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 dopamin-

ergic 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-

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Article ID: 1673-7067(2008)03-0133-010

CLC number: R741

Document code: A

Received date: 2007-12-25

tion^[7]. Uncontrolled activation of microglia may cause direct toxicity to neurons by releasing various cytotoxic compounds^[8]. Activated microglia have been found not only in post-mortem PD brain, but also in various PD models^[9-11]. Inhibition of microglia-mediated neuroinflammation, therefore, might become a promising therapeutic strategy in PD^[12].

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^[13-15]. In the CNS, triptolide is capable of blocking lipopolysaccharide (LPS)-induced activation of microglia^[16,17]. 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^[18]. 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 Health 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^[19]. 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 μ g of MPP⁺ (Sigma, USA) (5 μ g/ μ L) in a saline buffer was infused at a rate of 0.4 μ L/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^[17]. Briefly, triptolide was dissolved in saline containing 5% dimethylsulphoxide and applied intraperitoneally. Rats received a single dose of 5 μ g/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^[20].

2.4.2 Forelimb akinesia (stepping test) Movement initiation for each limb was assessed using the forelimb akinesia test protocol^[21-23]. 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

plus contralateral steps)].

2.4.3 Vibrissae-elicited forelimb placing A vibrissae-elicited forelimb-placing test was used to test reaction to sensory stimuli as described previously^[22-25]. At the assigned time points after surgery, rats were gently held by their torsos with the forelimbs hung free. The respective vibrissae of the animal were brushed against the corner edge of a countertop to elicit a forelimb placing response from the forelimb on the same side. Intact animals place the forelimb of both sides quickly onto the countertop. Animals with unilateral damage will show varying degrees of impaired limb placing ability, while still placing the unimpaired limb reliably. Each animal was tested 10 times on each side. Percentage of unsuccessful placing responses was determined [(number placed contralaterally divided by the number placed ipsilaterally) × 10].

2.5 Immunohistochemistry and quantification As previously described by our group^[26], rats were given an overdose of urethane (2 g/kg, i.p.) and perfused intracardially with 100 mL 0.9% saline followed by 400 mL cold 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB, pH 7.4). Brains were removed and immersed from 10%-30% gradient sucrose in PB for 24-48 h at 4 °C for cryoprotection. Coronal brain sections (free-floating, 30 µm) were cut by a freezing microtome (Leica 1900, German) and processed for immunofluorescence. All the sections were blocked with 10% normal donkey serum in 0.01 mol/L phosphate buffered saline (PBS, pH 7.4) with 0.3% Triton-X-100 for 1 h at room temperature and incubated overnight at 4 °C with mouse anti-OX-42 (1:1000, Sigma, USA), rabbit anti-tyrosine hydroxylase (TH) (1:1000, Sigma, USA) primary antibody. The sections were then incubated with fluorescein isothiocyanate (FITC)-conjugated donkey anti-mouse IgG (1:200, Chemicon, USA) or rhodamine-conjugated donkey anti-rabbit IgG (1:200, Chemicon, USA) for 90 min at 4 °C, then washed in PBS. Omission of primary antibody served as negative control. The stained sections were examined with a Leica fluorescence microscope, and images were captured with a CCD spot camera.

Because the morphology of microglia is complex and the immunoreactive staining includes both cell bodies and their processes, cell counts may not sufficiently quantify activation. Therefore, the integrated densities (pixelwise integration of density) of OX-42 immunoreactivity (OX-42-IR) within the SN were measured with the Leica Qwin 500 image analysis

system (Germany) at six randomly selected sections for each animal.

Quantification of viable dopaminergic neurons were principally consistent with a method previously described by Vaananen AJ *et al.* with slight modifications^[27]. TH is the rate-limiting enzyme in the synthesis of catecholamines, and specific for catecholaminergic neurons. Thus, quantification of TH-positive neurons in serial sections throughout the SN can be used as a reliable measure for the numbers of viable dopaminergic neurons. The relative numbers of TH-positive neurons were evaluated by counting TH-positive neurons at six randomly selected sections within the SN area in both ipsilateral (injected) and contralateral (non-injected) side under the fluorescence microscope. The results were expressed as a percentage of TH-positive neurons at the injected side compared to the non-injected side, and the percentages for each experimental group were pooled.

2.6 Experimental procedure

2.6.1 Effects of intranigral MPP⁺ injection on microglial activation and TH-positive cell loss in rats After baseline behavioral assessments, rats received intranigral injection of 10 µg/2 µL MPP⁺. On day 0, 1, 3, 7, 14, and 21 after surgery, rats were perfused with fixative and the brains were removed for immunohistochemistry analysis. Quantification of the density of OX-42-IR and percentage of TH-positive neurons were performed as described in the previous section. The rats of sham group received an intranigral injection of sterile NS (2 µL) and were killed at day 21 after NS injection.

2.6.2 Effects of MPP⁺ on behavioral performances in rats Behavioral performances were evaluated before sacrifice at different time points by performing forelimb akinesia, vibrissae-elicited forelimb placing and apomorphine induced rotation tests. Data in the MPP⁺ group were compared with those in saline group.

2.6.3 Effects of triptolide on MPP⁺-induced microglial activation and TH-positive cell loss After behavioral assessments on the day 21 post-surgery, rats were subjected to immunohistochemistry. The degree of microglial activation and TH-positive neuronal loss in the sham group, MPP⁺ group, triptolide treatment group, and vehicle treatment group was examined by detecting OX-42-IR density and the percentage of TH-positive neuronal number ipsilateral to the MPP⁺ injected side compared to the contralateral side.

2.6.4 Effects of triptolide on MPP⁺-induced behavioral de-

fects in rats Behavioral abnormalities of rats from all the experimental groups were examined at different time points after MPP⁺ intoxication. Items of testing were the same as the behavioral tests described previously.

2.7 Statistical analysis Differences of immunohistochemical and behavioral data between two groups at a single time point were performed by student's *t*-test. One-way ANOVA followed by Holm-Sidak *t*-test was employed for analyzing percentage of TH-positive neurons and behavioral data at different time points after MPP⁺ injection. Two-way ANOVA (treatment×time) followed by Newman-Keuls post hoc test was used for analyzing behavioral data between groups at different time points. Results have been presented as Means ± SEM. *P* < 0.05 were considered statistically significant.

3 Results

3.1 Intraneurally injected MPP⁺ induced microglial activation and dopaminergic neuronal loss in the SN area of rats

Activated microglia have been implicated in PD^[10,28]. In order to examine the time course of microglial activation during the 21 d after MPP⁺ injection, brain sections were immunostained with an antibody at different time points against OX-42 (CD-11b), a marker of activated microglia. In control animals, OX-42 immunopositive microglia in the intact SN exhibited the characteristics of inactive, resting microglia, and possessed small cell bodies. In rats treated with MPP⁺, however, there was a marked increase in the number of OX-42-immunopositive microglia with morphological changes typical of activation. Activated microglia exhibited large cell bodies with multiple,

Fig. 1 Microglia and dopaminergic neurons in the SN as revealed with OX-42 and TH immunoreactivity respectively. **A**, Morphological presentation of OX-42-immunopositive microglia in the SN of rats at different time points after MPP⁺ injection. Scale bar, 50 μ m. **B**, Density of OX-42-IR in the SN of rats following intraneural MPP⁺ injection. **C**, Representative photomicrographs showing TH immunopositivity in the SN of rats at different time points after MPP⁺ injection. Scale bar, 100 μ m. **D**, Quantification of the numbers of TH-positive neurons in the injected ipsilateral SN as compared with the contralateral non-injected SN, expressed as a percentage ratio. Data were represented as Means±SEM. **P* < 0.05, #*P* < 0.05 vs baseline (*n* = 6).

thick and short lamellipodia (Fig. 1A), which was consistent with previous studies^[29]. The density of OX-42-IR significantly increased at day 3 post-lesion ($t = 2.867$, $P < 0.05$), and reached the peak at day 7 ($t = 7.046$, $P < 0.05$). At day 14 and day 21 post-lesion, the increase in OX-42-IR became less marked ($t = 5.050$, $P < 0.05$; $t = 4.186$, $P < 0.05$; Fig. 1B).

Intranigral MPP⁺ injection induced ongoing reduction of dopaminergic neurons as assessed by their expression of TH in SN ipsilateral to the MPP⁺ injection site (Fig. 1C). The decrease in the percentage of TH-positive neurons became statistically significant at the third day post-lesion ($t = 3.731$, $P < 0.05$) and progressively manifested during the subsequent days of observation [One-way ANOVA, $F(5,30) = 81.453$, $P < 0.05$; Fig. 1D].

3.2 Intranigral injected MPP⁺ induced progressive behavioral abnormalities in rats Unilateral nigrostriatal lesions produce asymmetries in body posture and limb use in rats. Apomorphine induced rotational behavior, forelimb akinesia and vibrissae-elicited forelimb placing tests have been widely used in hemiparkinsonian rat model of PD as good indicators of damage to the nigrostriatal pathway^[20,22,23,25,30]. Here, statistically significant behavioral defects occurred from the seventh day following intranigral MPP⁺ injection (rotational behavior: $t = 5.485$, $P < 0.05$; forelimb akinesia: $t = 6.869$, $P < 0.05$; vibrissae-elicited forelimb placing: $t = 7.737$, $P < 0.05$). Such defects progressively worsened during the subsequent 14 d of observation [One-way ANOVA, rotational behavior: $F(2,15) = 20.441$, $P < 0.05$; forelimb akinesia: $F(2,15) = 11.141$, $P < 0.05$; vibrissae-elicited forelimb placing: $F(2,15) = 6.822$, $P < 0.05$; Fig. 2].

Our results are broadly in accordance with previous study by Banerjee R *et al.* who showed that intranigral MPP⁺ infusion cause long-lasting damage to the nigrostriatal dopamine pathway, and did not show any sign of recovery in striatal dopamine content even after 90 d^[31].

3.3 Triptolide attenuated MPP⁺-induced microglial activation and dopaminergic neuronal loss According to previous studies, triptolide is a potent anti-inflammatory and immunosuppressive agent, and is capable of inhibiting LPS-induced microglial activation and dopaminergic neuronal loss^[13,16]. Here, we extended these studies to examine whether triptolide could quench MPP⁺-induced microglial activation and protect dopaminergic cells from MPP⁺ neurotoxicity.

While the MPP⁺ group of rats exhibited increased OX-

Fig. 2 Behavioral performances at different time points after MPP⁺ intoxication. Unilateral, 10 μ g infusions of MPP⁺ resulted in significant behavioral disabilities as assessed by apomorphine induced rotational behavior (A), forelimb akinesia (B), and vibrissae-elicited forelimb placing tests (C). Data were represented as Mean $\pm$ SEM. * $P < 0.05$, vs the control group ($n = 6$).

42-IR in the ipsilateral SN at day 21 post-lesion, the density of OX-42-IR in the triptolide treatment group were much attenuated and were statistically not different from that in the sham group ($t = 1.043$, $P = 0.304$), corroborating an inhibitory effect of triptolide on microglial activation (Fig. 3A, B).

The percentage of TH-positive neurons decreased in the SN ipsilateral to the MPP⁺ injection site as compared with the contralateral side at day 21 post-lesion ($t = 11.008$, $P < 0.05$).

Fig. 3 Effect of triptolide on MPP⁺-induced microglial activation and dopaminergic neurotoxicity in rats. **A**, Representative photomicrographs showing OX-42 (microglial marker) and TH (dopaminergic neuronal marker) in the injected SN area. OX-42 immunostaining in green (Aa, b, c, d); Scale bar, 50 μ m; TH-positive neurons in red (Ae, f, g, h); Scale bar, 200 μ m. **B**, Quantification of OX-42 immunoreactivity of different groups. Data were expressed as density of OX-42-IR. **C**, Quantification of the TH-positive neurons in the injected SN as compared to those in the contralateral non-injected SN, expressed as a percentage ratio. Data were represented as Mean $\pm$ SEM. * P < 0.05, vs sham group; # P < 0.05, vs MPP⁺ group (n = 6).

Fig. 4 Effect of triptolide on MPP⁺-induced behavioral abnormalities in rats. While MPP⁺ led to progressive behavioral disabilities in rats, administration of triptolide resulted in attenuated locomotor defects as evaluated by apomorphine induced rotational behavior (**A**), forelimb akinesia (**B**), and vibrissae-elicited forelimb placing tests (**C**) between 7 to 21 d. Data were represented as Mean $\pm$ SEM. # P < 0.05, vs the MPP⁺ group (n = 6).

Triptolide provided partially neuroprotection against dopaminergic neuronal loss as indicated by a much higher percentage of TH-positive neurons in the triptolide treatment group compared with the MPP⁺ group ($t = 3.050$, $P < 0.05$; Fig. 3A, C). In the vehicle treatment group of rats, the density of OX-42-IR and the percentage of TH-positive neurons were principally not different from the MPP⁺ group ($t = 0.078$, $P = 0.939$; $t = 0.421$, $P = 0.683$; Fig. 3B, C).

3.4 Triptolide mitigated behavioral abnormalities in MPP⁺ intoxicated rats Since triptolide was capable of preserving dopaminergic neurons from MPP⁺-induced cell death, we then further our study to investigate whether triptolide could attenuate MPP⁺-induced behavioral defects in rats.

As shown in previous sections, intranigral injection of MPP⁺ resulted in progressive locomotor disabilities. In rats treated with triptolide, there was a noticeable attenuation of behavioral defects between 7 to 21 d (Fig. 4). Two-way ANOVA revealed significant effect of triptolide treatment (rotational behavior: $F(3,135) = 89.051$, $P < 0.05$; forelimb akinesia: $F(3,135) = 97.932$, $P < 0.05$; vibrissae-elicited forelimb placing: $F(3,135) = 105.091$, $P < 0.05$) and interaction between triptolide treatment and time (rotational behavior: $F(3,135) = 21.902$, $P < 0.05$; forelimb akinesia: $F(3,135) = 20.798$, $P < 0.05$; vibrissae-elicited forelimb placing: $F(3,135) = 20.906$, $P < 0.05$).

Such results are consistent with the suggestion that anti-inflammatory and immunosuppressive agents are neuroprotective and may improve motor function in PD animals^[32,33]. In the vehicle treated group, the behavioral defects were not ameliorated compared with the MPP⁺ group at all the time points observed.

4 Discussion

The search for neuroprotective agents in PD is primarily based on the ability of a specific drug to reduce dopaminergic neuronal loss. Agents that can provide such neuroprotective effects have the potential to become novel therapeutics to PD^[4,5].

Existing animal models of PD provide us with useful tools to test new neuroprotective agents and strategies. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its active metabolite, MPP⁺, represent ideal neurotoxins that can replicates most features of sporadic PD in various animals^[35]. However, if MPTP is systemically administered, not only dopaminergic neurons but also other cell types, particularly

the glial cells will be affected simultaneously^[29]. Because the enzymatic conversion of MPTP to MPP⁺ by monoamine oxidase (MAO)-B mainly occurs in astrocyte^[35-37], and drugs that can increase MAO activity have been shown to aggravate the neurotoxicity of MPTP by producing more MPP⁺^[29,36], study of neuroprotective agents using MPTP model of PD is open to multiple interpretations including the influence of astrocyte by the drugs tested. The MPP⁺-induced hemiparkinsonian rats, thus, can be used as a reliable tool to understand the pathogenic relationships between microglial reaction and dopaminergic neurons, without having to concern about possible interference of astrocyte-neuron interaction. After MPP⁺ enters into dopaminergic neurons through dopamine transporter (DAT), it is concentrated within the mitochondria and inhibit complex I activity, which leads to the decrease in ATP content and bring widespread damage to neurons. The inhibition of complex I is considered as the “primary injury” to dopaminergic neurons^[38,39]. However, more attention have recently been drawn to the “secondary damage” to neurons caused by inflammatory processes which have been suggested to have a major impact on the progressive dopaminergic cell death in PD^[38-40].

The hallmark of neuroinflammation in PD is the activation of microglia. From a neuropathological standpoint, microglial activation is indicative of an active, ongoing process of cell death^[40,41]. Activated microglia not only bring damage to neurons through releasing a variety of noxious compounds^[42,43], but also recruit and activate more microglial cells, forming a vicious cycle that leads to more dopaminergic neuronal death. In the present study, after MPP⁺ intoxication to SN, we observed a progressive TH-positive cell loss and behavioral defects in rats, which were consistent with previous studies^[23,31,44]. Activation of microglia was seen from the third day after MPP⁺ injection and lasted throughout our experiment. Together with the suggestion that in the etiology of PD, activation of microglia represent an important cause of dopaminergic neuronal death^[7,39], so it is postulated that microglia inhibitors might protect dopaminergic cell from injury induced by PD neurotoxins.

Extracts of the herb TWHF have been used for over two centuries as Traditional Chinese Medicine (TCM) to treat a variety of inflammatory and autoimmune diseases. Most of the pharmacological activity of TWHF can be attributed to triptolide and its most abundant and active component^[14,45].

Here, we showed that chronic administration of triptolide decreased the immunodensity of OX-42, attenuated TH-positive neuronal loss and ameliorated the behavioral abnormalities in MPP⁺-induced hemiparkinsonian rats. Our results are in agreement with previous reports, wherein triptolide inhibits LPS-induced microglial activation and dopaminergic neuronal loss^[13,16,17], providing further evidence for the involvement of microglia in PD pathogenesis and the therapeutic potential for microglia inhibitors.

One of the possible mechanisms underlying the pharmacological effect of triptolide may be suppressing the expression of cyclooxygenase-2 (Cox-2), a specific proinflammatory factor which has emerged as an important mediator of microglial activation^[40]. Neurons expressing COX-2 would cause their own demise through a harmful interplay with microglia^[46-48]. Studies using COX-2 knockout mice and COX-2 inhibitors demonstrated reduced microglial activation and attenuated dopaminergic cell loss in PD models^[40,49,50]. Since triptolide have been suggested to inhibit COX-2 expression in a variety of settings^[14,51], further researches are warranted to determine whether triptolide exerts its inhibitory effect on microglial activation in MPP⁺ rat model of PD through modulating COX-2 expression.

It is also worth mentioning that inhibition of microglia by triptolide, as used here, only partially blocked the dopaminergic cell death, suggesting that other mechanisms independent of microglia-related neuronal damage are also involved in MPP⁺ neurotoxicity. As mentioned above, after MPP⁺ is taken up by dopaminergic neurons, it is concentrated in mitochondria and inhibits complex I, resulting in ATP depletion and subsequent cell death^[52]. Thus, the inhibition of complex I may account for another aspect of the disease phenotype which can not be ameliorated by microglia inhibitors. Although this possibility cannot be confirmed with certainty, our observation that inhibition of microglia by triptolide preserve only a part of TH-positive neurons from injury rather support the view that dopaminergic neurons are damaged through more than one mechanism.

In summary, our result support the involvement of microglia in the progressive dopaminergic neuronal loss in MPP⁺ rat model of PD. Triptolide exerted neuroprotective effect on MPP⁺-induced dopaminergic neuronal injury and attenuated behavioral disabilities. Suppression of microglial activation may, at least in part, account for the mechanism un-

derlying the neuroprotective effect of triptolide. Control of microglia-related neuroinflammation, thus, may represent a promising therapeutic strategy in the future management of PD.

Acknowledgments: This work was supported by Scientific and Technical Supporting Program, Ministry of Science and Technology of China (No. 2006BAI04A11-3), Foundation of Doctor Unit at High School from Ministry of Education, China (No. 20060246072), and Medical Leader Sponsorship by Shanghai Municipal Government (No. 2007-057).

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雷公藤内酯可减轻 MPP⁺ 诱导的大鼠多巴胺神经元损伤

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摘要: 目的 以小胶质细胞激活为特征的神经炎性反应作为帕金森病(Parkinson's disease, PD) 一种重要的致病原因, 正受到越来越多的关注。本文的目的是研究 1- 甲基-4- 苯基- 吡啶(1-methyl-4-phenyl pyridinium, MPP⁺)所诱导的偏侧PD大鼠模型中小胶质细胞的激活, 观察雷公藤内酯作为一种小胶质细胞抑制剂对多巴胺神经元的保护作用及其对 MPP⁺ 所导致的大鼠行为学异常的改善效果。方法 通过黑质区域显微注射 MPP⁺ 制作偏侧 PD 大鼠模型。分别在基线、MPP⁺ 注射后第 1、3、7、14、21 天时通过测定黑质区域 OX-42 的免疫荧光强度评定小胶质细胞的激活程度, 测定酪氨酸羟化酶的表达情况评定存活多巴胺神经元的数量; 通过阿朴吗啡诱导的旋转行为、前肢跨步及触须引发的不对称放置试验得分测评行为学表现。结果 黑质区域 MPP⁺ 注射导致小胶质细胞的激活、多巴胺神经元进行性死亡以及行为学缺陷的不断加重。雷公藤内酯可以显著抑制小胶质细胞的激活, 部分阻止 MPP⁺ 对多巴胺神经元的毒性, 改善行为学异常。结论 上述结果显示雷公藤内酯对 MPP⁺ 诱导的偏侧 PD 大鼠模型多巴胺神经元具有保护作用, 其机制可能与抑制小胶质细胞的激活有关, 这为 PD 免疫抑制治疗提供了实验依据。

关键词: 帕金森病; 雷公藤内酯; 小胶质细胞; 神经元