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

T. Araki 1,4, T. Kumagai 2, M. Matsubara 1, T. Ido 2, Y. Imai 1 and Y. Itoyama 3

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The neuroprotective effects of riluzole (2-amino-6-trifluoromethoxy benzothiazole), a Na+ channel blocker with antinociceptive activity, MK-801, a blocker of N-methyl-D-aspartate (NMDA) receptors and monoamine oxidase (MAO) inhibitor pargyline were compared in the model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced depletion of dopamine and its metabolite 3, 4-dihydroxyphenylacetic acid (DOPAC) levels in mice. The mice received four intraperitoneal injections of MPTP (10 mg/kg) at 1-hr intervals and then the brains were analyzed at 1, 3 and 7 days after the treatments. Dopamine and DOPAC levels were significantly decreased in the striatum from 1 day after MPTP treatments. A severe depletion in dopamine and DOPAC levels was found in the striatum 3 and 7 days after MPTP treatments. Riluzole dose-dependently antagonized the MPTP-induced decrease in dopamine and DOPAC levels in the striatum. Pargyline also protected against MPTP-induced decrease in dopamine levels in the striatum. However, this drug showed no significant change in the striatal DOPAC levels. On the other hand, MK-801 failed to protect against MPTP-induced decrease in dopamine levels in the striatum. However, MK-801 reversed the MPTP-induced decrease in DOPAC levels. These results suggest that riluzole can protect against MPTP-induced striatal dopamine and DOPAC depletion in mice. This protective effect may be caused by inactivation of voltage-dependent Na+ channels by riluzole. Furthermore, the present study suggests that the activation of NMDA receptors does not mainly contribute to MPTP-induced neurodegeneration, whereas MAO, especially MAO type B (MAO-B) plays a crucial role in MPTP-induced degeneration of the nigrostriatal dopaminergic neuronal pathway.

Keywords: MPTP, riluzole, pargyline, MK-801, dopamine, mice

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. This neurotoxin also leads to a decrease of dopamine content in the striatum and loss in the number of nigrostriatal dopaminergic neurons in several

1Department of Clinical Pharmacology and Therapeutics, Tohoku University Graduate School of Pharmaceutical Science and Medicine, Sendai, Japan.
2Cyclotron and Radioisotope Center, Tohoku University, Sendai, Japan.
3Department of Neurology, Tohoku University School of Medicine, Sendai, Japan.
4To whom correspondence should be addressed at Department of Clinical Pharmacology and Therapeutics, Tohoku University Graduate School of Pharmaceutical Science and Medicine, Aoba-yama, Sendai 980-8578, Japan; Tel: +81-22-217-6807; Fax: +81-22-217-6807; E-mail: tsuaraki@mail.cc.tohoku.ac.jp
species including monkeys, dogs, cats and mice. The neurotoxic effects of MPTP are thought to be initiated by MPP⁺ which is a major metabolite formed by the monoamine oxidase (MAO) 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. Therefore, the MPTP-treated mouse is widely used as a rodent model of Parkinson's disease.

Riluzole has been reported as an antagonist of excitatory amino acid neurotransmission (Benavides et al., 1985). This compound, unlike the MK-801, does not bind to any glutamate receptor subtypes, but stabilizes voltage-dependent Na⁺ 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 (Jones-Humble et al., 1994). In contrast, Boireau et al., (1994a) 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. The aim of the present study, therefore, was to investigate, in the striatum of MPTP-treated mice, a possible effect of riluzole in comparison with the MAO inhibitor pargyline and NMDA receptor antagonist MK-801 (Dizocilpine).

**MATERIALS and METHODS**

Male C57BL/6 mice (22-28 g) were used in this study. The mice received intraperitoneal four injections of MPTP (10 mg/kg) at 1 h intervals, the total dose per mouse being 40 mg/kg, as described previously (Tanji et al., 1999). The mice were sacrificed by cervical dislocation at 1, 3 and 7 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-dihydroxyphenylacetic 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