Original Research Article

Aloperine attenuates high glucose-induced oxidative injury in Schwann cells via activation of NRF2/HO-1 pathway

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Abstract

Purpose: To determine the involvement of nuclear factor erythroid 2-related factor 2 (NRF2) and heme oxygenase-1 (HO-1) in the action of aloperine on Schwann cell injury caused by high glucose (HG).
Methods: Cell viability was determined using MTT assay while the release of lactate dehydrogenase (LDH) was determined by biochemical assay. Apoptosis was assessed using flow cytometry, while the levels of malondialdehyde (MDA) were determined by Annexin V-FIT staining. Glutathione S-transferase (GST), glutathione peroxidase (GPX), and reactive oxygen species (ROS) were determined using enzyme-linked immunosorbent assay.
Results: Treatment with HG suppressed RSC96 cell viability and increased LDH release, while aloperine reversed these results (p < 0.05). Apoptosis of RSC96 cells was induced by HG stimulation, but was abolished by aloperine. The levels of ROS, MDA, and GST were enhanced in cells following treatment with HG, but was reversed by aloperine (p < 0.05). The decreased level of GPX caused by HG in RSC96 cells was elevated by aloperine. Moreover, aloperine upregulated NRF2 and HO-1 in RSC96 cells treated with HG (p < 0.05).
Conclusion: Aloperine attenuates HG-induced oxidative injury in Schwann cells via activation of NRF2/HO-1 pathway, suggesting its potential as a potent drug for the management of diabetic peripheral neuropathy.

Keywords: Aloperine, Schwann cells, High glucose, Oxidative stress, NRF2, HO-1

INTRODUCTION

Diabetic peripheral neuropathy (DPN) is considered a common complication of diabetes mellitus (DM, type 2), which is characterized by peripheral nerve dysfunction [1,2]. The symptoms of DPN include pain, paresthesia, and neurological deficits [3]. Schwann cells exert a vital role in peripheral nerves [4], which are also sensitive to hyperglycemia, and the injury in Schwann cells induced by oxidative stress under long-term hyperglycemia contributes to DPN [5]. Schwan cell damage caused by high glucose (HG) may affect nerve conduction velocity, cause

atrophy of axons, and alter axonal regeneration [6]. Thus, it is essential to identify the underlying mechanism of Schwann cell injury caused by HG and to develop promising therapeutic strategies for DPN treatment and prevention.

Oxidative stress is a critical mechanism for HG-induced Schwan cell injury [7]. Some studies have suggested that the nuclear factor erythroid 2-related factor 2 (NRF2)/heme oxygenase-1 (HO-1) pathway is involved in the modulation of oxidative responses [8-10]. In retinal pigment epithelial cells, salvianolic acid A can prevent oxidative stress through activation of the NRF2/HO-1 pathway [8]. This signaling pathway also mediates the HG-induced Schwan cell injury [11], and also participates in the regulatory process of Schwan cell injury induced by HG [11]. Therefore, this signaling pathway is essential for alleviating oxidative injury in Schwann cells.

Aloperine is an alkaloid extracted from *Sophora alopecuroides* [12]. Aloperine has multiple functions including anti-tumor, anti-inflammatory, and antiallergenic activities in some diseases [12,13]. In addition, aloperine also modulates oxidative stress [14]. Xu *et al* reported that aloperine alleviated neuropathic pain by suppressing oxidative stress and NF-κB pathway activation [14].

In a subarachnoid hemorrhage model, aloperine attenuated brain injury by inhibiting oxidative stress through activating the NRF2/HO-1 pathway [15]. Therefore, aloperine may reduce oxidative injury of Schwann cells caused by HG via the NRF2/HO-1 pathway.

Thus, the objective of this study was to investigate the influence of aloperine on the oxidative injury of Schwann cells caused by HG, and to determine the involvement of the NRF2/HO-1 pathway.

EXPERIMENTAL

Cell culture

RSC96 Schwann cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained in DMEM medium with fetal bovine serum (FBS; 10%; Gibco, Grand Island, NY, USA) in an incubator (5% CO_2; 37°C). The cells were cultured for 12 h and were co-treated with 25 mM (low glucose) or 150 mM glucose (high glucose; HG) and 1, 10, or 50 μM aloperine. The chemical formula of aloperine is shown in Figure 1 A.

Cell viability assay

Cells after co-treatments with glucose and aloperine were inoculated into 96-well plates (3 × 10^3 cells/well) for 24 h and added with 20 μL of MTT for another 4 h. Subsequently, the formazan crystals were solubilized by adding 200 μL of dimethyl sulfoxide. Absorbance was determined at 490 nm.

Lactate dehydrogenase (LDH) assay

LDH release from RSC96 cells after co-treatments with glucose and aloperine was measured by an LDH assay kit (Abcam, Cambridge, UK). The absorbance was determined at 490 nm.

Flow cytometry

RSC96 cells after co-treatments with glucose and aloperine were collected, washed, centrifuged, and re-suspended in binding buffer, and then treated with Annexin V-FITC (5 μL), stained with propidium iodide (PI, 5 μL), and quantitated using flow cytometry (BD Biosciences, San Jose, CA, USA).

qRT-PCR

Total RNA was isolated, cDNA was produced, and the qRT-PCR was performed using a SYBR Green Master Mix (Thermo Fisher Scientific, Scotts Valley, CA, USA) with the primer sequences for NRF2 [16] and HO-1 [17] (see Table 1 for primer sequences). The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels [18].

Western blotting

Proteins were extracted, quantified, and the lysates were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes. After blocking, the membranes were probed with anti-NRF2 (dilution, 1:500) (Abcam, Cambridge, UK), anti-HO-1 (dilution, 1:500) (Abcam), and anti-β-actin (dilution, 1:1,000) (Abcam) for 12 h at 4°C, and then incubated with anti-IgG secondary antibody (Abcam). The blots were visualized using an ECL chemiluminescence reagent kit (Beyotime, Shanghai, China). The relative protein levels were determined by normalizing to β-actin.

Enzyme-linked immunosorbent assay (ELISA) and ROS release analyses

After co-treatment with glucose and aloperine, RSC96 cells were harvested and

malondialdehyde (MDA), glutathione S-transferase (GST), and glutathione peroxidase (GPX) levels were determined using ELISA assay kits. ROS production was evaluated using a Cellular ROS assay kit (Abcam).

Statistical analysis

Statistical analysis was conducted using SPSS statistical software (SPSS, Chicago, IL, USA). All the data are shown as mean ± standard deviation (SD). One-way analysis of variance with the least significant difference post hoc test was used to compare differences among multiple groups. $P < 0.05$ was regarded as statistically significant.

RESULTS

Aloperine alleviated HG-induced RSC96 cell injury

To investigate the influence of aloperine on HG-induced cell injury, Schwann cells (RSC96) were co-treated with HG and aloperine. Treatment with HG (150 mM) reduced the viability of RSC96 cells compared to cells treated with 25 mM glucose. However, aloperine dose-dependently increased cell viability ($p < 0.05$; Figure 1 B). Furthermore, LDH release was induced by HG treatment ($p < 0.01$), but was reversed by aloperine ($p < 0.05$; Figure 1 C). These results indicated that aloperine alleviated HG-induced RSC96 cell injury.
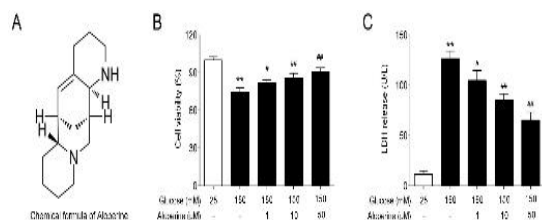

Figure 1: Aloperine reduced RSC96 cell injury induced by high glucose (150 mM). A. The chemical formula of aloperine. **B.** The cell viability was determined using the MTT assay. **C.** The lactate dehydrogenase release in different cell groups; $^{**}p < 0.01$ *vs.* the 25 mM glucose group; $^{#}p < 0.05$ and $^{##}p < 0.01$ *vs.* the 150 mM glucose group

Aloperine inhibited HG-induced RSC96 cell apoptosis.

The apoptosis of RSC96 cells was significantly induced by HG treatment ($p < 0.01$), while this was reversed by 10 µM ($p < 0.01$) and 50 µM ($p < 0.01$) aloperine treatment (**Figure 2**). These results revealed that aloperine inhibited HG-induced RSC96 cell apoptosis.
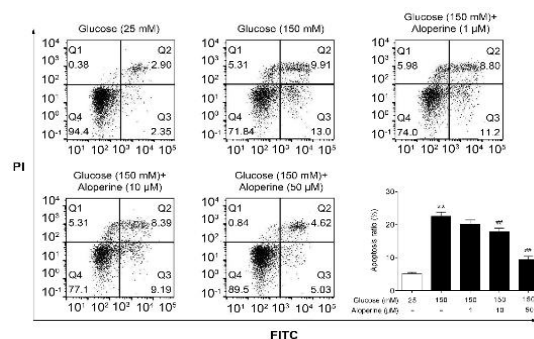

Figure 2: Aloperine inhibited high glucose-induced RSC96 cell apoptosis. Apoptosis was determined using flow cytometry; $^{**}p < 0.01$ *vs.* the 25 mM glucose group; $^{#}p < 0.05$ and $^{##}p < 0.01$ *vs.* the 150 mM glucose group (high glucose)

Aloperine reduced HG-induced oxidative stress in RSC96 cells.

To further investigate the influence of aloperine on HG-induced Schwann cell injury, oxidative stress was evaluated. The levels of ROS and MDA in RSC96 cells were increased by HG ($p < 0.01$), which were dose-dependently inhibited by aloperine (all, $p < 0.01$, Figure 3 A and B). Moreover, the levels of GST were increased by HG ($p < 0.01$), and were dose-dependently reversed by aloperine ($p < 0.05$; Figure 3 C). The level of GPX was decreased in RSC96 cells by HG, and was also dose-dependently reversed by aloperine ($p < 0.05$; Figure 3 D). Together, these results showed that aloperine remediated HG-induced oxidative stress in RSC96 cells.
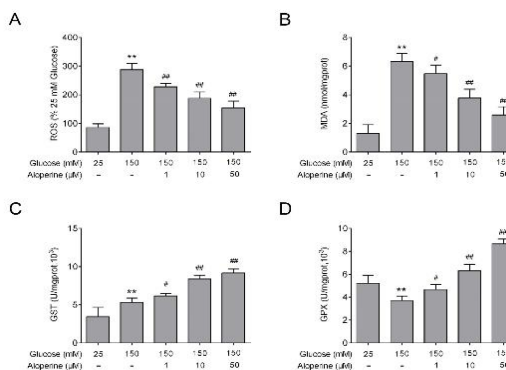

Figure 3: Aloperine reduced the oxidative stress in RSC96 cells caused by high glucose (150 mM). Levels of ROS (**A**), MDA (**B**), GST (**C**), and GPX (**D**) in RSC96 cells were evaluated using ELISA assays; $^{**}p < 0.01$ *vs.* the 25 mM glucose group; $^{#}p < 0.05$, and $^{##}p < 0.01$ *vs.* the 150 mM glucose group. ROS = reactive oxygen species; MDA = malondialdehyde; GST = glutathione S-transferase; GPX = glutathione peroxidase

Aloperine increased NRF2/HO-1 pathway activities in RSC96 cells caused by HG.

To determine the underlying mechanism of aloperine on HG-induced cell injury, the levels of NRF2 and HO-1 were determined. The mRNA levels of NRF2 and HO-1 in RSC96 cells were increased by HG treatment (all $p < 0.01$), and were further increased after treatment with 1 μM, 10 μM, and 50 μM aloperine (Figure 4A and 4B, $p < 0.05$ or $p < 0.01$). In addition, western blotting results showed that the protein levels of NRF2 and HO-1 were increased in RSC96 cells by HG treatment ($p < 0.01$), and were further increased after treatment with 1 μM, 10 μM, and 50 μM aloperine (Figure 4C and 4D, $p < 0.05$ or $p < 0.01$). Hence, aloperine activated the NRF2/HO-1 pathway in RSC96 cells cultured with HG.
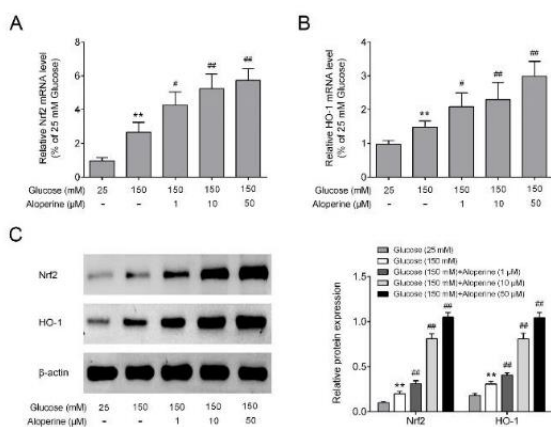

Figure 4: Aloperine activated NRF2/HO-1 pathway in RSC96 cells induced by high glucose (150 mM). The mRNA (A, B) and protein levels (C, D) were determined using qRT-PCR assays or western blotting; [**]$p < 0.01$ *vs.* the 25 mM glucose group; [#]$p < 0.05$ and [##]$p < 0.01$ *vs.* 150 mM glucose group. NRF2 = nuclear factor erythroid 2-related factor 2; HO-1 = heme oxygenase-1

DISCUSSION

Schwann cell injury caused by oxidative stress under long-term hyperglycemia is a critical factor for DPN [5,6]. Hence, it is essential to find effective therapeutic methods to treat DPN.

In our study, the role of aloperine in Schwann cell injury caused by HG was investigated, showing that aloperine increased cell viability and decreased LDH release in RSC96 cells cultured in HG. These results were consistent with previous reports [19,20]. A study showed that aloperine elevated cell viability and reduced neuronal injury stimulated by oxygen-glucose deprivation and reperfusion [19]. The increased LDH release induced by H_2O_2 exposure was

blocked by aloperine in a cell model of Alzheimer's disease [20]. Likewise, our study revealed that aloperine alleviated RSC96 cell injury induced by HG.

Studies have further revealed that HG leads to apoptosis of Schwann cells [21,22]. The progression of DPN can be alleviated by inhibiting the apoptosis of Schwann cells [22]. Therefore, the protection of aloperine from apoptosis of RSC96 cells caused by HG was determined in this study. The results indicated that increased apoptosis of RSC96 cells caused by HG was dose-dependently reversed by aloperine. A study reported that aloperine decreased the levels of apoptosis-related proteins, Bax and Bcl-2, in pulmonary hypertension induced by monocline [23]. However, aloperine was found to induce cancer cell apoptosis [24]. For example, aloperine inhibited cancer progression by inducing prostate cancer cell apoptosis [24]. This result indicated that the influences of aloperine on cell apoptosis might vary among different cells.

Oxidative stress plays critical roles in the Schwan cell injury induced by HG [7]. The effects of aloperine on oxidative stress were thus investigated. The results showed that the major indicators of oxidative stress, including ROS, MDA, GST, were increased, while GPX was decreased in RSC96 cells after HG treatment, but this was reversed by aloperine. Thus, in Schwan cells aloperine alleviated oxidative stress induced by HG, which was similar to previous studies [23,25]. Aloperine attenuated alterations in oxidative stress biomarkers induced by monocrotaline in pulmonary hypertensive rats [23]. Aloperine also reduced oxidation activity induced by H_2O_2 [25]. Therefore, these results indicated that aloperine alleviated HG-induced oxidative stress in RSC96 cells.

It is known that NRF2/HO-1 signaling also participates in the regulatory process of Schwan cell injury induced by HG [11]. Thus, the NRF2/HO-1 pathway might mediate the effect of aloperine on Schwann cell injury caused by HG. In our study, aloperine increased NRF2/HO-1 pathway activity in RSC96 cells induced by HG. In previous studies, aloperine inhibited allergic airway inflammation via activating the NRF2/HO-1 pathway [26]. In the subarachnoid hemorrhage model, aloperine activated the NRF2/ARE pathway and ameliorated early brain injury [15].

CONCLUSION

Aloperine attenuated HG-induced oxidative injury in Schwann cells via activating the NRF2/HO-1

pathway. Thus, aloperine may be an effective drug for the treatment of DPN.

DECLARATIONS

Acknowledgement

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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