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PHARMACOKINETICS OF REPEATED SINGLE ORAL DOSES OF ENALAPRIL MALEATE (MK-421) IN NORMAL VOLUNTEERS

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ABSTRACT

Enalapril, the ethyl ester of a potent angiotensin converting enzyme inhibitor, enalaprilat, was administered to healthy volunteers as a capsule containing 10 mg of the maleate salt, every 24 h for eight doses. Serum profiles show little accumulation of enalaprilat following eight daily doses of enalapril maleate. An average effective half-life for accumulation of approximately 11 h was calculated from urine data. Comparison of observed 24-h urinary recoveries of enalaprilat to predicted steady-state recovery indicates that an 'average' steady state for enalaprilat is attained by the third or fourth dose of enalapril maleate. Statistical comparison of daily urinary recoveries, as well as C_{min} values for enalaprilat, confirm this. Observed fluctuations in serum and urine data during apparent steady state suggest some day-to-day variability in the absorption of enalapril maleate and/or its hydrolysis to enalaprilat. An accumulation ratio of 1·3 for enalaprilat was calculated from the predicted steady-state urinary recovery and observed urinary recovery for dose one.

KEY WORDS Enalapril maleate Enalaprilat Steady state Accumulation

INTRODUCTION

Enalapril maleate (MK-421) is the maleate salt of the ethyl ester of enalaprilat (N-[S]-1-carboxy-3-phenylpropyl]-L-Ala-L-Pro), a potent angiotensin-converting enzyme inhibitor, with antihypertensive activity both in laboratory animals[1, 2] and man.[3–6] In contrast to enalaprilat, enalapril maleate is well absorbed orally, and is rapidly and extensively hydrolysed to the active diacid.[7] Following oral administration of a 10 mg capsule of enalapril maleate to normal

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volunteers in three separate studies,[7, 8, *] mean urinary recovery of total drug (enalaprilat plus enalapril, expressed as enalaprilat equivalents) was 60–77 per cent of the administered enalaprilat equivalents, while mean recovery of enalaprilat was 43–56 per cent of the administered enalaprilat equivalents. The ratio of enalaprilat to total drug in urine was approximately 0·70. There was no detectable metabolism of enalapril beyond its hydrolysis to enalaprilat. Peak serum concentrations of total drug occurred at approximately 1 h, while peak serum concentrations of enalaprilat occurred 3–4 h post drug administration. Serum profiles of enalaprilat were polyphasic with a prolonged terminal phase. Low concentrations of enalaprilat were detected as late as 72 h after drug administration. Following intravenous administration of enalaprilat (2·5, 5, and 10 mg) to healthy subjects,[9] serum concentration curves for enalaprilat were also polyphasic, with a prolonged terminal phase; all doses converged to the same terminal serum concentrations. Area under the serum concentration–time curve (AUC) was linearly related to dose with a positive intercept which was eliminated by factoring out the extrapolated terminal phase from the total AUC.

The purpose of the present study was to evaluate the impact of the observed terminal serum phase for enalaprilat on accumulation of enalaprilat in normal subjects receiving single daily doses of enalapril maleate 10 mg p.o. for 8 days.

METHODS

Experimental protocol

Twelve healthy male volunteers between the ages of 20 and 23, weighing between 60 and 103 kg, participated in this study. Subjects were judged to be in good health on the basis of history, physical examination, ECG, and routine laboratory data (haematology, blood chemistry, urinalysis). Subjects with a history of cardiac disease, renal insufficiency or other renal disorder, gastrointestinal disease (including GI ulcers and GI intolerance to drugs), subjects who used drugs regularly, had a history of drug and/or alcohol abuse, and subjects who had previously taken captopril were excluded.

After an overnight fast, one 10 mg enalapril maleate capsule was administered orally to each subject with 250 ml of water at 8 am daily, in the clinic, for 8 days.

On days 1 and 8, serum samples were obtained at 0 (predrug), 0·5, 1, 2, 3, 4, 6, 12, 15, 22, and 24 h. Additional serum samples were obtained at 36, 48, 60, 72, and 96 h after the eighth dose. On days 2–7, a predrug serum sample was obtained. Urine collections were −1–0, 0–1, 1–2, 2–4, 4–8, 8–12, and 12–24 h for day 1, 0–24 h for days 2–7, and 0–1, 1–2, 2–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, and 96–120 h after the eighth dose.

* Unpublished data.

Assay procedures

Enalaprilat in serum, and enalaprilat and total drug in urine were determined by radioimmunoassay. The method, described elsewhere,[8, 10] is specific for enalaprilat. 'Total drug' refers to measurement of enalaprilat following enzymic hydrolysis of any intact enalapril, and represents the sum of enalapril and enalaprilat expressed in terms of enalaprilat equivalents. Lower limits for reliable measurement are 0·36 ng ml^{-1} for serum and 36 ng ml^{-1} for urine. The interassay coefficient of variation is 6–8 per cent.

Data analysis

The value of half-life lies in the determination of drug accumulation upon multiple dosing. The effective half-life for accumulation is that half-life consistent with observed accumulation. This half-life may or may not be (and often is not) obtainable from the plasma concentration profile following drug administration. For a given (known) dosing regimen, an estimate of the effective half-life for accumulation can be made from the mean plasma concentration, AUC, or urinary recovery obtained for two dosing intervals during chronic drug administration. The ratios of the respective parameters for the two dosing intervals are measures of the rate of drug accumulation. Further, the effective half-life so obtained can be used to predict the average steady-state parameters and the average time course of accumulation by another regimen. Based on these concepts as presented by Kwan *et al.*,[11] effective half-life for accumulation, steady-state urinary recovery of enalaprilat, and accumulation ratio were calculated from the following relationships:

$$\frac{U_\tau^n}{U_\tau^1} = \frac{1-e^{-n\omega\tau}}{1-e^{-\omega\tau}} \quad (n = 2, 3, ..., 8) \tag{1}$$

$$U_\tau^{ss} = \frac{U_\tau^n}{1-e^{-n\omega\tau}} \quad (n = 2, 3, ..., 8) \tag{2}$$

$$R = \frac{U_\tau^{ss}}{U_\tau^1} \tag{3}$$

$$\bar{\omega} = \sum_{i=1}^{(n-1)} \omega_i \Big/ (n-1) \tag{4}$$

$$\bar{\omega}_{t_{\frac{1}{2}}} = \frac{0{\cdot}693}{\bar{\omega}} \tag{5}$$

where U_τ^n, U_τ^1, and U_τ^{ss} are the urinary recoveries of enalaprilat for the nth dosing interval, the first dosing interval, and a dosing interval at steady state, respectively; τ is the dosing interval and n is the number of doses; ω is the rate constant associated with the effective half-life for accumulation; $\bar{\omega}_{t_{\frac{1}{2}}}$ is the effective half-life for accumulation, averaged over all doses; and R is the accumulation ratio for enalaprilat at steady state.

Changes in minimum serum concentration of enalaprilat and urinary recovery of enalaprilat and total drug were analysed using the paired *t*-test; the results were corroborated with the Wilcoxon signed-rank test. All tests were two-tailed at the $\alpha = 0{\cdot}05$ level. Standard regression analysis techniques were used to evaluate the trends over days in the above parameters. It was verified that no significant interaction existed between the subject and day effects; hence, the model included only different intercepts for the various subjects as well as the overall linear and quadratic effects over time.

RESULTS

Figure 1 contains the mean 0–24-h serum profiles of enalaprilat for dose 1 and dose 8. This figure suggests that there is little accumulation of enalaprilat following eight daily doses of enalapril maleate. Also included in the figure are the mean trough serum concentrations (C_{min}) of enalaprilat for doses 2–6 (C_{min}, dose 7 equals 0 h, dose 8; C_{min}, doses 1 and 8 are their respective 24-h values). Mean serum and urine parameter values are in Table 1. Mean C_{min} increased significantly from dose 1 to 2 to 3, then levelled off through the seventh dose with a slope not significantly different from zero. The C_{min} value for day 8, however, was lower than for day 7 ($p = 0{\cdot}06$). Figure 2 contains the mean 0–96-h serum profile for dose 8. The prolonged terminal phase seen in prior single-dose studies (p.o. administration of enalapril maleate, i.v. administration of enalaprilat)[7–9] is also evident here.

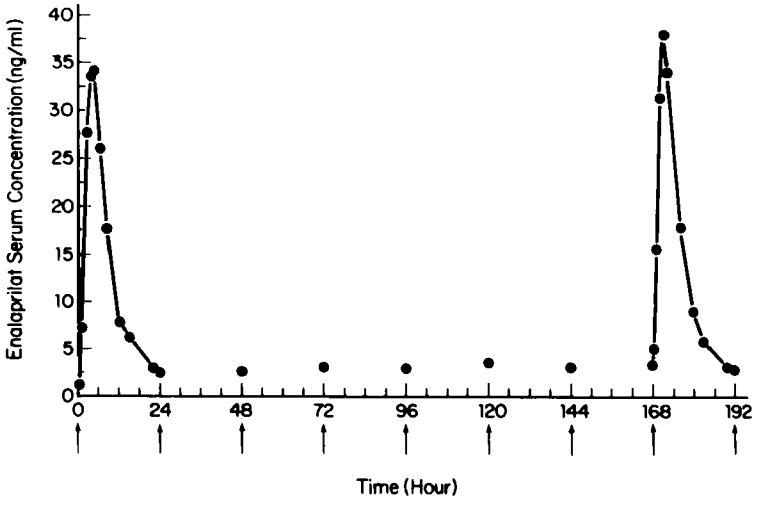

Figure 1. Mean enalaprilat serum profile following once daily administration (↑) of enalapril maleate 10 mg capsules p.o. for eight doses. Minimum values (C_{min}) only are shown for doses 2–7

Table 1. Mean serum and urine parameters for enalaprilat and total drug following multiple doses of enalapril maleate (1 × 10 mg capsule q.d. × 8) in 12 normal subjects

	Day (dose)							
	1	2	3	4	5	6	7	8
C_{min} (ng ml^{-1})*‖	2·4	2·6	3·1‡	3·0	3·6	3·1‡	3·4	2·9
Urinary recovery (mg)†¶								
Enalaprilat	2·41	3·28	3·15‡	3·06	3·13‡	3·53‡	3·03	2·67§
Total drug	3·69	4·84	4·54‡	4·23	4·40‡	5·00‡	4·39	3·95§

* Trough serum concentration of enalaprilat (24 h post drug administration).
† For a dosing interval, i.e. 0–24 h.
‡ $n = 11$.
§ $n = 10$.
‖ Significant increase from dose 1 to 2 to 3 ($p < 0·05$); no significant differences for third to seventh dose.
¶ Significant increase from dose 1 to 2 ($p < 0·05$); no significant differences from second to seventh dose.

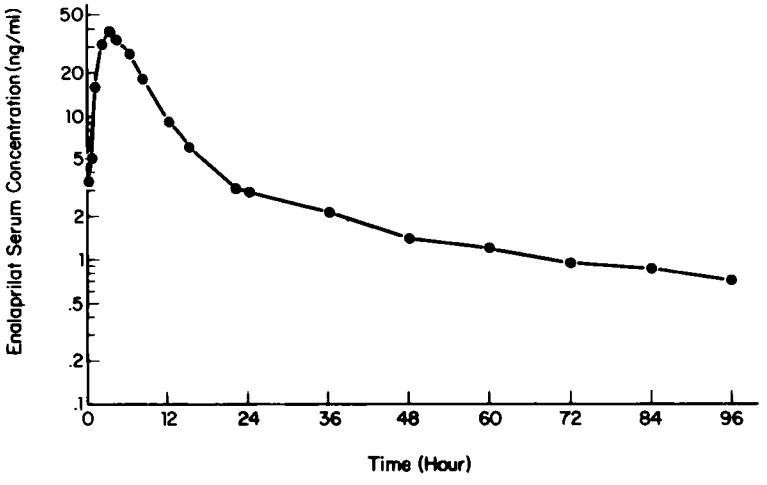

Figure 2. Mean enalaprilat serum profile following the eighth dose of enalapril maleate 10 mg capsules, administered once daily (q24 h)

Mean urinary recovery of enalaprilat increased significantly from day 1 to day 2 and levelled off through day 7 (slope not significantly different from zero). As with C_{min}, the urinary recovery value for day 8 was lower than for day 7 ($p = 0·07$). Urinary recovery of enalaprilat for day 8 (0–24 h), expressed as per cent of administered enalaprilat equivalents, was less ($p < 0·05$) than total recovery of enalaprilat, expressed as per cent of total (all doses) enalaprilat equivalents administered (Table 2). The pattern of urinary recovery of total drug (enalaprilat plus enalapril, expressed as enalaprilat equivalents) is similar to that seen for enalaprilat.

Mean urinary recoveries reported in Table 1 were used to calculate a rate constant for accumulation (ω) for doses 2–8 (compared to the first dose) according to equation (1). Steady-state urinary recoveries (U_τ^{ss}) and accumulation ratios (R) were also predicted (equations (2) and (3)). Values for these calculated parameters are given in Table 3. An average effective half-life for accumulation of 11·1 h was calculated from equations (4) and (5).

Table 2. Mean urinary recovery* of enalaprilat and total drug for dose 8 and all doses combined (total recovery)

	Recovery dose 8 (0–24 h)	Total recovery (All doses)†
Enalaprilat¶	39‡	45§
Total drug**	58‡	62‖

* Expressed as per cent of administered enalaprilat equivalents (based on dosage form assay value).
† From zero hour dose 1 to 96 or 120 h following dose 8.
‡ $n = 10$.
§ $n = 9$.
‖ $n = 8$.
¶ Significant difference between dose 8 and total, $p < 0{\cdot}05$.
** Difference between dose 8 and total, $p = 0{\cdot}062$.

Table 3. Calculated accumulation parameters and predicted steady-state urinary recovery

Day	U_τ^{obs}* (mg)	ω (h^{-1})	U_τ^{ss} (mg)	R	$\bar{\omega}_{t_{\frac{1}{2}}}$ (h)
1	2·41	—	—	—	
2	3·28	0·0425	3·77	1·56	
3	3·15	0·0584	3·20	1·33	
4	3·06	0·0642	3·07	1·27	
5	3·13	0·0611	3·13	1·30	
6	3·53	0·0477	3·53	1·46	
7	3·03	0·0661	3·03	1·26	
8	2·67	0·0970	2·67	1·11	
Mean		0·0624	3·20	1·3	11·1

* U_τ^{obs} = mean urinary recovery of enalaprilat (mg) for a dosing interval (see Table 1).

DISCUSSION

Following a single oral dose of enalapril maleate,[8] a terminal half-life for enalaprilat of approximately 35 h was estimated from the 48- and 72-h serum concentrations. Serum profiles following i.v. administration of enalaprilat[9] exhibited similarly prolonged terminal half-lives (*c.* 36 h). Based on observations

in the i.v. study in humans and further work in animals,* it is postulated that the terminal phase of the enalaprilat serum profile represents binding of enalaprilat to angiotensin-converting enzyme and that this binding involves a fixed amount of drug, regardless of the dose administered. If this is so, the terminal phase would effectively have a fixed or 'one-time' contribution to accumulation, unlike the additive contribution of a linear process with successive doses; conventional determination of a half-life predictive of accumulation is precluded. The present study, however, allows the estimation of an effective half-life consistent with the observed accumulation of enalaprilat in subjects with normal renal function following chronic dosing of enalapril maleate. From the effective half-life (11·1 h) so obtained, one would predict that steady state for enalaprilat would be reached in 2–3 days, or after two or three daily doses of enalapril maleate.

Comparison of steady-state urinary recovery (U_τ^{ss}) with observed urinary recovery (U_τ^{obs}) suggests that an underlying steady state was attained for enalaprilat by the third or fourth daily dose of enalapril maleate. This was confirmed by statistical analysis of C_{min} and urinary recovery data. The accumulation ratio indicates that on the average, a 1·3-fold accumulation of enalaprilat can be expected following repeated single (q24 h) doses of enalapril maleate.

It is obvious that the prolonged terminal phase of the enalaprilat serum profile observed following single i.v. doses of enalaprilat or oral doses of enalapril maleate contributes little to the accumulation of enalaprilat, and that steady-state predictions based on the half-life associated with this phase would not be valid. The effective half-life determined in this study, in fact, more closely approximates the half-life of the phase immediately preceding the terminal phase.

The approach to, and attainment of, a true steady state for a particular chemical species is characterized by (among other things): a progressive increase in C_{min} to a maximum value at steady state, with a constant C_{min} for subsequent doses; a progressive increase in urinary recovery for the compound for each successive dosing interval to a maximum recovery at steady state, with constant recovery for subsequent dosing intervals; equality between the per cent of administered dose recovered during a dosing interval at steady state and total recovery as a per cent of all doses administered. Although an underlying steady state for enalaprilat was observed in this study, deviations from theoretical steady-state conditions were seen as a drop in C_{min} and urinary recovery values on day 8, and a urinary recovery on day 8 which was less than total recovery, expressed as per cent of administered enalaprilat equivalents. These observed deviations likely reflect variability in the absorption and/or disposition of enalapril. Similar urinary recovery patterns for enalaprilat and total drug across doses indicate that this variability cannot be attributed solely to variability in hydrolysis of enalapril to enalaprilat, but that variable absorption may also be a

* Unpublished data.

contributing factor. The fact that day 7 was essentially an 'outpatient' day, while subjects were maintained in the clinic throughout day 8 may be related in some undetermined fashion to the above observation. Estimating the effective half-life for enalaprilat as an average overall dose minimized the influence of day-to-day variability in absorption or disposition of enalapril on this parameter and, thus, a half-life useful for predictive purposes was obtained.

REFERENCES

1. D. M. Gross, C. S. Sweet, E. H. Ulm, E. P. Backlund, A. A. Morris, D. Weitz, D. L. Bohn, H. C. Wenger, T. C. Vassil and C. A. Stone, *J. Pharmacol. Exp. Ther.*, **216**, 552 (1981).
2. C. S. Sweet, D. M. Gross, P. T. Arbegast, S. L. Gaul, P. M. Britt, C. T. Ludden, D. Weitz and C. A. Stone, *J. Pharmacol. Exp. Ther.*, **216**, 558 (1981).
3. H. Gavras, B. Waeber, I. Gavras, J. Biollaz, H. R. Brunner and R. O. Davies, *Lancet*, **2**, 543 (1981).
4. G. P. Hodsman, J. J. Brown, D. L. Davies, F. Fraser, A. F. Lever, G. D. Morton and J. I. S. Robertson, *Br. Med. J.*, **285**, 1697 (1982).
5. S. G. Chrysant, R. D. Brown, D. C. Kim and J. L. Brown, *Clin. Pharmacol. Ther.*, **33**, 741 (1983).
6. H. J. Gomez, V. J. Cirillo and K. H. Jones, *J. Hypertens.*, **1**, Suppl. 1, 65 (1983).
7. E. H. Ulm, *Drug. Metab. Rev.*, **14**, 99 (1983).
8. E. H. Ulm, M. Hichens, H. J. Gomez, A. E. Till, E. Hand, T. C. Vassil, J. Biollaz, H. R. Brunner and J. L. Schelling, *Br. J. Clin. Pharmacol.*, **14**, 357 (1982).
9. A. E. Till, J. D. Irvin, M. Hichens, R. B. Lee, R. O. Davies, B. Swanson and P. H. Vlasses, presented at *Amer. Soc. Clin. Pharmacol. Ther., 83rd Annual Meeting*, Lake Buena Vista, Florida, 17–20 March 1982.
10. M. Hichens, E. L. Hand and W. S. Mulcahy, *Ligand*, **4**, 43 (1981).
11. K. C. Kwan, N. R. Bohidar and S. S. Hwang, in *Pharmacokinetics—A Modern View*, L. Z. Benet and G. Levy (Eds.), Plenum, New York (in press).