III. 3. Protective Effect of Riluzole on MPTP-Induced Depletion of Dopamine and Its Metabolite Content in Mice

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INTRODUCTION

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is well known to produce clinical, biochemical and neuropathological changes analogous to those observed in idiopathic Parkinson's disease\textsuperscript{1,2}. This neurotoxin also leads to a decrease of dopamine content in the striatum and loss in the number of nigrostriatal dopaminergic neurons in several species including monkeys, dogs, cats and mice. The neurotoxic effects of MPTP are thought to be initiated by MPP\textsuperscript{+} which is a major metabolite formed by the monoamine oxidase (MAO) B-mediated oxidation of MPTP. MPP\textsuperscript{+} is taken up by high-affinity dopamine and noradrenaline uptake systems and is subsequently accumulated within mitochondria of nigrostriatal dopaminergic cells\textsuperscript{3}. There it disrupts oxidative phosphorylation by inhibiting complex I of the electron transport chain\textsuperscript{4}. This can lead to a number of deleterious effects on cellular function, resulting in neuronal cell death. Therefore, the MPTP-treated mouse is widely used as a rodent model of Parkinson's disease.

Riluzole (2-amino-6-trifluoromethoxy benzothiazole) has been reported as an antagonist of excitatory amino acid neurotransmission\textsuperscript{5}. This compound stabilizes voltage-dependent Na\textsuperscript{+} channels in their inactivated state and inhibits the release of glutamate. A previous study demonstrated that riluzole did not prevent MPTP-induced dopamine depletion in the mouse striatum\textsuperscript{6}. In contrast, Boireau et al.\textsuperscript{7} reported that riluzole antagonized the MPTP-induced decrease in dopamine levels in mice. Thus, there is no consensus in the literature whether riluzole has neuroprotective activity in the brain of MPTP-treated mice. To make it clear whether riluzole is useful, we investigated a possible effect of riluzole.

MATERIALS and METHODS

Male C57BL/6J mice (22-28 g) were used in this study. The mice received intraperitoneal four injections of MPTP (10 mg/kg) at 1h intervals, the total dose per mouse
being 40 mg/kg, as described previously). The mice were sacrificed by cervical dislocation at 3 days after the last injection of MPTP for biochemical study as described below.

After decapitation, brains were quickly removed and the two striata were rapidly dissected out freehand on an ice-cold glass Petri dish. Samples were immediately weighed, then frozen and stored at -80°C until assay. The dissection procedure was performed in less than 2 min. Striata were sonicated in ice-cold 0.2M perchloric acid containing 100 ng/ml isoproterenol as internal standard. Homogenates were centrifuged at 3,000 rpm for 15 min at 4°C. The supernatant was filtered (pore size 0.45 μm, Millipore filter) and a 30-μl aliquot of the supernatant was used for determination of the dopamine, 3,4-dihydroxyphenyl acetic acid (DOPAC) and isoproterenol by high-performance liquid chromatography (HPLC) with an electrochemical detector (ECD) (Eicom, Japan). The mobile phase consisted of 0.1M sodium citrate-0.1M sodium acetate solution (pH 3.5) including 1.064 M octane sulfonic acid and 0.013 mM Na₂EDTA and 15% (v/v) methanol. The recoveries of dopamine, DOPAC and isoproterenol through the present procedures were > 93%. Levels of dopamine and its metabolite were calculated from the comparison of sample peak area with internal standard peak region and are expressed as μg/g tissue weight.

For the effect of riluzole, the animals were divided into 6 groups; (1) Vehicle (0.5 % carboxymethyl-cellulose, CMC)-treated group; (2) Riluzole (10 mg/kg)-treated group; (3) MPTP- and 0.5% CMC-treated group; (4) MPTP- and riluzole (3 mg/kg)-treated group; (5) MPTP-and riluzole(10 mg/kg)-treated group. The mice were injected intraperitoneally (i.p) with riluzole or 0.5% CMC 30 min before and 90 min after the first administration of MPTP (Groups 3, 4 and 5). For groups 1 and 2, 0.5% CMC-treated or riluzole (10 mg/kg)-treated animals were injected i.p. in the same manner with saline treatment instead of MPTP. Each group contained 5 animals. For additional study, the animals were divided into 2 groups; (6) MPTP- and 0.5% CMC-treated group; (7) MPTP- and riluzole (20mg/kg) -treated group. The mice were injected i.p. with riluzole or 0.5% CMC 30 min before and 90 min after the first administration of MPTP (Groups 6 and 7). Riluzole was generously provided by Rhone-Poulene-Rorer. Each group contained 5-7 animals.

The mice were killed by cervical dislocation at 3 days after the last MPTP treatment. As described above, striatal extracts were prepared for HPLC monoamine measurements.

All values were expressed as means±S.E. and statistical significance was evaluated using an analysis of variance (ANOVA) followed by Williams multiple range test.

RESULTS

Riluzole dose-dependently prevented a significant reduction in striatal dopamine and DOPAC levels of mice 3 days after MPTP treatments (Table 1). Furthermore, riluzole at a higher dose of 20 mg/kg prevented a significant reduction in striatal dopamine, DOPAC, and HVA levels of MPTP-treated mice (Table 2). In addition, rectal temperature showed no significant changes in riluzole (10 mg/kg)-treated mice, as compared to vehicle-treated
animals, although the temperature showed approximately 0.5°C decrease in mice 1 hr after riluzole injection.

DISCUSSION

In the present study, MPTP caused a significant reduction in dopamine and DOPAC levels from 3 days after MPTP treatments. In the present study, therefore, we evaluated the effects of riluzole on the striatal dopamine and DOPAC levels at 3 days after MPTP treatments.

The present study showed that riluzole antagonized the MPTP-induced decrease in dopamine and DOPAC levels in the striatum of mice in a dose-dependent manner. The present results demonstrate that riluzole can protect against MPTP-induced decrease in dopamine levels.

Riluzole is an inhibitor of glutamatergic transmission in the central nervous system. This drug is currently given to patients with amyotrophic lateral sclerosis (ALS) in an attempt to improve their prognosis, possibly via blockade of the glutamate neurotoxic effects9. Interestingly, a previous study suggested that riluzole can partially antagonize the increase in the release of dopamine induced by superfusion with MPP+, the active metabolite of MPTP10. Furthermore, they reported that riluzole can protect against MPTP-induced striatal dopamine depletion in mice either by blocking the entry of Na+ or by reducing the release of glutamate7. In contrast, Jones-Humble et al.6 demonstrated that riluzole had no significant effect on dopamine depletion in the striatum of mice. Therefore, the neuroprotective effect of riluzole against MPTP-induced striatal dopamine depletion in mice is still unclear. However, a recent interesting study reported that riluzole delayed the appearance of parkinsonian motor abnormalities in a chronic monkey model of MPTP toxicity, designed to resemble more closely Parkinson’s disease11. Furthermore, this drug was shown to alleviate the circling behavior in 6-hydroxydopamine-treated rats and to decrease the suppression of dopamine metabolism, at both striatal and nigral levels12. Both neuroprotective and palliative effects of riluzole have also been obtained in an acute model of MPTP intoxication in monkeys13. These observations are, at least in part, consistent with our present findings.

In the present study, of interest is that riluzole showed a significant effect on MPTP-induced striatal dopamine and DOPAC depletion in a dose-dependent manner. Interestingly, a previous study in mice indicated that voltage-dependent Na+ channel blockers can prevent MPTP-induced dopamine depletion in the striatum9. The blockade of Na+ entry is known to prevent the cascade of events that follows the neuronal depolarization after ATP (adenosine triphosphate) depletion. Furthermore, the neurotoxic effects of MPP+ increase the release of neurotransmitters, by a mechanism which was proposed to be Na+ dependent. Thus, inactivation of voltage-dependent Na+ channels by riluzole might be involved in any protective effects on MPTP-induced striatal dopamine and DOPAC depletion. However, we cannot
rule out the possibility that riluzole exerts its protective effect through a modulation of glutamate release.

In conclusion, our results show that riluzole can protect against MPTP-induced striatal dopamine and DOPAC depletion in mice. The protective effect may be caused by inactivation of voltage-dependent Na’ channels of riluzole. These findings demonstrate that riluzole with ability to block voltage-dependent Na’ channels may be useful in the treatment of neurodegenerative diseases.

REFERENCES


Table 1. Effects of riluzole on the striatal dopamine and its metabolites content in MPTP-treated mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dopamine (μg/g tissue)</th>
<th>DOPAC (μg/g tissue)</th>
<th>HVA (μg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (0.5% CMC)</td>
<td>13.44 ±0.75**</td>
<td>4.23 ±0.51**</td>
<td>2.83 ±0.24</td>
</tr>
<tr>
<td>Riluzole (10 mg/kg)</td>
<td>12.09 ±0.73**</td>
<td>4.92 ±0.28**</td>
<td>2.73 ±0.07</td>
</tr>
<tr>
<td>MPTP+0.5% CMC</td>
<td>3.81 ±0.13</td>
<td>2.01 ±0.31</td>
<td>2.46 ±0.36</td>
</tr>
<tr>
<td>MPTP+riluzole (3 mg/kg)</td>
<td>4.71 ±1.26</td>
<td>3.49 ±0.38**</td>
<td>2.73 ±0.23</td>
</tr>
<tr>
<td>MPTP+riluzole (10 mg/kg)</td>
<td>6.27 ±0.64*</td>
<td>4.27 ±0.17**</td>
<td>2.48 ±0.16</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE. *p<0.05, **p<0.01 compared with MPTP+0.5% CMC group (Williams multiple range test). n=5 mice. Drug treatment schedules were expressed in experimental design section.
Table 2. Effects of riluzole (20 mg/kg) on the striatal dopamine and its metabolites content in MPTP-treated mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dopamine (μg/g tissue)</th>
<th>DOPAC (μg/g tissue)</th>
<th>HVA (μg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (0.5% CMC)</td>
<td>13.44±0.75**</td>
<td>4.23±0.51**</td>
<td>2.83±0.24**</td>
</tr>
<tr>
<td>MPTP+0.5% CMC</td>
<td>2.70±0.37</td>
<td>0.93±0.06</td>
<td>1.38±0.16</td>
</tr>
<tr>
<td>MPTP+riluzole (20 mg/kg)</td>
<td>5.36±0.44**</td>
<td>1.39±0.08**</td>
<td>1.86±0.14**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE. **p<0.01 compared with MPTP+0.5% CMC group (Williams multiple range test). Drug treatment schedules were expressed in experimental design section. n = 5-7.