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Enalapril (MK421) and its lysine analogue (MK521): A comparison of acute and chronic effects on blood pressure, renin-angiotensin system and sodium excretion in normal man

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1 The immediate and long-term effects of enalapril (MK421) and its lysine analogue (MK521) in once-daily dosage, were compared in a study of 12 normal subjects.
2 Both compounds lowered blood pressure equally throughout 24 h without causing tachycardia.
3 The biochemical changes with MK521 were more sustained than with MK421, but this did not affect the magnitude of blood pressure reduction.
4 Twenty-four hours after the previous dose, with both active drugs, plasma renin concentration was significantly higher on day 8 than on day 1, though angiotensin I did not increase in proportion; this probably reflects a fall in renin-substrate with prolonged converting enzyme inhibition.
5 There was an early natriuresis with each compound but this effect was no longer apparent after 8 days of continuous therapy.
6 Both MK421 and MK521 were well tolerated with no serious side effects.

Keywords enalapril MK 521 blood pressure renin-angiotensin sodium excretion

Introduction

Captopril, the first orally active converting enzyme inhibitor, is effective in the treatment of hypertension and congestive cardiac failure (Atkinson & Robertson, 1979; Dargie *et al.*, 1983). Recently, a new group of proline derivatives, lacking a sulphydryl group, has been developed (Patchett *et al.*, 1980). This present study was designed to examine the effects of two of these inhibitors, enalapril maleate (MK421) and its lysine analogue (MK521). Single dose studies have shown them to be potent inhibitors with a long duration of action. This study compares both their immediate and long-term effects on blood pressure, electrolyte excretion and the renin-angiotensin system in normal subjects.

Methods

Twelve healthy, normotensive male volunteers, aged 23–39 years, weighing between 61 and 90 kg (mean 73 ± 2 kg) were included in the study, which was approved by the hospital ethical supervisory committee. The nature of the experiment was explained to each volunteer and written informed consent was obtained. Each had a medical history taken and a physical examination performed. None had a family history of hypertension. For safety purposes, routine laboratory investigations were performed before and during administration of the experimental drugs.

For 3 days before, and for the 8 days of the experiment, the subjects were maintained on a

fixed metabolic diet containing sodium 150 mEq and potassium 70 mEq daily. Meals were given at 09.00, 12.00 and 18.00 h, and the constituents were the same throughout each treatment period. Alcohol and strenuous exertion were prohibited.

On the morning of days 1 and 8 the subjects attended the ward at 08.00 h after an overnight fast and lay supine. At 08.50 h a blood sample was drawn. Blood pressure and pulse rate were then measured, supine, and after 2 min standing, using a Hawksley random zero sphygmomanometer. The bladder was then emptied and the urine discarded. The experimental drug was given at 09.00 h, following which the subjects were mobilised and given a light breakfast. At 14.00 h the subjects again lay supine. At 15.00 h a further blood sample was drawn, followed by measurement of pulse rate and blood pressure. The subjects were again mobilised to attend the ward at 08.00 h on the following morning for repeat measurements as above. Drug was given at 09.00 h on all study days for 8 days.

On days 1 and 8 at times 0 and again 6 h and 24 h after drug administration, measurements were made of plasma active renin concentration (normal range 10–50 mu/l) (Millar *et al.*, 1978), blood angiotensin I (normal range 3–15 pmol/l) (Waite, 1973), plasma angiotensin II (normal range 5–35 pmol/l) (Atkinson *et al.*, 1980), plasma aldosterone (normal range < 500 pmol/l) (Fraser *et al.*, 1973), plasma cortisol (normal range 140–700 nmol/l) and serum converting enzyme activity (ACE) (Cushman & Cheung, 1971; Anderson *et al.*, 1981). As the antibody used for the angiotensin II assay reacts mol for mol with angiotensin III, the angiotensin II assay gives also some assessment of angiotensin III. Urine was collected for 24 h, from 09.00 h to 09.00 h on days 1 and 8, and each collection divided into two time periods, 0–8 h and 8–24 h. Measurement was made of urine volume, sodium, potassium, creatinine and protein. Five subjects completed continuous 24 h urine collections for each of the 8 days of the experiment.

Drug administration

Capsules containing 10 mg of enalapril maleate (MK421), lysine analogue (MK521) or placebo were supplied by Merck, Sharp and Dohme and Company, Rahway, New Jersey. Each subject received an 8-day course of all three drugs allocated to a Latin Square design in strictly double-blind fashion. A single oral dose was given at 09.00 h for 8 days with a minimum period of 3 weeks between each experimental period.

Statistical methods

The results were initially analysed using repeated measures analysis of variance, and the data presented are the follow-up multiple comparisons which are based on paired *t*-tests after logarithmic transformation of the data where appropriate. Each test compares the changes on one drug with the changes on another drug, with one test, for example, comparing the change in supine systolic blood pressure from day 1, 0 h, to day 1, 6 h, on MK421 with the corresponding change on placebo. Significant results are stated either as being significant as an individual test or as being significant after making a Bonferroni correction to allow for the fact that 15 comparisons are made on each variable (Miller, 1966). The analysis of the electrolyte results was based entirely upon paired *t*-tests, comparing each set of results on an active drug back to the corresponding placebo results. No correction was made to allow for the multiple comparisons.

Results

Blood pressure and heart rate

Measurements of pulse rate and blood pressure obtained with both inhibitors are presented in Tables 1, 2 and 3. With both compounds there was a fall of approximately 10% in systolic and diastolic blood pressure 6 h and 24 h after the first dose. After 8 days of continuous administration, blood pressure remained slightly lower than on placebo at all time points. There was no further fall in blood pressure on standing with either compound.

Supine heart rate did not change with treatment and there were similar rises of pulse rate with both compounds and with placebo on standing.

Plasma converting enzyme activity

Figure 1a illustrates the reduction in plasma converting enzyme activity following the administration of both compounds. Activity decreased markedly to under 10% of basal values 6 h after the first dose. At 24 h 40% recovery of enzyme activity was apparent with MK421 but recovery was minimal with MK521. These differences at 24 h were significant ($P < 0.05$). After 8 days of continued therapy, the pattern of diurnal variation was still apparent.

Table 1 Mean (s.e. mean) values of blood pressure, pulse and biochemical data during treatment with placebo.

	Day 1			*Day 8*		
	Basal	*6 h*	*24 h*	*Basal*	*6 h*	*24 h*
ACE (μmol hippurate $ml^{-1} h^{-1}$)	1.66 (0.10)	1.62 (0.09)	1.63 (0.09)	1.63 (0.11)	1.62 (0.11)	1.64 (0.10)
Renin (mu/l)	27 (5)	31 (8)	24 (4)	20 (3)	19 (3)	23 (3)
Angiotensin I (pmol/l)	7.2 (0.8)	8.8 (1.2)	7.9 (0.7)	7.5 (1.0)	7.8 (0.8)	6.8 (0.8)
Angiotensin II (pmol/l)	15.5 (4.2)	12.9 (1.3)	11.3 (1.2)	11.3 (1.2)	12.2 (1.5)	9.6 (0.8)
Aldosterone (pmol/l)	130 (18)	125 (15)	138 (20)	138 (18)	103 (13)	138 (23)
Cortisol (nmol/l)	423 (41)	228 (17)	379 (39)	404 (52)	240 (19)	400 (26)
Supine systolic BP (mm Hg)	108.5 (2.9)	112.3 (1.7)	107.7 (2.6)	106.2 (2.7)	107.8 (2.6)	108.8 (2.4)
Supine diastolic BP (mm Hg)	66.0 (1.9)	69.0 (2.5)	64.8 (1.6)	61.5 (2.6)	66.3 (2.3)	64.0 (2.0)
Supine pulse (beats min^{-1})	63.0 (2.9)	65.2 (3.5)	61.0 (1.9)	62.2 (2.4)	58.8 (1.9)	60.7 (2.7)
Erect systolic BP (mm Hg)	107.0 (3.2)	112.8 (3.0)	111.8 (3.2)	110.0 (3.9)	105.8 (2.6)	109.3 (2.5)
Erect diastolic BP (mm Hg)	71.0 (2.3)	76.7 (2.3)	75.5 (2.1)	72.7 (3.0)	67.0 (4.5)	72.2 (2.1)
Erect pulse (beats min^{-1})	77.7 (4.2)	80.0 (4.0)	83.5 (3.8)	79.3 (4.5)	81.6 (3.5)	86.3 (5.5)

Plasma renin and angiotensin I

Figure 1b and c illustrates the rise in plasma active renin and blood angiotensin I with the first dose and after 8 days respectively of each compound. In each case the levels of renin and angiotensin I had failed to return to basal values after 24 h. The rise associated with MK521 was greater than that with MK421 and for both drugs the rise at 6 h was much greater at 8 days than on day 1.

At 24 h after the last dose of each drug, plasma renin concentration was significantly higher on day 8 than on day 1 ($P < 0.025$), although there was no significant change in the concentration of angiotensin I.

Plasma angiotensin II

Figure 1d illustrates the fall in plasma angiotensin II with each compound. Angiotensin II concentrations fell by approximately 50% 6 h after the first dose of each drug, with return to basal values after 24 h. The same pattern was apparent after 8 days of treatment, although at all time points suppression appeared more complete with MK521.

Plasma angiotensin III

The low levels of plasma angiotensin II during treatment with each drug similarly indicate low concentrations of angiotensin III (see **Discussion**).

Plasma aldosterone

Figure 1e illustrates the fall in plasma aldosterone with each compound. Significant suppression, similar with each drug, occurred at 6 h, with return to basal values after the first dose and after 8 days' treatment.

Plasma cortisol

Plasma cortisol concentrations were significantly and similarly lower after 6 h (15.00 h) with placebo and active drug in keeping with the known diurnal variation in cortisol secretion.

Electrolyte excretion

Table 4 illustrates the changes in electrolyte excretion occurring with each compound. With

Table 2 Mean (s.e. mean) values of blood pressure, pulse and biochemical data during treatment with MK421, together with statistical comparison of changes on MK421 with corresponding change on placebo.

	Day 1						*Day 8*					
	Basal		*6 h*		*24 h*		*Basal*		*6 h*		*24 h*	
ACE (μmol hippurate $ml^{-1} h^{-1}$)	1.68	(0.10)	0.15	(0.02)**	0.69	(0.08)**	0.80	(0.12)**	0.15	(0.02)††	0.78	(0.07)
Renin (mu/l)	24	(3)	147	(24)**	59	(5)**	93	(15)**	336	(87)††	92	(10)
Angiotensin I (pmol/l)	7.2	(0.6)	46.6	(8.5)**	17.3	(2.5)**	21	(3.7)**	102	(32)†	19.4	(3.1)
Angiotensin II (pmol/l)	10.5	(1.3)	5.5	(1.0)*	9.3	(0.9)	10.0	(1.0)	7.3	(0.9)†	12.2	(0.9)†
Aldosterone (pmol/l)	155	(25)	103	(23)	153	(25)	160	(35)	113	(33)	158	(30)
Cortisol (nmol/l)	442	(37)	218	(16)	392	(42)	455	(44)	224	(19)	425	(30)
Supine systolic BP (mm Hg)	113.5	(3.0)	102.3	(2.2)**	102.8	(2.4)**	101.5	(1.8)*	102.8	(2.9)	102.8	(1.6)
Supine diastolic BP (mm Hg)	66.7	(3.5)	61.5	(1.6)*	58.7	(1.9)	59.3	(2.0)	60.5	(2.7)	62.0	(2.3)
Supine pulse (beats min^{-1})	64.0	(2.2)	65.5	(2.9)	62.3	(1.9)	63.3	(2.6)	66.7	(2.2)	62.2	(2.9)
Erect systolic BP (mm Hg)	103.8	(3.3)	97.0	(3.8)*	102.0	(3.6)	102.8	(2.7)	98.0	(3.1)	104.7	(2.6)
Erect diastolic BP (mm Hg)	73.8	(2.5)	69.2	(3.1)*	68.8	(3.0)	69.3	(2.7)	69.8	(2.9)	69.5	(2.1)
Erect pulse (beats min^{-1})	82.3	(3.7)	84.5	(3.3)	86.5	(4.1)	86.7	(3.4)	90.9	(4.5)	85.7	(3.8)†

Asterisks (*) indicate changes from Day 1, 0 h, and daggers (†) indicate changes from Day 8, 0 h.
*, † Significant at 0.05 level as an individual test.
**, †† Significant at 0.05 level allowing for multiple comparisons.

each drug there was a significant increase in urinary sodium excretion within 24 h of the first dose, although after 8 days this effect within a few hours of administration had largely disappeared. There was no change in the diurnal pattern of sodium excretion and there were no significant differences in urine creatinine or potassium excretion over any time period.

In five subjects cumulative measurements of urinary sodium excretion over the 8-day period showed a net sodium loss of 48 mmol (not significant) in excess of placebo with each drug (mean ± s.e. mean daily sodium excretion 133 ± 5 mmol on placebo compared with 139 ± 4 mmol on MK421 and 139 ± 5 mmol on MK521).

Serum electrolytes

During long term treatment there was a significant fall in serum sodium concentration during MK521 (141.4 ± 0.5 pre-treatment 139.8 ± 0.5 mmol/l after 8 days, mean ± s.e. mean, $P < 0.05$) and a significant rise in serum potassium concentration during MK421 (3.89 ± 0.08 pre-treatment, 3.99 ± 0.06 mmol/l after 8 days, mean ± s.e. mean, $P < 0.01$).

Side effects

The drugs were well tolerated, although one subject noted slight gastric flatulence throughout the period of MK421 administration. Two subjects complained of light-headedness related to posture during administration of both active drugs, although blood pressure did not fall measurably on standing. In each subject blood pressure was distinctly lowered and the symptoms were not considered to be specific drug side effects.

There were no instances of taste loss, skin rash, glycosuria, leucopenia or proteinuria, nor were any abnormalities detected on routine biochemical screening.

Table 3 Mean (s.e. mean) values of blood pressure, pulse and biochemical data during treatment with MK521, together with statistical comparison of changes on MK521 with corresponding change on placebo.

	Day 1						*Day 8*					
	Basal		*6 h*		*24 h*		*Basal*		*6 h*		*24 h*	
ACE (μmol hippurate $ml^{-1} h^{-1}$)	1.67	(0.12)	0.15	(0.02)**	0.27	(0.04)**	0.30	(0.04)**	0.13	(0.02)††	0.31	(0.03)
Renin (mu/l)	26	(3)	226	(52)**	96	(12)**	158	(38) **	450	(135)††	191	(35)
Angiotensin I (pmol/l)	8.0	(0.6)	69	(21.8)**	32.2	(3.9)**	32.7	(6.2) **	90.2	(24.3)††	36.1	(5.3)†
Angiotensin II (pmol/l)	11.9	(1.4)	5.7	(1.1)*	10.0	(2.6)	8.5	(0.8)	5.7	(1.0)†	8.1	(0.8)
Aldosterone (pmol/l)	150	(23)	83	(20)*	128	(18)	135	(18)	68	(18)	178	(43)
Cortisol (nmol/l)	390	(20)	209	(18)	383	(40)	441	(29)	250	(28)	412	(31)
Supine systolic BP (mm Hg)	110.2	(2.8)	102.5	(16)*	99.0	(2.1)*	100.8	(3.4)	102.8	(2.6)	103.2	(2.5)
Supine diastolic BP (mm Hg)	68.8	(2.5)	61.8	(2.2)*	58.8	(2.4)*	56.5	(4.1)	60.3	(1.4)	62.2	(1.4)
Supine pulse (beats min^{-1})	62.8	(2.1)	63.8	(2.4)	62.0	(2.7)	61.7	(2.3)	61.5	(2.1)	61.0	(3.0)
Erect systolic BP (mm Hg)	110.0	(3.1)	96.0	(3.3)*	101.5	(2.6)*	96.8	(3.8)*	97.3	(2.0)	104.0	(2.3)
Erect diastolic BP (mm Hg)	78.2	(2.5)	63.7	(3.1)**	69.0	(3.2)*	65.8	(4.3)*	67.0	(2.9)	69.7	(2.0)
Erect pulse (beats min^{-1})	83.3	(4.2)	86.2	(4.5)	85.5	(4.4)	84.5	(4.7)	87.3	(4.2)	84.5	(3.8)

Asterisks (*) indicate changes from Day 1, 0 h, and daggers (†) indicate changes from Day 8, 0 h.
*, † Significant at 0.05 level as an individual test.
**, †† Significant at 0.05 level allowing for multiple comparisons.

Discussion

MK421 (enalapril maleate) is a substituted N-carboxymethyldipeptide (Figure 2). It is largely inactive until absorbed and de-esterified, yielding the highly active, but poorly absorbed diacid (MK422) (Ulm *et al.*, 1982). MK521 (the lysine analogue of MK422) is orally absorbed and requires no bioactivation. MK421 has an absorption peak at about 1 h with a minimum absorption of about 60%. MK521 is absorbed more slowly with a peak of 6–8 h and a minimum absorption of about 30%. Apart from bioactivation of MK421, the compounds are not significantly metabolised and are excreted largely unchanged in the urine.

Both compounds block the pressor effects of infused angiotensin I in animals (Patchett *et al.*, 1980; Gross *et al.*, 1980) and man (Biollaz *et al.*, 1981; Burnier *et al.*, 1982) although this effect is not apparent 24 h after drug administration. The effects on converting enzyme activity are still apparent after 72 h, although the rate of disappearance of drug is more rapid (Biollaz *et al.*, 1982). Despite this, there is a close relationship between the levels of active metabolite and plasma converting enzyme activity.

In the present study, blood pressure was similarly lowered by both active drugs and this effect was sustained after 24 h and with long-term treatment. Other placebo-controlled, single-dose studies have suggested a similar effect in normal subjects given captopril, MK421 (MacGregor *et al.*, 1980, 1981; Millar *et al.*, 1982 a, b) and MK521 (Millar *et al.*, 1982 a, b). While the hypotensive action of these drugs is possibly multifactorial, these results do suggest an important role for the renin-angiotensin system in the maintenance of blood pressure in sodium replete man. Studies in small numbers of hyper-

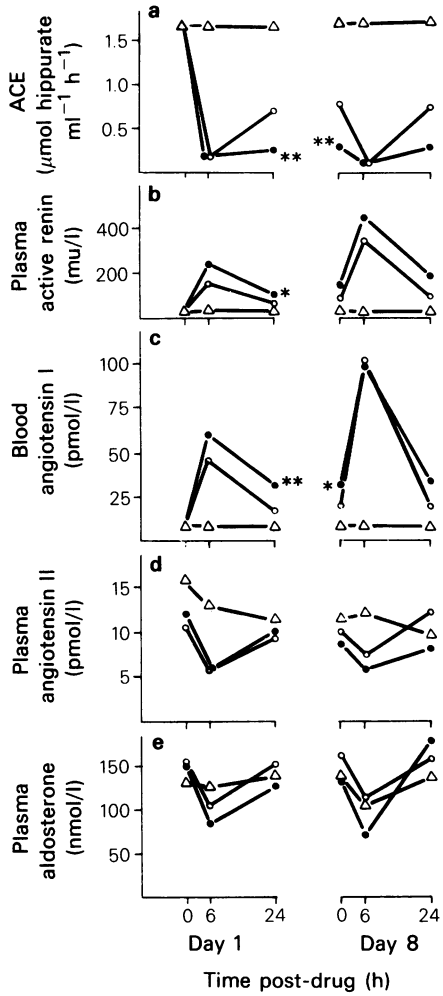

Figure 1 Mean changes in components of the renin-angiotensin system during treatment with placebo △——△, MK421 ○——○ and MK521 ●——●. For clarity, standard errors have been omitted.
Asterisks indicate statistical comparison of changes on MK521 with corresponding changes on MK421.
* $P < 0.05$ as an individual test.
** $P < 0.05$ allowing for multiple comparisons.

tensive patients have shown MK421 to be an effective agent in essential hypertension (Gavras *et al.*, 1981), and hypertension associated with renovascular disease (Hodsman *et al.*, 1982, 1983). MK421 appears to be equally effective given once or twice daily (Bergstrand *et al.*, 1982).

Despite the fall in blood pressure with both MK421 and MK521, there were no differences in supine or standing pulse rates when compared with placebo. This finding is consistent with previous studies which have shown baroreceptor resetting, but not impairment, during treatment with captopril, MK421 and MK521 (Millar *et al.*, 1982 a, b; Mancia *et al.*, 1982).

Plasma converting enzyme activity was distinctly suppressed by both MK421 and MK521, although the effects were more prolonged with MK521. The expected rise in plasma renin and angiotensin I was seen and was greater with MK521. Plasma concentrations of aldosterone and angiotensin II were significantly reduced after 6 h but had returned to basal values after 24 h. The same patterns of diurnal variation in all the above measurements were seen after 8 days of continuous treatment.

At 24 h after the last dose of each drug the concentration of plasma renin was significantly higher on day 8 than on day 1, although angiotensin I levels did not increase in proportion. This apparent dissociation between renin and angiotensin I during long-term treatment presumably reflects the fall in renin-substrate which is a feature of prolonged inhibition of converting enzyme (Rasmussen *et al.*, 1981). We have observed a similar lack of parallelism between the increase in plasma renin and blood angiotensin I concentrations during long-term treatment with captopril and enalapril in renovascular hypertension (Atkinson *et al.*, 1982b; Hodsman *et al.*, 1983).

Despite the very high circulating levels of renin and angiotensin I, there is no evidence of the converting enzyme inhibition being overcome during long-term drug administration and angiotensin II remains low.

Des-Asp^1-angiotensin II (angiotensin III) accounts for less than 10% of the total angiotensin II immunoreactivity in plasma from man (Semple *et al.*, 1976). Consequently, although it is considered to be a potent stimulus to aldosterone secretion, its low concentration in human plasma would suggest a relatively unimportant role. The possibility that the residual concentration of angiotensin II immunoreactive material detected following ACE inhibition with MK421 or MK521 may contain a higher concentration of angiotensin III than normal, with a consequently increased effect on aldosterone secretion, seems unlikely. Angiotensin III is formed primarily by the action of aminopeptidase A on angiotensin II; consequently any suppression of plasma angiotensin II following converting enzyme inhibition will result in a proportional fall in angiotensin III.

The possibility that angiotensin III may be formed by an alternative pathway such as the action of converting enzyme on des-Asp^1-angiotensin I is also unlikely, as animal studies

Table 4 Mean (s.e. mean) values of urine electrolyte and creatinine excretion (mmol/volume).

			Na^+		K^+		*Creatinine*	
Day 1	0–8 h	Placebo	47	(5)	40	(3)	6.3	(0.6)
		MK421	71	(8)**	39	(3)	6.4	(0.5)
		MK521	63	(8)*	37	(2)	6.6	(0.7)
	8–24 h	Placebo	63	(9)	22	(2)	9.9	(1.1)
		MK421	64	(6)	22	(2)	10.3	(0.8)
		MK521	77	(9)	25	(2)	12.4	(1.5)
Day 8	0–8 h	Placebo	53	(6)	36	(2)	6.1	(0.3)
		MK421	64	(2)	41	(3)	7.5	(0.5)
		MK521	67	(10)*	38	(3)	5.9	(0.4)
	8–24 h	Placebo	73	(5)	21	(2)	10.9	(0.5)
		MK421	67	(6)	24	(2)	11.1	(0.6)
		MK521	69	(9)	25	(3)	10.1	(0.7)

* Significant at 0.05 level.
** Significant at 0.01 level.

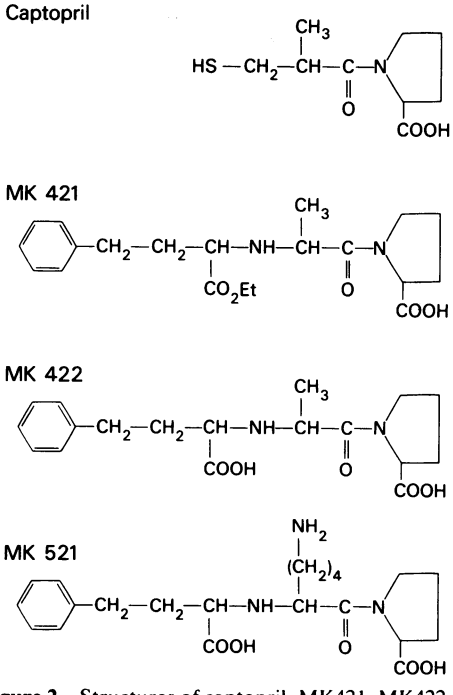

Figure 2 Structures of captopril, MK421, MK422 and MK521.

have shown that converting enzyme inhibitors are very effective in blocking this reaction (Garcia del Rio *et al.*, 1981) even at very high concentrations of des-Asp-angiotensin I.

Although plasma concentrations of angiotensin II and aldosterone had returned to basal levels after 24 h, with sustained elevation of renin and angiotensin I, blood pressure did not rise with either drug at 24 h after the last dose. We have shown a similar diurnal change in components of the renin-angiotensin system in patients with renal artery stenosis (Hodsman *et al.*, 1982, 1983) and this again was not associated with loss of blood pressure control. We have also shown that by using doses of MK421 in excess of 10 mg, more prolonged, though not necessarily more profound, suppression of converting enzyme activity and angiotensin II is induced.

Previous studies in subjects on free dietary intake (Brunner *et al.*, 1981; Millar *et al.*, 1982b) have suggested a natriuresis following a single dose of both MK421 and MK521. The present study, in which the sodium intake was fixed, confirms a significant natriuresis. The natriuretic effect of MK521 appeared later in keeping with the known pattern of drug absorption. After 8 days of continuous therapy the natriuretic effects was no longer apparent, despite continued suppression of plasma aldosterone and angiotensin II. Cumulative measurements of urinary sodium excretion suggest a net sodium loss of up to 50 mEq in excess of placebo over the 8-day period. It is thus unlikely that sodium excretion contributes greatly to the long-term hypotensive effects of either drug, at least in normal subjects.

The potency of each drug in a dose of 10 mg was comparable, although the effects on components of the renin-angiotensin system appeared more prolonged with MK521. These differences were still apparent after 8 days of continuous treatment, but there was no evidence that this affected blood pressure control. Thus, MK421 and MK521 are potent inhibitors with a prolonged duration of action. They are well tolerated, although further testing is required to exclude significant toxicity.

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