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Assessment of bioavailability of experimental single-unit sustained release tablets of verapamil hydrochloride using the stable isotope technique

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A stable isotope technique has been used to assess the bioavailability of sustained release verapamil products. The test formulations were tablets with a core containing 90 mg of verapamil hydrochloride coated with ethylcellulose film, the permeability of which was controlled using different amounts of hydroxypropyl methylcellulose. A product containing ethylcellulose 75% hydroxypropyl methylcellulose 25% w/w gave a single-unit sustained release tablet of verapamil hydrochloride that allowed a dose interval of 24 h. There was no loss in bioavailability, even though verapamil had extensive first-pass metabolism.

Verapamil is an anti-arrhythmic calcium ion antagonist which is usually administered as conventional tablets containing 40–120 mg three times daily. Therapeutically, less frequent dosing would be desirable; the drug, as hydrochloride, is readily soluble in water and its elimination half-life is short enough for it to be a suitable candidate for a sustained release formulation. On the other hand, it undergoes extensive first-pass metabolism which is often regarded as a disadvantage for a drug in sustained release form (Notari 1980).

We have attempted to develop an adequate sustained release preparation by coating tablets with a variety of ethyl cellulose films containing hydroxypropyl methylcellulose, the details of which and results of in-vitro studies have been reported by Kannikoski (1984). In the present text we describe the biopharmaceutical behaviour of these formulations as evaluated in man given a reference tablet containing a stable isotope of verapamil simultaneously with the test preparation.

Verapamil exhibits extensive first-pass metabolism and saturation of the first-pass effect may cause an increase in bioavailability with a sustained release product as shown with methoxalen, e.g. Schmid et al (1980). However, Eichelbaum et al (1981) have shown that no problem arises in this respect with verapamil if doses are as low as those we used.

MATERIALS AND METHODS

Drug products

A rapidly disintegrating, uncoated tablet containing 40 mg of deuterium-labelled verapamil (d_7-verapamil) hydrochloride was used as a reference tablet. The sustained release products (I, II and III) were of the single-unit type. Core tablets containing 90 mg of verapamil hydrochloride were coated with ethyl cellulose film (3 mg cm^{-2}), the permeability of which was controlled using different amounts of hydroxypropyl methylcellulose. The proportions of ethylcellulose/hydroxypropyl methylcellulose in the coatings were: I 60/40, II 70/30 and III 75/25% w/v. The core tablets were composed of 10 parts verapamil hydrochloride, 10 parts lactose and 1 part gelatin. The $t_{50\%}$-values for the formulations in a rotating paddle dissolution test using water as medium were, for the reference tablet 0·07 h, for I 1·0 h, for II 2·7 h and for III 4·3 h (Kannikoski 1984).

Absorption test

Volunteers, 7 female, 10 male, aged 19 to 33 years, 55 to 77 kg were informed about possible side effects and their written consent was obtained. Routine haematological tests showed that all subjects had values within normal range. Groups of three subjects were used for each formulation studied, in single dose tests. Drug administration was at 8 am, all subjects having fasted overnight, for at least 10 h. Each subject took one tablet of the reference preparation and one tablet of the test formulation simultaneously (40 mg + 90 mg of verapamil hydrochloride), with 100 ml of tap water. Food was subsequently withheld for 3 h. Blood samples (10 ml) were collected immediately before dosing, following the time schedule shown in Fig. 1. Serum was separated and frozen (−18 °C) for assay of the drug. The steady state test using formulation III was carried out in five volunteers who on the first day

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took the preparations as in the single-dose tests but from the second to the sixth days only one tablet of the test formulation was taken, at 8 am, blood samples being taken on the sixth day.

Measurement of drug concentrations

Verapamil, d_7-verapamil (labelled in the isopropyl group) and norverapamil (as hydrochlorides) and d_6-verapamil (labelled in the aromatic rings) and d_6-norverapamil (both as free bases) were provided by the Orion Pharmaceuticals (Espoo, Finland). The amounts of undeuterated compounds in d_6-preparations were less than 2% and in d_7-verapamil less than 1%. No d_7-verapamil was available as pure reference material.

Assay

To 1 ml of serum 20 ng of internal standards (d_6-analogues) were added in 50 µl of methanol. After 15 min the sample was made alkaline (pH = 9·6) with aqueous ammonia, vortexed (4 min) with 4 ml of freshly distilled diethyl ether and the ether extract vortexed with 2 ml of 0·1 M HCl for 1 min. The ether layer was discarded, and the aqueous phase was made alkaline (pH = 10) then vortexed (2 min) with 4 ml of diethyl ether. The ether extract was evaporated to dryness in nitrogen at 40 °C and the residue dissolved in 30 µl of methanol. Aliquots of 1–5 µl were analysed using GC/MS.

Serum samples for calibration curves were processed as above after adding the appropriate amounts of verapamil, d_7-verapamil and norverapamil (as the hydrochlorides) in 50 µl of water.

The sample extracts were analysed by GC/MS with a selected ion monitoring technique (Carlo Erba 2300 gas chromatograph coupled via a jet separator to a JEOL JMS-D300 mass spectrometer). The column (1 m × 2 mm 2% SE-30) was held at 285 °C. Ion currents were monitored at m/z 303 (the base peak in the mass spectrum of verapamil), at m/z 310 (d_7-verapamil), at m/z 306 (d_6-verapamil), at m/z 289 (norverapamil), at m/z 296 (d_7-norverapamil) and at m/z 292 (d_6-norverapamil). The electron energy used was 70 eV and the ionization current 300 µA.

Quantitation of analytes was by using the Mass Fragmentographic program of the JEOL JMA-2000 Data System. Calibration curves for verapamil, d_7-verapamil and norverapamil were constructed daily using peak heights in the concentration ranges 0·5–5 and 5–50 ng ml^{-1} of serum. For the concentration range 0·5–5 ng ml^{-1}, 2 ng of internal standard were used. Two calibration curves were prepared to maintain linearity of calibration (correlation coefficient typically >0·999). Quantitation of d_7-norverapamil was achieved using the calibration curves for norverapamil. This was considered to be correct since no significant difference was observed between the calibration curves for verapamil and d_7-verapamil.

Precision after studying replicate spiked serum samples at the level of 5 ng ml^{-1} was for verapamil and d_7-verapamil 4% (c.v., n = 6) and for norverapamil 6% (c.v., n = 6). Recovery for verapamil and d_7-verapamil was 96 ± 8% (n = 6) and for norverapamil 92 ± 11% (n = 6). Detection limits were about 0·2 ng ml^{-1}.

Pharmacokinetic calculations

In calculations of times of the peak concentration (t_{max}), of peak concentrations (C_{max}) and of minimum serum concentrations (C_{min}) the measured values for each subject were used. The absorption rate constant (k_a), its half-life ($t_{½a}$) and the time lag relating to absorption (t_{lag}) were calculated using the back-projection technique and a two-compartment, open model.

The rate constant of the terminal disposition phase (λ_z) and its half-life (elimination half-life, $t_{½}$) was calculated from the serum levels of the isotope d_7 4, 6, 8, 12 and 24 h after dosing. The areas under the concentration-time curves (AUC_{0-24h}) were calculated by the trapezoidal method and extrapolated to infinity ($AUC_{0-\infty}$). The relative bioavailability of the test product was obtained by comparing the AUC-value with the AUC-value of the simultaneously administered reference tablet. Corrections were made for the differences in drug amounts in the preparations during calculations. Statistical evaluations were carried out using the paired *t*-test.

RESULTS AND DISCUSSION

Initially, the in-vivo characteristics of the reference tablet were compared with those of an aqueous solution of verapamil hydrochloride. The concentration-time curves are shown in Fig. 1 and the calculated pharmacokinetic parameters in Table 1. In every subject, absorption was faster and C_{max} values higher after the water solution than after the reference tablet. However, no significant differences in AUC values were found.

The difference between the mean t_{lag} values of the two formulations was 8 min, which is an approximation of the in-vivo dissolution time of the reference tablet. The difference between the $t_{½a}$ values was 3 min. Thus, the formulation used as the reference tablet can be regarded as a rapid release formulation.

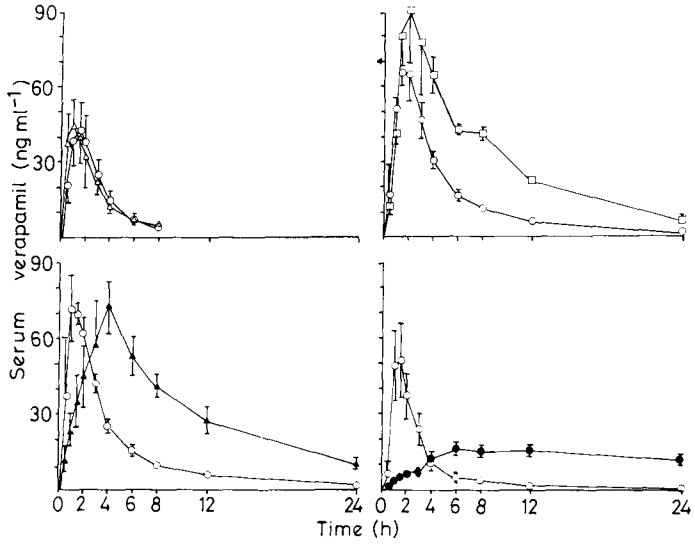

FIG. 1. Absorption of verapamil and its stable isotope d_7 after oral single dose in healthy volunteers. Each point represents a mean ± s.e.m. (n = 3). Key: ○ = reference tablet containing 40 mg of isotope d_7 of verapamil hydrochloride; △ = aqueous solution containing 40 mg of verapamil hydrochloride; □ = sustained release tablet I (90 mg); ▲ = sustained release tablet II (90 mg); ● = sustained release tablet III (90 mg).

The rate of absorption of verapamil is so high that it should be possible to regulate the whole absorption phase through controlled release of the drug.

Considerable inter-individual variability in the pharmacokinetics of verapamil after oral dosing has been reported (Koike et al 1979; Eichelbaum et al 1981; Woodcock et al 1982; Asthana et al 1984). We, too, noticed the same phenomenon. The pharmacokinetic parameters in Table 2 were obtained from the 14 subjects who took both the reference tablet and a sustained release product simultaneously.

Table 1. Pharmacokinetic parameters of verapamil administered simultaneously as an uncoated tablet (containing isotope d_7) and as an aqueous solution. The amount of drug in both was 40 mg of verapamil hydrochloride. Means ± s.e.m., n = 3.

Parameter	Aqueous solution	Tablet
k_a h^{-1}	4·44 ± 0·71	3·39 ± 1·04
$t_{\frac{1}{2}a}$ min	9 ± 1	12 ± 2
t_{lag} min	13 ± 1	21 ± 2
$AUC_{0-\infty}$ ng ml^{-1} h	128 ± 40	129 ± 38

The values in Table 2 are in agreement with the earlier reports. We found on average, 5-fold differences in C_{max} values and in AUC values and the AUC values (Table 1, Table 2) significantly ($P < 0·001$) differed from each other although the three subjects in Table 1 were also among the 14 subjects

Table 2. The pharmacokinetic parameters of verapamil administered in rapidly disintegrating tablets. Oral single dose of 40 mg of d_7-verapamil hydrochloride (n = 14). Also shown are the pharmacokinetic parameters of norverapamil after administration of rapidly disintegrating reference tablets containing 40 mg of verapamil hydrochloride isotope d_7. In the paired *t*-test, the values of norverapamil were compared with the simultaneously measured values for unchanged verapamil (n = 14).

	Verapamil		Norverapamil		
Parameter	Range	Mean ± s.e.m.	Range	Mean ± s.e.m.	*t*-test
t_{max} (h)	1–2	1·5 ± 0·4	1–2	1·9 ± 0·2	$P<0·05$
C_{max} (ng ml^{-1})	20–101	58 ± 6·5	15–52	36 ± 3	$P<0·001$
AUC_{0-24} (ng ml^{-1} h)	87–421	221 ± 30	82–444	283 ± 26	$P<0·01$
$AUC_{0-\infty}$ (ng ml^{-1} h)	88–455	237 ± 32	85–473	297 ± 28	$P<0·01$
$t_{\frac{1}{2}}$ (h)	2·5–8·0	5·3 ± 0·4	2·5–8·0	5·7 ± 0·3	$P>0·05$

in Table 2. Woodcock et al (1982) have reported differences in C_{max} values as great as 17-fold after a single dose of 80 mg of verapamil. Accordingly, it is difficult, and may even be misleading, to draw conclusions from traditional absorption studies of verapamil formulations if the numbers of subjects are low and a crossover design has not been possible.

One way of minimizing variation and allowing more reliable conclusions about bioavailability is to use a stable isotope-labelled drug as an internal standard (Wolen & Gruber 1980). We used a rapidly disintegrating d_7-verapamil tablet for this purpose, and assumed linear pharmacokinetics.

Fig. 2 has been generated from the results of 14 subjects simultaneously administered with the refer-

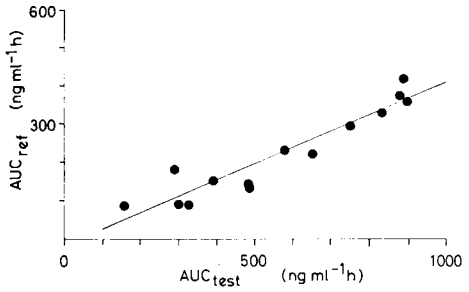

FIG. 2. Linear correlation between the $AUC_{0-\infty}$ values of the simultaneously-administered reference tablet and the sustained release tablets in 14 healthy volunteers. Regression line: y = 0·464 x −14·1; r = 0·924.

ence tablet and some of the three sustained release formulations. The equation of the regression line was y = 0·464 x −14. Statistical evaluation revealed a highly significant linear correlation ($P < 0{\cdot}001$) between the $AUC_{0-\infty}$ values of the reference tablet and the test tablets. In Kendall's rank order correlation test, the z-value was 0·00026, which also denotes a highly significant correlation. It was therefore considered that conclusions could justifiably be drawn using the present experimental procedure. The slope of the regression line was 0·464, which is very near to the ratio of the doses (40 mg : 90 mg = 0·444). This also indicates that no loss in bioavailability has occurred with the sustained release products as compared with the reference tablet.

Table 3. Pharmacokinetic parameters of verapamil after single dose of 90 mg in sustained release formulation III. Bioavailability was calculated from the $AUC_{0-\infty}$-value of the simultaneously ingested isotope tablet.

Subject	t_{max} h	C_{max} ng ml^{-1}	AUC_{0-24} ng ml^{-1} h	$AUC_{0-\infty}$ ng ml^{-1} h	Bioavailability %
1	8	37·6	445	478	152
2	6	33·6	308	329	163
3	4	37·7	451	481	158
4	8	20·6	266	288	70
5	8	12·5	150	162	82
Mean	6·8	28·4	324	348	125
s.e.m.	0·8	5·1	57	60	20

Serum concentrations of norverapamil, the active metabolite of verapamil, were also measured and the pharmacokinetic parameters are shown in Table 2. The t_{max} and AUC values for norverapamil were higher than those for verapamil. In contrast, the C_{max} value was lower than the corresponding value for verapamil. The difference in the elimination half-life was not statistically significant. These observations are in agreement with those of Kates (1983).

Drug release from test formulation I was only slightly retarded (dissolution $t_{50\%}$ 1·0 h). As shown in Fig. 1, this was not enough to cause any marked changes in-vivo compared with the reference tablet. The relative bioavailability of I was 104 ± 11% (mean ± s.e.m.). The dissolution $t_{50\%}$ of II was 2·7 h. With formulation II, a clear retardation in absorption was evident from the delayed t_{max} and lower C_{max} values (Fig. 1). The relative bioavailability of II was 106 ± 1%. When formulations I and II were evaluated on the basis of norverapamil concentrations, the conclusions were the same as those reached on the basis of verapamil concentrations.

The dissolution $t_{50\%}$ of III was 4·3 h, the concentration-time curve obtained in the absorption study (Fig. 1) showed the mean verapamil serum levels between 4 and 24 h to be in the range 11·2 to 15·8 ng ml^{-1}. Relative bioavailabilities were 93 ± 6% on the basis of 0 to 24 h data and 123 + 12% on the basis of extrapolation to ∞ compared with the values of the rapidly disintegrating isotope tablet.

To obtain more definite information about the in-vivo properties of formulation III, five more subjects were included in the absorption study; the results of single-dose tests are shown in Table 3. Drug administration in these five volunteers was continued for six days (see Table 4). Figs 3 and 4 show the concentration-time curves for verapamil and norverapamil after both the first and sixth doses.

The results in Tables 3 and 4 and Figs 3 and 4 confirm the findings after the single-dose study in three subjects. Between 4 and 24 h, the mean verapamil level fluctuated between 8·1 and

Table 4. Pharmacokinetic parameters of verapamil after the sixth dose of 90 mg in sustained release formulation III. Bioavailability was calculated from the AUC_{0-24}-value of the isotope tablet ingested simultaneously with the first dose.

Subject	t_{max} h	t_{min} h	C_{max} ng ml^{-1}	C_{min} ng ml^{-1}	C_{max}/C_{min}	AUC_{0-24} ng ml^{-1} h	Bioavailability %
1	8	0	47·7	9·8	4·9	714	227
2	6	0	32·5	7·0	4·6	460	228
3	12	1	20·0	5·8	3·4	376	123
4	6	0	13·4	5·1	2·6	224	54
5	6	0	16·7	3·6	4·6	201	102
Mean	7·6	0·2	26·1	6·3	4·0	395	147
s.e.m.	1·2	0·2	6·3	1·0	0·4	93	35

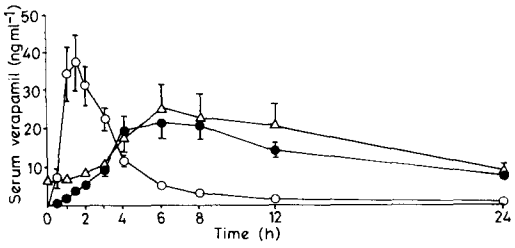

FIG. 3. Concentration-time curves of verapamil and its isotope d_7 after peroral administration. Each point represents a mean ± s.e.m. Key: ○ = reference tablet (40 mg) on first day (n = 8); ● = sustained release tablet III (90 mg) on first day (n = 8); △ = sustained release tablet III on sixth day (n = 5).

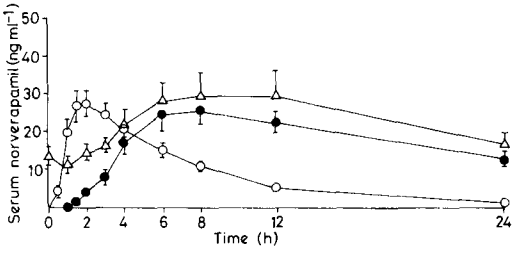

FIG. 4. Concentration-time curves of norverapamil and its isotope d_7 after peroral administration of verapamil hydrochloride. For explanation see Fig. 3.

21·7 ng ml^{-1} (after single doses) and between 9·1 and 25·2 ng ml^{-1} (sixth doses). The corresponding values for norverapamil were 13·1 to 25·9 ng ml^{-1} and 16·8 to 29·5 ng ml^{-1}. The relative bioavailability of verapamil was 124 ± 13% in the single dose study and 147 ± 35% after the sixth dose. The corresponding values for norverapamil were 99 ± 7% and 98 ± 10%. None of these values differed significantly ($P > 0·05$) from 100%. Thus, it is evident that there is no decline in bioavailability if verapamil is administered in a sustained release formulation, even though it has large first-pass metabolism. This is in accordance with results of Bhamra et al (1983).

It has also been claimed that drug released from the formulation 8 to 12 h after oral administration cannot be utilized in-vivo (Koch-Weser & Schechter 1981; Welling 1983). However, our results show that absorption of verapamil must also have occurred 8 and even 12 h after dosing. If there was no absorption after 8 h, the calculated mean drug levels at 24 h would be 2·5 ng ml^{-1} (single-dose) or 2·8 ng ml^{-1} (sixth dose). The measured concentrations were 8·1 and 9·1 ng ml^{-1}, or more than three times higher than the calculated levels. If there was no absorption after 12 h, the corresponding concentrations at 24 h would be 3·4 and 4·8 ng ml^{-1}, i.e. the levels found were some two-fold higher than those calculated. It is evident that absorption of verapamil has also occurred after 8 or 12 h. With the present procedure it is not possible to say from which part of the GI-tract absorption has occurred.

It has also been claimed that use of a single-unit to deliver a drug at a controlled rate cannot lead to once daily treatment without loss of bioavailability unless the drug has a long biological half-life (Beckett 1983). However, in our study, single-unit products were used, the biological half-life of verapamil was 5·3 h, but no loss in bioavailability was noted.

CONCLUSIONS

The stable isotope technique for assessment of bioavailability was successfully applied to verapamil formulations. Although inter-individual variation in the pharmacokinetics of verapamil was considerable, good correlations in relation to intraindividual values of, e.g. AUC were obtained.

No loss in bioavailability was noted with the sustained release tablets, even though verapamil is a drug with a substantial first-pass metabolism.

The results show that it is possible to make a single-unit sustained release tablet for administration at 24 h from verapamil hydrochloride.

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