatment against cerebral IR damage in rats and elucidating the underlying mechanisms involved. The findings presented in this work demonstrated that GMC1 pretreatment markedly decreased infarct volume and improved neurological function following cerebral IR injury in rats. Notably, GMC1 pretreatment effectively improved mitochondrial function and prevented IR-induced cerebral oxidative stress and inflammatory response. These protective effects of GMC1 were attributed partly to the modulation of AMPK/SIRT1 signaling pathway.

Stroke constitutes a significant contributor to global disability and mortality, and there is a need for potent neuroprotective strategies to improve outcomes in stroke patients [13]. Mitochondrial dysfunction is a hallmark of cerebral IR injury, contributing to energy depletion, oxidative stress, and neuronal death [14]. Previous studies have demonstrated that AMPK activation improves mitochondrial function by promoting mitochondrial biogenesis, enhancing ATP production, and reducing mitochondrial ROS generation. AMPK activation stimulates mitochondrial respiration and improves the efficiency of oxidative phosphorylation, leading to enhanced cellular energy status and protection against ischemic stroke. AMPK activation can stimulate SIRT1 activity, and in turn, SIRT1 can potentiate AMPK signaling to promote cellular energy balance and mitochondrial health [15-17]. In this study, GMC1 pretreatment resulted in improved mitochondrial function, as evidenced by decreased mitochondrial ROS levels, enhanced mitochondrial membrane potential, and increased ATP levels. These findings show that GMC1 may preserve the integrity and performance of mitochondria, providing neuroprotection against cerebral ischemic injury. The AMPK-dependent mechanism underlying the mitochondrial improvements observed in this study is in line with previous reports that have highlighted the role of AMPK activation in maintaining mitochondrial homeostasis and function [18-21].

Oxidative stress is an important pathological factor in cerebral IR damage, leading to cellular damage and neuronal death [22]. During ischemia, the lack of oxygen and energy supply results in impaired mitochondrial function, leading to increased ROS production. Reperfusion following ischemia leads to the rapid influx of oxygen, resulting in the generation of additional ROS. The sudden increase in ROS production overwhelms the endogenous antioxidant defenses, leading to oxidative damage [23,24]. Activation of AMPK during cerebral IR injury can modulate oxidative stress by inhibiting ROS production and promoting antioxidant defenses. AMPK activation can stimulate SIRT1 activity

by increasing the cellular nicotinamide-adenine dinucleotide (NAD^+)/NADH ratio, while SIRT1 activation can enhance AMPK activity through deacetylation and activation of AMPK kinase (AMPKK) [25,26]. This reciprocal interaction between AMPK and SIRT1 creates a positive feedback loop, amplifying their protective effects against oxidative stress and cerebral IR injury [27]. Overall, the AMPK/SIRT1 pathway plays a significant role in mitigating oxidative stress and protecting against cerebral IR injury. Activation of AMPK and SIRT1 can modulate multiple cellular processes involved in oxidative stress regulation, mitochondrial function, and inflammation, thereby promoting neuronal survival and reducing tissue damage following stroke [28,29]. Our data revealed that GMC1 pretreatment exhibited potent antioxidant effects by enhancing enzymatic antioxidant GSH. Moreover, GMC1 reduced the level of 8-isoprostane, a marker of lipid peroxidation and oxidative stress. These results indicated that GMC1 exerts significant antioxidant effects, thereby protecting against oxidative damage induced by cerebral IR injury. The AMPK-mediated antioxidant effects observed in this study further emphasize the involvement of AMPK signaling in neuroprotection against oxidative stress.

Inflammation assumes a crucial role in the development of cerebral IR damage, contributing to neuronal injury and exacerbating the ischemic cascade [30]. Inflammatory responses play a dual role in cerebral IR injury. While acute inflammation contributes to tissue damage and exacerbates the ischemic injury, resolution of inflammation is crucial for the recovery and repair processes [31]. AMPK activation has been shown to exert anti-inflammatory effects via suppressing the activation of NF-kB and inhibiting the production of pro-inflammatory cytokines. By inhibiting NF-kB signaling, AMPK reduces the transcriptional activity of pro-inflammatory genes, resulting in decreased production of inflammatory mediators [32]. This anti-inflammatory action is further supported by the upregulation of SIRT1, a downstream target of AMPK, which exhibits anti-inflammatory properties by deacetylating NF-kB and inhibiting its transcriptional activity [33]. In the present work, GMC1 pretreatment resulted in decreased levels of pro-inflammatory cytokines, TNF-α and IL-6, and an upregulation of the anti-inflammatory cytokine IL-10. These findings indicate that GMC1 possesses potent anti-inflammatory properties and modulates the inflammatory response in the brain tissue. The findings of this study, where GMC1 pretreatment suppressed the expression of pro-inflammatory cytokines and upregulated the anti-inflammatory cytokine IL-10, are consistent with the reported anti-inflammatory effects of AMPK activation. The AMPK-dependent anti-inflammatory actions observed in this study suggest that GMC1 may suppress the activation of NF-kB, a key regulator of inflammatory responses, through the AMPK/SIRT1 signaling pathway.

AMPK activation has emerged as a promising therapeutic target due to its multifaceted effects on cellular processes that promote neuronal survival and recovery [34]. Through its effects on mitochondrial function and energy metabolism, AMPK activation enhances neuronal resilience and attenuates ischemic damage [35,36]. The improvement in mitochondrial function observed in this study, coupled with the decreased oxidative stress and inflammatory response, underscores the neuroprotective actions of GMC1 through AMPK activation. Furthermore, the anti-inflammatory effects of AMPK activation contribute to neuroprotection by reducing secondary neuronal injury and promoting tissue repair. The mechanistic insights gained from this study indicate that the neuroprotective effects of GMC1 are mediated through the activation of the AMPK/SIRT1 signaling pathway. GMC1 pretreatment resulted in the activation of AMPK and upregulation of SIRT1, a downstream target of AMPK that plays a crucial role in cellular stress responses. Additionally, GMC1 pretreatment decreased NF-kB protein, which is known to regulate inflammatory gene expression. These findings suggest that GMC1 exerts its neuroprotective effects by activating the AMPK/SIRT1 axis, leading to the inhibition of NF-kB signaling and subsequent improvement of mitochondrial function and reduction of inflammation and oxidative stress. Overall, the interrelationship between the AMPK signaling pathway, mitochondrial function, oxidative stress, inflammatory responses, and neuroprotection is tightly intertwined in the context of cerebral IR injury. AMPK activation exerts beneficial effects on mitochondrial function, attenuates oxidative stress and inflammatory responses, and promotes neuroprotection. The findings of this study support the existing literature, highlighting the importance of AMPK activation in mediating the neuroprotective effects of GMC1, ultimately improving outcomes in cerebral IR injury.

Limitations and suggestions

Although the study identified the involvement of

the AMPK/SIRT1 signaling pathway in mediating the neuroprotective effects of GMC1, the precise molecular mechanisms underlying this pathway and its interactions with other signaling cascades remain to be fully elucidated. Additional experiments, such as genetic or pharmacological manipulation of specific pathway components, would provide a more detailed understanding of the molecular mechanisms involved. Also, additional research are required for determining the translational potential of GMC1 in clinical settings. The efficacy, safety, and optimal dosing of GMC1 should be evaluated in preclinical models that better represent the complexity of human stroke. Additionally, the potential interactions of GMC1 with other medications commonly used in stroke treatment should be considered. It should be noted that understanding the transformation of polyphenols into microbial metabolites and their implications for neuroprotection is indeed a crucial area for comprehensive study. The involvement of the gut microbiome in neurodegenerative diseases and stroke, influencing risk factors and disease progression, highlights the significance of unraveling these complex interactions. Moreover, exploring the impact of polyphenol metabolites on the gut microbiome, neurodegenerative diseases, and stroke presents exciting opportunities for developing innovative preventive and therapeutic strategies. Investigating the gut-brain axis and its multifaceted role in health and disease represents a compelling frontier in biological research, offering potential interventions that could revolutionize our approach to managing these conditions [37,38].

Conclusion

To summarize, the present study demonstrated that GMC1 pretreatment exerts significant neuroprotective effects in a rat model of cerebral IR injury. The findings revealed that GMC1 pretreatment reduced cerebral infarct volume and improved neurological function, which were associated with the enhancement of mitochondrial function and attenuation of oxidative stress and inflammatory response. The neuroprotective effects of GMC1 preconditioning were mediated to some extent by activation of the AMPK/SIRT1 pathway in IR brains. It can be suggested that GMC1 holds promise as a therapeutic agent for cerebral IR injury by targeting multiple cellular pathways involved in neuroprotection. By further elucidating the mechanisms and optimizing its therapeutic regimen, GMC1 may contribute to improved outcomes and the quality of life of stroke patients.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The research protocol and all experimental procedures were approved by the National Key Research and Development Program of China (Ethics approval number: 2020YFC2005404).

This study was funded by the National Key Research and Development Program of China.

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