

Neuroprotective Effect of Riluzole Against MPTP Neurotoxicity in Mice

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INTRODUCTION

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes degeneration of mesencephalic dopaminergic neurons in several species including monkeys, dogs, cats and mice. The neurotoxic effects of MPTP are thought to be initiated by 1-methyl-4-phenylpyridinium ion (MPP⁺) which is a major metabolite formed by the monoamine oxidase B-mediated oxidation of MPTP. MPP⁺ is taken up by high-affinity dopamine and noradrenaline uptake systems and is subsequently accumulated within mitochondria of nigrostriatal dopaminergic cells^{1,2)}. This can lead to a number of deleterious effects on cellular function, resulting in neuronal cell death.

2-Amino-6-trifluoromethoxy benzothiazole (riluzole) has been reported to antagonize excitatory amino acid neurotransmission³⁾. This compound stabilizes voltage-dependent Na⁺ channels in their inactivated state and inhibits the release of glutamate. A previous study showed that riluzole can not prevent MPTP-induced dopamine depletion in the mouse striatum⁴⁾. In contrast, Boireau et al.⁵⁾ demonstrated that riluzole can antagonize the MPTP-induced decrease in dopamine levels in mice. Thus, there is no consensus in the literature whether riluzole has a neuroprotective effect in the brain of MPTP-treated mice. Furthermore, the mechanisms for the neuroprotection of riluzole against MPTP neurotoxicity are not fully understood. In the present experiments, therefore, we examined the effect of riluzole on MPTP-induced neurotoxicity, utilizing immunohistochemical markers.

MATERIALS AND METHODS

Male C57BL/6 mice (22-28 g) were used in this study. The mice were injected intraperitoneally (i.p.) with four administrations of MPTP (10 mg/kg) at 1-h intervals, the total dose per mice being 40 mg/kg, as described previously^{6,7}.

The animals were divided into 3 groups; (1) Vehicle (0.5% carboxymethyl-cellulose, CMC)-treated group; (2) MPTP- and 0.5% CMC-treated group; (3) MPTP- and riluzole (10 mg/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 2 and 3). The animals of group 1 were injected i.p. in the same manner with saline treatment instead of MPTP. Each group contained five animals. For the immunohistochemical study, the mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) at 3 and 7 days after MPTP treatment, and the brains were perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) following a heparinized saline flush. The brains were removed 1-h after perfusion-fixation at 4°C and were immersed in the same fixative until they were embedded in paraffin. Paraffin sections, 5 µm in thickness, were used for immunohistochemistry.

For tyrosine hydroxylase (TH) immunostaining, a polyclonal anti-TH antibody (Chemicon International, Temecula, USA) and a Vectastain elite ABC kit (Vector Lab., Burlingame, USA) were used. The immunohistochemical staining with anti-TH antibody (1:200) was performed as described previously⁶.

For microtubule-associated protein 2 (MAP 2) immunostaining, a monoclonal mouse anti-MAP 2a,b antibody (Chemicon International Inc., Temecula, USA) and a Vector M.O.M. Immunodetection Kit (Vector Lab., Burlingame, USA) were used. In brief, the paraffin sections were washed for 5 min in 0.01 M phosphate-buffered saline (PBS, pH 7.4) and treated with 3% hydrogen peroxidase in 0.01 M PBS. The paraffin sections were then washed two times for 2 min in 0.01 M PBS, followed by 60 min of pre-incubation with mouse Ig G blocking reagent. The brain sections were then incubated with anti-MAP 2a,b antibody (1:500) including M.O.M. diluent overnight at 4°C. After a 4 min rinse in 0.01 M PBS, the sections were incubated with biotinylated secondary Ig G antibody for 10 min and then with avidin-biotin peroxidase complex for 30 min at room temperature. Immunoreactions were visualized using 0.05% diaminobenzidine and 0.01% hydrogen peroxidase in 0.05% Tris-HCl buffer (pH 7.6) and then the sections were counterstained with hematoxylin. Negative control sections were treated in the same way as described

above except that the antibody against anti-MAP 2a,b was omitted.

For glial fibrillary acidic protein (GFAP) immunostaining, a polyclonal anti-GFAP antibody (Labsystems, Helsinki, Finland) and a Vectastain elite ABC kit (Vector Lab., Burlingame, USA) were used. The immunohistochemical staining with anti-GFAP antibody (1:200) was performed as described previously⁶.

RESULTS

Representative microphotographs of TH and MAP 2 immunostaining in the substantia nigra are shown in Fig. 1. Dopaminergic neurons with TH antibody or MAP 2 antibody were easily detectable in the substantia nigra of vehicle-treated mice. The bodies and fibers of dopaminergic cells were intensely stained with evident immunopositive processes. A decrease in the number of these cells was noticed in mice 3 and 7 days after MPTP treatment. In contrast, the administration of riluzole at a dose of 10 mg/kg prevented the decrease in the number of dopaminergic neurons 3 and 7 days after MPTP treatment.

Representative microphotographs of GFAP immunostaining in the striatum are shown in Fig. 2. GFAP-positive astrocytes were absent in the striatum of vehicle-treated mice. The levels of staining for GFAP-positive astrocytes were mildly increased in the striatum 3 days after MPTP treatment. Seven days after MPTP treatment, GFAP-positive astrocytes exhibited a ramified form with many fine processes in the striatum, which showed marked increases in this area. On the other hand, a marked increase in GFAP-positive astrocytes in the striatum was found in riluzole-treated mice 3 days after MPTP treatment. Seven days after MPTP treatment, however, a marked increase in GFAP-positive astrocytes in the striatum was not observed in riluzole-treated mice, as compared with vehicle-treated mice.

DISCUSSION

Our immunohistochemical study indicated that riluzole can protect against MPTP-induced neuronal damage in the substantia nigra.

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 effects⁸. Interestingly, a previous study suggested that riluzole can partially antagonize the increase in the release of striatal dopamine induced by superfusion with MPP⁺, the

active metabolite of MPTP⁹⁾. Furthermore, Boireau et al.⁵⁾ 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 glutamate. In contrast, Jones-Humble et al.⁴⁾ demonstrated that riluzole had no significant effect on dopamine depletion in the striatum of mice. We have no explanation for these discrepancies at present. However, these discrepancies might be the results of differences in dosing, timing, animal strain, animal age or experimental protocol including dose-response studies. Therefore, the neuroprotective effect of riluzole against MPTP-induced striatal dopamine depletion in mice is controversial. 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 disease¹⁰⁾. 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 levels¹¹⁾. Both neuroprotective and palliative effects of riluzole have also been obtained in an acute model of MPTP intoxication in monkeys¹²⁾. These observations are, at least in part, consistent with our present findings.

In the present study, it is of interest that riluzole in the present study markedly increased GFAP-positive astrocytes in the mouse striatum 3 days after MPTP treatment, as compared with vehicle-treated animals. The reason for this phenomenon is presently unclear. However, several studies reported that astrocytes can confer neuronal protection by synthesizing and releasing the free-radical scavenger glutathione and its precursors glutamate, cysteine and glycine^{13,14)}. A recent interesting study also indicated that GFAP-null mice were more sensitive to cerebral ischemia than wild-type mice and that astrocytes can provide a protective function after ischemia¹⁵⁾. These observations suggest that astrocytes may play an important role for the neuroprotective function in the brain. Therefore, it is conceivable that this protective effect of riluzole may be caused by the activation of astrocytes.

In conclusion, our results show that riluzole can protect against MPTP-induced neuronal damage. The protective effect may be, at least in part, caused by the activation of astrocytes. These results demonstrate that riluzole is effective against MPTP-induced neurodegeneration of the nigrostriatal dopaminergic neuronal pathway. Our findings also provide a rationale for the identification of astrocytes as a prominent target for the development of new therapies of Parkinson's disease.

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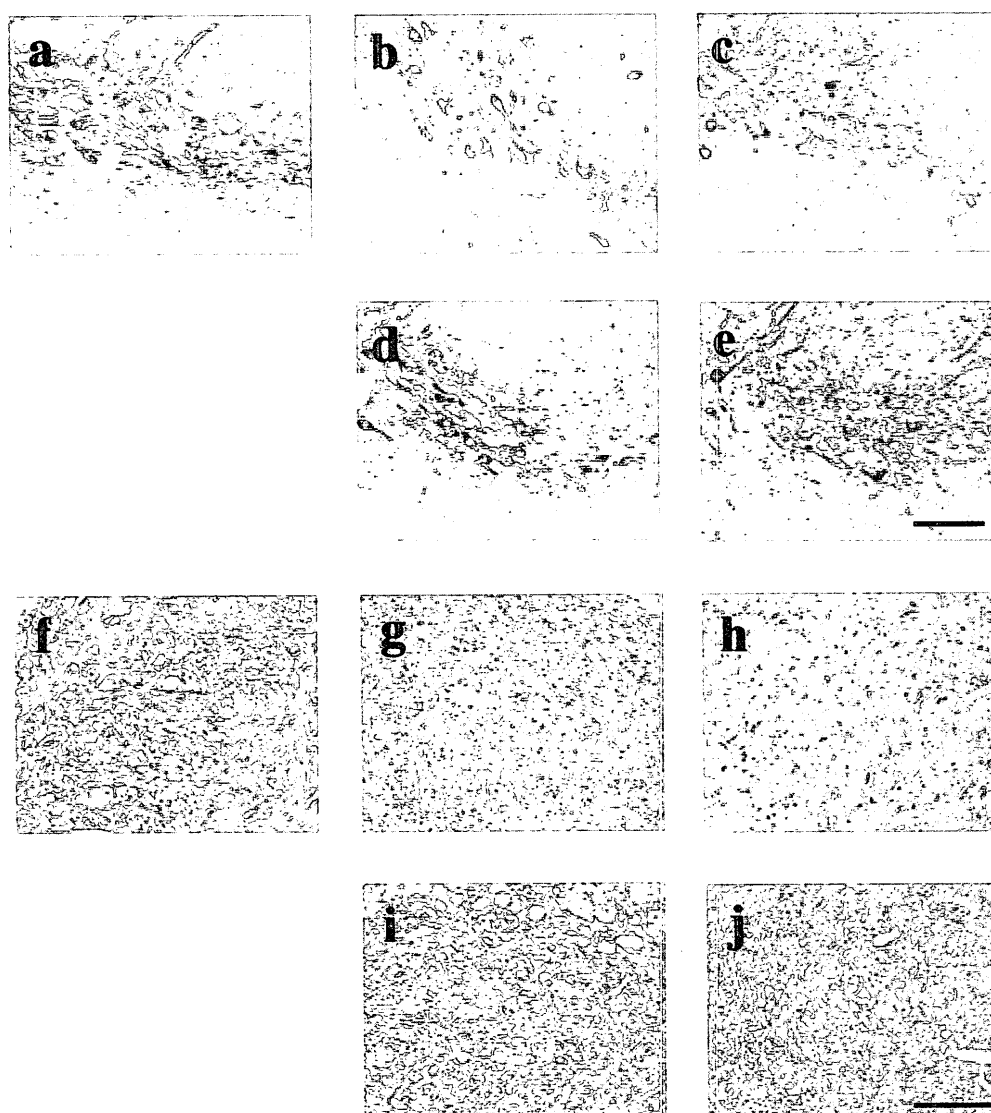


Fig. 1. Dopaminergic neurons stained with antibodies for TH or MAP 2 in the substantia nigra of vehicle-, MPTP- and MPTP + riluzole (10 mg/kg)-treated mice. TH staining (a-e). MAP 2 staining (f-j). (a,f) Vehicle (0.5% CMC)-treated mice. (b,g) Mice 3 days after MPTP treatment. (c,h) Mice 7 days after MPTP treatment. (d,i) Riluzole (10 mg/kg)-treated mice 3 days after MPTP treatment. (e,j) Riluzole (10 mg/kg)-treated mice 7 days after MPTP treatment. Bar (a-e and f-j) = 100 μ m. $n = 5$.

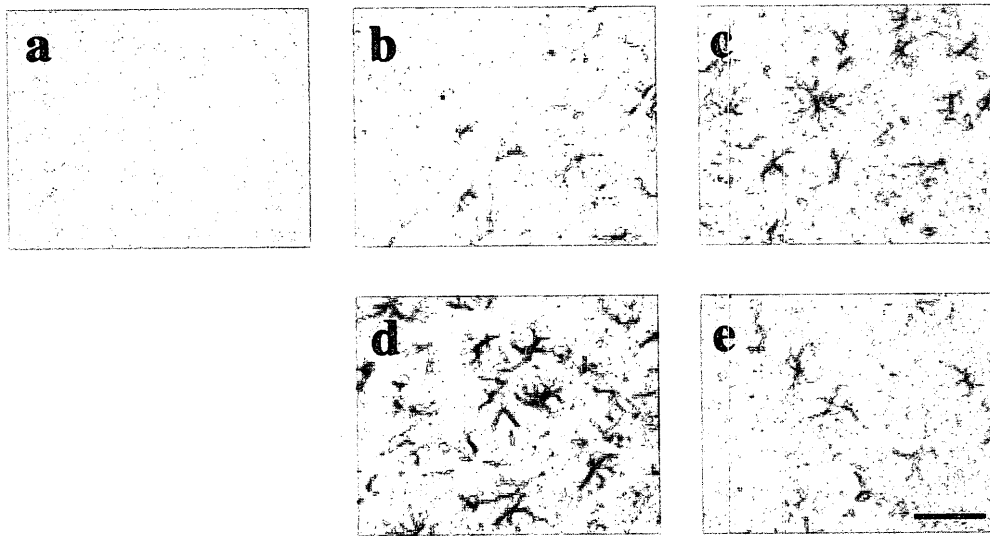


Fig. 2. Astrocytes stained with antibodies for GFAP in the striatum of vehicle-, MPTP- and MPTP + riluzole (10 mg/kg)-treated mice. (a) Vehicle (0.5% CMC)-treated mice. (b) Mice 3 days after MPTP treatment. (c) Mice 7 days after MPTP treatment. (d) Riluzole (10 mg/kg)-treated mice 3 days after MPTP treatment. (e) Riluzole (10 mg/kg)-treated mice 7 days after MPTP treatment. Bar = 50 μ m. $n = 5$.