Ginsenoside protects against AKI via activation of HIF-1α and VEGF-A in the kidney-brain axis

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Received June 17, 2019; Accepted November 29, 2019

DOI: 10.3892/ijmm.2020.4466

Abstract. Acute kidney injury (AKI) is characterized by abrupt kidney dysfunction. It results in remote organ dysfunction, including the brain. The underlying mechanism of the kidney-brain axis in AKI and effective protective approaches remain unknown. The present study aimed to investigate the potential protective effect of ginsenoside (GS) on AKI induced by glycerol in rats. Kidney function was initially assessed by blood urea nitrogen (BUN) and creatinine (Cre) tests, and was identified to be severely impaired following glycerol treatment, based on significant increases in BUN and Cre levels observed. Severe extensive necrosis of the majority of the renal tubules was observed by hematoxylin and eosin staining, additionally confirming that glycerol induced AKI. GS was identified to ameliorate the impairment of kidney function in the context of AKI. Further investigation of the mechanism revealed that GS may induce protection against oxidative stress via a kidney-brain axis. Furthermore, GS improved the activation of hypoxia-inducible factor 1α (HIF-1α) and vascular endothelial growth factor A (VEGF-A) in the hypothalamus response to AKI, and in the kidney tissues. The protective effect of GS in AKI may be associated with the interaction between the kidney and the brain. Taken together, these results suggested that GS was involved in the protective effects against AKI by decreasing oxidative damage to the kidney and brain, and by upregulating HIF-1α and VEGF-A levels in the kidney-brain axis.

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Key words: ginsenoside, hypoxia-inducible factor 1α, vascular endothelial growth factor A, acute kidney injury, kidney-brain axis

Introduction

Acute kidney injury (AKI) is a disease with a high mortality, not only due to renal dysfunction, but also due to remote organ dysfunction, including the brain (1-3). There is an urgent requirement to identify more effective therapies to prevent or treat AKI. The induction of AKI by glycerol has been widely used as a model in experimental studies (4,5). The major pathophysiological process of AKI induced by glycerol is rhabdomyolysis, which induces severe renal toxicity due to oxidative damage, inflammation, endothelial dysfunction, ischemia, cellular and tissue edema, vasoconstriction and apoptosis (3,5,6). It has been demonstrated previously that renal disease may cause oxidative stress, and biochemical and structural changes of the brain through humoral and non-humoral crosstalk (1,7). However, the molecular mechanisms underlying the crosstalk between the kidney and the brain in AKI remain poorly understood.

Ginsenoside (GS) has also been demonstrated to exhibit beneficial effects on the human body, including in brain and renal tissues. GS has been previously investigated as a protective agent against ischemia/reperfusion injury, metabolic disorders, endothelial dysfunction, cardiotoxicity (8-11). Recently, GS has also been demonstrated to be useful to neuronal functions, and to protect the CNS against a variety of types of brain injury (12-14). However, the mechanisms for the protective effects of GS against AKI in the kidney-brain axis remain unclear.

Hypoxia-inducible factor 1α (HIF-1α) is an important modulator of cellular transcriptional response to low oxygen conditions and is actively involved in the hypoxia induced by kidney injury (15) and ischemic brain damages (16). Specifically, the predominant distribution of HIF-1α protein in both the kidneys and brain highlights its essential role in protecting against dysfunction of the kidney-brain axis (17,18). The production of HIF-1α markedly increases in response to stimulation such as renal ischemia/reperfusion injury (19), while knockdown of HIF-1α aggravates ischemic damage (15,20). Collectively, these studies imply that HIF-1α may be involved in the development of renal and brain dysfunction induced by AKI.

Vascular endothelial growth factor A (VEGF-A) is an angiogenesis and vascular permeability factor induced by

hypoxia. The increase in VEGF-A protein levels under hypoxic conditions is partially due to the regulation of HIF-1α (21,22). Several studies have indicated the beneficial effects of VEGF in animal models of brain injury such as ischemic stroke (23) and Alzheimer's disease (24), including antiapoptotic, anti-inflammatory, antioxidant and angiogenic effects. Furthermore, the expression of VEGF and VEGF receptor (VEGFR), which has been observed in the hypothalamus, was observed to be increased following hypoxia (24,25). However, it remains unclear whether HIF-1α and VEGF-A are more extensively involved in the protective effect of GS against AKI in rats.

Accordingly, in the present study, the effect of GS on kidney function and oxidative stress in AKI rats was investigated in the kidney-brain axis. It was hypothesized that GS may rescue the impairment of oxidative stress induced by glycerol injection in the kidney and hypothalamus of rats. Investigation of the protective effect of GS in the kidney-brain axis and the molecular mechanism of its action may suggest a potential therapeutic intervention, and assist in designing novel agents that may attenuate or prevent renal injury.

Materials and methods

Animals and groups. Adult male Sprague Dawley (SD) rats (age, 6-8 weeks) were obtained from the Experimental Animal Center of Dalian Medical University [permit no. SYXK (Liao) 2013-0006] and were housed under controlled temperature (20-25˚C), humidity (40-70%), specific pathogen-free and 12 h light/dark cycle conditions with free access to food and water. All treatment protocols were approved by the Animal Care and Ethics Committee of Dalian Medical University. GS was obtained from Hongjiu Biotech Co., Ltd., and is referred to as the total saponins of *Panax ginseng* in the present study. As described in previous studies (11,26), concentration gradient optimization experiments were performed. By measuring renal function and morphology, the dose of 250 mg/kg/day was selected, based on 4 ml/day, and GS was dissolved in normal saline (NS).

All the rats (n=138) were randomly allocated into 3 groups: NS + NS; AKI + NS; and AKI + GS. The AKI groups received intramuscular injection of 50% glycerol [10 ml/kg body weight (BW)] and the NS + NS group received an injection of NS. GS or NS (2 ml) was administrated intragastrically for 2 consecutive days (twice/day) following glycerol injection, as described in our previous study (26). Rats were sacrificed under general anesthesia using intraperitoneal injection of 4% chloral hydrate (400 mg/kg BW). A total of 30 rats among all 138 rats were involved in the renal function, renal histology and malondialdehyde (MDA) and superoxide dismutase (SOD) analyses at 48 h following GS or NS treatment (3 groups; n=10 per group). Blood samples (1 ml) were collected from the posterior orbital venous plexus. The right kidneys and brains were collected for MDA and SOD analyses. The left kidneys were removed for histopathological analysis, and 18 rats among all 138 rats included in immunohistochemistry analyses (3 groups; n=6 per group). For the western blot analysis, each group was subdivided into 5 subgroups at 0, 6, 12, 24 and 48 h following GS or NS treatment (n=6 per subgroup).

Renal function assays. Blood urea nitrogen (BUN), creatinine (Cre) levels were measured by the Fearon and the Jaffe methods (26), using a urea assay kit (cat. no. C013-1-1) and creatinine assay kit (cat. no. C011-1-1), respectively (both from Nanjing Jiancheng Bioengineering Institute), according to the manufacturer's protocol.

Renal histology. The kidneys were fixed in 10% formalin at room temperature (RT) for 24 h and embedded in paraffin. Paraffinized kidney sections (4 μm) were stained with Hansen's hematoxylin and eosin at RT. After deparaffinization and rehydration, nuclei were stained with hematoxylin at RT for 10 min and the cytoplasm were counterstained with eosin at RT for 5 min. The sections were washed with distilled water in both steps. Then, the sections were dehydrated in graded alcohol, cleared in xylene and mounted. The extent of renal tissue damages was evaluated by the extent of tubular injury, dilatation, vacuolation and necrosis. A total of 5 fields of each slice (3 slides/animal) were randomly selected for a blinded assessment of expression (n=10 per group) using a light microscope (x20 magnification).

MDA and SOD analysis. Renal and hypothalamus tissues were collected at 48 h following GS or NS treatment. Tissue MDA levels were measured using a commercial assay kit (cat. no. A003-1-1; Nanjing Jiancheng Bioengineering Institute). Tissue superoxide dismutase (SOD) activity was determined using the Xanthine oxidase method with a commercial assay kit (cat. no. A001-1-1; Nanjing Jiancheng Bioengineering Institute).

Western blot analysis. Total protein was isolated from brain or kidney tissues at 5 time points following GS or NS treatment using a Total Protein Extraction kit (Nanjing Keygen Biotech Co., Ltd.). Total protein concentrations were quantified by BCA Protein Assay kit (Nanjing Keygen Biotech Co., Ltd.). An equal amount of protein (40 μg) was separated by 10% SDS-PAGE, and transferred to a polyvinylidene difluoride membrane following the manufacturer's protocol. Membranes were incubated with 5% nonfat milk in TBS with 1% Tween-20 (TBST) for 60 min at RT. The primary antibodies were as follows: Rabbit-HIF-1α antibody (1:1,000; cat. no. PB0245; Wuhan Boster Biological Technology, Ltd.); rabbit VEGF-A antibody (1:1,000; cat. no. PB9071; Wuhan Boster Biological Technology, Ltd.); and rabbit β-actin antibody (1:1,000; cat. no. 20536-1-AP; Wuhan Sanying Biotechnology). The membranes were incubated with the primary antibodies at 4˚C overnight, subsequently washed with TBST 3 times (10 min each), and then incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:5,000; cat. no. 31460; Thermo Fisher Scientific, Inc.) for 2 h at RT. The membranes were incubated with ECL reagent (Thermo Fisher Scientific, Inc.) in the dark for 10 min at RT, and the bands were visualized using a Universal Hood II Gel Doc system (Bio-Rad Laboratories, Inc.). The mean grey values of the bands were quantitatively analyzed using Image Lab software (v4.0; Bio-Rad Laboratories, Inc.), and the band values were expressed as target protein/β-actin ratio for each sample.

Immunohistochemical staining. Frozen kidney slices (7 μm) were permeabilized with 3% H_2O_2/methanol for 10 min at

Table I. Effect of GS treatment on renal function in rats.

Biomarkers	NS + NS	AKI + NS	AKI + GS
BUN (mmol/l)	8.94±1.00	23.51±1.47[a]	10.04±0.95[b]
Cre (mmol/l)	95.68±4.32	157.34±11.22[a]	80.00±6.33[b]

AKI rats exhibited a significant increase in BUN and Cre compared with the control group. GS treatment significantly attenuated glycerol-induced rise in BUN and Cre. Data are expressed as mean ± standard error of the mean (n=10). [a]P<0.05 vs. NS + NS group; [b]P<0.05 vs. AKI + NS group. BUN, blood urea nitrogen; Cre, creatinine; NS, normal saline; AKI, acute kidney injury; GS, ginsenoside.

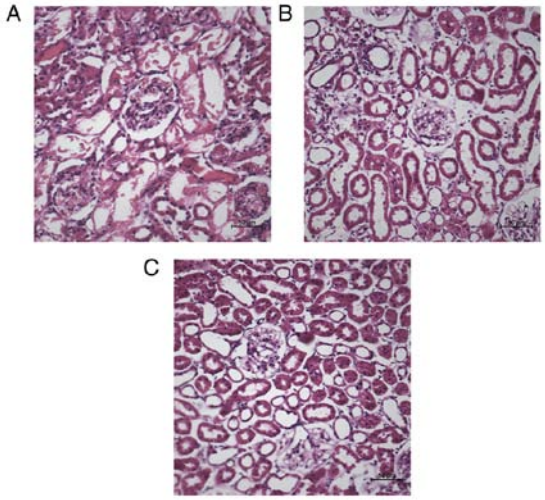

Figure 1. Effect of GS on kidney histological changes by hematoxylin and eosin staining. (A) AKI + NS group, (B) AKI + GS group and (C) NS + NS group. Kidney sections of AKI rats exhibited severe tubular necrosis. GS treatment significantly improved renal morphology compared with that in the AKI + NS group. Scale bar=50 μm. GS, ginsenoside; AKI, acute kidney injury; NS, normal saline.

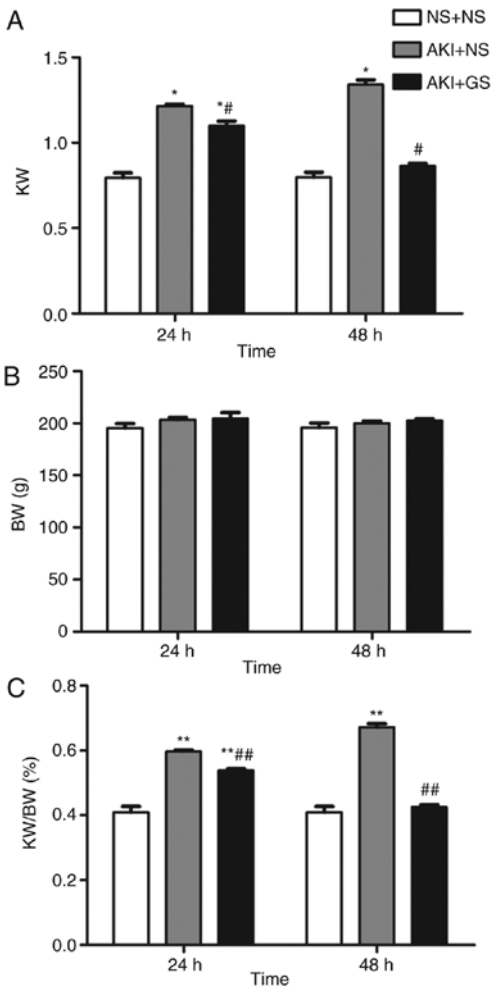

Figure 2. Effect of GS on KW, BW and KW/BW induced by AKI in rats. (A) KW and (C) KW/BW remarkably increased in the AKI + NS group. GS treatment resulted in a significant attenuation of the glycerol-induced increase in KW and KW/BW. (B) BW was unchanged in all groups. Data are expressed as mean ± standard error of the mean (n=10). *P<0.05 and **P<0.01 vs. NS + NS group. #P<0.05 and ##P<0.01 vs. AKI + NS group. NS, normal saline; AKI, acute kidney injury; GS, ginsenoside; KW, kidney weight; BW, body weight.

RT, and blocked with 2% BSA (Sigma-Aldrich; Merck KGaA) for 30 min at RT. Next, sections were incubated at 4˚C overnight with rabbit anti-HIF-1α (1:100) or rabbit anti-VEGF-A (1:100) primary antibodies and then rinsed twice in PBS, and incubated with a HRP-conjugated secondary antibody (ready to use; cat. no. SA1022; Wuhan Boster Biological Technology, Ltd.) for 2 h at RT. The sections were then counterstained with Mayer's hematoxylin for 1 min at RT. The number of positive granules in tissue sections were imaged with a light microscope (x20 magnification; Leica DM 4000 B; Leica Microsystems GmbH) and were semi-quantified by Image-Pro Plus software (v6.0; Media Cybernetics, Inc.). The evaluation method was the same as the aforementioned renal histology analysis (n=6 per group).

Statistical analysis. Each experiment was replicated 3 times. All data are presented as mean ± standard error of the mean and were performed using the SPSS v17 software (SPSS, Inc.). Statistical significance was determined by one-way analysis of variance followed by a Student-Newman-Keuls post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of GS on renal function and structure in glycerol-induced AKI rats. The results presented in Table I indicated that glycerol injection in rats induced a significant increase in the levels of renal function, BUN and Cre compared with the control group (P<0.05). In addition, GS attenuated the changes in BUN and Cre levels induced by glycerol, but the levels of BUN and Cre in the AKI + GS group were significantly increased compared with those in the control group (Table I; P<0.05). Overall, these data suggested that GS may alleviate glycerol-induced renal impairment.

Histologic examination in AKI rats indicated that glycerol treatment induced widespread degeneration and severe necrosis in the majority of renal tubules, disintegration of the tubular epithelial cells, tubular vacuolation and dilatation (Fig. 1A). Concomitantly, GS treatment resulted in decreased

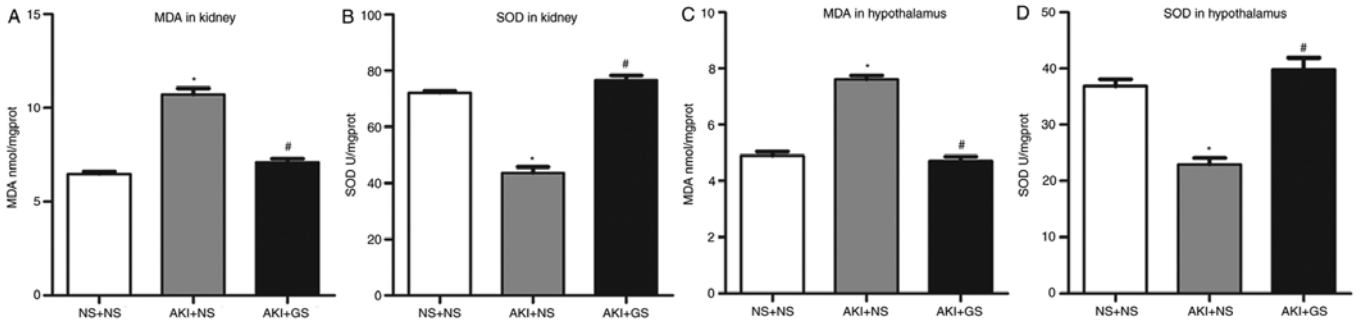

Figure 3. Effect of GS on MDA and SOD of the kidney and the hypothalamus in rats. (A and C) GS significantly decreased the MDA levels in the (A) kidney and (C) hypothalamus tissues following glycerol injection. (B and D) GS treatment markedly increased SOD levels in the (B) kidney and (D) hypothalamus tissues compared with the AKI + NS group. Data were expressed as mean ± standard error of the mean (n=10). *P<0.05 vs. NS + NS group. #P<0.05 vs. AKI + NS group. NS, normal saline; AKI, acute kidney injury; GS, ginsenoside; MDA, malondialdehyde; SOD, superoxide dismutase.

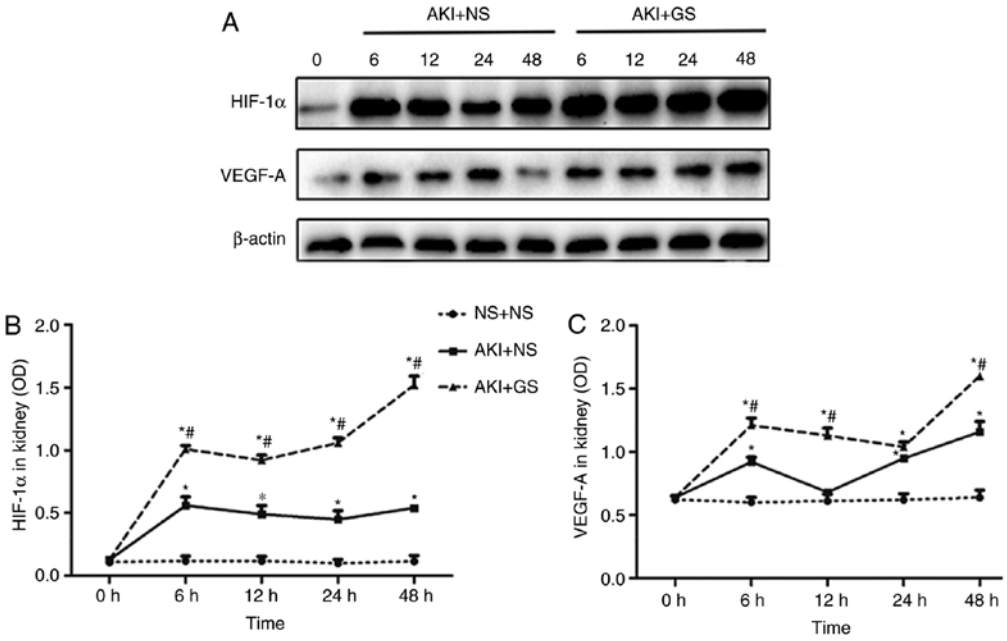

Figure 4. Effect of GS on HIF-1α and VEGF-A protein levels in kidney tissues examined by western blot analysis at 5 timepoints following glycerol injection. (A) The protein levels of HIF-1α and VEGF-A in kidney tissue between 0 and 48 h. (B) Western blot analysis demonstrated that GS treatment significantly increased the alteration of HIF-1α induced by AKI at each time point in the 48 h time frame. (C) Western blot analysis also indicated that the patterns of VEGF-A expression were similar to that of HIF-1α in the kidney tissues. Data are expressed as mean ± standard error of the mean (n=6 at each time point). *P<0.05 vs. NS + NS group. #P<0.05 vs. AKI + NS group. NS, normal saline; AKI, acute kidney injury; GS, ginsenoside; HIF-1α, hypoxia-inducible factor 1α; VEGF-A, vascular endothelial growth factor A; OD, optical density.

pathological changes, renal tubular repair and protected renal tubules from morphological alterations (Fig. 1B). Histological evaluation suggested that glycerol treatment produced significant renal structural abnormalities and functional impairment, and GS may prevent the damage induced by AKI.

Effect of GS on kidney weight (KW), BW and KW/BW ratio. In addition to the morphological changes observed, KW and the ratio of KW to BW (KW/BW) were also altered. The results presented in Fig. 2 indicated that BW was unchanged in all groups at 24 and 48 h. At 24 h, there were significant increases in KW and KW/BW in AKI + NS and AKI + GS groups compared with the control group (P<0.05). GS treatment also decreased KW and KW/BW in the AKI + GS 24 h group compared with the AKI + NS 24 h group (P<0.05), but the KW and KW/BW in AKI + GS 24 h group remained increased compared with those in the control group 24 h (Fig. 2A and C). Similarly, GS treatment inhibited the increase of KW and KW/BW at 48 h (P<0.05), and there was no difference compared with the control group 48 h (Fig. 2B).

Effect of GS on the antioxidative capacity in AKI rats. Kidney MDA level, an index of oxidant capacity, was significantly increased in the AKI + NS group. Rats treated with GS exhibited a significant decrease in kidney MDA level (Fig. 3A).

AKI rats exhibited a significant attenuation of kidney SOD level compared with the AKI + NS and NS + NS groups. GS treatment resulted in a significant increase of SOD levels in the kidney tissues (Fig. 3B). There were no significant differences in kidney MDA and SOD levels between the NS + NS group and AKI + GS group (P>0.05). These data demonstrated that oxidative stress is involved in the impairment of kidney tissues

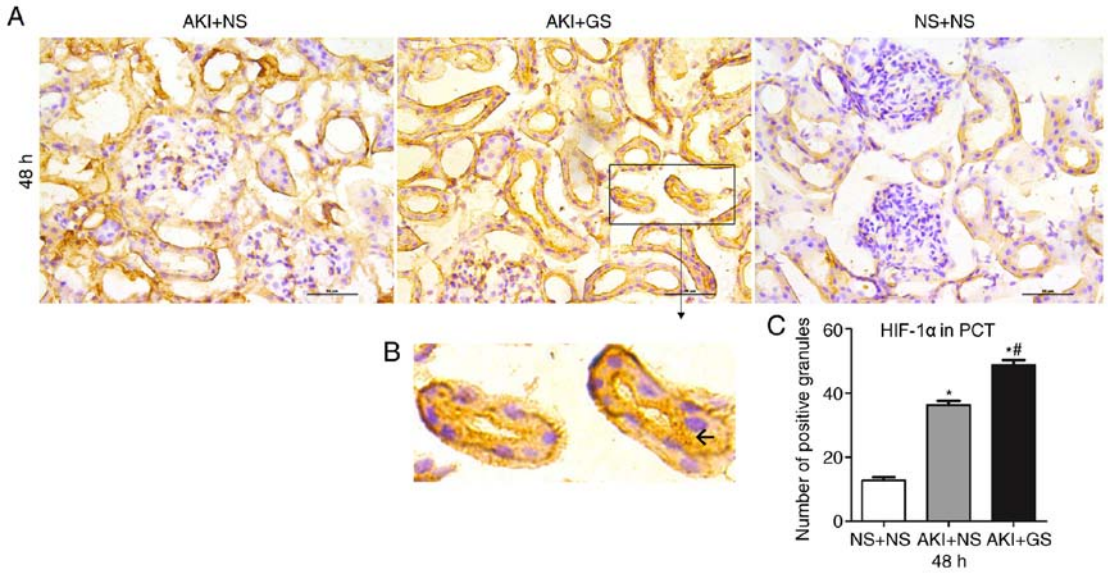

Figure 5. Analysis of the effect of GS on the expression of HIF-1α in the kidney tissues using immunohistochemistry. (A) Expression of HIF-1α in PCT at 48 h. GS treatment significantly increased the alteration of HIF-1α induced by AKI at 48 h. (B) Enlargement of the image inside the black insert shown in (A). Arrows indicate HIF-1α-positive granules in PCT. (C) Quantitative analysis of HIF-1α-positive granules in PCT at 48 h. Scale bar=50 μm. Data are expressed as mean ± standard error of the mean (n=6). *P<0.05 vs. NS + NS group; #P<0.05 vs. AKI + NS group. GS, ginsenoside; HIF-1α, hypoxia-inducible factor 1α; NS, normal saline; AKI, acute kidney injury; PCT, proximal convoluted tubule.

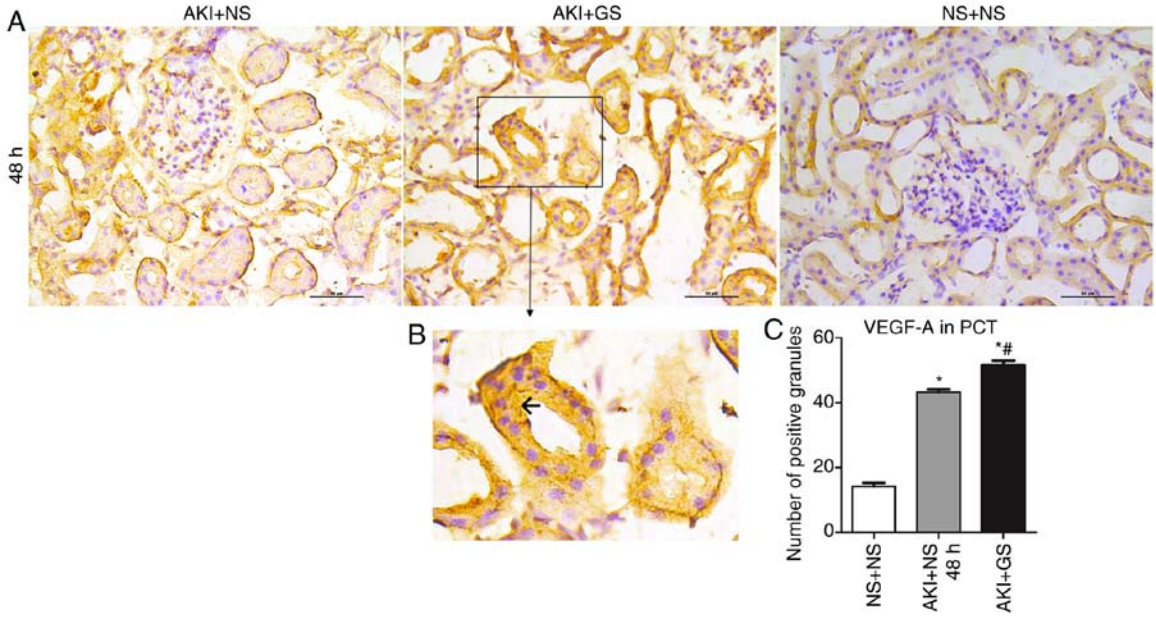

Figure 6. Analysis of the effect of GS on the expression of VEGF-A in the kidney tissues using immunohistochemistry. (A) Expression of VEGF-A in PCT at 48 h. GS treatment significantly increased the alteration of VEGF-A induced by AKI at 48 h. (B) Enlargement of the images inside the black insert shown in (A). Arrow indicates VEGF-A-positive granules in PCT. (C) Quantitative analysis of VEGF-A-positive granules in PCT at 48 h. Scale bar=50 μm. Data are expressed as mean ± standard error of the mean (n=6). *P<0.05 vs. NS + NS group. #P<0.05 vs. AKI + NS group. GS, ginsenoside; VEGF-A, vascular endothelial growth factor A; NS, normal saline; AKI, acute kidney injury; PCT, proximal convoluted tubule.

following glycerol injection, and that GS treatment attenuated the impairment.

In the hypothalamus, the level of MDA and SOD exhibited a similar pattern. MDA levels were increased, whereas SOD levels were decreased in the AKI + NS group. GS reversed the impairments observed in the AKI + GS group (Fig. 3C and D; P<0.05). The results suggested that GS serves a neuroprotective role in AKI rats, presumably by attenuating damage caused by oxidative stress.

Effect of GS on the level of HIF-1α and VEGF-A in kidney tissues of AKI rats. To fully understand the roles of HIF-1α and VEGF-A during AKI, the changes in HIF-1α and VEGF-A expression levels in kidney were investigated by western blot analysis at various time points. A low expression of HIF-1α protein was observed in the kidney at 0 h in all groups, whereas the expression of HIF-1α was markedly increased in the AKI + GS group between 6 and 48 h, compared with the AKI + NS group (Fig. 4A and B). HIF-1α expression in

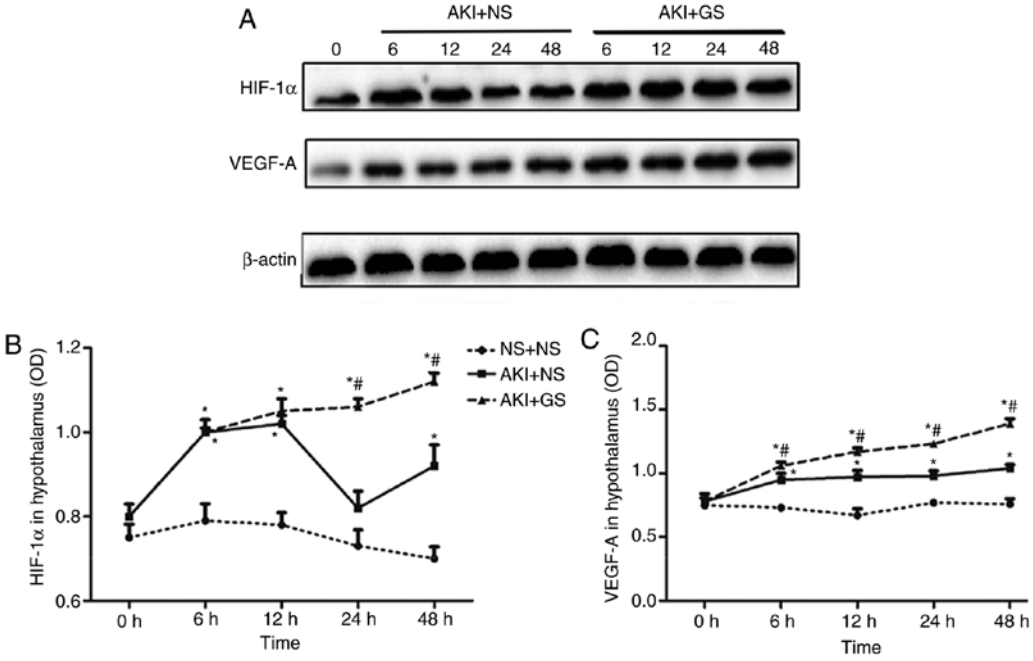

Figure 7. Effect of GS on HIF-1α and VEGF-A protein in the hypothalamus by western blot analysis at 5 time points following glycerol injection. (A) Protein levels of HIF-1α and VEGF-A in the hypothalamus between 0 and 48 h. (B) Western blot analysis demonstrated that GS treatment significantly increased the alteration of HIF-1α induced by AKI in a time-dependent manner. (C) Western blot analysis also revealed that VEGF-A expression was consistent with that of HIF-1α in the kidney tissues. Data are expressed as mean ± standard error of the mean (n=6 at each time point). *P<0.05 vs. NS + NS group. #P<0.05 vs. AKI + NS group. GS, ginsenoside; HIF-1α, hypoxia-inducible factor 1α; VEGF-A, vascular endothelial growth factor A; NS, normal saline; AKI, acute kidney injury; PCT, proximal convoluted tubule; OD, optical density.

the AKI + GS group was increased compared with that of the AKI + NS group at 6 h, and this expression level was sustained between 12 and 24 h, peaking at 48 h (Fig. 4B; P<0.05).

Consistent with the changes in HIF-1α protein levels, the expression levels of VEGF-A protein in the kidney also exhibited similar changes (Fig. 4A and C). The expression of VEGF-A protein in the AKI + GS group was increased compared with the AKI + NS group at 6 h. VEGF-A expression began to decrease slightly at 12 h, increasing again at 24 h, and peaking at 48 h (Fig. 4C). The results demonstrated that the HIF-1α and VEGF-A expression levels were significantly increased in the AKI + NS group, but that they were further increased following GS treatment at 48 h.

In addition, the expression of HIF-1α and VEGF-A in kidney tissues was assessed using immunohistochemistry at 48 h. HIF-1α-positive granules were stained brown and observed primarily in the renal epithelial cells of proximal convoluted tubule (PCT) (Fig. 5B). There were more positive granules in the AKI + GS group compared with the AKI + NS group, and the number of positive granules in the AKI + NS group was markedly increased compared with the control group at 48 h (Fig. 5A and C). Furthermore, VEGF-A expression was also observed in the renal tubular epithelial cells (Fig. 6B). These changes were similar to those observed in HIF-1α expression (Fig. 6A and C).

Effect of GS on the levels of HIF-1α and VEGF-A in the hypothalamus of AKI rats. In the present study, the expression levels of HIF-1α and VEGF-A in the hypothalamus were examined following GS exposure at 6, 12, 24 and 48 h after AKI. The expression levels of the two proteins were increased in the AKI + NS group, and further upregulated in the AKI + GS group at every time point (Fig. 7A-C), similar to the expression of these proteins in the kidney tissues (Fig. 4A-C). These data suggested that upregulation of HIF-1α and VEGF-A in the hypothalamus may contribute to the protective effect of GS against kidney dysfunctions following at 48 h after AKI.

Discussion

The results of the present study confirmed that glycerol impaired renal function and induced AKI, as evidenced by increased BUN and Cre levels and exacerbated renal structural damage. In addition, rats with AKI exhibited notable kidney weight abnormalities. Consistent with previous studies, the results of the present study demonstrated that glycerol injection induced oxidative stress damages, indicated by increased kidney MDA levels and attenuated kidney SOD levels. Oxidative damages induced by glycerol was also observed in the hypothalamus in the present study. These results suggested that AKI not only induced renal dysfunction, but also caused oxidative damages to the brain. The present study provided novel evidence suggesting that AKI may progress from single-organ failure to a multi-organ dysfunction syndrome.

Previous studies have explored the protective role of GS in AKI rats. The neuroprotective and renoprotective effects of GS have been demonstrated in various studies (8,10). GS protected neuron function against oxidative damage, inflammation, ischemia and apoptosis (12,13) and improved cognitive function in memory-impaired mice (12), diabetic mice (27) and ageing mice (14). In the present study, GS was identified to attenuate

AKI-induced oxidative neurotoxicity in the kidney and in the hypothalamus. These data suggested that GS may serve a protective role against AKI in the kidney-brain axis, primarily in an antioxidative capacity.

To further investigate the protective effect of GS in the kidney-brain axis, the potential molecular mechanisms were investigated in the present study.

The response to ischemic or hypoxic conditions may have a causal association with HIF-1α in the development and progression of AKI. The results of the present study indicated that HIF-1α and VEGF-A expression levels were increased in the kidney and hypothalamus tissues during the processes of AKI. HIF-1α regulates the adaptive response to hypoxia and other stresses including glycolysis and angiogenesis (15,17,18,28). VEGF-A is a target gene of HIF-1α, is a survival factor for renal tubule cells and has been implicated in mediating protection against hypoxia and hypoglycemia (29). Recently, HIF-1α/VEGF-A activation has also been demonstrated to have protective effects in multiple animal models of renal injury, and in animal models of cerebral ischemia (28,30,31). VEGF confers neuroprotection by decreasing infarct size, delaying neuronal injury and stimulating angiogenesis in ischemia brain injury (32), diabetic (33). The present study demonstrated that the interaction between HIF-1α and VEGF-A may be involved in the response of the kidney-brain axis to hypoxia following AKI. In addition, HIF-1α and VEGF-A induction in the kidney-brain axis may promote tissue adaptation and survival during renal injury, and this effect may be a self-protective mechanism. However, it is important to note that there were differences between the changes in HIF-1α levels in kidney and hypothalamus tissues in the AKI rats during the 48 h time period. The HIF-1α protein levels in the AKI + NS group in kidney peaked at 6 h, and the hypothalamus HIF-1α protein levels in the AKI + NS group peaked at 12 h. As the interaction between kidney and brain has not been fully clarified, additional studies are required.

In the present study, GS was identified to enhance glycerol-induced upregulation of HIF-1α and VEGF-A in the hypothalamus and kidney. The results indicated that, apart from inhibiting the oxidative stress, the protective effect of GS may partially be attributed to the involvement of HIF-1α and its downstream gene VEGF-A in kidney-brain axis.

The molecular mechanisms underlying these results are not completely understood. Previous studies have demonstrated that the PI3K/Akt pathway was activated in response to hypoxia, resulting in anti-apoptosis and renal cell survival (28,30,31). Whether GS promotes HIF-1α/VEGF-A activation via the PI3K/Akt pathway, anti-apoptosis or mitochondrial involvement requires further investigation.

In summary, the results of the present study demonstrated that GS is a natural inducer of HIF-1α expression, and that it protected the kidney and brain against AKI by decreasing oxidative stress and upregulating VEGF-A. These data provide an improved understanding of the neuroprotective and renal protective role of GS in AKI, and indicate that HIF-1α may be a promising therapeutic target for treating patients with AKI.

Acknowledgements

Not applicable.

Funding

The present study was supported by Scientific Research Cultivation Project, School of Medicine, Jiaxing University and Scientific Research Project of Education Department of Zhejiang Province (grant no. Y201942486).

Availability of data and materials

The data and materials generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

HM performed the majority of the experiments. CJ designed the experiments, interpreted the data and reviewed the manuscript. LX performed the experiments and analyzed the data. DC, HL and YX provided the reagents/materials and technical assistance. KM provided technical assistance. MW designed the experiments, interpreted the data and wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Animal Care and Ethics Committee of Dalian Medical University and performed according to the National Institute of Health Guide for the care and Use of Laboratory Animals.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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