Br. J. clin. Pharmac. (1982), **14**, 357–362

ENALAPRIL MALEATE AND A LYSINE ANALOGUE (MK-521): DISPOSITION IN MAN

E.H. ULM, M. HICHENS, H.J. GOMEZ, A.E. TILL, E. HAND, T.C. VASSIL, J. BIOLLAZ, H.R. BRUNNER & J.L. SCHELLING

Merck Sharp & Dohme Research Laboratories, West Point, PA and Rahway, NJ, USA and Department of Medicine, Centre Hospitalier Universitaire Lausanne, Switzerland

1 The disposition of two angiotensin converting-enzyme inhibitor drugs was studied in normal volunteers. One drug was enalapril maleate (MK-421), which requires *in vivo* esterolysis to yield active inhibitor (MK-422). The other was a lysine analogue of MK-422 (MK-521), which requires no bioactivation.

2 Absorption of enalapril maleate (10 mg, p.o.) was rapid, with peak serum concentrations of enalapril observed 0.5–1.5 h after administration. Based upon urinary recovery of total drug (enalapril plus MK-422), absorption was at least 61%. Bioactivation appeared to be largely post-absorptive. From the ratio of MK-422 to total drug in urine, the minimum extent of bioactivation was estimated at 0.7.

3 A similar dose of MK-521 was absorbed more slowly, reaching peak serum concentrations 6–8 h following drug administration. Minimum absorption, based upon urinary recovery, was 29%.

4 Serum concentration *v* time profiles for both drugs were polyphasic and exhibited prolonged terminal phases.

5 Recovery in urine and faeces of administered enalapril maleate (intact and as MK-422) was 94%. Recovery of MK-521 was 97%. These results indicate lack of significant metabolism of these agents, apart from the bioactivation of enalapril.

Introduction

Angiotensin converting enzyme inhibition has proved to be an effective therapy for many forms of hypertension (Gavras *et al.*, 1981; Horowitz, 1981).

Enalapril maleate (1-[*N*-[1-(S)-)ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butene dioate(1:1) salt), a relatively poor converting enzyme inhibitor, gives rise, via esterolysis, to MK-422, a very potent inhibitor (Figure 1, a and b) (Gross *et al.*, 1981; Patchett *et al.*, 1980; Tocco *et al.*, 1982). Unlike its ester, MK-422 is poorly absorbed when administered orally to animals (Tocco *et al.*, 1982). MK-521, a lysine analogue of MK-422 (Figure 1, c), is at least as potent a converting enzyme inhibitor *in vitro* as MK-422, and its oral absorption properties in animals are intermediate between MK-422 and enalapril (Merck Sharp & Dohme Research Laboratories, unpublished observations and Patchett *et al.*, 1980). Both enalapril maleate and MK-521 inhibit converting enzyme *in vivo* after oral administration to animals and man (Biollaz *et al.*, 1981; Gross *et al.*, 1981). In addition, enalapril maleate was demonstrated to be antihypertensive in man (Gavras *et al.*, 1981).

The present study was designed to study the disposition of enalapril maleate and MK-521 in man.

Methods

Twelve healthy male volunteers each received 10 mg capsules of enalapril maleate and MK-521 in an open, randomized, crossover design. Details of the procedures employed have been presented (Brunner *et al.*, 1981).

The dosage forms, supplied by Merck Sharp & Dohme Research Laboratories, Rahway, N.J., were size 3 opaque, white, hard gelatin capsules containing drug with lactose as diluent. Enalapril maleate capsules contained 9.8 mg of drug (20 μmol), which corresponded to 6.9 mg equivalents of MK-422. The

0306–5251/82/090357–06 $01.00

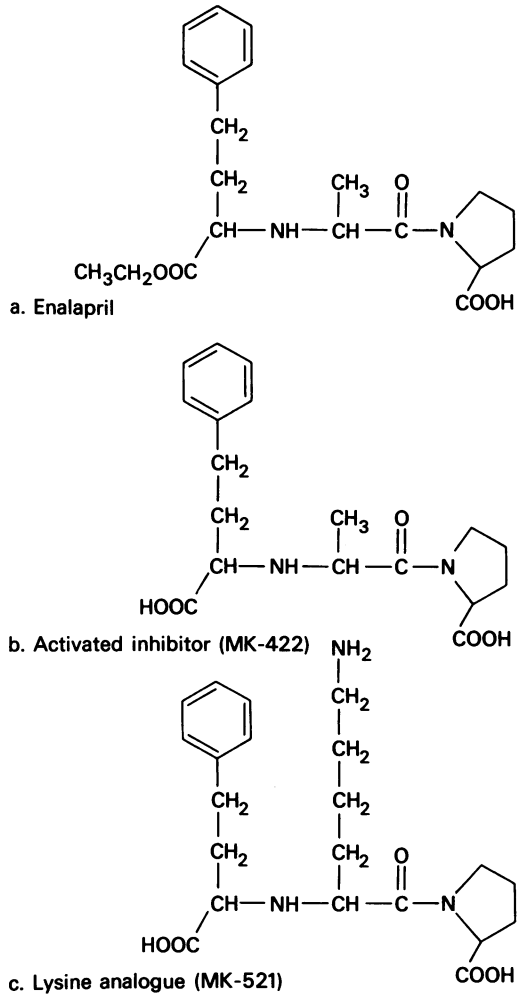

Figure 1 Chemical structure of agents.
a. Enalapril was administered as its 1:1 maleate salt. It gives rise to the corresponding converting enzyme inhibitor **b** upon esterolysis. MK-521, lysine analogue of MK-422, was administered as the free base.

lysine analogue capsules contained 10 mg of drug (25 μmol).

Serum, urine and faeces were collected at the indicated intervals post dose and stored frozen prior to analysis.

Drug measurements in serum and urine were made by radioimmunoassay (Hichens *et al.*, 1981). The assay employs an antiserum produced to a conjugate in which MK-521 is linked to albumin via a dinitrophenylene bridge, and a radio-iodinated derivative of MK-521. While MK-422 and MK-521 are almost equipotent, MK-421 lacks reactivity in the assay, but can be measured as MK-422 following esterolysis by a crude enzyme preparation from rat liver. The lowest reliably measured concentration of MK-422 (MW 348) is 0.4 ng/nl or 1.2 nM (85% of control binding), using 10 μl of serum per tube. Urines are diluted 100-fold prior to assays, affording a lower limit of 115 nM. Intra- and inter-assay percent coefficients of variation are respectively 6.0, 7.5 (7.2 nM); 4.5, 7.5 (72 nM); 7.0, 8.5 (430 nM) for samples assayed in triplicate.

Drug content of faeces was determined by isotope dilution with *in vitro* inhibition of angiotensin converting enzyme serving to quantitate drug (Tocco *et al.*, 1982). Faeces were homogenized in *circa* 2 volumes of ethanol using a Polytron homogenizer (Brinkman Instruments). As appropriate, either 4700 d min^{-1} ml^{-1} of MK-521-[^{14}C]-(2,3 phenylpropyl) or 66,500 $dmin^{-1}$ ml^{-1} enalapril maleate-[^{3}H]-(2,3 phenylpropyl) plus 2,440 d min^{-1} ml^{-1} MK-422-[^{14}C]-(2,3 phenylpropyl) was added to a 10 ml aliquot of homogenate.

After removal of the solids by centrifugation, a 1 ml aliquot of the supernatant, reduced to dryness *in vacuo*, was reconstituted with aqueous detergent (1% cutscum, Fisher Scientific, Pittsburgh, PA). Radioactivity was determined by liquid scintillation counting after combustion in a Packard B-306 sample oxidizer.

Mean ($\pm$ s.d.) recovery of added radioactive enalapril was 106 $\pm$ 6% (n = 76) and no corrections for recovery were made for this entity. For MK-422 and MK-521 where mean recoveries were 77 $\pm$ 9% (n = 76) and 76 $\pm$ 14% (n = 66), respectively, the individual recovery factors were used to calculate faecal content.

Results

The mean serum concentrations of enalapril and MK-422 following administration of enalapril maleate are plotted versus time in Figure 2. Relevant absorption and excretion parameters for enalapril maleate and MK-521 are presented in Table 1. The observed maximum serum concentration of enalapril occurred at 1 $\pm$ 0.03 h and that of MK-422, at 4 $\pm$ 1.5 h post dose. The serum profile for active inhibitor was polyphasic, with a prolonged terminal phase, MK-422 being detectable as late as 96 h post dose. From the 48 and 72 h data points, a terminal half-life of approximately 35 h was estimated.

Similarly, the mean serum concentration of MK-521 versus time (Figure 3) was polyphasic, with a prolonged terminal phase. The observed maximum serum concentration occurred at 7 $\pm$ 1.0 h post dose, with drug detectable at 96 h post dose. Serum concentrations at 48 and 72 h suggested a terminal half-life of approximately 30 h.

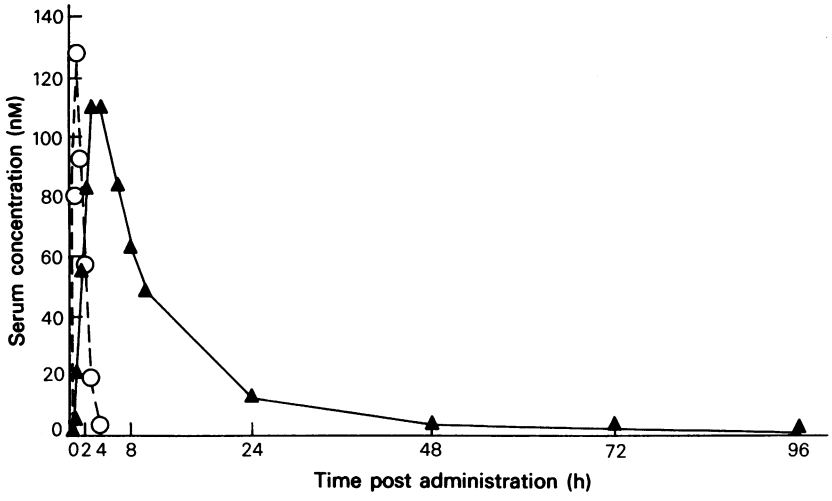

Figure 2 Mean serum concentrations of enalapril (○) and MK-422 (▲) *v* time after dose of 10 mg enalapril maleate (20 μmol) to twelve subjects.

Table 1 Summary of mean absorption and excretion parameters for enalapril maleate (20 μmol) and MK-521 (25 μmol) in 12 subjects.

	Enalapril maleate		*MK-521*
	Enalapril	*MK-422*	
Observed peak serum concentration (nM)	140(52)	116(50)	95(55)
range	77–232	54–220	36–216
Time to peak (h)	1(0.3)	4(1.5)	7(1.0)
Range	0.5–1.5	2–8	6–8
AUC_0^∞ nmol l^{-1} h	198(26)	1409(437)	1694(808)
Range		750–2121	866–3620
Renal clearance* (ml/min)	–	158(46)	106(13)
Range		97–256	77–127
Urinary recovery (% dose)	18(2)	43(10)	29(15)
Faecal recovery (% dose)	6(10)†	27(16)	69(23)
Total accountability	94(10)		97(15)

Values in parentheses are s.d.

*Renal clearance = $\frac{[\text{Urinary recovery}]_{2\,h}^{10\,h}}{AUC_{2\,h}^{10\,h}}$

† One sample missing; $n = 11$.

Mean urinary recovery of total inhibitor (MK-422 plus enalapril) was 61% of the administered dose of enalapril maleate, expressed as equivalents of MK-422. Mean recovery of MK-422 was 43%. The ratio of MK-422 to total inhibitor in the urine was 0.70. Mean urinary recovery of MK-521 was 29% of the dose. Mean renal clearances of MK-422 and MK-521 were 158 ± 46 and 106 ± 13.32 ml/min, respectively. Following enalapril maleate administration, a significant fraction of total drug excreted in the first urine collection interval was intact enalapril (Table 2). Intact enalapril also appeared in small quantities in the faeces of most subjects; and, although MK-422 comprised the major portion of drug in the faeces, one subject eliminated 47% of the dose as intact enalapril. Mean faecal recovery of total drug was 31%

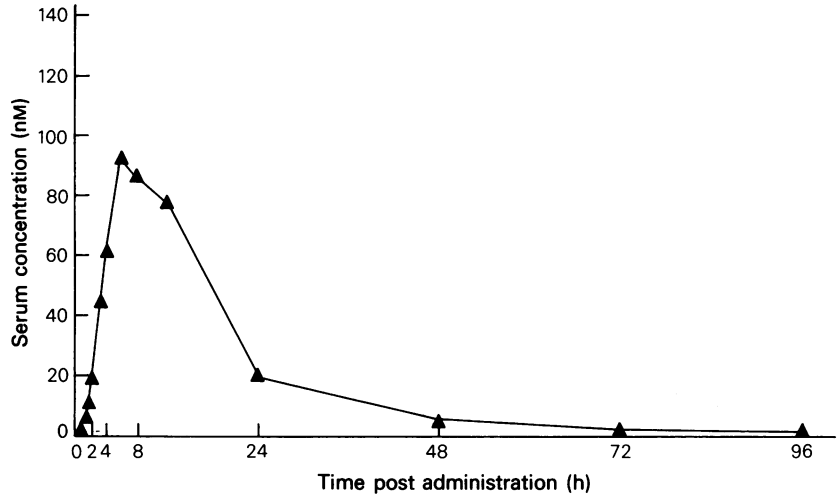

Figure 3 Mean serum concentrations of MK-521 *v* time after dose of 10 mg (25 μmol) to twelve subjects.

of the administered dose of enalapril maleate. Mean faecal recovery of MK-521 was 69% of the administered dose. Accountabilities of enalapril, intact and as MK-422, and MK-521 was nearly quantitative.

Discussion

Enalapril maleate, virtually inactive as a converting enzyme inhibitor, must be absorbed and bioactivated in order to inhibit converting enzyme *in vivo*. Biollaz *et al.* (1981) and Brunner *et al.* (1981) have presented evidence that enalapril maleate behaves pharmacologically in man as would have been predicted by animal studies and prior experience with captopril. However, the duration of the responses seen with enalapril was considerably longer than that of captopril. The pressor response to angiotensin I was found to be depressed, for the duration of their experiment (up to 5 h) and aldosterone and angiotensin II concentrations were lowered for at least 10 h following a dose of 10 mg (Brunner *et al*, 1981). Inhibition of plasma converting enzyme was virtually complete through 10 h. Compared to predose activity levels, significant inhibition persisted, even at 72 h post dose. MK-521, in the same studies, differed from enalapril in having a slower onset and a somewhat longer duration of activity. However, MK-521 was administered at a

Table 2 Urinary excretion of enalapril and MK-521 by collection period

	Time post dose (h)								
	0–2	*2–4*	*4–6*	*6–8*	*8–20*	*20–24*	*24–48*	*48–72*	*Total*
Enalapril									
Total drug*	2680 ±300	3130 ±260	1860 ±130	1290 ±60	880 ±50	1590 ±140	530 ±60	180 ±10	12150 ±670
MK-422	440 ±110	1900 ±250	1700 ±120	1300 ±60	880 ±50	1610 ±180	480 ±70	190 ±10	8520 ±570
Enalapril†	2240 ±250	1230 ±170	1600 ±80	–	–	–	–	–	3630 ±350
MK-521	20 ±20	520 ±510	940 ±550	1190 ±680	1030 ±560	2430 ±1260	710 ±210	210 ±80	7060 ±3620

Subjects ($n = 12$) received 20 μmol of enalapril maleate and 25 μmol of MK-521 in a random cross-over design.

* Total inhibitor after hydrolysis of enalapril to MK-422.

† Difference between 'total drug' and MK-422.

somewhat higher molar dose (25 μmol *v* 20 μmol of enalapril maleate), as noted previously.

Enalapril was rapidly absorbed after oral administration. At the earliest time points enalapril was the predominant form, indicating that a substantial portion was absorbed intact. Its subsequent hydrolysis to MK-422 was seen, with the observed peak concentrations of MK-422 lagging those of enalapril by an average 2.7 h.

With respect to plasma converting enzyme activity, it has been reported that about 80% inhibition was required to obtain significant blockade of an angiotensin I induced pressor response (Biollaz *et al.*, 1981; Brunner *et al.*, 1981). This level of inhibition occurred about 1 h after enalapril maleate administration and about 2 h after MK-521 (Biollaz *et al.*, 1982). From the serum concentration versus time profiles (Figures 2 and 3), the corresponding inhibitor concentrations were 20 and 18 nM. Serum concentrations of inhibitor at or above these levels were maintained for 19 and 22 h for enalapril maleate and MK-521, respectively. Even considering the difference in molar doses of the two agents in this study, it is clear that both are long acting.

The time course of MK-422 and MK-521 in serum closely paralleled their pharmacological and biochemical effects (Biollaz *et al.*, 1982). In addition to the results of Biollaz *et al* (1982), Swanson *et al.* (1981) reported an inverse relationship between plasma converting enzyme activity and the serum concentration of MK-422 in subjects who had received enalapril maleate.

Clearance of enalapril from serum was rapid and clearly due to more than one mechanism. Metabolic clearance (bioactivation to MK-422) and renal clearance were major routes. A significant fraction of the drug excreted in the first urine collection interval was intact enalapril.

A probable site of bioactivation of enalapril is the liver. Human plasma was reported to be devoid of enalapril esterolytic activity; whereas, human liver homogenates contained the requisite activity (Tocco *et al.*, 1982). Studies of the disposition of i.v. enalapril maleate in rats and dogs have indicated biliary excretion as another possible route of elimination (Tocco *et al.*, 1982). Whether this is operative in man must await appropriate studies. Clearance of MK-521 occurred via the urine and, based on available data, no other routes were implicated.

The excellent overall accountability in urine and faeces of MK-521 ruled out any significant metabolism either systemically or in the gut. Beyond bioactivation, enalapril appeared to undergo no other significant metabolism. The recovery of total inhibitor in urine and faeces was 94 ± 10%. Recovery of enalapril in some of the faeces samples indicated that enalapril also can escape metabolism in the gut. The relatively simple metabolic fate is in sharp contrast to that reported for the sulphhydryl-containing converting enzyme inhibitor, captopril (Ikeda *et al.*, 1981; Kripalani *et al.*, 1980; Singvi *et al.*, 1981). By virtue of its free sulphhydrul group, captopril forms disulphides with a variety of endogenous sulphhydryl-containing compounds, including proteins. These kinds of metabolites may have some importance with regard to the side effect profile of captopril (Igic *et al.*, 1981).

With the advent of enalapril maleate and MK-521, there exist potent converting enzyme inhibitors with metabolic stability. As a consequence of this stability, accurate determination of their concentration in biological samples can be made. They should therefore serve as valuable research tools in the study of the renin angiotensin system, as well as provide effective therapy for the treatment of some forms of hypertension.

References

BIOLLAZ, J., BURNIER, M., TURINI, G.A., BRUNNER, D.B., PORCHET, M., GOMEZ, H.J., JONES, K.H., FERBER, F., ABRAMS, W.B., GAVRAS, H. & BRUNNER, H.R. (1981). Three new long-acting converting-enzyme inhibitors: Relationship between plasma converting-enzyme activity and response to angiotensin I. *Clin. Pharmac. Ther.*, **29**, 665–670.

BIOLLAZ, J., SCHELLING, J.L., JACOT DES COMBES, B., BRUNNER, D.B., DESPONDS, G., BRUNNER, H.R., ULM, E.H., HICHENS, M. & GOMEZ, H.J. (1982). Enalapril maleate and a lysine analogue (MK-521) in normal volunteers; relationship between serum drug levels and the renin angiotensin system. *Br. J. clin. Pharmac.*, **14**, 363–368.

BRUNNER, D.B., DESPONDS, G., BIOLLAZ, J., KELLER, I., FERBER, F., GAVRAS, H., BRUNNER, H.R. & SCHELLING, J.L. (1981). Effect of a new angiotensin converting-enzyme inhibitor MK-421 and its lysine analogue on the components of the renin system in healthy subjects. *Br. J. clin. Pharmac.*, **11**, 461–467.

GAVRAS, H., BIOLLAZ, J., WAEBER, B., BRUNNER, H.R., GAVRAS, I. & DAVIES, R.O. (1981). Antihypertensive effect of a new angiotensin converting enzyme inhibitor MK-421. *Lancet*, **ii**, 543–547.

GROSS, D.M., SWEET, C.S., ULM, E.H., BACKLUND, E.P., MORRIS, A.A., WEITZ, D., BOHN, D.L., WENGER, H.C., VASSIL, T.C. & STONE, C.A. (1981). Effect of N-[(S)-1-carboxy-3-phenylpropyl]-L-ala-L-pro and its ethyl ester

(MK-421) on angiotensin converting enzyme *in vitro* and angiotensin pressor responses *in vivo*. *J. Pharmac. exp. Ther.*, **216**, 552–557.

HICHENS, M., HAND, E.L. & MULCAHY, W.S. (1981). Radioimmunoassay for angiotensin converting enzyme inhibitors. *Ligand Quarterly*, **4**, 43.

HOROWITZ, Z.P. (1981). *Angiotensins Converting Enzyme Inhibitors, Mechanisms of Action and Clinical Implications*. Baltimore-Munich: Urban and Schwarzenberg.

IGIC., R.P., GAFFORD, J.T. & ERDÖS, E.G. (1981). Effect of captopril on proteins and peptide hormones. *Biochem. Pharmac.*, **30**, 683–685.

IKEDA, T., KOMAI, T., KAWAI, K. & SHINDO, H. (1981). Urinary metabolites of 1-(3-mercapto-2-D-methyl-1-oxopropyl)-L-proline (SQ-14225) a new antihypertensive agent in rats and dogs. *Chem. Pharmac. Bull.*, 1416–1422.

KRIPALANI, K.J., McKINSTRY, D.N., SINGVI, S.M., WILLARD, D.A., VUKOVICH, R.A. & MIGDALOF, B.H. (1980). Disposition of captopril in normal subjects. *Clin. Pharmac. Ther.*, **27**, 636–641.

PATCHETT, A.A., HARRIS, E., TRISTRAM, E.W., WYVRATT, M.J., WU, M.T., TAUB, D., PETERSON, E.R. IKELER, T.J., tenBROEKE, J., PAYNE, L.G., ONDEYKA, D.L., THORSETT, E.D., GREENLEE, W.J., LOHR, N.S., HOFFSOMMER, R.D., JOSHUA, H., RUYLE, W.V., ROTHROCK, J.W., ASTER, S.D., MAYCOCK, A.L., ROBINSON, F.M., HIRSCHMANN, R., SWEET, C.S., ULM, E.H., GROSS, D.M., VASSIL, T.C. & STONE, C.A. (1980). A new class of angiotensin-converting enzyme inhibitors. *Nature*, **288**, 280–283.

SINGVI, S.M., KRIPALANI, K.J., DEAN, A.V., KEIM, G.R., KULESZA, J.S., MEEKER, F.S., ROSS, J.J., SHAW, J.M. & MIGDALOF, B.H. (1981). Absorption and bioavailability of captopril in mice and rats after administration by gavage and in the diet. *J. pharm. Sci.*, **70**, 885–888.

SWANSON, B.N., HICHENS, M., MOJAVERIAN, P., FERGUSON, R.K., VLASSES, P.H. & DUDASH, M. (1981). *Res. Comm. Chem. Path. Pharm.*, **33**, 525–536.

SWEET, C.S., GROSS, D.M., ARBEGAST, P.T., GAUL, S.L., BRITT, P.M., LUDDEN, C.T., WEITZ, D. & STONE, C.A. (1981). Antihypertensive activity of N-[(S)-1-(ethyoxycarboxyl)-3-phenylpropyl]-L-ala-L-pro (MK-421). An orally active converting enzyme inhibitor. *J. Pharmac. exp. Ther.*, **216**, 558–566.

TOCCO, D.J., deLUNA, F.A., DUNCAN, A.E.W., VASSIL, T.C. & ULM, E.H. (1982). The physiological disposition and metabolism of enalapril maleate (MK-421) in laboratory animals. *Drug Metab. Dispos.*, **10**, 15–19.

(Received January 22, 1982, accepted June 9, 1982)