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· Original article ·

Catalytic metalloporphyrin protects against MPTP-induced Parkinson's disease in mice

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[**ABSTRACT**] **Objective**: To observe the effects of manganese(Ⅲ) meso-tetrakis(N, N′-diethylimidazolium-2-yl) porphyrin (MnTDM) in treatment of early Parkinson's disease(PD) mouse model induced by subcutaneous injection of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine(MPTP) and to discuss its possible mechanism. **Methods**: Forty male C57BL/6 mice were evenly randomized into 4 groups: MPTP model group(subcutaneous injection of 25 mg/kg MPTP for 3 days), MnTDM+MPTP group (15 mg/kg MnTDM was subcutaneously injected 1 h before MPTP injection), MnTDM control group, and normal saline group. Performance of animals in the pole and swimming test was observed 3 days after the last injection. Levels of dopamine (DA) and its metabolites(3,4-dihydroxyphenylacetic acid [DOPAC] and homovanillic acid [HVA]) in the striatum of animals were measured by high-performance liquid chromatography with an electrochemical detector(HPLC-ECD). Thiobarbituric acid (TBA) method was used to examine the levels of malondialdehyde(MDA). **Results**: Acute injection of MPTP could be used for establishment of PD model. The striatal levels of DA, DOPAC and HVA in MPTP group were significantly lower($P<0.01$) and the striatal level of MDA was significantly higher($P<0.05$) than those of the control group. MPTP had no obvious effect on the behavioral performance of the animals in a short term. MnTDM could partly inhibit the above effects of MPTP. Compared with MPTP group, MnTDM+MPTP group had significantly higher DA, DOPAC, and HVA levels and significantly lower MDA level(all $P<0.05$). There was no significant difference in the behavioral indices of animals between the 4 groups. **Conclusion**: MnTDM can inhibit lipid peroxidation and promote DA production; it has preventive and therapeutic effects on MPTP-induced PD.

[**KEY WORDS**] Parkinson disease; MPTP; MnTDM; oxidative stress

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Parkinson's disease(PD) is a neurodegenerative disorder characterized by tremor, rigidity and akinesia, with selective degeneration of the nigrostriatal dopaminergic neurons in the central nervous system as the prominent pathological changes. The neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine(MPTP) can induce, in humans, monkeys and mice, the neurochemical, neurological and pathological changes that are analogous to those observed in PD[1]. It is well known that MPTP causes behavioral, electrocortical and neurodegenerative changes when injected into several areas of the rat brain or given systemically[2]; these effects can be prevented by the injection of Cu-free superoxide dismutase(SOD)[3]. MnTDM is a new generation manganese porphyrin analog, which is designed to optimize biologically important properties and minimize the potential toxicities[4]. The aim of this study was to verify whether MnTDM could prevent behavioral impairment and nigrostriatal degeneration in MPTP-induced PD in C57BL/6 mice. Further investigation is warranted to investigate whether MnTDM has neuroprotective effect *in vitro* and whether it can benefit patients with PD.

1 MATERIALS AND METHODS

1.1 Animals and establishment of PD model Male C57BL/6 mice(8 weeks old, weighing 20-25 g, specific

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pathogen free[SPF]) were purchased from Shanghai SIPPR-BK Lab Animal Co. Ltd. The mice were randomly divided into 4 groups (n_{saline} [control] = 10, $n_{MPTP\ 25\ mg/kg}$[MPTP] = 10, $n_{MnTDM\ 15\ mg/kg}$[MnTDM] = 10, $n_{MnTDM\ 15\ mg/kg+\ MPTP\ 25\ mg/kg}$[MnTDM+MPTP]=10). Mice were subcutaneously injected with saline(vehicle) 25 mg/kg MPTP(Sigma, St. Louis, MO, USA) for 3 days once daily[5]; saline (vehicle) MnTDM (15 mg/kg) was subcutaneously injected 1 h before treatment with the MPTP, while similar amounts of saline were added to relevant other groups.

1.2 Pole and swim test Mice were subjected to pole test 3 days after the last administration as described above. The pole test was performed according to the method established by Okuda *et al*[6]. Briefly, animals were positioned head upwards near the top of a rough-surfaced iron pole (10 mm in diameter and 100 cm high). The time periods for the animals to cover the first 25 cm distance of the pole(defined as T_{25cm}) and the second 25 cm distance(T_{50cm}) were recorded. Triplicate pole tests were carried out and the average values were calculated.

Swimming test was carried out after the pole test in water tubs (40 cm length, 25 cm width, 16 cm height). The depth of water was kept at 12 cm and the temperature was maintained at (27±2) ℃. Swim-score scales were: 0, hind part sinks with head floating, occasional swimming using hind limbs while floating on one side; 3, occasional floating/swimming only; 5, continuous swimming[7]. The score would be deducted 0.5 every floating time of 10 seconds as the score between 3 and 5.

1.3 Brain tissue preparation All mice were sacrificed 3 days after the last drug treatment or saline injection. After decapitation, brains were quickly removed and divided into 2 parts; one for the analysis of dopamine (DA) and its metabolites; the other was embedded on the dry-ice and then stored at −80℃ for determination of malondialdehyde(MDA).

1.4 Analysis of striatal dopamine and its metabolites Mice were sacrificed by cervical dislocation. The 2 striatal parts were rapidly isolated from the cerebrum on an ice-cold glass plate, weighed and homogenized in chilled $HClO_4$ (0.2 mol/L) and centrifuged at 16 000×g for 20 min. DA, 3, 4-dihydroxyphenyl acetic acid (DOPAC), and homovanillic acid (HVA) were measured by high-performance liquid chromatography (HPLC) (Hitachi, Japan) with an electrochemical detector (ECD) (Eicom, Japan). Fifteen microlitres of the clear supernatant was injected directly into the HPLC system. The mobile phase consisted of 0.1 mol/L NaH_2PO_4, 1 mmol/L L-octane sulfonic acid sodium salt, 0.5 mmol/L EDTA and 10% (V/V) methanol, pH 3.2. The mobile phase was delivered at a constant rate of 1 ml/min by an EICOM Model EP-10 pump through a C18 Phase analytical column (5 μm × 4.6 mm×250 mm; Beckman, CA) placed in a heater box(50℃). Electrodetection was performed at 0.8 V. The signal from the detector was recorded and the data were analyzed by using a N2000 chromatography data system software; the contents of DA and its metabolites were calculated by comparing the simple peak area with the internal standard peak region, and were expressed as microgram per gram of tissue weight[1].

1.5 Measurement of MDA content The contents of MDA in the striatum were measured by the thiobarbituric acid-reaction to assay the lipid peroxide (LPO). Tissue samples frozen at −80°C were well irrigated with normal saline, and by admixing with the KCl (1.5%), homogenization at a ratio of 1 : 10 was achieved. LPO level in the centrifuged tissue homogenate was measured according to the method described by Kaymaz *et al*[8]. The reaction product was assayed spectophotometrically at 532 nm. LPO level was expressed as nmol of MDA per mg protein of tissue[9].

1.6 Statistical analysis All data were statistically analyzed with one-way analysis of variance (ANOVA) followed by Dunnett test for multiple comparisons among different groups (SAS, Version 6.12). Results were given as mean ± S.E.M. values. Values of $P<0.05$ were considered significant.

2 RESULTS

2.1 Behavioral test There was no difference in behavioral ability(pole and swimming score) in mice between the control and MPTP group, the MnTDM+MPTP group and MPTP group, or the MnTDM group and control group. Concretely, in the pole test, the T_{25cm} and T_{50cm} in MPTP group and MnTDM+MPTP group were not prolonged compared with those in the saline

group. Animals in the control group and MnTDM＋MPTP group had higher scores in the swimming test than those in the MPTP group did; there was no significant difference between the 4 groups(Fig 1).

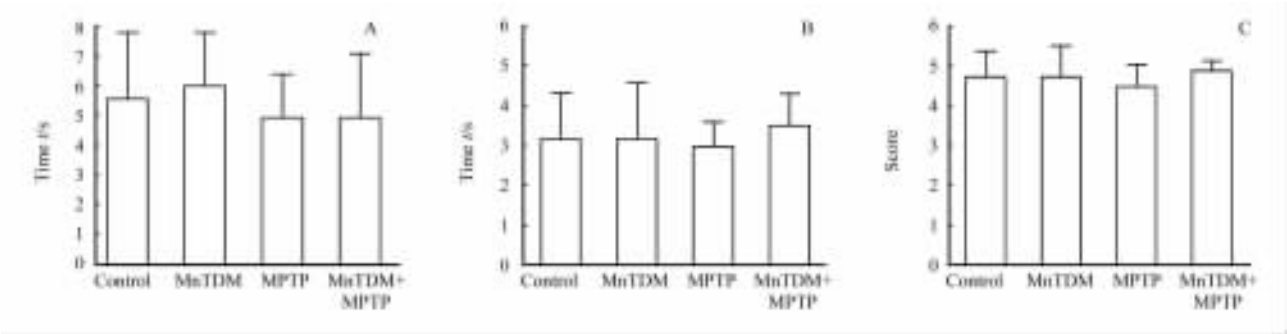

Fig 1　Behavioral performance of mice in each group

A: The time period to cover the first 25 cm of the pole; B: The time period to cover the second 25 cm of the pole; C: Score of swimming test in the mice. $n=8$, $\bar{x}\pm s$

2.2　Striatal DA and its metabolites in each group　Injection of MPTP resulted in significant decreases in contents of DA and its metabolites(DOPAC, HVA) in the striatum. MPTP(25 mg/kg) decreased DA content by 50% compared with the control group($P<0.01$). Significant decreases in DOPAC (by 70%) and HVA (by 38%) were observed($P<0.01$) in mice treated with MPTP compared with those in the control group. Compared with MPTP group, the contents of DA, DOPAC and HVA in the MnTDM＋MPTP group increased by 62.6%, 63.5% and 40.7%, respectively ($P<0.05$, Fig 2).

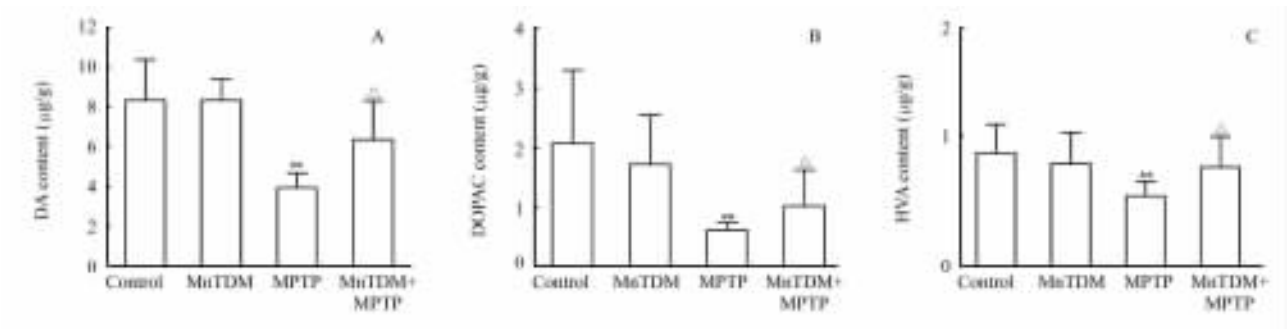

Fig 2　DA(A), DOPAC(B) and HVA(C) contents in each group

$^{**}P<0.01$ *vs* control group, $^{\triangle}P<0.05$ *vs* MPTP group. $n=7$, $\bar{x}\pm s$

2.3　Striatal MDA content in each group　The content of striatal MDA in the MPTP group increased by 44.5% compared with that in the control group($P<0.05$). The striatal MDA content in the MnTDM＋MPTP group decreased significantly (by 34.9%) compared with that in the MPTP group($P<0.05$, Fig 3). MDA content was expressed as nmol of per mg protein of tissue.

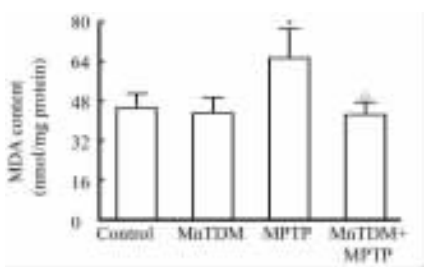

Fig 3　Striatal MDA content in each group

$^{*}P<0.05$ *vs* control group; $^{\triangle}P<0.05$ *vs* MPTP group. $n=5$, $\bar{x}\pm s$

3 DISCUSSION

In the present study, we demonstrated that systemic treatment of mice with synthetic catalytic scavengers of reactive oxygen species (ROS) meso-porphyrin mimetic MnTDM conferred neuroprotection against MPTP-mediated neurotoxicity in C57BL/6 mice. MPTP treated C57BL/6 mice in this study demonstrated pronounced early PD characters, including unchanged behavioral performance, decreased dopamine content, and increased MDA content in the striatum. The MPTP neurotoxicity observed in this study agreed with that reported previously[10]. We also examined the effects of MnTDM on the striatal dopamine level and found that pretreatment with MnTDM 1 h before MPTP injection prevented not only the down-regulation of dopamine, DOPAC and HVA contents in the striatum, but also the up-regulation of MDA content. These findings indicate that MnTDM has a protective effect against MPTP-induced neurotoxicity in mice.

Brain is particularly sensitive to oxidative stress-mediated damage due to high oxygen consumption. It is well known that antioxidant defense systems exist in a balance with endogenous reactive oxygen species under normal condition. Disruption of this balance appears to be one of the major factors involved in the selective neuropathogenesis associated with PD[11]. Other factors that exacerbate this sensitivity are the presence of autooxidizable neurotransmitters and a high concentration of polyunsaturated fatty acids in neuronal membranes. Furthermore, the brain has only low level of endogenous antioxidants to protect itself from oxidative damage. Studies have shown that excitotoxicity (neuronal death resulting from excessive stimulation of glutamate receptors) could lead to increased production of ROS, which is a key pathological process in neurological disorders. Mice that lack SOD2 develop a fatal neurodegenerative phenotype that can be rescued by catalytic antioxidants[12]. Moreover, mice overexpressing SOD2 are protected from excitotoxic injury induced by kainate, whereas heterozygous SOD2 knockout mice showed exacerbated damage[13].

MPTP is a lipophilic molecule able to cross the blood-brain barrier (BBB). MPTP exposure can cause irreversible and severe parkinsonism clinically and pathologically similar to idiopathic PD, *i. e.* marked reduction in the dopaminergic neurons within the substantia nigra compacta (SNc) and decrease in striatal tyrosine hydroxylase (TH) activity and dopamine level[14]. Although the precise mechanisms by which MPTP destroys dopaminergic neurons remain controversial, the toxic effects of MPTP appear to depend on its biotransformation to N-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase (MAO). The generated MPP^+ was shown to be taken up by the dopaminergic neurons *via* the dopamine re-uptake system, a process that can be blocked by DA uptake blockers. It has been suggested that ROS may be generated during the conversion of MPTP to MPP^+; this is supported by the increased superoxide ($O_2^{\cdot-}$) and hydroxyl radical ($\cdot OH$)[15] concentrations during the biotransformation of MPTP. Further supporting evidence included that pretreatment of mice with diethyldithiocarbamate, a SOD inhibitor, enhanced MPTP-induced neurotoxicity, whereas transgenic mice overexpressing human Cu, Zn-SOD with increased SOD activity, were reported to be more resistant to MPTP than non-transgenic mice[16].

As a catalytic SOD/catalase mimetic, MnTDM can scavenge a wide range of ROS, not only $O_2^{\cdot-}$ and H_2O_2, but also peroxynitrite ($ONOO^-$) and lipid peroxyl radicals as well. The newly developed series (MnTDM) of metalloporphyrins are small molecule antioxidant analogous to the catalytic site of SOD; they show much higher SOD and catalase activities and much higher potencies as inhibitors of lipid peroxidation than native Cu, Zn-SOD. The high solubility of the series is expected to allow these compounds to better penetrate the BBB and therefore be more bioavailable[17-18]. In this study, the administration of MnTDM prevented MPTP-induced up-regulation of MDA, suggesting that ROS acts in the upstream of the dopaminergic neurons loss pathway following exposure to MPTP.

In conclusion, PD character and biochemical response induced by MPTP involve an exaggerated formation of ROS and can be antagonised by pretreatment with the SOD mimetic MnTDM at low concentration (15 mg/kg). Our findings provide a novel and potentially valuable approach for studying the oxidative process in neurodegenerative disorders, including PD.

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锰卟啉络合物对 MPTP 诱导的帕金森病小鼠的防治作用

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[摘要] **目的**: 观察锰卟啉络合物[manganese(Ⅲ) meso-tetrakis(N, N′-diethylimidazolium-2-yl) porphyrin, MnTDM]对1-甲基-4-苯基-1, 2, 3, 6-四氢吡啶(1-methyl-4-phenyl-1, 2, 3, 6- tetrahydropyridine, MPTP)诱导的早期帕金森病模型小鼠的防治效果, 探讨其可能的作用机制。**方法**: C57BL/6雄性小鼠随机分为MPTP模型组(连续3 d皮下注射25 mg/kg MPTP), MnTDM+MPTP组(于MPTP注射前1 h皮下注射15 mg/kg MnTDM)以及MnTDM对照组、生理盐水对照组, 每组10只。末次注射后第3日进行爬杆和游泳等行为学检测; HPLC-ECD法检测各组小鼠纹状体多巴胺(dopamine, DA)及其代谢产物3, 4-二羟基苯乙酸(DOPAC)和高香草酸(HVA)水平; 硫代巴比妥酸(thiobarbituric acid, TBA)法测定各组小鼠纹状体丙二醛(malondialdehyde, MDA)水平。**结果**: 急性注射MPTP可建立早期帕金森病小鼠模型; 与对照组相比, MPTP组小鼠纹状体DA、DOPAC、HVA水平明显下降($P<0.01$), MDA水平明显升高($P<0.05$); 但短期对小鼠行为学指标影响不大。MnTDM能部

分抑制 MPTP 的上述作用;与 MPTP 组相比,MnTDM+MPTP 组小鼠纹状体 DA、DOPAC、HVA 水平明显上升,MDA 水平明显下降(P 均<0.05)。各组小鼠间行为学指标无统计学差异。**结论:** MnTDM 能抑制脂质过氧化,促进多巴胺类神经递质分泌,对 MPTP 诱导的帕金森病小鼠有一定防治作用。

[关键词] 帕金森病;MPTP;锰卟啉络合物;氧化应激

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