The pharmacodynamics and dose-response relationships of the angiotensin converting enzyme inhibitor, cilazapril, in essential hypertension

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1 In a double-blind, placebo controlled, crossover study 12 patients with essential hypertension received single doses of 5, 10 and 20 mg of cilazapril, a new angiotensin converting enzyme (ACE) inhibitor.

2 All doses similarly and significantly ($P < 0.05$) reduced supine and erect blood pressure without increasing heart rate. The hypotensive effect was evident within 1 h, maintained for up to 8 h, with a maximal effect at 6 h. There was no discernible effect on blood pressure at 24 h after dosing.

3 Plasma ACE activity was markedly inhibited to the same extent after all doses, with a peak inhibition of 94–96% at 2–3 h. At 24 h residual inhibition of ACE was 49–54%.

4 Plasma renin activity increased in a dose-dependent manner with a peak at 6 h, and returned to baseline at 24 h. No correlation was found between the reduction in blood pressure and plasma renin activity, either at baseline or following cilazapril.

5 There were no significant changes in plasma noradrenaline and the responses to upright posture and to dynamic exercise were preserved. There was no evidence of impaired exercise performance.

6 Cilazapril is a potent ACE inhibitor with a rapid onset and a prolonged duration of action. These results suggest that peak ACE inhibition is achieved by 5 mg and that lower doses may be useful in clinical practice.

Keywords cilazapril angiotensin converting enzyme inhibitor blood pressure pharmacodynamics

Introduction

Cilazapril (RO 312848) is the mono-ethyl ester of a new nonthiol, long-acting and potent inhibitor of angiotensin converting enzyme. Cilazapril itself is inactive, but following oral administration it is rapidly de-esterified to the active diacid metabolite cilazaprilat (RO 31 3113) (Figure 1). Preliminary studies, both *in vitro* and in a variety of animal species, confirm that cilazapril inhibits angiotensin converting enzyme, attenuates the

pressor response to infused angiotensin 1, and exhibits an antihypertensive effect of greater magnitude than captopril or enalapril (Natoff *et al.*, 1985).

This study in patients with essential hypertension, investigates the cardiovascular, autonomic and humoral effects of cilazapril and examines the dose-response relationships.

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Figure 1 Comparison of the chemical structures of enalapril and cilazapril and their respective active metabolites, enalaprilat and cilazaprilat.

Methods

The study was undertaken in 12 patients (seven males, five females) with mild to moderate essential hypertension who were otherwise healthy. The blood pressure criteria were confirmed during the run-in period with average supine diastolic pressures in the range 90–110 mmHg. The patients were aged 49 ± 14 years and weighed 71 ± 10 kg (mean $\pm$ s.d.). The clinical details of the patients are summarised in Table 1.

Patients gave written informed consent before entry to the study, which received the prior approval of the local Ethics Review Committee. After a 4 week drug free interval, the patients received single doses of 5, 10 and 20 mg of

cilazapril and matching placebo in a double-blind crossover study. Study days were at least 1 week apart and the treatment order was randomised to a Latin square design.

On each study day, the patients arrived fasted at the Clinical Pharmacology Research Unit at 08.30 h, having avoided coffee, tea, alcohol and cigarettes from 22.00 h of the preceding night. A small cannula (Venflon) was inserted into an antecubital vein to permit blood sampling. A manometer cuff was attached to an arm for blood pressure measurement using a Sentron semi-automatic sphygmomanometer (Bard, Biomedical). Precordial E.C.G. leads were attached for the measurement of heart rate and heart period on a Grass polygraph model 7D1D (Grass Instruments Co. Mass. U.S.A.).

Table 1 Clinical details

Patient	Sex (M/F)	Age (years)	Weight (kg)	*Supine basal BP (mmHg)	Plasma creatinine ($\mu\text{mol l}^{-1}$)
1	M	20	61.2	172/89	85
2	F	43	71.0	164/103	101
3	M	55	85.0	156/96	110
4	F	63	71.2	154/94	106
5	M	52	80.9	138/92	106
6	F	48	60.0	164/106	81
7	F	50	60.0	220/118	95
8	M	55	80.6	156/94	89
9	M	55	86.4	149/97	68
10	F	55	62.2	152/94	66
11	M	23	72.7	148/93	74
12	M	66	63.1	152/92	84
Mean $\pm$ s.d.	7M/5F	48.8 ± 14.3	71.2 ± 10.2	$160 \pm 21/97 \pm 9$	88.8 ± 15.1

*as recorded on the first study day after 30 min supine rest.

After 30 min of supine bedrest, blood pressure and heart rate were recorded in duplicate in the supine position and after 1, 2 and 5 min of standing. Baseline blood samples for plasma angiotensin converting enzyme (ACE) activity, plasma renin activity (PRA), aldosterone and noradrenaline were taken. The patients received the appropriate capsules with 200 ml of tap water and blood pressure and heart rate measurements were repeated after 30, 60 and 90 min, and 2,3,4,6,8,24,48 and 72 h after oral dosing. Blood samples were taken for plasma ACE activity at the same times as the blood pressure measurement. Additional samples were collected for PRA, noradrenaline and aldosterone at 3, 6 and 24 h after dosing.

Plasma angiotensin converting enzyme activity was assayed by the method of Cushman & Cheung (1971) as modified by Chiknas (1979). One enzyme unit generates 1 nmol of hippuric acid per minute from the tripeptide substrate Hip-His-Leu under standard assay conditions. The inter and intra assay coefficients of variation were 6% and 2% respectively with a limit of detection of 0.1 enzyme unit. PRA was analysed using the radioimmunoassay method of Derkx *et al.* (1978). The normal range in our laboratory is 4–12 ngA1 ml⁻¹ h⁻¹, with a limit of detection of 1 ngA1 ml⁻¹ h⁻¹, the inter and intra assay coefficients of variation being 7.0% and 5.5%, respectively. Plasma aldosterone concentrations were determined by the radioimmunoassay technique developed by McKenzie & Clements (1974). The limit of detection was 10 pg ml⁻¹ and inter and intra assay coefficients of variation were 11% and 7% respectively. Plasma catecholamines were assayed using a radioenzymatic method (Da Prada & Zurcher, 1976).

Tests of autonomic reflex function

The following autonomic function tests were undertaken 6 h after oral dosing with placebo and cilazapril.

Orthostatic test: The cardiovascular (blood pressure and heart rate) and catecholamine (adrenaline and noradrenaline) response to upright posture (1 min standing) was used as an indirect measure of baroreceptor reflex function.

Dynamic exercise: A dynamic exercise test by bicycle ergometry was undertaken for 2 min. Male patients were exercised at 125W and the females at 65W. Blood pressure and heart rate were recorded and samples for plasma catecholamines collected before and at the end of the

2 min exercise period. The exercise induced tachycardia and increase in systolic blood pressure and catecholamines were used as indices of sympathetic integrity.

Data analysis

Results throughout are expressed as mean $\pm$ s.d. The blood pressure and heart rate changes were subjected to repeated measures analysis of variance which included day effect, treatment effect, and time/treatment interaction, followed by pairwise comparison. The hormonal responses were evaluated by paired *t*-tests with Bonferroni correction. The autonomic results were compared by paired *t*-test (two-tailed). The results were considered significant at $P < 0.05$.

Results

Cilazapril was generally well tolerated and no adverse biochemical or haematological abnormalities were detected.

Blood pressure and heart rate

Cilazapril significantly reduced systolic and diastolic blood pressure in both the supine and erect postures ($P < 0.05$ ANOVA) (Figures 2 and 3). The hypotensive effect was apparent within 1 h, maintained up to 8 h, with a peak effect at 6 h. For supine blood pressure, comparing placebo with 20 mg cilazapril, the recordings were respectively 150/88 $\pm$ 11/10 and 137/85 $\pm$ 11/9 mmHg at 1 h; 138/88 $\pm$ 14/10 and 123/70 $\pm$ 14/11 mmHg at 6 h; 142/83 $\pm$ 14/8 and 131/73 $\pm$ 14/7 mmHg at 8 h. At 24 h after dosing, no discernible effect on blood pressure was detected. The effects of the different doses of cilazapril on supine and erect heart rate are shown in Figure 4. The fall in blood pressure was comparable in both the supine and erect postures and was not associated with a significant increase in heart rate or impairment of orthostatic tolerance. All active doses produced similar antihypertensive effects and there were no significant differences between the different doses. However, the rank order of effects indicated that the 20 mg dose caused the greatest reduction in blood pressure.

Plasma angiotensin converting enzyme inhibition

Plasma angiotensin-1-converting enzyme activity was markedly (>90%) inhibited to the same extent after all doses of the active drug with

Figure 2 The effects of cilazapril (5 (●), 10 (□) and 20 (▲) mg) and placebo (○) on supine systolic and diastolic blood pressure.

Figure 3 The effect of cilazapril (5 (●), 10 (□) and 20 (▲) mg) and placebo (○) on erect systolic and diastolic blood pressure after 5 min standing.

Figure 4 The effect of cilazapril (5 (●), 10 (□), 20 (▲) mg) and placebo (○) on supine and erect heart rate after 5 min standing.

Figure 5 The % inhibition of plasma angiotensin converting enzyme activity by placebo (○) and cilazapril 5 mg (●), 10 mg (□) and 20 mg (▲).

Table 2 a) Plasma renin activity (ng AI ml⁻¹ h⁻¹) and b) plasma aldosterone concentration (pg ml⁻¹) (mean $\pm$ s.d.)

Treatment	Time (h)			
	0	3	6	24
Plasma renin activity				
Placebo	2.43 $\pm$ 1.38	2.17 $\pm$ 2.43	3.23 $\pm$ 2.13	3.49 $\pm$ 1.70
Cilazapril (5 mg)	2.34 $\pm$ 1.60	7.85 $\pm$ 13.20	*13.40 $\pm$ 12.30	2.83 $\pm$ 1.70
Cilazapril (10 mg)	2.60 $\pm$ 1.80	3.3 $\pm$ 2.70	*16.90 $\pm$ 21.20	6.60 $\pm$ 5.30
Cilazapril (20 mg)	2.50 $\pm$ 1.39	6.50 $\pm$ 5.0	*32.1 $\pm$ 20.0	5.80 $\pm$ 2.60
Plasma aldosterone concentration				
Placebo	97.30 $\pm$ 24.6	75.4 $\pm$ 40.9	90.9 $\pm$ 37.8	109.4 $\pm$ 39.5
Cilazapril (5 mg)	94.9 $\pm$ 31.8	46.3 $\pm$ 22.9	49.1 $\pm$ 14.4	105.0 $\pm$ 51.9
Cilazapril (10 mg)	93.7 $\pm$ 38.0	57.3 $\pm$ 16.1	52.7 $\pm$ 17.1	114.0 $\pm$ 39.9
Cilazapril (20 mg)	102.5 $\pm$ 50.5	67.3 $\pm$ 23.0	64.7 $\pm$ 20.3	88.6 $\pm$ 40.2

* $P < 0.005$ (paired to test with Bonferroni correction relative to placebo).

a peak effect at 2–3 h. There were no significant differences between the doses but again the rank order of ACE inhibition suggested that the 20 mg dose had the greatest effect. The % peak ACE inhibition after the 5 mg dose was 93.8 ± 9.6 , after 10 mg was 95.3 ± 10.5 and following the 20 mg dose was 96.0 ± 8.9 . At 24 h ACE activity was still significantly inhibited: $49.4 \pm 12.8\%$ with 5 mg, $53.2 \pm 22.5\%$ with 10 mg and $54.2 \pm 14.3\%$ with 20 mg. These results are shown in Figure 5.

Plasma renin activity and plasma aldosterone concentrations

The results of the plasma renin activity and aldosterone response to converting enzyme inhibition are shown in Table 2. There was a dose dependent increase in plasma renin activity which was maximal at 6 h and had returned to baseline values at 24 h after all active doses. There was no significant correlation between the basal plasma renin activity ($r = 0.4$, $P > 0.1$) or the reactive increases in PRA and the antihypertensive effect. There was a concomitant fall in plasma aldosterone on all active treatments with a peak effect at 6 h after dosing, but the results were not statistically significant.

Autonomic function tests

The results for placebo and 20 mg cilazapril were compared. Plasma noradrenaline concentrations were unaffected by cilazapril even at the time of the maximum fall in blood pressure.

Orthostatic test: Baseline blood pressure was significantly reduced by cilazapril. On placebo, assumption of upright posture was associated with a definite rise in heart rate and noradrenaline. Following cilazapril the postural increases in heart rate and catecholamines were preserved and comparable to placebo (Figure 6).

Dynamic exercise: After placebo, there were significant increases in systolic blood pressure and heart rate (the respective increments were 16 ± 34 mmHg and 55 ± 13 beats min⁻¹). Circulating noradrenaline was also markedly increased by 5.6 ± 4.1 nmol l⁻¹. Cilazapril treatment neither influenced the exercise induced tachycardia nor the systolic blood pressure increases nor the exercise induced rise in circulating catecholamines. The increases were, respectively, 19 ± 23 mmHg, 48 ± 15 beats min⁻¹ and 4.7 ± 3.3 nmol l⁻¹. Further, the subjective perception of exercise effort was not changed.

Figure 6 The effects of placebo and 20 mg cilazapril on the cardiovascular and catecholamine response to postural stress (1 min standing).

Discussion

We have examined the cardiovascular and humoral effects of the new nonthiol angiotensin converting enzyme (ACE) inhibitor, cilazapril, in patients with essential hypertension. There were significant reductions in both systolic and diastolic blood pressures in the supine and erect postures following all active doses, with no clearly defined dose-response relationship for anti-hypertensive effect. The hypotensive effect was apparent within 1 h of dosing and maintained for at least 8 h with a maximal effect at 6 h. No significant effect on blood pressure was apparent 24 h after any of the active doses.

The fall in blood pressure was not associated with a significant increase in heart rate or plasma catecholamines, in accord with previous findings with other ACE inhibitors (Niarchos *et al.*, 1982;

Zanella *et al.*, 1981; Miller *et al.*, 1982). This is unlikely to be related to a gross sympatho-inhibitory effect or to an impairment of baro-reflex function, as the pressor, the chronotropic and catecholamine responses in the supine position, following upright posture and after dynamic exercise were unaltered by cilazapril. The absence of reflex tachycardia with blood pressure reduction after ACE inhibitors appears, in part, to be related to an enhancement of cardiac parasympathetic tone (Sturani *et al.*, 1982; Campbell *et al.*, 1985; Ajayi *et al.*, 1985a) following the withdrawal of the vagolytic actions of angiotensin II (Ajayi *et al.*, 1985b).

Plasma angiotensin converting enzyme activity was markedly inhibited to a similar extent after all active doses with a peak effect at 2–3 h. The residual inhibition at 24 h was also comparable with the different doses of cilazapril.

Although no significant differences between doses were seen, the rank order of peak inhibition was consistent with the 20 mg dose exhibiting the greatest inhibition. However, a clearer dose-response relationship was apparent with the changes in plasma renin activity (PRA) whereby the 20 mg dose of cilazapril caused the greatest increase in PRA. The reactive rises in PRA were maximal at 6 h and coincident with the lowest blood pressures. No correlation, however, was found between baseline or reactive PRA increases and the antihypertensive efficacy of cilazapril. Although a predictive value of basal PRA for the hypotensive action of captopril has been reported (Case *et al.*, 1980) this has not been a universal finding (Waeber *et al.*, 1980; Gavras *et al.*, 1978).

Plasma aldosterone concentrations tended to fall with active treatment to a nadir at 6 h.

Although no clear dose-response relationship was evident, only the 20 mg dose of cilazapril appeared to suppress the aldosterone concentration at 24 h.

In conclusion, these results suggest that cilazapril is a potent ACE inhibitor, with a rapid onset and a prolonged duration of humoral and antihypertensive actions. The results of this study suggest that the upper part of the dose-response relationships for its therapeutic effect is already achieved at 5 mg. For the long term treatment of hypertension further studies should be undertaken with doses of 5 mg or less.

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