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AMPK/Nrf2/NF-κB Pathway-Dependent Modulation of Oxidative/Inflammatory Responses Mediate Ginsenoside Compound Mc1 Neuroprotective Effect in Diabetic Rats

Qiang Gao[1], Zilun Gao[2], Yaguang Jiao[3], Baoshan Tan[3] and Zhenhui Gao[3]*

[1]*Department of Neurosurgery, Baoji People's Hospital, Baoji, 721000, China*
[2]*Department of Neurosurgery, Xi'an Medical University, Xi'an, 710000, China*
[3]*Department of Neurosurgery, The Jintai Hospital, Baoji, 721000, China*

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QG, and ZH designed the project. All authors performed the experimentations, analyzed and interpreted the data. ZH major contributor in writing the manuscript. All authors read and approved the final manuscript.

Key words
Diabetes, Stroke, Ischemia-reperfusion, AMPK, Oxidative stress, Inflammation

ABSTRACT

Cerebral ischemia-reperfusion (IR) injury is more severe in diabetics, involving various pathophysiology. Ginsenoside compound-Mc1 (GMC1) is a deglycosylated ginsenoside having multi-organ benefits. The purpose of this study was to investigate the neuroprotective effects of GMC1 after IR injury in diabetic rats, and the involvement of inflammatory and oxidative pathways via activation of the 5'-adenosine monophosphate-activated protein kinase (AMPK)/nuclear factor-erythroid 2-related factor-2 (Nrf2) signaling. Type-1 diabetes was induced via streptozotocin injection in male Sprague-Dawley rats. GMc1, 10 mg/kg, was administered intraperitoneally for one month before exposing rats to cerebral IR injury. Cerebral IR was performed through temporary (30 min) blocking of cerebral middle artery followed by 24 h reperfusion. Brain tissue was collected to assess cerebral infarct volumes and histological injury. Pro-inflammatory cytokines and oxidative stress indicators were tested using ELISA and fluorometric techniques. Western blotting was performed to detect the expression of Nrf2 and AMPK activity. GMC1 administration significantly reduced IR-induced cerebral dysfunction, as well as the extent of infarct volumes and histological injury. GMC1 significantly lowered tumor necrosis factor-alpha (TNF-α), interlekin-1β, nuclear factor-κB (NF-κB)-p65, intracellular reactive oxygen species (ROS), and 8-isoprostane levels while raising manganese-superoxide dismutase (mnSOD) levels and Nrf2 and AMPK protein expression. However, inhibiting AMPK activity with compound C substantially attenuated the protective effects of GMC1 in diabetic rats subjected to cerebral IR injury. GMC1 prevented IR-induced cerebral injury in diabetic rats by modulating oxidative stress and inflammatory responses, and AMPK/Nrf2/NF-κB signaling is the key regulator of these neuroprotective actions.

INTRODUCTION

Cerebral ischemia-reperfusion (IR) injury is the result of blood returning to the brain tissues after some duration of ischemia (Lin *et al*., 2016; Wang *et al*., 2020) Oxidative stress and inflammation are particularly important in the initiation of IR and cerebral diseases (Wu *et al*., 2020). Due to the rapid recovery of oxygen during reperfusion, significant reactive oxygen species (ROS) are created, causing substantial damage to the intracellular organelles like mitochondria and subsequent promotion of necrosis and apoptosis (Wu *et al*., 2020; Tauffenberger and Magistretti, 2021). Diabetes mellitus, characterized by hyperglycemia, is a prevalent disorder among patients and has been associated with a poor prognosis following IR injury (De Ponte *et al*., 2021). IR damage to the cerebral tissue is more severe in diabetes patients, resulting in chronic oxidative stress and inflammatory responses (Wang *et al*., 2020). Diabetes impairs cerebral antioxidant capacity, which reduces the cerebro-protective effects of conditioning therapies against cerebral IR injury (De Ponte *et al*., 2021; Wang *et al*., 2020). There are currently no effective treatment options for reducing oxidative stress and inflammation in cerebral cells following IR injury and hence preventing cerebral infarction in diabetic conditions.

The activation of 5' adenosine monophosphate activated protein kinase (AMPK) may minimize the consequences of cerebral IR injury. AMPK dysregulation

* Corresponding author: dr.medpaper45@gmail.com
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also contributes to the onset and progression of diabetic neuropathy (Kim *et al*., 2016). Increased AMPK activity in the cells causes anti-inflammatory and antioxidative phenotypes by targeting the nucleus and mitochondria (Jeon, 2016).

Ginsenoside compounds appears to have anti-diabetic, anti-obesity, and anti-infarction properties (Bai *et al*., 2018; Fan *et al*., 2020). Numerous investigations have focused on individual chemical components generated from ginsenoside and their biological effects (Bai *et al*., 2018; Fan *et al*., 2020). In contrast to glycosylated ginsenosides, deglycosylated ginsenosides such as ginsenoside compound Mc1 (GMC1) are highly absorbed and have higher bioavailability. GMC1 is a newly synthetized ginsenoside that is produced by cloned ginsenosidase from the main ginsenoside Rc (Hong *et al*., 2020). The antioxidative and anti-inflammatory properties of GMC1 have not been fully explored in cerebral disorders. Moreover, recent report suggests that ginsenosides' antioxidative impacts occur via an AMPK-dependent mechanism in hepatic and skeletal muscle cells (Shen *et al*., 2013; Huang *et al*., 2017).

Thus, this study aims to investigate the therapeutic potential of GMC1 in diabetic rats with cerebral IR injury. The study will explore whether GMC1 activates AMPK and nuclear factor-erythroid factor 2-related factor 2 (Nrf2) pathway, and exerts its anti-inflammatory and antioxidative effects in diabetic rats against cerebral IR injury. The novelty of this study lies in the exploration of GMC1, a newly synthesized ginsenoside compound, and its potential therapeutic effects in the diabetic context of cerebral IR injury. The study also focuses on investigating the underlying mechanisms and may provide new treatment options for reducing IR injury outcomes in diabetic conditions, ultimately preventing cerebral infarction.

MATERIALS AND METHODS

Study area

This study was done at the Department of Neurosurgery, Baoji People's Hospital, China from May 2021 to July 2022.

Animals

Sixty male Sprague-Dawley rats (220±25 g) were housed in an animal house with a constant temperature (22±2°C) and 45% humidity, and they were subjected to a 12:12-h light-dark cycle. All rats had free access to normal chow and water. After one week of acclimation, rats were fasted for 10 h to induce diabetes.

Induction of diabetes and experimental grouping

A single 60 mg/kg intraperitoneal injection of streptozotocin (STZ; S0130, Sigma-Aldrich, MO, USA) was used to develop experimental type 1 diabetes. STZ was instantly prepared in 0.1 mol/l citrate buffer (pH 4.5). The tail vein was used to collect blood samples 24 h following STZ injection. Diabetic rats were defined as having a glucose levels more than 16.7 mmol/l. The glucometry (Nova Max Plus, USA) was repeated at the end of diabetic period (after 30 days). The diabetic rats were then randomly allocated into five groups (12 rats per group, 6 rats for assessing infarct volumes and 6 rats for other parameters):Sham, IR, IR+GMC1, IR+CC, and IR+CC+GMC1. GMC1 was intraperitoneally given to the diabetic IR rats for one week at the concentration of 10 mg/dl as the effective dose of this compound in previous reports (Hong *et al*., 2020; Yanwei *et al*., 2022). Compound C (CC) (866405-64-3, Sigma-Aldrich, MO, USA) is a selective inhibitor of AMPK and was injected at 5 mg/dl during GMC1 treatment. The groups receiving no treatments received the same amount of vehicle saline solution. The injection solutions were prepared freshly every day.

Induction of cerebral IR injury

Rats were anesthetized via intraperitoneal injection of 40 mg/kg sodium pentobarbital. Then, the right common carotid artery was exposed, and a nylon thread was inserted into the internal carotid artery through a subtle incision, and the thread was advanced to occlude the origin of middle cerebral artery (MCA). After 30 min of occlusion, the thread was removed and successful reperfusion was confirmed. Finally, the incised areas in the neck were ligated and reperfusion was continued for 24 h. Sham-operated animals underwent the identical procedure but did not have MCA occlusion. Rats were kept on a heating pad until they awoke after the procedure. After a 24-h reperfusion period, the motor function test was performed, blood samples were taken and then the animals were sacrificed. For histological and molecular study, the right cerebral penumbra tissues were removed and split cut into two parts. One part was fixed in 10% formalin for histological evaluations, while the other was stored at -80°C.

Measurement of cerebral infarct volumes

The brains of animals were instantly removed under anesthesia, and were immediately frozen at -20°C. The brain samples were sliced with a thickness of 2mm, stained for 30 min at 37°C with 2,3,5-triphenyl tetrazolium chloride (17779, Sigma-Aldrich, USA) and then left in 4 % (w/v) paraformaldehyde (818715, Merck, Germany) overnight.

The images of slices were captured and examined using Image J software (National Institutes of Health, Bethesda, USA). The infarct regions were represented by the white zones on each slice. Brain infarct volumes were calculated and normalized as percentages of the animal's total brain volume.

Quantification of motor function

The motor function of the animals was tested and quantified using a neurological deficit scoring system at the end of 24-h reperfusion time. More serious brain locomotor damage is indicated by higher scores in this method. Neurological findings were graded using a five-point scale, with zero representing no neurological deficit symptoms, 1 representing failing to fully extend the left paw, 2 representing circling to the left, 3 representing falling to the left, and 4 representing no spontaneous walking and having low levels of consciousness. To lessen experiment bias, each animal was rated by two observers blinded to research groups, and the average values were reported as the final score.

Quantification of inflammatory mediators and oxidative stress markers

The cerebral samples were isolated and homogenized in lysing buffer (Beyotime, Jiangsu, China) and centrifuged at 10,000 rpm. Following the collection of samples' supernatants, the levels of pro-inflammatory cytokines and mediators including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and nuclear factor kappa-light chain enhancer of activated B cells (NF-κB), as well as the levels of oxidative stress markers including 8-isoprostane and manganese superoxide dismutase (mnSOD) were determined using appropriate ELISA kits according to manufacturers' instructions (MyBioSource, Inc., USA). The levels inflammatory mediators and oxidative stress markers were normalized with sample's protein content measured by Bradford method, and the results were presented as mg of sample's protein.

Determination of intracellular ROS levels

The cerebral samples' fresh samples were incubated in a 2 micromole DCFDA dye solved in phosphate buffer solution (287810, Sigma-Aldrich, USA) for 30 min at 37 °C to evaluate intracellular ROS levels. Following that, the excitation and emission absorbance of solutions were fluorometrically measured at 480 and 530 nm, respectively. The resulting values were normalized according to the protein concentrations in the samples.

Western blot

The Western blotting method was used to detect the protein expression of NRF2, AMPK, and GAPDH in cerebral samples. Approximately 20 µg of proteins from each sample were electrophoresed on sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (IPVH00010, Merck Millipore, England). The membranes were subsequently blocked in 5% skim milk for an h with 0.1% Tween-20. The blocked membranes were then exposed to the primary antibodies of target proteins (1:1500, Cell Signaling Technology, USA) at 4°C overnight, followed by washing the membranes with Tris buffer saline (TBS) three times. Then, the horseradish peroxidase (HRP)-conjugated secondary antibody (1:2500, Cell Signaling Technology, USA) was applied for an h to develop specific binding. The membrane was then rinsed in TBS and incubated in the darkroom with the enhanced chemiluminescence reagents (32106, ThermoFisher Scientific, Germany) before being subjected to the X-ray film. The protein bands and their corresponding intensities were observed. Image J software (NIH, USA) was used to detect the protein bands of Nrf2, AMPK which their intensities were then normalized to GAPDH intensity of the samples.

Statistical analysis

The data were presented as mean ± standard deviation. The differences between the groups were evaluated using one-way analysis of variance and the Tukey post hoc test once the normal distribution of data was confirmed. The alpha level of 0.05 was considered as the minimal level of statistical significance.

RESULTS

GMC1 reduced cerebral dysfunction and histology, while CC reversed its effect

We developed a STZ-induced type 1 diabetes model in rats to explore the influence of GMC1 on cerebral IR injury in this model. We confirmed that diabetic rats had hyperglycemia and weight loss compared to normal rats 30 days following STZ treatment (Table I). Figure 1A, B depicts that induction of IR injury in diabetic rats significantly exacerbated rats motor function and increased cerebral infarct volume in comparison to the diabetic sham group ($p<0.001$). On the other hand, treatment of these rats with GMC1 significantly restored the IR-induced changes of cerebral function, and infarct volume as compared to IR group. Importantly, inhibition of AMPK activity through CC considerably abolished the neuroprotective effects of GMC1 in diabetic IR rats ($p<0.05$).

Table I. Blood glucose levels and body weights of diabetic and nondiabetic control rats.

Rats	Blood glucose levels (mM)	Initial body weights (g)	Final body weights (g)
Nondiabetic controls	6.7±1.0	226.3±9.7	277.9±13.0
Diabetics	23.4±2.5***	231.4±10.1	205.8±8.6***

The data was provided as mean ± SD. ***p<0.001 vs. nondiabetic control rats.

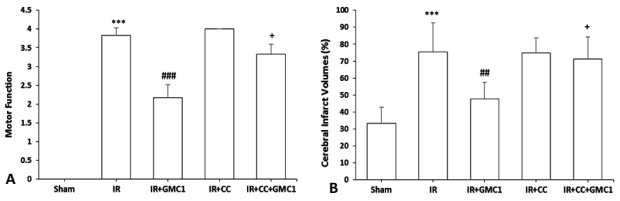

Fig. 1. Cerebral IR injury was prevented by GMC1 treatment in diabetic rats. (A) Motor function, (B) infarct volumes. The data was provided as mean ± SD. ***p<0.001 vs. Sham group, ##p<0.01 and ###p<0.001 vs. IR group, +p<0.05 vs. IR+GMC1 group.

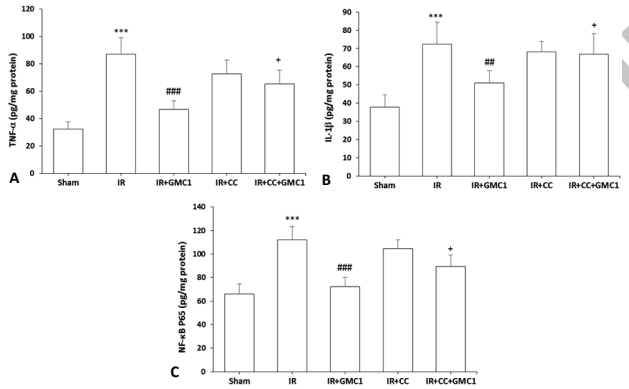

Fig. 2. GMC1 treatment elicited anti-inflammatory properties in diabetic rats with cerebral IR injury. (A) TNF-α, (B) IL-1β, (C) NF-κB-p65. The data was provided as mean ± SD. ***p<0.001 vs. Sham group, ##p<0.01 and ###p<0.001 vs. IR group, +p<0.05 vs. IR+GMC1 group.

GMC1 reduced cerebral inflammatory mediators, while CC reversed its effect

We found a significant rise in the cerebral levels of proinflammatory mediators including TNF-α, IL-1β, and NF-κB-p65 after induction of IR injury in diabetic rats (p<0.001) (Fig. 2). GMC1 treatment substantially reduced TNF-α (p<0.01), IL-1β, and NF-κB-p65 (p<0.001) levels in comparison to those of IR rats. However, co-administration of CC significantly reversed the anti-inflammatory effect of GMC1 in these diabetic rats (p<0.05).

GMC1 reduced cerebral oxidative stress markers, while CC reversed its effect

After inducing IR injury in diabetic rats, cerebral levels of 8-isoprostane increased while levels of mnSOD decreased (p<0.001) as shown in Figure 3A, B. Furthermore, when compared to the IR group, GMC1 significantly lowered 8-isoprostane levels (p<0.05) and increased mnSOD levels (p<0.001). Blockade of AMPK activity considerably attenuated the protective impacts of GMC1 on cerebral oxidative stress markers as compared to the IR+GMC1 group (p<0.05) (Fig. 3).

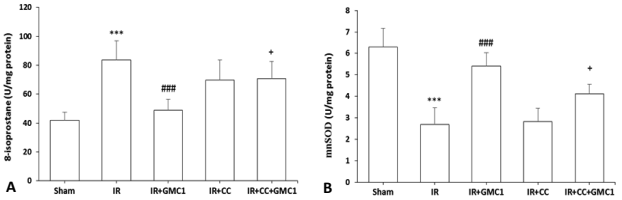

Fig. 3. GMC1 treatment elicited anti-oxidative properties in diabetic rats with cerebral IR injury. (A) 8-isoprostane, (B) mnSOD. The data was provided as mean ± SD. ***p<0.001 vs. Sham group, #p<0.05 and ###p<0.001 vs. IR group, +p<0.05 vs. IR+GMC1 group.

GMC1 reduced cerebral ROS levels, while CC reversed its effect

The oxidative state of the cerebral tissue was also assessed using intracellular ROS measurement through DCFDA method. As shown in Figure 4, the IR group had significantly greater ROS levels than the Sham group (p<0.001). GMC1 treatment effectively decreased IR-induced intracellular ROS increase in diabetic rats (p<0.001). Nonetheless, simultaneous administration of CC blocked the effect of GMC1 on ROS levels (p<0.05).

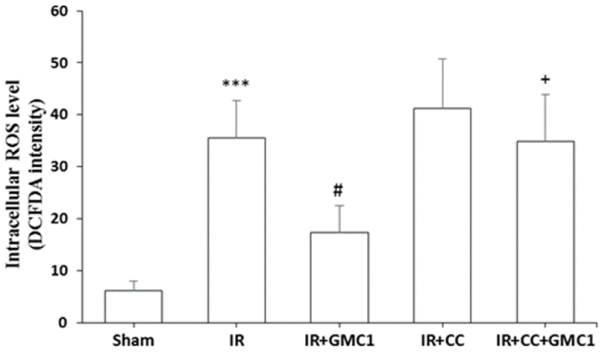

Fig. 4. GMC1 treatment reduced intracellular ROS production in diabetic rats with cerebral IR injury. The data was provided as mean ± SD. ***p<0.001 vs. Sham group, ###p<0.001 vs. IR group, +p<0.05 vs. IR+GMC1 group.

GMC1 upregulated cerebral expression of AMPK and Nrf2 proteins, while CC reversed its effect

Since the protective properties of GMC1 was AMPK signaling-dependent, we decided to measure the effect of GMC1 on the expression of phosphorylated AMPK and its downstream protein Nrf2 in diabetic rats under cerebral IR injury. After IR injury in diabetic rats, phosphorylated AMPK and Nrf2 expression were significantly reduced compared to the Sham group ($p<0.001$). GMC1 treatment upregulated the expression of phosphorylated AMPK in the brain of diabetic rats ($p<0.001$) (Fig. 5A). Nrf2 is a redox-sensitive transcription factor that protects cells from oxidative damage by inducing the production of antioxidant enzymes such as SOD. The expression level of Nrf2 was also enhanced substantially in IR+GMC1 group as compared to the IR group ($p<0.001$) (Fig. 5B). Finally, inhibition of AMPK activity by CC administration significantly reversed the expression of Nrf2 in GMC1-treated diabetic rats ($p<0.01$).

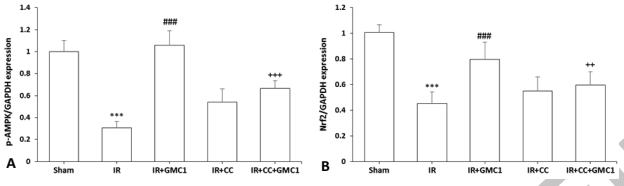

Fig. 5. GMC1 treatment upregulated the expression of p-AMPK and Nrf2 in diabetic rats with cerebral IR injury. (A) p-AMPK expression, (B) Nrf2 expression. The data was provided as mean ± SD. ***$p<0.001$ vs. Sham group, ###$p<0.001$ vs. IR group, ++$p<0.01$, and +++$p<0.001$ vs. IR+GMC1 group.

DISCUSSION

In the current study, we revealed that GMC1 protected cerebral tissue of diabetic rats from IR-induced oxidative stress and inflammatory responses through an AMPK/Nrf2-dependent mechanism. GMC1 treatment reduced brain damage, motor dysfunction and infarct volume in association with increasing cerebral antioxidant capacity and positively regulating the inflammatory response, resulting in decreased pro-inflammatory cytokines and oxidative markers production. Inhibition of AMPK activity substantially reversed the protective impacts of GMC1 on cerebral tissue, suggesting the involvement of AMPK signaling in this protection.

The antioxidative and anti-inflammatory actions of GMC1 have previously been described, to some extent, in other organs such as the heart, skeletal muscle, and liver (Hong *et al*., 2020; Shen *et al*., 2013; Huang *et al*., 2017; Yanwei *et al*., 2022). Our findings further support the idea that this compound possesses antioxidative and anti-inflammatory properties in the brain of diabetic rats suffering from IR injury. Cerebral IR damage has been linked to increased oxidative stress (Lin *et al*., 2016). Diabetes can enhance chronic oxidative stress and boost the basal level of ROS in tissues, exacerbating this condition (De Ponte *et al*., 2021; Wang *et al*., 2020). Following the administration of GMC1 to diabetic rats, the main lipid peroxidation index, 8-isoprostane, as well as intracellular ROS levels, were reduced in the brain tissue, whereas endogenous antioxidant mnSOD was increased, demonstrating the attenuation of IR-induced cerebral oxidative damage in diabetic rats. Inflammatory responses and oxidative stress have essential cross talk in the setting of cerebral IR injury process (Lin *et al*., 2016; De Ponte *et al*., 2021). Excessive ROS generated upon reperfusion might initiate the production of pro-inflammatory cytokines such as TNF-α, and IL-1β, exacerbating IR injury (Wang *et al*., 2020). In addition, previous studies found that the inflammatory responses generated by IR damage are prompted in diabetic rats (De Ponte *et al*., 2021; Zhang *et al*., 2017). Along with these findings, we found that GMC1 administration reduced the production of the pro-inflammatory cytokines TNF-α, and IL-1β, as well as NF-κB-p65, all of which were raised in the brain of diabetic rats following IR damage. NF-κB is a transcription factor, activated by a variety of harmful signals, and regulates central physiological processes including inflammation, oxidative stress, and apoptosis (Liu *et al*., 2017). NF-κB signaling is engaged during the early stages of diabetic neuropathy (Suryavanshi and Kulkarni, 2017), which promotes more cytokine release and inflammatory reactions along with more oxidative stress, augmenting the sensitivity of cerebral cells to IR in diabetic rats.

Another noteworthy aspect of this study was that the neuroprotective effects of GMC1 in diabetic rats are AMPK-dependent. AMPK functions as a primary energy sensor in cells, and its dysregulation leads to the development and progression of diabetic neuropathy and IR injury (Almabrouk *et al*., 2014). AMPK suppresses inflammatory responses by regulating numerous downstream signaling pathways like NF-κB/TNF-α/IL-1β through promoting SIRT1 activity (Chen *et al*., 2016). AMPK can also diminish TNF-dependent leukocyte-endothelial cell interactions and endoplasmic reticulum stress-induced lipid peroxidation, resulting in the improvement of endothelial dysfunction (Chen *et al*., 2016). A recent study found that GMC1 phosphorylates the AMPK protein in rat cardiomyocytes (Hong *et al*., 2020), which is in accord with our finding. As a result, it is reasonable to conclude that the antioxidative and anti-inflammatory effects of

this compound in the brain of diabetic rats subjected to IR injury necessitate proper activation of the AMPK signaling pathway's components. Nrf2 expression was upregulated in diabetic rats with cerebral IR damage after GMC1 treatment. It is activated following AMPK activation and primarily upregulated antioxidant protein production and reduces oxidative insults (Joo *et al*., 2016; Fischhuber *et al*., 2020). Normally, the cytoplasmic protein Kelch-like ECH-associated protein 1 (KEAP1) binds Nrf2 to create a complex that retains Nrf2 at a reasonably stable low-level (Hu *et al*., 2016). When a cell is subjected to oxidative stress, Nrf2 dissociates from KEAP1 and reaches the nucleus, where it activates antioxidant-related genes (Hu *et al*., 2016). As a result, AMPK/Nrf2 signaling is critical in the defense against oxidative stress.

Importantly, basal expression of Nrf2 and AMPK found to be low in diabetic rat tissues, which were further reduced following cerebral IR injury (Wardyn *et al*., 2015). Similarly, we discovered that Nrf2 expression was reduced in diabetic rats with cerebral IR injury, together with its downstream target, mnSOD, signifying that hyperglycemia reduces the antioxidative potential of the Nrf2 pathway. GMC1 on the other hand, completely reversed these effects of IR damage. Furthermore, new evidence suggests that the NF-κB and Nrf2 compete for one other's transcriptional inhibition. For example, Nrf2 activation inhibited the Toll-like receptor-4/NF-κB pathway in a mouse model of hepatic IR (Rao *et al*., 2015). Also, an Nrf2 agonist boosted Nrf2 levels while decreasing NF-κB levels in hyperglycemia (Zhang *et al*., 2016). These findings suggest that Nrf2 activation decreased NF-κB signaling. As a result, we may infer that the proportion of NF-κB and Nrf2 in the cell plays an important role in defining the status of cellular and tissue inflammatory and oxidative responses. Given how the AMPK/Nrf2/NF-κB pathway is disturbed in diabetes, we applied CC to inhibit AMPK activity and then evaluated GMC1 actions. The addition of CC substantially suppressed GMC1's protective effects on the production of Nrf2 and NF-κB-p65, as well as other inflammatory cytokines, oxidative stress, cerebral function, and tissue damage. As a result, our findings indicate that GMC1-induced modulation of the AMPK/Nrf2/NF-κB pathway is responsible for its neuroprotective properties in diabetic rats. However, the effects of GMC1 on additional actors involved in the progression of IR injury, such as cell-cell adhesion molecules, protein kinase C and intracellular Ca^{2+} signaling, autophagy, and mitochondrial dysfunction, need further investigation. Another limitation of this study is that we only used GMC1 after we had confirmed the diabetes incidence. Based on promising studies on its metabolic activities (Roh *et al*., 2020), GMC1 appears to have the potential to lower the occurrence of diabetes in subjects, but further research is needed to fully understand the contribution of this issue on the IR injury outcome. Furthermore, mitochondrial biogenesis and intra-mitochondrial pathways are two of the most critical players in AMPK's cellular impacts, which we did not look at in this work.

CONCLUSION

The findings of this investigation revealed that GMC1 had protective benefits against cerebral IR injury in diabetic rats by improving cerebral function and reducing inflammatory responses and oxidative stress. The modification of the components of Nrf2/NF-κB pathways by GMC1 was linked to these promising outcomes, and AMPK was a key regulator of this protective signaling. However, additional research with a larger sample size is necessary to confirm the neuroprotective impacts of GMC1.

DECLARATIONS

Acknowledgement

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Ethics statement

All animal experiments in this study were carried out in line with the university's guidelines of Laboratory Animal Care and were approved by the Committee for the Use of Animals in Research (ethical No: BH2021-012). This research was approved by Baoji People's Hospital Animal Ethical Committee, approved No: BH2021-012.

Availability of data and material

The data supporting the findings of this study are available upon the request from corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Almabrouk, T.A., Ewart, M.A., Salt, I.P. and Kennedy, S., 2014. Perivascular fat, AMP-activated protein kinase and vascular diseases. *Br. J. Pharmacol*., **171**: 595–617. https://doi.org/10.1111/bph.12479

Bai, L., Gao, J., Wei, F., Zhao, J., Wang, D. and Wei, J., 2018. Therapeutic potential of ginsenosides as an adjuvant treatment for diabetes. *Front. Pharmacol*., **9**: 423. https://doi.org/10.3389/fphar.2018.00423

Chen, B., Li, J. and Zhu, H., 2016. AMP-activated protein kinase attenuates oxLDL uptake in macrophages through PP2A/NF-κB/LOX-1 pathway. *Vasc. Pharmacol*., **85**: 1–10. https://doi.org/10.1016/j.vph.2015.08.012

De Ponte, M.C., Cardoso, V.G. and Gonçalves, G.L., 2021. Early type 1 diabetes aggravates renal ischemia/reperfusion-induced acute kidney injury. *Sci. Rep*., **11**: 19028. https://doi.org/10.1038/s41598-021-97839-7

Fan, W., Huang, Y. and Zheng, H., 2020. Ginsenosides for the treatment of metabolic syndrome and cardiovascular diseases: Pharmacology and mechanisms. *Biomed. Pharmacother*., **132**: 110915. https://doi.org/10.1016/j.biopha.2020.110915

Fischhuber, K., Matzinger, M. and Heiss, E.H., 2020. AMPK enhances transcription of selected Nrf2 target genes via negative regulation of Bach 1. *Front. Cell. Dev. Biol*., **8**: 628. https://doi.org/10.3389/fcell.2020.00628

Hong, S.H., Hwang, H.J. and Kim, J.W., 2020. Ginsenoside compound-Mc1 attenuates oxidative stress and apoptosis in cardiomyocytes through an AMP-activated protein kinase–dependent mechanism. *J. Ginseng. Res*., **44**: 664-671. https://doi.org/10.1016/j.jgr.2019.08.006

Hu, B., Wu, Y. and Liu, J., 2016. Tempol protects against acute renal injury by regulating PI3K/Akt/mTOR and GSK3β signaling cascades and afferent arteriolar activity. *Kidney Blood Press. Res*., **41**: 937–946.

Huang, Q., Wang, T., Yang, L. and Wang, H.Y., 2017. Ginsenoside Rb2 alleviates hepatic lipid accumulation by restoring autophagy via induction of sirt1 and activation of AMPK. *Int. J. mol. Sci*., **18**: 1063. https://doi.org/10.3390/ijms18051063

Jeon, S.M., 2016. Regulation and function of AMPK in physiology and diseases. *Exp. mol. Med*., **48**: e245. https://doi.org/10.1038/emm.2016.81

Joo, M.S., Kim, W.D., Lee, K.Y., Kim, J.H., Koo, J.H. and Kim, S.G., 2016. AMPK facilitates nuclear accumulation of Nrf2 by phosphorylating at serine 550. *Mol. Cell Biol*., **36**: 1931–1942. https://doi.org/10.1128/MCB.00118-16

Kim, Y. and Park, C.W., 2016. Adenosine monophosphate–activated protein kinase in diabetic nephropathy. *Kidney Res. clin. Pract*., **35**: 69-77. https://doi.org/10.1016/j.krcp.2016.02.004

Lin, L., Wang, X. and Yu, Z., 2016. Ischemia-reperfusion injury in the Brain: Mechanisms and potential therapeutic strategies. *Biochem. Pharmacol. Los Angel*., **5**: 213. https://doi.org/10.4172/2167-0501.1000213

Liu, T., Zhang, L., Joo, D. and Sun, S.C., 2017. NF-κB signaling in inflammation. *Signal. Transduct. Target. Ther*., **2**: 17023. https://doi.org/10.1038/sigtrans.2017.23

Rao, J., Qian, X. and Li, G., 2015. ATF3-Mediated NRF2/HO-1 Signaling regulates TLR4 innate immune responses in mouse liver ischemia/reperfusion injury. *Am. J. Transplant*., **15**: 76–87. https://doi.org/10.1111/ajt.12954

Roh, E., Hwang, H.J. and Kim, J.W., 2020. Ginsenoside Mc1 improves liver steatosis and insulin resistance by attenuating ER stress. *J. Ethnopharmacozzl*., **259**: 112927. https://doi.org/10.1016/j.jep.2020.112927

Shen, L., Xiong, Y. and Wang, D.Q., 2013. Ginsenoside Rb1 reduces fatty liver by activating AMP-activated protein kinase in obese rats. *J. Lipid. Res*., **54**: 1430-1438. https://doi.org/10.1194/jlr.M035907

Suryavanshi, S.V. and Kulkarni, Y.A., 2017. NF-κβ: A potential target in the management of vascular complications of diabetes. *Front. Pharmacol.*, **8**: 798. https://doi.org/10.3389/fphar.2017.00798

Tauffenberger, A. and Magistretti, P.J., 2021. Reactive oxygen species: Beyond their reactive behavior. *Neurochem. Res.,* **46**: 77-87. https://doi.org/10.1007/s11064-020-03208-7

Wang, M., Weng, X., Chen, H., Chen, Z. and Liu, X., 2020. Resveratrol inhibits TNF-α-induced inflammation to protect against renal ischemia/reperfusion injury in diabetic rats 1. *Acta Cir. Bras*., **35**: e202000506. https://doi.org/10.1590/s0102-865020200050000006

Wang, Y., Xiao, G., He, S., Liu, X., Zhu, L., Yang, X., Zhang, Y., Orgah, J., Feng, Y., Wang, X., Zhang, B. and Zhu, Y., 2020. Galectin-3 mediated inflammatory response contributes to neurological recovery by QiShen YiQi in subacute stroke model. *Biomed. Pharmacother*., **125**: 109945. https://doi.org/10.3389/fphar.2021.588587

Wardyn, J.D., Ponsford, A.H. and Sanderson, C.M., 2015. Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways. *Biochem. Soc. Trans*., **43**: 621–626. https://doi.org/10.1042/BST20150014

Wu, L., Xiong, X., Wu, X., Ye, Y., Jian, Z., Zhi, Z. and Gu, L., 2020. Targeting oxidative stress and inflammation to prevent ischemia-reperfusion injury. *Front. mol. Neurosci*., **13**: 28. https://doi.org/10.3389/fnmol.2020.00028

Yanwei, Z., Kun, X. and Yanqing, Z., 2022. Ginsenoside-Mc1 reduces ischemia/reperfusion-induced cardiac arrhythmias through activating JAK2/STAT3

pathway and attenuating oxidative/endoplasmic reticulum stress in hyperlipidemic rats. *Turk. J. Biochem*., **2022**.

Zhang, Y., Hu, F. and Wen, J., 2017. Effects of sevoflurane on NF-κB and TNF-α expression in renal ischemia-reperfusion diabetic rats. *Inflamm. Res*., **66**: 901-910. https://doi.org/10.1007/s00011-017-1071-1

Zhang, Y., Yuan, D., Yao, W., Zhu, Q., Liu, Y., Huang, F., Feng, J., Chen, X., Huang, Y., Chi, X. and Hei Z., 2016. Hyperglycemia aggravates hepatic ischemia reperfusion injury by inducing chronic oxidative stress and inflammation. *Oxid. Med. Cell Longev*., **2016**: 3919627. https://doi.org/10.1155/2016/3919627