Establishment and evaluation of the sdf-1α over-expressing mouse parkinson disease model

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In the present study, to establish a stromal cell derived factor-1α (SDF-1α) mouse Parkinson's disease (PD) model, low-dose 1-methyl-4-phenyl-tetrahydropyridine (MPTP) was subcutaneously injected at different time points into mice which had been intracerebrally treated with pDsRed2-N1-SDF-1α, and behaviors were observed at different time points. The pod-grabbing time, the number of tyrosine hydroxylase (TH) positive cells and the expression of SDF-1α in the brain were determined at different time points. PD-like behaviors were observed in the mice of MPTP group and could last for at least 40 days. At different time points, the pod-grabbing time in MPTP group was longer than that in control group, but the TH positive cells and DA content were markedly decreased when compared with control group. In addition, plasmid transcription was noted in MPTP group and SDF-1α expression was detectable. Intermittent and multiple subcutaneous injections of MPTP can be used to establish a PD model in SDF-1α over-expressing mice, which provides an experimental basis for the investigation of the role of SDF-1α induced chemotaxis and migration in the treatment of PD.

Key words: SDF-1α, Parkinson's disease, mouse model.

INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disease of the central nervous system and a common degenerative disease with motor dysfunction. Nowadays, the pathogenesis of PD is still unclear, and is considered as a result of interaction between genetic factors and environment factors (Klodowska et al., 2005). Currently, the commonly used clinical strategies can not effectively delay the nature course of PD.

Clinical application of cell and tissue transplantation techniques makes the cure of PD possible. Harrower et al. (2006) transplanted the primary porcine fetal neural tissue into unilateral 6-OHDA lesioned rats and grafts survived for up to 5 months. In addition, there was evidence of host vascularization and myelinated fibers within the graft area extending into the surrounding host tissue accompanied by synapsin expression. Kim et al. (2002) transplanted embryo-derived stem cells into the striatum of PD animals and results confirmed the survival of grafts which then differentiated into dopaminergic neurons. In 1996, Bianchi et al. (1996) found that male fetal progenitor cells persisted in maternal blood for as long as 27 years postpartum. In 2002, Anto et al. (2002) reported intra-thyroidal fetal cells carrying chromosome Y survived in Graves' disease mother postpartum and they speculated fetal cells can enter the maternal specific parts through pregnancy and delivery. Fetal cells can migrate into and survive in the mother and these cells can cross the brain blood barrier and reside in the brain, which further confirm the results aforementioned (Tan et al., 2005). However, whether fetal cells can migrate into the maternal specific parts and its exact mechanisms are still poorly understood.

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Studies have demonstrated SDF-1α plays important roles in the development of nervous system and brain injury through regulation (McGrath et al., 1999; Luo et al., 2005) and chemotaxis (Nanki and Lipsky, 2000; Spanno et al., 2004). However, there is no study reporting whether fetal cells can migrate into substantia nigra striatum through vertical transplantation in the presence of SDF-1α induction, whether these cells can differentiate and mature in local micro-environment and whether this transplantation can improve symptoms of PD and increase the intracerebral DA content.

In studies on PD models, 1-methyl-4-phenyl-tetrahydropyridine (MPTP) induced monkey PD has similarities in the symptoms and biochemical and pathological characteristics to human PD, and it is also reported that Lewy body-like structure can be found in 20% of PD Rhesus monkey even half a year after establishment of animal model (Beal, 2001). However, the expense of this model is high, and the cycles for observation and treatment is prolonged which limit its wide application. Systemic injection of high dose of MPTP can also induce pathological and biochemical changes in mice which are similar to presentations in human PD. Furthermore, the expense is significant reduced and cycles for observation and treatment are shortened. Thus, this mouse PD model has widely been used in drug development and gene therapy (Dawson et al., 2002). In the mouse PD model, C57BL mouse is a preferred species. Evidence shows C57BL mouse is high sensitive to MPTP with high success rate. In addition, the pathophysiological features are more similar to human PD (Irwin et al., 1992). To investigate the survival and migration of fetal cells in PD mother, it is necessary to establish an animal model which has advantages in simplicity in establishment and stability. In this study, through stereotactic technique, low dose of MPTP was repeatedly injected into mouse to establish a mouse PD model in which mice can stably express SDF-1α.

MATERIALS AND METHODS

Animals and reagents

Female C57BL/6j mice (n=52) (specific pathogen free) weighing 20 to 24 g (age: 8 to 12 weeks) were purchased from the Animal Center of Southern Medical University (Certification NO: scxk [Guangzhou, 2006-0015]). Animals were housed in separate cages in a quiet environment under a 12:12 h light-dark cycle and given ad libitum access to water and food. This study was approved by the Animal Care and Ethics Committee. MPTP (Sigma, USA), rabbit anti-mouse TH antibody (Boster, China) and DA standard (Sigma, USA) were used in this study.

Identification of plasmid pDsRed2-N1-SDF-1α

The plasmid pDsRed2-N1-SDF-1α was incubated with a solution (20 µl) containing Xhol and EcoR I at 37°C for 2 h, and the products were subjected to 1% agarose gel electrophoresis followed by identification.

Establishment of SDF-1α expressing Mouse PD model

Injection of pDsRed2-N1-SDF-1α into right caudate putamen (CPU) with stereotactic technique

Healthy C57BL/6j mice (n = 52) were randomly divided into MPTP group (n = 26) and control group (n = 26). (1) MPTP group: Mice were anesthetized with intraperitoneal injection of 10% chloral hydrate (40 mg/kg) and fixed on an operation table. The mouse head was maintained horizontal and at the middle line (difference in the heights of anterior and posterior fontanelles < 0.4 mm, and that of left and right brains < 1 mm). The coordinates of right CPU was determined according to the stereotaxic atlas of mouse: -0.5±0.1 mm behind anterior fontanel, 2.0±0.1 mm lateral to sagittal suture (right) and 3.0±0.1 mm under the dura. A total of 4 µl of plasmid solution (0.25 µg of LP2000 and 0.1 µg of pDsRed2-N1-SDF-1α in HBS) were injected at a speed of 0.5 µl/min. The needle was maintained for 2 min and then retracted at a speed of 1 mm/min. Post-operative injection of penicillin (1000 U) was performed for infection prevention. (2) Control group: The procedures were identical to those previously mentioned, and 4 µl of CPU (0.25 µg of LP200 in HBS) were injected.

Establishment of the PD model through MPTP injection

One week after plasmid injection, PD model was established. In the MPTP group, MPTP (2 mg/ml) was subcutaneously injected into mice at 9:00 am at days 1, 4, 7, 10, 13, and 16 at a dose of 25 mg/kg. In the control group, identical volume of normal saline was injected.

Evaluation of the PD model

Observation of behaviors

After MPTP injection, the behaviors of these treated mice were observed and duration of time spent in each behavior was recorded. Observation was performed for 1 h at 9:00 am twice weekly, and the behaviors observed are the symptoms of PD including tremor, postural instability, bradykinesia, hypokinesia, myotonia etc.

Pod-grabbing time

Five mice were randomly selected from each group and used to detect the pod-grabbing time at 30 min before MPTP/saline injection and 30 min after MPTP/saline injection at days 4, 10, 7, 13 and 16. A soft wood ball with 1.5 cm in diameter was fixed at the end of an iron rod (50 cm in length and 1 cm in diameter). The rod was wrapped with gauze and mice were put on the top of the rod with head upward. The interval from the bilateral hind limbs of mice touching the rod to the mice reaching the other end and leaving the rod was recorded. Before testing, mice were trained 5 times and testing was carried out three times. Data were input into a computer and analyzed.

Immunohistochemistry for TH and counting of TH positive cells in the SN

Five mice were randomly selected from each group after one injection of MPTP, 6 injections of MPTP and 30 days after the last injection of MPTP, and anesthetized with intraperitoneal injection of
10% chlora hydrate (40 mg/kg). The heart was exposed after thoracotomy and the right auricle was opened. Perfusion was performed with 50 ml of normal saline and then with 40 ml of cold 4% paraformaldehyde (0.01 mmol/L, pH 7.4) within 2 to 4 h. The brain was obtained and brain tissues at 3.0 to 4.5 mm behind anterior fontanel (tissues between middle epithalamus and middle hypothalamus) were used for immunohistochemistry. These tissues were fixed in 4% paraformaldehyde again for 24 h and then embedded in paraffin followed by consecutively cutting into 5 μm sections. Immunohistochemistry was conducted with SABC method according to manufacturer's instructions. In brief, sections were deparaffinized and permeabilized with 0.3% Triton-X100 followed by incubation with 3% H2O2 at room temperature. Then, antigen retrieval was performed and sections were blocked with 5% BSA followed by incubation with rabbit anti-mouse TH polyclonal antibody (1:50) at 4°C overnight in a humidified environment. Sections were thereafter treated with biotin conjugated goat anti-rabbit secondary antibody and SABC compound followed by development with DAB. Reaction was terminated by washing with distilled water followed by counterstaining with hematoxylin. After dehydration and transparentization, sections were mounted. In addition, the primary antibody was replaced by PBS serving as negative control. Positive cells present brown. Three sections were selected and the number of TH positive cells in the bilateral SNs was counted followed by summing.

Detection of DA content in the SN

Five mice were randomly selected from each group after three injections of MPTP, 6 injections of MPTP and 30 days after the last injection of MPTP, anesthetized with intraperitoneal injection of 10% chloral hydrate (40 mg/kg) and then decapitated. Bilateral substantia nigra-stra-triums were isolated, weighed after water removal and stored at -80°C. Before detection of DA content, tissues were put into 0.5 ml of 0.05 mol/L perchloric acid for protein removal followed by homogenation. Then, centrifugation was performed at 4°C for 30 min at 12000 r/min and 30 µl of supernatant were collected for high performance liquid chromatography (Shimadzu LC-20A). Conditions for chromatography were: chromatographic column (Shim-pack VP-ODS C18; 250 x 4.6 mm; 5 µm), mobile phase buffer (0.05 mol/L sodium dihydrogen phosphate + 0.02 mol/L sodium citrate): methanol (90:10), flow rate of 0.8 ml/min, column temperature of 35°C, emission wavelength (EM) of 333 nm, and excitation wavelength (EX) of 285nm. The detection sensitivity was 0.01 AU and recording were performed for 20 min. The peak area was compared with that under standard curve and concentration was determined. Then, the total content of DA was calculated and expressed as total content/wet weight of brain (µg/g).

Detection of SDF-1α in the brain

Expression of SDF-1α carrying fluorescence

Forty days after PD model establishment, one mouse was selected randomly from each group, anesthetized, perfused with cold normal saline and decapitated. The brains were immediately collected followed by frozen sectioning (25 µm in thickness) at -20°C. The whole procedures were carried out in dark and sections were observed under a confocal microscope. Representative photographs were obtained and the expression of SDF-1α carrying fluorescence was determined.

Detection of SDF-1α expression by western blot assay

The brains and the spleen of mice of control group were collected and put into liquid nitrogen followed by grinding. Then, 800 µl of 1× SDS loading buffer were added and the mixture was boiled for 5 min. Centrifugation was performed at 10000 g/min for 5 min and 40 µl of supernatant were subjected to SDS-PAGE. Then, the proteins were transferred onto PVDF membranes which were blocked in 5% non-fat milk in TBST at 4°C overnight. The membranes were washed with TTBS three times and then incubated with rabbit anti-mouse SDF-1α monoclonal antibody (1:10000, Abcam) at room temperature for 1 h. Following washing with TTBS thrice (3×10 min), these membranes were treated with HRP conjugated goat anti-rabbit IgG polyclonal antibody (1:5000) at room temperature for 1 h followed by washing with TTBS thrice (3×10 min). Then, 1.4 ml of substrate solution (SuperSignal®) (A:B=1:1) were added onto the membranes followed by incubation in dark for 2 to 3 min. Excessive liquid was removed and membranes were put on a plastic wrap followed by development in dark.

Statistical analysis

Results were expressed as charts or tables. The quantitative data were presented as means ± standard deviation (±s) and statistical analysis was performed SPSS version 13.0 statistic software package. Analysis of variance with repeated measurement and factorial design was used and comparisons between two groups were performed with t test. A value of P<0.05 was considered statistically significant.

RESULTS

Identification of plasmid pDsRed2-N1-SDF1α

Lane 3 represents plasmid pDsRed2-N1-SDF1α after digestion. Two bands were found at 270 bp and 4.7 kbp which were identical to the mouse SDF1α gene and vector pDsRed2-N1. Lane 4 represents plasmid pDsRed2-N1-SDF1α without digestion by restriction enzymes and a band was noted at 5.0 kbp which is the sum of the sizes of vector pDsRed2-N1 and mouse SDF1α. These findings suggest plasmid pDsRed2-N1-SDF1α carries mouse SDF1α gene (Figure 1).

Behavior changes after MPTP injection

There were no mice dead, and all mice presented tremor, hunchbacked, hair erecting, hindlimb opening, bradykinesia, unsteady gait, etc at 5 to 10 min after MPTP injection, and several mice had epileptic attack (2/25, 8.0%). About 30 to 90 min later, most symptoms were improved gradually, but bradykinesia, unsteady gait and poor spirit were still observed and mice presented limb rigidity and significantly reduced activities. Twelve hours later, these symptoms resolved. After 3 injections of MPTP, the symptoms deteriorated over the times of injection and the duration of each symptom was prolonged. Forty days after animal model establishment, the hindlimb opening was still obvious and the activities were very poor in the cages. However, these manifestations were not noted in mice of control group.
Figure 1. Electrophoresis of pDsRed2-N1-SDF1α after restriction enzyme digestion. (1) DNA marker DL2000. (2) DNA Marker λ/HindIII. (3) Plasmid pDsRed2-N1-SDF1α after restriction enzyme digestion. (4) Plasmid pDsRed2-N1-SDF1α.

**Observation of pod-grabbing time**

As shown in Table 1, there was interaction effect between time points and grouping (F = 39.741, P < 0.001). There was significant difference in the pod-grabbing times determined at different time points (F = 39.532, P < 0.001), and the pod-grabbing time decreased over time. However, no marked difference was found between mice at different time points (F = 0.578, P = 0.716). Furthermore, the pod-grabbing time of mice in MPTP group was remarkably different from that in control group at different time points (P < 0.005) except before MPTP injection (t = 0.430, P = 0.679).

**Immunohistochemistry for dopaminergic neurons in the substantia nigra**

As shown in Figure 2, a lot of TH positive cells were found in the SN of the control group. These cells were big, round and plump and positive staining was characterized by rough, big and brown granules in the cytoplasm. These cells had morphology integrity, even staining was found in the cytoplasm and cell processes were thin, long and clear. In the sections of the MPTP group, the TH positive cells were characterized by shrinkage and pleomorphic changes. The staining in the cytoplasm was uneven with the absence of cell processes suggesting damage to these cells. In addition, the less TH positive cells were found. As shown in Figure 2, the time did not affect the number of TH positive cells (F = 2.545, P = 0.130) and the number of TH positive cells in control group was significantly higher than in MPTP group (F = 712.128, P < 0.001). Moreover, the number of TH positive cells in the SN at 30 days after last injection of MPTP was lower than that immediately after last injection (t = 7.387, P < 0.001) (Table 2).

**DA content in the SN**

As shown in Figure 3, the standard curve equation was $y=1773858x + 10477.63$ (X: peak area) suggesting favorable linear correlation (R = 0.9998). Figure 2 shows the DA expression in the SN of control group and MPTP group. The DA content was calculated according to standard curve and wet weight showing in Table 3. In addition, in the MPTP group, there was significant difference in the DA content of bilateral striatums between different time points (F = 433.320, P < 0.001). Furthermore, the DA content of bilateral striatums after 3 injections of MPTP was higher than that after 6 injections and 30 days after last injection (P < 0.05). Additionally, the DA content of bilateral striatums in MPTP group was significantly lower than in control group at different time points (P < 0.001).

**Expression of fluorescence protein in the brain**

Thirty-seven days after plasmid injection, scar was found in the needle passage under confocal microscope, and a lot of proteins with red fluorescence were identified in the needle passage. These findings suggested the mice were transfected with plasmid pDsRed2-N1-SDF1α and the SDF1α was expressed.

**Detection of SDF1α expression by western blot assay**

Protein expression was found in the lane 1 of MPTP group, and lane 2 and 3 showed no protein expression. The molecular weight of target protein was 11 kb which was identical to that of SDF1α. Therefore, we speculated that SDF1α was expressed in the brain of mice with transfection. In addition, no bands were noted in the lane 3 of the spleen of control group.

**DISCUSSION**

MPTP is frequently used to establish the PD model in animals. MPTP itself is not toxic but highly lipophilic. Therefore, MPTP can cross the brain blood barrier and membrane of neurons. The MPTP entering the astrocytes and 5-hydroxytryptamine (5-HT) neurons can be oxidized into an active toxin (1-methyl-4-phenyl pyridinium, MPP+)
Table 1. Pod-grabbing after MPTP injection (Second, mean ± SD, n = 5).

<table>
<thead>
<tr>
<th>Group</th>
<th>0 day</th>
<th>4 day</th>
<th>7 day</th>
<th>10 day</th>
<th>13 day</th>
<th>16 day</th>
<th>Total</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPTP</td>
<td>5.26</td>
<td>10.00</td>
<td>13.64</td>
<td>15.72</td>
<td>17.16</td>
<td>19.36</td>
<td>13.52</td>
<td>39.532</td>
<td>0.000</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>s</td>
<td>0.35</td>
<td>1.94</td>
<td>2.67</td>
<td>2.49</td>
<td>2.19</td>
<td>1.68</td>
<td>5.13</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.36</td>
<td>5.24</td>
<td>5.28</td>
<td>5.18</td>
<td>5.14</td>
<td>5.26</td>
<td>5.24</td>
<td>0.578</td>
<td>0.716</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>s</td>
<td>0.38</td>
<td>0.40</td>
<td>0.31</td>
<td>0.40</td>
<td>0.46</td>
<td>0.48</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.31</td>
<td>7.62</td>
<td>9.46</td>
<td>10.45</td>
<td>11.15</td>
<td>12.31</td>
<td>9.38</td>
<td>38.140*</td>
<td>0.000*</td>
</tr>
<tr>
<td>s</td>
<td>0.35</td>
<td>2.83</td>
<td>4.76</td>
<td>5.80</td>
<td>6.51</td>
<td>7.52</td>
<td>5.52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ t/F = 0.430 \quad 5.384 \quad 6.952 \quad 9.365 \quad 12.001 \quad 18.035 \quad 234.896^* \quad (F = 39.741, \quad P = 0.000)#

*F statistic and P value of main effect; # F statistic and P value of interaction effect.

Figure 2. TH-positive cells in the SNc (Up) TH positive cells in the substantia nigra pars compacta (SNc) of MPTP treated mice. (A) SNc at 40 ×; (B) Regional magnification of A (200 ×); (C) Regional magnification of B (400 ×). Arrow represents TH positive cells. (Down) TH positive cells in the substantia nigra pars compacta (SNc) of control mice. (A) SNc at 40 ×; (B) Regional magnification of A (200 ×); (C) Regional magnification of B (400 ×). Arrow represents TH positive cells.

In the presence of monoamine oxidase-B (Mao-B). The MPP+ is similar to DA in structure and can be transported into dopaminergic neurons by dopamine transporter (DAT). On one hand, MPP+ can inhibit the activity of tyrosine hydroxylase (TH) decreasing the DA synthesis, on the other hand, MPP+ can inhibit the activity of mitochondrial complex 1 (NADH dehydrogenase). The consequence of these processes is to result in death of dopaminergic neurons. Therefore, the manifestations are as a result of dopaminergic neuron specific toxicity and similar to those of human PD. Studies have shown C57BL/6 mouse aged 8 or 64 weeks is the most sensitive to MPTP. In addition, MPTP treatment in different ways can establish PD models with distinct features: in acute
PD model (establishment was completed within 1 week) and subacute model (establishment was completed within 5 to 10 days), mice have symptoms similar to acute PD and degradation and necrosis of neurons. In addition, the activation and proliferation of glial cells were also noted. But the typical pathological features of PD (α-synuclein aggregation and Lewy body) are absent. Therefore, in recent years, increasing researchers apply intermittent administration of MPTP or continuous administration of low-dose MPTP through a pump to establish PD model in animals.

Therefore, female C57BL/6j mice which are sensitive to MPTP were recruited for investigation. Low dose MPTP was subcutaneously injected at different time points to establish a mouse PD model. After MPTP injection, typical symptoms of PD including tremor, hunchbacked, hair

### Table 2. Number of TH-positive cells in the SN of both groups (number/field at high magnification).

<table>
<thead>
<tr>
<th>Group</th>
<th>16 day</th>
<th>30 day</th>
<th>Total</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPTP</td>
<td>x</td>
<td>82.00</td>
<td>54.40</td>
<td>68.20</td>
<td>7.387</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>6.04</td>
<td>5.77</td>
<td>15.58</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>x</td>
<td>311.60</td>
<td>313.20</td>
<td>312.40</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>31.20</td>
<td>25.13</td>
<td>26.72</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>x</td>
<td>196.80</td>
<td>183.80</td>
<td>190.30</td>
<td>2.018*</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>122.85</td>
<td>137.48</td>
<td>127.08</td>
<td></td>
</tr>
<tr>
<td>t/F</td>
<td>16.156</td>
<td>22.441</td>
<td>712.128*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* F statistic and P value of main effect; # F statistic and P value of interaction effect.

### Table 3. DA contents in the SN of both groups (ng/g wet weight).

<table>
<thead>
<tr>
<th>Group</th>
<th>10 day</th>
<th>16 day</th>
<th>30 day</th>
<th>Total</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPTP</td>
<td>x</td>
<td>116.22</td>
<td>51.19*</td>
<td>26.86*</td>
<td>64.75</td>
<td>433.320</td>
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<tr>
<td></td>
<td>s</td>
<td>3.81</td>
<td>6.58</td>
<td>3.80</td>
<td>39.31</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>x</td>
<td>142.60</td>
<td>142.09</td>
<td>143.87</td>
<td>142.85</td>
<td>0.135</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>5.67</td>
<td>6.15</td>
<td>4.85</td>
<td>5.23</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>x</td>
<td>129.40</td>
<td>96.64</td>
<td>85.36</td>
<td>103.80</td>
<td>189.464*</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>14.63</td>
<td>48.29</td>
<td>61.81</td>
<td>48.34</td>
<td></td>
</tr>
<tr>
<td>t/F</td>
<td>8.636</td>
<td>22.569</td>
<td>42.444</td>
<td>1655.877*</td>
<td></td>
<td></td>
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<tr>
<td>P</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000*</td>
<td></td>
<td></td>
<td>(F = 197.006, P = 0.000)*</td>
</tr>
</tbody>
</table>

* F statistic and P value of main effect; # F statistic and P value of crossover effect. *P<0.05, vs. 10 d.
erecting, hindlimb opening, bradykinesia, limb rigidity, significantly reduced activities, etc, were observed and

Figure 3. DA content in the SN of MPTP group and control group determined by HPLC. Left: the standard curve of DA content and linear correlation coefficient; Right: DA standard and representative chromatograms from DA chromatography; A: chromatogram of DA
this model effectively mimics the presentations of human PD. Furthermore, the latency to onset of these symptoms was shortened with the increase of times of injection, and the duration of each symptom was prolonged. After PD model establishment, the bradykinesia, reduced activities and instability of hindlimb activity were still observed 60 days after last injection of MPTP, even the typical symptoms were improved. The duration of certain symptoms (reduced activities, limb rigidity, unsteady gait, slow response, etc.) was longer than previously reported, which may be relevant with the way in which medication was performed, the dose of MPTP and the time to observation, the species (C57BL/6j mouse), gender, age, etc. The pod-grabbing time can be used to evaluate the coordinated movement. In this study, the pod-grabbing time of PD mice in MPTP group was 2 times longer than that of mice without PD (before MPTP injection) and mice in control group. This result suggests the coordinated movement is markedly compromised, which is a critical manifestation of PD.

After MPTP injection, the TH positive cells and the DA content in the SN were decreased with the increase of times of injection and this decrease could be observed not only during the MPTP injection, but after the last injection of MPTP. With the decrease in the TH positive cells, the DA content was gradually reduced due to TH as a rate-limiting enzyme of DA synthesis. Thirty days after last injection of MPTP, the number of TH positive neurons was decreased by about 70% and DA content by about 65%. These decreases also explained the compromised coordinated movement in MPTP treated mice. But the symptoms were improved to a certain extent, which was not consistent with the decrease of TH positive cells and DA content. These findings indicated there might be changes in certain neurotransmitter which partially offsets the impaired function due to decreased DA content. Nevertheless, the mechanism of this compensation is unknown.

SDF1α plays important roles in the brain injury via its chemotaxis and regulation (Pujol et al., 2005). CXCR4 is the unique receptor of SDF-1 and one CXCR4 can only bind to one SDF-1. Peng et al. (2007) found that CXCR4 expressing bone marrow stem cells had the characteristic of directed migration along the concentration gradient of SDF-1. In the presence of tissue or organ injury, SDF-1 can promote the recruitment of bone marrow stem cells and inflammatory cells into lesioned sites. Therefore, SDF1α is a critical chemokine regulating the post-injury repair and local inflammation (Kucia et al., 2004; Nanki and Lipsky, 2000). CXCR4 is highly expressed in the primitive / naïve cells. Study showed sequential transcription of CXCR4 was detected in the neuroepithelial cells of fetal mouse at gestation day 8.5 to 9.5 (McGrath et al., 1999). Therefore, CXCR4 expressing fetal cells might cross the placenta and migrate into maternal parts with high expression of SDF1α. Thus, in this study, before PD model was established, mice were intracerebrally treated with plasmid carrying mouse SDF1α gene resulting in over-expression of SDF1α, a secretory protein (Aiuti et al., 1997), in the brain. Because the plasmid also carried a protein with red fluorescence, expression of protein with red fluorescence also confirmed the transcription and expression of mouse SDF1α. In the present study, proteins with red fluorescence were found in the needle passage under a confocal microscope suggesting the mice of MPTP group were successfully transfected with plasmid pDsRed2-N1-SDF1α and mouse SDF1α was transcribed and expressed in the brain. Furthermore, Western blot assay revealed SDF1α protein expression in the brain.

However, these findings were not observed in the spleen of control group. Taken together, these results confirmed the PD mice had high expression of SDF1α in the CPU.

Taken together, in the present study, low dose MPTP was subcutaneously injected into C57BL/6j mice multiple times to establish a PD model. These mice had PD like symptoms and pathological changes in TH positive neurons were identified in the SN accompanied by decreased DA content in the SN. These results imply this model effectively mimics the pathophysiological characteristics of human PD and can be used in studies on PD. At the same time, plasmid pDsRed2-N1-SDF1α was injected to the right CPU of PD mouse and results showed these mice were successfully transfected and SDF1α was over-expressed in the brain. Our study provides experimental basis for further investigation of the role of SDF1α induced chemotaxis and migration in the treatment of PD.

REFERENCES


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