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Neuroprotective effects of ginsenoside Rg1 through the Wnt/β-catenin signaling pathway in both in vivo and in vitro models of Parkinson's disease

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ABSTRACT

Ginsenoside Rg1 (Rg1) is a major bioactive ingredient in *Panax ginseng* that has low toxicity and has been shown to have neuroprotective effects. The objectives of the present study were to explore the potential of the application of Rg1 for the treatment of Parkinson's disease (PD) and to determine whether its neuroprotective effects are exerted through the Wnt/β-catenin signaling pathway by using in vivo and in vitro models of PD. In the in vivo study, Rg1 treatment ameliorated the behavioral deficits of "Pole test", and reduced dopaminergic cell loss that were induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP) in a dose-dependent manner in an in vivo model of PD. In the in vitro study, cell viability was increased and cell apoptosis induced by 1-methyl-4-phenylpyridinium(MPP+) was decreased by Rg1 pretreatment. Rg1 induced protective effects on the protein and mRNA expression levels of markers of the Wnt/β-catenin signaling pathway in both the in vivo and the in vitro studies, and these neuroprotective effects were blocked by DKK1 in the in vitro study. Our results provide evidence that Rg1 has neuroprotective effects in both in vivo and in vitro PD models, and these effects act through the Wnt/β-catenin signaling pathway. Taken together, these results indicate that Rg1 may exert therapeutic effects on PD via the Wnt/β-catenin signaling pathway and may therefore provide a novel approach for the treatment of PD.

1. Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disorder that is characterized by the gradual, progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and decreased dopamine levels in the striatum (caudate and putamen) of the basal ganglia (Lees et al., 2009; Badger et al., 2014). Despite decades of research, the further investigation of molecular mechanisms underlying the progressive loss of dopaminergic neurons in PD remain indispensable (Jenner and Olanow, 2006; Yang et al., 2009). Understanding these mechanisms is essential because it may provide the means for future therapeutic strategies. Current pharmacological treatments for PD provide only symptomatic treatment and do not prevent the progressive loss of dopaminergic neurons in PD patients (Schapira, 2009; Olanow and Schapira, 2013). Thus, further insights into the molecular mechanisms of PD and the discovery of new therapeutic agents for PD that have higher efficacy are needed.

While the underlying mechanisms of PD are not yet completely understood, accumulating evidence indicates that dysfunction in the Wnt/β-catenin signaling pathway may be an important aspect

Abbreviations: Rg1, ginsenoside Rg1; PD, Parkinson's disease; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP+, 1-methyl-4-phenylpyridinium; DKK1, Dickkopf-1; PFA, paraformaldehyde; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; CKK-8, cell counting kit-8; RT-PCR, Reverse transcription polymerase chain reaction; CNS, central nervous system.

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(Castelo-Branco and Arenas, 2006). The Wnt/β-catenin signaling pathway is also called the canonical Wnt signaling pathway, and it controls neuronal survival (Castelo-Branco et al., 2003), participates in the development of the central nervous system (CNS) and regulates the function of the adult nervous system (Inestrosa and Arenas, 2010). Evidence has indicated that dysfunction in the Wnt/β-catenin signaling pathway plays an important role in the pathophysiology of nigrostriatal dopaminergic neurons in PD models (L'Episcopo et al., 2014). The canonical Wnt signaling pathway can be blocked by the extracellular protein Dickkopf-1 (Dkk1), which binds to LRP5/6 and contributes to neurotoxicity in PC12 cells and in neurodegenerative disease (Dun et al., 2013; Scott and Brann, 2013). Because many of the components of this signaling pathway have been identified in the adult brain, Wnt/β-catenin signaling may be important for maintaining neuronal survival. Therefore, the activation of the Wnt/β-catenin signaling pathway in PD models may be crucial to modify disease progression (Parish and Thompson, 2014).

Ginsenoside Rg1 (Rg1), one of the most active ingredients in ginseng, has been suggested to have potential therapeutic effects for neurodegenerative diseases. Its chemical structure is shown in Fig. 1. Some studies have investigated the beneficial effects of Rg1 on the CNS in animal models and cultured neuronal cells (Radad et al., 2004; Shen and Zhang, 2007). In recent years, some studies have shown that ginsenosides such as Rg1 have beneficial effects in PD models that are associated with antiapoptotic, antioxidant and other molecular mechanisms (Chen et al., 2005, 2003; Xu et al., 2009). However, the mechanisms underlying the neuroprotective effects of Rg1 on dopaminergic neurons remain to be elucidated. To date, no report has investigated whether the neuroprotective effects of Rg1 are exerted through the Wnt/β-catenin signaling pathway in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP)-induced and 1-methyl-4-phenylpyridinium(MPP+)-induced PD models.

In this study, we established an in vivo PD model by using MPTP, a type of neurotoxin that can cause the loss of dopaminergic neurons in the SNpc of animals, leading to a parkinsonian-like syndrome (Przedborski et al., 2000). In an in vitro PD model, MPP+ was chosen. MPP+ is the active metabolite of MPTP, and it induces cell death in a rat adrenal gland pheochromocytoma cell line (PC12 cells) (Itano et al., 1994).

In the present study, we wished to investigate whether Rg1 treatment exerts neuroprotective effects in PD models and whether these effects were associated with the activation of the Wnt/β-catenin signaling pathway. Our results indicated that the neuroprotective effects of Rg1 in both in vivo an in vitro PD models might be mediated through the activation of the Wnt/β-catenin pathway.

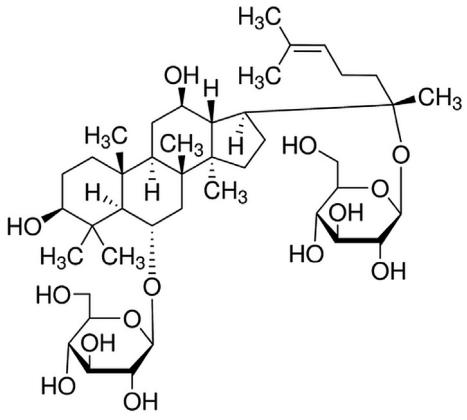

Fig. 1. Chemical structure of ginsenoside Rg1.

2. Materials and methods

2.1. Ethics statement

All animal experiments were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All animal studies complied with the ARRIVE guidelines. The animal protocols were approved by the Committee on the Ethics of Animal Experiments of the Dalian Medical University. All efforts were made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to in vivo techniques, if available.

2.2. Animals and treatment

C57BL/6J mice (6–8 weeks old, male, weighing 16–25 g), purchased from the Experimental Animal Center of Dalian Medical University (SPF level), were used for this study. The mice were maintained in a constant environment with a temperature of 20–22 °C and 50–60% humidity under a 12-h light/dark cycle of artificial light (lights on at 08:00) with free access to food and water. After adaptive feeding for one week, the mice were randomly divided into six groups: (i) control group; (ii) Rg1 20 mg/kg group; (iii) MPTP 30 mg/kg group; (iv) MPTP 30 mg/kg + Rg1 5 mg/kg group; (v) MPTP 30 mg/kg + Rg1 10 mg/kg group; and (vi) MPTP 30 mg/kg + Rg1 20 mg/kg group. Each experimental group consisted of 10 mice, and a total of 60 mice were used.

The subchronic method of an MPTP-induced PD model was established. Groups receiving MPTP injections (groups iii, iv, v, and vi) were administered an intraperitoneal injection (i.p.) with MPTP-HCl (Sigma–Aldrich, USA) in saline at a dosage of 30 mg/kg/day for each mouse for 5 consecutive days (Jackson-Lewis and Przedborski, 2007). Groups with Rg1 administration (groups iv, v, and vi) were treated i.p. with Rg1 2 h before the MPTP injection and then treated with Rg1 for another 10 days post-treatment. The group with only Rg1 (group ii) was treated with Rg1 for 15 consecutive days. The control group (i) was administered i.p. with the same volume of saline for 15 consecutive days.

2.3. Drugs

Rg1 (HPLC > 98%) was purchased from Dalian Melonepharma Biological Technology Co., Ltd. (Dalian, China). MPTP and MPP+ were purchased from Sigma–Aldrich (Sigma, USA). Monoclonal anti-mouse TH was purchased from ImmunoStar, Inc. (ImmunoStar, USA). Polyclonal anti-mouse/rat Wnt1 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz Biotechnology, USA). Polyclonal anti-mouse/rat β-catenin, GSK-3β and p-GSK-3β were purchased from Cell Signaling Technology, Inc. (Beverly, USA). The Wnt/β-catenin signaling pathway inhibitor DKK1 was purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). The second antibodies for immunofluorescence were purchased from Jackson ImmunoResearch Laboratories, Inc. (Jackson ImmunoResearch Laboratories, USA). The reagents for reverse transcription polymerase chain reaction (RT-PCR) were purchased from Life Technologies Inc. (Carlsbad, CA, USA). TUNEL staining kits were purchased from Boehringer Mannheim (detection kit, BM, Germany). The cell counting kit-8 (CKK-8) kit was purchased from Biosynthesis Biotechnology Co. Ltd. (Beijing, China). The BCA kit and Enhanced Chemiluminescence (ECL) were purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco BRL (Gaithersburg, MD, USA).