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The Disposition of the Tolcapone 3-*O*-Methylated Metabolite is Affected by the Route of Administration in Rats

TOMOO FUNAKI, HIDEKO ONODERA, NAOMI USHIYAMA, YUKO TSUKAMOTO, CHIEKO TAGAMI, HIDEO FUKAZAWA AND ISAMI KURUMA

Nippon Roche Research Center, 200 Kajiwara, Kamakura-shi, Kanagawa 247, Japan

Abstract—Catechol-*O*-methyltransferase (COMT) catalyses the transfer of the methyl group from *S*-adenyl-L-methionine (SAM) to one of the hydroxy groups of a catechol, usually the hydroxy group in position 3. COMT is present mainly in a soluble form (S-COMT) in the cytosol, but a small fraction is bound to cell membranes (MB-COMT). MB-COMT has higher affinity for the catechol substrate than does S-COMT by a factor of > 10, and high MB-COMT activity is observed in the intestinal muscle layer. The present study investigates the effect of the administration route on the disposition of the tolcapone 3-*O*-methylated metabolite following intravenous and oral tolcapone administration in rats. Tolcapone is a substrate for COMT although the 3-*O*-methylated metabolite produced has no pharmacological actions. The 3-*O*-methylated metabolite was eliminated very slowly following oral administration of tolcapone, and its concentration approached a plateau level, which was in contrast to the situation following intravenous administration of tolcapone. It is thought that the oral dose of tolcapone receives a high exposure to MB-COMT in the intestinal muscle layer during its absorption, and tolcapone seems to form a complex with MB-COMT having a high affinity constant (i.e. a very low K_i). The fraction of the intravenous dose of tolcapone metabolized to the 3-*O*-methylated metabolite at 10 mg kg^{-1} was 2·6%, whereas those of the oral doses, which were corrected by the bioavailability, were 5·4% for 20 mg kg^{-1} and 2·7% for 40 mg kg^{-1}.

The current treatment of choice for Parkinson's disease combines L-dopa with a peripheral decarboxylase inhibitor, such as benserazide (Madopar) or carbidopa (Sinemet) (Da Prada et al 1987). When the decarboxylation route of L-dopa is blocked, however, the drug is effectively metabolized by catechol-*O*-methyltransferase (COMT) to form high concentrations of 3-*O*-methyldopa, a biologically inactive compound (Melega et al 1990; Rose et al 1991). COMT catalyses the transfer of the methyl group from *S*-adenyl-L-methionine (SAM) to one of the hydroxy groups of a catechol, usually the hydroxy group in position 3. This form is mainly present in a soluble form (S-COMT) in the cytosol and only a small fraction is bound to cell membranes (MB-COMT) (Kopin 1985). However, the binding affinity of MB-COMT for the catechol substrate is more than 10 times higher than that of S-COMT (Malherbe et al 1992); and high MB-COMT activity is observed in the intestinal muscle layer (Nissinen et al 1988).

The combination of Madopar or Sinemet with COMT inhibitors improves the relative bioavailability of L-dopa by reducing the conversion of L-dopa to 3-*O*-methyldopa (Kaakkola et al 1990; Zürcher et al 1990). Tolcapone (Ro 40-7592; 3, 4-dihydroxy-4′-methyl-5-nitrobenzophenone) is a new selective inhibitor of COMT (Zürcher et al 1990) and is presently in clinical trials for Parkinson's disease. In-vitro studies showed that tolcapone inhibited COMT activity more effectively in the duodenum than in the liver (Zürcher et al 1990). Taking into account the difference in catechol substrate affinity between S-COMT and MB-COMT, it can be anticipated that the route of tolcapone administration will affect the disposition of its 3-*O*-methylated metabolite, because tolcapone is also a substrate for COMT. This study aims to clarify the effect of administration route on the disposition of the 3-*O*-methylated metabolite following intravenous and oral administration of tolcapone in rats, although the 3-*O*-methylated metabolite has no pharmacological effect.

Correspondence: T. Funaki, Nippon Roche Research Center, 200 Kajiwara, Kamakura-shi, Kanagawa 247, Japan.

Materials and Methods

Materials

Tolcapone, its 3-*O*-methylated metabolite (Ro 40-7591; 3-*O*-methyl-4-hydroxy-4′-methyl-5-nitrobenzophenone) and Ro 40-6031 (3,4-dihydroxy-4′-chloro-5-nitrobenzophenone) were synthesized by Hoffman-La Roche (Basle, Switzerland). All other chemicals were reagent grade.

Animal experiments

Male Sprague-Dawley rats, 195–215 g (7 weeks of age), were placed in four groups of four animals each. Each group received either 20 or 40 mg kg^{-1} tolcapone orally, 10 mg kg^{-1} tolcapone intravenously, or 10 mg kg^{-1} 3-*O*-methylated metabolite (Ro 40-7591) intravenously. The rats were fasted overnight but were allowed free access to water. Food was given 4 h after administration of the drug. For the intravenous administration, tolcapone was dissolved in 60% macrogol 200 in normal saline and the 3-*O*-methylated metabolite in a mixture of 20% dimethylsulphoxide and 60% macrogol 200 in 0·9% saline. Those solutions were administered by bolus injection at a volume of 1 mL kg^{-1} into the penis vein under light ether anaesthesia. For the oral administration, a suspension of tolcapone was made using a vehicle comprising 0·5% carmellose sodium salt, 0·4%

polyoxyethylene (20) sorbitan monooleate and 0·5% benzyl alcohol in 0.9% saline. The suspension was administered via a stomach tube at a volume of 5 mL kg^{-1}. Each rat was held in a separate metabolic cage after the above administrations and blood samples (100 μL) were taken during the ensuing 6–8 h with a heparinized micropipette from an incision made in the tail, thus allowing the rats to move freely during the experiment.

Analytical method

The concentrations of tolcapone and the 3-*O*-methylated metabolite in blood were determined by reversed-phase HPLC. One hundred microlitre of blood was mixed with 0·1 mL saline and 0·1 μg internal standard (Ro 40-6031, 0·167 μg mL^{-1} in acetonitrile). The mixture was allowed to stand for 2 h at 4°C. After centrifugation (6000 rev min^{-1}, 5 min), 0·6 mL organic layer was evaporated under a gentle stream of nitrogen. The residue was dissolved in 100 μL eluent. After filtration by passage through a 0·45 μm pore-size membrane filter, 50 μL was injected into the HPLC.

The HPLC system consisted of an LC-6A pump (Shimadzu Co. Ltd, Kyoto, Japan), a KSST-601 autoinjector (Kyowa Seimitsu Co. Ltd, Tokyo, Japan), a Jasco 875-UV spectrophotometric detector (Japan Spectroscopic Co. Ltd, Tokyo, Japan) operated at 330 nm, and YMC ODS AM-314 column (300 × 6 mm i.d., particle size 5 μm, YMC Co. Ltd, Kyoto, Japan). The mobile phase was prepared by dissolving 2·3 g *N*-hexylmethylamine in 1300 mL acetonitrile, to which was then added 700 mL 0·05 M sodium dihydrogen phosphate (pH 2·2). The flow rate of the mobile phase was 1·0 mL min^{-1}. The limit of detection for tolcapone and the 3-*O*-methylated metabolite was 50 ng mL^{-1} each.

Data analysis

The terminal elimination rate constant (k) was assessed by applying logarithmic regression analysis to the terminal part of the concentration–time profile. The half-life ($t_{\frac{1}{2}}$) was calculated as $t_{\frac{1}{2}} = 0{\cdot}693/k$. The area under the blood concentration (AUC) and the first moment (AUMC) time curves were calculated using the trapezoidal method and extrapolated to infinity. The systemic blood clearance (CL) was calculated as CL = D/AUC, in which D is the dose. The mean residence time (MRT) was calculated as MRT = AUMC/AUC. The mean residence time of the metabolite following administration of the parent drug was calculated as $MRT_m = AUMC_m/AUC_m - MRT$ (Veng-Pedersen & Gillespie 1987), in which the subscript m represents metabolite. The volume of distribution at steady-state (Vd_{ss}) was calculated as $Vd_{ss} = CL \cdot MRT$. The bioavailability (F) was calculated as $F = AUC_{po}/AUC_{iv} \cdot D_{iv}/D_{po}$, in which the subscript represents the administration route. The fraction of dose metabolized to the 3-*O*-methylated metabolite (f_m) was calculated as $f_m = (AUC_m)_p/(AUC_m)_m \cdot D_m/D_p$ (Lane & Levy 1980), in which the subscript p represents parent drug.

Results

The blood concentration–time curves following intravenous and oral administration of tolcapone and intravenous administration of the 3-*O*-methylated metabolite are shown in Fig. 1. Following the oral administration of tolcapone, the

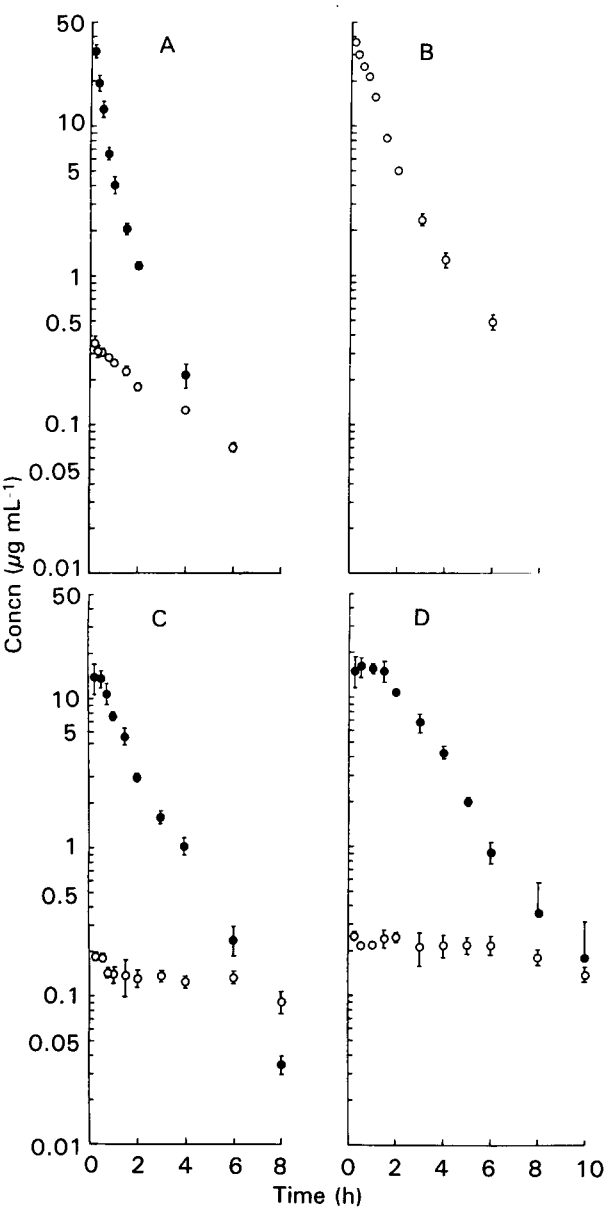

FIG. 1. The blood concentration–time curves following intravenous tolcapone administration at the dose of 10 mg kg^{-1} (A) and the 3-*O*-methylated metabolite at the dose of 10 mg kg^{-1} (B), and following oral tolcapone administration at doses of 20 mg kg^{-1} (C) and 40 mg kg^{-1} (D). The closed and open circles represent the concentrations of tolcapone and the 3-*O*-methylated metabolite, respectively. Data show the mean ± s.e.m.

3-*O*-methylated metabolite was eliminated very slowly, and its concentration approached a plateau, in contrast to that following intravenous administration of the 3-*O*-methylated metabolite itself. The 3-*O*-methylated metabolite elimination following intravenous administration of tolcapone was also not so slow in contrast with that for orally administered tolcapone; thus an effect of route of administration on the disposition of the 3-*O*-methylated metabolite was observed. The pharmacokinetic parameters following intravenous and oral tolcapone administration and those following intravenous administration of the 3-*O*-methylated metabolite are shown in Table 1. The $t_{\frac{1}{2}}$, CL and MRT following oral tolcapone administration were statistically different between

Table 1. Pharmacokinetic parameters following oral and intravenous administration of tolcapone and intravenous administration of its 3-*O*-methylated metabolite. Values are expressed as mean ± s.e.m.

Compound administered	Dose (mg kg^{-1})	Route	Compound measured	Pharmacokinetic parameter						
				C_{max} (μg mL^{-1})	T_{max} (h)	$t_{\frac{1}{2}}$ (h)	AUC (μg h mL^{-1})	MRT (h)	CL (mL min^{-1} kg^{-1})	Vd_{ss} (L kg^{-1})
Tolcapone	10	i.v.	Tolcapone	—	—	0·861 ±0·092	21·8 ±1·8	0·610 ±0·037	7·84 ±0·63	0·291 ±0·036
			3-*O*-methylated metabolite	0·37 ±0·03	0·31 ±0·15	2·15 ±0·10	1·26 ±0·05	3·11* ±0·13		
3-*O*-Methylated metabolite	10	i.v.	3-*O*-Methylated metabolite	—	—	1·34 ±0·06	45·1 ±1·4	1·20 ±0·04	3·71 ±0·11	0·267 ±0·009
Tolcapone	20	p.o.	Tolcapone	15·9 ±2·5	0·38 ±0·06	0·812 ±0·029	21·1 ±1·2	1·44 ±0·03	16·0 ±1·0	
			3-*O*-Methylated metabolite	0·19 ±0·01	0·44 ±0·06	9·81 ±1·88	2·50 ±0·51	13·0* ±3·0		
Tolcapone	40	p.o.	Tolcapone	20·2 ±1·6	0·63 ±0·26	1·31 ±0·31	49·0 ±1·4	2·24 ±0·18	13·7 ±0·4	
			3-*O*-Methylated metabolite	0·28 ±0·03	1·56 ±0·86	8·85 ±2·86	3·79 ±0·57	11·5* ±4·3		

*The value was calculated as $MRT_m = AUMC_m/AUC_m - MRT$.

the two oral doses ($P < 0·05$). However, there was no statistically significant difference between those oral doses for $t_{\frac{1}{2}}$ or MRT of the 3-*O*-methylated metabolite. In contrast, there were statistically different results between the different routes of tolcapone administration ($P < 0·05$). The fraction of an intravenous tolcapone dose metabolized to the 3-*O*-methylated metabolite was 2·6% at 10 mg kg^{-1}, whereas those fractions for the oral doses were 2·6% for 20 mg kg^{-1} and 1·5% for 40 mg kg^{-1}. The bioavailability of tolcapone was 0·48 for 20 mg kg^{-1} and 0·56 for 40 mg kg^{-1}. When corrected for bioavailability, the fractions of the oral tolcapone doses metabolized to the 3-*O*-methylated metabolite were 5·4% for 20 mg kg^{-1} and 2·7% for 40 mg kg^{-1}.

Discussion

Although a slight dose-dependency was observed in the present study, the data were considered to correspond to linear pharmacokinetics for simplicity, and this does not obstruct our purpose. As we expected, the effect of administration route on the disposition of the 3-*O*-methylated metabolite, i.e. the difference in the blood concentration–time curves of the 3-*O*-methylated metabolite, was observed in rats following intravenous and oral administration of tolcapone. The difference due to the route of administration could be explained by the difference between S-COMT and MB-COMT in the affinity for the catechol substrate. As described above, MB-COMT has more than 10 times the affinity for the catechol substrate than does S-COMT (Malherbe et al 1992), and high MB-COMT activity is observed in the intestinal muscle layer (Nissinen et al 1988). Thus, when administered orally, tolcapone is highly exposed to the MB-COMT in the intestinal muscle layer during its absorption process, and it seems to form a complex with MB-COMT having a high affinity constant (i.e. a very low K_i). The fraction of the oral tolcapone dose that passes through the intestinal muscle layer and reaches the general circulation would then be exposed to S-COMT and MB-COMT in many tissues in the same way as is the intravenous tolcapone dose.

The reason for the slow elimination rate of the 3-*O*-methylated metabolite following oral tolcapone administration was considered not to be due to the slow elimination rate of the 3-*O*-methylated metabolite itself, as the elimination rate of intravenously administered 3-*O*-methylated metabolite was not so slow. Tolcapone was used in suspension form for the toxicology studies. In the present study, therefore, the suspension form was used for the oral tolcapone dose in contrast with the solution form for the intravenous dose. This difference in dosage forms between oral and intravenous administrations, however, is unlikely to be the reason for the difference in the disposition of the 3-*O*-methylated metabolite between these two administration routes, because the absorption rate-limited process could not be observed in the blood concentration–time course of tolcapone following oral administration.

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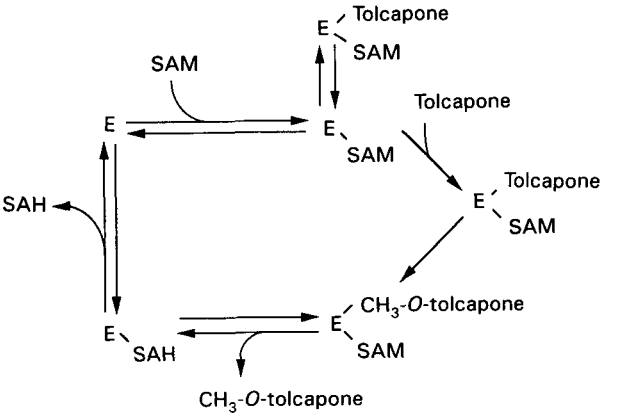

FIG. 2. Proposed kinetic reaction mechanism for tolcapone. Once *S*-adenyl-L-methionine (SAM) is bound to the enzyme (E), tolcapone is bound in an ordered sequence. Some tolcapone molecules bind to E but do not give a product (non-productive binding) and methyl transfer takes place in other tolcapone molecules. 3-*O*-Methylated metabolite (CH_3-*O*-tolcapone) is released from the enzyme before the release of *S*-adenosylhomocysteine (SAH).

oral study, classical Michaelis–Menten-type kinetics failed to depict a similar plateau concentration–time profile of the metabolite. The near plateau concentration–time profile of the 3-*O*-methylated metabolite observed following the oral tolcapone administration may be explained by the difference in inhibition constant (K_i) of tolcapone complexes with S-COMT or MB-COMT. The kinetic reaction mechanism for COMT and tropolone, an inhibitor of COMT, has been reported (Rivett & Roth 1982), in which a compulsory-ordered ternary complex mechanism was described. The kinetic scheme for tolcapone might be described in terms of the compulsory-ordered ternary complex mechanism, with the addition of non-productive binding, as shown in Fig. 2. The kinetics for a non-productive binding model have been reported (Funaki et al 1993). The blood concentration–time curves of tolcapone and its *O*-methylated metabolite following intravenous and oral administration were well described by the pharmacokinetic model based on the reaction mechanism in Fig. 2 (data not shown). However, in-vitro evidence for the non-productive binding of tolcapone has not yet been reported. Therefore, further study may be necessary to explain the near plateau concentration–time profile of the 3-*O*-methylated metabolite following oral administration of tolcapone.

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