Effects of repeated doses of enalapril on renal function in man

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1 Ten healthy subjects received 10 mg oral enalapril (MK 421) daily for a period of 8 days. Renal clearances of electrolytes, urate and phosphate were monitored and factored for glomerular filtration rate, as measured by creatinine clearance, with particular emphasis on the first and eighth day of treatment. Apart from a fall of around 10% in creatinine clearance between 1–2 h on both days 1 and 8, GFR remained unchanged throughout the study.

2 Fractional sodium excretion increased in a biphasic manner by approximately 50% over control between 1–2 h and 4–8 h on day 1. Significant chloruresis ($+39.0 \pm 12.9\%$) and kaluresis ($+26.5 \pm 10.3\%$) occurred between 4–8 h. Urinary pH increased between 0–1 h ($+0.29 \pm 0.12$; $P < 0.05$), and between 4–8 h ($+0.50 \pm 0.08$; $P < 0.01$). The biphasic saluretic effect was also seen between 1–2 h and 4–8 h on day 8.

3 Enalapril caused significant increases in urate and phosphate excretion on day 8 of therapy. There was a biphasic increase in fractional urate excretion at 1–2 h ($+28.1 \pm 6.9\%$; $P < 0.05$) and at 4–8 h ($+21.0 \pm 6.0\%$ $P < 0.01$). Significant phosphaturia ($+36.8 \pm 5.2\%$; $P < 0.05$) was also observed at 4–8 h on day 8.

4 Urinary drug excretion was also biphasic; over the first 2 h the predominant drug form was unchanged enalapril, whilst the peak excretion of the diacid metabolite, enalaprilat, occurred at 4–8 h. 24 h renal clearance of enalaprilat (ml/min) remained constant throughout the study [136.0 ± 6.5 (day 1), and 135.4 ± 4.9 (day 8)]. It is suggested that both enalapril and enalaprilat contribute separately to the acute renal effects observed.

Keywords enalapril renal function angiotensin converting enzyme inhibitor

Introduction

Enalapril [*N*-(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl-L-alanyl-L-proline] is a long acting, non-sulphydryl containing, angiotensin converting enzyme (ACE) inhibitor which has been shown to inhibit the pressor effect of angiotensin 1 in animals (Patchett *et al.*, 1980), and to be an effective antihypertensive agent in man (Brunner *et al.*, 1981; Gavras *et al.*, 1981). It has been demonstrated that, whilst enalapril possesses some *in vitro* ACE inhibitory activity

(Gross *et al.*, 1981), it is the de-esterified diacid (MK 422; enalaprilat; [*N*-(*S*)-1-carboxy-3-phenylpropyl-L-alanyl-L-proline], which is the active species both *in vivo* and *in vitro* (Gross *et al.*, 1981). Animal experiments with enalaprilat showed the compound to have extremely poor bioavailability when compared with the parent mono-ethyl ester, enalapril (Tocco *et al.*, 1982).

Whereas considerable information has accumulated on the cardiovascular, hormonal

and haemodynamic consequences of ACE inhibition (Creager *et al.*, 1981; Guillevin *et al.*, 1983), little has been reported on the renal effects of either captopril (Hollenberg *et al.*, 1981; Antonaccio, 1982) or enalapril (de Zeeuw *et al.*, 1983). Weinberger (1982) reported that chronic captopril therapy (75 mg daily), in hypertensive patients, resulted in blunting of hyperuricaemic effects of concurrent hydrochlorothiazide therapy. Other long term experience with variable dosage of captopril (up to 450 mg daily) did not show these effects (Karlberg *et al.*, 1982).

These divergent findings prompted us to investigate the effects of enalapril on renal handling of electrolytes, phosphate and urate, under controlled conditions, in a group of 10 healthy subjects. Studies were carried out observing acute effects as well as those after repeated exposure to enalapril for 1 week. Accompanying changes in urinary excretion and renal clearance of the active metabolite, enalaprilat, were also followed.

Methods

Subjects

Ten healthy male volunteers took part in this study lasting 11 days. Prior to inclusion in the study, and following completion, subjects underwent a physical examination and laboratory screening, and gave informed written consent. Subjects were given dietary counselling with guidance on regulation of fluid intake and precision of urine collection. They refrained from consuming high-salt containing foods, caffeine, smoking, drinking ethanol and taking other medication for the duration of the study. Mean age was 21.3 (range 20–23) years and mean weight 73 (range 60–97) kg. The study was approved by the Westminster Hospital Ethics Committee.

Design of study

The study was carried out over a period of approximately 3 months. Urine and plasma analyses with the exception of drug, were all carried out blind, immediately after collection. Serum and urine samples for drug estimation were batched and retained at -20°C for analysis at the conclusion of the study. Subjects received enalapril 10 mg once daily for 8 consecutive days. Drug was administered orally at 08.00 h. Fractional collections of urine were undertaken on three consecutive control days prior to treatment and on treatment days 1 and 8. These

fractions consisted of 0–1 h, 1–2 h, 2–4 h, 4–8 h, and 8–24 h periods. On control and all study days, subjects were encouraged to drink 250 ml water/h to ensure steady urine flow of around 4–5 ml/min. Plasma samples were taken at 08.00 h, 12.00 h and 16.00 h on control days and treatment days 1 and 8. For drug assay, serum samples were obtained at 0 (pre-drug), 0.5, 1, 2, 3, 4, 6, 12, 15, 22 and 24 h after drug administration on days 1 and 8, and prior to dosing on intervening days 2 to 7.

In order to monitor completeness of urine collections and examine the influence of the drug on tubular handling of creatinine, 24 h urinary creatinine excretion was monitored for the duration of the study. The 24 h creatinine excretion values for control days, day 1 and day 8 were (mean $\pm$ s.e. mean; mmol/24 h): 15.60 ± 0.6 ; 16.84 ± 0.8 and 16.23 ± 0.9 (NS) respectively. This consistency in the observed urinary output of creatinine indicates that the drug had no effect on the renal tubular handling of creatinine for the duration of the study.

Analytical methods

- (a) *Chemical analysis* Plasma electrolytes, creatinine, uric acid and routine biochemical parameters were measured by standard automated procedures. Urinary electrolytes were measured by atomic absorption spectrophotometry (Perkin-Elmer spectrophotometer model 603), chloride by titrimetric method (Buchler), uric acid by a uricase method (Boehringer) osmolality cryoscopically (Advanced Instruments), and pH with a Radiometer PHM 26 pH meter. Urinary inorganic phosphates were measured by the method of Fiske & Subbarow (1925) and creatinine by the alkaline picrate method (Bonsnes & Taussky, 1945).
- (b) *Drug assay* Serum and urine samples for drug analysis were frozen at -20°C immediately after collection. Serum and urine enalaprilat concentrations were measured by radioimmunoassay (Hichens *et al.*, 1981). Intact enalapril in the urine was estimated as the difference between the concentration of enalaprilat before and after *in vitro* hydrolysis using a rat liver homogenate (Tocco *et al.*, 1982). The intra-assay coefficient of variation for enalaprilat over the concentrations range (ng/ml), 2.5 to 150 for both serum and urine was 4–8%; the inter-assay coefficient of variation over the same concentration range of enalaprilat was 7–9%.
- (c) *Clearance data* The excretion of electro-

lytes, urate and phosphate were expressed as fractional excretions ($E_x\%$) where

$$E_x = \frac{\text{clearance of } x}{\text{creatinine clearance}} \times 100\%$$

The renal clearance of enalaprilat (CL_{EN}) was determined from the relationship $CL_{EN} = \sum_{0-24} U_{drug} \times V/AUC$ where $U_{drug} \times V$ = urinary excretion of enalaprilat, and AUC = the total area under the serum enalaprilat concentration versus time curve between 0–24 h.

- (d) *Statistical analysis* All results were expressed as mean $\pm$ s.e. mean or percentage changes over baseline ($\Delta\%$). Observed changes were consistent enough to allow two-tailed paired Student's *t*-test to be applied to compare days 1 and 8 with the mean of three control day measurements for each subject. Results were considered significant at $P < 0.05$ level.

Results

Creatinine clearance, fractional urinary electrolyte excretion and pH

Between 1–2 h, a small but significant fall in creatinine clearance (CL_{Cr}) was observed, $CL_{Cr} = -11.9 \pm 2.6\%$ (day 1; $P < 0.05$), and $-9.0 \pm 2.4\%$ (day 8; $P < 0.05$). Changes in creatinine clearance within the first hour after enalapril were such that the 0–2 h creatinine clearance, CL_{Cr} (ml/min), did not differ from control; 127.5 ± 8.4 (control) vs 126.5 ± 9.1 (day 1) and 119.5 ± 6.8 (day 8).

For urinary electrolytes, results are presented as fractional excretions of each collection period on days 1 and 8 of enalapril in comparison with matched values on control days. The 0–1 h, 1–2 h and 2–4 h results are tabulated (Table 1), and 0–4, 4–8, 8–24 and 0–24 h results are shown in Figure 1.

0–8 h (Table 1; Figure 1) A biphasic response occurred with fractional sodium excretion (E_{Na+}) on both day 1 and day 8. On day 1, E_{Na+} increased by $+53.5 \pm 26.4\%$ (1–2 h; $P < 0.05$) and by $+50.3 \pm 19.3\%$ (4–8 h; $P < 0.01$). On day 8, parallel increases were noted, $\Delta E_{Na+} = +79.0 \pm 30.1\%$ (1–2 h; $P < 0.01$) and $+77.7 \pm 12.5\%$ (4–8 h; $P < 0.001$). Chloruresis was only significant at 1–2 h on day 8 ($+76.8 \pm 29.1\%$; $P < 0.05$) but was significant at 4–8 h on both day 1 and day 8 ($+38.0 \pm 12.9\%$; $P < 0.05$, and $+38.5 \pm 9.5\%$; $P < 0.01$, respectively). There was an increased trend toward kaluresis at 1–2 h on both day 1 and day 8; this just failed to reach significance ($+65.5 \pm 17.3\%$, day 8; $P < 0.06$).

Table 1 Fractional excretions ($E\% \times 10$) of sodium, potassium, chloride, urate and phosphate, over 0–1, 1–2 and 2–4 h periods on day 1 and day 8 in 10 normal subjects taking oral enalapril (10 mg), once daily for 8 days. Control = mean of measurements on three pre-drug days in each subject. Results are expressed as mean $\pm$ s.e. mean; * $P < 0.05$; ** $P < 0.01$.

Urinary parameter $E\% \times 10$	Period (h)			2–4	
	1–2			Day 1	
	Control	Day 1	Day 8	Control	Day 8
Sodium	5.74 $\pm$ 0.74	7.04 $\pm$ 1.26	7.06 $\pm$ 1.35	7.24 $\pm$ 1.02	11.25 $\pm$ 1.60**
Potassium	129.1 $\pm$ 15.5	151.0 $\pm$ 21.9	169.3 $\pm$ 22.0	183.5 $\pm$ 21.2	258.9 $\pm$ 19.5
Chloride	9.83 $\pm$ 1.12	10.75 $\pm$ 2.05	12.24 $\pm$ 1.76	12.95 $\pm$ 1.52	19.99 $\pm$ 1.98*
Urate	59.0 $\pm$ 3.3	54.3 $\pm$ 4.4	53.4 $\pm$ 3.9	65.0 $\pm$ 2.5	80.8 $\pm$ 4.3*
Phosphate	96.3 $\pm$ 11.4	77.3 $\pm$ 15.3	85.0 $\pm$ 7.5	93.8 $\pm$ 9.3	80.4 $\pm$ 12.4
				7.28 $\pm$ 0.78	9.45 $\pm$ 1.49
				196.3 $\pm$ 21.7	150.3 $\pm$ 13.6
				13.69 $\pm$ 1.18	13.68 $\pm$ 0.73
				64.8 $\pm$ 2.4	63.2 $\pm$ 5.4
				95.1 $\pm$ 11.8	86.4 $\pm$ 12.8

Figure 1 Fractional excretions ($E\% \times 10$) of sodium, chloride, urate, phosphate over 0-4, 4-8, 8-24, and 0-24 h periods. For further details see legend to Table 1. $\square$ = Control; $\square$ = day 1; $\blacksquare$ = day 8. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

However, between 4-8 h, kaluresis was significant ($+26.5 \pm 10.3\%$, day 1; $P < 0.05$).

Urinary pH increased on day 1, $\Delta \text{pH} = +0.29 \pm 0.12$ (0-1 h; $P < 0.05$) and $+0.50 \pm 0.08$ (4-8 h; $P < 0.01$), but not on day 8.

0-24 h (Figure 1) Creatinine clearance did not change over 24 h; CL_{Cr} (ml/min) = 119.1 ± 4.5 (Control) vs 120.3 ± 5.2 (day 1) and 117.7 ± 6.4 (day 8). The only positive change in monovalent ion excretion over 24 h related to sodium; $\Delta E_{\text{Na}^+} = +36.8 \pm 14.5\%$ (day 1; $P < 0.05$) and $+41.8 \pm 15.6\%$ (day 8; $P < 0.05$).

Fractional urate and phosphate excretion (Table 1; Figure 1) 0-8 h changes in urate excretion became significant only on day 8 and again showed a biphasic pattern; $\Delta E_{\text{urate}} = +28.1 \pm 6.9\%$ (1-2 h; $P < 0.05$) and $+21.0 \pm 6.0\%$ (4-

8 h; $P < 0.01$). The latter change was also accompanied by significant phosphaturia; $\Delta E_{\text{PO}_4} = +36.8 \pm 5.2\%$ (4-8 h; $P < 0.05$).

0-24 h (Figure 1) Over 24 h, on day 8, urate excretion remained significantly above control; $\Delta E_{\text{urate}} = +9.8 \pm 3.0\%$ ($P < 0.05$), whereas the increase in phosphate excretion did not attain significance compared with control.

Plasma data (Table 2)

Plasma creatinine, electrolyte and urate concentrations, 4 and 8 h after drug administration, were compared with the mean of three time-matched control measurements for each subject.

4 h post-drug A small but significant fall in plasma creatinine of $-5.3 \pm 2.0 \mu\text{mol/l}$ ($P <$

Table 2 Plasma creatinine, sodium, potassium, chloride and urate concentrations at 4 and 8 h after daily administration at 08.00 h of oral enalapril (10 mg) for 8 days in 10 normal subjects. Control = mean of measurements on three pre-drug days in each subject taken at 12.00 h and 16.00 h. Results, in mmol/l except creatinine ($\mu\text{mol/l}$), are expressed as mean $\pm$ s.e. mean.; * $P < 0.05$; ** $P < 0.01$.

	Time after drug administration					
	Control	4 h Day 1	Day 8	Control	8 h Day 1	Day 8
Creatinine ($\mu\text{mol/l}$)	96.8 $\pm$ 3.7	93.5 $\pm$ 3.7	91.5 $\pm$ 3.0*	95.8 $\pm$ 3.1	96.1 $\pm$ 3.3	100.0 $\pm$ 3.7
Sodium (mmol/l)	140.3 $\pm$ 0.2	139.7 $\pm$ 0.3	138.2 $\pm$ 0.4**	139.9 $\pm$ 0.3	138.6 $\pm$ 0.4*	137.6 $\pm$ 0.6**
Potassium (mmol/l)	4.06 $\pm$ 0.08	3.85 $\pm$ 0.05	3.86 $\pm$ 0.02	4.03 $\pm$ 0.04	4.05 $\pm$ 0.05	3.91 $\pm$ 0.05
Chloride (mmol/l)	101.1 $\pm$ 0.5	103.6 $\pm$ 0.7*	103.1 $\pm$ 0.7*	100.8 $\pm$ 0.4	101.4 $\pm$ 0.8	101.5 $\pm$ 0.5
Urate (mmol/l)	0.36 $\pm$ 0.02	0.35 $\pm$ 0.02	0.33 $\pm$ 0.02**	0.36 $\pm$ 0.02	0.34 $\pm$ 0.02*	0.34 $\pm$ 0.02

0.05) was observed on day 8. At this time, plasma sodium concentration had fallen by -2.1 ± 0.3 mmol/l ($P < 0.01$). Chloride concentration increased by 2.5 ± 0.8 mmol/l and $+2.0 \pm 0.7$ mmol/l on days 1 and 8 respectively ($P < 0.05$). A fall occurred in mean potassium concentration from 4.06 to 3.85 and 3.86 mmol/l on days 1 and 8 respectively, but failed to attain significance. The uricosuria which occurred between 1–2 h on day 8 (Table 1) was accompanied by a fall in plasma urate concentration of -0.03 ± 0.01 mmol/l ($P < 0.01$).

8 h post-drug Plasma sodium concentration fell significantly at 8 h on day 1 and day 8 by -1.3 ± 0.2 mmol/l ($P < 0.05$) and -2.3 ± 0.5 mmol/l ($P < 0.01$) respectively, reflecting the 4–8 h natriuretic response (Figure 1). No changes were seen in plasma creatinine, chloride or potassium. Plasma urate concentration fell from a control value of 0.36 ± 0.02 to 0.34 ± 0.02 mmol/l at 8 h though this fall only reached significance on day 1.

Urinary drug excretion (Figure 2)

Urinary excretion of unchanged enalapril reached a maximum over the first 2 h [mean total 0–2 h excretion = 972 ± 107 μg (day 1) and 921 ± 56 μg (day 8)] and was largely complete within 4 h. Mean urinary excretion of enalaprilat peaked at 4–8 h to a similar extent on both days 1 and 8 [1009 ± 93 μg (day 1) and 875 ± 80 μg (day 8)]. Renal clearance of

enalaprilat over the 24 h period was 136.0 ± 6.5 (day 1), and 135.3 ± 4.9 ml/min (day 8).

Serum enalaprilat

There was no evidence to suggest any appreciable degree of drug accumulation after repeated daily dosing for 8 days. The mean trough serum enalaprilat concentration (ng/ml) measured on each day of the study, increased from 2.43 ± 0.28 (day 1) to 3.03 ± 0.37 (day 3), and remained unaltered thereafter (2.93 ± 0.32 ; day 8).

Discussion

Reported changes in glomerular filtration rate following ACE inhibition have been variable. Thus GFR may increase (Hollenberg *et al.*, 1979; Dzau *et al.*, 1980), remain unchanged (de Leeuw *et al.*, 1983) or even fall (Aldigier *et al.*, 1982). Concern has been expressed about the safety of converting enzyme inhibitors in conditions of low renal perfusion such as bilateral renal artery stenosis when an acute fall in GFR may result (Hricik *et al.*, 1983). The changes in creatinine clearance which we observed in our group of healthy subjects were of small magnitude.

The increases in fractional sodium excretion, which we noted over and above any change in GFR, imply a direct intrarenal effect. It appears to have biphasic characteristics with increases over 1–2 h and 4–8 h periods. Natriuresis after

Figure 2 Sequential urinary excretion of enalapril and enalaprilat ($\mu\text{g}/\text{period}$) over 24 h after drug administration on day 1 (—) and day 8 (----) in 10 normal subjects taking oral enalapril (10 mg) once daily for 8 days. Results are expressed as mean $\pm$ s.e. mean.

ACE inhibition has been noted in healthy subjects (Brunner *et al.*, 1981), hypertensives (Hollenberg *et al.*, 1979; de Zeeuw *et al.*, 1983) and in patients with cardiac failure (Creager *et al.*, 1981). Our observed natriuresis was not accompanied by potassium retention. Furthermore at times of peak saluresis, at 1–2 h and 4–8 h, increases in potassium excretion were observed. This implies that the natriuretic effect of enalapril is not simply mediated via reduced aldosterone secretion, a conclusion which is supported by the finding that enalapril can induce significant natriuresis in adrenal-

ectomized animals (Sweet *et al.*, 1983). There are reports that angiotensin II can induce tubular reabsorption of sodium independently of changes in GFR, renal blood flow or intracortical redistribution of blood flow (Johnson & Malvin, 1977; Borresen *et al.*, 1982). It is therefore possible that this early enalapril induced natriuresis could reflect reduction in angiotensin II activity. Since ACE has been located in the brush border of proximal renal tubules (Ward *et al.*, 1975; Caldwell *et al.*, 1976), it is nevertheless conceivable that enalapril has direct tubular effects independent of

any change in renal haemodynamics, or of any reduction in angiotensin II or of aldosterone activity.

Our detailed analysis revealed significant phosphaturia and uricosuria on the eighth day of enalapril administration. De Zeeuw *et al.* (1983) also reported phosphaturia following enalapril. Since phosphate is primarily re-absorbed in the proximal tubule (Knox *et al.*, 1977), this suggests that enalapril or its metabolite, enalaprilat, may interfere with proximal tubular electrolyte and phosphate transport. The absolute magnitude of the enalapril-induced sodium loss is small with an increase from 0.64% control to 1.08% of the filtered load at 4–8 h after drug administration. This is of the same order as follows administration of other drugs which are both natriuretic and uricosuric. Thus, for example, we have found an increase in fractional sodium excretion of $0.62 \pm 0.12\%$ to $1.56 \pm 0.15\%$ in a similar cohort of subjects given racemic indacrinone (Noormohamed *et al.*, 1984). The blunting of the hyperuricaemic effect of hydrochlorothiazide therapy by captopril (Weinberger, 1982) is of particular interest in view of the synergistic antihypertensive action of diuretics and converting enzyme inhibitors, and their likely combination in the long term treatment of both hypertension and cardiac failure. Furthermore a recent report (Malini *et al.*, 1984) has shown increased uric acid clearance and reduced plasma urate levels in a group of hypertensive patients treated long term with enalapril alone. We have also observed an enalapril-induced reduction in plasma urate levels in the present study.

It is difficult to explain the observed increase in urinary pH as urinary bicarbonate and titratable acidity were not measured in this study. Plasma bicarbonate did not change. It is possible that enalapril and/or its metabolite may inhibit tubular Na^+/H^+ exchange thus increasing urinary pH whilst at the same time causing a significant natriuresis.

Enalapril is unusual in being a pro-drug (Biollaz *et al.*, 1982; Gomez *et al.*, 1983). Serum enalapril concentrations reach a peak between 0.5 h and 1.5 h after oral administration with little unchanged drug in the circulation by 4 h (Biollaz *et al.*, 1982). We have previously

reported that peak serum concentrations of the active metabolite, enalaprilat, occur at 3–4 h (Till *et al.*, 1984) by which time inhibition of plasma converting enzyme is maximal (Brunner *et al.*, 1981). We have found no evidence of drug accumulation during 8 days of therapy with attainment of steady state within 3 days. This is consistent with an accumulation half-life of approximately 11 h, calculated from the urinary recovery of enalaprilat (Till *et al.*, 1984). We have shown that the predominant form of the drug in the urine to be initially enalapril (up to 2 h); after 8 h drug excretion is totally in the form of enalaprilat. In this study, the renal clearance of enalaprilat is of the same order as GFR, agreeing with the findings of Ulm *et al.* (1982). By contrast, the renal clearance of unchanged enalapril, calculated from the data presented by Ulm *et al.* (1982), is of the order of 300 ml/min. This implies tubular secretion of unchanged drug. A renal clearance of around 400 ml/min has been reported for captopril and has been reduced by the concurrent administration of probenecid (Singhvi *et al.*, 1982). Such findings imply that renal transport of both captopril and unchanged enalapril may be occurring via the tubular secretory pathway for organic acids.

The effects of enalapril on creatinine, sodium, chloride, phosphate and urate clearances may not be totally due to the metabolite enalaprilat. Thus, although the maximal changes coincide with the maximal urinary excretion of enalaprilat between 4–8 h, an increase in sodium excretion is already apparent within 2 h. This suggests some of the observed changes in renal tubular function may be due to unchanged enalapril. Other studies are currently in progress in our laboratory to define more accurately the relative contributions of enalapril and enalaprilat to the observed changes in renal tubular function.

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