Research Report

Elevated interleukin-1β induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine aggravating dopaminergic neurodegeneration in old male mice

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ABSTRACT

IL-1β is a potent pro-inflammatory cytokine that regulates neuroinflammation during brain damage. The expression of IL-1β has been reported to be elevated in the striatum and substantia nigra of patients with Parkinson’s disease (PD). Moreover, greater prevalence of PD in men than in women is described previously. Here, by using a sensitive mice model in which the expression of luciferase reporter gene is under the control of human IL-1β gene promoter, we examined IL-1β gene expression pattern in vivo after subacute MPTP toxication in old male and female mice and found that MPTP elicited greater dopaminergic toxicity in old male than in female mice. Old male mice showed dramatically elevated luciferase signals in a flexuous manner at early period of time, meanwhile, the changes in female were more moderate. However, no significant difference in astroglial reaction was observed at later time point between sexes. In conclusion, the present study demonstrated that elevated IL-1β gene expression at early period of time may be in part responsible for the DA neuron susceptibility to MPTP in old male mice.

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1. Introduction

Parkinson’s disease is a chronic and age-related neurodegenerative disorder well characterized by a progressive loss of dopaminergic neurons projecting from the SNpc to the striatum. Epidemiological studies have demonstrated a higher prevalence of PD in men than in women (Diamond et al., 1990; Van Den Eeden et al., 2003; Wooten et al., 2004). Meanwhile, in vivo studies on experimental animal models also report greater MPTP neurotoxic effect in male than in female mice (Miller et al., 1998). Estrogen, which is revealed to exert dopaminergic neuroprotection both in human (Tsang et al., 2000) and PD mouse model (Dluzen et al., 1996, 2001; Ramirez et al., 2003; D’Astous et al., 2006), may contribute to the gender difference in PD.

Glial reaction is observed in brain tissues of postmortem PD patients and MPTP-induced animal models (Langston et al.,...
indicating that activation of microglia and astrocytes might play a role in the neurodegenerative process. Activated microglia may damage the CNS by releasing various kinds of cytokines and pro-inflammatory molecules (Hunot et al., 1996; Cassarino et al., 1997; Kohutnicka et al., 1998; McGuire et al., 2001; Wu et al., 2002), or on the other hand play neuroprotective roles by producing neurotrophic factors such as BDNF and GDNF (Batchelor et al., 1999). Astrocytes in CNS either possess neurotrophic properties through producing large amount of molecules which may benefit the injured nervous system or play toxic effect by releasing cytokines (Eddleston and Mucke, 1993). Previous studies have demonstrated that glial reaction also implicates in the neuroprotective effect of estrogen (Garcia-Segura et al., 1999; Rozovsky et al., 2002; Sortino et al., 2004; Tripanichkul et al., 2006).

IL-1β is a cytokine mainly released from glial cells that plays important roles in mediating cellular responses to injuries in the CNS. Elevated IL-1β was detected in the striatum (Mogi et al., 1994, 1996), and SN (Hunot et al., 1999) of PD patients, suggesting its association with PD. However, contradictory findings about the role of IL-1β in brain damages were reported (Mason et al., 2001; Arai et al., 2004; Jones et al., 2005). The reaction pattern of IL-1β in subacute PD animal model at early time has not been investigated before. Therefore, in our study, we used a transgenic mouse model cHS4I-hIL-1βP-Luc, in which the expression of luciferase reporter gene was under the control of human IL-1β gene promoter (Li et al., 2008) to monitor IL-1β gene expression in vivo following subacute MPTP intoxication in old male and female mice. Astrocitic reaction, transcriptional expression of inflammatory molecule TNF-α, IL-6, iNOS and apoptotic molecule bcl-2, bax were also examined at later time in both sexes.

2. Results

2.1. Old male mice showed more dramatically elevated luciferase activities after MPTP intoxication, while changes in female mice were moderate

Old male and female mice were screened for luciferase expression at different time points (Fig. 1A). All animals showed low but detectable basal level of luciferase activity at the position of head. MPTP induced elevated transcriptional expression of IL-1β at 2 h in the cortex, striatum and substantia nigra, which was in line with luciferase signals at 2 h (Fig. 1B). The luciferase expression elevated significantly at 2 h, 8 h, 32 h and 49 h in MPTP-treated old male mice, the inducible signals peaked at 8 h, then gradually declined. The old female mice showed marked increase at 4 h and 26 h following MPTP administration, while the signals from saline groups showed slight variations around the basal level (Fig. 1C). By 49 h, the enzyme activity had
returned back to baseline in MPTP-treated female mice, while MPTP-treated male mice revealed sustained higher level (Fig. 1C). As quantified by the LivingImage software, male and female showed different patterns of MPTP-induced luciferase signals, both sexes revealed significant higher levels of luciferase expression after MPTP administration; the changes in male mice were especially vigorous, but quite moderate in female (Fig. 1D). IL-1β protein expression in the striatum also remained at higher level in old male mice even at 96 h (Supplementary Fig. A).

2.2. MPTP elicited less dopaminergic toxicity in old female than in male mice

MPTP produced distinguished depletion of striatal TH protein level in both sexes at 96 h, moreover, TH reduction was
significantly greater in male than in female (Figs. 2A, B). The TH positive neurons in the SNpc showed similar tendency to striatal TH protein. There was no prominent difference of TH positive cell number between the saline and MPTP groups in the SNpc of old female mice, however, male mice showed significant decrease of TH positive cells induced by MPTP (Figs. 2C, D). Altogether, MPTP elicited less dopaminergic toxicity in old female than in male mice.

2.3. The transcriptions of some inflammatory and apoptotic molecules in the substantia nigra showed no significant changes between groups

The mRNA levels of cytokine TNF-α and IL-6 in the substantia nigra were detected by real-time PCR at 96 h and revealed no significant alteration between MPTP and saline groups, although the p-value of IL-6 between the control and MPTP groups in female mice was 0.07 (Figs. 3A, B). The transcription of pro-inflammatory molecule iNOS also showed no distinguished changes within groups (Fig. 3C), neither were the apoptosis related molecules bcl-2 and bax (Figs. 3D, E). Cox-2, another pro-inflammatory molecule, is non-detectable in our model, while Timm22, a non-affected gene (Lepagnol-Bestel et al., 2007), showed no changes (Supplementary Fig. B).

2.4. MPTP intoxication increased GFAP protein expression and GFAP positive cells in the striatum of male and female mice

Measured by Western blot analysis, striatal GFAP protein expression revealed robust increase induced by MPTP in both sexes at 96 h, while the control groups remained in a relatively lower level (Figs. 4A, B). Intensively more GFAP positive cells were also observed in the striata of old male and female mice after MPTP administration compared to control groups (Fig. 4C), without distinguished changes within sexes. We also detected the expression of neurotrophic
factor BDNF as it may support dopaminergic neurons (Hyman et al., 1991; Batchelor et al., 1999). Slightly higher expression of BDNF was found in old female mice, however, no significant difference was observed between groups (Supplementary Figs. C, D).

3. Discussion

Using an in vivo bioluminescence imaging system, it is the first study to investigate the expression pattern of IL-1β at early period of time after multiple MPTP administration in old mice. The luciferase signals in the brain of MPTP-treated old male mice were more dramatically increased compared with female mice, meanwhile, the two groups clearly demonstrated distinct reaction patterns. Multiple MPTP intoxication initiated greater neurotoxicity in old male than in female mice. The transcriptional expression of TNF-α, IL-6 and iNOS in the SN at 96 h revealed no significant change within groups. Reactive astrocytes were highly evoked in the striatum of MPTP-treated mice, irrespective of gender. The present study indicated that quickly profound increase of IL-1β at early time points may be in part responsible for the increased susceptibility of dopaminergic neurons to MPTP exposure in old male mice.

Experimental studies have reported greater MPTP neurotoxic effect in male than in female mice both in young and old mice (Miller et al., 1998; Ciesielska et al., 2007), which was also found in the present study that loss of striatal TH protein expression and TH positive neurons in the SNpc induced by MPTP was much more severe in old male than in female mice. In line with TH protein expression, significantly decreased level of striatal DA was previously observed in male mice than in female (Joniec et al., 2009). The benefit effect of estrogen on DA neuron survival may be an explanation.

Inflammatory processes have been shown to be associated with the pathogenesis of PD. Non-steroidal anti-inflammatory drug (NSAID) users were reported to possess lower risk of developing PD (Wahner et al., 2007). IL-1β, a very important cytokine which can be released from active microglia or astrocytes, is a potent regulator of inflammation. The luciferase signals detected in the brain of transgenic mice were proved to be correlated with transcriptional expression of IL-1β in the brain in our study. Moreover, the sensitivity and continuity for monitoring IL-1β gene expression during the disease process has made it distinguished from conventional methods. There was elevated inducible luciferase expression after MPTP administration in old mice, this finding is in agreement with elevated IL-1β in the striatum and SN of PD patients (Mogi et al., 1994, 1996; Hunot et al., 1999). As revealed in previous study that increased IL-1β mRNA is observed only in early stage after single MPTP toxication (Hebert et al., 2003), the flexuous manner of IL-1β expression in old male mice may be reflect the reaction of brain to repeated MPTP intoxication. The luciferase expression in male was much stronger than in female, this is also consistent with previous findings showing that additional administration of estrogen down-regulates...
microglial activation in male mice following MPTP intoxication (Tripanichkul et al., 2006). Activated microglia are thought to aggravate neurodegeneration rather than neuroprotection by producing numerous cytokines, such as TNF-α and IL-6, and pro-inflammation molecule iNOS (Wu et al., 2002). We examined the transcriptional expression of TNF-α, IL-6 and iNOS in the SN at 96 h following MPTP administration, however, these molecules did not display significant changes. These findings are in line with previous in which up-regulation of TNF-α, IL-6 and iNOS occurs very early (Hebert et al., 2003).

Besides microglial activation, reactive astrocytes also occur extensively in the basal ganglia of PD animals (McGeer et al., 2001). As astrocytic activation is characterized by an increased expression of GFAP in the injured area (Eddleston and Mucke, 1993), thus, robust GFAP expression and massive GFAP positive cells observed at 96 h after MPTP treatment indicated strong activation of astrocytes in the striatum of aged brains. These astroglia synthesize cytokines and neurotrophic factors which may either damage or benefit nearby cells. Synthesized in both microglia and astrocytes (Batchelor et al., 1999), BDNF is a neurotrophic factor that protects dopaminergic neuron from neurotoxins MPTP and 6-OHDA (Spina et al., 1992). The unaltered striatal BDNF protein expression examined in the present study was also explored in MPTP, 6-OHDA or the HIV protein gp120 insulted models (Mocchetti et al., 2007). However, we did not observe astroglial reaction at earlier time points. It is likely that the protective role of astroglial activation could not compensate for the fatal pathways which have already started in neurons.

In conclusion, the present study demonstrated that different changes in reactive IL-1β gene expression at early time after MPTP administration may be in part responsible for the DA neuron susceptibility to MPTP in old male and female mice. cHS4I-hIL-1β-P-Luc transgenic mice are proved to be a valuable model to study the inflammatory processes in PD.

4. Experimental procedures

4.1. Animals

cHS4I-hIL-1β-P-Luc transgenic mice were used in the research. Generation of the mice has been described previously (Li et al., 2008). The transgenic mice were bred to C57BL/6 for 3 generations before testing. Transgenic offsprings were identified by PCR using the forward-luc (5′ TTCCGCCCTTCTTGGCCTTTATG 3′) and reverse-luc (5′ CAGCTATTCTGATTACACCCGAGG 3′), primers specific for luciferase gene. Transgenic male and female mice at the age of 12–14 months were for the experiments. All animals were housed three to four per cage at 22 °C under a 12 h light/dark cycle with free access to food and water.

4.2. Materials and drug treatment

Luciferin (Biosynth, Switzerland) was dissolved in PBS at 15 mg/ml and stored at –20 °C. MPTP was purchased from Sigma-Aldrich (St. Louis, USA). Old male and female transgenic mice were injected intraperitoneally with MPTP at the dose of 15 mg/kg for 2 days with 2 injections every 6 h within a day, or
0.9% saline. Mice were sacrificed at 2 h or 96 h as indicated in Fig. 1A.

4.3. In vivo imaging

In vivo bioluminescent imaging was performed using an IVIS imaging system (Xenogen, CA, USA) as previously described (Li et al., 2008). 150 μl sodium salt luciferin was injected intraperitoneally, mice were then anesthetized with isoflurane/oxygen and placed on the imaging stage. 12 min after luciferin injection, mice were imaged for 1 min. Photons emitted from specific regions were quantified using a Living-Image software (Xenogen, CA, USA). In vivo luciferase activity was presented in photons emitted per second.

4.4. Tissue preparation

Mice were anesthetized with 10% chloral hydrate to minimize suffering. After being perfused intracardially with 0.9% saline solution, brains were carefully removed. For mice killed at 2 h, the cortex, hippocampus, striatum and substantia nigra were isolated on ice for total RNA extraction. The right cerebral hemisphere of mice killed at 96 h was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for section preparation, the striatum and substantia nigra of the left cerebral hemisphere were isolated on ice for protein preparation and total RNA extraction.

4.5. Immunobloting

The method of protein extraction and Western blot analysis has been described elsewhere (Bian et al., 2008). The primary antibodies used here were: mouse anti-tyrosine hydroxylase (1:4000; Sigma, USA); mouse monoclonal GFAP antibody (1:1000; Lab Vision, USA); mouse monoclonal β-actin antibody (1:1000; Lab Vision, USA); monoclonal mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:10,000; Kangcheng, China) and rabbit BDNF (1:200; Santa Cruz, USA). Signals were detected with chemiluminescence detection system (Santa Cruz, USA). The protein levels were quantified by densitometry using Quantity One 4.5.2 software (Bio-Rad, USA).

4.6. Immunohistochemistry

The right cerebral hemisphere was post-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Frozen sections were then cut at 30 μm on freezing microtome (Leica, Germany). Immunohistochemistry of brain tissues was carried out according to the previously published methods (Bian et al., 2008) with minor modifications. Briefly, sections were subjected to microwave for 15 min in 0.1 M Tris buffer and 0.03% H$_2$O$_2$. After being rinsed with 0.9% saline, Mice were sacrificed at 2 h or 96 h as indicated in Fig. 1A.

4.7. Cell counting

For measurement of the density of TH positive cells in the SNpc, we performed total cell counting and stereological counting based on the previous description (Zhang et al., 2004). Cell counting was under light microscope in a double-blind fashion. Four 30 μm sections were captured under light microscope per mouse, and then positive-labeled cells were counted manually. Sections were selected every 120 μm per mouse (n=7–10 per group).

4.8. Quantitative real-time PCR

Total RNA was extracted from the SN of the left hemisphere using Trizol reagent (Invitrogen, USA). Reverse transcription was carried out using random primer and Moloney murine leukemia virus reverse transcriptase (Promega, USA), and real-time PCR was carried out for quantification of IL-1β, TNF-α, IL-6, iNOS, bcl-2 and bax mRNA on ABI 7300 PCR machine (Applied Biosystems, Foster City, USA). For plotting a standard curve, serially diluted cDNA fragments were used in each experiment. Expression of target gene or GAPDH was quantified to the standard curve, and the relative expression value was calculated as the ratio of target cDNA to GAPDH. The primers used in the real-time PCR were:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>5′ CAACGCTCAGCTACACAGGCTCGAGATG 3′</td>
<td>5′ TTGTGAAGAAACACGGTTTTCAC 3′</td>
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<tr>
<td>IL-1β</td>
<td>5′ CAGGCCTCTTGTCTACGAACCTC3′</td>
<td>5′ TGGACGTCTCCAGTTATCTGTTAG 3′</td>
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<tr>
<td>TNFα</td>
<td>5′ CGAGCCTTGTGCTCTGGAAGAACC 3′</td>
<td>5′ IL-6 forward: 5′ GTAGTTCCGCTCCCTTCTCTTCTGTCG 3′</td>
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<tr>
<td>TNFα</td>
<td>5′ CGAGCCTTGTGCTCTGGAAGAACC 3′</td>
<td>5′ IL-6 forward: 5′ CTCTGACTCCAGTTATCTGTTAG 3′</td>
</tr>
<tr>
<td>iNOS</td>
<td>5′ TGGCCAC CCTTGTCAGCTACG 3′</td>
<td>5′ IL-6 reverse: 5′ TGGACGTCTCCAGTTATCTGTTAG 3′</td>
</tr>
<tr>
<td>iNOS</td>
<td>5′ TGGCCAC CCTTGTCAGCTACG 3′</td>
<td>5′ iNOS reverse: 5′ CATATTTGTTTGGGGCAGGTTTGTC 3′</td>
</tr>
<tr>
<td>β-actin</td>
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<td>5′ iNOS reverse: 5′ CATATTTGTTTGGGGCAGGTTTGTC 3′</td>
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<tr>
<td>GFAP</td>
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<td>5′ bcl-2 forward: 5′ GGATTCTGGCCTCTTGGAGTCCGG 3′</td>
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<tr>
<td>GAPDH</td>
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<td>5′ bcl-2 reverse: 5′ GGATTCTGGCCTCTTGGAGTCCGG 3′</td>
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<td>bcl-2</td>
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<tr>
<td>bax</td>
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<tr>
<td>bax</td>
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<td>5′ bax reverse: 5′ GGATTCTGGCCTCTTGGAGTCCGG 3′</td>
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<tr>
<td>TH</td>
<td>5′ CACGCTCTTGTCTACGAACCTC3′</td>
<td>5′ TH forward: 5′ GGATTCTGGCCTCTTGGAGTCCGG 3′</td>
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</tbody>
</table>

4.9. Data analysis

Data were analyzed using SPSS software (version 11.5; SPSS, Chicago, USA). All values are expressed as mean±S.E. Statistical analysis of group differences was assessed by ANOVA followed by multiple comparisons with the LSD post-hoc test. p<0.05 was considered significant.

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Appendix A. Supplementary data


REFERENCES


