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Single- and Multiple-dose Mibefradil Pharmacokinetics in Normal and Hypertensive Subjects

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Abstract

Mibefradil is a single-enantiomer calcium antagonist belonging to a new class, the tetralol derivatives. The recommended doses for treatment of hypertension and chronic stable angina pectoris are 50 or 100 mg. Mibefradil is metabolized via parallel pathways of esterase-catalysed hydrolysis and cytochrome P450 3A4-mediated oxidation. Mibefradil also inhibits cytochrome P450 3A4 and consequently inhibits its own metabolism, a property illustrated by three studies performed early in the drug's development.

After single intravenous doses of 2·5 to 80 mg to healthy male subjects, pharmacokinetics are linear. At the representative 40 mg intravenous dose mean pharmacokinetic parameters were clearance 241 ± 76 mL min^{-1}, terminal exponential half-life 15·0 h and volume of distribution at steady state 213 L. After single oral doses of 10 to 320 mg, reduced first-pass metabolism occurs with increasing dose. This effect is accompanied by increasing absolute bioavailability as the dose is increased. In an oral multiple-dose study of healthy male volunteers mibefradil doses of 100, 150 or 250 mg (from tablets) were administered once daily for 28 days. Reduction of the first-pass effect was noted, although the data suggested that a maximum was reached for doses of 150 mg or more. In a study of the effect of hypertension on mibefradil pharmacokinetics, 12 patients received oral mibefradil once daily at doses of 50, 100, 150, or 200 mg in 100 mL orange juice for 8 days. Steady state was reached within 3 days and accumulation generally ranged from three- to sevenfold. Single-dose non-linearity was observed in the first-pass effect, although for multiple dosing oral clearance values were dose-independent and lower than for single doses. After multiple dosing at the recommended dosage of 50 and 100 mg once daily, oral clearance of mibefradil stabilizes to approximately the same value for both doses. Hence, the single-dose non-linearity has little clinical relevance but demonstrates the self-inhibition of metabolism seen with mibefradil.

Studies so far suggest that self-inhibition of its oxidative metabolic pathway leads to a low clearance and long half-life, enabling once-daily dosing and conferring low intra- and inter-patient variability in pharmacokinetics.

Mibefradil, a tetralol derivative, is a new single-enantiomer calcium antagonist administered once daily at 50 or 100 mg for the treatment of hypertension or chronic stable angina pectoris. Mibefradil selectively inhibits T-type Ca^{2+} channels, in contrast with established calcium antagonists that block L-type calcium channels only (Mishra & Hermsmeyer 1994).

The drug is metabolized via parallel pathways which can be divided into two broad categories—esterase-catalysed hydrolysis and cytochrome P450 3A4 (CYP3A4)-mediated oxidation (*O*-demethylation, *N*-demethylation and ring-hydroxylation). In addition to being a substrate for CYP3A4, mibefradil is a CYP3A4 inhibitor. The extent of plasma-protein binding, predominantly to α_1-acid glycoprotein, is approximately 99·5%. Most of the pharmacodynamic activity of mibefradil is believed to reside in the intact compound.

This paper summarizes pharmacokinetic results for mibefradil from five studies: a single-dose intravenous and oral study in normal subjects; a multiple-dose comparative bioequivalence study comparing five dosage forms; a single-dose pharmacokinetic and relative bioavailability study of the 50- and 100-mg tablet formulations of mibefradil; an oral multiple-dose study using once-daily doses of 100, 150 or 250 mg for 28 days in healthy

subjects; and an 8-day multiple-dose study using 50, 100, 150 or 200 mg once-daily doses in hypertensive patients.

Methods

Pharmacokinetic analyses were conducted by model independent methods (Gibaldi & Perrier 1982). All studies used accurate and specific high-performance liquid-chromatography (HPLC) for determination of plasma mibefradil. All studies were approved by local human-experimentation committees and were in compliance with the Declaration of Helsinki and its amendments. Written informed consent was obtained from all participants.

Single-dose studies in normal subjects
Thirty healthy males, 18–35 years, ±20% of ideal body weight, participated in this ascending single-dose study. After overnight fast intravenous doses of 2·5, 5, 10, 20, 40 or 80 mg were infused over a period of 30 min. Model independent parameters of CL (clearance), $t_{1/2z}$ (terminal exponential half-life), and V_{SS} (volume of distribution at steady state) were determined.

Forty-two subjects participated in the oral studies; many of these had also participated in the intravenous study. Subjects did not necessarily receive the same intravenous and oral doses. After overnight fast subjects were given mibefradil dissolved in 100 mL orange juice. Absolute bioavailability (F) was calculated for subjects who received both intravenous and oral doses.

Multiple-dose comparative bioequivalence study
A randomized, five-way, complete cross-over study was used to investigate the comparative bioequivalence of four mibefradil formulations in 25 male volunteers: four × 6·25 mg tablets, one × 25 mg tablet, two × 25 mg tablets, one × 50 mg tablet, and 43 mg in solution in orange juice. The average (standard deviation) age and weight of the subjects were 28·6 ± 8·0 years and 75·6 ± 11·6 kg, respectively; races were 23 white, 1 black, 1 other. Each treatment was administered once daily for 7 days. Trough blood samples for accumulation monitoring were obtained on days 5 and 6. Complete steady-state profiles were obtained on day 7, with continued sampling on day 8 (up to 48 h post-dose). Solid dosage forms were administered with 240 mL tap water; the 43 mg of drug in 100 mL orange-juice solution was ingested with an additional 140 mL tap water. After overnight fast, dosing on day 7 was at 0700 h, with breakfast served at approximately 0930 h.

Single-dose pharmacokinetic and relative bioavailability study
Twenty-four healthy white men, 21 to 40 years, participated in this three-way, cross-over randomized study. The volunteers received each of three treatments as a single dose in 100 mL water: two × 50-mg tablets (reference formulation); two × 50-mg tablets (commercial formulation); one × 100-mg tablet (commercial formulation). Doses were given on study days 1, 8 and 15 to accommodate a wash-out period between treatments. Blood samples (5 mL) were collected before administration of the dose on the day of drug intake and 15 and 30 min and 1·5, 2, 3, 4, 6, 8, 12, 16, 24, 33 and 48 h thereafter. Standard methods were used to calculate 90% confidence intervals for the ratio between the test and reference formulations (Schuirmann 1987).

Oral multiple-dose study in normal subjects
In this 28-day, multiple-dose oral study using tablets given once daily, doses of 100, 150 or 250 mg were administered to 3–5 subjects at each dosage level. Healthy, non-smoking male subjects, 32·4 ± 5·4 years, 73·7 ± 6·7 kg (mean ± standard deviation), received all doses as 50-mg tablets taken with 240 mL tap water after overnight fasting. After the first dose 48-h plasma mibefradil concentration–time profiles were constructed. Dosing was re-instated after collection of the 48-h blood sample for a total of 28 doses. After dose 28, mibefradil plasma concentration–time profiles were obtained over 48-h periods.

Single- and multiple-dose study in hypertensive patients
To investigate intra-subject single- and multiple-dose mibefradil pharmacokinetics, investigators gave 12 hypertensive patients oral mibefradil once daily at doses of 50, 100, 150 or 200 mg in 100 mL orange-juice solutions for 8 days. Doses were administered after overnight fast, with standardized light meals served 4 h post-dose. Complete plasma concentration–time profiles were obtained on days 1 and 8.

Results and Discussion

Model-independent parameters for the single-dose studies in normal subjects are summarized in Table 1. Oral clearance (CL/F) is clearly dose-dependent (decreasing at higher doses). Values of F for the 10-, 20-, 40-, and 80-mg oral doses were calculated on the basis of area analysis with the respective intravenous dose data of 2·5, 5, 10 and 20 mg. Mean F values were 35·9%, 55·2%, 73·8% and

Table 1. Mibefradil pharmacokinetic parameters after 30-min intravenous infusion and single-oral-dose administration.

Dose (mg)	n	Route	CL (mL min^{-1})	CL/F (mL min^{-1})	t½ (h)	V_{SS} (L)	C_{max} (ng mL^{-1})	t_{max} (h)	F (%)
2·5	6	Intravenous	275 ± 92	–	14·9	255 ± 130	–	–	–
5·0	6	Intravenous	303 ± 75	–	14·6	220 ± 61	–	–	–
10	6	Intravenous	321 ± 93	–	13·4	241 ± 128	–	–	–
	6	Oral	–	1190 ± 397	11·2	–	26·7 ± 13	1·00 ± 0·42	35·9 ± 29
20	6	Intravenous	285 ± 54	–	12·3	196 ± 65	–	–	–
	6	Oral	–	642 ± 147	13·3	–	67·8 ± 14·5	0·96 ± 0·53	55·2 ± 18·6
40	4	Intravenous	241 ± 76	–	14·8	213 ± 40	–	–	–
	6	Oral	–	712 ± 149	9·8	–	115 ± 32·4	0·86 ± 0·14	73·8 ± 56·7
80	4	Intravenous	251 ± 33	–	10·3	176 ± 19	–	–	–
	6	Oral	–	369 ± 124	14·9	–	61·0 ± 50	0·87 ± 0·34	70·4 ± 28·7
120	6	Oral	–	265 ± 58	13·2	–	495 ± 57·9	1·38 ± 0·54	–
160	6	Oral	–	179 ± 62	17·1	–	777 ± 137	1·50 ± 0·32	109 ± 15·5
320	6	Oral	–	162 ± 20	16·9	–	1490 ± 218	2·00 ± 0·56	91 ± 1·6

Data are means ± s.d. n—number of subjects; CL—clearance; t½—half-life; V_{SS}—steady state volume of distribution; C_{max}—maximum plasma mibefradil concentration; t_{max}—time to C_{max}; F—bioavailability.

70·4%, respectively. The Tukey test was unable to detect differences between pharmacokinetic parameters as a function of dose. Visual inspection of the parameters also indicated the absence of dose-dependent kinetics. The observation that intravenous systemic clearance is unaffected by dose indicates that this oral phenomenon is a first-pass effect. As the oral mibefradil dose increases, first-pass saturable metabolism occurs, increasing F, which in turn reduces oral clearance. One postulated mechanism is that mibefradil, by virtue of its ability to inhibit CYP3A4, might inhibit its own hepatic first-pass metabolism, with or without linkage to the P-glycoprotein transporter system (Wacher et al 1995). Another possibility is that mibefradil might inhibit CYP3A4 activity in the gut wall (Wu et al 1995), thus increasing its own bioavailability.

Pharmacokinetic parameters for the multiple-dose comparative bioequivalence study are summarized in Table 2. Standard methods were used to calculate 90% confidence intervals for the ratio between the test and reference formulations (Schuirmann 1987). In accordance with US Food and Drug Administration guidelines (Chen & Patnaik 1992), two × 25-mg tablets were assumed to be bioequivalent to the one × 50-mg tablet and four × 6·25-mg tablets were assumed to be equivalent to a 25-mg tablet. The bioavailability of the 25-mg doses relative to that of the 50-mg doses was only 50%. As noted previously, this is consistent with first-pass dose-dependent kinetics for the 25- and 50-mg doses (Figure 1). Because all doses were normalized to 50 mg, the enhanced area under the plasma concentration–time curve (AUC) values associated with the 50-mg doses is indicative of non-linearity. However, 25 mg is not a recommended dose for clinical use.

The AUC values for the solution were lower than those for the 50-mg doses. This is not uncommon, because solid dosage forms often contain excipients that enhance disintegration and dissolution, increase wetability, and inhibit first-pass drug-metabolizing enzymes.

Pharmacokinetic parameters for all three formulations in the single-dose pharmacokinetic and relative bioavailability studies were almost identical. Mean maximum plasma concentrations (C_{max}) of 617, 641 and 643 ng mL^{-1}, respectively, were found 1·6–1·8 h (t_{max}) after drug intake. The

Table 2. Mibefradil once-daily 7-day pharmacokinetic parameters from a five-way cross-over bioequivalence study.

Dose	CL/F (mL min^{-1})	$AUC_{0\rightarrow\infty}$ (ng h mL^{-1})	$t_{\frac{1}{2}}$ (h)	C_{max} (ng mL^{-1})	C_{min} (ng mL^{-1})	t_{max} (h)	$C_{SS,ave}$ (ng mL^{-1})
4 × 6·25-mg tablets	237 ± 100	2846 ± 1270	14·1 ± 2·98	182 ± 61·4	38·9 ± 21·2	2·3 ± 1·2	86·6 ± 35·4
1 × 25-mg tablet	238 ± 109	2875 ± 1643	12·7 ± 2·90	185 ± 72·7	37·6 ± 24·3	2·1 ± 1·2	88·0 ± 40·3
43-mg solution	147 ± 59	8518 ± 3924	15·2 ± 4·39	399 ± 122	122 ± 57·3	2·5 ± 1·4	232 ± 82·7
2 × 25-mg tablets	120 ± 37	12388 ± 4125	18·2 ± 4·94	536 ± 147	184 ± 63·6	2·6 ± 1·1	312 ± 85·1
1 × 50-mg tablet	111 ± 35	13535 ± 4333	18·2 ± 4·19	588 ± 151	195 ± 68·5	2·6 ± 1·4	340 ± 94·3

Data are means ± s.d. (n = 25). CL/F = oral clearance; $t_{\frac{1}{2}}$ = half-life; $AUC_{0\rightarrow\infty}$ = area under the plasma concentration-time curve; C_{max} = maximum plasma mibefradil concentration; C_{min} = minimum plasma concentration; t_{max} = time of C_{max}; $C_{SS,ave}$ = average steady state plasma concentration.

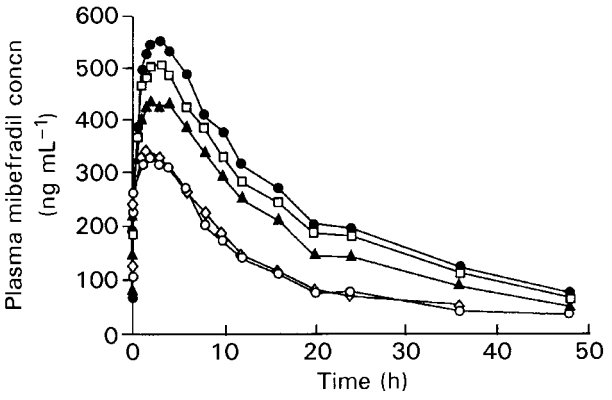

Figure 1. Mean steady-state and fall-off mibefradil plasma concentration–time profiles after five single oral doses: ●, 1 × 50-mg tablet; □, 2 × 25-mg tablet; ▲, 1 × 43-mg solution; ○, 1 × 25-mg tablet; ◇, 4 × 6·25-mg tablet. Data on the ordinate were normalized to a 50-mg dose by multiplying by plasma concentration (50 mg mg^{-1} administered dose). For a system with dose proportionality all curves would have been superimposable. Note that comparative bioavailability from the 25-mg subtherapeutic doses is approximately 50% that from the 50-mg doses.

mean elimination half-lives (t½) were 14·7, 15·4 and 15·2 h, respectively, and the $AUC_{0\rightarrow\infty}$ were 9940, 10400 and 10500 ng h mL^{-1}, respectively. CL/F ranged from 181 to 192 mL min^{-1}, and the volume of distribution (Vd/F) from 243 to 248 L. All three formulations were bioequivalent with regard to C_{max} and AUC. The pharmacokinetic parameters determined in this study were in good agreement with those measured in other studies after single oral doses of 100 mg mibefradil.

Table 3 summarizes the non-compartmental pharmacokinetic parameters for the oral multiple-dose study of normal subjects. With the 100-mg dose mean CL/F decreased substantially between dose 1 to dose 28. With the 150- and 250-mg doses, CL/F for dose 28 was no lower than for dose 1. These findings indicate that mibefradil pharmacokinetics are concentration-dependent irrespective of whether the concentration results from a single large dose or smaller multiple doses.

Table 4 summarizes the pharmacokinetic parameters obtained from the single- and multiple-dose studies of hypertensive patients. Trough plasma concentrations indicated that the steady state was generally reached within 1 week. After multiple dosing oral clearance was dose-independent, because of self-inhibition of metabolism. Mean multiple-dose oral clearance values were all lower than for corresponding single doses (Figure 2). With multiple dosing at the recommended dosages of 50 and 100 mg once daily oral clearance of mibefradil stabilizes to approximately the same value for both doses, hence the single-dose non-linearity has little clinical relevance but indicates the self-inhibition of metabolism that is seen with mibefradil.

These five studies characterized the pharmacokinetics of mibefradil mostly in normal volunteers. Single oral doses covered a range from 10 to 320 mg and multiple oral doses ranged from 25 to 250 mg once daily for 28 days. The influence of impaired renal function, impaired liver function and concomitant administration of certain other drugs on the pharmacokinetics of mibefradil have been investigated in other studies. Population pharmacokinetic analysis of data from 315 patients with hypertension did not provide evidence of a clinically relevant influence of age, race or body weight on the pharmacokinetics of mibefradil (Welker & Banken 1998).

Mibefradil emerges as having a pharmacokinetic profile different from that of other calcium antagonists. Because of self-inhibition of CYP3A4-mediated oxidation, with multiple dosing metabolism of mibefradil is dependent on de-esterification through esterases (a high-capacity metabolic system) and not via the CYP-mediated pathway. As a result, predicted oral bioavailability is approximately 80%, higher than for most other calcium antagonists (Kelly & O'Malley 1992). Oral clearance of mibefradil at steady state for the recommended doses of 50 and 100 mg is approximately

Table 3. Mibefradil pharmacokinetic parameters after 28 days of once-daily dosing with 100-, 150- or 250-mg, as 50-mg tablets, with 240 mL water.

Dose	Dosing day	CL/F (mL min^{-1})	$AUC_{0\rightarrow 24\,h}$	$t_{\frac{1}{2}}$ (h)	C_{max} (ng mL^{-1})	C_{min} (ng mL^{-1})	t_{max} (h)
100 mg	1	216 ± 80·9	–	17·0 ± 6·76	520 ± 130	–	2·33 ± 0·58
n = 3	28	138 ± 38·0	12574 ± 3039	35·4 ± 3·93	718 ± 150	393 ± 112	2·33 ± 1·16
150 mg	1	181 ± 25·6	–	17·9 ± 1·51	739 ± 40·4	–	1·17 ± 0·29
n = 3	28	180 ± 62·5	14850 ± 4315	37·4 ± 11·6	914 ± 70·3	594 ± 192	1·67 ± 1·26
250 mg	1	134 ± 27·5	–	23·9 ± 9·81	1322 ± 251	–	2·33 ± 0·99
n = 5	28	157 ± 22·7	26967 ± 4033	38·5 ± 7·21	1506 ± 163	886 ± 191	2·20 ± 1·10

Data are means ± s.d. CL/F = oral clearance; $t_{\frac{1}{2}}$ = half-life; $AUC_{0\rightarrow 24\,h}$ = area under the plasma concentration–time curve; C_{max} = maximum plasma concentration; C_{min} = minimum plasma concentration; t_{max} = time of C_{max}.

Table 4. Mibefradil single- and multiple-dose pharmacokinetic parameters for hypertensive patients after once-daily oral doses of 50, 100, 150 or 200 mg for 8 days.

Dose (mg)	Dosing day	CL/F ($mL\,min^{-1}$)	t½ (h)	V_{area} ($L\,kg^{-1}$)	C_{max} ($ng\,mL^{-1}$)	t_{max} (h)
50	1	445±183	11·8±3·34	5·70±2·63	220±76·0	0·938±0·496
	8	173±71*	18·6±4·54*	3·41±1·19*	402±185*	1·72±1·75
100	1	218±116	20·5±7·02	4·10±1·76	478±145	1·09±0·344
	8	126±41*	25·0±7·69	5·47±1·52*	929±196	1·62±0·443*
150	1	233±153	16·0±4·75	3·47±1·26	952±586*	1·54±1·04
	8	119±37*	34·0±11·1*	4·32±1·12	1373±371	2·14±1·66
200	1	174±751	19·4±4·03	3·62±1·30	1116±329	1·67±0·661
	8	155±442	24·4±3·71*	4·28±0·824	1538±433*	1·33±0·443

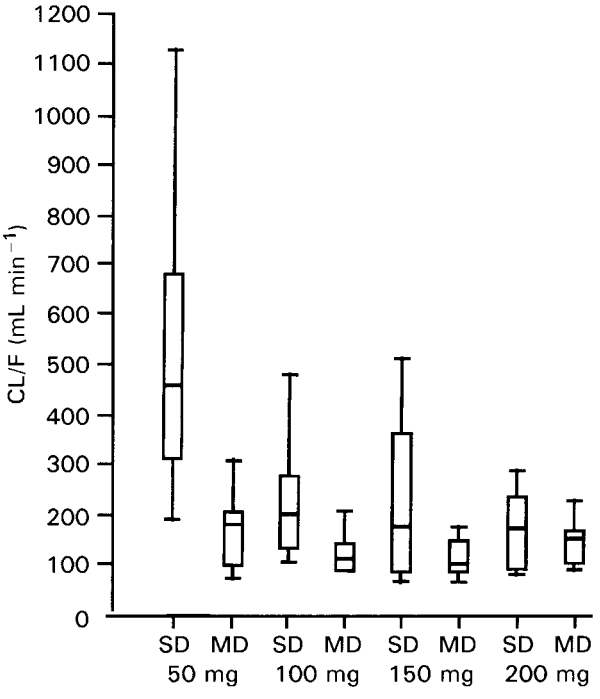

Figure 2. Box-whisker plot of mibefradil oral clearance (CL/F) for doses of 50–200 mg in hypertensive patients on day 1 (single dose = SD) and day 8 (multiple dose = MD). The two horizontals of each box show the range within which the middle 50% of all values lie, i.e. between the first and third quartiles. The horizontal lines outside the box (whiskers) represent the lowest and highest values and define the range.

150 $mL\,min^{-1}$. Low clearance with a coefficient of variation of approximately 35% leads to a narrow range of plasma mibefradil concentrations for a given dose in most of the population. All other calcium antagonists have larger clearances (Kelly & O'Malley 1992). At steady state mibefradil has a long half-life of 17–25 h; this enables once-daily dosing without the requirement for slow-release formulations.

The inhibition by mibefradil of CYP3A4 and CYP2D6 does reduce the metabolizing capacity for drugs metabolized by these CYP isozymes. As a consequence, mibefradil, like other drugs including many calcium antagonists, has the potential to cause clinically significant alterations in the pharmacokinetics of some concurrently administered drugs.

In summary, with multiple doses of 50 to 100 mg mibefradil orally once daily the time of maximum plasma concentration was reached in approximately 2·4 h, absolute bioavailability was predicted to be approximately 80%, clearance was 95–125 $mL\,min^{-1}$, oral terminal exponential volume of distribution was 180 L and terminal exponential half-life was 22 h (range 17–25 h).

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