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Research Article

Multitarget effects of Korean red ginseng in animal model of Parkinson's disease: antiapoptosis, antioxidant, antiinflammation, and maintenance of blood–brain barrier integrity

Jong Hee Choi [1,2], Minhee Jang [2,3], Seung-Yeol Nah [4], Seikwan Oh [5], Ik-Hyun Cho [1,2,6,*]

[1] Department of Science in Korean Medicine, Graduate School, Kyung Hee University, Seoul 02447, Korea
[2] Brain Korea 21 Plus Program and Institute of Korean Medicine, Graduate School, Kyung Hee University, Seoul 02447, Korea
[3] Department of Cancer Preventive Material Development, Graduate School, Kyung Hee University, Seoul 02447, Korea
[4] Ginsentology Research Laboratory, Department of Physiology, College of Veterinary Medicine and Bio/Molecular Informatics Center, Konkuk University, Seoul 05029, Korea
[5] Department of Neuroscience, Tissue Injury Defense Research Center, School of Medicine, Ewha Womans University, Seoul 03760, Korea
[6] Department of Convergence Medical Science, College of Korean Medicine, Kyung Hee University, Seoul 02447, Korea

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ABSTRACT

Background: Ginsenosides are the main ingredients of Korean red ginseng. They have extensively been studied for their beneficial value in neurodegenerative diseases such as Parkinson's disease (PD). However, the multitarget effects of Korean red ginseng extract (KRGE) with various components are unclear.
Methods: We investigated the multitarget activities of KRGE on neurological dysfunction and neurotoxicity in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–induced mouse model of PD. KRGE (37.5 mg/kg/day, 75 mg/kg/day, or 150 mg/kg/day, per os (p.o.)) was given daily before or after MPTP intoxication.
Results: Pretreatment with 150 mg/kg/day KRGE produced the greatest positive effect on motor dysfunction as assessed using rotarod, pole, and nesting tests, and on the survival rate. KRGE displayed a wide therapeutic time window. These effects were related to reductions in the loss of tyrosine hydroxylase–immunoreactive dopaminergic neurons, apoptosis, microglial activation, and activation of inflammatory factors in the substantia nigra pars compacta and/or striatum after MPTP intoxication. In addition, pretreatment with KRGE activated the nuclear factor erythroid 2–related factor 2 pathways and inhibited phosphorylation of the mitogen-activated protein kinases and nuclear factor-kappa B signaling pathways, as well as blocked the alteration of blood–brain barrier integrity.
Conclusion: These results suggest that KRGE may effectively reduce MPTP-induced neurotoxicity with a wide therapeutic time window through multitarget effects including antiapoptosis, antiinflammation, antioxidant, and maintenance of blood–brain barrier integrity. KRGE has potential as a multitarget drug or functional food for safe preventive and therapeutic strategies for PD.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder. The main symptoms of PD are motor dysfunctions including bradykinesia, tremor, and muscular rigidity and nonmotor-related disorders including genitourinary problems, emotional changes, and cognitive problems [1–3]. Neuropathological hallmarks of PD are serious loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the midbrain and accumulation of α-synuclein aggregates into Lewy bodies and Lewy neuritis [4,5]. The loss of dopaminergic neurons is implicated in various other pathogenic mechanisms, including neuroinflammation, glutamate excitotoxicity, oxidative stress, mitochondrial dysfunction, and protein aggregation. The mechanisms share overlapping and complicated features [1–5]. Although most PD therapies, including levodopa, provide only symptomatic relief in reducing the motor symptoms, patients receiving long-term levodopa therapy must contend with side effects including

* Corresponding author. Department of Convergence Medical Science, Brain Korea 21 Plus Program, Institute of Korean Medicine, College of Korean Medicine, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Korea.
E-mail address: ihcho@khu.ac.kr (I.-H. Cho).

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levodopa-induced dyskinesias and response oscillations [6]. Therefore, development of efficient and safe therapeutic approaches that delay the onset or forestall the progression of PD is critical.

So far, most neuroprotection trials have produced unmet (not satisfied) results in PD when they have targeted only one of the multifactorial pathways that engaged in regulating synaptic function and neuronal survival [7]. On the other hand, the recent advances in medical biology including neuropharmacology have been responsible for new insights into the multifactorial and highly complex pathological hallmarks of various neurodegenerative disorders [7–10]. The development of multitargeted drugs to prevent and cure PD might be a new paradigm in the discovery and design for safe, effective, and innovative drugs. Although accumulating reports has been emphasized on characterizing and identifying potentially active natural material–derived pharmaceutical components to congruent this unmet demand [11], no approved PD-protective medicines are currently available [12]. Natural products remain an enormous untapped resource for the incessant development and discovery of therapeutic approaches for a wide range of disease conditions [13].

Panax (*P.*) *ginseng* Meyer, a perennial herb of the family Araliaceae, has been widely used as an adaptogen, particularly in the countries of East Asia for millennia. The major active constituents of *P. ginseng* are ginsenosides, which are derivatives of triterpenoid dammarane [14,15]. More than 100 ginsenosides have been isolated. The most frequently studied ones in PD are ginsenoside Rb1, Rd, and Rg1 [14,15]. *In vitro*, ginsenosides Rb1 and Rg1 have protective effects on mesencephalic dopaminergic cells stressed with glutamate [16]. Ginsenoside Rd mitigates neuroinflammation of dopaminergic cells that was evoked by lipopolysaccharide exposure by inhibiting inducible nitric oxide (iNOS) and cyclooxygenase (COX)-2 expressions [17]. Ginsenoside Rg1 mitigates dopamine-induced apoptosis in PC12 cells by reducing oxidative stress [18]. *In vivo*, Rg1 has positive effects on dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)– and lipopolysaccharide-induced rodent models of PD through antiapoptosis [19] and antiinflammation [20]. Each ginsenoside has different pharmacological effects *in vivo* and *in vitro* models of PD, which depend on the diversity of the sugar components and the number and position of the sugar moieties [14,21,22]. Korean red ginseng extract (KRGE) protected dopaminergic neurons by suppressing the cleavage of p35 to p25 [23] or by alleviating protein expression profiles related to enhancing energy metabolism in the brain of MPTP-treated mice [24]. However, the neuroprotective activities of ginseng extract in PD remain unclear. The purpose of the present study is to evaluate the multitarget effects of KRGE in a MPTP-induced mouse model of PD.

2. Materials and methods

2.1. Animals and ethical statement

Adult male C57BL/6J mice (Narabiotec Co., Ltd., Seoul, Korea, 7–9 weeks old, 22–24 g) were housed at a constant temperature of 23 ± 2°C with a 12-h light–dark cycle (lights on from 07:00 to 19:00) and were provided with food and water *ad libitum*. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Kyung Hee University. In this process, proper randomization of laboratory animals and data handling were performed in a blinded manner in accordance with recent recommendations from an National Institutes of Health (NIH) workshop on preclinical models of neurological diseases [25].

2.2. Preparation of KRGE

KRGE (Korea Ginseng Corporation, Daejeon, Korea) was prepared, as previously described [26,27], from the roots of 6-year-old fresh *P. ginseng* Meyer. KRGE contained major ginsenosides Rb1 (7.44 mg/g), Rb2 (2.59 mg/g), Rc (3.04 mg/g), Rd (0.91 mg/g), Re (1.86 mg/g), Rf (1.24 mg/g), Rg1 (1.79 mg/g), Rg2s (1.24 mg/g), Rg3s (1.39 mg/g), and Rh1 (1.01 mg/g) and other minor ginsenosides, as determined by high-performance liquid chromatography.

2.3. Experimental groups

First, to confirm the most effective dose and mechanism of administration of KRGE for pretreatment, mice were randomly divided into sham, MPTP, MPTP + KRGE pretreatment (35.7 mg/kg, 75 mg/kg, and 150 mg/kg), and KRGE groups ($n = 5$–15 per group). Second, to investigate the therapeutic time window of KRGE use, mice were randomly divided into sham, MPTP, MPTP + KRGE treatment pre-, onset-, progression-, and peak-treatment, and KRGE groups ($n = 5$ per group).

2.4. Treatment with MPTP and KRGE

For MPTP intoxication, mice received four intraperitoneal injection of MPTP-HCl (20 mg/kg body weight; Sigma-Aldrich, St. Louis, MO, USA) dissolved in phosphate-buffered saline at 2 h intervals in accordance with published guidelines [28]. KRGE was dissolved in physiological saline and treated orally at doses of 37.5 mg/kg, 75 mg/kg, or 150 mg/kg once daily from 1 h before the first MPTP intoxication. In addition, mice were treated with 150 mg/kg KRGE (the most effective dose) once daily from 1 h before the first MPTP intoxication and at 24, 72, or 120 h after the last MPTP intoxication. Each experiment was repeated >three times using the same protocol. The total daily dose of KRGE given to mice was determined after considering body weight, metabolic rate, and traditional dose given to humans [26,27].

2.5. Behavioral assessments

Forelimb and hindlimb motor performance and balance were measured by the pole and rotarod tests. Briefly, mice ($n = 5$–10 per group) were placed head down near the top of a rough-surfaced wooden pole (40 mm in diameter and 50 cm in height). The time taken to reach the bottom of the pole was measured. One hour after the pole test, the mice were placed on a rotating cylinder (4 cm in diameter) with a coarse surface for firm grip and tested for three trials with an accelerating speed of 16 rpm/s. A cut-off time of 5 min and an intertrial interval of 15 min were used. Latency on the rod before falling was measured. The utility of nest-building behavior was measured as an indicator of health and welfare in mice. Briefly, to test the individual nest-building behavior, mice were individually housed in cages containing wood chip bedding and one square of pressed cotton (Nestlets; Ancare Corp., Bellmore, NY, USA). The following morning, the manipulation of the Nestlet and the constitution of the built nest were assessed manually according to a five-point scale: 0, no nest (>90% intact); 1, flat nest (50–90% remaining intact); 2, nest covering the mouse (<50% remains intact); 4, an identifiable but flat nest (using >90% of Nestlet); 5, perfect nest [29]. The behavioral tests were accomplished by an experimenter who was unaware of the experimental conditions and were done under constant temperature (23 ± 3°C) and humidity (55 ± 4%) in a quiet room, 1, 3, and 7 days after the final MPTP intoxication.

2.6. Histological evaluation

Seven days after the last MPTP intoxication, the brains ($n = 5$ per group) for histological evaluation were prepared as described previously [26,27]. Sequential coronal sections (30 μm in thickness)

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