Triptolide protects against 1-methyl-4-phenyl pyridinium-induced dopaminergic neurotoxicity in rats: Implication for immunosuppressive therapy in Parkinson’s disease

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Abstract: Objective Neuroinflammation with microglial activation has been implicated to have a strong association with the progressive dopaminergic neuronal loss in Parkinson’s disease (PD). The present study was undertaken to evaluate the activation profile of microglia in 1-methyl-4-phenyl pyridinium (MPP+)–induced hemiparkinsonian rats. Triptolide, a potent immunosuppressant and microglia inhibitor, was then examined for its efficacy in protecting dopaminergic neurons from injury and ameliorating behavioral disabilities induced by MPP+.

Methods The rat model of PD was established by intranigral microinjection of MPP+. At baseline and on day 1, 3, 7, 14, 21 following MPP+ injection, the degree of microglial activation was examined by detecting the immunodensity of OX-42 (microglia marker) in the substantia nigra (SN). The number of viable dopaminergic neurons was determined by measuring tyrosine hydroxylase (TH) positive neurons in the SN. Behavioral performances were evaluated by counting the number of rotations induced by apomorphine, calculating scores of forelimb akinesia and vibrissae-elicited forelimb placing asymmetry.

Results Intranigral injection of MPP+ resulted in robust activation of microglia, progressive depletion of dopaminergic neurons, and ongoing aggravation of behavioral disabilities in rats. Triptolide significantly inhibited microglial activation, partially prevented dopaminergic cells from death and improved behavioral performances.

Conclusion These data demonstrated for the first time a neuroprotective effect of triptolide on dopaminergic neurons in MPP+-induced hemiparkinsonian rats. The protective effect of triptolide may, at least partially, be related to the inhibition of MPP+-induced microglial activation. Our results lend strong support to the use of immunosuppressive agents in the management of PD.

Keywords: Parkinson's disease; triptolide; microglia; neurons

1 Introduction

Parkinson’s disease (PD) is one of the most common age-related neurodegenerative diseases[1]. Clinically, most patients suffer from slowness of movement, rest tremor, rigidity, and disturbances in balance. Pathologically, it is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra (SN)[2,3]. Although symptomatic treatments of PD are of proven efficacy, no drugs have been established to have a clinically validated effect on slowing down its progression[4,5]. Better understanding of these processes leading to progressive dopaminergic neuronal loss may provide effective targets for the design of novel therapeutics to PD.

Neuroinflammation have currently been implicated as a driving force in PD pathogenesis[6]. Microglia is suspected to play a major role in this process. As constitutive immune cells in the brain, microglia is sensitive to even minor disturbances in central nervous system (CNS) homeostasis and become readily activated in response to abnormal stimula-
tion. Uncontrolled activation of microglia may cause direct toxicity to neurons by releasing various cytotoxic compounds. Activated microglia have been found not only in post-mortem PD brain, but also in various PD models. Inhibition of microglia-mediated neuroinflammation, therefore, might become a promising therapeutic strategy in PD.

We focused on triptolide, an active component derived from the traditional Chinese medicinal plant Tripterygium Wilfordii Hook F (TWHF), which has anti-inflammatory and immunosuppressive activities. In the CNS, triptolide is capable of blocking lipopolysaccharide (LPS)-induced activation of microglia. However, whether triptolide could inhibit microglial activation and exert neuroprotective effect in 1-methyl-4-phenyl pyridinium (MPP⁺)-induced hemiparkinsonian rats still remain unknown.

The aim of the present study was, first, to evaluate the activation profile of microglia in MPP⁺-induced hemiparkinsonian rats, an ideal animal model of PD. Then, we sought to investigate the effect of triptolide on microglial activation, and examined whether it could exert neuroprotective effect against MPP⁺ neurotoxicity.

2 Materials and methods

2.1 Animals

Experiments were performed on adult male Sprague-Dawley rats (Experimental Animal Center, Shanghai Medical College of Fudan University, China) weighing 250-280 g. Rats were housed in suspended cages and maintained on a 12:12 h light-dark cycle with free access to food and water. All experimental protocols and animal handling procedures were approved by Animal Care and Use Committee (ACUC) of Fudan University, and were consistent with the National Institutes of Healthy Guide for the Care and Use of laboratory animals. All efforts were made to minimize the number of animals used and their suffering.

2.2 Intranigral injection of MPP⁺

Before surgery, 54 rats were randomly divided into nine groups (6 animals each). Six groups received MPP⁺ alone and were subjected to behavioral and immunohistochemical analysis at baseline (day 0) and on day 1, 3, 7, 14, 21 after surgery. One group received saline instead of MPP⁺ and served as sham control. The remaining two groups were treated by intraperitoneal (i.p.) injection of triptolide and normal saline (NS) respectively following MPP⁺ injection. During surgery, all animals were securely placed into a stereotaxic device (Jiangwan, Shanghai, China) under Chloral Hydrate (400 mg/kg, i.p.) anesthesia with bregma and lambda at a horizontal level. All stereotaxic coordinates were determined according to the brain atlas of Paxinos and Watson. For intranigral microinjection, a small hole was drilled in the skull above the left SN (5.0 mm anteroposterior, 2.1 mm lateral). A microsyringe was then lowered (7.7 mm dorsoventral), and 10 μg of MPP⁺ (Sigma, USA) (5 μg/μL) in a saline buffer was infused at a rate of 0.4 μL/min for 5 min. After infusion of the toxin, the probe was kept in the same position for a further 5 min for complete diffusion of the drug and then slowly retracted. The post-operative animals were left in a temperature-controlled chamber until they recovered from anesthesia, then they returned to their home cages.

2.3 Administration of triptolide

Triptolide (purity: 98%) was provided by National Institute for the Control of Pharmaceutical and Biological Products (No. 111567-200502). Administration of triptolide was principally consistent with the method described previously. Briefly, triptolide was dissolved in saline containing 5% dimethylsulphoxide and applied intraperitoneally. Rats received a single dose of 5 μg/kg triptolide or vehicle per day from day 3 before MPP⁺ injection and continued for 21 d post-lesion (24 d in total).

2.4 Behavioral tests

The degree of damage to nigrostriatal pathway can be assessed using sensitive drug and non-drug tests. A series of tests was performed before and on day 1, 3, 7, 14 and 21 after surgery.

2.4.1 Rotational behavior analysis

At all the above mentioned time points, rats were injected subcutaneously with 0.3 mg/kg apomorphine hydrochloride (Sigma, USA) dissolved in 0.1% ascorbate saline solution and allowed to sit in a separate cage for 15 min. Animals were then placed in an iron bowl, and the net number of turns (made towards the side of lesion minus those made away from the lesioned side) in a 30-min trial was calculated.

2.4.2 Forelimb akinesia (stepping test)

Movement initiation for each limb was assessed using the forelimb akinesia test protocol. In short, the rat was held by the experimenter with one hand fixing the hindlimbs and slightly raising the hind part above the surface. The animal was allowed to initiate stepping movements in a 10 s period for the left forelimb and then the right one for a balanced order. An ipsilateral asymmetry score was derived (ipsilateral steps/ipsilateral plus contralateral steps) – (contralateral steps/ipsilateral steps) – 1.