Parkinsonism-Preventing Activity of 1-Methyl-1,2,3,4-tetrahydroisoquinoline Derivatives in C57BL Mouse in Vivo

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Received February 28, 2006; accepted April 17, 2006; published online April 21, 2006

Parkinson’s disease (PD) is a neurodegenerative disease characterized by degeneration of nigro-striatal dopaminergic neurons and reduction in the level of dopamine in this area. Since the discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could induce parkinsonism in humans,1) 1,2,3,4-tetrahydroisoquinoline (TIQ) derivatives have been considered as candidate endogenous causative factors of idioopathic Parkinson’s disease (PD), because of their structural similarity to MPTP. We have reported that tetrahydroisoquinoline derivatives (TIQ) are characterized by degeneration of nigro-striatal dopaminergic neurons and reduction in the level of dopamine in this area. While the content of TIQ derivatives tends to be increased in cerebrospinal fluid (CSF) of PD patients,2) 1MeTIQ is significantly decreased in the parkinsonian brain and MPTP-injected mouse brain.3,4) Bradykinesia, one of the symptoms of PD, was induced by injection of TIQ, 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ),4) and 1-(3’,4’-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (3’,4’DHBnTIQ),4) exist in the brain of mammals of several species. While the content of 1BnTIQ tends to be increased in cerebrospinal fluid (CSF) of PD patients,3) 1MeTIQ is significantly decreased in the parkinsonian brain and MPTP-injected mouse brain.5,6) Bradynkinesia, one of the symptoms of PD, was induced by injection of TIQ, 1BnTIQ, or 3’,4’DHBnTIQ in mice,5,6) so these TIQ derivatives are considered to be parkinsonism-inducing substances. MPTP and TIQ derivatives have inhibitory activities towards complex I of the mitochondrial respiratory chain and tyrosine hydroxylase.7,8)

We have synthesized derivatives of 1MeTIQ (Fig. 1) and evaluated their in vitro neurotoxicity and protective activity against toxicity due to salsolinol in SH-SY5Y human neuroblastoma cells. In the present study, we tested the parkinsonism-preventing potential of these derivatives by means of the pole test in C57BL mice in vivo, and measured brain dopamine contents by liquid chromatography-tandem mass spectrometry. Parkinsonism was induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydroisoquinoline (MPTP), and pretreatment with any of the 1MeTIQ derivatives prevented its induction. 6-Hydroxy-1MeTIQ showed the greatest protective activity. The amount of dopamine in the brain was reduced by MPTP treatment, and this reduction was suppressed by pretreatment with 1MeTIQ derivatives. These hydroxy-1MeTIQ derivatives may have potential for the treatment of Parkinson’s disease as well as 1MeTIQ itself.

Key words Parkinson’s disease; neuroprotection; tetrahydroisoquinoline derivative

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Fig. 1. Structures of 1,2,3,4-Tetrahydroisoquinoline (TIQ), 1-Benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ), 1-(3’,4’-Dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (3’,4’DHBnTIQ) and 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) Derivatives

Table 1. Structures of 1,2,3,4-Tetrahydroisoquinoline (TIQ), 1-Benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ), 1-(3’,4’-Dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (3’,4’DHBnTIQ) and 1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) Derivatives

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<tr>
<td>1MeTIQ</td>
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<td>7-hydroxy-1MeTIQ</td>
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Reagents 1MeTIQ, 5-hydroxy-1MeTIQ, 6-hydroxy-1MeTIQ and 7-hydroxy-1MeTIQ were synthesized according to the literature.5,12,13)

Treatment of the Reagents Twenty-four C57BL/6N male mice were divided into six groups (n=4) with the aid of a table of random numbers. Saline or a 1MeTIQ derivative...
80 mg/kg in saline was intraperitoneally injected twice daily for 5 d. After a 2-d interval, MPTP 30 mg/kg in saline was intraperitoneally injected once daily for 4 d. The pole test was performed 2 d after the final dosage.

**Pole Test** The pole test for bradykinasia was conducted by using a modification of the reported method. A mouse was positioned upward at the top of a rough-surfaced pole (8 mm diameter and 55 cm height), and $T_{\text{turn}}$ and $T_{\text{LA}}$ were measured. $T_{\text{turn}}$ is the time from the beginning of movement until the mouse turns completely downward, and $T_{\text{LA}}$ is the time until it arrives at the floor. The elongation of these parameters is considered to reflect bradykinasia. This test was performed five times successively for each mouse.

**Sample Preparation for Determination of Dopamine Contents** Whole brain was removed on the day after the pole test and homogenized with 2 ml of homogenizing solution (0.32 M sucrose, 400 μM EDTA-2K, 0.1% acetic acid), then 2.5 nmol of 6-methoxy-1MeTIQ was added as an internal standard. The homogenate was extracted with dichloromethane to remove lipids and hydrophobic compounds. The aqueous layer was filtered and the filtrate was subjected to liquid chromatography-tandem mass spectrometry (LC/MS/MS).

**LC/MS/MS Conditions** LC/MS/MS analysis was conducted with an API2000 (Applied Biosystems) triple-stage quadrupole-mass spectrometer coupled to an Agilent 1100 HPLC system (Agilent, CA, U.S.A.), and the electrospray ionization method was used for measurement. Reversed-phase Mightysil RP-18 GP (10 cm×4.6 mm I.D., 5 μm particle diameter, Kanto Chemicals, Tokyo, Japan) was used as the separation column, and the mobile phase was 15% acetonitrile and 0.1% acetic acid in water (flow rate 0.6 ml/min). Analytical conditions were automatically determined by Analyst® (application software for quantitative determination with the API2000). We tried to detect dopamine in brain by LC/MS/MS. Automatic optimisation indicated that the product ion $m/z$ 137 of $m/z$ 154 of dopamine was the most suitable ion for quantitative determination by multiple reactions monitoring (MRM). Similarly, the product ion $m/z$ 146 of $m/z$ 178 of 6-methoxy-1MeTIQ was selected for quantitative determination by MRM.

**RESULT AND DISCUSSION**

Parkinsonism was induced by MPTP, resulting in extension tendencies of $T_{\text{turn}}$ and $T_{\text{LA}}$, and pretreatment with any of the 1MeTIQ derivatives prevented these changes (Fig. 2). 1MeTIQ and 7-hydroxy-1MeTIQ shortened $T_{\text{turn}}$ (0.7—0.8 s) and $T_{\text{LA}}$ (2.0—2.3 s) compared with MPTP treated mice, but these were not significant. 5-Hydroxy-1MeTIQ and 6-hydroxy-1MeTIQ shortened $T_{\text{turn}}$ and $T_{\text{LA}}$ to the control level. 6-Hydroxy-1MeTIQ showed the greatest preventive activity, followed by 5-hydroxy-1MeTIQ, 7-hydroxy-1MeTIQ and 1MeTIQ in that order, at equal concentration. We have reported that the administration of 1MeTIQ did not affect the $T_{\text{turn}}$ and $T_{\text{LA}}$ values in C57BL mice. In *in vitro* assay, hydroxy-1MeTIQs were less toxic than 1MeTIQ; the LD_{50} value in SH-SY5Y cells was 3.59 mM. The introduction of a hydroxyl group into 1MeTIQ is likely to reduce permeation into the brain. Nevertheless, hydroxy-1MeTIQ derivatives have stronger parkinsonism-preventing activity than 1MeTIQ upon peripheral administration. This suggests that hydroxy-1MeTIQ derivatives work at lower concentration in the brain, compared with 1MeTIQ. The most effective compound for *in vitro* neuroprotection was 7-hydroxy-1MeTIQ. In this study, its activity was almost the same as that of 1MeTIQ, and it was less effective than 5- and 6-hydroxy-1MeTIQ. A possible explanation of the difference is that we used different toxic compounds, salsolinol and MPTP, in the two experiments. These two compounds both generate reactive oxygen species (ROS) as mediators of toxicity, and both inhibit α-ketoglutarate dehydrogenase, but MPTP inhibits complex I of the mitochondrial respiratory chain, while salsolinol inhibits complex II.

The amount of dopamine in the brain was reduced to 45% by MPTP treatment. Smaller reductions to 62—90% were seen after pretreatment with 1MeTIQ derivatives (Fig. 3). 6-Hydroxy-1MeTIQ completely suppressed the decrease of dopamine by MPTP. Such a recovery of dopamine content is considered to be an index of protective effects.

We have reported that 1MeTIQ has a neuroprotective effect *in vitro* against five dopaminergic neurotoxins, MPP^+, 6-hydroxydopamine, rotenone, salsolinol and 1BnTIQ, in SH-SY5Y cells and in cultured rat mesencephalic neurons. Other authors have used tyrosine hydroxylase immunohistochemistry to establish a potential protective effect of 1MeTIQ *in vivo* by measuring the effect of 1MeTIQ on dopaminergic neuronal death induced by MPP^+. TIQ, or...
rotenone.\textsuperscript{9,22,23} We have reported parkinsonism-preventing substance having a TIQ moiety was only 1MeTIQ, but that three novel compounds were found in its derivatives. The present report is the first to show that 1MeTIQ and its derivatives reverse the decrease of dopamine induced by MPTP. These compounds may exist in the brain as hydroxylated metabolites of 1MeTIQ biosynthesized from β-phenethylamine and pyruvate.\textsuperscript{10} 7-Hydroxy-1MeTIQ might be biosynthesized from tyrosine. We consider that these hydroxy-1MeTIQ derivatives may have potential for the treatment of Parkinson’s disease.

REFERENCES