69. Piroheptine Prevents Loss of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-Induced Striatal Loss of Dopamine in Mice

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1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been known to produce irreversible Parkinsonism in humans and monkeys (Davis et al. 1979; Burns et al. 1983; Langston et al. 1983). Subsequently, it was shown that MPTP induced depletion of striatal dopamine in mice (Heikkila et al. 1984). These animal models have been considered to be the best model of Parkinson's disease available at present. Although exact cause of Parkinson's disease still remains to be elucidated, newer approaches to this disorder have been attempted using these models. Monoamine oxidase inhibitors (Langston et al. 1984a) and dopamine uptake inhibitors (Ricaurte et al. 1985) thus have been shown to be effective in preventing MPTP-induced loss of striatal dopamine.

Recently, we found that pretreatment with 3-(10,11-dihydro-5H-dibenz[a,d]cyclohepten-5-ylidene)-1-ethyl-2-methylpyrrolidine (Piroheptine), an anticholinergic agent having a dopamine-uptake inhibiting property, prevented loss of striatal dopamine in MPTP-treated mice. It is the purpose of this communication to report our results.

Materials and methods. Male 8-week-old C57BL/6J mice (20–30 g) were used. They were kept in an animal room for at least one week prior to the experiment. They had free access to food and water, and were maintained in a 12 h light/dark cycle.

MPTP (free base, Aldrich) was dissolved in a small amount of ethanol, and diluted with 0.14 M sodium chloride to a final concentration of 1 mg/ml. MPTP (10 mg/kg) was injected intraperitoneally (i.p.) four times at one hour interval. Other group received piroheptine (a generous gift from Fujisawa Pharmaceutical Company, Tokyo, Japan) 20 mg/kg i.p. 30 min before each MPTP injection. Control mice received saline i.p., and another group received piroheptine alone i.p.

Animals were sacrificed by cervical dislocation under light anesthesia with sodium pentobarbital 24 hr after the last injection of MPTP. The brains were immediately removed, frozen on dry ice, and the striata were dissected out. The striata were homogenized in 0.5 N perchloric acid containing α-methyldopamine hydrobromide as an internal standard, and centrifuged at 15,000 g for 10 min. The supernatant was used for assays of dopamine and its major metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), by a high-performance liquid chromatography (Shimadzu LC3A) with an electrochemical detector (Kotaki ECP-1) according to the method of Nagatsu et al. (1979). The column used was a 15 cm × 0.6 cm i.d. stainless-steel column packed with Shim-packed-CLC-ODS (Shimadzu). The mobile phase consisted of 0.1 M monosodium phosphate pH 3.0
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containing 1 mM 1-heptanesulfonic acid sodium salt, 1 mM EDTA and 0.7% methanol. Peak areas were calculated electrically by a recording unit (Shimadzu C-RIA). The results were analyzed statistically using Student's t-test.

**Results.** Results were summarized in Table I. Striatal dopamine content decreased to 20% of the control 24 hr after the last injection of MPTP. DOPAC was reduced by approximately 50%. Pre-treatment with piroheptine almost completely prevented the loss of dopamine and DOPAC in MPTP-injected mice. Piroheptine alone caused approximately 10% increase in dopamine content, however, this increase could not account for the near complete recovery of dopamine when piroheptine was given prior to MPTP.

**Discussion.** Since the original report on MPTP induced Parkinsonism by Davis et al. (1979), MPTP has been considered as one of the best tools to investigate various aspects of Parkinson's disease. It has been postulated that MPTP has to be metabolized to 1-methyl-4-phenylpyridinium ion (MPP⁺) by monoamine oxidase B (MAO B) before it exerts dopaminergic neurotoxicity (Langston et al. 1984b). MPP⁺ selectively gains entrance into dopaminergic neurons by a high affinity dopamine transport system (Javitch et al. 1985).

In the present study, piroheptine was shown to effectively prevent loss of striatal dopamine and DOPAC in MPTP-treated mice. Piroheptine is an anticholinergic agent (Hitomi et al. 1972) having a dopamine-uptake inhibiting property in addition (Ohashi et al. 1972). Other dopamine uptake blockers such as amfonelic acid or mazindol have been shown to prevent MPTP-induced loss of striatal dopamine in animals (Ricaurte et al. 1985). Therefore, observed effects of piroheptine may be due to its dopamine-uptake inhibiting property.

However, recently MPTP was shown to have a cholinergic action on cultured adrenal chromaffin cells (Hotchkiss et al. 1984). Therefore, anticholinergic property may in part be responsible for the prevention of MPTP-induced loss of striatal dopamine. This question has to be elucidated by further studies. Piroheptine is a relatively non-toxic agent having been used in the treatment of Parkinson's disease. It appears to be important to investigate prospectively if piroheptine alters the natural course of Parkinson's disease or not. Also, further studies using primate models are very important.

**Summary.** Effect of piroheptine, an anticholinergic agent having a dopamine-uptake inhibiting property, on MPTP-induced striatal loss of dopamine was studied in C57BL/6J mice. Pre-treatment with piroheptine almost completely prevented loss of striatal dopamine in MPTP-injected mice. Piroheptine is another agent which prevents MPTP neurotoxicity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Dopamine</th>
<th>DOPAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10</td>
<td>9.2±0.3</td>
<td>1.0±0.5</td>
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<tr>
<td>MPTP</td>
<td>10</td>
<td>1.9±0.2a</td>
<td>0.5±0.1a</td>
</tr>
<tr>
<td>Piroheptine</td>
<td>10</td>
<td>10.2±1.2</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td>Piroheptine + MPTP</td>
<td>10</td>
<td>9.5±0.9a</td>
<td>0.8±0.2**</td>
</tr>
</tbody>
</table>

Expressed as microgram per gram of wet tissue (Mean±SD). *P<0.01 as compared with respective values of saline treatment. **P<0.01 as compared with respective values of MPTP treatment. See the text for the details of treatment schedules.
References