NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION
RESEARCH ARTICLE
D. Huang et al. / Neuroscience xxx (2017) xxx–xxx

Long-term Changes in the Nigrostriatal Pathway in the MPTP Mouse Model of Parkinson's Disease

Dongping Huang,[a†] Zishan Wang,[a†] Jiabin Tong,[a] Mo Wang,[a] Jinghui Wang,[a] Jing Xu,[b] Xiaochen Bai,[a] Heng Li,[a] Yulu Huang,[c] Yufei Wu,[c] Yuanyuan Ma,[a] Mei Yu[a*] and Fang Huang[a*]

[a] The State Key Laboratory of Medical Neurobiology, The Institutes of Brain Science and the Collaborative Innovation Center for Brain Science, School of Basic Medical Sciences, Shanghai Medical College, Fudan University, 138 Yixueyuan Road, Shanghai 200032, China

[b] School of Life Science and Technology, Tongji University, 1239 Siping Road, Shanghai 200092, China

[c] School of Basic Medical Sciences, Fudan University, 138 Yixueyuan Road, Shanghai 200032, China

Abstract—Parkinson's disease (PD) is a common and progressive neurodegenerative disorder. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD is widely used to study the progression of this disease. Behavior impairment is closely related to the damage of the dopaminergic system in the basal ganglia. Here, MPTP-induced changes in mouse behavior and glial activation were evaluated at different time points after the treatment and the long-term changes in the nigrostriatal pathway were analyzed. We found that mice exposed to MPTP displayed a full recovery in the rotarod test and the pole test but not in the wire hanging test at 65 days post-injection. A biphasic activation of microglial cells was revealed in the nigrostriatal pathway of MPTP-treated mice. However, activation of astrocytes displayed an approximately bell-shaped kinetics and an approximately S-shaped kinetics in the striatum and the substantia nigra, respectively. In addition, the numbers of complement component 3 (C3)-positive neurotoxic astrocytes in the substantia nigra of MPTP-treated mice increased with time and reached a maximum at 42 days, and declined at 74 days, after the treatment. Three months later, the dopaminergic system was partially recovered from the lesion of MPTP. The time course of pathophysiological events has important implications for the interventions or treatment of PD.

Key words: Parkinson's disease, behavior tests, glial activation, C3.

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disease, affecting 1–2% of people aged above 65 years worldwide (Toulouse and Sullivan, 2008; Felice et al., 2016). The pathological hallmarks of PD are the progressive loss of dopaminergic neurons in the midbrain substantia nigra (SN) and the presence of Lewy bodies (Dauer and Przedborski, 2003; Tieu, 2011). When the loss of dopaminergic neurons results in a threshold depletion of 70–80% dopamine in the striatum, motor symptoms of PD occur (Zigmond et al., 2002; Dauer and Przedborski, 2003; Tieu, 2011). The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model is the most commonly used model for elucidating damages to the nigrostriatal pathway in PD. In this model, glial activation is an early event and plays a detrimental role in the progression of the disease.

Activation of microglial cells at the striatum emerges as early as 90 min after acute MPTP intoxication (Liu et al., 2015). The activation of microglia in the nigrostriatal pathway autonomously decreases to a level similar to the resting state approximately 9–14 days after MPTP administration (Kohutnicka et al., 1998; Liu et al., 2015). Whether MPTP elicits a second cascade of microglial activation in the nigrostriatal pathway, particularly at the late phase, remains unclear. Reactive astrocytes are observed accompanying the progression of PD, although the initiation of astrocyte activation is later than microglial activation. Recently, Liddelow et al. have reported that activated microglia induce neurotoxic reactive astrocytes, and complement component 3 (C3)-positive ($C3^+$) neurotoxic astrocytes are present in the SN of PD patients (Liddelow et al., 2017). Dynamic changes in $C3^+$ astrocytes in the nigrostriatal pathway in the MPTP mouse model of PD warrant further investigation.

To this end, in this study, an acute regimen of MPTP was administered to adult C57BL/6 mice. The behaviors

*Corresponding authors. Fax: +86-21-64174579.
E-mail addresses: yumei@fudan.edu.cn (M. Yu), huangf@shmu.edu.cn (F. Huang).
† These authors contributed equally to this work.
Abbreviations: C3, component 3; DOPAC, 3,4-dihydroxyphenylacetic acid; $GFAP^+$, GFAP-positive; HVA, homovanillic acid; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ORP, overall rod performance; PBS, phosphate-buffered saline; PD, Parkinson's disease; SN, substantia nigra; SNpc, substantia nigra pars compacta; TH^+, TH-positive.

https://doi.org/10.1016/j.neuroscience.2017.11.041
0306-4522/

of the animals and the changes in glial activation and dopaminergic pathways were assessed at different time points after the treatment of MPTP. The glial activation was also evaluated in mice at 4 months after exposure to a subchronic regimen of MPTP.

EXPERIMENTAL PROCEDURES

MPTP treatments of mice

Ten- or 16-week-old male C57BL/6 mice were injected intraperitoneally with MPTP-HCl (Sigma, USA) in 0.9% NaCl, using an acute dosing regimen of 18 mg/kg every 2 h for four doses (Jackson-Lewis and Przedborski, 2007) or subchronic regimen of 20 mg/kg, once per day for 5 consecutive days (Selvaraj et al., 2012), respectively. Mice were subjected to behavioral tests or euthanized at different time points after MPTP administration (see Fig. 1A). All experimental protocols were approved by the Institutional Animal Care and Use Committee of Fudan University, Shanghai Medical College. All surgeries were performed under general anesthesia, and all efforts were made to minimize adverse effects.

Behavioral tests

Rotarod test. The rotarod test was conducted in reference to previous studies (Liu et al., 2015; Suo et al., 2015). The overall rod performance (ORP) for each animal was evaluated by the trapezoidal method as the area under the curve in a plot of time on the rod versus rotation speed (Tieu et al., 2003).

Pole test. A stand with a rough-surfaced pole (1 cm in diameter and 50 cm in height) was used. A training trial was administered to the mice one day before the experiment. The next day, each mouse was placed head-up near the top of the pole and time to turn and time to reach the bottom were recorded. The test was performed three times for each animal, and the results presented were the average values of the three trials (Yu et al., 2013).

Wire hanging test. Mice suspended by their forelimbs from a horizontal wire (1.6 mm in diameter, 50 cm long, 30 cm high between two poles) tend to support themselves with their hindlimbs to avoid falling and to aid in progression along the wire. The latency to fall down and the ability to grip the wire was scored as described by Kaizaki et al (Kaizaki et al., 2014). This test was carried out three times, the final results were an average of the three trials.

HPLC

The striatum was weighed and sonicated in 0.4 M $HClO_4$ (10 μl per milligram tissue) on ice and then centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatants were removed for determining the concentration of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) using the chromatograph (ESA, Chelmsford, MA, USA) with a 5014B electrochemical detector (Suo et al., 2015).

Protein extraction and western blot analysis

Tissues were homogenized in the lysis buffer containing a protease inhibitor cocktail (Thermo Fisher, USA) on ice. Protein samples (20 μg) were separated by sodium dodecyl sulfate-polyacrylamide gels and then transferred onto polyvinylidene difluoride membranes (Millipore, USA). The primary antibodies used were as follows: mouse anti-TH (1:41000; Sigma, USA), rabbit anti-GFAP (1:4000; Millipore, USA) and mouse anti-β-actin (1:10,000; Sigma-Aldrich, USA). The membrane-bound proteins were detected with an Odyssey infrared imaging system (Li-Cor, USA).The protein levels were quantified by densitometry analysis using ImageJ (National Institutes of Health, USA).

Immunohistochemical staining and immunofluorescence staining

Brain sections (30 μm thick) were permeabilized, quenched the endogenous peroxidases with 0.1% H_2O_2 and blocked in phosphate-buffered saline (PBS) containing 0.2% Triton X-100 and 10% normal goat serum 37 °C for 45 min. The sections were then incubated with mouse anti-TH (1:1000; Sigma, USA) in PBS with 1% goat serum at 4 °C overnight. After washing, the sections were incubated with biotinylated anti-mouse secondary antibodies (1:200; Vector Laboratories, USA) at 37 °C for 45 min and then with AB peroxidase (1:200; Vector Laboratories, USA) at 37 °C for 45 min. The peroxidase reaction was detected with 0.05% DAB (Sigma, USA) in 0.1 M Tris buffer and 0.03% H_2O_2.

For immunofluorescence staining, brain sections were blocked and then incubated at 4 °C overnight with the primary antibodies as follows: mouse anti-TH (1:500; Sigma, USA) and rabbit anti-Iba1 (1:500; Wako, Japan), rabbit anti-GFAP (1:1000; Millipore, USA) and rat anti-complement component 3 (1:50; Millipore, USA). After washing, the sections were incubated with secondary antibodies: donkey anti-mouse Alexa Fluor 594 and donkey anti-rabbit Alexa Fluor 488, or donkey anti-rabbit Alexa Fluor 488 and anti-rat Alexa Fluor 594 (1:1000; Invitrogen, USA). Slides were coverslipped and imaged with a Leica confocal microscope (TCS SP-2, Leica, Germany).

Quantification of Iba1-, GFAP- and C3-positive cells

Quantification of $GFAP^+$, $Iba1^+$ or $C3^+$ cells was carried out by combining the methods described in the references (Baiguera et al., 2012; Shao et al., 2013) with modification and analyzed with Image-Pro Plus 6.0 (Media Cybernetics, USA). Briefly, images were captured through a camera mounted on the fluorescence microscope (Olympus, Japan) as used in many studies (Gao et al., 2011; Sevc et al., 2014). All the parameters for the microscope and image capture system were held constant. Since the cell bodies of dopaminergic neurons that reside in the

Please cite this article in press as: Huang D et al. Long-term Changes in the Nigrostriatal Pathway in the MPTP Mouse Model of Parkinson's Disease. Neuroscience (2017), https://doi.org/10.1016/j.neuroscience.2017.11.041