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Inhibition of soluble catechol-*O*-methyltransferase and single-dose pharmacokinetics after oral and intravenous administration of entacapone

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Abstract. The inhibition of soluble catechol-*O*-methyltransferase (S-COMT) in red blood cells (RBCs) by entacapone, and the pharmacokinetics of entacapone after single oral (5–800 mg) and IV (25 mg) doses have been examined in an open study in 12 healthy young male volunteers.

Oral entacapone dose-dependently decreased the activity of S-COMT in RBCs with a maximum inhibition of 82 % after the highest dose (800 mg). The inhibition of S-COMT in RBCs was reversible and the activity recovered within 4–8 h.

Entacapone showed linear pharmacokinetics over the dose range studied: C_{max} and AUC were correlated with the dose of the drug. Oral absorption of entacapone was fast, with a t_{max} ranging from 0.4 to 0.9 h, depending on the dose. Systemic availability of entacapone varied between 30 and 46 %. Entacapone was rapidly eliminated by metabolism with a half-life of 0.27–0.30 h after oral doses of 5 to 50 mg. After doses from 100 to 800 mg the disposition was best described by two phases with a $t_{1/2\alpha}$ of 0.27–0.37 h and $t_{1/2\beta}$ of 1.59–3.44 h.

Over the dose range studied, the single oral and IV doses of entacapone were well tolerated. No haematological, biochemical or haemodynamic adverse effects were seen.

The results show that entacapone is an orally effective and reversible COMT inhibitor in man and has simple, linear pharmacokinetics.

Key words: Entacapone; catechol-*O*-methyltransferase; pharmacokinetics; healthy volunteers, adverse effects, metabolism

Catechol-*O*-methyltransferase (COMT) is an enzyme that catalyses the metabolic inactivation of endogenous catechols and xenobiotics with a catechol structure by *O*-methylation (Guldberg and Marsden 1975). COMT accepts catecholamine neurotransmitters, their metabolites, catechol steroids and drugs such as levodopa, alpha-methyldopa and isoprenaline as substrates (Guldberg and Marsden 1975).

The physiological role of COMT, both within the CNS and in peripheral organs, is relatively poorly understood, mainly due to the lack of specific COMT inhibitors. The discovery of a new generation of potent, specific, and orally effective COMT inhibitors has now started progress in understanding the physiological role of COMT in the metabolism of catechols (Bäckström et al. 1989; Borgulya et al. 1989; Waldmeier et al. 1990) and it has also revitalised interest in the possible therapeutic use of COMT inhibitors (Männistö and Kaakkola 1990; Männistö et al. 1992). A potential indication for COMT inhibitors is their adjunctive use in the treatment of Parkinson's disease with levodopa (Männistö and Kaakkola 1990). When levodopa is used with peripherally acting dopadecarboxylase inhibitors (DCI), e. g. carbidopa or benserazide, methylation of levodopa to 3-*O*-methyldopa (3-OMD) becomes its most important metabolic pathway which leads to the accumulation of 3-OMD in plasma.

3-OMD competes with levodopa for transport into the brain and so may reduce the clinical efficacy of levodopa (Calne et al. 1972). The rationale for the use of COMT inhibitors in the treatment of Parkinson's disease is that blocking the metabolism of levodopa to 3-OMD should increase the availability of levodopa and hence the fraction reaching the brain. In addition, the elimination

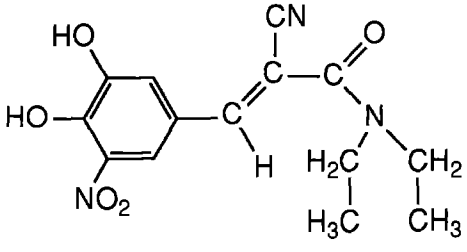

Fig. 1. Structural formula of entacapone

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half-life of levodopa would probably be prolonged (Männistö and Kaakkola 1990).

Entacapone (OR-611, (*E*)-2-cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-5-nitrophenyl) propenamide Fig. 1), is a potent and highly selective inhibitor of COMT in vitro, which is orally effective in vivo (Nissinen et al. 1992). Entacapone penetrates poorly into the brain and is considered principally to be a peripherally acting COMT inhibitor (Nissinen et al. 1992; Kaakkola and Wurtman 1993). Entacapone is currently undergoing clinical trials as a potential antiparkinsonian agent.

Determination of the activity of S-COMT in RBCs provides a means for direct evaluation of the inhibition of COMT in vivo in humans (Schultz et al. 1991; Weinshilboum 1978). In the present study we evaluated the effect of entacapone on the activity of S-COMT in RBCs of healthy volunteers after single oral doses of entacapone. We also assessed the single-dose pharmacokinetics and bioavailability of entacapone and its (*Z*)-isomer, the only metabolite of entacapone found in human plasma (Wikberg et al. 1993).

Subjects and methods

Subjects

Twelve healthy male subjects took part in the study. Their mean age was 24 years (range 22–28 years), mean height 179 cm (range 172–184 cm) and mean weight 73 kg (range 66–80 kg). Prior to entering the study the subjects underwent clinical examination including a laboratory screen and ECG. Subjects with history or clinical or laboratory signs of cardiovascular, renal, hepatic, neurological or psychiatric disorders were excluded.

The subjects were informed both verbally and in writing about the purpose and design of the study, and they gave written consent to it. The study was conducted at the Deaconess Hospital in Helsinki, Helsinki, Finland. It was approved by the ethics committee of the Deaconess Hospital in Helsinki and it followed the guidelines of the Declaration of Helsinki.

Study design

Single oral doses (5, 25, 50, 100, 200, 400, and 800 mg) and an intravenous dose (25 mg) of entacapone were administered to healthy volunteers. The study used an open design and the doses were given in increasing order. Between the doses of 5–100 mg of entacapone there was a wash-out period of at least 2 days, and between the doses from 200 to 800 mg the wash-out period was at least 1 week. The intravenous dose of entacapone was administered after all the subjects had completed the oral study.

Entacapone, supplied by Orion-Farmos Pharmaceuticals, Espoo, Finland, was administered as 5, 25, 50 or 100 mg tablets. For intravenous administration of entacapone, a sterile, concentrate solution (1 mg·ml^{-1}) was prepared. The concentrate consisted of 100 mg entacapone, 150 mg sodium phosphate dihydrate and Ringer's infusion solution (pH 6.5–7.5, Orion-Farmos Pharmaceuticals) to 100 ml. The final infusion solution (25 mg·100 ml^{-1}) was prepared by diluting the concentrate with Ringer's solution. This infusion solution was separately prepared at Orion-Farmos Pharmaceuticals for each subject, and was administered within 3 h of preparation. The solution was infused at a constant rate over 15 min.

The subjects came to the hospital in the morning after an overnight fast and they continued to fast up to 3 h after intake of the study drug.

The oral study medication was admistered with 200 ml water. The subjects were served a standard lunch after about 3 h and a snack 6 and 12 h after the intake of the study drug.

Blood pressure, heart rate and ECG were followed throughout the study day. Adverse experiences were sought with a questionnaire before and 24 h after intake of the study medication.

Blood sampling

In the oral study venous blood samples for determination of the plasma concentrations of entacapone and its (*Z*)-isomer were taken before drug intake at 8.00 h (0-sample) and 10, 20, 30, 45, 60, and 90 min, and 2, 3, 4, 5, 6, 8, 10, and 12 h after intake of the drug. *In the IV study*, venous samples were taken before starting the infusion, on completion of it (0 min) and 2, 5, 10, 15, 20, 30, 45, 60, and 90 min, and 2, 3, 4, 6, and 8 h after the infusion of entacapone.

Blood samples (10 ml) were collected from an antecubital vein without stasis, using vacuum system, and were placed in chilled, polycarbonate EDTA tubes. The plasma was promptly separated by centrifugation for 10 min at + 4 °C, stored at –70 °C and protected from light until analysis.

For the determination of S-COMT in RBCs in 6 subjects, venous blood samples were taken before the oral intake of entacapone, and 10, 20, 30, 45, 60, 90 min, and 2, 3, 4, and 8 h after the study drug. The samples (1 ml) were collected in EDTA tubes and stored overnight at + 4 °C. On the following day RBCs were washed and stored at –70 °C until determination of COMT activity.

A venous blood sample for the determination of haematological and clinical chemistry safety parameters was taken before and 24 h after intake of the medication.

Bioanalytical determinations

Plasma concentrations of entacapone and its (*Z*)-isomer were determined by reversed-phase high-performance liquid chromatography (RP-HPLC) with electrochemical detection (Karlsson and Wikberg 1992). In brief, the analytes were extracted into 6 ml *n*-hexane-ethyl acetate (1:1, v/v) after acidification with hydrochloric acid. An aliquot (5 ml) of the organic phase was evaporated. The residue was reconstituted in 500 µl dimethyl sulphoxide, and a 20 µl aliquot was injected into the HPLC system. Chromatographic peak height was linear over the concentration range 10–4000 ng·ml^{-1}. For both analytes, the intra-assay coefficient of variation (CV) was less than 11 % at the limit of quantitation (10 ng/ml) and less than 6 % at higher concentrations ($n = 8$).

The activity of S-COMT in RBCs was determined using HPLC with electrochemical detection (Schultz et al. 1989). The washed RBCs were haemolysed with water and the mixture was centrifuged. An aliquot of 100 µl of the clear supernatant was incubated at 37 °C in 50 mM phosphate buffer, pH 7.8, in the presence of 200 µM *S*-adenosyl-l-methionine, 2.0 mM magnesium chloride and the substrate, 400 µM 3,4-dihydroxybenzoic acid, in a volume of 250 µl. After 60 min incubation the reaction was terminated with perchloric acid, the protein precipitate was removed by centrifugation and 20 µl supernatant was injected into the HPLC system. An electrochemical detector was used for quantitation of the 3-*O*-methylated product. The protein concentration of the enzyme preparation was determined by a modified Lowry procedure (Markwell et al. 1978). The enzyme activity was expressed as pmol product·mg protein^{-1}·min^{-1} and was used in further calculations. The intra-assay CV of the method was 5 % and the between-day CV was 8 %.

Pharmacokinetic analysis

Iterative, nonlinear, least squares regression analysis was used to fit the plasma concentration-time data (Metzler and Weiner 1989). For *entacapone after oral administration*, a one-compartment (for the 5–

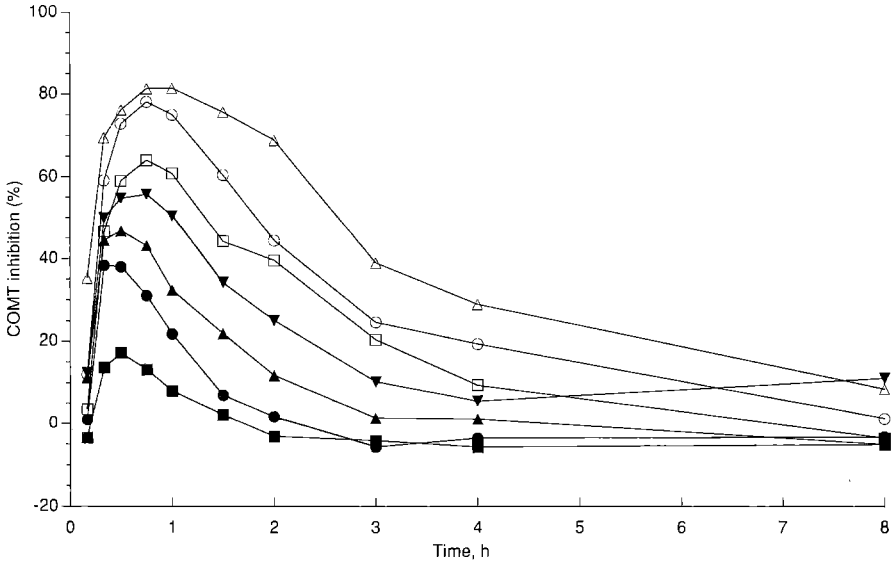

Fig. 2. Mean inhibition (%) of soluble COMT activity in red blood cells after oral administration of entacapone 5 (■), 25 (●), 50 (▲), 100 (▼), 200 (□), 400 (○), and 800 mg (△) in healthy volunteers ($n = 5$–6)

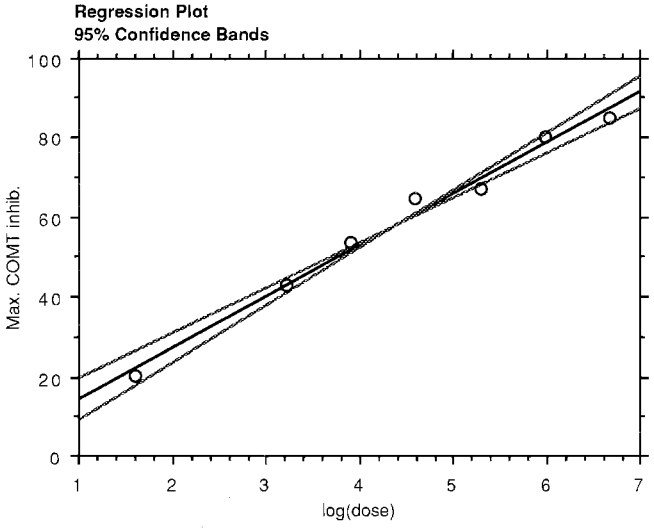

Fig. 3. Analysis of maximum inhibition (%) of soluble COMT activity in erythrocytes after increasing single oral doses (5–800 mg) of entacapone. $Y = 1.613 + 12.848 * X$, $r^2 = 0.989$

50 mg doses) or a two-compartment (for the 100–800 mg doses) open model was applied to calculate the rate constant of each phase. The reciprocal of the squares of the concentrations was used as a weighting factor. Half-life ($t_{1/2\alpha}$ and $t_{1/2\beta}$) values were calculated by dividing ln2 by the rate constant of the corresponding phase. The peak concentration of entacapone in plasma (C_{max}) and the time to the peak concentration (t_{max}) were recorded by visual inspection of the individual plasma concentration-time data. The area under plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method up to the last quantifiable concentration. The area beyond that time point was extrapolated by dividing the last concentration by the terminal rate constant. The systemic availability (f) of entacapone was calculated using the formula $f = AUC_{p.o.} \times D_{i.v.}/(AUC_{i.v.} \times D_{p.o.})$, where $D_{i.v.}$ and $D_{p.o.}$ are the intravenous and oral doses, respectively.

For *the (Z)-isomer of entacapone*, the pharmacokinetic parameters (C_{max}, t_{max} and AUC) were calculated as described for entacapone. The proportion of the AUC of the (*Z*)-isomer of the summed AUC values for the (*E*)- and (*Z*)-isomers was also determined.

The plasma concentration of *entacapone* decreased biexponentially *after the intravenous dose*, so, a two-compartment open model was applied to curve fitting. The reciprocal of the concentrations was used to weight the data. Correction for the infusion time was done according to Loo and Riegelman (1970). The half-life values for distribution and elimination were calculated by standard methods, as well as the volumes of distribution (the volume of central compartment and the volume of peripheral compartment). The total plasma clearance (CL) of entacapone was obtained from the formula $CL = D_{i.v.}/AUC_{i.v.}$.

For *the (Z)-isomer of entacapone*, the AUC and its proportion of the summed AUC values for both isomers were calculated.

Statistical analysis

The activity of S-COMT in RBC was determined at separate time points after oral administration of entacapone by calculating the percentage change from baseline in the activity. Maximal inhibition was analysed using two-way ANOVA with subject and dose of entacapone as independent factors. If a significant effect of the dose was observed in the ANOVA, pairwise comparisons between doses were performed with Tukey's test. The dependence of the inhibition on the dose of entacapone was analysed by fitting a linear regression for the means of the maximum inhibition to the logarithm of the dose. The dependence of the inhibition on the plasma concentration of entacapone was analysed by regression analysis.

For the means of the pharmacokinetic parameters, regression equations were fitted and correlations calculated for the different oral doses of entacapone. For the laboratory safety variables, intraindividual changes were calculated and the average changes were estimated with 95% confidence intervals. Statistical analysis was carried out using SAS software in a VAX mainframe computer.

Results

All 12 subjects completed the oral study. As one of the subjects was unable to participate in the IV study for personal reasons, 11 people completed it.

Inhibition of S-COMT in red blood cells after oral administration of entacapone

The inhibition of S-COMT in RBCs in 6 subjects at different time points is shown in Fig. 2. A reduction in the activity of up to 17% was found after the 5 mg dose and the

Table 1. Pharmacokinetic parameters of entacapone and its (*Z*)-isomer after a single oral dose (5–800 mg) of entacapone in healthy volunteers

	Entacapone						(*Z*)-Isomer of entacapone			
Dose of entacapone (mg)	C_{max} (ng/ml)	t_{max} (h)	AUC (h·ng/ml)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	f[a] [%]	C_{max} (ng/ml)	t_{max} (h)	AUC (h·ng/ml)	$(Z)/(E+Z)$[c]
5	62	0.46	34	0.27	[b]	29	–	–	–	–
	(25)	(0.12)	(7)	(0.14)	–	(9)	–	–	–	–
25	324	0.40	161	0.28	[b]	29	–	–	–	–
	(115)	(0.09)	(51)	(0.11)	–	(10)	–	–	–	–
50	560	0.59	335	0.30	[b]	29	29	0.60	16	4.4
	(192)	(0.23)	(74)	(0.11)	–	(7)	(11)	(0.22)	(6)	(1.5)
100	1116	0.60	724	0.27	1.59	32	63	0.62	44	5.6
	(244)	(0.22)	(135)	(0.09)	(1.13)	(9)	(17)	(0.20)	(13)	(1.2)
200	1808	0.73	1646	0.32	3.44	36	114	0.85	102	5.8
	(763)	(0.20)	(279)	(0.14)	(2.65)	(11)	(50)	(0.34)	(28)	(1.0)
400	4332	0.77	3812	0.28	2.90	42	238	0.79	238	5.9
	(2053)	(0.20)	(487)	(0.06)	(2.02)	(9)	(105)	(0.21)	(57)	(1.4)
800	7284	0.88	8388	0.37	3.10	46	413	1.06	572	6.3
	(2667)	(0.33)	(796)	(0.08)	(1.69)	(11)	(131)	(0.47)	(139)	(1.1)

Data expressed as mean (SD); $n = 12$
[a] $n = 11$
[b] β-phase not observed
[c] $AUC_{(Z)}/(AUC_{(E)} + AUC_{(Z)})$

maximum inhibition (82 %) was obtained after the 800 mg dose. The maximum inhibition occurred within 1 h of the oral administration of entacapone. The inhibition was reversible and the activity of S-COMT recovered within 8 h after all the doses of entacapone.

A linear relationship between the maximum inhibition of S-COMT in RBCs and the entacapone dose was indicated by the results of the regression and correlation analyses (Fig. 3). The regression coefficient was 12.848 ($P < 0.001$) and the intercept was 1.613 (NS), $r = 0.995$ ($P < 0.001$).

Pharmacokinetic parameters of entacapone after oral administration

The plasma concentration-time profiles and the mean pharmacokinetic parameters of entacapone after oral administration of 5–800 mg doses are presented in Fig. 3 and Table 1, respectively.

Entacapone was rapidly absorbed after oral administration; t_{max} values varied from 0.40 to 0.88 h after different doses. There was a trend towards a delay in t_{max} values when the dose of entacapone was increased. Regression and correlation analyses showed a positive linear relationship with the dose of entacapone and the mean C_{max}, with a regression coefficient of 9.21 ($P = 0.0001$) and an intercept of 132.86 (NS), $r = 0.996$ ($P < 0.001$). The mean AUC of entacapone calculated from zero to 2 h increased linearly with the dose; the regression coefficient was 8.85 ($P = 0.0001$) and the intercept was –127.09 (NS), $r = 0.999$ ($P = 0.0001$). The regression equation was fitted using the AUC from zero to 2 h to eliminate the contribution of the terminal elimination phase to the AUC after higher oral doses.

The disposition of entacapone after the oral doses of 100 to 800 mg was mainly described by two phases. The half-life of the initial elimination ($t_{1/2\alpha}$) was 0.27–0.37 h and the terminal elimination half-life ($t_{1/2\beta}$) was 1.59–3.44 h. At the lower entacapone doses (5–50 mg) only one disposition phase, with a half-life of 0.27–0.30 h, was observed. This half-life describes the rate of the initial elimination.

The systemic availability of entacapone was 29–46 % and it appeared to increase with the dose (Table 1).

Pharmacokinetic parameters of the (Z)-isomer of entacapone after oral administration of entacapone

The pharmacokinetics of the (*Z*)-isomer of entacapone after oral administration of entacapone 50–800 mg is presented in Table 1. After entacapone 5 mg no quantifiable concentration was observed in plasma and after 25 mg there were insufficient concentration values for the pharmacokinetic analysis.

The peak concentration of the (*Z*)-isomer was reached after 0.62–1.06 h. After the various oral doses, the proportion of the AUC of the (*Z*)-isomer was 4.4.–6.3 % of the summed AUC values for entacapone and the (*Z*)-isomer.

Pharmacokinetic parameters of entacapone and its (Z)-isomer after intravenous administration

The plasma concentration-time curve of entacapone after 25 mg IV is shown in Fig. 4. Entacapone was first distributed into the central volume of 4.14 (SD 0.86) l and then it rapidly entered the peripheral volume of 29.2 (11.5) l. The half-life of the β-phase was 0.506 (0.146) h. The total plasma clearance of entacapone was 750 (127) $ml \cdot min^{-1}$. After the intravenous dose the proportion of the AUC of the (*Z*)-isomer of entacapone of the summed AUC, of both isomers was 2.6 %.

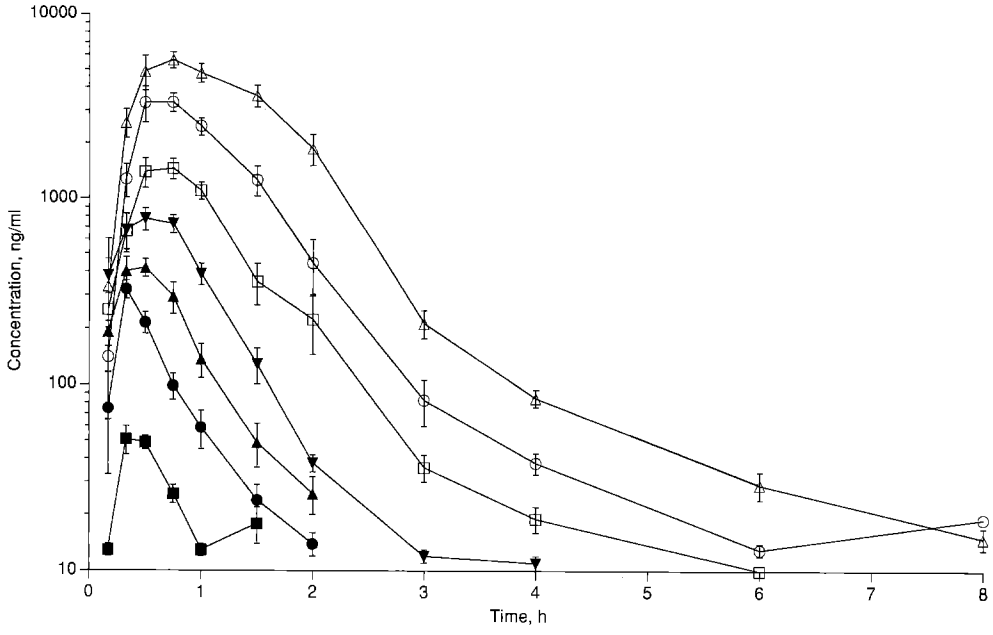

Fig. 4. Mean (SEM) plasma concentrations of entacapone in 12 healthy volunteers after oral administration of entacapone 5 (■), 25 (●), 50 (▲), 100 (▼), 200 (□), 400 (○), and 800 mg (△)

Safety parameters and adverse experiences

No clinically significant deviations in the haematological or clinical chemistry safety parameters were observed after the administration of entacapone. Neither blood pressure nor heart rate was influenced by any of the oral doses of entacapone or the IV infusion.

One subject had transient nausea after 800 mg entacapone. After the 50–800 mg doses, the urine was turn transiently dark yellow or orange. There were no other drug-related adverse experiences.

Discussion

The present study has revealed that entacapone is an orally effective COMT inhibitor in humans; it dose-dependently inhibited S-COMT in RBCs and the inhibition was reversed within 4–8 h, depending on the dose administered. It is not clear whether the degree of inhibition of S-COMT in human RBCs by entacapone is correlated with that in peripheral organs. The activity of S-COMT in human RBCs is low compared to that in the liver, kidney, lung and gut wall (Ball et al. 1971; Nissinen et al. 1988; Schultz et al. 1989), but it is correlated with that in the lung and kidney (Weinshilboum 1978). Animal findings with entacapone suggest that, due to its better penetration into the gut wall compared to RBCs, S-COMT was more strongly and inhibited for a longer time in the gut than in the RBCs (Nissinen et al. 1992). It has been proposed that the gut is the main site of the *O*-methylation of levodopa (Nissinen et al. 1988). Thus, the degree and duration of COMT inhibition in RBCs due to entacapone may differ from its action on other organs, or in the human body as a whole.

We have previously shown that oral entacapone dose-dependently reduced the formation of 3-OMD when coadministered with levodopa and carbidopa to healthy

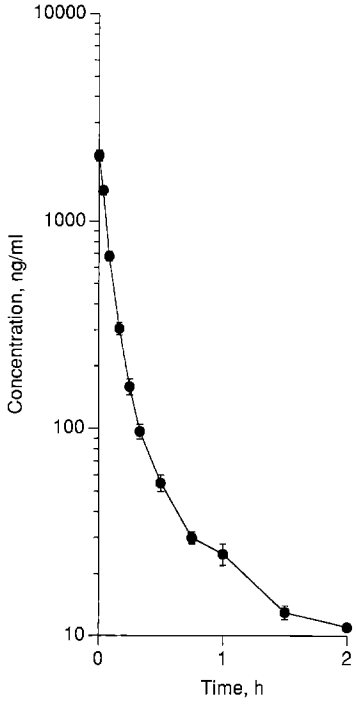

Fig. 5. Mean (SEM) plasma concentrations of entacapone in 11 healthy volunteers after a 15 min infusion of 25 mg entacapone

volunteers (Keränen et al. 1993). Both in volunteers and parkinsonian patients entacapone increased the AUC of levodopa and reduced urinary excretion of homovanillic acid, a COMT-dependent metabolite of dopamine (Keränen et al. 1993; Myllylä et al. 1993). Those findings and the present results strongly suggest that oral administration of entacapone inhibits COMT in various human organs.

The gastrointestinal absorption of entacapone was fast. It is quite possible that the absorption starts in the stomach, but the major part occurs in the proximal small bowel. The increase in t_{max} with the dose of entacapone may be a consequence of the dissolution of the entacapone tablets

(the higher doses were administered as 100 mg tablets), but we cannot exclude an effect of entacapone on the emptying rate of the stomach. The co-administration of entacapone appeared to increase the t_{max} of levodopa (Keränen et al. 1993; Myllylä et al. 1993).

The systemic availability of entacapone averaged about 30% after doses of 5–50 mg, and it seemed to increase to 46% after the higher doses (100–800 mg). The AUC values after IV administration of 25 mg and after smaller oral doses may have been underestimated, because the terminal elimination phase could not be detected due to the low plasma concentrations of entacapone. The terminal elimination phase of entacapone was shown to contribute markedly to the AUC values after higher oral doses. Thus, the apparent increase in the systemic availability of entacapone with the dose is probably related to the method of pharmacokinetic calculation used and not to any dose-related change in the metabolic processes of entacapone. Thus, the total clearance of entacapone may have been somewhat overestimated. The AUC entacapone was lower in young volunteers than in parkinsonian patients in another study (Myllylä et al. 1993). This finding suggests that there may be an age-related decrease in the metabolism of entacapone. However, due to the short duration of action and its good tolerability, the age-related change in the elimination of entacapone does not seem to be clinically important.

The results of the IV administration of entacapone indicated that the drug was initially distributed to a small volume, roughly corresponding to the blood volume. The half-life of the β-phase was about 0.5 h. Because concentrations remained below the determination limit after 2 h, the half-life of the terminal elimination phase may been longer.

The only metabolite of entacapone, which itself is the (*E*)-isomer, found in human plasma is the (*Z*)-isomer (Wikberg et al. 1993). The plasma concentration-time profiles of entacapone and its (*Z*)-isomer seemed to be similar. The plasma concentrations of the (*Z*)-isomer remained rather low both after oral and intravenous administration of entacapone. The AUC of the (*Z*)-isomer accounted for only about 5% of the total AUC of both isomers. The (*Z*)-isomer is mainly formed by isomerisation after the intake of entacapone since the entacapone tablets contain less than 0.2% of the (*Z*)-isomer. The (*Z*)-isomer is as effective a COMT-inhibitor in vitro as entacapone (I.-B. Lindén, oral communication). Due to its low plasma levels in man, the (*Z*)-isomer obviously contributes very little to the COMT inhibition after entacapone.

The metabolic pathways of entacapone in man have been characterised. Although entacapone has a catechol structure, it does not seem to be a substrate for human COMT (Wikberg et al. 1993). About 10% of an oral dose is excreted in urine within 8 h after administration as glucuronides of entacapone and its (*Z*)-isomer. Thus, a large part of entacapone and its metabolites seem to be eliminated by biliary excretion (Wikberg et al. 1993). The clearance of entacapone is high, being roughly equal to the hepatic plasma flow in man.

Initially, the major impact of the adjunctive use of a COMT inhibitor with levodopa/DCI in Parkinson's disease was thought to be reduction of the plasma concentrations of 3-OMD (Guldberg and Marsden 1975). Earlier experimental studies suggested that 3-OMD might reduce the fraction of levodopa reaching the brain and cause deterioration of the behavioural response to the drug (Wade and Katzman 1975; Reches and Fahn 1982; Reches et al. 1982; Gervas et al. 1983). However, recent human studies have shown that the plasma levels of 3-OMD causing a deleterious effect on the clinical response to levodopa in parkinsonian patients were much higher than those normally observed during long-term levodopa/DCI treatment (Nutt et al. 1987). Thus the major advantage of a COMT inhibitor is probably the increase in the availability of levodopa and possibly a reduction in its elimination rate. Entacapone does not penetrate into the brain to a significant degree (Nissinen et al. 1992). Animal data seem to suggest that the major impact of COMT inhibitors on the behavioural effects of levodopa can be attributed to the increased peripheral availability of levodopa (Törnwall and Männistö 1993). A peripherally acting COMT inhibitor may thus offer a novel therapeutic approach in the treatment of parkinsonian patients with fluctuating responses to levodopa predisdue to more critical dependence on constant delivery of levodopa from plasma to the brain than in clinically stable patients (Gancher et al. 1987). This concept is supported by preliminary results with entacapone in parkinsonian patients (Nutt et al. 1994; Ruottinen and Rinne 1993). Even if entacapone had a rather short elimination half-life, it is of the same magnitude as that of levodopa/DCI. On pharmacokinetic bases entacapone is well suited with concomitant administration of levodopa/DCI. This will be tested in further clinical trials in patients with Parkinson's disease.

References

Ball P, Knuppen R, Breuer H (1971) Purification and properties of catechol-O-methyltransferase of human liver. Eur J Biochem 21: 517–525

Borgulya J, Bruderer H, Bernauer K, Zürcher G, Da Prada M (1989) Catechol-O-methyltransferase-inhibiting pyrocatechol derivatives – synthesis and structure activity studies. Helv Chim Acta 72: 952–968

Bäckström R, Honkanen E, Pippuri A, Kairisalo P, Pystynen J, Heinola K, Nissinen E, Lindén I-B, Männistö PT, Kaakkola S, Pohto P (1989) Synthesis of some novel potent and selective catechol-O-methyltransferase (COMT) inhibitors. J Med Chem 32: 841–846

Calne DB, Reid JL, Vakil SD (1972) Parkinsonism treated with 3-O-methyldopa. Clin Pharmacol Ther 14: 386–389

Gancher ST, Nutt JG, Woodward WR (1987) Peripheral pharmacokinetics of levodopa in untreated, stable and fluctuating patients. Neurology 37: 940–944

Gervas J, Muradas B, Bazan E, Aguado E, de Yebenes J (1983) Effects of 3-OM-dopa on monoamine metabolism in rat brain. Neurology 33: 278–282

Guldberg HC, Marsden CA (1975) Catechol-O-methyltransferase: Pharmacological aspects and physiological role. Pharmacol Rev 27: 135–206

Kaakkola S, Wurtman R (1993) Effects of catechol-O-methyltransferase inhibitors and l-3, 4-dihydroxyphenylalanine with or without carbidopa on extracellular dopamine in rat striatum. J Neurochem 60: 137–144

Karlsson M, Wikberg T (1992) Liquid chromatographic determination of a new catechol-O-methyltransferase inhibitor, entacapone,

and its Z-isomer in human plasma and urine. J Pharm Biomed Anal 10: 593–600

Keränen T, Gordin A, Harjola V-P, Karlsson M, Korpela K, Pentikäinen PJ, Rita H, Seppälä L, Wikberg T (1993) The effect of catechol-O-methyltransferase inhibition by entacapone, on the pharmacokinetics and metabolism of levodopa in healthy volunteers. Clin Neuropharmacol 16: 145–156

Loo JCK, Riegelman S (1970) Assessment of pharmacokinetic constants from postinfusion blood curves obtained after iv infusion. J Pharm Sci 59: 53–55

Markwell MAK, Haas SM, Bieber LL, Tolbert NE (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 87: 206–210

Metzler CM, Weiner DL (1989) PCNONLIN Version 30 Software for the statistical analysis of nonlinear models on micros, PCNONLIN user guide. Statistical Consultants Inc, Lexington, Ky

Männistö PT, Kaakkola S (1990) Rationale for selective COMT inhibitors as adjuncts in the drug treatment of Parkinson's disease. Pharmacol Toxicol 66: 317–323

Männistö PT, Ulmanen I, Lundström K, Taskinen J, Tenhunen J, Tilgmann C, Kaakkola S (1992) Characteristics of catechol-O-methyltransferase (COMT) and properties of selective COMT-inhibitors. Prog in Drug Res 39: 291–350

Myllylä VV, Sotaniemi KA, Illi A, Suominen K, Keränen T (1993) Effects of entacapone, a COMT inhibitor, on the pharmacokinetics of levodopa and on cardiovascular responses in patients with Parkinson's disease. Eur J Clin Pharmacol 45: 419–423

Nissinen E, Tuominen R, Perhoniemi V, Kaakkola S (1988) Catechol-O-methyltransferase in human and rat small intestine. Life Sci 42: 2609–2614

Nissinen E, Lindén I-B, Schultz E, Pohto P (1992) Biochemical and pharmacological properties of a peripherally acting catechol-O-methyltransferase inhibitor entacapone. Naunyn Schmiedeberg's Arch Pharmacol 346: 262–266

Nutt JG, Woodward WR, Gancher ST, Merrick D (1987) 3-O-methyldopa and the response to levodopa in Parkinson's disease. Ann Neurol 21: 584–588

Nutt JG, Woodward WR, Stone CK, Beckner RM, Carter JH, Gancher ST, Hammerstad JP, Gordin A (1994) Effects of peripheral catechol-O-methyltransferase (COMT) inhibitor on the pharmacokinetics and pharmacodynamics of levodopa in parkinsonian patients. Neurology (in press)

Reches A, Fahn S (1982) 3-O-methyldopa blocks dopa metabolism in rat corpus striatum. Ann Neurol 12: 267–271

Reches A, Miekle L, Fahn S (1982) 3-O-methyldopa inhibits rotations induced by levodopa in rats after unilateral destruction of the nigrostriatal pathway. Neurology 32: 887–888

Ruottinen H, Rinne UK (1993) Effect of COMT inhibition with entacapone on clinical response, pharmacokinetics and metabolism of levodopa in Parkinson's disease. Neurology 43 [Suppl 2]: 685

Schultz E, Nissinen E, Kaakkola S (1989) Determination of catechol-O-methyltransferase activity in erythrocytes by high performance liquid chromatography with electrochemical detection. Biomed Chromatogr 3: 64–67

Schultz E, Tarpila S, Bäckström A-C, Gordin A, Nissinen E, Pohto P (1991) Inhibition of human erythrocyte and gastroduodenal catechol-O-methyltransferase activity by nitecapone. Eur J Clin Pharmacol 40: 577–580

Törnwall M, Männistö PT (1993) Effects of three types of catechol *O*-methylation inhibitors on l-3,4-dihydroxyphenylalanine-induced circling behaviour in rats. Eur J Pharmacol 250: 77–84

Wade L, Katzman R (1975) 3-O-methyldopa inhibition of L-dopa at the blood-brain barrier. Life Sci 17: 131–136

Waldmeier PC, Baumann PA, Feldtrauer JJ, Hauser K, Bittiger H, Bischoff S, Von Sprecher G (1990) CGP 28014, a new inhibitor of cerebral catechol-O-methylation with a non-catechol structure. Naunyn-Schmiedeberg's Arch Pharmacol 342: 305–311

Weinshilboum RM (1978) Human erythrocyte catechol-O-methyltransferase: correlation with lung and kidney activity. Life Sci 22: 625–630

Wikberg T, Vuorela A, Ottoila P, Taskinen J (1993) Identification of major metabolites of the catechol-O-methyltransferase inhibitor entacapone in the rat and in humans. Drug Metab Dispos 21: 81–92