Effects of nicotine on the microglia of Parkinson’s disease mice

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Abstract  Objective To investigate the protective effects of nicotine on dopaminergic neurons. Methods C57BL/6J mice were injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20mg/kg) to establish Parkinson’s disease (PD) model after pretreated with nicotine (0.25 mg/kg). The effects of nicotine to PD mice were observed by their behavior test and immunocytochemistry staining (ICC) for tyrosine hydroxylase (TH) and OX-42 in the compact part of the substantia nigra (SNc). Results The results showed that nicotine markedly attenuated the dyskinesia of PD mice, increased the number of TH-positive dopaminergic neurons and simultaneously inhibited the activation of microglia in the SNc. Conclusion These findings suggest that nicotine has protective effects on dopaminergic neurons in the MPTP-induced PD mice. It may be related to inhibiting the activation of microglia.

Key words nicotine; Parkinson disease; dopaminergic neuron; microglia

Introduction

Parkinson disease (PD) is a common chronic neurodegenerative disorder which is characterized by a progressive degeneration and necrosis of dopaminergic (DAergic) neurons in substantia nigra (SN), as well as the decrease of dopamine (DA) level in striatum. The clinical features of the disease include tremor, rigidity and postural instability. Although several approved drugs may alleviate PD symptoms, long term use of these drugs is often associated with aggravating side effects, and none of these drugs can slow down, prevent and even reverse the progress of PD[1]. So, to explore a neuroprotective drug is very essential.

Nicotine administration and cigarette smoke have been shown to attenuate MPTP-induced degenerative effects in the mouse striatum and substantia nigra[2-4]. But the contradictory results of nicotine on dopaminergic neuron were also reported and it may be because of differing routes and schedules of administration[5-7]. So more studies are needed to assess the usefulness of nicotine and the mechanism of nicotine for PD.

Microglia were considered the resident immune cells of the central nervous system (CNS). In physiology conditions, microglia acted as immunologic surveillance. When some injury happened, microglia was activated and became the major composition of neuron inflammation. Activated microglia could secrete many factors which may closely related to the neuronal injury[8]. So, the present study was designed to investigate the effect of nicotine on the behavior and dopaminergic neuron of the MPTP-induced parkinsonism. Meanwhile in order to investigate the possible mechanism of nicotine, we used immunohistochemistry to examine the changes of the microglia in the substantia nigra of mice.
Materials and Methods

Animal model

C57BL/6J healthy mice (Dalian Medical University Animal Center, China) weighing 18-22g/body were randomly divided into four groups. The model group was injected with MPTP (20mg/kg) for 8 day, the treated group was injected nicotine (0.5mg/kg/day, i.p, twice) for 10 day and then injected MPTP and nicotine for 8 day, the control group and the treated-control group were given the same volume of physiological saline.

Behavior Tests

After the beginning of MPTP treatment 4 and 8 days, the pole test and the traction test were performed to evaluate the motor function of the mice.

The pole test The animal was placed head upward near the top of a rough-surfaced wood pole (5 cm in diameter and 100 cm in height), and the following time was recorded: 1. the mouse climb from the top to the half of the whole length. 2. the mouse turn from the half of the whole length to the floor. 3. the animal turn completely downward and climb down to the floor. The test score is the sum of the above three times.

The traction test The mice were suspended by their front paws to a wire placed horizontally. Scoring was as follows: 3 = the mouse grasp the wire with two hind paw. 2 = the mouse grasp with one hind paw. 1= the mouse can’t grasp the wire with neither of hind paw.

Immunohistochemistry

Animals were deeply anesthetized with 4% chloral hydrate (400 mg/kg, i.p) their chest cavities were opened and perfused transcardially with physiological saline and 4% paraformaldehyde. The brain was post-fixed by using 4% paraformaldehyde and was put in phosphate buffer saline (PBS) containing 30% sucrose. When the brain was submerged, 50-μ m-thick brain sections were sliced on a vibratome. The above sections were rinsed first in PBS 10 min × 3, then were incubated with bovine serum albumin (BSA) containing 0.1%Triton-x-100 for 1 hour. The sections were incubated in the primary antibody (TH-Ab, 1:1000, OX-42-Ab,1:400) overnight at 4°C. The sections were rinsed in PBS 10 min × 3 and further incubated in the biotinylated-second antibody at room temperature for 1 h. Then the sections were rinsed in PBS 10 min × 3 and then being incubated with avidin–biotin complex A:B;PBS (1:1:400) (Avidin Biotin Complex Kit, Sigma Company, USA) at room temperature for 2 hs. Diaminobenzidine (DAB; Sigma Company, USA) was used to detect signals. The control sections were incubated with PBS instead of primary antibody.

HPIAS series colorful pathology photograph system was used to analyze TH immunoreactive DA neurons and OX-42 immunoreactive microglia. The brain sections were observed with 10× microscope. The number of TH immunoreactive DA neurons, and OX-42 immunoreactive microglia was measured.

Statistical analysis

All data were expressed as mean ± standard deviation. Differences between groups were assessed using an one-way factorial analysis of variance (ANOVA) with post hoc test of LSD in Equar Variances Assumed. In all comparisons, P < 0.05 were considered significant.

Results

Behavior test

Animals were examined behaviorally with the pole test and the traction test on 4th and 8th day after the first injected MPTP. The model group showed dyskinesia: the time of the pole test were significantly prolonged and the score of the traction test was obviously decreased compared with the control group. Nicotine treated group displayed a shorter time on the pole test and a higher score in the traction test compared with the model group. No significant changes were found in the nicotine treated alone group (Table 1).
Table 1  The effects of nicotine on MPTP mice at pole test and traction test

<table>
<thead>
<tr>
<th>Group</th>
<th>Pole test Day4</th>
<th>Traction test Day4</th>
<th>Pole test Day8</th>
<th>Traction test Day8</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS+NS</td>
<td>12.25±0.89</td>
<td>3.00±0.00</td>
<td>12.13±1.25</td>
<td>3.00±0.00</td>
</tr>
<tr>
<td>NS+MPTP</td>
<td>40.88±11.06**</td>
<td>1.50±0.76**</td>
<td>38.00±8.88**</td>
<td>1.38±0.53**</td>
</tr>
<tr>
<td>Nicotine+MPTP</td>
<td>19.63±4.81***</td>
<td>2.75±0.46**</td>
<td>28.13±7.50***</td>
<td>2.13±0.99***</td>
</tr>
<tr>
<td>Nicotine+NS</td>
<td>11.75±0.89</td>
<td>3.00±0.00</td>
<td>11.63±0.92</td>
<td>3.00±0.00</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 compared with group NS+NS. ¶ p<0.05, ¶¶ p<0.01 compared with group NS+MPTP

**Immunohistochemistry**

**The effects of nicotine on the DA neurons in the substantia nigra compact**  Immunohistochemical analysis of TH, the marker for dopaminergic neurons, indicated dopaminergic degeneration in the substantia nigra pars compacta (SNc) after the administration of MPTP. TH-positive cells in the SNc of the PD mice were significantly decreased compared to the control mice (P<0.01). The administration of nicotine suppressed the MPTP-induced dopaminergic degeneration in the SNc (P<0.01) (Fig. 1, 2).

![Fig 1. Dopaminergic neurons in the SNc of the mice. A and D, NS+NS group; B and E, NS+MPTP group; C and F, Nicotine+MPTP group](image)

**The effects of nicotine on the microglia in the substantia nigra reticular**  The mice were measured for OX-42 immunoreactive microglia in the SNr. Compared with saline-treated group, the model group showed a markedly increased (p<0.01). While of the nicotine pretreated group OX-42 immunoreactive microglia were much less than that of the model mice (p<0.05) (Fig. 3, 4).

![Fig 3. Microglia in the SN of mice. A, NS+NS group; B, NS+MPTP group; C, Nicotine+MPTP group](image)
Fig 4. The number of OX-42-IR positive microglia in SN of mice. # p<0.05, ## p<0.01 compared with group NS+NS; * p<0.05, ** p<0.01 compared with group NS+MPTP

Discussion

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) provides one of the most valuable approaches to analyze critical aspects of Parkinson’s disease (PD) in the animal model. We treated C57BL/6J mice with MPTP (20mg/kg, sc) for 8 days to establish PD model. On the 4th and 8th day, behavior change was tested including the pole test and the traction test. The model group exhibited a serious motor disability. After the injection of MPTP, immunohistochemistry also found a large loss of TH-ir dopaminergic neuron in SN of the model mice. The behavior outcome and immunohistochemistry results were consistent with PD feature and confirmed that our model was reliable. We demonstrated that chronic pretreatment of nicotine with the dose of 0.5mg/kg may significantly improve the dyskinesia of the mice and attenuate MPTP-induced TH-ir dopaminergic neurons loss in the SN, which indicated the potent protective effects of nicotine on dopaminergic depletion.

To identify the underlying mechanism for the neuroprotective effect of nicotine on dopaminergic neuron, we studied the effect of nicotine on microglia in MPTP-treated mice. We found the OX-42 immunoreactive microglia in SN of nicotine-pretreated group was significantly lower \((P<0.05)\) than that of MPTP-treated group. Microglia-mediated immunoinjury was crucial for neuronal death. The postmortem analysis of the brains of patients with Parkinson’s disease (PD) showed that reactive microglia were aggregation in the substantia nigra (SN) \(^{[10]}\) and suggested inflammation in the brain may plays an role in the pathogenesis of PD. Microglia were considered the resident immune cells of the central nervous system (CNS). In physiology conditions, microglia acted as immunologic surveillance. When some injury happened, microglia was activatied and became the major composition of neuron inflammation. In the MPTP mouse model, microglial activation occurred as early as 1 day after the MPTP injection, while the depletion of dopaminergic neurons was not pronounced until 7 days after the MPTP injection \(^{[11]}\). So microglial activation may plays a role in the initiation stage of disease progression and was not merely as a response to neuronal death. Using PET in human also supported that neuroinflammatory responses by intrinsic microglia contribute significantly to the progressive degeneration process of the disease\(^{[12]}\). Because of the important role of microglial activation in the PD, inhibit microglial activation may constituted an effective manner of neuroprotection against microglial activation-induced neuronal injury and ultimately impede the development of these diseases. Liu et al found the LPS-induced degeneration of the midbrain neurons was significantly reduced by cotreatment with naloxone and further study showed that naloxone interfered in the binding of LPS to cell membranes so that inhibited microglia activation and protected dopaminergic neurons as well as other neurons in the midbrain cultures from inflammatory damage\(^{[13]}\). Du et al confirmed that blockade of microglial activation was neuroprotective in the PD
model\textsuperscript{[14]}. So we supposed that nicotine inhibiting microglia activation perhaps was a neuroprotective mechanism of nicotine. Studies found alpha7 nicotinic receptor existed in rat microglial cells and in cultured microglia nicotine exhibited a inhibit microglia activation effect. Therefore it was possible that nicotine inhibited microglia via alpha7 nicotinic receptor\textsuperscript{[15,16]}

Many studies of mechanisms of microglial activation-induced neuronal injury has been done. Activated microglia could secrete many factors. A number of these factors, such as the glia-derived neurotrophic factor, are potentially beneficial to the survival of neurons. However, the majority of factors produced by activated microglia, are proinflammatory and neurotoxic. These include the cytokines tumor necrosis factor (TNF) and interleukin-1(IL-1), free radicals such as nitric oxide (NO) and superoxide, fatty acid metabolites such as eicosanoids, and quinolinic acid. These factors may closely related to the neuronal injury\textsuperscript{[8]}. It is a pity that we haven’t a further evidence on how nicotine affect microglia activation and whether some neurotoxic factors were involve in the protect effect.

Our study showed that nicotine could improve the symptom of PD mice and protected dopaminergic neurons in the SN which was via inhibiting the microglia activation.

References

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