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RESEARCH ARTICLE

Ginsenoside Rb1 prevents high glucose-induced Schwann cell injury through the mitochondrial apoptosis pathway

Li Xiaogang, Zhang Zhe, Wang Rui, Xia Xinxin, Liu Yonghui, Sun Lianqing

Li Xiaogang, Department of Anesthesiology, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, China

Zhang Zhe, Wang Rui, Xia Xinxin, Liu Yonghui, Sun Lianqing, Department of Traditional Chinese Medicine, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, China

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Correspondence to: Prof. Sun Lianqing, Department of Traditional Chinese Medicine, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, China. sunlianqing1@126.com

Telephone: +86-18991232784

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Abstract

OBJECTIVE: To investigate the effects of ginsenoside Rb1 on high glucose-induced neurotoxicity and the underlying molecular mechanism in primary cultured Schwann cells (SCs).

METHODS: Cultured SCs were divided into six groups that received (a) normal glucose, (b) osmotic control, (c) high glucose, (d) high glucose plus 1 μM ginsenoside Rb1, (e) high glucose plus 10 μM ginsenoside Rb1, or (f) high glucose plus 100 μM alpha lipoic acid (ALA). Intracellular reactive oxygen species (ROS) generation and mitochondrial transmembrane potential (ΔΨm) were detected by flow cytometric analyses. Apoptosis was confirmed by the annexin V-FITC/propidium iodide (PI) method, and the concentration of 8-hydroxy-2-deoxy guanosine (8-OHdG) was detected by an enzyme-linked immunosorbent assay. Western blotting was performed to analyze the expression levels of important transcription factors such as cytochrome c, bcl-2, bax, activated caspase-3, and activated poly (ADP-ribose) polymerase (PARP).

RESULTS: Ginsenoside Rb1 inhibited high glucose-induced oxidative stress by decreasing ROS and 8-OHdG levels as well as mitochondrial depolarization in SCs. 3-(4,5)-dimethylthiahiazo(-z-y1)-3, 5-di-phenytetrazoliumromide and annexin V-FITC/PI assays showed that incubating SCs with high glucose decreased cell viability and increased the number of apoptotic cells, whereas treatment with ginsenoside Rb1 protected SCs against high glucose-induced cell damage. Furthermore, ginsenoside Rb1 down-regulated the expression of high glucose-induced bax and cytochrome c release but up-regulated bcl-2 expression. In addition, ginsenoside Rb1 attenuated high glucose-induced activation of caspase-3 and minimized cleavage of PARP in SCs.

CONCLUSION: These results suggest that ginsenoside Rb1 antagonizes high glucose-induced oxidative stress and activation of the mitochondrial apoptosis pathway in SCs.

Keywords: Diabetic neuropathies; Oxidative stress;

Ginsenosides; Apoptosis inducing factor; Schwann cells

INTRODUCTION

Numerous epidemiological studies indicate increased incidence of diabetes annually, suggesting that diabetes is becoming a predominate disease in China.[1,2] As a common and chronic metabolic disease, diabetes has serious microvascular complications such as diabetic peripheral neuropathy (DPN), diabetic nephropathy, and diabetic retinopathy. DPN is characterized by peripheral nerve dysfunction and is the most common neuropathic syndrome in patients with diabetes.[3,4] Although DPN has recently attracted attention because of an alarming rise in morbidity and mortality, its precise pathogenesis is still unclear. Recent evidence indicates that hyperglycemia-induced production of reactive oxygen species (ROS) in mitochondria leads to neuropathy through multiple pathways, including polyol, advanced glycation end-product, protein kinase C, and hexosamine pathways.[5,6] Consistent with this viewpoint, inhibition of ROS may block the initiation and progression of neuropathy.[7,8]

Renshen (*Radix Ginseng*), the root of Panax ginseng CA Meyer, is a well-known traditional herb and has been widely used in China for more than 2000 years. Ginsenoside Rb1 (Figure 1), one of the principle bioactive components of Renshen (*Radix Ginseng*), has received a great deal of attention and is considered to have multiple pharmacological functions, especially various neuroprotective effects.[9-14] However, the benefit of ginsenoside Rb1 in DPN patients and the molecular mechanism involved in the neuroprotective effects of DPN have not been explored.

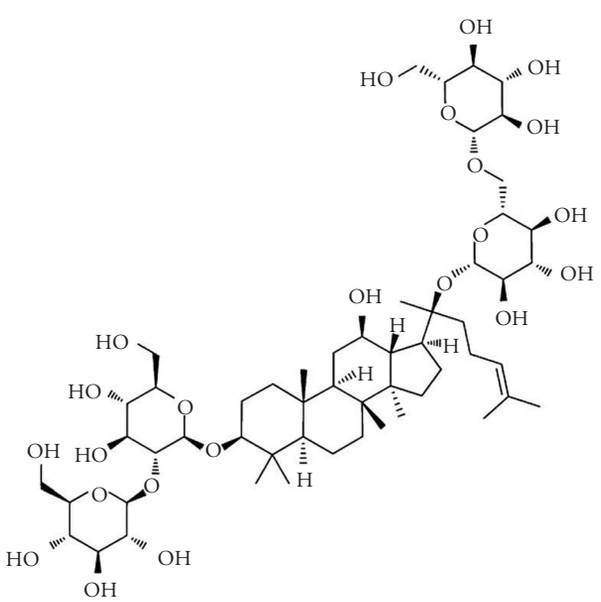

Figure 1 Chemical structure of ginsenoside Rb1

Schwann cells (SCs) are the most important myelin-forming and supporting cells in the peripheral nervous system. These cells play central roles in the pathogenesis of peripheral neuropathy by contributing to peripheral nerve regeneration and providing myelination.[8] SCs may be a target for treatment of DPN because of reversible defects.[15] It has been shown that hyperglycemia is a potent initiator of apoptosis in SCs of diabetic models.[16] In fact, hyperglycemia-induced oxidative stress in mitochondria plays a central role in the apoptotic pathway, triggering a marked loss of mitochondrial transmembrane potential ($\Delta\Psi m$) and the release of cytochrome C. It is widely acknowledged that the apoptotic process is initiated by activation of caspase-3 and mainly regulated by the BcL-2 family.

In the present study, we investigated the effects of ginsenoside Rb1 on high glucose-induced neurotoxicity, the underlying molecular mechanism of oxidative stress, and mitochondrial dysfunction in primary cultured SCs.

MATERIALS AND METHODS

Animals

Sprague Dawley rats, 3-5 d old, weighing (6.4 ± 1.1) g, were supplied by Wei-tong Lihua Experimental Animal Center (Beijing, China, license No. SCXK 2009-0013).

Cell culture and treatments

SCs were obtained from sciatic nerves and brachial plexus nerves under aseptic conditions. Briefly, both sciatic nerves and brachial plexus nerves were dissected out, and the whole epineurium was stripped off and placed into D-Hanks solution with 100 U/mL penicillin and 100 μg/mL streptomycin. Then, the nerves were minced into roughly 1 mm × 1 mm short segments and cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, Island, NY, USA,) supplemented with 20% heat-inactivated fetal bovine serum (Hyclone, Logan, UT, USA) at 37 ℃ in a humidified 5% CO_2 incubator. The medium was changed every 3 d. When adherent cells reached confluency, the cells were passaged by digestion with 0.05% trypsin-EDTA (GIBCO). The cultured cells were identified as SCs by their morphology and immunohistochemistry using an anti-S-100 protein antibody (Boster, Wuhan, China). Second and third passages of SCs with a purity of ≥ 95% were used in this study.

SCs were treated in duplicate as described previously.[17] Cells were treated as follows: control (con; 5.6 mM glucose), high glucose (HG; 50 mM glucose), and 50 mM glucose plus alpha lipoic acid (ALA) (100 μM) with HG in the presence of 1 or 10 μM ginsenoside Rb1. In addition, cells were treated with 5.6 mM glucose plus 44.4 mM mannitol as an osmotic control. Cultures were continued for 48 h.

Cell viability assay

Cell viability of SCs was determined by 3-(4,5)-di-