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Immunomodulatory and neuroprotective effects of ginsenoside Rg1 in the MPTP(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) -induced mouse model of Parkinson's disease

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ABSTRACT

Ginsenoside Rg1, one of the biologically active ingredients of ginseng, has been considered to be a candidate neuroprotective drug. The objective of the study was to study the protective effects of Rg1 through the peripheral and central inflammation in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease (PD) mouse model. Rg1 treatment protected TH-positive cells in the SNpc region from MPTP toxicity measured with immunofluoresence. The protein expression levels of TH in the SNpc region of MPTP-induced mice following treatment with Rg1 were higher than MPTP-induced mice which were tested with Western blot. The ratio of $CD3^+CD4^+$ to $CD3^+CD8^+$ T cells and $CD4^+CD25^+Foxp3^+$ regulatory T cells in the blood increased in MPTP-induced mice following treatment with Rg1 which were detected by flow cytometry analysis. Moreover, Rg1 reduced the serum concentrations of proinflammatory cytokines TNF-α, IFN-γ, IL-1β and IL-6 which were tested with enzyme-linked immunosorbent assay (ELISA). In addition, Rg1 inhibited the activation of microglia and reduced the infiltration of $CD3^+$ T cells into the SNpc region which were measured by immunofluorescence. Our results indicated that Rg1 may represent a promising drug for the treatment of PD via the regulation of the peripheral and central inflammation.

1. Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease which is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and decreased dopamine levels in the striatum of the basal ganglia [1]. Several prevalent mechanisms responsible for neural degeneration in PD include age, environmental toxins, gene mutations, mitochondrial dysfunction and free radical-mediated cell death [2]. However the causes of the dopaminergic neuronal cell death in PD is not fully known, the precise mechanism of PD has not yet been completely clarified.

In last decades, neuroinflammation and peripheral immunity have been considered to play an important role during the initiation and progression of PD [3,4]. Currently, the evidence that supports inflammation in PD includes inflammation in the peripheral immune system, an altered composition and phenotype of peripheral immune cells [4], microglial activation [5], and infiltration of peripheral immune cells into the central nervous system (CNS) [6]. Recently, the interaction between the peripheral and central immune responses in neurodegenerative processes has become increasingly recognized [7]. If inflammation were truly a necessary factor in the neurodegenerative process, then the suppression of cytotoxic T lymphocytes, downregulation of proinflammatory cytokines, inhibition of activated microglia and reduction of the infiltration of T cells into the brain may be meaningful therapeutic approaches to alleviate this disease.

Panax ginseng is one of the most widely applied herbal medicines in human medical history. Last decades, beneficial effects of ginseng to the central nervous system (CNS) have been investigated [8,9]. Ginsenoside Rg1, one of the most active ingredients of ginseng has been studied for potential neuroprotective effects on the mesencephalic dopaminergic cells [10,11,12]. Its chemical structure is shown in Fig. 1. But not a single study has reported the immunoprotective effects of Rg1 in PD mouse model. So here we reported the protective effects of Rg1 in MPTP-induced mouse model of PD from the peripheral and central inflammation perspective.

We established a PD model in C57BL/6J mice using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is a neurotoxin that causes the loss of dopaminergic neurons in the SNpc [13]. The

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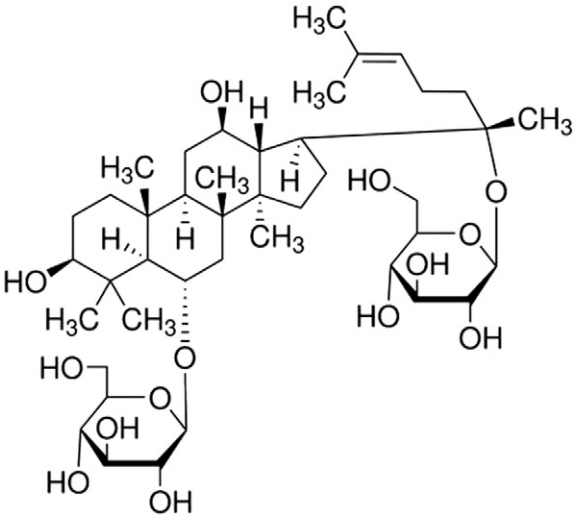

Fig. 1. Chemical structure of ginsenoside Rg1.

active metabolite of MPTP is considered to be 1-methyl-4-phenyl-pyridinium (MPP+), which leads to the necrosis or apoptosis of dopaminergic neurons [14]. This model remains the gold standard for the assessment of protective treatment methods for PD.

The current pharmacological treatments for PD are primarily dopamine replacement therapies, which can only alleviate certain symptoms and may cause side effects [15]. Our results indicated that Rg1 exerts therapeutic effects of PD through immune perspective and provides substantial evidence for preclinical application. We hope modulation of defects caused by a damaged immune system may lead to a new PD therapy and slow PD process in the long run.

2. Materials and methods

2.1. Chemicals

Ginsenoside Rg1 (HPLC > 98%) was purchased from Dalian Melonepharma Biological Technology, Ltd. (Dalian, China). 1-Methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP), paraformaldehyde (PFA), pentobarbital sodium were phased from Sigma-Aldrich (St. Loui, MO, USA). PE-Cy5-conjugated anti-CD3$^+$, FITC-conjugated anti-CD4$^+$, PE-conjugated anti-CD8$^+$ antibodies; APC-conjugated anti-CD4$^+$, PE-conjugated anti-CD25$^+$, Alexa Fluor 488-conjugated anti-Foxp3+ and PE-conjugated anti-CD3+ were purchased from BioLegend, Inc. (San Diego, CA, USA). The tumor necrosis factor (TNF-α), interferon–gamma (IFN-γ), interleukin-1β (IL-1β) and interleukin-6 (IL-6) kits were purchased from R&D Systems, Inc. (Minneapolis, MN, USA). The mouse anti-tyrosine hydroxylase (TH) antibody was purchased from ImmunoStar, Inc. (Hudson, WI, USA). The rabbit anti-Iba1 antibody was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The TRITC-conjugated goat anti-mouse and Alexa Fluor 488-conjugated goat anti-rabbit antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA). The BCA kit was purchased from Transgen biotech, Co., Ltd. (Beijing, China). The RIPA buffer and Enhanced Chemiluminescence (ECL) was purchased from Beyotime (Shanghai, China).

2.2. Ethics statement

All animal experiments were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal protocols were approved by the Committee on the Ethics of Animal Experiments of the Dalian Medical University. All surgeries were performed under general anesthesia with 2% pentobarbital sodium, 40 mg/kg, and efforts were made to minimize animal pain and discomfort.

2.3. 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. Mice were maintained at a constant temperature of 20–22 °C and 50–60% humidity under a 12-h light/dark cycle of artificial light (lights on at 08:00, lights off at 20:00) and had free access to food and water. After adaptive feeding for one week, the mice were randomly divided into 6 groups with 5 per group: (i) Control (saline) 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, (vi) MPTP 30 mg/kg + Rg1 20 mg/kg group.

We established the subchronic method of MPTP-induce PD mouse model. Groups with MPTP injection (group iii, iv, v, vi) were administered intraperitoneal injection (ip.) with MPTP-HCl in saline at the dosage of 30 mg/kg/day each mouse for 5 consecutive days [16]. Groups with Rg1 (group iv, v, vi) administration were treated ip. with Rg1 2 h before MPTP injection, and treated with Rg1 for another 10 days of post-treatment. Group with Rg1 (group ii) were treated with Rg1 for 15 days consecutively. Group with control (i) were administered ip. with 0.3 mL 0.9% saline for consecutive 15 days.

2.4. Blood sample preparation

All of the mice were anesthetized and 0.5–0.6 mL of peripheral blood was drawn through the angular vein. Serum was separated from the whole blood by centrifugation at 400 g (centrifugal force) for 10 min, and stored at −80 °C until processed for ELISA analysis. Peripheral blood mononuclear cells (PBMCs) were isolated with lysate, incubated with fluorescently labeled antibodies against CD3$^+$, CD4$^+$, CD8$^+$ T cells and CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells according to the manufacturer's instructions, washed twice with 0.01 M PBS (pH = 7.4) and prepared for flow cytometric analysis.

2.5. Brain tissue preparation

All of the mice were anesthetized and were rapidly perfused through the aorta with 100 mL saline for 10 min, followed by 60 mL 4 °C precooled 4% paraformaldehyde (PFA) for 10 min. The mice were then decapitated, and their brains were rapidly removed and post-fixed by immersion in 4% PFA at 4 °C for 12 h. Finally, the brain was sequentially dehydrated with 20% and 30% sucrose in 0.1 M PBS for immunofluorescence.

2.6. Flow cytometric analysis

Following centrifugation, each sample was resuspended in 500 μl of 0.01 M PBS (pH = 7.4 at a density of 1 × 106 cells/100 μL) and analyzed using a FACScan flow cytometer (BD Biosciences, Franklin Lakes, SD, USA). PBMCs were stained with PE-Cy5-conjugated anti-CD3$^+$, FITC-conjugated anti-CD4$^+$, PE-conjugated anti-CD8$^+$ antibodies; APC-conjugated anti-CD4$^+$, PE-conjugated anti-CD25$^+$ and Alexa Fluor 488-conjugated anti-Foxp3$^+$. Relevant negative-control and single-stained samples were used to identify the populations of interest and to exclude others. The data analysis was performed using CellQuest software. CD4$^+$ and CD8$^+$ T cells were gated on CD3$^+$ T cells. CD25$^+$ and Foxp3$^+$ T cells were gated on CD4$^+$ T cells.

2.7. ELISA analysis

Concentrations of tumor necrosis factor (TNF-α), interferon–gamma (IFN-γ), interleukin-1β (IL-1β) and interleukin-6 (IL-6) in the blood