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ORIGINAL ARTICLE

Salidroside Protects Against 6-Hydroxydopamine-Induced Cytotoxicity by Attenuating ER Stress

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Abstract Parkinson's disease (PD) is a neurodegenerative disease characterized by a persistent decline of dopaminergic (DA) neurons in the substantia nigra pars compacta. Despite its frequency, effective therapeutic strategies that halt the neurodegenerative processes are lacking, reinforcing the need to better understand the molecular drivers of this disease. Importantly, increasing evidence suggests that the endoplasmic reticulum (ER) stress-induced unfolded protein response is likely involved in DA neuronal death. Salidroside, a major compound isolated from *Rhodiola rosea L.*, possesses potent antioxidative stress properties and protects against DA neuronal death. However, the underlying mechanisms are not well understood. In the present study, we demonstrate that salidroside prevents 6-hydroxydopamine (6-OHDA)-induced cytotoxicity by attenuating ER stress. Furthermore, treatment of a DA neuronal cell line (SN4741) and primary cortical neurons with salidroside significantly reduced neurotoxin-induced increases in cytoplasmic reactive oxygen species and calcium, both of which cause ER stress, and cleaved caspase-12, which is responsible for ER stress-induced cell death. Together, these results suggest that salidroside protects SN4741 cells and primary cortical neurons from 6-OHDA-induced neurotoxicity by attenuating ER stress. This provides a rationale for the investigation of salidroside as a potential therapeutic agent in animal models of PD.

Keywords Parkinson's disease · ER stress · Salidroside · 6-OHDA · Neuroprotection

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a slow, progressive decrease in the number of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and an accumulation of intracellular Lewy body inclusions. Recently, accumulating evidence in PD models has indicated that protein folding and cytoplasmic Ca^{2+} homeostasis are disrupted in the development of PD, suggesting that endoplasmic reticulum (ER) stress may be involved in driving the progression of PD [1].

The ER is an essential organelle that regulates protein folding, protein maturation, cytoplasmic Ca^{2+} homeostasis, and cell survival [2]. ER dysfunction has been implicated in the pathological processes of many diseases [3]. In response to stress signals that disturb ER function and cellular Ca^{2+} homeostasis, an accumulation of misfolded proteins in the ER triggers an unfolded protein response (UPR) [4]. The UPR initially occurs as a response to the accumulation of unfolded proteins in the ER lumen to restore the protein folding ability of the ER, but a progressive UPR may trigger both protective effects and cell death [5]. Three independent UPR signaling pathways have been discovered. These pathways are mediated by the RNA-activated protein kinase R-like ER kinase (PERK), transcription factor 6, and inositol-requiring enzyme 1 (IRE1). Each pathway is activated during ER stress and requires the ER chaperone binding immunoglobulin

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protein (BiP, also known as GRP78). Chronic or severe activation of the UPR promotes the induction of caspase-12-mediated apoptosis [6].

Increasing evidence indicates that persistent oxidative stress results in apoptosis *via* the induction of ER stress. Oxidative stress is considered to be a disruption of the balance between reactive oxygen species (ROS) production and antioxidant capacity and can trigger apoptosis by activation of the ER stress response [7]. ER stress has been described as an early or initial response of cells to stress or damage [8]. ROS accumulation also results in disruption of cytosolic Ca^{2+} regulation. As Ca^{2+} plays a crucial role in neuronal excitability, functional protein structure, and neurotransmitter release, while also acting as a second messenger [9], this disruption can be disastrous. ROS accumulation induces Ca^{2+} release from the ER. The increased cytosolic Ca^{2+} in turn stimulates mitochondrial metabolism to produce more ROS [10]. Finally, increased Ca^{2+} concentration and accumulated ROS promote activation of the UPR pathway and, in certain contexts, promote caspase-12-mediated apoptosis [11]. 6-OHDA is a neurotoxin widely used to build PD models and has been reported to induce ROS release and disturb Ca^{2+} homeostasis, resulting in ER stress and decreased cell viability [12, 13].

Salidroside (p-hydroxyphenethyl-β-*D*-glucoside; $C_{14}H_{20}O_7$) is extracted from *Rhodiola rosea* L. and has many pharmacological actions, including anti-oxidative, anti-aging, anti-cancer, and neuroprotective effects [14, 15]. Previous studies have reported that salidroside protects against apoptosis in both PC12 cells with MPP^+-induced mitochondrial dysfunction [16] and *in vivo* models of PD [17]. However, few studies have investigated whether salidroside has a protective effect against 6-OHDA-induced ER dysfunction. In this study, we addressed this question in a DA neuronal cell line (SN4741), and dissected the underlying mechanisms.

Materials and Methods

Chemicals and Antibodies

The chemicals used were salidroside (25 μmol/L, 10 h, National Institute for the Control of Pharmaceutical and Biological Products, China), 6-OHDA (10 μmol/L, 4 h, MH116, Sigma-Aldrich, St. Louis, MO), tunicamycin (5 μmol/L, 24 h, #0652, Sigma-Aldrich), 4-phenylbutyrate (4-PBA, 2 mmol/L, 1 h, sc-200652, Santa Cruz, CA), DAPI (1:500, D9542, Sigma-Aldrich), and Fluo-3 AM (5 μmol/L, 30 min, s1056; Beyotime, Shanghai, China). A TUNEL staining kit (QIA39, Merck, Darmstadt, Germany) was used for apoptosis detection. The antibodies used for Western blotting and immunocytochemistry were anti-BiP (1:1000, ab108613, Abcam, Cambridge, UK), anti-PERK (1:1000, 3192, Cell Signaling, Boston, MA), anti-p-PERK (1:500, orb6693, Biorbyt, UK), anti-IRE1 (1:1000, 3294, Cell Signaling), anti-p-IRE1 (1:1000, Novus Biologicals, Littleton, CO), anti-cleaved caspase-12 (1:1000, ab13970, Abcam), and anti-β-actin (1:2000, 4967; Cell Signaling).

Cell Culture

SN4741 cells derived from mouse embryonic SNpc were cultured at 33 °C with 5% CO_2 in high-glucose Dulbecco's minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum, 1% *D*-glucose, 1% penicillin–streptomycin, and 2 mmol/L *L*-glutamine.

Measurement of Cell Viability

Cell viability was measured by the MTT assay (Sigma-Aldrich). Briefly, SN4741 cells were seeded in a 96-well plate at a concentration of 1.0×10^4/well. After overnight incubation, plates were incubated with salidroside or 6-OHDA. Subsequently, MTT (0.5 μg/mL) was added. Four hours later, the medium was removed, and 150 μL dimethyl sulfoxide was added. The absorbance was read at 570 nm with a microplate reader (Bio-Rad, Hercules, CA), and the absorbance value was expressed as a percentage to that of the untreated control cells.

Western Blotting

Western blotting was performed as previously described [18]. After treatment with 6-OHDA for 4 h, SN4741 cells were washed three times with room-temperature PBS and suspended in lysis buffer (100 μL buffer per 6-cm plate). The lysed samples were separated by SDS-PAGE and then transferred to polyvinylidene fluoride (PVDF) membranes. The PVDF membranes were blocked with 5% bovine serum albumin for 2 h at room temperature and then incubated with the indicated antibodies with gentle shaking at 4 °C overnight, followed by incubation with specific animal HRP-conjugated secondary antibodies. Protein bands were visualized using ECL. The band densities were quantified using ImageJ software (NIH, Bettthesda, MD).

Immunocytochemistry

SN4741 cells were plated on glass-bottomed dishes and allowed to reach 50%–60% confluence. Salidroside was added 10 h prior to 6-OHDA treatment. The cells were then washed three times with PBS, fixed in 4% paraformaldehyde (pH 7.4) for 20–30 min, and permeabilized with 0.1% Triton X-100 for 15–20 min. Cells were

then washed with PBS and incubated at 4 °C overnight with antibody against BiP. On the following day, the cells were washed with PBS and labeled with FITC-conjugated anti-rabbit secondary antibody, and the nuclei were stained with DAPI. Fluorescent images were captured using a confocal microscope (C2; Nikon, Japan). The relative fluorescence intensity was quantified using ImageJ.

Measurement of Intracellular ROS

The CM-H2DCFDA indicator (Life Technologies, Carlsbad, CA) was used to measure ROS production. SN4741 cells were pretreated with salidroside for 10 h and then 6-OHDA (10 μmol/L) was added for an additional 4-h incubation. The cells were then incubated with 100 μmol/L CM-H2DCFDA dye for 30 min at 33 °C, washed with PBS to remove the excess dye, and observed under a laser scanning confocal microscope (C2 Si; Nikon, Japan). The relative fluorescence intensity was quantified using ImageJ.

TUNEL Staining

Apoptosis was measured using the One-Step TUNEL Apoptosis Kit (QIA39, Merck). SN4741 cells were pretreated with salidroside for 10 h and then incubated with 6-OHDA (10 μmol/L) for an additional 4 h. After fixation and permeabilization, the cells were incubated with the TUNEL reaction mixture at 37 °C for 1 h according to the manufacturer's protocol. The cells were then washed three times with PBS (pH 7.4), stained with DAPI, and observed under a laser scanning confocal microscope (C2 Si; Nikon, Japan). The TUNEL-positive cells were counted using ImageJ.

Intracellular Ca^{2+} Measurement

The fluorescent calcium indicator Fluo-3 AM (Beyotime) was used to measure the level of intracellular free Ca^{2+} [19]. Cells at 50%–60% confluence were pretreated with salidroside for 10 h, and then the medium was replaced with Hanks' buffer (in mmol/L: 132 NaCl, 5 KCl, 10 dextrose, 10 HEPES, and 1.05 $MgCl_2$) containing Fluo-3 AM (5 μmol/L) for 30 min at 33 °C. Intracellular Ca^{2+} fluorescence images were obtained using a C2 Si spectral imaging confocal laser scanning system and the emission at 515 nm was collected after excitation with an argon laser at 488 nm. Relative fluorescence intensity analysis was quantified using ImageJ.

Primary Neuronal Culture

Adult Sprague–Dawley rats were obtained from the Experimental Animal Center of The Fourth Military Medical University in Xi'an, China. All animal care protocols were approved by the Animal Care and Use Committee of The Fourth Military Medical University. Cerebral cortical neurons from rat embryos were isolated and cultured as previously reported [20]. Briefly, 18-day-old embryonic cerebral cortices were obtained and dissociated. Single cells were suspended in Neurobasal medium with 3% B27. The cortical neuron suspension was then plated on 6-well plates (2×10^5–3×10^5 cells/cm^2) coated with poly-*D*-lysine and fibronectin. The primary cortical neurons were incubated at 37 °C in a moist atmosphere for 14 days with 95% O_2 and 5% CO_2. Neuronal ER stress was induced by exposure to H_2O_2 (300 μmol/L) for 24 h.

Statistical Analysis

Data were analyzed using one-way ANOVA. All values are presented as the mean ± SEM. $P < 0.05$ was considered to be statistically significant.

Results

Salidroside Attenuates Tunicamycin-Induced ER Stress

Salidroside has been shown to abolish MPTP-induced cytotoxicity through mitochondria-related pathways [16, 17]. However, whether it has a similar protective effect on ER stress is still unclear. To test this, SN4741 cells were pretreated with salidroside for 10 h, and then 5 μmol/L tunicamycin, a well-known ER stress inducer [21], was added for an additional 24 h. Tunicamycin significantly increased the level of ER stress, as measured by the activation of BiP, p-PERK, and p-IRE1, and this was significantly reversed by salidroside (25 μmol/L) (Fig. 1). Our data demonstrated that salidroside significantly attenuates tunicamycin-induced ER stress.

The ER Stress Inhibitor 4-PBA Protects SN4741 Cells from 6-OHDA-Induced Cytotoxicity

6-OHDA is a neurotoxin used in cellular models of PD and has been reported to induce apoptosis. Consistently, treatment of SN4741 cells with a range of 6-OHDA concentrations for various periods of time led to cell death as measured by MTT assays (data not shown). We found that treatment with 10 μmol/L (or >10 μmol/L) 6-OHDA for 4 h had anti-proliferative effects. Further, exposure to 6-OHDA significantly increased the levels of the ER stress markers BiP, p-PERK, and p-IRE1 (Fig. 2A), and these increases were reversed by the ER stress inhibitor 4-PBA (Fig. 2B). MTT assays showed that 4-PBA (2 mmol/L)

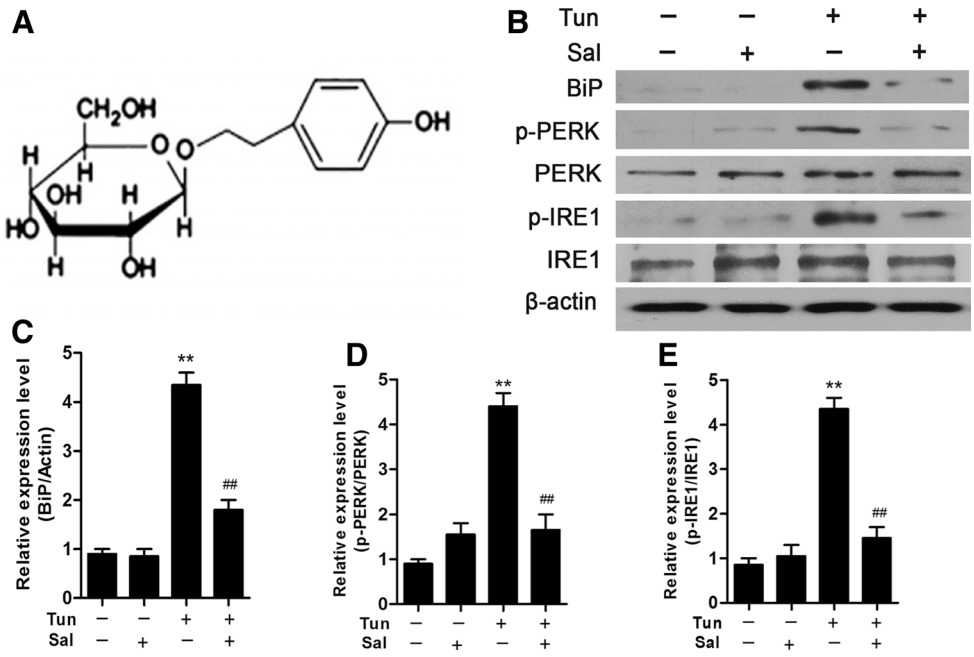

Fig. 1 Salidroside attenuates tunicamycin-induced ER stress. **A** Chemical structure of salidroside. **B** Western blots of ER stress sensors (BiP, p-PERK, and p-IRE1). SN4741 cells were pretreated with salidroside (Sal; 25 μmol/L) for 10 h, and then tunicamycin (Tun; 5 μmol/L) was added for an additional 24 h. (**C**–**E**) Quantification of band densities of p-IRE1, BiP, and p-PERK from B (mean ± SEM, $n = 3$; $^{**}P < 0.01$ compared with control group, $^{\#\#}P < 0.01$ compared with tunicamycin-treated group; one-way ANOVA).

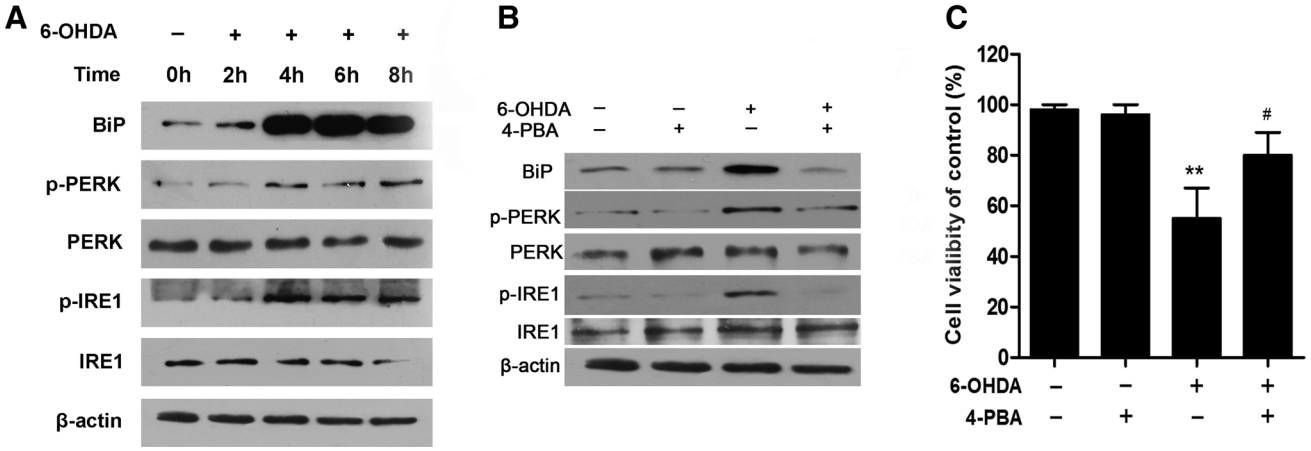

Fig. 2 The ER stress inhibitor 4-PBA blocks 6-OHDA-induced ER stress in SN4741 cells. **A**, **B** Western blots of ER stress sensors (BiP, p-PERK, and p-IRE1) in SN4741 cells after treatment with 6-OHDA (10 μmol/L) for 0–8 h (**A**), and after pretreatment with 4-PBA (2 mmol/L) for 1 h prior to treatment with 6-OHDA (10 μmol/L; 4 h) (**B**). **C** Protective effects of 4-PBA on 6-OHDA-induced neurotoxicity in SN4741 cells as assessed using MTT assays in cells treated as in **A** and **B** (mean ± SEM, $n = 3$; $^{**}P < 0.01$ compared with control group; $^{\#}P < 0.05$ compared with 6-OHDA-treated group).

clearly protected SN4741 cells against 6-OHDA-associated neurotoxicity (Fig. 2C). Together, our data suggested that the 6-OHDA-induced decrease in DA neuronal viability is mediated *via* the ER stress pathway.

Salidroside Alleviates 6-OHDA-Induced ER Stress

To further explore the possibility that salidroside inhibits 6-OHDA-induced ER stress, SN4741 cells were pretreated with salidroside for 10 h, then 6-OHDA (10 μmol/L) was added for an additional 4 h. Western blot analysis showed that salidroside pretreatment decreased the levels of ER stress markers (BiP, p-PERK, and p-IRE1) compared with 6-OHDA treatment alone (Fig. 3).

Salidroside Exposure Prevents 6-OHDA-Induced Accumulation of ROS and Ca^{2+}

Previous studies have suggested that the accumulation of ROS and intracellular Ca^{2+} promotes ER stress [12, 13]. Thus, we tested whether salidroside treatment alters the 6-OHDA-induced increases in ROS and Ca^{2+} levels to

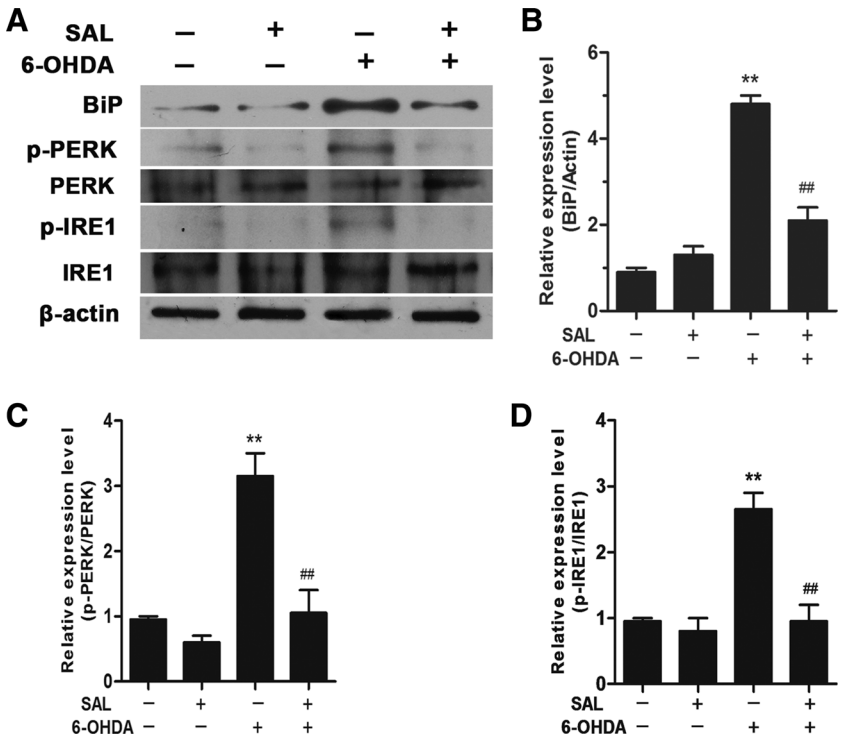

Fig. 3 Salidroside alleviates 6-OHDA-induced ER stress. **A** Western blots of ER stress sensors (BiP, p-PERK, and p-IRE1) in SN4741 cells pretreated with salidroside (SAL; 25 μmol/L) for 10 h prior to incubation with 10 μmol/L 6-OHDA for an additional 4 h. **B–D** Quantification of band densities of BiP, p-PERK, and p-IRE1 as in **A** (mean ± SEM, $n = 3$; $^{**}P < 0.01$ compared with control group; $^{\#\#}P < 0.01$ compared with 6-OHDA-treated group).

attenuate ER stress. Fluorescence staining revealed that salidroside reversed the accumulation of intracellular ROS and Ca^{2+} induced by 6-OHDA treatment (Fig. 4).

Salidroside Attenuates ER Stress-Related Cell Death in SN4741 Cells

As noted above, ER stress pathways are initially activated in response to disturbed ER homeostasis and function to restore proper protein folding. However, persistent ER stress can lead to cell death through activation of the caspase-12-mediated apoptosis pathway [6]. Thus, we next tested whether salidroside treatment specifically reduces ER stress-induced cell death. We showed that pretreatment with salidroside led to a significant reduction in the level of cleaved caspase-12, which was stimulated following 6-OHDA treatment (Fig. 5A). MTT and TUNEL assays showed that salidroside exposure reversed the 6-OHDA-induced decrease in SN4741 cell viability (Fig. 5B) and reduced the percentage of TUNEL-positive cells (Fig. 5C).

Salidroside Alleviates H_2O_2-Induced ER Stress in Primary Cortical Neurons

The data above suggested that salidroside has an extensive anti-ER stress effect. To determine whether salidroside protects against ER stress in the cerebral cortex, primary cerebral cortical neurons harvested from rat embryos were treated with H_2O_2 (300 μmol/L), a well-known oxidant that induces oxidative and ER stress [22]. Western blots (Fig. 6A), ROS (Fig. 6B) and Ca^{2+} fluorescence (Fig. 6C), and immunofluorescence analysis of BiP (Fig. 6D) in primary cortical neurons pretreated with salidroside prior to H_2O_2 exposure showed a significant reduction in the levels of ER stress markers, ROS, and Ca^{2+} compared with H_2O_2 alone. Together, our data suggested that salidroside alleviates H_2O_2-induced ER stress in primary cortical neurons.

Discussion

PD is a neurodegenerative disease characterized by progressive loss of DA neurons in the SNpc. Patients with PD lack effective therapies that halt neurodegenerative processes and can only be treated for clinical symptoms [23], reinforcing the need to better understand the biological processes that underlie the neurodegeneration [24]. Increasing evidence suggests that salidroside reduces the levels of ROS, which have been linked to the pathogenesis of many diseases [25] including diabetes, cardiovascular disease, and degenerative disease. Inhibition of ROS accumulation by salidroside has been reported to involve the upregulation of anti-oxidative enzymes and thioredoxin-1 activity, which suppress oxidative stress [26]. Importantly, previous studies using PD models have reported that salidroside prevents apoptosis due to MPP^+-induced mitochondrial dysfunction in PC12 cells [16, 17]. Based on these findings, we investigated whether

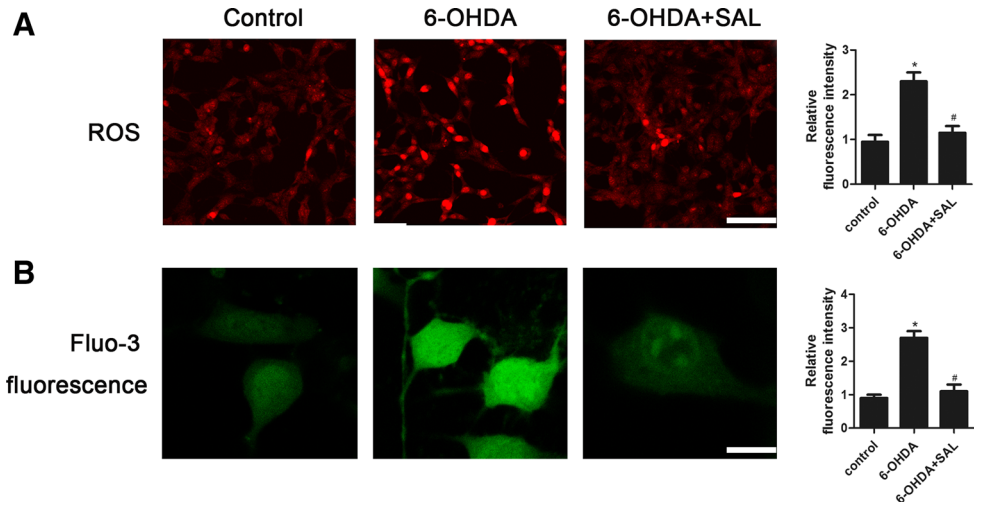

Fig. 4 Salidroside decreases the 6-OHDA-induced accumulation of ROS and intracellular calcium. **A** *Left panels* confocal microscopic images of ROS fluorescence (*red*) detected by DCF. SN4741 cells were pretreated with salidroside for 10 h, followed by addition of 10 μmol/L 6-OHDA for 4 h, then incubated with 100 μmol/L DCF for 30 min at 33 °C. *Scale bar*, 50 μm. *Right panel* relative fluorescence intensity of intracellular ROS levels. **B** *Left panels* confocal microscopic images of intracellular free Ca^{2+} fluorescence (*green*) detected by Fluo-3 AM (5 μmol/L). SN4741 cells were treated as described in **A**, but incubated with Fluo-3 AM. *Scale bar*, 10 μm. *Right panel* relative fluorescence intensity of intracellular free Ca^{2+} levels (mean ± SEM, $n = 3$; $^{*}P < 0.05$ compared with control group, $^{\#}P < 0.05$ compared with 6-OHDA-treated group).

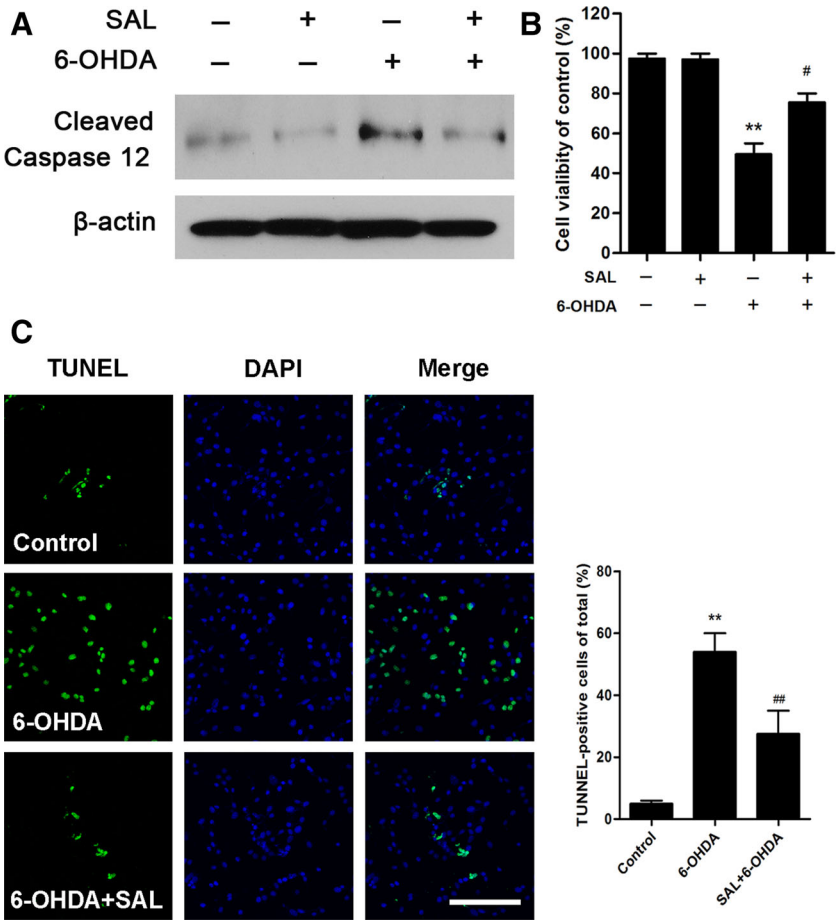

Fig. 5 Salidroside attenuates 6-OHDA-induced cell death in SN4741 cells. **A** Western blots of activated caspase-12 levels in SN4741 cells pretreated with salidroside (SAL; 25 μmol/L) for 10 h prior to incubation with 10 μmol/L 6-OHDA for an additional 4 h. **B** Analysis of SN4741 cells treated as described in **A** using an MTT assay to determine the effect of salidroside on 6-OHDA-induced cytotoxicity. **C** *Left panels* images of SN4741 cells treated as described in **A** assessed for apoptosis using a TUNEL assay. *Scale bar*, 50 μm. *Right panel* quantitative analysis of the number of TUNEL-positive cells (mean ± SEM, $n = 3$; $^{**}P < 0.01$ compared with control group; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ compared with 6-OHDA-treated group).

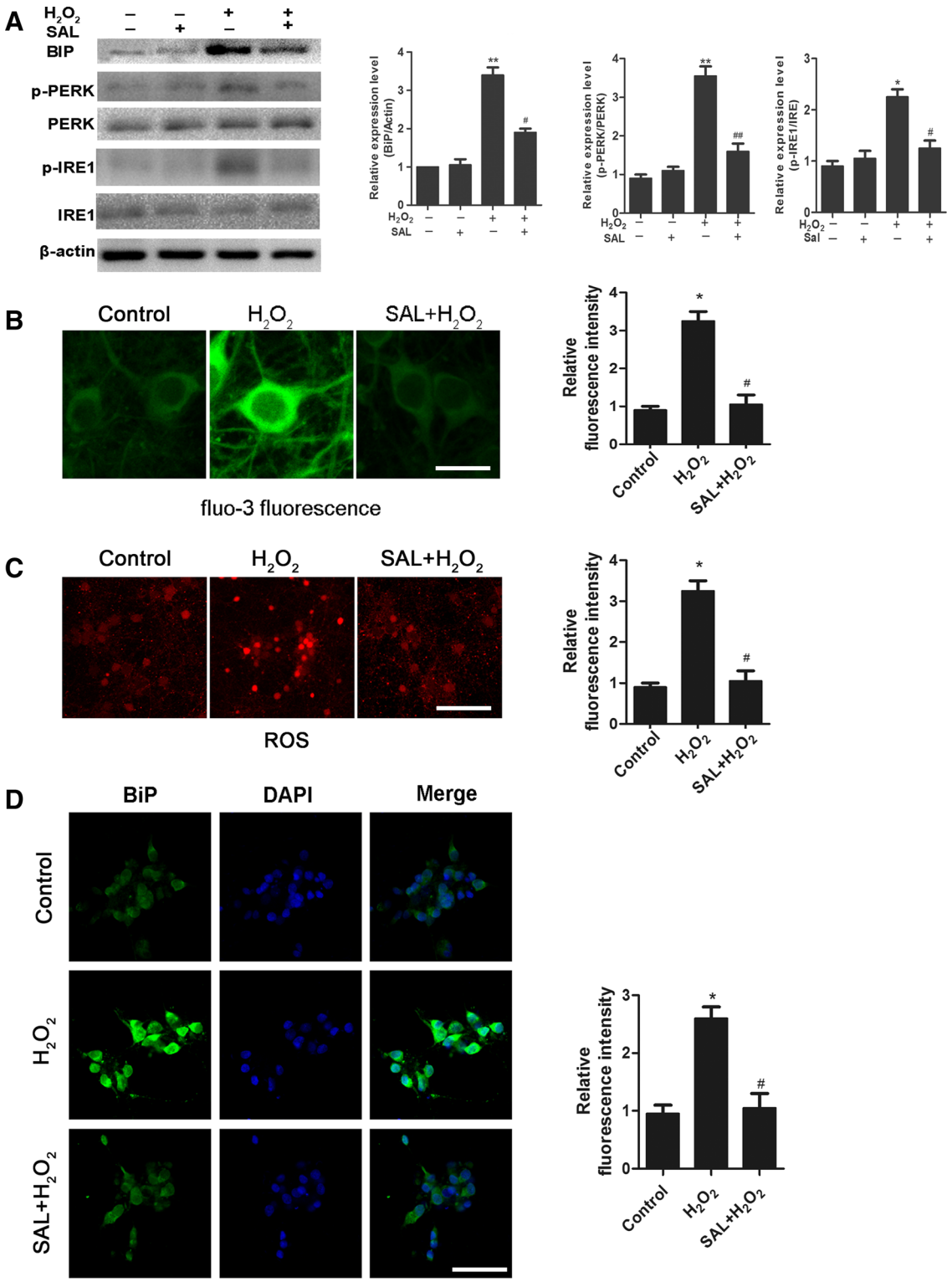

Fig. 6 Salidroside alleviates H_2O_2-induced ER stress in cultured primary cortical neurons. **A** *Left panel* western blots of BiP, p-PERK, and p-IRE1. Primary cortical neurons were pretreated with salidroside (SAL; 25 μmol/L) prior to incubation with H_2O_2 (300 μmol/L) for an additional 24 h. *Right panels* quantification of band densities of BiP, p-PERK, and p-IRE1. **B** *Left* intracellular free Ca^{2+} as detected by Fluo-3 AM. *Scale bar*, 10 μm. *Right* relative fluorescence intensity of intracellular free Ca^{2+} levels. **C** *Left* ROS as detected by DCF. *Scale bar*, 50 μm. *Right* relative fluorescence intensity of intracellular ROS levels. **D** *Left* immunofluorescence images of BiP. Nuclei were stained with DAPI (*green* BiP; *blue* DAPI). *Scale bar*, 50 μm. *Right* relative fluorescence intensity. Data are mean ± SEM, $n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control group; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ compared with H_2O_2-treated group.

salidroside similarly protects against 6-OHDA-induced ER dysfunction.

The accumulation of misfolded proteins disturbs cellular redox regulation and stimulates the production of endogenous ROS [27], leading to the activation of ER stress signals and ER dysfunction. In response to increased ROS levels, the ER elicits a series of effector signals known as the UPR to adapt to the changing environment and restore ER function and homeostasis. Signaling through the UPR is largely mediated by activation of the IRE1, PERK, and AFT6 pathways. However, in cases in which the stress signals are prolonged or too severe, the ER may trigger cell death through activation of the caspase-12 pathway [6].

As misfolded proteins accumulate, ROS are generated in the ER and pose a threat to the local environment, causing damage to ER chaperones, resident and "client" proteins, enzymes, and ER-related Ca^{2+} channels. Indeed, release of Ca^{2+} from the ER into the intracellular compartment has been linked to the activation of ER stress pathways [28]. When targeted to the mitochondria, this increase in intracellular Ca^{2+} can lead to further ROS generation [10]. Ultimately, persistent ER stress can lead to increased protein folding requirements and cell death due to changes in Ca^{2+} levels or ROS signaling [11].

In the current study, we demonstrated that salidroside protects against 6-OHDA-induced cytotoxicity *via* attenuation of ER stress. In addition, our results suggest that the protective effects of salidroside on neurotoxin-treated DA neurons and primary cortical neurons involve the inhibition of increases in intracellular ROS and Ca^{2+} levels. Taken together, our study suggests that salidroside has protective effects *via* the attenuation of ER stress through disrupting ROS and Ca^{2+} accumulation and provides a rationale for further investigation of salidroside as a potential therapeutic agent in animal models of PD.

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References

1. Mercado G, Valdes P, Hetz C. An ERcentric view of Parkinson's disease. Trends Mol Med 2013, 19: 165–175.
2. Hetz C, Mollereau B. Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. Nat Rev Neurosci 2014, 15: 233–249.
3. Placido AI, Pereira C, Duarte AI, Candeias E, Correia SC, Carvalho C, *et al.* Modulation of endoplasmic reticulum stress: an opportunity to prevent neurodegeneration? CNS Neurol Disord Drug Targets 2015, 29: 29.
4. Rojas-Rivera D, Armisen R, Colombo A, Martinez G, Eguiguren AL, Diaz A, *et al.* TMBIM3/GRINA is a novel unfolded protein response (UPR) target gene that controls apoptosis through the modulation of ER calcium homeostasis. FEBS J 2012, 279: 52–53.
5. Srivastava R, Deng Y, Shah S, Rao AG, Howell SH. BINDING PROTEIN Is a Master Regulator of the Endoplasmic Reticulum Stress Sensor/Transducer bZIP28 in Arabidopsis. Plant Cell 2013, 25: 1416–1429.
6. Mockel A, Obringer C, Hakvoort TBM, Seeliger M, Lamers WH, Stoetzel C, *et al.* Pharmacological modulation of the retinal unfolded protein response in Bardet-Biedl Syndrome reduces apoptosis and preserves light detection ability. J Biol Chem 2012, 287: 37483–37494.
7. Kitamura M, Hiramatsu N. The oxidative stress: endoplasmic reticulum stress axis in cadmium toxicity. Biometals 2010, 23: 941–950.
8. Doyle KM, Kennedy D, Gorman AM, Gupta S, Healy SJM, Samali A. Unfolded proteins and endoplasmic reticulum stress in neurodegenerative disorders. J Cell Mol Med 2011, 15: 2025–2039.
9. Verkhratsky A, Parpura V. Store-operated calcium entry in neuroglia. Neurosci Bull 2014, 30: 125–133.
10. Holtz WA, O'Malley KL. Parkinsonian mimetics induce aspects of unfolded protein response in death of dopaminergic neurons. J Biol Chem 2003, 278: 19367-19377.
11. Chaudhari N, Talwar P, Parimisetty A, Lefebvre d'Hellencourt C, Ravanan P. A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress. Front Cell Neurosci 2014, 8: 213.
12. Li B, Xiao L, Wang ZY, Zheng PS. Knockdown of STIM1 inhibits 6-hydroxydopamine-induced oxidative stress through attenuating calcium-dependent ER stress and mitochondrial dysfunction in undifferentiated PC12 cells. Free Radic Res 2014, 48: 758–768.
13. Oh YM, Jang EH, Ko JH, Kang JH, Park CS, Han SB, *et al.* Inhibition of 6-hydroxydopamine-induced endoplasmic reticulum stress by L-carnosine in SH-SY5Y cells. Neurosci Lett 2009, 459: 7–10.
14. Guan S, Feng HH, Song BC, Guo WX, Xiong Y, Huang GR, *et al.* Salidroside attenuates LPS-induced pro-inflammatory cytokine responses and improves survival in murine endotoxemia. Int Immunopharmacol 2011, 11: 2194–2199.
15. Wang J, Li JZ, Lu AX, Zhang KF, Li BJ. Anticancer effect of salidroside on A549 lung cancer cells through inhibition of oxidative stress and phospho-p38 expression. Oncol Lett 2014, 7: 1159–1164.
16. Li XF, Ye XL, Li XB, Sun X, Liang QA, Tao LZ, *et al.* Salidroside protects against MPP+-induced apoptosis in PC12 cells by inhibiting the NO pathway. Brain Res 2011, 1382: 9–18.
17. Wang SH, He H, Chen L, Zhang W, Zhang XJ, Chen JZ. Protective effects of salidroside in the MPTP/MPP+-induced model of Parkinson's disease through ROS-NO-related mitochondrion pathway. Mol Neurobiol 2015, 51: 718–728.
18. Yang Q, She H, Gearing M, Colla E, Lee M, Shacka JJ, *et al.* Regulation of neuronal survival factor MEF2D by chaperone-mediated autophagy. Science 2009, 323: 124–127.
19. Kojima A, Kitagawa H, Omatsu-Kanbe M, Matsuura H, Nosaka S. Presence of store-operated Ca-2 entry in C57BL/6J mouse ventricular myocytes and its suppression by sevoflurane. Br J Anaesth 2012, 109: 352–360.
20. Yan YG, Zhang J, Xu SJ, Luo JH, Qiu S, Wang W. Clustering of surface NMDA receptors is mainly mediated by the C-terminus of GluN2A in cultured rat hippocampal neurons. Neurosci Bull 2014, 30: 655–666.
21. Wang HP, Wang X, Ke ZJ, Comer AL, Xu M, Frank JA, *et al.* Tunicamycin-induced unfolded protein response in the developing mouse brain. Toxicol Appl Pharmacol 2015, 283: 157–167.

22. Pierre N, Barbe C, Gilson H, Deldicque L, Raymackers JM, Francaux M. Activation of ER stress by hydrogen peroxide in C2C12 myotubes. Biochem Biophys Res Commun 2014, 450: 459–463.
23. Toulouse A, Sullivan AM. Progress in Parkinson's disease-where do we stand? Prog Neurobiol 2008, 85: 376–392.
24. Fukui M, Choi HJ, Zhu BT. Mechanism for the protective effect of resveratrol against oxidative stress-induced neuronal death. Free Radic Biol Med 2010, 49: 800–813.
25. Cao SS, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. Antioxid Redox Signal 2014, 21: 396–413.
26. Qian EW, Ge DT, Kong SK. Salidroside promotes erythropoiesis and protects erythroblasts against oxidative stress by up-regulating glutathione peroxidase and thioredoxin. J Ethnopharmacol 2011, 133: 308–314.
27. Feldman DE, Chauhan V, Koong AC. The unfolded protein response: a novel component of the hypoxic stress response in tumors. Mol Cancer Res 2005, 3: 597–605.
28. Lindholm D, Wootz H, Korhonen L. ER stress and neurodegenerative diseases. Cell Death Differ 2006, 13: 385–392.