Rg1 reduces nigral iron levels of MPTP-treated C57BL6 mice by regulating certain iron transport proteins

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1. Introduction

Parkinson’s disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra (SN), giving rise to dopamine (DA) depletion in the striatum. A number of factors have been implicated in the pathogenesis of cell death in PD, which include elevated iron levels (Rouault, 2001; Ke and Qian, 2003; Zecca et al., 2004; Youdim et al., 2004; Jiang et al., 2007), oxidative stress, mitochondrial and dative stress inhibition.

Elevated iron levels in the substantia nigra (SN) participate in neuronal death in Parkinson’s disease, in which the misregulation of iron transporters such as divalent metal transporter (DMT1) and ferroportin1 (FP1) are involved. Our previous work observed that nigral iron levels were increased in MPTP-treated mice and Ginsenoside Rg1 which is one of the main components of ginseng, had neuroprotective effects against MPTP toxicity. Whether Rg1 could reduce nigral iron levels to protect the dopaminergic neurons? And whether its neuroprotective effect is achieved by regulating certain iron transporters? The present studies showed that Rg1 pre-treatment increased the dopamine and its metabolites contents in the striatum, as well as increased tyrosine hydroxylase expression in the SN. Further experiments observed that Rg1 pre-treatment substantially attenuated MPTP-elevated iron levels, decreased DMT1 expression and increased FP1 expression in the SN. These results suggest that the neuroprotective effect of Rg1 on dopaminergic neurons against MPTP is due to the ability to reduce nigral iron levels, which is achieved by regulating the expressions of DMT1 and FP1.

Ginsenosides, the principle active components of ginseng (Liu and Xia, 1992), have been shown to possess a variety of beneficial effects to human health, including anti-inflammatory, antioxidant and anticancer effects (Mochizuki et al., 1995; Kim et al., 1998; Wakabayashi et al., 1998). There are two major classes of ginsenosides, namely protopanaxatriol (Rg1, Rg2, Re, Rf, and Rb1) and protopanaxadiol (Rb1, Rb2, Rd, Rg3, Rh2, and Rh3). Numerous in vitro studies have demonstrated that the ginsenoside Rg1, asteroidal saponin of high abundance in ginseng, attenuates DA-induced apoptosis in PC12 cells by suppressing oxidative stress (Chen et al., 2003); inhibits the mitochondrial apoptotic pathway and increases the survival chance of primary cultured nigral neurons against rotenone toxicity (Leung et al., 2007). Ginsenoside Rg1 showed protective effect on MPTP-induced apoptosis in the mouse nigral neurons and this effect may be attributable to reducing the expression of iNOS and inhibiting the activation of caspase-3 (Chen and Chen, 2002; Chen et al., 2002). Ginsenoside Rg1 also exerts anti-inflammation properties on brain (Joo et al., 2005). Therefore, Rg1 is a kind of multifunctional drug possessing antiapoptosis, nitric oxide inhibition, anti-inflammation and oxidative stress inhibition.

Extensive studies have indicated that iron levels increase in the SN of patients with PD and animal models (Zecca et al., 2004; Youdim et al., 2004), but the mechanism is unclear. Iron may play a key role in the development of PD by enhancing the generation of suitable (Grunblatt et al., 2004; Youdim and Buccafusco, 2005; Van der Schyf et al., 2006).

Keywords:
Parkinson’s disease
Ginsenoside Rg1
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)
Dopamine
Iron
Divalent metal transporter (DMT1)
Ferroportin1 (FP1)
oxidative stress in cells (Rouault, 2001; Ke and Qian, 2003). Elevated brain iron levels may result from either increased import or decreased export (Qian and Shen, 2001). Divalent metal transporter (DMT1) is a widely expressed iron import protein which differ in both the C-terminus (with iron response element, IRE; and without IRE) and the N-terminus (two different promoters) (Lee et al., 1998; Hubert and Hentze, 2002; Mackenzie et al., 2007). Elevated expression of DMT1 is found in the SN of PD (Jiang et al., 2003; Moos and Morgan, 2004), indicating that disrupted expression of DMT1 might be involved in the nigral iron accumulation. Ferroportin (FP1) is a recently discovered transmembrane iron export protein (Donovan et al., 2000; McKie et al., 2000; Abboud and Haile, 2000). Decreased expression of FP1 in the SN can account for the increased iron levels in PD (Wang et al., 2007). Our previous work observed that nigral iron levels were increased in MPTP-treated C57BL6 mice (Jiang et al., 2003) and Rg1 had neuroprotective effects on dopaminergic neurons against MPTP toxicity (Yang et al., 2007). However, the mechanism of the neuroprotective effect of Rg1 on dopaminergic neurons against MPTP neurotoxicity still needs to be elucidated. Whether Rg1 could reduce nigral iron levels to protect the dopaminergic neurons? And whether its neuroprotective effect is achieved by regulating certain iron transporters? In the present study, we evaluated the actions of ginsenoside Rg1 on MPTP-treated C57BL6 mice, observed the effect of Rg1 on iron levels in the SN, as well as the expression of DMT1 and FP1 in order to test our hypothesis that the neuroprotective effect of Rg1 on dopaminergic neurons in the SN of MPTP-treated C57BL6 mice is due to the ability to reduce nigral iron levels, which is achieved by regulating the expressions of DMT1 and FP1.

2. Materials and methods

2.1. Materials

Rg1 was purchased from LKT Laboratories (USA), MPTP, tyrosine hydroxylase (TH), DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and other standard chemicals for preparation of buffers were obtained from Sigma (St. Louis, MO, USA). Reverse-transcription system kit was from Promega Corporation. TRIZol reagent was from GBCO. The antibodies for DMT1 and FP1 were from ADI (San Antonio, TX, USA).

2.2. Animal treatment

All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of Qingdao University. Male C57BL6 mice were kept under 19 ± 2 °C with a 12 h light-dark cycle and with free access to food and water. The mice were divided into three groups: (1) Rg1 pre-treated group: adult mice, weighing 20–22 g, were injected Rg1 (5 mg/kg, i.p. q.d.) for 3 days, on the forth day, the mice were pre-treated with Rg1 (5 mg/kg, i.p. q.d.) 2 h before MPTP (30 mg/kg, i.p. q.d.) injection for 5 days. (2) MPTP-treated group: the same procedures as above were followed, except that saline replaced Rg1. (3) Control group: normal adult mice weighing 20–22 g undergoing the same procedures with normal saline treatment. After 24 h of MPTP lesions, the mice were sacrificed and the brains were isolated for further experiments.

2.3. Iron staining

Animals were anesthetized (chloral hydrate, 400 mg/kg, i.p.) and killed by perfusion with 0.9% saline followed by 4% paraformaldehyde. Brains were removed and stored in 25% sucrose until sectioning. Sections (20 μm) through the SN were cut on a freezing microtome and alternate sections were stained for TH, DMT1, FP1 or iron. Iron staining was by Perls’ Prussian blue reaction: sections were fixed in 4% formaldehyde for 5 min, and washed for 30 s in Milli-Q water prior to staining, then incubated for 30 min in a freshly prepared solution of equal parts 2% HCl and 2% potassium ferrocyanide. Negative control sections were prepared by omitting the freshly prepared solution. After washing with PBS, sections were immersed in 95% methanol containing 1% hydrogen peroxide for 20 min, to quench endogenous peroxidase activity, and then incubated in a solution of DAB. All incubation and washing was performed in propylene glycol troughts that had been washed in 10% HCl (<0.02 ppm Fe) overnight and rinsed in Milli-Q water (Wang et al., 2004). Iron staining was analyzed by counting the number of positive cells at 400× magnification on an Olympus microscope. The average number of positive cells in four fields per slide was used to represent iron-staining density.

2.4. Immunohistochemistry

The sections were warmed to room temperature and washed in 0.01 M phosphate-buffered saline (PBS) for 10 min and then placed in 0.9% methanol containing 0.3% hydrogen peroxide for 30 min to eliminate endogenous peroxidase. After washing three times in PBS, sections were incubated in PBS containing 1% normal goat serum for 15 min at 37 °C, and then incubated overnight with rabbit anti-α TSH (1:10,000); rabbit anti-α TSH DMT1 + IRE (1:300); or rabbit anti-α TSH FP1 (1:300) at 4 °C in a humidified chamber. The sections were washed in PBS and incubated with biotinylated goat anti-rabbit IgG for 30 min at 37 °C, followed by amplification with streptavidin peroxidase for 30 min at 37 °C. Next, sections were rinsed with PBS and incubated for 10–15 min with DAB. Staining was absent in sections where the primary antibodies were omitted. Immunocytochemical staining was analyzed as for the iron staining above.

2.5. High-performance liquid chromatography with electrochemical detection

It was performed as previously described in our laboratory (Jiang et al., 2006). Briefly, both sides of the striatum were isolated carefully and transferred to liquid nitrogen. Samples were weighed and then homogenized in 0.3 ml liquid A (0.4 M perchloric acid). After centrifugation at 4 °C, 12,000 rpm for 20 min, 80 μl of the supernatant were transferred to new eppendorf tubes with 40 μl liquid B (20 mM citromalic acid–potassium, 300 mM dipotassium phosphate, 2 mM EDTA 2Na) addition. Another centrifugation 12,000 rpm for 20 min at 4 °C was conducted and then 100 μl of the supernatant were assayed for content of DA and its metabolites in striatum by HPLC-ED. Separation was achieved on a PE C18 reversed-phase column. The mobile phase (20 mM citromalic acid, 50 mM sodium caproate, 0.134 mM EDTA 2Na, 1.75 mM sodium octane sulphonic acid, 1 mM di-sec-butylamine and containing 5% (v/v) methanol) was used at a flow-rate of 1 ml/min. A 2465 electrochemical detector (Waters, USA) was employed and was operated in screen mode. Results were expressed as ng/mg wet weight of brain tissue.

2.6. Semi-quantitative RT-PCR

Total RNA from mice SN of each group was isolated using TRizol reagent according to the manufacturer’s instructions, and then 2 μg RNA was reverse-transcribed in a 20 μl reaction with oligo-DT primers using a reverse-transcription system. We amplified TH (467 bp) with the primers (forward: 5’-AAATCCACCACCTTAGAACGAC-3’; reverse: 5’-TACCCACTACCGTCCTCC-3’); FP1 (449 bp) with the primers (forward: 5’-TGGCTCTTGGGACTCTGCCG-3’; reverse: 5’-TCAGGATTTGGGGCAAGATGAC-3’); DMT1 + IRE (404 bp) with the primers (forward: 5’-CTGCGCTGCTGCGTCTG-3’; reverse: 5’-CACCGCTGTATCTGCC-3’); DMT1–IRE (227 bp) with the primers (forward: 5’-TCGCCGTGCTGCTGCGTCTG-3’; reverse: 5’-GCGTTTGAGTGTCGGTGCTGCCT-3’); GAPDH (236 bp) with the primers (forward: 5’-TTACACACCATGAGAAGGCC-3’; reverse: 5’-GGCTGAGACTTGTCGCTGATCA-3’); GAPDH (265 bp) with the primers (forward: 5’-TGTCCTCCATGTACTCCA-3’; reverse: 5’-GGTACGAAACGCGAGAGG-3’). Thermocycling for TH and GAPDH was carried out as follows: 94 °C for 4 min, then 35 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min, followed by 72 °C for 10 min. Thermocycling for DMT1 + IRE, FP1, GAPDH and GAPDH were carried out as follows: 94 °C for 5 min, then 32 cycles of 94 °C for 30 s, 62 °C for 30 s, 72 °C for 1 min, followed by 72 °C for 10 min. Ethidium bromide stained gels were scanned and qualified using Tanon Image Software.

2.7. Spectrophotometric assay of iron chelating activity

1,10-Phenanthroline can form a colored complex with Fe (II) (Yegorov et al., 1993), the spectrophotometric method was used to measure iron chelating activity. The aliquots of standard iron (II) solutions containing of Rg1 or DFO were transferred separately into a series of 10 ml-calibrated flasks, and then 1,10-phenanthroline, 10% hydroxylamine hydrochloride and 1 mol/l sodium acetate were added, respectively. The resulting mixtures were mixed well and absorbance was measured at 510 nm against blank solution.

2.8. Statistical analysis

The results were expressed as the mean ± S.D. The data were evaluated using a one-way ANOVA followed by the Student–Newman–Keuls test. Probability value of P < 0.05 was taken to be statistically significant.

3. Results

3.1. Rg1 increased the DA and its metabolites contents in the striatum of MPTP-treated mice

Using high-performance liquid chromatography with electrochemical detection, the DA contents and its metabolites, DOPAC and HVA in the striatum were investigated. MPTP significantly
decreased striatal DA, DOPAC and HVA levels in C57BL6 mice. Rg1 pre-treatment reduced the toxic effects of MPTP, with almost a complete reversal in the levels of DA and its metabolites (Table 1).

### 3.2. Rg1 increased the TH expression and mRNA expression in the SN of MPTP-treated mice

Using immunohistochemistry, we investigated TH expression in SN of mice. TH-immunoreactive (TH-ir) cells were significantly decreased by MPTP administration. Rg1 pre-treatment attenuated MPTP-lowered expression of TH. We also measured TH mRNA expression by semi-quantitative RT-PCR. Consistent with the TH expression, the TH mRNA was greatly decreased by MPTP. However, Rg1 pre-treatment significantly reduced MPTP toxicity, with less reduction of TH mRNA expression (Table 2).

### 3.3. Rg1 decreased the iron levels in the SN of MPTP-treated mice

To explore the possible iron chelating activity of Rg1, we measured the iron levels in the SN using Perl’s iron staining method. The results showed that the numbers of iron-staining cells were increased after MPTP administration. Rg1 pre-treatment substantially attenuated MPTP-elevated iron levels (Table 3).

### 3.4. Rg1 decreased the DMT1 + IRE expression and mRNA expression in the SN of MPTP-treated mice

To clarify the mechanism of iron chelating potentials of Rg1, we measured the expression of iron import protein—DMT1 + IRE expression using immunohistochemistry. DMT1 + IRE mRNA expression was increased in Rg1 pre-treated group compared with MPTP-treated group (Table 4). The semi-quantitative RT-PCR result also showed that Rg1 pre-treatment increased the DMT1 + IRE mRNA expression in the SN of MPTP-treated mice (Fig. 1).

### 3.5. Rg1 increased the FP1 expression and mRNA expression in the SN of MPTP-treated mice

To clarify the mechanism of iron chelating potentials of Rg1, we also measured the expression of iron export protein—FP1 expression using immunohistochemistry. MPTP significantly decreased nigral FP1 expression in C57BL6 mice. Again, Rg1 pre-treatment reduced the toxic effects of MPTP and FP1 expression was increased in Rg1 pre-treated group compared with MPTP-treated group (Table 5). The semi-quantitative RT-PCR result also showed that Rg1 pre-treatment increased the FP1 mRNA expression in the SN of MPTP-treated mice (Fig. 2).

### 3.6. Rg1 had no iron chelating activity

The iron chelating activity of Rg1 was detected using 1,10-phenanthroline. 10 mmol/l deferoxamine decreased densitometry significantly compared with the control. 10 mmol/l Rg1 had no effect on the densitometry compared with the control. This indicated deferoxamine had apparent iron chelating effect; however, Rg1 had no iron chelating effect (Table 6).

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### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>DA</th>
<th>DOPAC</th>
<th>HVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>17.67 ± 1.10</td>
<td>2.148 ± 0.14</td>
<td>1.202 ± 0.12</td>
</tr>
<tr>
<td>MPTP</td>
<td>6</td>
<td>4.857 ± 0.55a</td>
<td>1.206 ± 0.07a</td>
<td>0.410 ± 0.05a</td>
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<tr>
<td>MPTP + Rg1</td>
<td>6</td>
<td>12.025 ± 1.16ab</td>
<td>1.638 ± 0.40ab</td>
<td>0.911 ± 0.09ab</td>
</tr>
</tbody>
</table>

DA: *F* = 365.71; DOPAC: *F* = 132.16; HVA: *F* = 195.70.

* P < 0.01 compared with the control.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>TH-ir cell numbers</th>
<th>TH/GAPDH mRNA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>41.83 ± 2.86</td>
<td>0.911 ± 0.16</td>
</tr>
<tr>
<td>MPTP</td>
<td>6</td>
<td>9.50 ± 2.43</td>
<td>0.442 ± 0.17a</td>
</tr>
<tr>
<td>MPTP + Rg1</td>
<td>6</td>
<td>27.00 ± 3.35ab</td>
<td>0.775 ± 0.14d</td>
</tr>
</tbody>
</table>


* P < 0.01 compared with the control.

### Table 3

<table>
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<tr>
<th>Group</th>
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<th>Cell numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>12.04 ± 1.35</td>
</tr>
<tr>
<td>MPTP</td>
<td>6</td>
<td>33.18 ± 2.56a</td>
</tr>
<tr>
<td>MPTP + Rg1</td>
<td>6</td>
<td>17.54 ± 2.09ab</td>
</tr>
</tbody>
</table>

*F* = 394.80.

* P < 0.01 compared with control group.

### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>DMT1 + IRE positive cell numbers</th>
<th>DMT1–IRE positive cell numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>32.37 ± 3.41</td>
<td>30.08 ± 2.02</td>
</tr>
<tr>
<td>MPTP</td>
<td>6</td>
<td>44.89 ± 3.72</td>
<td>48.82 ± 2.24</td>
</tr>
<tr>
<td>MPTP + Rg1</td>
<td>6</td>
<td>35.06 ± 2.24</td>
<td>34.26 ± 0.97</td>
</tr>
</tbody>
</table>

DMT1 + IRE positive cell numbers: *F* = 25.63; DMT1–IRE positive cell numbers: *F* = 173.37.

* P < 0.01 compared with control group.

### Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>DMT1 + IRE mRNA expression (relative to GAPDH)</th>
<th>DMT1–IRE mRNA expression (relative to GAPDH)</th>
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<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>1.16a,b</td>
<td>1.202</td>
</tr>
<tr>
<td>MPTP</td>
<td>6</td>
<td>2.43a</td>
<td>1.206</td>
</tr>
<tr>
<td>MPTP + Rg1</td>
<td>6</td>
<td>2.35a,b</td>
<td>1.202</td>
</tr>
</tbody>
</table>

* P < 0.01 compared with control group.

### Table 6

The iron chelating activity of Rg1 was detected using 1,10-phenanthroline. 10 mmol/l deferoxamine decreased densitometry significantly compared with the control. 10 mmol/l Rg1 had no effect on the densitometry compared with the control. This indicated deferoxamine had apparent iron chelating effect; however, Rg1 had no iron chelating effect (Table 6).
4. Discussion

To our knowledge, this is the first report to demonstrate Rg1 could reduce nigral iron levels although it is not an iron chelator. Also, the mechanism of Rg1 reducing nigral iron levels is achieved by regulating certain iron transport proteins, quite different from the iron chelator deferoxamine.

In this study, we observed that Rg1 could increase DA and its metabolites levels in the striatum compared with the MPTP-treated group; Rg1 could also inhibit the MPTP-induced decrease of TH-ir cells in SN, indicating that Rg1 could directly protect against the toxic effects of MPTP on dopaminergic neurons. Results also confirmed that the MPTP-induced decrease in TH mRNA expression in the midbrain could be restored by Rg1 treatment. These results indicate the significant neuroprotective effects of Rg1 on dopaminergic neurons.

The neurotoxin MPTP has been widely used as an agent for inducing animal models of PD (Heikkila et al., 1984; Langston et al., 1983; Nagatsu and Sawada, 2006). Administration of MPTP in primates and mice can replicate the selective degeneration of dopaminergic neurons of the nigrostriatal pathway (Burns et al., 1983; Heikkila et al., 1984), thereby mimicking the clinical and pathological features of PD. It is well known that elevated nigral iron levels have been suggested as the underlying mechanisms of pathological features of PD. It is well known that elevated nigral iron levels have been suggested as the underlying mechanisms of pathogenesis of PD (Gerlach et al., 1994). Iron plays a key role in the oxidative stress, which is capable to catalyze H2O2 and form the highly reactive hydroxyl radicals resulting in increased oxidative damage (Jellinger et al., 1990). Free radical related oxidative stress promotes considerable damage to DNA, lipids and proteins (Whitnall and Richardson, 2006; Joshi et al., 2006). Iron chelators have been shown to be neuroprotective in MPTP models of PD (Youdim et al., 2004; Gal et al., 2005). In this study, we observed that Rg1 had no iron chelating activity using 1,10-phenanthroline, indicating that Rg1 could not chelate iron directly to decrease the nigral iron levels.

High levels of brain iron were related to the imbalance of some iron transport proteins (Galazka-Friedman et al., 2004; Sayre et al., 2000), such as iron import protein–DMT1, as well as iron export protein–FP1. DMT1, also known as natural resistance associated macrophage protein 2 (Nrm2p), is an iron import protein, responsible for iron import. There are at least four distinct isoforms of DMT1, which differ in both the C-terminus (with IRE and without IRE) and the N-terminus (two different promoters) (Lee et al., 1998; Hubert and Hentze, 2002; Mackenzie et al., 2007). High levels of DMT1 in the neurons of the SN in PD, may account for the high levels of iron in these regions (Burdo et al., 2001; Huang et al., 2004; Williams et al., 2000). Our previous studies showed increased DMT1 expression and elevated iron levels in the SNpc of PD mouse model (jiang et al., 2003), over expressed DMT1 could induce iron accumulation and cell injury (Xu et al., 2008). These suggested that the upregulation of DMT1 might be involved in the neurotoxicity of PD by increasing cellular iron levels. In the present study, we detected Rg1 decreased the DMT1 ± IRE expression and mRNA expression in the SN of MPTP-treated mice, indicating that Rg1 could suppress iron import caused by MPTP. For maintenance of a balanced iron homeostasis, the cellular iron import, as well as iron export must be tightly regulated. FP1, also known as metal transport protein1 (Abboud and Haile, 2000) or IREG1 (McKie et al., 2000) is a recently discovered transmembrane iron export protein (Donovan et al., 2000), which is expressed at the basolateral surfaces of duodenal enterocytes, in macrophages of the spleen and liver, in placental syncytiotrophoblasts as well as in the brain (Abboud and Haile, 2000; Donovan et al., 2000; Burdo et al., 2001). The partial loss of FP1 function might lead to an imbalance in iron distribution (Montosi et al., 2001) makes it possible that misregulation of FP1 in the brain might result in a consequent increase in tissue iron accumulation followed by neuronal death. Wang et al. reported that decreased expression of FP1 in the SN can account for the increased iron levels in the brain (Wang et al., 2007). After MPTP treatment, FP1 expression in the mice SN decreased. It was the decreased FP1 expression that caused the decreased iron export from the cells, leading to the elevated iron level in SN. Rg1 pre-treatment substantially attenuated MPTP toxicity and increased the FP1 expression.

Deferoxamine could chelate iron directly to decrease the iron levels; decreased iron upregulated DMT1 expression and downregulated FP1 expression according to the regulation of IRP/IRE system. This is confirmed by the data in our previous experiments and other studies that cells treated with the iron chelator deferoxamine displayed higher levels of DMT1 mRNA and protein (Cheong et al., 2004). Rg1 could downregulate DMT1 expression and upregulate FP1 expression, which is quite different from deferoxamine. The precise mechanism of the regulatory expression of DMT1 and FP1 are not fully understood up to now. Evidence has proved the IRE existed in the mRNA of DMT1 ± IRE and FP1. IRE-binding protein (IRE-BP) or iron regulation protein (IRP) could bind with the IRE (Youdim and Ben-Shachar, 1990; Jellinger et al., 1992;...
Double et al., 1999). This binding could regulate the expression of iron transport proteins and then regulate the iron metabolism (Kidd, 2000; Jenner, 2003; Andersen, 2004). However the exact mechanism of the regulation of IRPs is not clear. Some signal molecules are involved in the regulation of IRPs, including NO or oxidative stress. Dauer had demonstrated that oxidative stress could active the expression of IRP1, and then regulate the expression of iron transporters (Dauer and Przedborski, 2003).

The function of IRPs is affected by the intracellular oxidizing materials (Sohal and Weindruch, 1996). H2O2 has been proved to active IRP1 (Harley et al., 1993) in vitro study. The signal molecular NO can also active the expression of IRP1 and IRP2 (Harley et al., 1993; Hasbani et al., 2005). And the later experiments confirmed that oxidant stress-induced activation of IRP1 could be abolished by radical scavenger NAC (Sveinbjornsdottir et al., 2000). This suggested that the oxidative stress is involved in the activation of IRPs. Many literatures had reported that Rg1 possessed obvious antioxidant and radical scavenging effect (Chen et al., 2005; Bloomer et al., 2008), which might be involved in the activation of IRPs and the expression of iron transporters. This indicated that the neuroprotective effect of Rg1 on iron levels might be achieved by the regulation of DMT1 + IRE and FP1 by the activation of IRPs due to its antioxidant effect.

On the other hand, there was no IRE in the mRNA of DMT1–IRE, indicating the regulation of DMT1–IRE is independent on IRE/IRPs system. It is postulated that transcription factors acting on putative AP-1, NF-kappaB binding sites, or gamma-interferon responsive elements on the DMT1 promoter may also play a role in upregulating of DMT1–IRE. This could lead to increased iron influx (Huang et al., 2006). However the exact mechanisms of Rg1 on the expression of iron transport proteins remained unclear. In summary, the present study has demonstrated that Rg1 exerts neuroprotective effects against MPTP-induced dopaminergic degeneration in C57BL6 mice, by a mechanism, believed to reducing nigral iron levels, which is achieved by regulating the expression of DMT1 and FP1. The experiment provided new pharmacology target to the prevention and treatment of PD. Further studies are required to elucidate other possible mechanisms of Rg1, such as estrogen-like activities. Considering the multifunctional drugs possessing various central nervous system targets, the present findings provide greater symptomatic efficacy and indicate a potential treatment alternative which could be used readily to rescue and prolong the survival of neurons in patients with PD.

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