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The COMT inhibitor tolcapone potentiates the anticataleptic effect of Madopar in MPP$^+$-lesioned mice

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Abstract. Orally administered Madopar (levodopa/benserazide 4:1) dose-dependently antagonized haloperidol-induced (1 mg/kg s.c.) catalepsy in MPP$^+$-lesioned mice. Pretreatment with a new selective catechol-O-methyltransferase (COMT) inhibitor, tolcapone (30 mg/kg p.o.), slightly potentiated the antagonistic effect of Madopar (15 mg/kg p.o.) on haloperidol-induced catalepsy. The ability of tolcapone to increase the Madopar effect was significantly attenuated by high doses of 3-O-methyldopa (3-OMD) (800 mg/kg i.p.). This might suggest a competitive blockade of the active transport of levodopa through the blood-brain barrier. In conclusion, the inhibitory effect of tolcapone on the O-methylation of levodopa to 3-OMD by COMT is largely due to improved levodopa and dopamine availability in the brain, and to the reduced formation of 3-OMD.
Key words. Catalepsy; Madopar; tolcapone; 3-O-methyldopa.

Preclinical and clinical reports have suggested that in Parkinson's disease (PD) patients, high plasma concentrations of 3-OMD are associated with the occurrence of on-off phenomenon or dyskinesias during treatment with levodopa in combination with benserazide (Madopar)[1–3]. It is well known that 3-OMD competitively inhibits the transport of levodopa, since both amino acids are transported into the brain by the same saturable carrier system located within the blood-brain barrier. The synaptosomal uptake of levodopa in vitro[3–5] involves the same transport system, suggesting that 3-OMD impedes the penetration of levodopa in both cases[3,6,7]. Our recent study[8] provided further evidence that the decrease of 3-OMD formation may be important in the treatment of PD with Madopar, since attenuation of the Madopar effect by 3-OMD was statistically significant.

Even though levodopa may be metabolized along several pathways, it is known that COMT plays a major role in the O-methylation of levodopa to 3-OMD, and dopamine (DA) to 3-methoxytyramine. The conversion of levodopa to 3-OMD by catechol-O-methyltransferase (COMT, EC 2.1.1.6) constitutes the main metabolic pathway when the aromatic amino acid decarboxylase (AADC, EC 2.1.1.6) is blocked by peripheral AADC inhibitors, e.g. benserazide[5,6]. In this paper we investigate whether tolcapone, a reversible and competitive COMT inhibitor, potentiates the antagonistic effect of Madopar on haloperidol-induced catalepsy, and whether this effect is attenuated by the administration of 3-OMD. These studies used the mouse MPP$^+$ model to mimic the parkinsonian state.

Materials and methods

Animals. Male C57BL/6 mice (20–25 g) were used. They were housed in groups of 7–8 animals per cage in a room with controlled temperature of 22 ± 2 °C and relative humidity of 50–60% and with lighting from 07.30 to 19.30 h.

Intracerebroventricular injection of MPP$^+$. All the test animals were first given MPP$^+$ (30 µg/mouse) intracerebroventricularly. The i.c.v. injection was performed manually with a microsyringe, aimed at the lateral ventricle of the animals' brain, using a procedure similar to that described by Clark et al.[9] or Akaike and Himori[10]; the injection site was 2 mm lateral from the midsagittal line and 2 mm posterior from an imaginary midsagittal line drawn through the anterior base of the ears, and the volume injected was 10 µl/mouse over approximately 10 s. Three weeks after the MPP$^+$ injection, these animals were randomly tested with the following test paradigms.

Cataleptic behavior. Cataleptic behavior was determined according to the method of Himori et al.[8]. Mice were allowed to grasp a horizontal bar (2 mm in diameter) positioned 4 cm above the floor, with their forelimbs. Those mice that hung by their forelimbs from the horizontal bar for at least 30 s after placement were scored as showing cataleptic behavior. Madopar (p.o.) or 3-OMD (i.p.) were administered 1 h after the administration of s.c. haloperidol (1 mg/kg). Tolcapone was given p.o. 0.5 h after the administration of haloperidol. The incidence of cataleptic behavior was measured every 0.5 h up to 6 h after the administration of haloperidol. No cataleptic behavior was observed in the absence of haloperidol.

Drugs. Tolcapone (3,4-dihydroxy-4'-methyl-5-nitrobenzophenone, F. Hoffmann-La Roche Inc., Basle, Switzerland), 3-O-methyldopa (3-OMD, Sigma Chemical Co., St. Louis, Missouri, USA), levodopa (Sigma Chemical Co.), and benserazide hydrochloride (F. Hoffmann-La Roche Inc.) were suspended in 0.25%

CMC containing Tween 80, and the volume of solution injected was 10 ml/kg. Haloperidol (serenace® [injectable], Dainippon Pharmaceuticals Inc., Osaka, Japan) and MPP^+ iodide (1-methyl-4-phenylpyridinium iodide, Research Biochemicals Inc., Natick, Massachusetts, USA) were dissolved in 0.9% NaCl solution.

Statistical analysis. The values are expressed throughout the text as the percentage incidence of cataleptic behavioral symptoms. Statistical analysis of data was performed by Fisher's exact probability test.

Results

Antagonistic effects of Madopar on haloperidol-induced cataleptic behavior. Orally administered Madopar significantly antagonized haloperidol-induced catalepsy. The antagonism by Madopar (levodopa:benserazide 4:1) was reproducible and dose-dependent over the dose range employed in our experiments (6.25, 25, and 100 mg/kg). The antagonistic effect caused by 25 mg/kg Madopar lasted for approximately 3 h, but at a dose of 100 mg/kg effects lasted 3.5 h or longer (fig. 1). At 6.25 mg/kg Madopar showed only a transient antagonism of haloperidol-induced catalepsy. Based on these experiments, 15 mg/kg Madopar was selected as an appropriate threshold dose for determining tolcapone effects on haloperidol-induced catalepsy.

Effects of tolcapone alone on haloperidol-induced catalepsy. Tolcapone (30 mg/kg p.o.) neither antagonized nor potentiated haloperidol-induced catalepsy (data not shown).

Tolcapone potentiates the anticataleptic effect of Madopar on haloperidol-induced catalepsy. Madopar, at the threshold dose of 15 mg/kg p.o., slightly attenuated haloperidol-induced catalepsy. This slight antagonism became more consistent and statistically significant in the presence of the COMT inhibitor tolcapone (30 mg/kg p.o.) (table).

Tolcapone-induced increase of the Madopar effect is reversed by 3-OMD. Tolcapone (30 mg/kg p.o.)-induced increases of the anticataleptic effects of Madopar (15 mg/kg p.o.) on haloperidol-induced catalepsy was reversed by 3-OMD. This reversal became significant only at very high doses of 3-OMD (800 mg/kg i.p.) (fig. 2). No statistically significant reversal was observed at a lower dose of 3-OMD (400 mg/kg) (data not shown).

Discussion

All the studies described here were carried out using the mouse MPP^+ model of parkinsonism, where many of

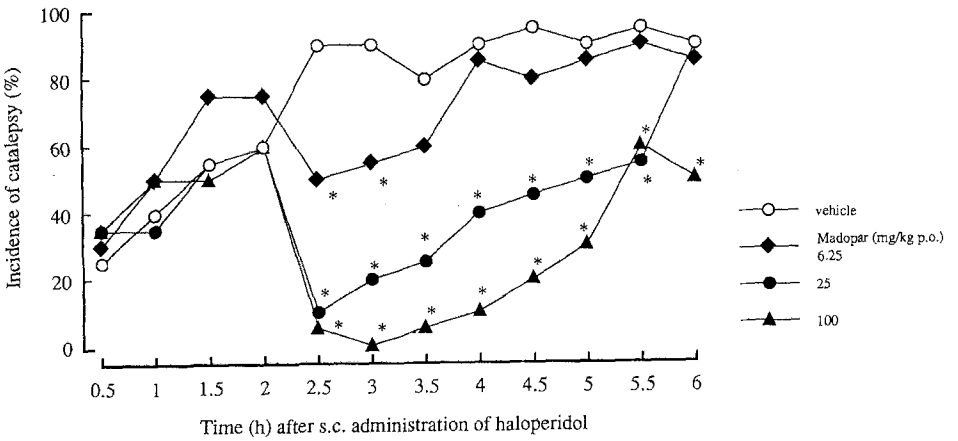

Figure 1. Inhibitory actions of Madopar on haloperidol-induced cataleptic behavior in MPP^+-treated mice. Test animals were given haloperidol (1 mg/kg s.c.) 1 h before p.o. administration of Madopar at time 1 h, and measurement of their cataleptic behavior started 0.5 h after s.c. administration of haloperidol. The number of animals for each group was 20. * $p < 0.05$ vs. their respective vehicle-treated groups.

Table. Augmentation by tolcapone of the Madopar effect on cataleptic behavior in response to haloperidol.

Treatment	No. of animals	Incidence of catalepsy (%)				
		Time after Madopar administration, h				
		0.5	1.5	2.5	3.5	4.5
Madopar alone	40	17.5	57.5	60.0	62.5	67.5
Madopar plus tolcapone	40	22.5	37.5	20.0**	32.5*	45.0*

Madopar (15 mg/kg) was orally administered to MPP^+-treated mice 1 h after s.c. administration of haloperidol (1 mg/kg). Tolcapone (30 mg/kg) was orally administered 0.5 h prior to p.o. Madopar. The incidence of catalepsy in mice given haloperidol (1 mg/kg s.c.) was approximately 80–90% during the period of 1.5–4.5 h after dosing. * $p < 0.01$, ** $p < 0.001$ vs. Madopar alone.

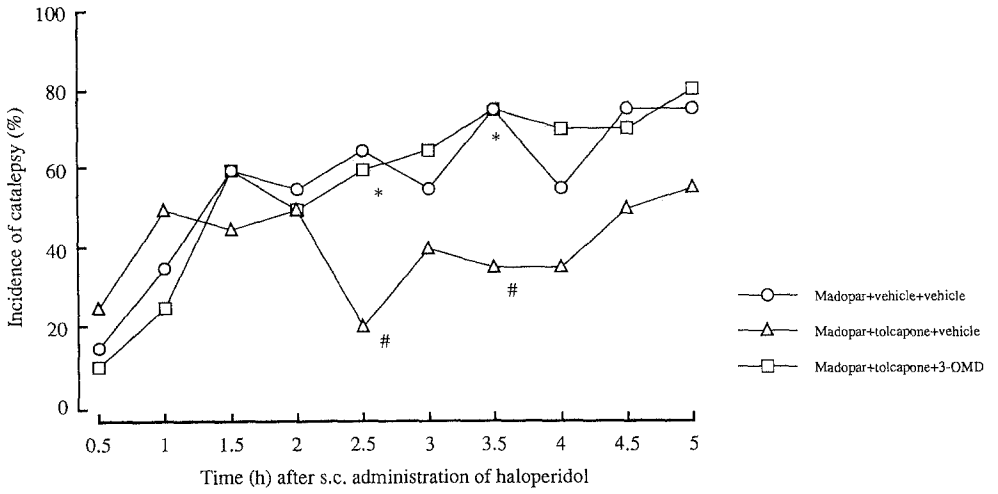

Figure 2. Reversal by 3-OMD of tolcapone-induced augmentation of the Madopar effect on haloperidol-induced cataleptic behavior. Madopar (15 mg/kg, p.o.) or 3-OMD (800 mg/kg, i.p.) was administered to MPP^+-treated animals 1 h after administration of s.c. haloperidol. Tolcapone (30 mg/kg) was given p.o. at time 0.5 h. The number of test animals for each group was 20. * $p < 0.05$ vs. their respective tolcapone and Madopar-treated groups (open triangles). # $p < 0.05$ vs. their respective vehicle and Madopar-treated groups (open circles).

the biochemical and pathological deficits associated with the human disorder are seen. Under conditions resembling parkinsonism, we tried to analyze the potentiation by tolcapone of the Madopar effect (anti-catalepsy), in order to see whether 3-OMD interference with transport of levodopa into the brain affects the behaviour of MPP^+-lesioned mice. Dose-response curves for madopar in normal mice (50–400 mg/kg p.o.)[8] were shifted to the left in the MPP^+-lesioned mice (6.25–100 mg/kg p.o.), suggesting behavioral supersensitivity. However, it seems unlikely that the doses required for the potentiation by tolcapone of the Madopar effect become less and the reversal by 3-OMD of the Madopar effect is augmented in MPP^+-lesioned mice.

The novel nitrocatechol derivative tolcapone has been well characterized at both the pharmacological and biochemical levels by Da Prada and his colleagues[11–13] and Maj et al.[14]. Tolcapone is efficiently absorbed from the gastrointestinal tract and apparently penetrates the blood-brain barrier. It elicits reversible and competitive COMT inhibition both in the periphery and in the central nervous system. This leads to increased levodopa availability, since the 3-O-methylation of levodopa to 3-OMD is blocked. Present and previous results suggest that the combination of Madopar with a COMT inhibitor should have beneficial effects in therapy for PD. Moreover, the results in the table show that tolcapone was able to potentiate the antagonistic effects of Madopar (levodopa plus benserazide) in MPP^+-lesioned mice. The coadministration of Madopar and tolcapone might allow lower doses of Madopar to be used in the treatment of Parkinsonian patients.

The present results are consistent with those of Maj et al.[14] where it was demonstrated using several different behavioral paradigms that tolcapone potentiated the effect of levodopa plus benserazide. This potentiation is probably primarily due to an increase in DA levels in the brain. Since this potentiation was found at low doses of Madopar (15 mg/kg i.p.), only minor interference of 3-OMD with transport of levodopa into the brain can be expected under these conditions. In fact, exogenously administered 3-OMD markedly reversed the potentiation by tolcapone only at very high doses (800 mg/kg i.p.) (fig. 2). The tolcapone-mediated potentiation of levodopa effects in our model is probably due to increased bioavailability of levodopa in the brain, and a corresponding increase in production of the neurotransmitter dopamine in the central nervous system.

By blocking COMT, tolcapone produces both a levodopa and S-adenosyl-L-methionine (SAM)-sparing effect, which could be of paramount importance in the treatment of PD-associated depression or dementia[13,15–17]. It remains to be determined whether tolcapone-mediated modulation of levodopa and SAM utilization in the brain also cause other changes, such as increased membrane fluidity[18,19], positive modulation of cholinergic, serotonergic, and catecholaminergic neurons[20–22], and inhibition of phospholipase A_2 activity possibly related to changes in levels of cAMP[23].

In conclusion, the peripherally and centrally active COMT inhibitor tolcapone, when given in combination with levodopa/AADC inhibitor formulations, e.g. Madopar, may enable therapeutic strategies for PD treatment to be improved by allowing levodopa doses to be reduced or dosing intervals to be lengthened, resulting in a lower incidence of on-off phenomena and/or dyskinesia.

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