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Stereoselective first-pass metabolism of highly cleared drugs: studies of the bioavailability of L- and D-verapamil examined with a stable isotope technique

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1 The pharmacokinetics of dextro(+)- and levo(−)-verapamil were studied in five healthy volunteers following oral administration of pseudoracemic verapamil containing equal amounts of unlabelled (−)- and dideuterated (+)-isomer.

2 (+)-verapamil exhibited approximately five times greater C_{max} (+): 240 ± 81.1 ng/ml, (−): 46.1 ± 15.7 ng/ml, $P < 0.001$) and AUC than (−)-verapamil. The apparent oral clearance (CL_o) for (+)-verapamil was significantly smaller than that for (−)-verapamil (+): 1.72 ± 0.57 l/min, (−): 7.46 ± 2.16 l/min, $P < 0.001$). The bioavailability of (+)-verapamil (50%) was 2.5 times greater than that of (−)-verapamil (20%), $P < 0.005$). Thus following oral administration verapamil exhibited a stereoselective first-pass metabolism. Neither t_{max} nor the elimination $t_{½,z}$ were different between the isomers.

3 The elimination of $t_{½,z}$ for each verapamil isomer obtained following oral administration (+): 4.03 h, (−): 5.38 h) were similar to those previously obtained following intravenous administration (+): 4.15 h, (−): 5.38 h, respectively).

4 Whereas the (+)- to (−)-verapamil plasma concentration ratio following oral administration was 4.92 ± 0.48, the ratio following i.v. administration was approximately 2. (−)-verapamil has been demonstrated to possess 8 to 10 times more potent negative dromotropic effect on AV conduction than (+)-verapamil. Therefore, following oral administration the same concentration of plasma verapamil consisting of a two to three times smaller proportion of the more potent (−)-isomer appeared to be less potent than that following i.v. administration with regard to the negative dromotropic effects on the AV conduction.

Keywords verapamil isomers stereoselective first-pass metabolism stable isotope techniques

Introduction

Following oral administration the bioavailability (*F*) of verapamil is low owing to hepatic first-pass metabolism (Schomerus *et al.*, 1976; Eichelbaum *et al.*, 1981 a,b; Freedman *et al.*, 1981; Kates *et al.*, 1981; Anderson *et al.*, 1982; McAllister *et al.*, 1982). The average *F* obtained in single oral dose studies was 20 to 30. Therefore, it might be expected that oral doses of 25 to 50 mg should elicit clinical effects comparable to those of i.v. doses of 5 to 10 mg. However, oral doses 10 to 15 times greater than i.v. doses are usually required to produce comparable clinical effects (Singh *et al.*, 1983). Verapamil has been shown to possess strong negative dromotropic effects on atrioventricular (AV) conduction. A significant relationship has

been demonstrated between the plasma verapamil concentration and the prolongation of PR interval in the surface ECG following i.v. and oral administration (Eichelbaum *et al.*, 1980; McAllister *et al.*, 1982; Reiter *et al.*, 1982). However, these studies have demonstrated that the concentration-effect curve following oral administration appeared to be shifted to the right of that following i.v. administration. On average, two to three times higher verapamil concentrations were required to produce an equivalent effect on AV conduction following oral administration. Clinically available verapamil is a racemic mixture of equal amounts of the (+)- and (−)-isomer. Electrophysiological experiments carried out in dogs have demonstrated that the (−)-isomer possessed 8 to 10 times more potent dromotropic effects on the AV nodal conduction than the (+)-isomer (Raschack, 1976; Satoh *et al.*, 1979). Recent studies carried out in healthy volunteers demonstrated that the (−)-isomer was 18 times more potent than the (+)-isomer in producing 10% PR interval prolongation in man (Echizen *et al.*, submitted for publication). The above findings prompted us to hypothesize that the first-pass metabolism of verapamil might be stereoselective (Eichelbaum *et al.*, 1980). If the more potent (−)-isomer is preferentially eliminated during first-pass metabolism, the same concentration of plasma verapamil following oral administration would consist of a smaller proportion of the more potent (−)-isomer and, therefore, would appear to be less potent than that following i.v. administration. In support of this hypothesis the plasma clearance of the (−)-isomer (1400 ml/min) has been demonstrated to be almost two times greater than that of the (+)-isomer (800 ml/min) following i.v. administration (Eichelbaum *et al.*, 1984). A difference in systemic clearance predicts that the hepatic extraction ratio (E) of the (−)-isomer would be greater than that of the (+)-isomer and, therefore, F of the (−)-isomer would be lower than that of the (+)-isomer. Since verapamil is a highly cleared drug, its E and F are subject to intra- and inter-subject variations (Eichelbaum & Somogyi, 1984). Therefore, we employed a stable isotope technique in order to investigate the differences in oral pharmacokinetics of verapamil isomers following simultaneous administration.

Methods

Subjects

Five healthy male volunteers (aged 23 to 27 years, weight 68 to 91 kg) consented freely to participate in the present study. The study protocol had been approved by the Ethics Committee of the University of Bonn. All subjects had normal renin and hepatic function as assessed by physical examination and appropriate biochemical tests. Electrocardiographic examinations (standard 12 leads) revealed no signs of conduction abnormalities. None of them regularly took any drugs. Two were smokers. They refrained from smoking 12 h prior to and throughout the study. Beverages containing ethanol or caffeine were prohibited prior to and throughout the study. All subjects had participated in a previous i.v. pharmacokinetic study with (+)- and (−)-verapamil (Eichelbaum *et al.*, 1984).

Synthesis of stable labelled verapamil isomers

Dideuterated verapamil isomers were synthesized by substituting two hydrogens by deuteriums at C-5. Optical isomers of 2-isopropyl-2-(3,4-dimethoxyphenyl)-pentane diacid nitrile were separately converted to their methylesters with diazomethane, then reduced with lithium aluminium hydride or with deuteride to the corresponding alcohols. On reaction with thionyl chloride and then with *N*-methylhomoveratrylamine, these alcohols were converted to unlabelled and dideuterated verapamil (2-isopropyl-2-(3,4-dimethoxyphenyl)-5-(*N*-methyl-*N*- (3,4-dimethoxyphenethyl)-aminopentane acid nitrile). Purification by column chromatography and subsequent crystallization yielded enantiomers of unlabelled and dideuterated verapamil hydrochloride. The isotopic purity of dideuterated verapamil was 98.5% and the optical purity of (+)- and (−)-verapamil was 99 and 95%, respectively.

Drug administration and blood sampling

After an overnight fast the subjects ingested 160 mg of pseudoracemic verapamil containing equal amounts of unlabelled (−)- and dideuterated (+)-verapamil dissolved in 100 ml of carbonated water. Two hours later they had a normal hospital breakfast. Blood samples were obtained through an indwelling cannula into glass tubes at the following times: 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 9.0, 11.0, 12.0, 15.0 and 24 h postdose. In order to exclude an isotope effect of the deuterium on the pharmacokinetics of (+)-verapamil, one volunteer received equal amounts of unlabelled and dideuterated (+)-verapamil at a total dose of 250 mg. Drug administration and blood samplings were carried out in the same manner as those aforementioned. Blood samples were centrifuged immediately at

3000 *g* and separated plasma was stored at −20°C until analyzed.

Drug analysis

Plasma verapamil was analyzed by gas chromatography with specific mass fragmentographic detection using d7-verapamil deuterated in the isopropyl group as an internal standard (Spiegelhalder & Eichelbaum, 1977) with a recently described modification (Eichelbaum *et al.*, 1984).

Pharmacokinetics

For each isomer the following pharmacokinetic parameters were calculated: the maximum plasma concentration (C_{max}), the time of its occurrence (t_{max}), the terminal half-life ($t_{½,z}$), the apparent oral clearance (CL_o) and the absolute bioavailability (F). The elimination half-life was calculated from the slope of the terminal log-linear portion of the plasma concentration-time plot (including at least four measured points) determined by linear regression analysis. The apparent CL_o of each isomer was derived from the equation:

$$CL_o = D_o/AUC_o$$

where D_o is the oral dose and AUC_o is the resulting area under the plasma concentration-time curve which was calculated by trapezoidal rule and extrapolated to infinity by dividing the last plasma concentration by the elimination rate constant. F was calculated by dividing the dose-adjusted area under the oral plasma concentration-time curve by the area under the intravenous plasma concentration-time curve, which had been obtained for each subject in a previous study (Eichelbaum *et al.*, 1984). According to the methods described in detail by Somogyi *et al.* (1982), we analyzed the relationship between the reciprocal of F ($1/F$) and CL_o. Briefly, the venous equilibrium model predicts that the hepatic metabolism of drugs with high hepatic clearance is described by the following equation:

$$E = CL_o/(Q + CL_o)$$

where E is the hepatic extraction ratio, CL_o is the apparent oral clearance and Q is liver blood flow (Rowland, 1972). This equation can be rearranged as follows.

$$1/F = CL_o/Q + 1$$

Therefore, a linear relationship is expected between the reciprocal of F and CL_o with an intercept of unity. All available data including those for (+)- and (−)-verapamil obtained in the present study, for racemic verapamil obtained in healthy subjects (Eichelbaum *et al.*, 1981) and for racemic verapamil in patients with liver cirrhosis (Somogyi *et al.*, 1981) were analyzed by least squares regression.

Statistical analysis

Results are presented as mean ± s.d.

Data were analysed by ANOVA, if not otherwise mentioned. Multiple comparisons were carried out by the Bonferroni t-test. A P value of less than 0.05 was considered statistically significant.

Results

The plasma concentration-time curves of unlabelled and dideuterated (+)-verapamil following simultaneous oral administration are shown in Figure 1. The plasma concentration-time curve of dideuterated (+)-verapamil was superimposable on that of the unlabelled (+)-verapamil. The $t_{½,z}$ (5.82 h) and CL_o (2.17 l/min) of dideuterated (+)-verapamil were almost identical to those of unlabelled (+)-verapamil ($t_{½,z}$: 6.27 h and CL_o 2.27 l/min).

The plasma concentration-time curves of (+)- and (−)-verapamil following simultaneous oral administration of the pseudoracemic mixture containing equal amounts of each isomer in a representative subject is shown in Figure 2. In all subjects plasma concentrations of the (+)-isomer were substantially higher than those of the (−)-isomer throughout the sampling period. The (+)- to (−)-isomer concentration ratio remained constant up to 12 h postdose (Figure 3). The pharmacokinetic parameters determined for each subject are summarized in Table 1. C_{max} for the (+)-isomer was approximately five times higher than that for the (−)-isomer ($P < 0.001$). Neither t_{max} nor $t_{½,z}$ were different between the isomers. CL_o for the (−)-isomer was four to five times greater than that for the (+)-isomer ($P < 0.001$), resulting in two to three times greater absolute F of the (+)-isomer as compared to that of the (−)-isomer.

There was strong linear relationship between $1/F$ and CL_o ($1/F = 0.606\ CL_o + 1.128$, $r^2 = 0.91$, $P < 0.001$). The y intercept of this regression line was approximately unity (Figure 4).

Discussion

Stable isotope techniques were used to study the pharmacokinetics of verapamil isomers following simultaneous oral administration. Since verapamil is a highly cleared drug, CL_o and F are subject not only to inter-individual

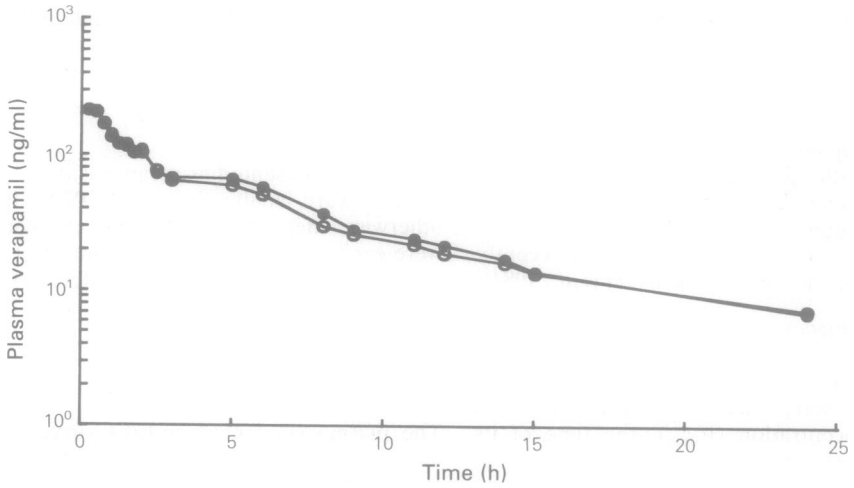

Figure 1 Plasma concentration-time curves of unlabelled (○) and dideuterated (●) (+)-verapamil following oral administration of 125 mg of unlabelled and dideuterated isomers.

but also to intra-individual variations (Eichelbaum & Somogyi, 1984). Without using the stable isotope technique a large number of subjects would have been required to obtain significant differences in CL_o and F between the isomers. In order to avoid isotope effects on the disposition of verapamil, deuterium was introduced at the C-5 atom, which is considered to be a metabolically inert position (Eichelbaum *et al.*, 1979). In addition, we studied the disposition of the unlabelled and stable labelled (+)-verapamil following simultaneous oral administration and confirmed that there was no isotope effect. Since all subjects enrolled in the present study had participated in the previous i.v. pharmacokinetic study with (+)- and (−)-verapamil, $t_{½,z}$ values for each isomer after oral administration were compared to those obtained after i.v. administration. The values of $t_{½,z}$ for the (+)- and (−)-isomer determined after i.v. and oral administration were similar (+): i.v. 4.08 h *vs* oral 4.03 h, (−): i.v. 4.86 h *vs* oral 5.38 h). The $t_{½,z}$ values of pseudoracemic verapamil obtained in the present study were also in good agreement with those of the racemic preparation previously reported by us and others (Anderson

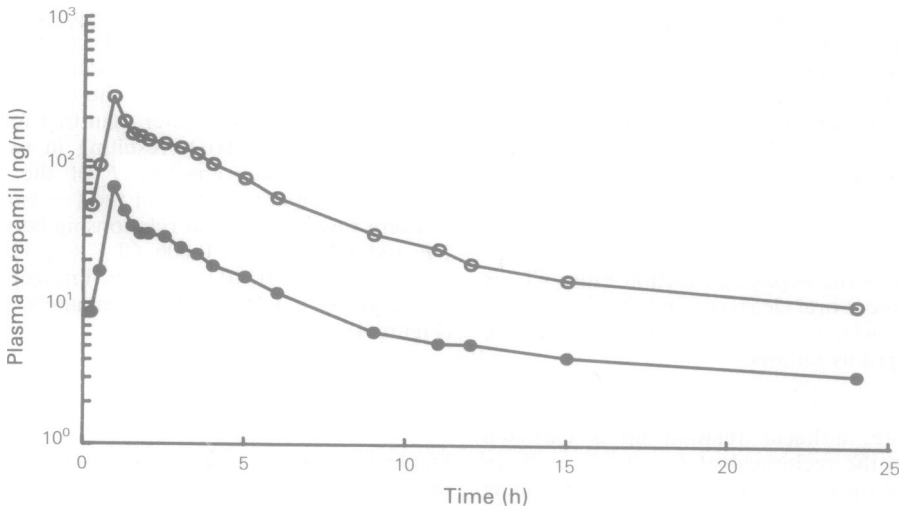

Figure 2 Plasma concentration of (+)- (○) and (−)- (●) verapamil following oral administration of 160 mg pseudoracemic verapamil in a representative subject.

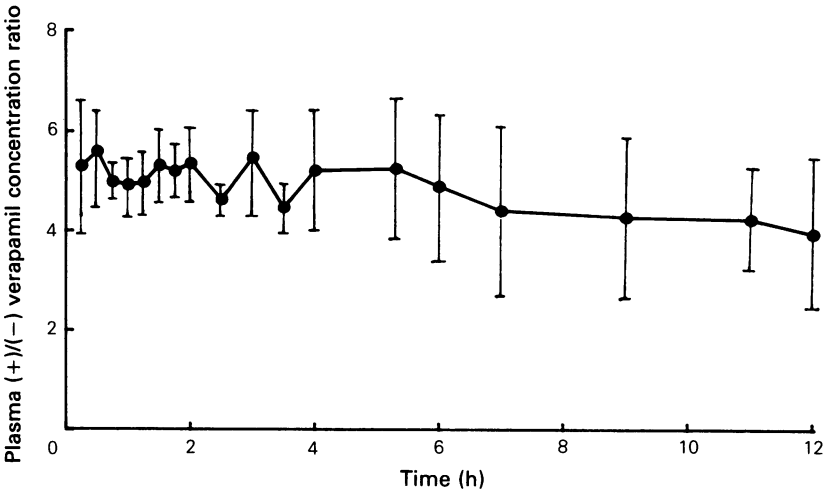

Figure 3 The plasma (+)- to (−)-verapamil concentration ratio (mean value ± s.d.) following oral administration of 160 mg pseudoracemic verapamil. There was no trend in this ratio with time up to 12 h postdose.

Table 1 Pharmacokinetic data and absolute bioavailability of D(+)-, L(−)- and pseudoracemic (±) verapamil following oral administration of 160 mg pseudoracemic verapamil.

Subject		C_{max} *(ng/ml)*	t_{max} *(min)*	$t_{½,z}$ *(h)*	CL_o *(l/min)*	F *(%)*
	(+)	201.4	30	3.47	2.11	41
1	(−)	36.8	30	5.53	9.10	13
	(±)	238.2	30	3.79	3.26	32
	(+)	349.1	30	3.80	1.10	65
2	(−)	49.9	30	4.51	7.20	18
	(±)	399.0	30	3.90	1.93	39
	(+)	130.9	30	4.03	2.41	33
3	(−)	24.0	30	4.95	10.22	16
	(±)	154.9	30	4.29	3.65	33
	(+)	275.6	60	4.07	1.21	64
4	(−)	64.6	60	4.99	5.10	34
	(±)	320.2	60	6.06	1.80	(45)b
	(+)	246.6	30	5.29	1.75	47
5	(−)	55.2	30	7.68	5.70	26
	(±)	301.8	30	5.73	2.43	(38)b
	(+)	240.7	—	4.03a	1.72	50·
		(34)	—		(33)	(28)
Mean	(−)	46.1 **	—	5.38a	7.46 **	20 *
		(34)	—		(29)	(31)
(CV%)	(±)	282.8	—	4.58a	2.61	38
		(32)	—		(31)	(14)

a; Harmonic mean
b; *F* in parentheses were calculated according to the equation. $1/F = 0.606\ CL_o + 1.128$
c; Coefficient variation
C_{max}; maximum plasma concentration
t_{max}; time of C_{max}
$t_{½,z}$; terminal half-life
CL_o; apparent oral clearance
F; absolute bioavailability
$^*P < 0.005$, $^{**}P < 0.001$

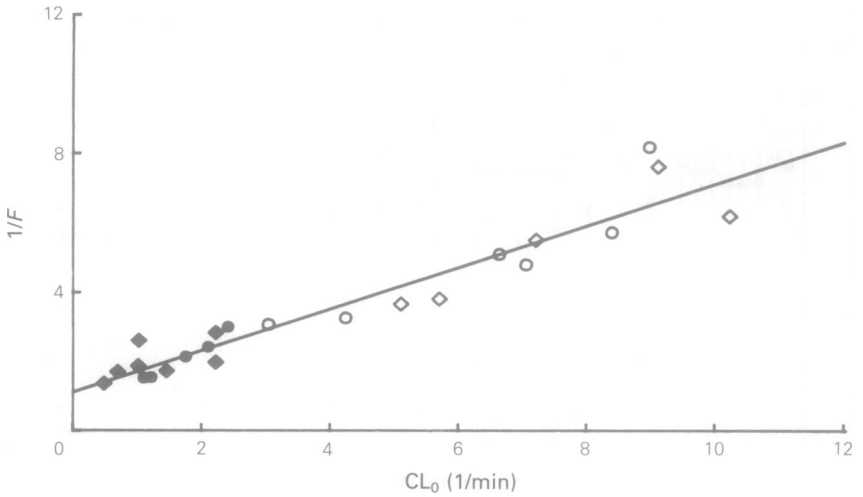

Figure 4 Plot of reciprocal of bioavailability ($1/F$) *vs* the apparent oral clearance (CL_o) for verapamil. Data include: racemic (○)-, (+) (●)- and (−) (◇)-verapamil in normal subjects as well as racemic verapamil (◆) in patients with liver cirrhosis.

et al., 1982; Eichelbaum *et al.*, 1980, 1981 a,b, 1984; McAllister & Kirsten 1982).

Previous studies have demonstrated that the same concentration of plasma verapamil following oral administration appeared to be two to three times less potent than that following i.v. administration with regard to the negative dromotropic effects on AV conduction (Eichelbaum *et al.*, 1980; McAllister *et al.*, 1982; Reiter *et al.*, 1982). This finding prompted us to hypothesize that the first-pass metabolism of verapamil might be stereoselective. The more active (−)-isomer of verapamil was assumed to be preferentially eliminated during first-pass metabolism (Eichelbaum *et al.*, 1980). The present study demonstrated stereoselective differences in the first-pass metabolism of the (+)- and (−)-isomer following single oral administration of racemic verapamil. Following oral administration of a pseudoracemic preparation containing equal amounts of each isomer larger AUC_o values for the (+)-isomer than those for the (−)-isomer were observed. The CL_o of the (−)-isomer was four to five times greater than that of the (+)-isomer. These findings might be anticipated from the results of our recent study which demonstrated that the plasma clearance of the (−)-isomer (*ca.* 1400 ml/min) was almost two times greater than that of the (+)-isomer (*ca.* 800 ml/min) (Eichelbaum *et al.*, 1984). The absorption of verapamil following oral administration of an aqueous solution has been shown to be almost complete (Schomerus *et al.*, 1976). Only traces of unchanged verapamil were recovered in urine (Eichelbaum *et al.*, 1979). Since the plasma clearance of racemic verapamil is high (*ca.* 1000 ml/min) and approaches liver blood flow, verapamil exhibits an extensive hepatic first-pass metabolism. This was supported by the finding of an hepatic extraction ratio of > 0.8 determined by direct measurement of plasma verapamil concentrations in arterial and hepatic venous blood (Woodcock *et al.*, 1981). Furthermore, we have observed that the construction of a mesocaval shunt in a patient with liver cirrhosis resulted in a substantial increase in F of verapamil from a preshunt value of 38% to 82% (Eichelbaum *et al.*, 1980). These data indicate that the liver is the site of the stereoselective first-pass metabolism.

It has been shown that stereoselective first-pass metabolism also occurs with propranolol in man (von Bahr *et al.*, 1982; Silber & Riegelman 1980); the more potent (−)-isomer exhibiting less extensive first-pass metabolism. Since (−)-propranolol is almost exclusively responsible for the β-adrenoceptor blocking effects (Barrett & Cullum, 1968), stereoselective first-pass metabolism would lead to the finding that an equivalent concentration of plasma propranolol following oral administration is more potent than that following i.v. administration. However, since the slopes of the concentration-effect curves for propranolol are relatively flat, it might be difficult to detect clinically meaningful differences in potency following i.v. and oral administration.

Somogyi *et al.* (1982) proposed an equation to predict F of highly cleared drugs with reasonable accuracy. Since their original equation for verapamil was obtained from a limited

number of subjects (n = 6), we added the present data as well as those obtained from patients with liver cirrhosis (Somogyi *et al.*, 1981) to the original data. The results of the analysis demonstrated an excellent linear relationship between $1/F$ and CL_o. This finding further validated the original concept. The F value of the racemate as well as of the isomers can be estimated from CL_o.

In a recent study we demonstrated that the volume of distribution for the (−)-isomer was two times greater than that of the (+)-isomer (Eichelbaum *et al.*, 1984). Therefore, the plasma concentration of the (+)-isomer was two times higher than that of the (−)-isomer following i.v. administration of racemic verapamil. In contrast, due to stereoselective first-pass metabolism the plasma (+)-isomer concentration was five times greater than that of the (−)-isomer following oral administration. Therefore, following oral dosing total verapamil concentrations consists of a smaller proportion of the more potent (−)-isomer. Thus, it would appear that an equivalent plasma verapamil concentration after oral administration appears to be less potent than that after i.v. administration. Since the plasma (+)- to (−)-verapamil ratio is different following i.v. and oral administration, the therapeutic concentrations following single and multiple oral dosing should not be extrapolated from those determined following single i.v. administration.

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