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PHARMACODYNAMICS

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Multiple-dose clinical pharmacology of the catechol-*O*-methyl-transferase inhibitor tolcapone in elderly subjects

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Abstract. *Objective*: The purpose of this study was to assess the multiple-dose clinical pharmacology of tolcapone, a novel catechol-*O*-methyltransferase (COMT) inhibitor, in elderly subjects.
Methods: The drug was administered orally t.i.d. for 7 days to four sequential groups of eight elderly subjects (gender ratio1 : 1) at doses of 100, 200, 400 and 800 mg in a double-blind, randomised, placebo-controlled, ascending-multiple-dose design. On days 2 and 7, a single dose of levodopa/benserazide 100/25 mg was given 1 h after the first intake of tolcapone. Plasma concentrations of tolcapone, its metabolite 3-*O*-methyltolcapone, levodopa and 3-*O*-methyldopa were determined during the course of the study in conjunction with COMT activity in erythrocytes.
Results: Tolcapone was well tolerated at all dose levels, with a slight increase in gastrointestinal adverse events in females at higher doses. The drug was rapidly absorbed and eliminated and showed no changes in pharmacokinetics with time during multiple doses of 100 and 200 mg t.i.d. At doses of 400 and 800 mg t.i.d., tolcapone accumulated moderately as reflected in increased C_{max} and AUC values. Despite the long half-life of 3-*O*-methyltolcapone (39 h), only minor accumulation occurred due to suppression of its formation by tolcapone.
The pharmacodynamics of tolcapone did not change during the week of treatment as reflected in inhibition of COMT activity in erythrocytes, the derived parameters of the plasma concentration-effect relationship (inhibitory E_{max} model with constant EC_{50} values) and the effect on levodopa pharmacokinetics (1.6 to 2.5-fold increase in bioavailability). This suggests the absence of tolerance development and the insignificance of the altered pharmacokinetics at 400 and 800 mg t.i.d. with regard to the pharmacodynamics.
Conclusion: The results of this study offer promising perspectives for the application of tolcapone as adjunct therapy to levodopa in the treatment of Parkinson's disease.

Key words Tolcapone, Elderly; levodopa, pharmacokinetics, pharmacodynamics, multiple-dose

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Tolcapone (3,4-dihydroxy-4′-methyl-5-nitrobenzophenone) is a novel, potent central and peripheral inhibitor of catechol-*O*-methyltransferase (COMT) and is active after oral administration (Zürcher et al. 1990, 1991). COMT plays an important role in the extraneuronal metabolism of both endogenous and exogenous compounds containing the catechol moiety (Guldberg and Marsden 1975). The antiparkinsonian drugs 3,4,-dihydroxyphenyl-L-alanine (levodopa) and apomorphine belong to the latter category (Coleman 1992). COMT inhibitors are under evaluation as adjunct therapy to levodopa in the symptomatic treatment of Parkinson's disease by increasing the availability of levodopa to the brain through the blockade of its degradation to 3-*O*-methyldopa (3-OMD) (Männistö 1994; Mizuno et al. 1994). An effective COMT inhibitor would reduce the problems inherent in long-term use of levodopa such as motor fluctuations and dyskinesias (Marsden 1994) due to a more smoothened delivery of levodopa to the brain.

In healthy male subjects, single doses of tolcapone were well tolerated up to and including 800 mg (Dingemanse et al. 1995a). Absorption and elimination occurred rapidly with an elimination half-life of about 2 h. Tolcapone pharmacodynamics have been

assessed by measurement of the inhibition of COMT activity in erythrocytes as well as the influence on the pharmacokinetics of concomitantly administered levodopa (Dingemanse et al. 1995b). For both pharmacodynamic markers, the increases in effect were dose dependent but not proportional to the dose of tolcapone. Tolcapone 200 mg, which appeared to be the optimal dose, increased both the area under the concentration-time curve and the elimination half-life of levodopa approximately twofold without changing the maximum concentration of levodopa and the time of its occurrence. These properties of tolcapone predict a prolongation of the therapeutic response to levodopa formulations in parkinsonian patients.

The objectives of this study were to assess the tolerability, pharmacokinetics and pharmacodynamics of multiple doses of tolcapone when administered alone and in combination with levodopa in elderly subjects. An elderly population was chosen because these subjects are more representative of the target population (patients with Parkinson's disease) than young males (Rochon and Gurwitz 1995). Drug disposition and sensitivity may be altered in the elderly (Crome and Patterson 1989) and age was shown to particularly influence the systemic availability of levodopa (Evans et al. 1981). A t.i.d. regimen of tolcapone was chosen because of its short elimination half-life (Dingemanse et al. 1995a,b). The doses investigated were around the optimal dose apparent from the single-dose study (Dingemanse et al. 1995b).

Methods

Subjects

Forty-eight volunteers (47 Caucasian, 1 Oriental) participated in this study after recruitment by the clinical investigator through advertising in newspapers. They were aged 55–80 years, stratified to a 1:1 male/female ratio and within –15%/+25% of their ideal body weight. Ethics Committee approval was obtained from the Stichting Beoordeling Ethiek Bio-Medisch Onderzoek, Assen, The Netherlands, and all subjects gave their written informed consent before any screening procedures were performed. The study was conducted in full conformity with the principles of the Declaration of Helsinki and its amendments. Subjects were considered to be healthy, as assessed by medical history, physical examination, vital signs, ECG, clinical laboratory determinations and absence of comedications. Tests for drugs of abuse (including benzodiazepines and tricyclic antidepressants) in blood and urine were also performed. During the study restrictions were applied regarding the intake of methylxanthine-containing beverages and food. Subjects were not allowed to smoke or consume alcohol for the entire length of the study.

Design

This was a double-blind, randomised, placebo-controlled, ascending-multiple-dose study of orally administered tolcapone. The dose levels were 100, 200, 400 and 800 mg t.i.d. studied sequentially. The decision to proceed to the next higher dose level was made on the basis of tolerability results of the previous dose level. In the dose group of 100 mg, two tablets of 50 mg were administered and in the other groups one, two or four tablets of 200 mg, respectively, were taken. Each dose level was investigated in a group of 12 subjects randomised to receive either active drug (n = 8) or placebo (n = 4). Tolcapone or placebo was administered t.i.d. for 7 days, with dosing intervals of 6 h/6 h/12 h, corresponding to anticipated therapeutic practice. Each subject received a single dose of Madopar 125 (levodopa 100 mg + benserazide 25 mg) 1 h after the first administration of tolcapone or placebo on study days 2 and 7, i.e. at the expected time of maximum tolcapone concentration (Dingemanse et al. 1995a,b). For safety reasons, tolcapone was given alone on day 1. In the morning, drug was administered after an overnight fast and on days 1, 2 and 7 fasting continued for a further 4 h. On the remaining days, breakfast was consumed 1/2 h after the first drug intake. On all study days, lunch and dinner were consumed 1.5 h before the second and third tolcapone/placebo intake, respectively. The subjects left the clinic on study day 13.

Assessments

Tolerability

Adverse events were assessed by spontaneous reports, observations and questioning at regular times. On study days 1, 2 and 7 these measurements were performed hourly. The intensity of the adverse events was rated on a three-point scale (mild, moderate, severe), and the potential relationship to test drug was assessed by the investigator prior to breaking the code. Supine and standing blood pressure, pulse rate, body temperature and ECG were recorded at frequent intervals. After taking the last blood sample, a physical examination as well as routine clinical laboratory tests was performed.

Pharmacokinetics/Pharmacodynamics

Blood samples of 10 ml were collected from each volunteer into EDTA-containing tubes via a polyethylene catheter inserted in a forearm vein, just before, and 1, 2, 4, 6, 7, 8, 10 and 12 h after first drug intake on day 1. Further samples were drawn just before, and 1, 2, 4, 6, 12, 16, 24, 48, 72 and 96 h after first drug intake on days 2 and 7. Two more blood samples were taken 120 and 144 h after the first dose on day 7.

Within 30 min of collection, the blood samples were centrifuged at 4° C and plasma was carefully separated from erythrocytes. The latter were washed twice with an equal volume of isotonic phosphate buffer (pH 7.4). Erythrocytes were stored at −20° C pending analysis. Plasma was divided into two aliquots and stored in glass tubes, one at −20° C for analysis of tolcapone and 3-*O*-methyltolcapone and the other at −70° C for analysis of levodopa and 3-OMD.

Plasma concentrations of tolcapone and its metabolite 3-*O*-methyltolcapone were determined by high-pressure liquid chromatography (HPLC) methods previously described (Timm and Erdin 1992; Dingemanse et al. 1995a). The limit of quantification was 0.04 $mg \cdot l^{-1}$ for both compounds with intra- and inter assay coefficients of variation lower than 5%. Plasma concentrations of levodopa and 3-OMD were determined by HPLC with column switching according to the method of Zürcher and Da Prada (1990). Endogenous levels of both compounds (about 0.01 $mg \cdot l^{-1}$) could be determined and the intra- and interassay coefficients of variation in the relevant concentration range were lower than 3% and 4%, respectively.

COMT activity in erythrocytes was assessed using a method described previously (Nohta et al. 1984; Dingemanse et al. 1995a). The assay involved the O-methylation of 4-(naphtho[1,2-*d*]thiazol-2-*yl*) pyrocatechol as substrate for COMT. It allowed the reliable fluorometric detection of COMT activity down to less than 1% of that normally present in human erythrocytes. The intra- and interas-

say coefficients of variation were lower than 4% and 6%, respectively. After determination of the haemoglobin (Hb) content in the original lysate with a standard test kit (Roche Diagnostics), COMT activity was expressed as a percentage of baseline activity.

Evaluation

When not specifically mentioned, results include both genders. The influence of multiple dosing on the pharmacokinetics and pharmacodynamics of tolcapone was evaluated by comparison of days 1, 2 and 7. Comparison of day 2 and day 7 is the most relevant because in therapeutic practice co-administration of tolcapone and levodopa is foreseen. Due to technical difficulties, no information on the pharmacokinetics of the 200-mg dose on day 1 was obtained.

Tolerability

The adverse events and clinical laboratory data were evaluated descriptively. On days 2 and 7, levodopa/benserazide was administered 1 h after the first daily dose of tolcapone or placebo. Adverse events with an onset time before administration of levodopa/benserazide were assigned to treatment groups as for days 1, 3–6 and 8–13, i.e. monotreatment with tolcapone or placebo. Adverse events with onset after additional administration of levodopa/benserazide on days 2 or 7 were assigned to the respective combination with levodopa/benserazide. Adverse events were therefore summarized for ten different treatment groups.

Clinical laboratory values were compared with the normal ranges supplied by the analysing laboratory. Individual vital signs were screened for values outside the predetermined normal ranges: systolic blood pressure, 90–200 mmHg; diastolic blood pressure, 55–110 mmHg; pulse rate, 40–120 beats $\cdot$ min^{-1}; body temperature, 35.0–37.5° C. In addition, the vital signs were screened for systematic trends conforming to the recommendations of the Joint National Committee (1993). Attention was particularly devoted to signs of increased dopaminergic activity, viz. postural hypotension, nausea and vomiting. The diagnosis of postural hypotension required the following three criteria to be met when the subjects changed from a supine to a standing position: spontaneously reported dizziness, a decrease of more than 10 mmHg in diastolic and/or a decrease of more than 20 mmHg in systolic blood pressure, and an increase of more than 25% in pulse rate.

Pharmacokinetics

Pharmacokinetic parameters were determined for both tolcapone and 3-*O*-methyltolcapone. The maximum plasma concentration (C_{max}) and the time to its occurrence (t_{max}) relative to dosing were read directly from the concentration-time data. The terminal elimination rate constant (λ_z) was obtained by log linear-regression analysis of the terminal portion of the third dosing interval on days 2 and 7 (on day 1 no samples were taken during the elimination phase of the compounds). The corresponding elimination half-life ($t_{1/2}$) was calculated using $\ln(2)/\lambda_z$. The area under the concentration-time curve (AUC) was calculated by linear-trapezoidal summation (Gibaldi and Perrier 1982). The same procedures were employed for estimation of the pharmacokinetic parameters of levodopa and 3-OMD, with the exception that the plasma concentrations of both compounds were corrected for the endogenous plasma concentration by subtraction of the latter. For levodopa, only concentrations measured up to 11–15 h after drug intake were used for calculation of λ_z, since concentrations measured thereafter were in the endogenous range.

To test for drug accumulation during multiple dosing, C_{max} and AUC (from the first daily dosing interval) on days 1, 2 and 7 were compared using analysis of variance (ANOVA).

COMT inhibitory activity

The pre-dose value of COMT activity in erythrocytes on day 1 was defined as the baseline value (E_0). From time plots of percentage COMT activity, the maximum inhibition of COMT activity (E_{max}) and the time to its occurrence (t_{Emax}) were directly read. The maximum degree of inhibition was defined as $(E_0–E_{max})/E_0$. The time to recovery of baseline activity (t_{rec}) was estimated by determination of the junction between linearly interpolated activity data and the baseline activity. The area under the effect-time curve (AUE) was calculated by linear-trapezoidal summation from the time of dosing (t_0), using E_0 as the value at t_0.

The influence of multiple dosing on pharmacodynamics was investigated by ANOVA of E_{max}, maximum degree of inhibition and AUE on days 1, 2 and 7. Gender differences between pharmacodynamic parameters were explored across dose levels using unpaired *t*-tests.

Pharmacokinetic–pharmacodynamic relationship

To describe the relationship between the tolcapone plasma concentration (C_p) and COMT activity in erythrocytes (E) at time t after drug administration in each individual subject, the inhibitory E_{max} model was employed (Holford and Sheiner 1981; Dingemanse et al. 1995a):

$$E = E_0 - \frac{I_{max} \cdot C_p}{EC_{50} + C_p}$$

where I_{max} is the maximum possible inhibitory effect on COMT activity attributable to the drug, and EC_{50} is the plasma tolcapone concentration associated with 50% of the maximum attainable inhibition. E_0, I_{max} and EC_{50} values were estimated for days 1, 2 and 7 by non-linear regression analysis using the program PCNONLIN, version 4.0, 1992 (Statistical Consultants, Lexington, Ky., USA). The EC_{50} values derived for days 1, 2 and 7 were compared by means of ANOVA, with the fixed factors dose and gender.

Results

Tolerability

All 48 subjects completed the study according to the protocol. The total and the most frequently reported adverse events are presented in Table 1. Only one adverse event (nausea) was rated as severe in a volunteer during combined treatment with tolcapone 200 mg and levodopa/benserazide on day 2. All adverse events resolved rapidly without sequelae. The incidence of adverse events displayed no clear relationship to the dose of tolcapone (with or without levodopa) and was, in general, higher in females than males. The female/male adverse events incidence ratio at tolcapone doses of 0, 100, 200, 400 and 800 mg (irrespective of the presence of levodopa) was 1.2, 5.5, 1.0, 2.0 and 1.4, respectively. A more detailed evaluation showed that the gender differences with respect to adverse events were only apparent in the gastrointestinal and, less clearly, in the dizziness/orthostasis category. The number of gastrointestinal adverse events in females/males at doses of 0, 100, 200, 400 and 800 mg tolcapone was 3/5, 3/0, 4/1, 4/0 and 8/4, respectively. For dizziness/orthostasis these

Table 1 Total and most frequently reported adverse events (–/+ absence/presence of levodopa + benserazide)

	Tolcapone dose (mg)				
	Placebo –/+	100 –/+	200 –/+	400 –/+	800 –/+
Number of subjects	16/16	8/8	8/8	8/8	8/8
Number of subjects reporting adverse events	13/11	5/3	6/7	5/5	7/6
Total number of adverse events reported	24/14	8/5	10/14	8/7	13/13
	Number of specific adverse events reported				
Nervous system disorders	12/10	3/2	4/8	3/5	1/6
Headache	6/4	2/1	2/4	3/1	–/2
Somnolence/fatigue	3/4	–/–	1/2	–/4	1/–
Dizziness/orthostasis	2/1	1/1	1/2	–/– –/	2
Gastrointestinal system disorders	8/–	–/3	2/3	3/1	9/3
Abdominal discomfort/pain	3/–	–/–	1/–	1/1	3/1
Nausea	1/–	–/2	–/2	–/–	1/1
Vomiting	1/–	–/–	€ /1	–/–	2/–
Diarrhoea	2/–	–/1	1/–	2/–	2/1
Urinary system disorders	–/1	–/–	2/2	–/– –/–	
Frequent micturition	–/1	–/–	2/2	–/– 1/–	

Table 2 Pharmacokinetic parameters of tolcapone during t.i.d. multiple dosing [Values are given as means (SD), $n = 8$]

Dose (mg)	C_{max} ($mg \cdot l^{-1}$)			t_{max} (h)			AUC [a] ($h \cdot mg \cdot l^{-1}$)			$t_{1/2}$ (h)	
	Day 1	Day 2	Day 7	Day 1	Day 2	Day 7	Day 1	Day 2	Day 7	Day 2	Day 7
100	4.9 (0.9)	5.5 (1.4)	5.1 (1.0)	1.1 (0.4)	1.2 (0.4)	1.2 (0.4)	12.0 (3.1)	14.9 (6.0)	13.1 (1.8)	2.2 (0.5)	2.8 (1.0)
200	–[b]	7.5 (2.2)	7.4 (3.3)	–[b]	2.1 (1.6)	1.2 (0.5)	–[b]	25.3 (5.7)	23.9 (7.9)	3.2 (0.9)	2.9 (0.5)
400	13.1 (3.0)	15.1 (2.8)	17.0 (4.5)	1.6 (0.5)	1.2 (0.4)	1.6 (1.1)	39.1 (9.7)	59.4 (30.3)	74.3 (48.1)	3.5 (2.1)	5.3 (3.7)
800	20.4 (7.1)	24.8 (9.3)	28.7 (8.2)	1.9 (1.0)	1.3 (0.4)	1.3 (0.5)	64.3 (20.8)	106.7 (44.2)	136.8 (46.4)	3.7 (1.1)	5.3 (1.5)

[a] Day 1, $AUC_{0-6\,h}$; days 2 and 7, $AUC_{0-\infty}$
[b] No reliable concentration data were available for the 200-mg dose group

numbers were 2/1, 1/1, 2/1, 0/0 and 2/0, respectively. The intensity of the adverse events after tolcapone 800 mg + levodopa/benserazide in females was greater than after other tolcapone doses.

There was no pattern of abnormal laboratory values or vital signs observed during the study, which would suggest a relationship to treatment with tolcapone. All ECGs recorded during the study were reported as normal. The examinations performed at the post-study screen did not reveal clinically relevant differences from the pre-study screen.

Pharmacokinetics of tolcapone and 3-*O*-methyltolcapone

A summary of the pharmacokinetic parameters of tolcapone in the different treatment groups on days 1, 2 and 7 is given in Table 2. After intake, the drug was rapidly absorbed at all dose levels with a t_{max} at 1–2 h. C_{max} and AUC increased approximately in proportion to dose. The plasma concentration-time courses of tolcapone 200 mg on days 2 and 7 are presented in Fig. 1. For the doses of 100 and 200 mg the pharmacokinetic parameters were constant during the course of the study (Table 2). However, at 400 and 800 mg a significant increase in C_{max} and AUC was observed during multiple dosing. The elimination of tolcapone after 100 and 200 mg t.i.d. was similar on days 2 and 7 with $t_{1/2}$ about 2–3 h. At doses of 400 and 800 mg, $t_{1/2}$ increased about 1.5-fold during multiple dosing from day 2 to day 7. Figure 2 shows the trough levels of tolcapone in the morning as a function of the tolcapone dose and the study day. Up to 200 mg t.i.d. no accumulation of tolcapone was observed. At 400 and 800 mg t.i.d. an increase in trough levels was observed which did not plateau during the treatment period at the latter dose. It is evident that variability in trough concentrations increases with increasing dose of tolcapone.

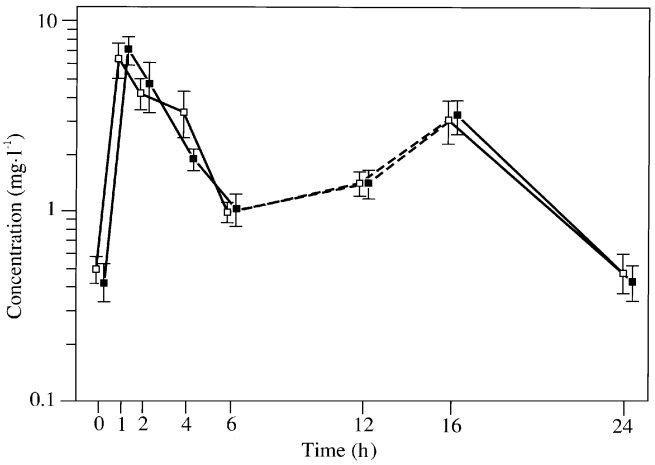

Fig. 1 Plasma concentration-time profiles of tolcapone 200 mg t.i.d. on day 2 (□) and day 7 (■). Data are presented as means with SEM (n = 8)

Trough plasma concentrations of 3-*O*-methyltolcapone accumulated about 1.7-fold at all doses up to days 4–5 and remained constant thereafter, with no clear dose dependence. The pharmacokinetic parameters of 3-*O*-methyltolcapone on day 7 were found to be independent of the dose of tolcapone. On average, values of 0.65 $mg \cdot l^{-1}$, 13.8 $h \cdot mg \cdot l^{-1}$ and 39 h were found for C_{max}, $AUC_{0-24\,h}$ and $t_{1/2}$, respectively. For all parameters the coefficient of variation was 21–23%.

COMT inhibitory activity

A summary of the pharmacodynamic parameters with respect to erythrocyte COMT activity on days 1, 2 and 7 (E_0, E_{max}, t_{Emax}, maximum degree of inhibition and AUE) is given in Table 3. The onset of COMT inhibition was rapid, with t_{Emax} = 1–2 h in most subjects. The maximum degree of COMT inhibition increased with increasing dose, and at doses of 400 mg and 800 mg more than 90% inhibition was attained. AUE tended to reach a plateau with 400–800 mg tolcapone.

The profiles of erythrocyte COMT activity vs time for tolcapone 200 mg on days 2 and 7 are presented in Fig. 3. The pharmacodynamic parameters were similar during the course of the study, the only exception being AUE, which increased significantly by approximately 20% in the 400- and 800-mg dose groups from

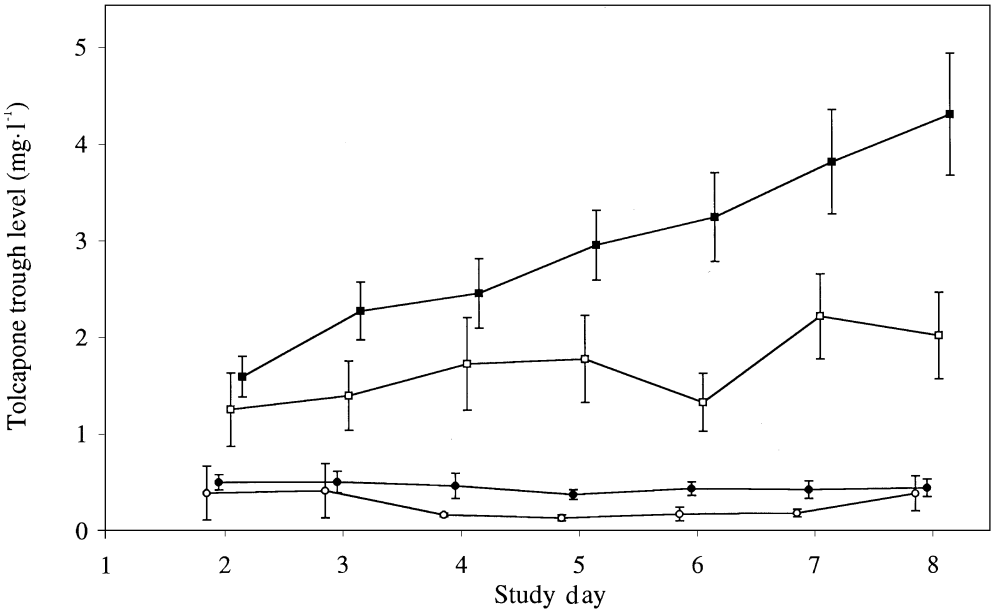

Fig. 2 Trough tolcapone plasma concentrations on study days 2–8 of treatment with different doses of tolcapone (t.i.d.): 100 (○), 200 (●), 400 (□) and 800 mg (■). Data are presented as means with SEM (n = 8)

Table 3 Pharmacodynamic parameters for inhibition of erythrocyte COMT activity by tolcapone during t.i.d. multiple dosing [Values are given as means (SD), n = 8]

Dose (mg)	E_0 ($pmol \cdot h^{-1} \cdot mgHb^{-1}$)	E_{max} ($pmol \cdot h^{-1} \cdot mgHb^{-1}$)			t_{Emax} (h)			$(E_0-E_{max})/E_0$ (%)			AUE (% baseline activity · h)		
	Day 1	Day 1	Day 2	Day 7	Day 1	Day 2	Day 7	Day 1	Day 2	Day 7	Day 1	Day 2	Day 7
100	39 (14)	11 (4)	8 (5)	8 (4)	2.0 (0.0)	1.1 (0.4)	1.2 (0.5)	72 (4)	79 (6)	79 (4)	260 (22)	331 (28)	301 (38)
200	36 (23)	6 (4)	5 (4)	6 (2)	2.0 (0.0)	2.4 (1.4)	1.4 (0.5)	83 (4)	86 (5)	81 (5)	356 (29)	418 (44)	376 (27)
400	41 (17)	4 (2)	3 (1)	3 (1)	2.0 (0.0)	1.1 (0.4)	1.7 (1.1)	91 (3)	92 (2)	91 (3)	406 (21)	483 (30)	482 (28)
800	36 (12)	2 (1)	2 (1)	2 (1)	2.5 (0.9)	1.7 (1.8)	1.4 (0.5)	94 (3)	94 (3)	94 (3)	430 (26)	516 (18)	520 (26)

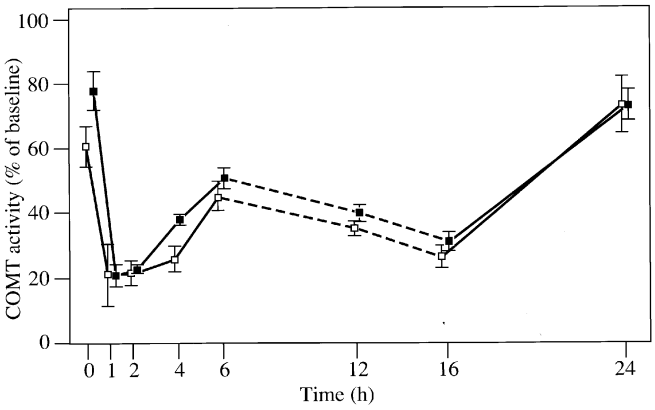

Fig. 3 Erythrocyte COMT activity-time profiles after 200 mg t.i.d. tolcapone on day 2 (□) and day 7 (■). Data are presented as means with SEM (n = 8)

day 1 to day 2. Thereafter, AUE remained stable until day 7.

The trough COMT activity in the morning (days 2–8) at the different doses of tolcapone was clearly dose dependent and virtually unchanged over the course of the study. On average, mean (SD) values of 93.1 (5.4), 72.8 (5.4), 43.7 (4.7) and 31.6 (2.9) were found for the 100-, 200-, 400- and 800-mg doses, respectively. After discontinuation of tolcapone treatment, COMT activity returned to baseline in a dose-dependent manner: t_{rec} [mean with (SD), n = 8] was 16 (8), 21 (4), 30 (4) and 48 (10) h after t.i.d. dosing of tolcapone 100, 200, 400 and 800 mg, respectively.

Pharmacokinetic–pharmacodynamic relationship

Table 4 lists the E_0, I_{max} and EC_{50} values for the effect of tolcapone on erythrocyte COMT activity, as estimated on the basis of the model given. For all treatments both E_0 and I_{max} were close to 100% and in most subjects EC_{50} was within the range 0.2–2.0 $mg \cdot l^{-1}$. On average, the EC_{50} was 0.8 ± 0.4 $mg \cdot l^{-1}$, with no differences between doses of tolcapone and days of treatment.

Pharmacokinetics of levodopa and 3-OMD

A summary of the effect of tolcapone on the pharmacokinetics of levodopa and 3-OMD (C_{max}, t_{max}, AUC and $t_{1/2}$) on study days 2 and 7 is given in Table 5. The plasma concentration-time courses of levodopa and 3-OMD in the 200-mg tolcapone group are presented in Fig. 4. The pharmacokinetics of levodopa and 3-OMD on days 2 and 7 were similar. Tolcapone

Table 4 Estimates of the pharmacokinetic–pharmacodynamic modelling [Values are means (SD), n = 8]

Dose (mg)	E_0 (%)			I_{max} (%)			EC_{50} $mg \cdot l^{-1}$		
	Day 1	Day 2	Day 7	Day 1	Day 2	Day 7	Day 1	Day 2	Day 7
100	99.0 (1.1)	101.0 (11.6)	124.1 (13.8)	94.6 (21.8)	88.3 (7.8)	114.7 (11.4)	0.9 (0.6)	0.7 (0.5)	0.6 (0.3)
200	–[a]	112.8 (28.9)	121.3 (16.1)	–[a]	105.7 (25.4)	109.6 (15.3)	–[a]	0.6 (0.3)	0.5 (0.1)
400	100.0 (0.5)	99.5 (18.8)	124.8 (24.7)	93.3 (2.2)	96.3 (17.1)	121.3 (22.5)	0.8 (0.4)	0.9 (0.4)	0.7 (0.3)
800	99.9 (0.1)	74.0 (20.3)	100.9 (38.4)	96.4 (2.2)	72.9 (18.7)	101.1 (36.5)	0.8 (0.2)	1.4 (0.5)	1.2 (0.3)

[a] No reliable concentration data were available for the 200-mg dose group on day 1

Table 5 Influence of tolcapone on the pharmacokinetics of levodopa (*upper panel*) and 3-OMD (*lower panel*) [Values are means (SD), n = 16 for placebo and n = 8 for the tolcapone doses]

Dose (mg)	C_{max} ($mg \cdot l^{-1}$)		t_{max} (h)		AUC[a] ($h \cdot mg \cdot l^{-1}$)		$t_{1/2}$ (h)	
	Day 2	Day 7	Day 2	Day 7	Day 2	Day 7	Day 2	Day 7
Placebo	0.8 (0.3)	1.0 (0.2)	1.3 (0.7)	1.0 (0.0)	2.2 (0.6)	2.5 (0.6)	2.0 (0.5)	1.8 (0.6)
100	1.3 (0.4)	1.4 (0.9)	1.0 (0.0)	1.0 (0.0)	3.8 (1.2)	4.5 (2.0)	2.6 (0.5)	2.2 (0.5)
200	1.0 (0.3)	1.2 (0.5)	1.8 (1.1)	1.0 (0.0)	4.6 (1.9)	4.1 (1.3)	2.2 (0.4)	2.1 (0.3)
400	1.4 (0.5)	1.2 (0.3)	1.0 (0.0)	1.3 (0.7)	5.5 (1.0)	5.1 (0.6)	3.2 (0.6)	3.1 (0.6)
800	1.3 (0.5)	1.2 (0.5)	1.0 (0.1)	1.3 (0.7)	5.6 (1.0)	5.2 (1.7)	3.0 (0.7)	3.2 (0.8)
Placebo	0.85 (0.40)	0.88 (0.33)	4.1 (1.0)	4.6 (2.1)	12 (4)	12 (3)	15 (4)	14 (4)
100	0.10 (0.05)	0.13 (0.05)	6.5 (2.8)	6.2 (3.0)	1.7 (0.9)	2.1 (0.8)	24 (19)	27 (22)
200	0.08 (0.04)	0.07 (0.03)	6.7 (4.1)	8.0 (4.9)	0.9 (0.6)	1.1 (0.4)	16 (12)	17 (10)
400	0.03 (0.01)	0.04 (0.03)	7.2 (3.1)	6.5 (2.8)	0.4 (0.2)	0.4 (0.2)	15 (9)	13 (4)
800	0.02 (0.01)	0.01 (0.01)	5.2 (2.5)	8.5 (4.0)	0.2 (0.1)	0.2 (0.2)	–	–

[a] For levodopa $AUC_{0\rightarrow\infty}$ and for 3-OMD $AUC_{0-23\,h}$ was calculated

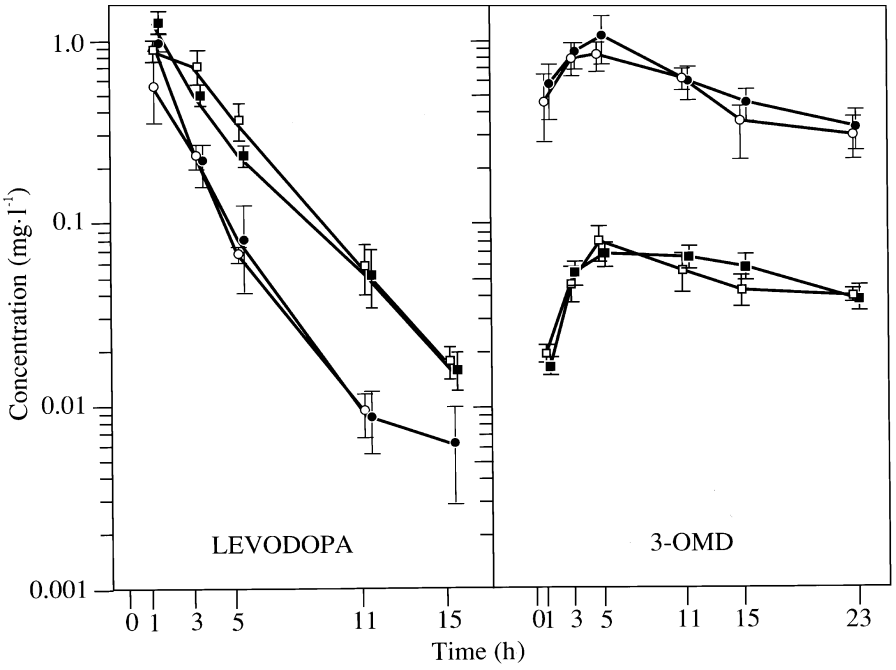

Fig. 4 Plasma concentration-time profiles of levodopa (*left panel*) and 3-OMD (*right panel*) on study days 2 (*open symbols*) and 7 (*closed symbols*). Data are presented as means with SEM with $n = 8$ for the 200 mg-dose of tolcapone (□, ■) and $n = 4$ for the corresponding placebo group (○, ●)

slightly increased C_{max} but did not influence t_{max} of levodopa, whereas it increased both AUC and $t_{1/2}$. The AUC of levodopa increased on average 1.6- to 2.5-fold and $t_{1/2}$ increased up to 1.8-fold, with no systematic differences between days 2 and 7 and with only small differences between the tolcapone doses. With respect to the effect of tolcapone on the pharmacokinetic parameters of 3-OMD, there was a marked dose-dependent decrease in C_{max} and AUC. At a dose of 800 mg tolcapone, formation of 3-OMD was virtually completely suppressed. The $t_{1/2}$ of 3-OMD was about 17 h and independent of the dose of tolcapone. No statistically significant differences were found in the pharmacokinetic parameters of 3-OMD between days 2 and 7.

Discussion

In this study, the COMT inhibitor tolcapone was administered for the first time in a multiple dosing regimen to elderly subjects and to females. In the light of the pharmacokinetic and pharmacodynamic findings with single doses of tolcapone (Dingemanse et al. 1995a,b), a treatment duration of 7 days should have been sufficient to detect possible changes in pharmacokinetics and/or pharmacodynamics due to multiple dosing.

Tolcapone showed a favourable tolerability profile on multiple administration at doses up to 800 mg t.i.d. Most adverse events were reported for the nervous system but in this category no difference to placebo and no tolcapone dose-dependence was observed. The incidence of gastrointestinal adverse events was slightly increased at a dose of 800 mg tolcapone. This phenomenon was not due to increased peripheral formation of dopamine from exogenous levodopa because it mainly occurred in the absence of levodopa/benserazide. In general, in this study females tended to be more sensitive than males to this type of adverse event. This was consistent with the observed slight gender differences in the incidence of dizziness/orthostasis. Since the presence of levodopa cannot explain this phenomenon, it is probably related to a local effect of tolcapone at high non-therapeutic doses. It is unknown whether this is related to higher local concentrations of tolcapone in females. Gastrointestinal side effects, such as nausea, epigastric pain, abdominal pain, loose stools and diarrhoea, have also been reported for the peripherally acting COMT inhibitor entacapone (Nutt et al. 1994). In general, the tolerability profile of tolcapone obtained in healthy male subjects after single dose administration (Dingemanse et al. 1995a,b) was very similar to that observed in elderly male and female subjects, also on multiple dosing.

During multiple dosing of tolcapone, there were no changes in tolcapone pharmacokinetic parameters up to 200 mg t.i.d. This indicates that the pharmacokinetics of tolcapone up to 200 mg are linear and that tolcapone plasma concentrations during multiple dosing can be predicted on the basis of single-dose pharmacokinetics. With 400 and 800 mg tolcapone, some drug accumulation was observed, possibly due to a reduced metabolic clearance with multiple high doses. However, the maximum tolcapone concentration attained in this study (43.1 $mg \cdot l^{-1}$) was well below those associated with toxicity in toxicokinetic studies in animals (100 $mg \cdot l^{-1}$).

In previous clinical studies with tolcapone (Dingemanse et al. 1995a,b), it was found that the

COMT-derived metabolite 3-*O*-methyltolcapone had a long half-life (30–40 h). This raised some safety concerns because on multiple dosing 3-*O*-methyltolcapone could theoretically accumulate to toxic concentrations. In this study its accumulation profile was much less than could be expected from its half-life of 39 h. The absence of marked accumulation can be explained by the fact that the formation of this metabolite is dependent on the activity of COMT as was hypothesised previously (Dingemanse et al. 1995a). The concentrations measured were approximately 35-fold lower than those eliciting first signs of toxicity in animal species.

The pharmacodynamics of tolcapone did not change during 1 week of t.i.d. treatment. This was shown by three different approaches. First, the COMT inhibitory activity of tolcapone expressed in COMT inhibition in erythrocytes did not change during the course of the study. Secondly, the concentration-effect relationship of tolcapone as described by a simple inhibitory E_{max} model did not change during multiple dosing (constant EC_{50} values). Finally, the influence of tolcapone on the pharmacokinetics of levodopa and 3-OMD did not differ between days 2 and 7. Altogether, these results indicate that on multiple dosing there is no slow accumulation of active metabolites and that there is no development of pharmacodynamic tolerance (Dingemanse et al. 1988). The accumulation of tolcapone plasma concentrations at 400 and 800 mg, therefore, was not reflected in altered pharmacodynamics with respect to inhibition of COMT activity in erythrocytes and effect on levodopa bioavailability.

The pharmacokinetic and pharmacodynamic characteristics as well as the concentration-effect relationship of tolcapone in elderly subjects in this study were very similar to those found previously in young male subjects (Dingemanse et al. 1995a,b). The drug showed a rapid absorption and elimination profile and the EC_{50} values were in a relatively narrow range.

In general, the effects of tolcapone on levodopa and 3-OMD pharmacokinetics were also similar to those reported previously in a single-dose study in young subjects (Dingemanse et al. 1995b). In young subjects simultaneous administration of levodopa with 400 and 800 mg tolcapone markedly delayed the t_{max} of levodopa (Dingemanse et al. 1995b). In this study, high doses of tolcapone were not associated with a delay in the absorption of levodopa, which is probably due to the interval of 1 h between the intake of tolcapone and levodopa. This finding supports the hypothesis of competition between levodopa and high doses of tolcapone for the active transport system across gut membranes (LeWitt 1989). Tolcapone induced a small increase in C_{max} of levodopa on both days 2 and 7, a phenomenon which had not been found previously (Dingemanse et al. 1995b).

Regarding the influence on levodopa pharmacokinetics, single-dose tolcapone appears to be markedly more potent than the other nitrocatechol COMT inhibitors nitecapone and entacapone (Kaakkola et al. 1990, 1994; Keränen et al. 1993). The same is true for chronic treatment, where chronic *simultaneous* administration of entacapone 200 mg and levodopa led to an increase of only about 45% in AUC of levodopa (Nutt et al. 1994). In this study tolcapone increased the AUC of levodopa up to 2.5-fold.

In conclusion, tolcapone exhibits a favourable tolerability profile during multiple dosing in elderly subjects of both genders. The pharmacodynamics of tolcapone do not change during multiple dosing and the pharmacokinetics are linear in the envisaged therapeutic dose range of 100–200 mg t.i.d. Further development of tolcapone for adjunct therapy in patients with Parkinson's disease is therefore warranted. Studies in which multiple doses of both tolcapone and levodopa/decarboxylase inhibitor are given as well as studies of longer duration in patients with Parkinson's disease treated with the triple combination of levodopa, a decarboxylase inhibitor (benserazide or carbidopa) and tolcapone are needed to confirm efficacy of tolcapone during chronic dosing (Tolosa et al. 1994; Limousin et al. 1995).

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