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Pharmacokinetic-pharmacodynamic interaction between the COMT inhibitor tolcapone and single-dose levodopa

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1 Single oral doses of the catechol-*O*-methyltransferase (COMT) inhibitor tolcapone (10–800 mg) or placebo were administered simultaneously with a dose of levodopa/benserazide 100/25 mg to seven sequential groups of six healthy male subjects in a two-way crossover study.

2 Plasma concentrations of tolcapone, its metabolite 3-*O*-methyltolcapone, levodopa and 3-*O*-methyldopa (3-OMD) were determined in conjunction with COMT activity in erythrocytes.

3 The drug combination was well tolerated at all dose levels and there were no signs indicative of an increase in dopaminergic stimulation.

4 Tolcapone caused a rapid and reversible inhibition of COMT activity in erythrocytes in parallel with a dose-dependent decrease in the formation of 3-OMD. Tolcapone increased the area under the concentration-time curve and elimination half-life of levodopa. The maximum effects were obtained at a dose of about 200 mg when both parameters increased approximately twofold. The drug had no influence on the maximum concentration of levodopa.

5 Tolcapone was rapidly absorbed and eliminated with, on average, a t_{max} of 1.5 h and a $t_{1/2}$ of 2.3 h. The drug showed dose-proportional pharmacokinetics, in contrast to 3-*O*-methyltolcapone whose formation was relatively decreased at higher doses.

6 Plasma concentrations of tolcapone correlated with inhibition of COMT activity in erythrocytes and suppression of 3-OMD levels, but not with changes in levodopa pharmacokinetics.

Keywords tolcapone levodopa COMT inhibition erythrocytes pharmacokinetics pharmacodynamics concentration-effect relationship modelling Parkinson's disease

Introduction

The cornerstone of the symptomatic therapy of Parkinson's disease is the administration of levodopa (3,4-dihydroxyphenyl-L-alanine), often in combination with an inhibitor of peripheral aromatic L-amino acid decarboxylase. The latter is given to reduce the peripheral conversion of levodopa to dopamine, thereby increasing the amount of levodopa reaching the brain [1]. After several years of treatment, complications such as motor fluctuations, dyskinesias and psychiatric side effects can arise [2]. These may partly be associated with the pharmacokinetic characteristics of levodopa, particularly its biotransformation to 3-*O*-methyldopa (3-OMD), which reaches far higher levels than levodopa upon chronic treatment [3, 4]. This transmethylation reaction is catalyzed by catechol-*O*-methyltransferase (COMT), which is virtually ubiquitous in human tissues and uses S-adenosyl-L-methionine as co-substrate [5]. COMT activity in gastrointestinal membranes and liver probably play an important role in the formation of 3-OMD [6].

Several strategies have been investigated to improve levodopa treatment. The use of controlled-release preparations of levodopa is well established,

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but these do not reduce the possible detrimental role of 3-OMD [7]. Inhibitors of COMT have clinical potential since they increase the availability of levodopa to the brain through the blockade of 3-OMD formation [8]. Additional beneficial effects are conceivable from central COMT inhibition leading to reduced metabolic degradation of dopamine in the striatum [9]. Tolcapone (3,4-dihydroxy-4'-methyl-5-nitrobenzophenone) is a potent COMT inhibitor which is active both centrally and peripherally [10]. It has been demonstrated to enhance markedly the bioavailability of levodopa in several animal species [11]. In man, tolcapone's tolerability, pharmacokinetic and pharmacodynamic properties after single-dose administration have recently been reported [12]. The results of this entry-into-man study permitted the initiation of interaction studies with levodopa.

The objectives of this study were to assess the tolerability of tolcapone when combined with a fixed formulation containing levodopa and a decarboxylase inhibitor, as well as its pharmacodynamics expressed as inhibition of COMT activity in erythrocytes and its influence on the pharmacokinetics of levodopa and 3-OMD.

Methods

Subjects

Forty-two healthy male volunteers (40 Caucasian, 1 black and 1 mulatto), aged 19–35 years and within 15% of their ideal body weight, participated in this study. 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 entire study was conducted in full conformity with the principles of the 'Declaration of Helsinki' and its amendments. Volunteers were considered to be healthy as assessed by medical history, physical examination, vital signs, ECG and clinical laboratory determinations. Tests for drugs of abuse in blood and urine were also performed. No concomitant medication was allowed during the study and restrictions were applied regarding the intake of methylxanthine-containing beverages and food.

Design

This was a double-blind, randomized, placebo-controlled two-way crossover study in which each subject received orally an unblinded single dose of Madopar® 125 (levodopa 100 mg + benserazide 25 mg) simultaneously with a single dose of tolcapone or placebo. After a washout period of 7–14 days, subjects received the alternative combination. Seven dose levels of tolcapone were sequentially studied in groups of six subjects: 10, 25, 50, 100, 200, 400 and 800 mg. Tablets of 5 and 50 mg were used and placebo tablets of identical appearance were added so that a total of five tablets were swallowed for 10–200 mg. At the dose levels of 400 and 800 mg, subjects took 8 and 16 tablets, respectively. The decision to proceed to the next higher dose level was made on the basis of tolerability results of the previous dose level. The drugs were administered in the morning with 200 ml tap water after an overnight fast, then fasting continued for a further 4 h. The subjects remained in the clinic until 48 h after drug intake.

Assessments

Tolerability Adverse events were assessed by spontaneous reports, observations and questioning at regular times. 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 withdrawal of the last blood sample, a physical examination as well as routine clinical laboratory tests were performed.

Pharmacokinetics/pharmacodynamics Blood samples of 10 ml were collected into tubes containing ethylenediaminetetraacetic acid as anticoagulant via a polyethylene catheter inserted into a forearm vein, just before, and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 28, 32 and 48 h after drug administration. Additional samples were collected 72 and 96 h after drug administration for subjects at dose levels of 200, 400 and 800 mg. 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 volumes 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.

COMT activity in erythrocytes was determined by a method based on the original work of Nohta *et al.* [13]. 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 inter-assay 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), and reference to the baseline COMT activity (pmol h^{-1} mg Hb^{-1}), COMT activity was expressed as a percentage of baseline activity.

Plasma concentrations of tolcapone and its metabolite 3-*O*-methyltolcapone were determined by high-pressure liquid chromatography (h.p.l.c.) methods previously described [12, 14]. Plasma concentrations of levodopa and 3-OMD were determined by h.p.l.c. with column switching according to the method of Zürcher & Da Prada [15]. Endogenous levels of both compounds could be determined

whereas the intra- and inter-assay coefficients of variation were lower than 3% and 4%, respectively.

Evaluation

Tolerability The adverse events and clinical laboratory data were evaluated descriptively. 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, 80–160 mm Hg; diastolic blood pressure, 50–95 mm Hg; pulse rate, 40–120 beats min^{-1}; body temperature, 35.0–37.5° C. Particular attention was devoted to signs of increased dopaminergic activity, *viz.* dizziness when standing up, nausea and vomiting. Postural hypotension required the following three criteria to be met when the subject changed from a supine to a standing position: a decrease of more than 10 and 20 mm Hg in diastolic and systolic blood pressure, respectively, and an increase of more than 30 beats min^{-1} in pulse rate. Mean vital signs data were screened for trends.

Pharmacodynamics The pre-dose value of COMT activity in erythrocytes was taken as the baseline value (E_0). From time plots of percentage COMT activity, relative to baseline, the maximum inhibition of COMT activity (E_{max}) and the time to its occurrence (t_{Emax}) were directly read. 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) to t_{rec}, taking E_0 as the value at t_0.

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 curve. The 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 and extrapolation to infinity [16]. 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 endogenous plasma concentration by subtraction of the latter. This should allow an accurate estimation of the pharmacokinetic parameters. For levodopa, only concentrations measured up to 8–10 h after drug intake were used for calculation of λ_z, since concentrations measured thereafter were in the endogenous range.

Pharmacokinetic-pharmacodynamic relationships 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 [12]:

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

where I_{max} is the maximum possible effect on COMT activity attributable to the drug, and EC_{50} is the plasma tolcapone concentration giving 50% of the maximum attainable inhibition. E_0 and EC_{50} values were estimated by non-linear regression analysis using the program PCNONLIN, version 3.0, 1989 (Statistical Consultants, Lexington, KY) with the simplifying assumption that COMT in erythrocytes could be completely inhibited, i.e. that I_{max} was equal to E_0 [12]. Relationships between several pharmacokinetic and pharmacodynamic parameters were explored using scatter plots of dynamic *vs* kinetic parameters. The reasoning for this was to investigate whether it was possible to select a variable, e.g. COMT activity in erythrocytes or tolcapone plasma concentration, to substitute for the relatively complex determination of levodopa concentrations. Moreover, it could theoretically help in defining the dose of tolcapone to be tested in patient studies. Pearson's correlation coefficient was calculated to evaluate linear relationships.

Results

Tolerability

All 42 subjects completed the study according to the protocol. The total and most frequently reported adverse events are presented in Table 1. No adverse event was rated as severe and all resolved quickly without sequelae. There was no pattern of abnormal laboratory values or vital signs observed during the study which suggested a treatment effect, and the abnormalities observed were judged to be clinically irrelevant except for two cases of postural hypotension in subjects at the lowest doses of tolcapone (10 and 25 mg). All ECGs recorded during the study were reported as normal. The investigations performed at discharge from the clinic did not reveal clinically relevant differences from the baseline situation.

Pharmacodynamics

The profiles of erythrocyte COMT activity/time for the different doses of tolcapone are presented in Figure 1. A summary of the pharmacodynamic parameters with respect to erythrocyte COMT activity (E_0, E_{max}, t_{Emax}, maximum degree of inhibition, AUE and t_{rec}) is given in Table 2. The onset of inhibition was rapid, with t_{Emax} shorter than 2 h for most subjects. Maximum degree of COMT inhibition increased with increasing dose, and at doses of 100 mg and over more than 80% inhibition was attained. The increase in AUE was not dose-proportional and

Table 1 Total and most frequently reported adverse events

	Levodopa/benserazide + placebo	*Levodopa/benserazide + tolcapone (mg)*						
		10	*25*	*50*	*100*	*200*	*400*	*800*
Number of subjects	42	6	6	6	6	6	6	6
Number of subjects reporting adverse events	24	4	5	2	6	3	4	4
Total number of adverse events reported	35	8	5	4	10	4	5	7
		Number of specific adverse events reported						
Nervous system disorders								
Headache	12	2	2	—	2	1	2	1
Somnolence/fatigue	12	2	2	3	5	—	2	2
Dizziness/orthostasis	4	—	—	—	2	1	—	1
Psychiatric disorders								
Apathy	—	3	—	—	—	—	—	—
Gastro-intestinal system disorders								
Nausea	—	—	—	—	1	—	—	1

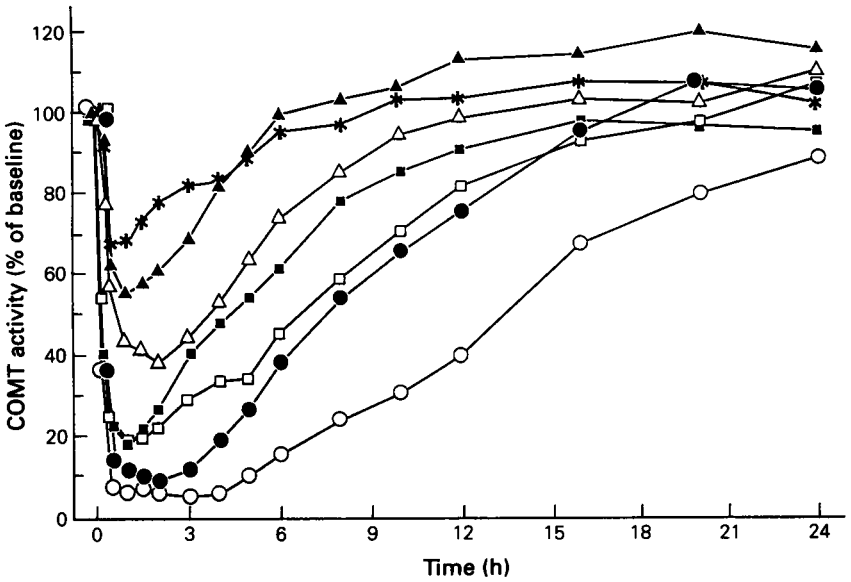

Figure 1 Erythrocyte COMT activity-time profile after tolcapone (* 10 mg, ▲ 25 mg, △ 50 mg, ■ 100 mg, □ 200 mg, ● 400 mg, ○ 800 mg). Data are presented as means of $n = 6$.

Table 2 Pharmacodynamic parameters for inhibition of erythrocyte COMT activity by tolcapone

Dose (mg)	E_0 *(pmol h^{-1} mg Hb^{-1})*	E_{max} *(pmol h^{-1} mg Hb^{-1})*	t_{Emax} *(h)*	$\frac{E_0 - E_{max}}{E_0} \cdot 100$ (%)	*AUE (% baseline activity h)*	t_{rec} (h)
10	55 ± 24	35 ± 14	0.8 ± 0.4	35 ± 4	127 ± 56	9.5 (4.8–11.9)
25	44 ± 8	22 ± 8	1.6 ± 0.7	50 ± 12	156 ± 45	5.7 (4.7–10.9)
50	48 ± 16	15 ± 8	1.3 ± 1.0	70 ± 6	349 ± 35	11.8 (9.8–15.2)
100	57 ± 21	9 ± 4	0.9 ± 0.4	85 ± 4	608 ± 253	15.4 (11.3–48.0)
200	43 ± 19	5 ± 3	2.2 ± 1.9	87 ± 8	711 ± 196	21.2 (15.1–23.9)
400	49 ± 19	4 ± 2	1.3 ± 0.7	93 ± 2	963 ± 505	16.8 (14.8–81.7)
800	43 ± 17	2 ± 1	2.3 ± 1.2	97 ± 2	1320 ± 500	23.5 (15.8–38.8)

Values are means ± s.d. ($n = 6$). For t_{rec} data are given as medians with range.

showed a high inter-individual variability. The t_{rec} roughly increased with dose, and median values were determined since in a few subjects COMT activity did not completely return to baseline within the sampling period, rendering t_{rec} not calculable.

Pharmacokinetics of levodopa and 3-OMD

The C_{max}, t_{max}, AUC and $t_{1/2}$ of levodopa after levodopa/benserazide in combination with placebo were (means ± s.d., n = 42) 1.3 ± 0.6 mg l^{-1}, 0.7 ± 0.3 h, 1.7 ± 0.4 mg l^{-1} h and 1.4 ± 0.2 h, respectively. For 3-OMD the respective values were 0.62 ± 0.11 mg l^{-1}, 4.3 ± 1.9 h, 16 ± 4 mg l^{-1} h and 14.8 ± 2.5 h. A summary of the effect of tolcapone on the pharmacokinetics of levodopa and 3-OMD, expressed as the ratio of the pharmacokinetic parameters determined in the presence and absence of tolcapone, is given in Table 3. The plasma concentration-time courses of levodopa and 3-OMD in a representative subject, in the presence and absence of 200 mg tolcapone, are presented in Figure 2. After administration of levodopa/benserazide 100/25 mg in combination with placebo, C_{max} of levodopa ranged over 0.4–3.6 mg l^{-1} (median 1.2 mg l^{-1}). After coadministration of tolcapone (10–800 mg), C_{max} of levodopa ranged over 0.4–2.8 mg l^{-1} (median 1.2 mg l^{-1}). There was no influence of tolcapone on C_{max} of levodopa and the extent of inter-individual variability in C_{max} was not altered.

Up to a dose of 200 mg, tolcapone did not change the t_{max} of levodopa. However, at 400 mg there was a trend for prolonged absorption and at 800 mg the t_{max} was significantly increased due to tolcapone (data not shown). At the latter dose, t_{max} ranged over 0.3–1.5 h without COMT inhibition, and over 1–3 h with COMT inhibition. For both doses the median t_{max} increased by 50 min.

Following administration of levodopa/benserazide with placebo the $t_{1/2}$ of levodopa ranged over 0.9–1.8 h. When combined with tolcapone, the $t_{1/2}$ of levodopa increased with increasing dose. The maximum

Table 3 Influence of tolcapone on the ratio of pharmacokinetic parameters of levodopa and 3-OMD

Dose	*Levodopa*			*3-OMD*		
(mg)	C_{max}	*AUC*	$t_{1/2}$	C_{max}	*AUC*	$t_{1/2}$
10	0.8 ± 0.3	1.2 ± 0.3	1.2 ± 0.2	0.60 ± 0.08	0.65 ± 0.10	1.1 ± 0.1
25	1.2 ± 0.6	1.5 ± 0.3	1.4 ± 0.3	0.42 ± 0.15	0.47 ± 0.11	1.1 ± 0.1
50	1.2 ± 0.5	1.7 ± 0.6	1.7 ± 0.4	0.31 ± 0.15	0.33 ± 0.12	1.1 ± 0.2
100	1.3 ± 0.6	1.9 ± 0.5	1.6 ± 0.2	0.21 ± 0.06	0.26 ± 0.06	1.2 ± 0.2
200	1.1 ± 0.2	2.0 ± 0.3	1.8 ± 0.2	0.14 ± 0.04	0.22 ± 0.04	1.3 ± 0.3
400	1.1 ± 0.5	1.9 ± 0.8	1.3 ± 0.4	0.09 ± 0.03	0.19 ± 0.04[a]	2.2 ± 0.5[a]
800	0.8 ± 0.5	1.7 ± 0.5	1.9 ± 0.2	0.03 ± 0.10	0.10 ± 0.01[b]	2.7 ± 0.6[b]

Values are ratios (parameter with tolcapone/parameter without tolcapone) and are presented as means ± s.d. (n = 6).
[a] n = 5, [b] n = 4.

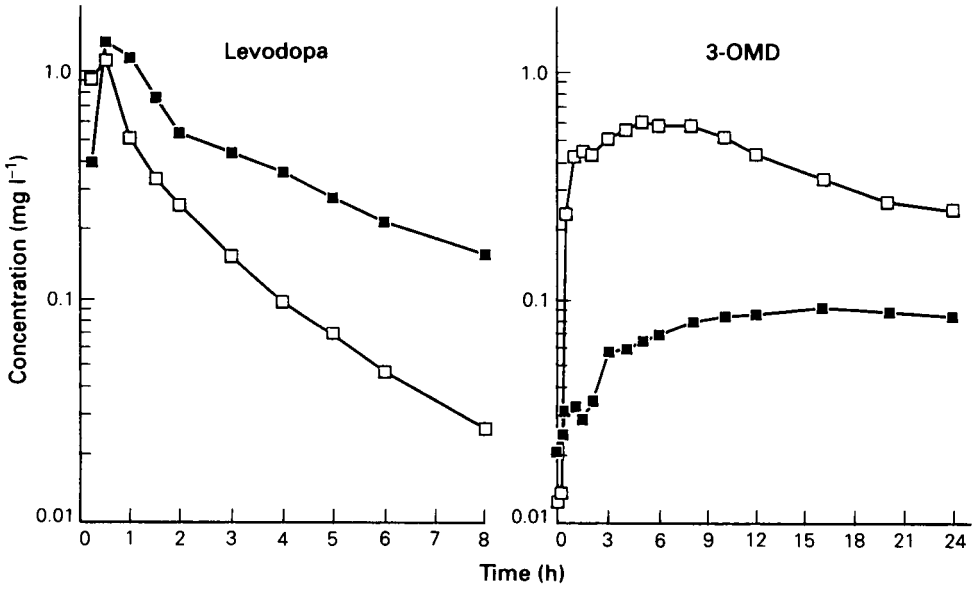

Figure 2 Plasma concentration-time profile of levodopa (left panel) and 3-OMD (right panel) in a subject who received levodopa/benserazide 100/25 mg in combination with placebo (□) and 200 mg tolcapone (■).

increase was attained at a dose of about 200 mg. The AUC of levodopa ranged over 1.0–2.9 mg l^{-1} h following administration of levodopa/benserazide + placebo. A maximum twofold increase in AUC of levodopa was induced by a dose of 200 mg tolcapone. Upon further increasing the tolcapone dose, no additional effect on AUC of levodopa was observed.

With respect to the pharmacokinetic parameters of 3-OMD, the COMT inhibitory effect of tolcapone was reflected by a marked dose-dependent decrease in C_{max} and AUC. The mean plasma concentration-time profiles of 3-OMD after the different treatments are presented in Figure 3. At a dose of 800 mg tolcapone, the plasma levels of 3-OMD were close to endogenous levels indicating almost complete suppression of its formation.

The time of peak concentration of 3-OMD was increased by tolcapone. The increase was most pronounced at a dose of 400 mg where a value of 12 ± 8 h (data not shown) was observed, compared with 4.3 ± 1.9 h when levodopa/benserazide was combined with placebo. At tolcapone doses higher than 200 mg, $t_{1/2}$ of 3-OMD was slightly increased. It should, however, be realized that due to plasma concentrations close to endogenous levels, $t_{1/2}$ of 3-OMD could be less accurately determined at doses of 400 and 800 mg tolcapone.

Pharmacokinetics of tolcapone and 3-O-methyltolcapone

The plasma concentration-time profiles of tolcapone after different doses are presented in Figure 4. A summary of the pharmacokinetic parameters of tolcapone in each treatment group is given in Table 4. The drug was rapidly absorbed at all dose levels with an average t_{max} of 1.5 ± 1.2 h (n = 42). At a dose of 800 mg, the absorption of tolcapone was slightly delayed. The C_{max} of tolcapone increased approximately dose-proportionally. The AUC data of tolcapone confirmed dose-proportional pharmacokinetics. The drug was rapidly eliminated from the body with an overall $t_{1/2}$ of 2.3 ± 0.7 h (n = 42). At doses of 400 and 800 mg there was a tendency for longer apparent $t_{1/2}$.

A summary of the pharmacokinetic parameters of 3-*O*-methyltolcapone for the different doses is given in Table 5. In several subjects, concentrations of the metabolite after 10 mg tolcapone were below the limit of quantification in a few samples. The t_{max} for 3-*O*-methyltolcapone increased with increasing dose of tolcapone and its plasma concentrations increased in a non-linear fashion, as reflected by both C_{max} and AUC. The $t_{1/2}$ of 3-*O*-methyltolcapone could most accurately be determined after doses of 200–800 mg tolcapone because of plasma sampling up to 96 h after drug intake. It appeared to be independent of dose and, on average, a value of 39 ± 10 h (n = 31) was found.

Pharmacokinetic-pharmacodynamic relationships

Table 6 lists the E_0 and EC_{50} values for the effect of tolcapone on erythrocyte COMT activity, as estimated on the basis of the model given. For all subjects and doses investigated, EC_{50} was within the range 0.5–1.3 mg l^{-1}. The fitted E_0 values were very close to the real baseline activity levels.

No simple correlations were found between tolcapone pharmacokinetics (AUC, C_{max}) or COMT activity in erythrocytes (AUE, maximum inhibition) and the effects on levodopa pharmacokinetics (data not shown).

Clear correlations ($r^2 > 0.6$) were obtained between 3-OMD AUC ratio and either AUC or C_{max} of

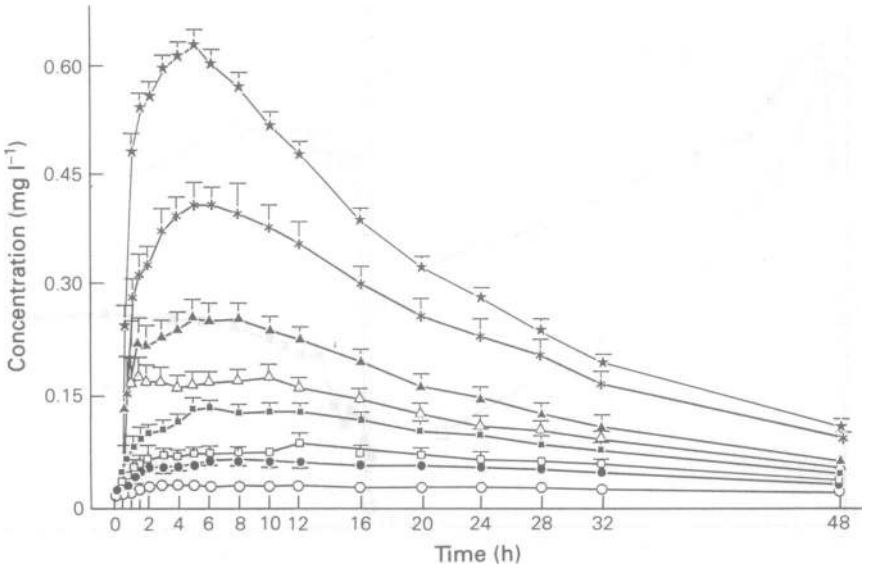

Figure 3 Plasma concentration-time profile of 3-OMD after combination of levodopa/benserazide 100/25 mg with placebo (★) and tolcapone (* 10 mg, ▲ 25 mg, △ 50 mg, ■ 100 mg, □ 200 mg, ● 400 mg, ○ 800 mg). Data are presented as means ± s.e. mean with n = 6 except for placebo where n = 42.

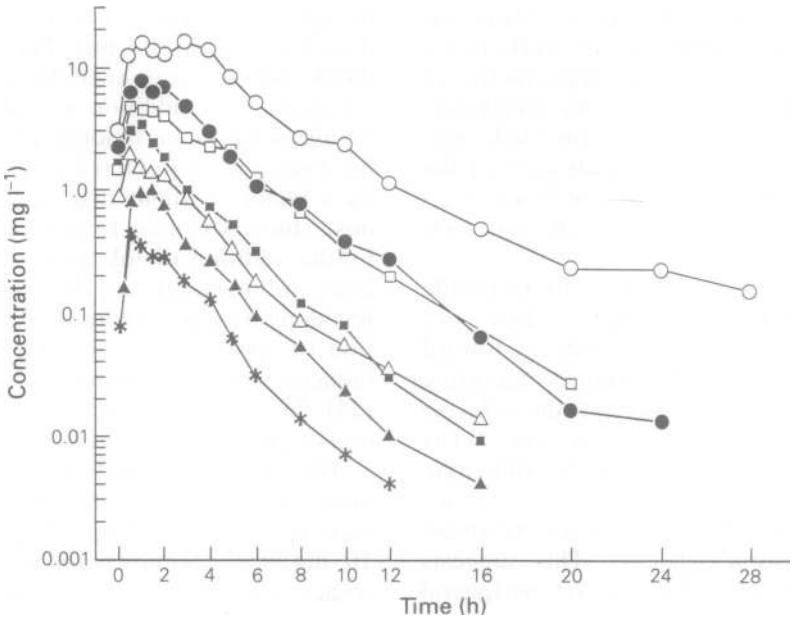

Figure 4 Plasma concentration-time profile of tolcapone after doses of 10–800 mg (* 10 mg, ▲ 25 mg, △ 50 mg, ■ 100 mg, □ 200 mg, ● 400 mg, ○ 800 mg). Data are presented as means of $n = 6$.

Table 4 Pharmacokinetic parameters of tolcapone

Dose (mg)	C_{max} *(mg l^{-1})*	t_{max} *(h)*	*AUC (mg l^{-1} h)*	$t_{1/2}$ *(h)*
10	0.5 ± 0.2	1.4 ± 1.4	1.3 ± 0.1	1.9 ± 0.2
25	1.3 ± 0.5	1.6 ± 1.3	3.3 ± 1.0	2.1 ± 0.4
50	2.3 ± 0.8	1.2 ± 1.1	6.2 ± 1.5	2.2 ± 0.4
100	4.5 ± 0.8	0.7 ± 0.3	10.2 ± 1.3	1.9 ± 0.3
200	7.5 ± 1.8	1.9 ± 1.8	24.0 ± 9.8	2.0 ± 0.3
400	11.3 ± 2.2	1.2 ± 0.5	34.6 ± 5.5	2.6 ± 0.8
800	24.3 ± 8.6	2.5 ± 1.3	109 ± 65	3.0 ± 1.0

Values are means ± s.d. ($n = 6$).

Table 5 Pharmacokinetic parameters of 3-*O*-methyltolcapone

Dose (mg)	C_{max} *(mg l^{-1})*	t_{max} *(h)*	*AUC (mg l^{-1} h)*	$t_{1/2}$ *(h)*
10	0.08 ± 0.02	8 ± 4	4.4 ± 0.6[a]	34 ± 5[a]
25	0.14 ± 0.06	10 ± 5	7.6 ± 2.6[a]	36 ± 7[a]
50	0.17 ± 0.03	14 ± 6	10.9 ± 1.9[c]	37 ± 9[c]
100	0.18 ± 0.05	12 ± 3	12.5 ± 3.5[b]	44 ± 14[b]
200	0.24 ± 0.04	18 ± 6	15.0 ± 4.1[b]	33 ± 1[b]
400	0.23 ± 0.04	19 ± 8	15.8 ± 2.8	36 ± 10
800	0.36 ± 0.04	26 ± 5	31.1 ± 8.4	43 ± 14

Values are means ± s.d. ($n = 6$).
[a] $n = 3$, [b] $n = 4$, [c] $n = 5$.

tolcapone or AUE of COMT inhibition. Figure 5 depicts the relationship between 3-OMD AUC ratio and maximum degree of COMT inhibition.

Table 6 Estimates of the pharmacokinetic-pharmacodynamic modelling

Dose (mg)	E_0 *(pmol h^{-1} mg Hb^{-1})* *actual*	*fitted*	EC_{50} *(mg l^{-1})*
10	55 ± 24	54 ± 23	1.0 ± 0.1 (0.8–1.1)
25	44 ± 8	47 ± 9	1.0 ± 0.1 (0.9–1.2)
50	48 ± 16	48 ± 15	0.8 ± 0.1 (0.7–0.8)
100	57 ± 21	55 ± 20	0.8 ± 0.1 (0.6–0.9)
200	43 ± 19	42 ± 17	1.0 ± 0.2 (0.8–1.3)
400	49 ± 19	52 ± 23	0.6 ± 0.1 (0.5–0.7)
800	43 ± 17	45 ± 16	0.7 ± 0.2 (0.5–1.0)

Values are means ± s.d. ($n = 6$). For EC_{50}, the range (min–max) is presented in parentheses.

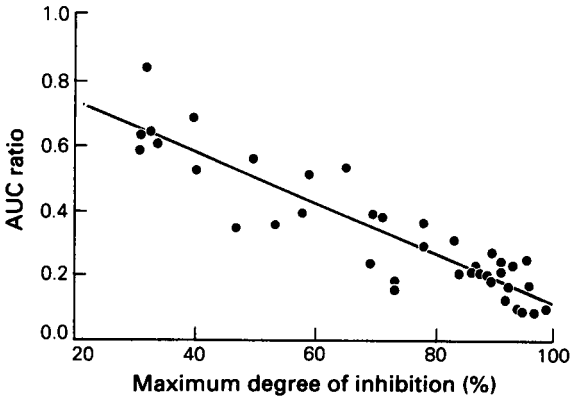

Figure 5 Relationship between 3-OMD AUC ratio and maximum degree of COMT inhibition in erythrocytes.

Discussion

The data reported in this paper constitute the second study in man with the novel COMT inhibitor tolcapone which is developed for the treatment of Parkinson's disease. In the entry-into-man study, tolcapone was shown to be a well tolerated, potent, reversible and orally active COMT inhibitor which inhibited COMT in erythrocytes in a concentration-dependent manner and showed a relatively high bioavailability compared with other COMT inhibitors

[12]. The present study was conducted to evaluate the therapeutic potential of tolcapone in Parkinson's disease, *viz.* its influence on the pharmacokinetics of levodopa in a fixed combination with the decarboxylase inhibitor benserazide. Moreover, this study was designed to shed more light on the usefulness of the measurement of COMT activity in erythrocytes as an alternative for the measurement of levodopa concentrations.

Tolcapone showed a favourable tolerability profile when combined with a single dose of levodopa/benserazide. There was no tendency toward an increase in the number of subjects reporting adverse events or in the intensity of the adverse events with increasing dose of tolcapone. The absence of any increase in signs of dopaminergic stimulation (orthostatic hypotension, nausea, vomiting) could be particularly important for the treatment of patients with Parkinson's disease. This suggests that there is no relevant increase in peripheral dopamine levels and that 25 mg benserazide is sufficient to block the decarboxylase during elevated levodopa bioavailability. It should, however, be realised that the criteria for postural hypotension were set conservatively and that only a single levodopa dose was tested in healthy subjects. These results remain to be confirmed in patients with Parkinson's disease treated chronically with tolcapone and higher doses of levodopa. Tolcapone's tolerability profile is quite different from that of the poorly tolerated first-generation COMT inhibitors [5], but similar to that of other nitrocatechol COMT inhibitors such as nitecapone and entacapone [8, 17].

Based on an historical comparison, simultaneous administration of levodopa/benserazide did not apear to influence the pharmacodynamics of tolcapone, as reflected by its inhibition of COMT activity in erythrocytes [12]. The same is true for the pharmacokinetics of tolcapone and 3-*O*-methyltolcapone [12].

The pharmacokinetic parameters of levodopa and 3-OMD determined after administration of levodopa/benserazide with placebo are in agreement with those reported previously [3, 18, 19]. Despite the fact that the levodopa/benserazide formulation was taken on an empty stomach and under standardized conditions (e.g. with respect to exercise [20]), absorption was quite variable. However, double-peak phenomena, reported to occur due to the influence of levodopa on gastric emptying [21], were observed in only 10 cases, two with levodopa/benserazide + placebo and eight with levodopa/benserazide + tolcapone. Most of the latter cases were in the 800 mg group. High doses of tolcapone delayed the absorption of levodopa. This might be explained by competition between levodopa and tolcapone for the saturable transport mechanism for aromatic and branched chain amino acids across gut membranes, since both compounds are catechol derivatives [22]. Tolcapone did not influence the C_{max} of levodopa, which may be relevant in the treatment of patients with Parkinson's disease to prevent peak-dose dyskinesia.

A dose of 10 mg tolcapone already induced increases in $t_{1/2}$ and AUC of levodopa. The effect of tolcapone on AUC of levodopa was similar in the dose range 100–800 mg. The maximum increase in bioavailability was twofold at a dose of 200 mg tolcapone. The addition of a decarboxylase inhibitor (benserazide or carbidopa) to levodopa formulations has been shown to increase the latter's bioavailability by a factor of about two [23]. Based on this single-dose study, a similar further benefit could be attained by the addition of tolcapone to levodopa/decarboxylase inhibitor regimens. Tolcapone increased the $t_{1/2}$ of levodopa by a maximum of 90%. The triple combination of levodopa, benserazide and tolcapone in rats induced a 3.5-fold increase in the AUC of levodopa [24]. In contrast, peripheral decarboxylase inhibitors do not prolong the $t_{1/2}$ of levodopa [25].

The mean C_{max} and AUC values of the COMT-derived levodopa metabolite 3-OMD were dose-dependently reduced by tolcapone. Even at a dose of 10 mg, the decreases amounted to 40% and 35%, respectively. In rats, the formation of 3-OMD was almost completely suppressed by tolcapone [24]. Reduction of the formation of 3-OMD indicates that tolcapone not only inhibits COMT in erythrocytes but also in other human organs, since it has been proposed that the gut is the main site of *O*-methylation of levodopa [26]. Tolcapone might therefore be an excellent probe to explore the anticipated role of 3-OMD in the late deterioration of patients with Parkinson's disease treated with levodopa [7]. In addition to a marked levodopa-sparing effect, it is expected that tolcapone will reduce the utilization of the universal methyl donor S-adenosyl-L-methionine [27]. S-Adenosyl-L-methionine has been reported to have a therapeutic effect in depression [28], and about 40% of patients with Parkinson's disease also suffer from depression [29].

The peripherally acting COMT inhibitor entacapone is clearly less potent than tolcapone since at a dose of 200 mg it increased the AUC of levodopa by only 40% [30]. Nitecapone proved to be even less potent than entacapone [17]. Entacapone up to doses of 400 mg did not increase the $t_{1/2}$ of levodopa whereas tolcapone 200 mg elicited an increase of 80% [30]. Also based on the suppression of formation of 3-OMD, tolcapone appears to be more potent than other COMT inhibitors [17, 30]. These comparative potency data are in agreement with *in vitro* and *in vivo* animal results [31].

The different pharmacokinetic-pharmacodynamic relationships explored indicate that COMT activity in erythrocytes is a marker which can predict the reduction of 3-OMD formation, but that both COMT activity in erythrocytes and 3-OMD levels are not clearly related to increases in levodopa bioavailability. This suggests that COMT activity in erythrocytes may reflect COMT activity in other tissues [32]. On the other hand, the poor correlations with levodopa bioavailability are probably related to alternative metabolic pathways of levodopa becoming more prominent when COMT is inhibited [3]. Since levodopa is the active moiety in the treatment of patients with Parkinson's disease, the value of the surrogate marker COMT activity in erythrocytes appears to be

limited. Comparison of the EC_{50} values, which were in a relatively narrow range, with those obtained upon treatment with tolcapone alone [12] suggests that the presence of the COMT substrate levodopa does not influence the pharmacodynamics of tolcapone as expressed in erythrocyte COMT inhibition.

In conclusion, the administration of the COMT inhibitor tolcapone with levodopa/benserazide increases the bioavailability of levodopa by suppressing the formation of 3-OMD. Investigation of the potential beneficial effects of tolcapone in Parkinson's disease is therefore warranted [33, 34]. A prolongation of the therapeutic effect of each levodopa dose is anticipated, but with a quicker onset of action than exhibited by slow-release formulations of levodopa. The interaction of tolcapone with levodopa/carbidopa, another widely used levodopa/decarboxylase inhibitor combination [35], and tolcapone's effects upon multiple dosing, should also be studied.

The clinical part of this study was conducted at Pharma Bio-Research Int., Assen, The Netherlands, with Dr J. H. G. Jonkman acting as the principal investigator.

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