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Immunomodulatory activities of polysaccharides from *Chlorella pyrenoidosa* in a mouse model of Parkinson's disease

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ABSTRACT

Neuro-inflammation is implicated as a major pathogenic factor in Parkinson's disease (PD). Dietary supplements of *Chlorella pyrenoidosa* possess great anti-inflammatory activities, but its neuroprotective effect remains unclear. The aim of this study was to investigate the effects of polysaccharides from *Chlorella pyrenoidosa* (CPS) on motor activity, dopamine expressions, microglial activation, and peripheral immunomodulatory responses in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of PD. The results of this study indicated that CPS reduced bradykinesia, inhibited the loss of striatal dopamine and its metabolites, and led to an increase in tyrosine hydroxylase in PD mice. In addition, CPS also suppressed the striatal Emr1 expression and tumor necrosis factor-α, interleukin-1β and IL-6 levels in serum. Furthermore, the gut immune biomarkers such as serum diamine oxidase and small intestinal secretory immunoglobulin A were enhanced by CPS treatment. These findings demonstrate that CPS has protective effect in MPTP-induced neurotoxicity in this model of PD *via* its immunomodulatory action.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with high prevalence of 1–2% among people older than 60 years old. Characteristic features of PD include resting tremor, bradykinesia, rigidity and postural instability. Although disease etiology remains elusive, genetic, environmental, and neuroinflammatory processes are factors influencing the development of early- or late-onset PD (Schapira, 2013). Clinical neurological examination identified a massive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of PD patients, correlating with striatal dopamine deficiency. Current treatment of PD with L-dihydroxyphenylalanine (L-dopa) aims at restoring dopamine, which only focuses on symptomatic relief. However, long-term use of L-dopa may add to the oxidative load and microglial activation, triggering chronic inflammatory response in disease progression (Smith, Parker, & Bennett, 1994).

To elucidate the molecular cascade of cell death in dopaminergic neurons, neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is widely used to generate animal models of PD (Bové & Perier, 2012). MPTP selectively damages the dopaminergic neurons and causes microglial activation

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consequent on increased levels of inflammatory cytokines, producing an irreversible parkinsonism syndrome in mice (Sugama et al., 2003). Numerous studies have shown that the regulation of inflammatory mediator in immune-brain communication plays a protective role in inflammation-induced dopaminergic cell loss (Gomez-Nicola, Teeling, Guaza, Godbout, & Taub, 2013; Wilms et al., 2007). Therefore, development of more preventive and therapeutic strategies for neuroprotection in PD has gained increasing attention.

Chlorella pyrenoidosa (*C. pyrenoidosa*), a species of the freshwater microalgae genus *Chlorella*, has been used in traditional food in Taiwan and Japan. Growing evidence indicates that *C. pyrenoidosa* possesses a number of health benefits, including regulating blood lipids and blood sugar, enhancing immune system, providing antioxidant activity and displaying anti-inflammatory effects (Lee, Choi, Cho, & Son, 2003; Merchant & Andre, 2001; Mizoguchi, Takehara, Masuzawa, Saito, & Naoki, 2008). There are many active components in *C. pyrenoidosa*, including carotenoids, polysaccharides and phytol. Moreover, no toxic effects have ever been observed in laboratory animals or humans who have consumed *C. pyrenoidosa* (Merchant & Andre, 2001). Polysaccharides from *C. pyrenoidosa* (CPS) are thought to possess immunological properties, which could induce TNF-α and IL-1β expression *via* TLR4-mediated protein kinase activation in macrophages (Hsu et al., 2010). Previous studies have revealed that polysaccharides reduced the severity of experimental neuronal damage and neuroinflammation (Cui, Jia, Zhang, Zhang, & Wang, 2012). However, there is little research related to the neuroprotective effects of CPS on neurotoxin-induced PD. In this study, we investigated the immunomodulatory effects of polysaccharides extract from *C. pyrenoidosa* in an MPTP-induced mouse PD model.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (8 weeks old, 18–22 g) were purchased from BioLasco Co. (Taipei, Taiwan) and housed under controlled temperature (25 ± 2 °C), relative humidity (50%), and 12 h on/off light cycle with *ad libitum* access to food and water at the Animal House Facility of Institute of Food Science and Technology. All animal experimental procedures were in strict accordance with the recommendation in the "Guidebook for the Care and Use of Laboratory Animals", published by The Chinese-Taipei Society of Laboratory Animal Sciences. The study was approved by the National Taiwan University Institutional Animal Care and Use Committee (Approval No. NTU-IACUC-99-53).

2.2. Materials

C. pyrenoidosa hot water extracts were provided by Taiwan Chlorella Manufacturing Co., Ltd (Taipei, Taiwan). MPTP, L-dopa, benserazide, glucose, fructose, xylose, arabinose, ribose and primers were purchased from Sigma Co. (St. Louis, MO, USA). Trizol® reagent was purchased from Invitrogen (Carlsbad, CA, USA). QuantiTect® Reverse Transcription Kit was purchased from Qiagen (Hilden, Germany). KAPA SYBR® FAST qPCR Kit Master Mix ABI Prism™ was purchased from KapaBiosystems (Woburn, MA, USA). Anti-tyrosine hydroxylase antibody was obtained from Millipore (Billerica, MA, USA). UltraVision LP Detection System: HRP Polymer/DAB Plus Chromogen was purchased from Thermo Scientific (Waltham, CA, USA). Mannose was purchased from Alfa Aesar (Heysham, UK), and rhamnose was purchased from Merck (Darmstadt, Germany). Mouse TNF-α, IL-1β, and IL-6 ELISA kits were purchased from eBioscience (San Diego, CA, USA). Mouse IgA ELISA kit was purchased from Bethyl Laboratories (Montgomery, TX, USA). Mouse DAO ELISA Kit was purchased from Immundiagnostik (Bensheim, Germany).

2.3. *Preparation of* C. pyrenoidosa *polysaccharides (CPS)*

C. pyrenoidosa hot water extracts were dissolved in 4-fold of ultrapure water, and precipitated with four fold of 95% (v/v) ethanol (4 °C, overnight). Subsequently, the mixture was centrifuged at 7500 *g* for 15 min at 4 °C to collect the precipitated polysaccharide fraction. The precipitate was dialyzed against ultrapure water (4 °C, overnight) and lyophilized for 24 hours to obtain polysaccharides from *C. pyrenoidosa*. CPS samples were stored at –20 °C until they were used.

2.4. *Mice model for testing CPS*

After one-week habituation, mice then were randomly divided into 5 groups (n = 6). Treatment groups received CPS (100 or 200 mg/kg, once per day) for 19 days, while control, negative and positive groups were administrated with water instead. Experimental parkinsonism was established by i.p. injections of MPTP (dissolved in 0.9% saline, 15 mg/kg four times at two-hour interval) on the 11th day 1 hour after oral administration of CPS or water, while control group received four injections of saline. Appropriate guidelines were abided in handling MPTP (Przedborski et al., 2001). Positive group was treated with MPTP followed by a daily dose of L-dopa (50 mg/kg) with benserazide (25 mg/kg) (i.p.) from the 12th day for the purpose of preventing the peripheral decarboxylation of L-dopa. Dose of L-dopa and benserazide was selected based on the literature (Szego, Gerhardt, Kermer, & Schulz, 2012). Animals were sacrificed 2 hours after treatments on day 19th. The experimental design is illustrated in Fig. 1.

2.5. *Behavioral test*

2.5.1. *Pole test*

The pole test is a practical method to detect the degree of bradykinesia in mice PD model (Ogawa, Hirose, Ohara, Ono, & Watanabe, 1985). The head of the mice were placed upside by sliding its forepaws on top of a vertical wooden pole (diameter 8 mm, height 50 cm), which was wrapped in gauze to prevent slipping (Yang et al., 2011). The time it took for the mice to turn down, climb down and four feet reached the floor was measured. Each mouse was required to perform three successive trials at 30-second intervals, with a cut-off limit of 30 seconds. This test was performed two days before and three days after the day of MPTP injection. All the mice were pretrained three times before the formal tests.