**Neuroprotective effects of stemazole in the MPTP-induced acute model of
Parkinson's disease: involvement of the dopamine system**

Zhirui Guo^{1#}, Shasha Xu^{1#}, Na Du¹, Jia Liu¹, Yiyun Huang², Mei Han^{1,*}

¹ Key Laboratory of Radiopharmaceuticals, Ministry of Education, Department of Chemistry, Beijing Normal University, Beijing 100875, China

² Yale PET Center, Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, CT 06516, USA

* Corresponding author: Mei Han

Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University

Address: No.19 Xin Jie Kou Wai Street, Hai Dian District, Beijing 100875, P.R. China

Tel.: +86 10 62207786;

Fax: +86 10 6220 6031;

E-mail: hanmei@bnu.edu.cn

[#] These two authors contributed to the work equally and should be regarded as co-first authors

Abstract

Parkinson's disease is a neurodegenerative disorder characterized by a loss of nigrostriata dopaminergic neurons, which has been thought, at least in part, to result from oxidative stress. The present study aims to investigate the neuroprotective effects of stemazole (ST) on the dopamine (DA) system and its possible mechanisms of action in a mouse model of PD. Mice were injected intraperitoneally with MPTP (20 mg/kg) four times at 2-h intervals for one day to induce Parkinsonism, and then treated with ST (10, 30 and 50 mg/kg) or Madopar (120 mg/kg) for 7 days. Behavioral analyses were performed with locomotor activity measures and rotarod test. Tyrosine hydroxylase (TH) and dopamine transporter (DAT) levels were detected by immunohistochemistry method. DA and its metabolites were determined by high-performance liquid chromatography with an electrochemical detector. Oxidative stress levels were assessed by measuring the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX). Our results demonstrated that ST treatment improved locomotor activity and motor coordination in MPTP mice. There was also a significant increase in TH-positive cells (~24%, $P<0.01$) and DAT levels (~26%, $P<0.01$) in MPTP mice treated with ST (50 mg/kg) compared with the vehicle group. Madopar treatment showed weaker effects on TH-positive cells (21%, $P<0.05$) and DAT levels (21%, $P<0.05$). DA and its metabolite levels were significantly increased with ST (50 mg/kg) treatment ($P<0.01$, compared with the vehicle group). In addition, SOD and GSH-PX activities were elevated notably in ST treatment groups compared with the vehicle group. In conclusion, these results suggest that ST has neuroprotective effect on the impaired DA system, potentially through enhancement of the cell's anti-oxidative capacity. Hence it may be used as a potential therapeutic agent for Parkinson's disease.

Key words: Parkinson's disease; Stemazole; Neuroprotection; Dopamine system;
Oxidative stress; MPTP

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder whose cardinal features include tremor, slowness of voluntary movement, muscle rigidity and postural instability [1]. PD predominantly causes damages in the nigrostriatal pathway, which results in the degeneration of pars compacta neurons in the substantia nigra (SN) and subsequent loss of their projecting nerve fibers in the striatum. This then leads to the depletion of striatal dopamine (DA) and loss of dopamine transporter (DAT). It has been reported that clinical symptoms appeared when approximately 80% of the striatal DA is lost [2]. Thus, treatment strategies for PD have primarily focused on DA supplementation, with drugs such as *L*-3,4-dihydroxyphenylalanine (*L*-DOPA, a dopamine precursor), DA receptor agonists and monoamine oxidase B (MAO-B) inhibitors [3]. However, these available therapeutic agents are not capable of halting the progression of the disease and always induce various side effects. As a result there is still an urgent need for PD therapies with improved efficacy and side effect profile.

Stemazole (ST, chemical name: *N*-(4-(4, 5-dihydro-5-thioxo-1, 3, 4-oxadiazol-2-yl) phenyl) hydrazinecarbothioamide) is a novel small molecule emerged from our drug screening program [4] and has been found to have protective effects against neurodegeneration in a rat model of Alzheimer's disease (AD) [5] . Since AD and PD share some pathophysiological features that are attributed to the degeneration of neurons, we carried out a study to investigate whether ST also has neuroprotective effects in a PD animal model.

The most widely used mouse model of PD is produced by systemic administration

of the neurotoxin MPTP, which reproduces several of PD symptoms and induces damage to the dopaminergic neurons [6]. MPTP is converted by MAO-B to MPP⁺, which is known to cause cell oxidative stress damage in the SN, thereby further lead to the impairment of dopamine system. Oxidative stress is considered one of the most common hallmarks in neurodegenerative diseases [7], and plays a pivotal role in PD-associated neurodegeneration [8, 9]. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) are the main antioxidant enzymes in the body, and the loss of their levels and activities could reflect the occurrence of oxidative stress in PD disease.

Therefore, we also measured the activities of SOD and GSH-PX.

In this study, we investigated the effects of ST on behavioral changes and characteristics of the DA system. We also measured the activities of SOD and GSH-PX in an MPTP-induced PD mouse model. Madopar was used as a positive control drug for comparison.

2. Materials and methods

2.1. Animals and treatment procedures

Male C57BL/6 mice aged 6-8 weeks (20-24 g) were randomly divided into the following groups: 1) control (intraperitoneally saline solution only), 2) ST alone (30 mg/kg/day), 3) vehicle (intraperitoneally saline solution after MPTP), 4) high dose of ST (50 mg/kg/day, ST-H), 5) middle dose of ST (30 mg/kg/day, ST-M), 6) low dose of ST (10 mg/kg/day, ST-L), and 7) Madopar (120 mg/kg/day) [10]. ST was synthesized and prepared as previously described [4]. All groups except for the control group and

the ST alone group were given MPTP intraperitoneally four times at 2 h interval at the dosage of 20 mg/kg on Day 1 [11]. ST treatment groups were given ST intraperitoneally to mice at dose of 10, 30 or 50 mg/kg/day for 7 days (0.1 mL/10 g body weight) after injections of MPTP. Madopar group was given Madopar intragastrically at dose of 120 mg/kg/day for 7 days after injections of MPTP. Animals had free access to food and drinking water. All animals were maintained according to the international guidelines for care and use of laboratory animals. Experimental procedures involving animals were approved by the Ethics Committee of Beijing Normal University (BNU/EC/01/2011).

2.2. Behavioral analyses and brain tissue preparation

Ten mice were randomly selected from each group and a number of behavioral tests were performed on Day 0 (1 day before MPTP), Day 2, 4 and 8 (1, 3 and 7 days after MPTP injection) respectively.

2.2.1. Locomotor activity and Rotarod test

Locomotor activity, including animals' movement distance and velocity, was assessed by a computer analyzer as previously described [12]. Rotarod test, known as the latent period, was recorded with the time spent on the rotating bar as previously described [13].

2.2.2. Brain tissue preparation

Mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) after the behavior test on Day 8 and rapidly perfusion-fixed with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer solution (PBS), pH 7.4. The frozen

brains were cut into 18 μm coronal sections for immunohistochemistry using a cryostat microtome (CM3000; Leica, Germany).

2.3. TH and DAT immunohistochemical staining

Analyses of TH immunoreactivity in the SN and striatum and DAT immunoreactivity in the striatum were conducted on brain sections as previously reported [14]. The striatal sections used for TH and DAT immunohistochemistry are from same brain tissue and the adjacent position. Sections were incubated with either mouse anti-TH antibody at 1:5000 dilutions or mouse anti-DAT antibody at 1:1000 dilutions at 4°C overnight. The slices were then incubated with peroxidase-conjugated secondary antibody for DAT or the Vectastain ABC kit for TH. All staining sections were visualized with DAB. The sections were counted using a bright-field microscope (M165FC, Leica), optical densities (OD) of TH and DAT staining were calculated by the Image-ProPlus software. TH-positive neurons were manually counted by researchers blinded to the treatment groups.

2.4. Measurement of DA and its metabolites levels in the striatum

Levels of DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured by high-performance liquid chromatography with an electrochemical detector (HPLC-ECD) as previously described [15].

2.5. Assay of SOD and GSH-PX activities

The SN was removed, blotted, weighed and homogenized in 0.1 M PBS (pH 7.4). The samples were centrifuged at 3,000 rpm/min for 30 min at 4°C. The supernatant

was collected and restored at -80°C. The protein concentration was determined by using bovine serine albumin as a standard [16]. The activities of SOD and GSH-PX were determined following the detection kit specifications. Results are presented as units of activity per mg of protein (wet weight).

2.6. Statistical analyses

Data of all the results were presented as mean $\pm$ S.E.M. The analysis of variance (ANOVA) was performed for all tests, followed by Dunnett's test. The results of immunohistochemical staining were indicated as a ratio compared to the control group.

3. Results

3.1 Behavioral analyses

3.1.1. Locomotor activity

Locomotor activity testing is a commonly used method to evaluate motor impairment. As shown in Fig. 1, on Day 2 all mice were found to have a significant reduction in total movement distance and mean velocity compared to the normal control ($P<0.01$). Mice treated with ST-H exhibited a marked improvement in locomotor activity compared to the vehicle group ($P<0.01$). The group of mice treated with Madopar, or ST-L and ST-M also showed an improvement, albeit not statistically significant. On Days 4 and 8, all animals treated with ST or Madopar displayed improved motor activity, although no significant difference was found when compared with the vehicle group. Mice treated with ST alone (without MPTP) showed no change in locomotor activity when compared to the control group.

3.1.2. Rotarod test

We used an accelerating rotarod test to determine motor coordination and balance. Results are presented in Fig. 2. One day after MPTP injection, there was a significant reduction in rotarod performance observed in vehicle group (~43 s) as compared to the control group (~108 s, $P<0.01$). On Day 4, a significant difference was observed between control and vehicle ($P<0.01$) mice. Mice treated with Madopar have a significant improvement in rotarod activity ($P<0.01$). On Day 8, groups treated with ST-L and ST-M showed slight improvement in rotarod activity, while MPTP mice treated with both ST-H and Madopar exhibited a significant increase in the latent period compared to the vehicle group ($P<0.01$). ST treatment alone did not affect the latent period as compared to the control group.

3.2. Effects of ST on TH-positive neurons in the SN and striatum

TH is a key enzyme for dopamine biosynthesis and is used as a marker for dopaminergic neurons. After 7 days of treatment with ST and the completion of behavioral assessment, half of the mice in each group were sacrificed for TH-immunoreactive detection. Representative coronal mesencephalon sections containing TH-positive neurons and fibers in the SN and striatum are shown in Fig. 3. MPTP exposure led to a marked loss of TH positive neurons in the SN (Fig. 3B) for vehicle group compared with the control group ($71.4 \pm 5.9\%$ vs. $100.0 \pm 17.6\%$, $P<0.01$). Mice treated with ST-L and ST-M had increased TH-positive neurons, reaching $74.9 \pm 9.2\%$ and $84.5 \pm 15.4\%$, respectively, of the control levels, and this increase reached significance in the mice treated with ST-H ($88.5 \pm 10.6\%$ of the control group,

$P<0.01$). A similar effect was also seen in the Madopar group ($86.2 \pm 7.4\%$, $P<0.05$).

Accompanying the loss of DA neurons in the MPTP model was a reduction in TH-positive fibers in the striatum. As shown in Fig. 3D, optical density (OD) analysis of TH-positive fibers revealed a significant loss of striatal DA terminals in the vehicle-treated MPTP group as compared to the control group ($69.4 \pm 3.3\%$ of the control levels, $P<0.01$). Significant elevations in the density of TH-positive fibers were observed in mice treated with ST-M and ST-H ($91.4 \pm 2.9\%$ and $92.8 \pm 5.4\%$ of the control group level, $P<0.01$), as well as ST-L ($85.3 \pm 6.5\%$ of the control group level, $P<0.05$). A significant improvement was also seen in the Madopar-treated group ($87.4 \pm 4.1\%$ of the control group level, $P<0.01$). ST treatment alone did not affect the TH expression levels in the SN and striatum compared to the control mice ($P>0.05$).

3.3. Effects of ST on DAT level in the striatum

DAT is a key biomarker in the diagnosis of PD. In this study, immunochemical staining was used to detect DAT levels in the striatum. Results are presented in Fig. 4. MPTP treatment elicited a significant reduction in DAT level in the striatum ($72.8 \pm 3.2\%$ of the level in the control group, $P<0.01$) (Fig. 4B). Treatment with ST-L, ST-M and ST-H inhibited the reduction of DAT protein in a dose-dependent manner, with levels of $83.5 \pm 4.6\%$, $88.9 \pm 5.1\%$ ($P<0.05$), $91.4 \pm 3.1\%$ ($P<0.01$), respectively, as compared to the control group. It appeared that treatment with ST-M and ST-H normalized DAT expression in MPTP mice (no significant difference from the control group, $P>0.05$). Madopar also showed a significant effect ($88.3 \pm 5.6\%$, $P<0.05$). ST treatment alone did not affect the DAT levels compared to the control mice ($P>0.05$).

3.4. Effects of ST on DA and its metabolites levels in the striatum

The effects of ST on the levels of DA and its metabolites in the striatum were measured by HPLC-ECD. Results are presented in Fig. 5. MPTP administration led to a marked reduction in striatal DA (49.04 ± 7.16 vs. 471.48 ± 29.04 ng/g), DOPAC (19.28 ± 1.62 vs. 139.42 ± 8.89 ng/g) and HVA levels (25.83 ± 5.42 vs. 90.91 ± 4.10 ng/g) compared to the control mice ($P < 0.01$). Treatment with ST inhibited MPTP-induced depletion of DA, with ST-H markedly prevented the decreases in DA (135.99 ± 6.77 ng/g), DOPAC (39.96 ± 5.28 ng/g) and HVA (52.72 ± 2.67 ng/g) in the striatum of MPTP mice ($P < 0.01$, compared with the vehicle mice). The ratio of DOPAC and HVA to DA, which is a measure of DA metabolism, was significantly higher ($P < 0.01$) in the vehicle-treated MPTP group than the control group (Fig. 5D). Treatment with ST effectively reversed the abnormally increased ratio of (DOPAC+HVA) to DA induced by MPTP ($P < 0.01$ for ST-L, ST-M, ST-H, respectively), indicating that ST inhibited the turnover of DA in the striatum of MPTP mice. In comparison, although Madopar treatment led to increases in DA, DOPAC and HVA levels similar to those in ST-H treated mice, it did not show a significant effect on the ratio of (DOPAC+HVA) to DA. ST treatment alone did not affect the levels of striatal DA and its metabolites compared to the control mice ($P > 0.05$).

3.5. Effects of ST on SOD and GSH-PX activities in the SN

Oxidative stress evaluation included the analysis of SOD and GSH-PX activities, which are the major players in the natural antioxidant defense system. Results are shown in Fig. 6. MPTP administration resulted in a significant reduction in SOD and

GSH-PX activities relative to the control group ($P < 0.01$ for all comparisons). The activity of SOD was reduced to $53.2 \pm 10.9\%$ of the level in the vehicle group ($P < 0.01$, compared with the control group). Significant increases in SOD activity were found in MPTP mice treated with ST-L (~31%), ST-M (~34%) and ST-H (~51%) ($P < 0.01$). Madopar treatment elicited similar increase in SOD activity (~28%) as compared to the vehicle group ($P < 0.01$), but ST-H treatment was more effective compared to Madopar treatment ($P < 0.05$).

The activity of GSH-PX was improved significantly after ST administration at 10, 30, or 50 mg/kg ($P < 0.01$), with levels of $86.2 \pm 6.0\%$, $88.3 \pm 5.4\%$ and $97.8 \pm 4.1\%$, respectively, as compared to the control group. In the Madopar group, the activity of GSH-PX was also improved to $89.2 \pm 6.7\%$ of the level in the control group, which was lower than that of ST-H group.

4. Discussion

Stemazole is a novel small molecule previously found to have neuroprotective effects against neurodegeneration [5]. In the present study we investigated the protective ability of ST against the neurotoxicity of MPTP in a mouse model of PD. Our findings indicated that ST effectively protected against the MPTP-induced deficits in locomotor activities and motor coordination. Moreover, we found that ST protected against the loss of TH-positive neurons in the SN and TH-positive fibers in the striatum, and attenuated the reduction in striatal DAT levels induced by MPTP. Treatment with ST-H returned both TH and DAT to normal levels. Furthermore, treatment with ST increased levels of DA and its metabolites in the striatum, and

decreased the ratio of (DOPAC+HVA) to DA in MPTP mice. As a possible mechanism of its neuroprotective action, ST increased the activities of SOD and GSH-PX, indicating that it may induce an increase in anti-oxidative capacity in MPTP mice.

The neurotoxin MPTP is known to cause the animals to develop symptoms of Parkinsonism. MPTP-treated mice are widely used for studies on PD. Movement deficits in PD result from dopaminergic dysfunction in the brain. In the present study, treatment with ST was shown to improve MPTP-induced motor deficits. In the first day after MPTP treatment, locomotor activities decreased by up to 55%, which was a direct result from the loss of nigral dopaminergic neurons induced by the acute neurotoxicity of MPTP. This reduction in locomotor activities was counteracted by ST treatment, as a marked improvement in locomotor activities was found in mice treated with ST-H compared to vehicle-treated animals. However, on Days 4 and 8, ST and Madopar treatment didn't show significant improvement compared with the vehicle group. This may be partly attributed to our acute MPTP model, which has also been shown to present natural fast recovery in behavior tests [17, 18]. It may conceal the regulating effect of the ST or Madopar on locomotor activity. For rotarod activity, Madopar exhibited superior protection for latency to fall compared to ST in a short period. This may result from the direct DA supplementation after Madopar administration, which is the primary neurotransmitter involved in motor functions and postural instability. Nevertheless, ST may work mainly through neuroprotective effect on the impaired DA system, instead of direct supplementation of DA.

TH is a key enzyme for DA biosynthesis and thus used as a marker for the integrity of dopaminergic neurons [19, 20]. Our study found that MPTP reduced TH-positive neurons by 29% and TH-positive fibers by 30%. Treatment with ST significantly attenuated the reduction in TH-positive neurons and effectively restored TH expression. This suggested that ST might offer protection against dopaminergic neurodegeneration induced by the neurotoxicity of MPTP. DAT is a key protein on the presynaptic membrane of DA neurons in the striatum, and its density is an index of the integrity and function of DA neurons. Therefore, a marked decrease in DAT is the gold standard by which clinical diagnosis of PD is made or ascertained [21, 22]. Our results indicated that MPTP reduced DAT density by 27% compared to control group and treatment with ST protected against MPTP-induced DAT reduction in a dose-dependent manner. Furthermore, ST-H showed a better effect than Madopar, as DAT level increased by 26% in MPTP mice treated with ST-H, and by 21% in those treated with Madopar. ST alone treatment in control mice had no effect on DAT expression. Hence, the increasing of DAT level observed in MPTP mice resulted from the counteracting effect of ST on the MPTP-induced DA neurons damage.

DA is the primary neurotransmitter involved in motor functions and its loss directly impacts the physical movements and contributes to the clinical symptoms of PD [23]. DA depletion is a cardinal feature of PD in humans and in animal models of the disease [24, 25]. MPTP causes a partial lesion of the nigrostriatal pathway and significant reduction in striatal DA levels [26, 27]. Results from the present study showed that treatment with ST markedly attenuated MPTP-induced DA depletion in

the striatum, and reduced the (DOPAC+HVA)/DA ratio, which was significantly altered by MPTP. The metabolites/neurotransmitter ratio is an index of the rate of neurotransmitter metabolism. The neuroprotective effect of ST may be due, in part, to a decrease in dopamine metabolism in the striatum.

Previous studies have confirmed the relationship between DA depletion and motor impairment in PD mice [28, 29]. In our present study, the motor coordination and balance ability (rotarod test) and DA system were damaged more seriously than the locomotor activity by the acute administration of MPTP. The recovery process of rotarod test and DA system was consistent, however, inconsistent with the recovery process of the locomotor activity. This inconsistency may be partly attributed to the acute model. The purpose of the present study is to investigate the neuroprotective effects of ST on the DA system. The acute model can induce the most dramatic changes to the DA system in a short period of time, however, present a fast recovery in behavior tests [17, 18], especially the locomotor activity. Furthermore, this inconsistency may also indicate that locomotor activity was also regulated by other systems that may have been affected by MPTP for which recovery is rapid, as PD is much more than a dopaminergic disorder [30]. Further studies using subacute PD model, of which the natural recovery was chronic, could be done to investigate the relationship between the mean locomotor activity and DA system action.

A defect in one or more of the naturally occurring antioxidant defense mechanisms could lead to neurodegeneration in PD [8]. Oxidative stress becomes more prominent in the process of ageing, and thus is the most significant risk factor for PD [31]. MPP^+

could cause abnormal energy metabolism and then lead to the lipid peroxidation, mitochondrial harm, SOD and GSH-PX depletion, and eventually cell degeneration. [32]. Therefore, antioxidant ability of ST was investigated to illustrate the neuroprotective mechanism of ST. Our studies showed that treatment with ST could counteract against the decreases of both SOD and GSH-PX activities induced by MPTP administration. This is consistent with our previous study, which has confirmed that ST could dose-dependently protect SH-SY5Y cells from H₂O₂-induced oxidative stress cytotoxicity in vitro [5]. Therefore, the dopaminergic neuroprotective effect of ST in PD mouse model may result from its resistance to oxidative stress by increasing the activities of antioxidant enzymes.

Madopar has been the “gold standard” and most widely used medication in PD treatment, and its mechanism of action was attributed to the supplementation of DA in the brain. Therefore, Madopar was chosen as a positive control to evaluate the therapeutic efficacy of ST. Our studies showed that ST offered stronger protective trend than Madopar in TH and DAT levels as well as antioxidant enzyme activities, which indicated that ST exerts its neuroprotective effect through the normalization of the DA system and the enhancement of the brain’s anti-oxidative capacity.

Limitation of this study should be noted. The acute model was chosen in our study due to its dramatic depletion to the dopamine system in a short period of time. However, this model presented a fast recovery in behavior [17, 18], which limits its use in the behavioral assessment of PD therapeutic strategy. More appropriate PD model should be chosen to evaluate the behavioral improvement effect of ST.

5. Conclusion

In conclusion, results from the present study indicate that stemazole has a protective effect against MPTP-induced neuronal damage and plays an important role in behavioral improvement in PD model. The protective effect appears to be related to the DA system, through stimulated increases in TH and DAT levels and decrease in DA metabolism. As a possible mechanism of action, stemazole treatment leads to enhanced anti-oxidative capacity, which may have resulted in the repair/restoration of dopaminergic neurons. Therefore, stemazole may be considered as a potential novel agent for PD treatment, and warrants clinical investigation of its therapeutic efficacy in PD patients.

Acknowledgements

The authors acknowledge the financial support from the China National Science Foundation (81173139), the Major Research Plan of NSFC (21233003), and the National Science and Technology Major Project of the Ministry of Science and Technology of China (2013ZX09J13110-11B).

References

1. Oida, Y., et al., *Rifampicin attenuates the MPTP-induced neurotoxicity in mouse brain*. Brain Research, 2006. **1082**: p. 196-204.
2. Elsworth, J.D. and R.H. Roth, *Dopamine synthesis, uptake, metabolism, and receptors: Relevance to gene therapy of Parkinson's disease*. Experimental Neurology, 1997. **144**(1): p. 4-9.
3. Davie, C.A., *A review of Parkinson's disease*. British medical bulletin, 2008. **86**: p. 109—127.
4. Sun, Y., et al., *Synthesis and biological evaluation of a novel human stem/progenitor cells proliferation activator: 4-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl) thiosemicarbazide (Stemazole)*. European Journal of Medicinal Chemistry, 2011. **46**(7): p. 2930-2936.
5. Han, M., et al., *Therapeutic efficacy of stemazole in a beta-amyloid injection rat model of*

- Alzheimer's disease*. European Journal of Pharmacology, 2011. **657**(1-3): p. 104-110.
6. Przedborski, S. and M. Vila, *MPTP: a review of its mechanisms of neurotoxicity*. Clinical Neuroscience Research, 2001. **1**(6): p. 407-418.
 7. Mc Guire, P.J., A. Parikh, and G.A. Diaz, *Profiling of oxidative stress in patients with inborn errors of metabolism*. Molecular Genetics and Metabolism, 2009. **98**(1-2): p. 173-180.
 8. Jenner, P. and C.W. Olanow, *Oxidative stress and the pathogenesis of Parkinson's disease*. Neurology, 1996. **47**(6): p. S161-S170.
 9. Zhang, J., et al., *Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons*. American Journal of Pathology, 1999. **154**(5): p. 1423-1429.
 10. Li, X.M., et al., *Ameliorative and neuroprotective effect in MPTP model of Parkinson's disease by Zhen-Wu-Tang (ZWT), a traditional Chinese medicine*. Journal of Ethnopharmacology, 2010. **130**(1): p. 19-27.
 11. Jackson-Lewis, V. and S. Przedborski, *Protocol for the MPTP mouse model of Parkinson's disease*. Nature Protocols, 2007. **2**(1): p. 141-151.
 12. Liu, J., et al., *Neuroprotective effects of Jitai Tablet, a traditional Chinese medicine, on the MPTP-induced acute model of Parkinson's disease: Involvement of the dopamine system*. Evidence-based complementary and alternative medicine : eCAM, 2014. **2014**: p. 542383-542383.
 13. Zhang, H.N., C.N. An, and X.P. Pu, *Protocatechuic acid inhibits neurotoxicity induced by MPTP in vivo*. Neuroscience Letters, 2010. **474**(2): p. 99-103.
 14. Chung, Y.C., et al., *Fluoxetine prevents MPTP-induced loss of dopaminergic neurons by inhibiting microglial activation*. Neuropharmacology, 2011. **60**(6): p. 963-974.
 15. Luo, D.Z., et al., *Fucoidan protects against dopaminergic neuron death in vivo and in vitro*. European Journal of Pharmacology, 2009. **617**(1-3): p. 33-40.
 16. Lowry, O.H., et al., *Protein measurement with the folin phenol reagent*. Journal of Biological Chemistry, 1951. **193**(1): p. 265-275.
 17. Taylor, T.N., J.G. Greene, and G.W. Miller, *Behavioral phenotyping of mouse models of Parkinson's disease*. Behavioural Brain Research, 2010. **211**(1): p. 1-10.
 18. Jacksonlewis, V., et al., *Time-course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine*. Neurodegeneration, 1995. **4**(3): p. 257-269.
 19. Ishikawa, S., et al., *Human DJ-1-specific transcriptional activation of tyrosine hydroxylase gene*. Journal of Biological Chemistry, 2010. **285**(51): p. 39718-39731.
 20. Hosp, J.A., et al., *Dopaminergic projections from midbrain to primary motor cortex mediate motor skill learning*. Journal of Neuroscience, 2011. **31**(7): p. 2481-2487.
 21. Winogrodzka, A., et al., *I-123 beta-CIT SPECT is a useful method for monitoring dopaminergic degeneration in early stage Parkinson's disease*. Journal of Neurology Neurosurgery and Psychiatry, 2003. **74**(3): p. 294-298.
 22. Ravina, B., et al., *Dopamine transporter imaging is associated with long-term outcomes in Parkinson's disease*. Movement Disorders, 2012. **27**(11): p. 1392-1397.
 23. Sowell, R.A., J.B. Owen, and D.A. Butterfield, *Proteomics in animal models of Alzheimer's and Parkinson's diseases*. Ageing Research Reviews, 2009. **8**(1): p. 1-17.
 24. Carder, R.K., et al., *Dopamine released from mesencephalic transplants restores modulation*

- of striatal acetylcholine-release after neonatal 6-hydroxydopamine-an invitro analysis.* Experimental Neurology, 1989. **105**(3): p. 251-259.
25. Bloem, B.R., et al., *The MPTP model - versatile contributions to the treatment of idiopathic Parkinsons-disease.* Journal of the Neurological Sciences, 1990. **97**(2-3): p. 273-293.
 26. Geng, X.C., et al., *Neuroprotective effects of echinacoside in the mouse MPTP model of Parkinson's disease.* European Journal of Pharmacology, 2007. **564**(1-3): p. 66-74.
 27. Heikkila, R.E., A. Hess, and R.C. Duvoisin, *Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice.* Science, 1984. **224**(4656): p. 1451-1453.
 28. Henderson, J.M., et al., *Relationships between various behavioural abnormalities and nigrostriatal dopamine depletion in the unilateral 6-OHDA-lesioned rat.* Behavioural Brain Research, 2003. **139**(1-2): p. 105-113.
 29. Haobam, R., et al., *Swim-test as a function of motor impairment in MPTP model of Parkinson's disease: A comparative study in two mouse strains.* Behavioural Brain Research, 2005. **163**(2): p. 159-167.
 30. Ahlskog, J.E., *Beating a dead horse - Dopamine and Parkinson disease.* Neurology, 2007. **69**(17): p. 1701-1711.
 31. Cui, H., Y. Kong, and H. Zhang, *Oxidative stress, mitochondrial dysfunction, and aging.* Journal of signal transduction, 2012. **2012**: p. 646354.
 32. Weydert, C.J. and J.J. Cullen, *Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue.* Nature Protocols, 2010. **5**(1): p. 51-66.

Figure legend:

Fig.1. Total movement distance (A) and mean velocity (B) on day 0 (1 day before injection of MPTP), day 2 (1 day after MPTP), day 4 (3 days after MPTP) and day 8 (7 days after MPTP).

Data are shown as mean $\pm$ S.E.M (n =9-10). **P < 0.01 vs. vehicle group.

Fig.2. Latent period on day 0, day 2, day 4 and day 8. Data are shown as mean $\pm$ S.E.M (n = 9-10).

**P < 0.01 vs. vehicle group.

Fig.3. Photomicrographs of representative SN (A) and striatum (C) sections stained with an antibody against TH. The number of TH-positive neurons in the SN (B) and the level of TH expression in the striatum (D) are expressed as the mean $\pm$ S.E.M (n = 5). *P < 0.05, **P < 0.01 vs. vehicle group.

Fig.4. Photomicrographs of representative striatum sections stained with a DAT antibody (A). Optical density of DAT-positive fibers in the striatum (B) is expressed as the mean $\pm$ S.E.M (n = 5).

*P < 0.05, **P < 0.01 vs. vehicle group.

Fig.5. Concentrations of DA, DOPAC and HVA were detected by HPLC-ECD and the ratio of (DOPAC + HVA) to DA was calculated. Data are expressed as the mean $\pm$ S.E.M (n = 4-5). *P < 0.05, **P < 0.01 vs. vehicle group.

Fig.6. Activities of SOD (A) and GSH-PX (B) in the substantia nigra of mice. Data were shown as the mean $\pm$ S.E.M (n = 5). **P < 0.01 vs. vehicle group. ⁺P < 0.05 vs. Madopar group.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6