A review of the preclinical cardiovascular pharmacology of cilazapril, a new angiotensin converting enzyme inhibitor

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1 Cilazapril is the monoethyl ester prodrug form of the di-acid cilazaprilat, a new angiotensin converting enzyme (ACE) inhibitor. Cilazaprilat has an IC_{50} of 1.9 nM as an inhibitor of rabbit lung ACE *in vitro* making it one of the most potent ACE inhibitors currently available. Studies on a wide range of other enzymes show that the inhibition is highly specific.

2 An oral dose of 0.1 mg kg⁻¹ cilazapril evoked the same maximum degree of plasma ACE inhibition (~76%) in the rat as 0.25 mg kg⁻¹ enalapril. Cilazapril (0.25 mg kg⁻¹ p.o.) inhibited plasma ACE by > 95%. The rate of recovery of ACE activity was slower with cilazapril (5–6% h⁻¹) than with enalapril (10% h⁻¹).

3 In anaesthetised rats cilazaprilat was equipotent with ramiprilat and slightly more potent (1.5×) than enalaprilat as an inhibitor of the angiotensin I pressor response.

4 Following oral administration to conscious rats and intravenous administration to anaesthetised dogs, cilazapril was 2–4.5× more potent than enalapril as an ACE inhibitor.

5 In cats cilazapril (0.1 and 0.3 mg kg⁻¹ p.o.) dose dependently decreased plasma ACE activity and the angiotensin pressor response. Peak effects occurred at 2 h after dosing and plasma ACE inhibition was maintained at ≥ 50% for up to 18 h. Mean arterial pressure was also decreased dose dependently with a peak effect at 3–4 h.

6 Daily oral dosing of cilazapril (30 mg kg⁻¹ p.o.) to spontaneously hypertensive rats evoked a progressive and prolonged (24 h) antihypertensive response with a maximum decrease in systolic blood pressure of 110 mm Hg.

7 Cilazapril (10 mg kg⁻¹ p.o. twice daily for 3.5 days) progressively decreased blood pressure in volume depleted renal hypertensive dogs. The maximum fall in systolic pressure was 39 ± 6 mm Hg.

8 Haemodynamic studies in open chest anaesthetised dogs showed that the hypotensive response to intravenous cilazapril was accompanied by a reduction in total peripheral resistance. Small decreases in cardiac output and myocardial contractile force were seen at high doses.

9 Cilazapril had no adverse effect on cardiovascular reflexes. There was no impairment of the baroreflex in rats. Exercise-induced tachycardia and pressor responses in conscious cats were unchanged.

10 Cilazapril is exceptionally well absorbed by the oral route (98% in rats).

Keywords angiotensin converting enzyme cilazapril blood pressure
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Introduction

Angiotensin converting enzyme (ACE) inhibitors have proved to be successful agents for the treatment of mild to moderate hypertension, normalising blood pressure in 40–50% of patients when given as monotherapy and in 80–90% when given in combination with a diuretic (De Felice & Kostis, 1987). Unlike other classes of antihypertensive drugs, ACE inhibitors have not been associated with sexual dysfunction, depression, fatigue and reflex tachycardia, which has widened their acceptance for first line therapy (Rasmussen *et al.*, 1986).

Synthesis of conformationally restricted structures that allow optimal binding to ACE has resulted in the non-sulphydryl ACE inhibitor cilazapril (Ro 31-2848), the monoethyl ester prodrug form of cilazaprilat [9(S)-[1(S)-carboxy-3-phenylpropylamino]-octahydro-10-oxo-6H-pyridazo[1,2-a] [1,2]diazepine-1(S)carboxylic acid] (Attwood *et al.*, 1984). The purpose of the present report is to review the preclinical cardiovascular pharmacology of cilazapril to determine whether it meets accepted criteria for a successful new antihypertensive drug. These criteria include potency, target selectivity, duration of antihypertensive action, absence of adverse effects on haemodynamics/cardiovascular reflexes and adequate oral absorption.

Inhibition of the renin angiotensin system

ACE inhibition in vitro

Enzyme activities were determined by Natoff *et al.* (1985) using an assay based on the release of hippuric acid from the substrate Hip-His-Leu, originally described by Cushman & Cheung (1971) with the modification of Hayakari *et al.* (1978). IC_{50} values were estimated from dose-response curves ($S \rightarrow E + I$) generated over a wide range of concentrations. Approximate K_i values for the binding of cilazaprilat and enalaprilat to rabbit lung ACE were determined from plots of IC_{50} values vs enzyme concentration (Cha *et al.*, 1975).

The IC_{50} values for cilazaprilat and eleven other ACE inhibitors are shown in Table 1.

Cilazaprilat was more potent than enalaprilat (1.6 $\times$), lisinopril (1.7 $\times$), ramiprilat (2 $\times$) and captopril (3.6 $\times$) in direct comparisons. Taken together with the literature values quoted for a number of recently developed ACE inhibitors, derived under similar assay conditions (Table 1), only the di-acid form of spirapril has potency significantly greater than that of cilazaprilat.

For a limited number of compounds the reaction was initiated by adding substrate to a mixture of enzyme and inhibitor ($S \rightarrow E + I$). Under these conditions both cilazaprilat and

Table 1 Inhibition of rabbit lung ACE using Hip-His-Leu substrate *in vitro*

Drug*	IC_{50} (nM)	Reference
Cilazapril ^a	1.93 (1.86–2.01)	Natoff <i>et al.</i> (1985)
Enalapril ^a	3.12 (2.98–3.26)	Natoff <i>et al.</i> (1985)
Lisinopril ^a	3.3	Nixon (unpublished)
Ramipril ^a	4.0	Nixon (unpublished)
Captopril ^a	6.93 (5.68–8.28)	Natoff <i>et al.</i> , (1985)
Spirapril	0.8	Sybertz <i>et al.</i> (1987)
Benazapril	1.7	Watthey <i>et al.</i> (1985)
Fentiapril	6.0	Iso <i>et al.</i> (1981)
Fosenopril	11.0	Powell <i>et al.</i> (1984)
Pivalopril	18.0	Schwab <i>et al.</i> (1983)
Pentopril	28.0	Goodman (1985)
Delapril	40.0	Nishikawa <i>et al.</i> (1983)

^a Reaction initiated by addition of enzyme to a mixture of substrate and inhibitor ($E \rightarrow I + S$).

* Di-acid form of ester prodrug used. IC_{50} values above the dotted line were derived under similar experimental conditions in this laboratory, as described in the text. Figures in parenthesis are 95% confidence limits. Values below the dotted line are from other laboratories using comparable assay conditions and where similar IC_{50} values for standard ACE inhibitors have been derived.

Table 2 *In vitro* potencies of cilazaprilat, enalaprilat and captopril as ACE inhibitors

Substrate	Source of ACE	Cilazaprilat	IC_{50} (nM)	
			Enalaprilat	Captopril
Hip-His-Leu	Human plasma	0.61 (0.58–0.64)	1.66 (1.54–1.80)	15.70 (12.99–18.87)
	Hog kidney	2.83 (2.54–3.13)	5.95 (5.64–6.27)	13.15 (11.48–15.09)
	Human lung	1.39 (1.31–1.47)	2.93 (2.79–3.07)	13.05 (12.45–13.67)
AI	Rabbit lung	0.78 (0.72–0.85)	1.44 (1.30–1.60)	

Figures in parenthesis are 95% confidence limits.

enalaprilat showed an increase in potency with IC_{50} 0.97 (0.90–1.04) and 1.29 (1.22–1.36) respectively (Natoff *et al.*, 1985). K_i values for cilazaprilat and enalaprilat (0.05 and 0.15 nM respectively) were around 10-fold lower.

The ranking order of potency cilazaprilat > enalaprilat > captopril derived for rabbit lung ACE was maintained regardless of tissue or enzyme source (Table 2) with cilazaprilat having a potency advantage of 1.3–2.7-fold over enalaprilat and 5–26-fold over captopril. There is therefore little evidence for preferential inhibition of ACE from one particular source by any of these compounds. It is interesting to note that the same potency order was maintained at the more clinically relevant level of 90% inhibition of plasma ACE where IC_{50} values were: cilazaprilat (10 nM), enalaprilat (21 nM) and captopril (50 nM) (Natoff *et al.*, 1985).

Specificity for ACE

Cilazaprilat (20 μ M) did not inhibit porcine pancreatic and human granulocyte elastase, cathepsins B, D and G, chymotrypsin, carboxypeptidase A, thermolysin and porcine pancreatic phospholipase A_2 . It was however a competitive inhibitor of *E. coli* dipeptidylcarboxypeptidase with an IC_{50} of 35 nM (Natoff *et al.*, 1985).

Thus at concentrations ~10,000-fold higher than its IC_{50} against ACE, cilazaprilat did not block the activities of a variety of lipolytic and proteolytic enzymes, showing high specificity for ACE. Deutch & Soffer (1978) have reported that *E. coli* dipeptidylcarboxypeptidase has properties similar to ACE and the activity of cilazaprilat against this enzyme supports their findings.

ACE inhibition ex vivo

Natoff *et al.* (1985) reported that when AP1 rats were dosed orally with cilazapril (0.1 mg kg⁻¹) and enalapril (0.25 mg kg⁻¹) the rates of onset

and peak ACE inhibitory activities (76–77% at ~1 h post-dose) were similar for both drugs (Figure 1). Peak inhibition for cilazapril (0.25 mg kg⁻¹) was greater (96%). The activity of cilazapril waned at a rate of 5–6% h⁻¹ whereas the rate of decrease for enalapril was more rapid (10% h⁻¹), indicating a shorter duration of action. Estimated times of return to 50% inhibition of plasma ACE were 5 h for cilazapril (0.1 mg kg⁻¹) and 4 h for the equiactive dose of enalapril (0.25 mg kg⁻¹). Ten hours recovery was required for cilazapril (0.25 mg kg⁻¹).

Cilazapril was also more effective than enalapril by the oral route as an inhibitor of plasma ACE activity in conscious baboons (S. W. Holmes, unpublished observations). Thus cilazapril (0.5 mg kg⁻¹) gave a peak inhibition of > 95% baboon plasma ACE within 30 min of administration whereas the same dose of enalapril gave a peak effect of 87% at 2 h. A dose of 5 mg kg⁻¹ p.o. cilazapril maintained plasma ACE inhibition at > 95% for at least 8 h. When cilazapril or enalapril (0.5 mg kg⁻¹) were given intravenously to baboons, however, the profiles of ACE inhibitory activity and recovery were superimposable. These data suggest that cilazapril and enalapril have similar potency as inhibitors of baboon plasma ACE but that the oral absorption of cilazapril is greater and the onset of action more rapid.

Inhibition of angiotensin pressor response in vivo

Anaesthetised rats Inhibition of the angiotensin I (AI) pressor response was determined in urethane anaesthetised spontaneously hypertensive rats that had been unilaterally adrenalectomised and contralaterally adrenal demedullated at 10 weeks of age (SHAD rats). The method employed compared the inhibition of the AI response relative to that of the AII response (angiotensin pressor response ratio, APRR) to allow for changes in post-synaptic vascular sensitivity (Natoff *et al.*, 1981). ACE

Figure 1 Effect of oral doses of cilazapril (0.1 mg kg^{-1} , $\bullet$; 0.25 mg kg^{-1} , $\blacktriangle$) and enalapril (0.25 mg kg^{-1} , $\circ$) on plasma ACE activity in conscious rats. Values shown are mean $\pm$ s.e. mean.

inhibitors were administered intravenously and the APRR calculated at various time points after dosing. Table 3 shows the median effective doses (ED_{50} values) derived at 15 min. The results show that cilazapril was equipotent with ramiprilat and slightly more potent than enalaprilat. Captopril was the least potent of the drugs

tested. The relatively high ED_{50} value of cilazapril vs cilazaprilat cannot be accounted for by incomplete hydrolysis of the prodrug at the 15 min time point since a greater degree of ACE inhibition was not apparent at subsequent time points. One possible explanation may be that after i.v. administration much of the prodrug is eliminated by non-renal pathways (see section on oral absorption).

Similar experiments were made following oral administration of the ACE inhibitors to conscious male AP1/R2 rats with indwelling right jugular venous cannulae for AI/AII administration and right carotid arterial cannulae for BP recording. Doses required to reduce the APRR by 30% were calculated by linear regression analysis at 15–150 min after administration. Cilazapril was rapidly absorbed showing a maximum effect at 60 min ($\text{ED}_{30} 0.02 \text{ mg kg}^{-1}$). The maximum inhibitory effect of enalapril was also produced at 1 h after dosing, but the compound was 4.5 times less potent ($\text{ED}_{30} 0.09 \text{ mg kg}^{-1}$). Captopril was also rapidly absorbed, but was eight times less potent than cilazapril ($\text{ED}_{30} 0.16 \text{ mg kg}^{-1}$ at 30 min). Lisinopril was very slowly absorbed with ACE inhibition continuing to develop at 150 min after dosing ($\text{ED}_{30} 0.08 \text{ mg kg}^{-1}$) (D. T. Burden, unpublished observations).

In the anaesthetised dog, cilazapril had an ED_{50} of 0.08 (0.06 – $0.12 \text{ } \mu\text{mol kg}^{-1}$ i.v.) against AI (300 ng kg^{-1} i.v.) induced pressor

Table 3 Median effective doses of ACE inhibitors evoking 50% reduction in the preinhibitor angiotensin pressor response ratio in anaesthetised rats

Compound	$\text{ED}_{50} (\text{mmol kg}^{-1})$ (95% confidence limits)
Cilazaprilat	0.06 (0.03–0.10)
Cilazapril	0.44 (0.25–0.92)
Ramiprilat	0.06 (0.05–0.07)
Enalaprilat	0.09 (0.08–0.11)
Enalapril	0.26 (0.19–0.45)
Captopril	0.49 (0.35–0.76)

Values measured 15 min after dosing;
 $n = 4$ – 6 for each dose level of inhibitor.
 Angiotensin pressor response ratio =
 AI pressor response (mm Hg)
 AII pressor response (mm Hg)

responses, the corresponding values for enalapril and captopril being 0.16 (0.07–0.34) and 0.41 (0.27–0.64) $\mu\text{mol kg}^{-1}$. Thus cilazapril was twice as potent as enalapril and five times as potent as captopril in this preparation (M. Gerold, unpublished observations). The dog was also more sensitive to the effects of the ester prodrug than the rat (compare values with those for cilazapril and enalapril in Table 3).

A comprehensive study made in conscious cats related the plasma ACE inhibitory activity of cilazapril to its effect on APRR and blood pressure in the same animal. Animals were prepared with indwelling carotid arterial and jugular venous cannulae for direct blood pressure recording and angiotensin administration.

Control pressor responses to AI (100 ng kg^{-1} i.v.) and AII (30 ng kg^{-1} i.v.) were obtained prior to oral administration of cilazapril (0.1 and 0.3 mg kg^{-1}) or placebo. AI and AII injections were repeated at 14 time intervals up to 96 h after dosing. Immediately before each AI/AII challenge blood samples were removed via the arterial catheter for plasma ACE activity determinations (Holmes, 1987). Cilazapril evoked a dose related inhibition in the APRR and plasma ACE activity. At the lower dose (0.1 mg kg^{-1}) plasma ACE was reduced to a nadir of $7.7 \pm 0.9\%$ of predose levels and APRR was inhibited by a maximum of $79.3 \pm 14.1\%$ at 2 h after dosing (Figure 2a). Corresponding peak effects at 0.3 mg kg^{-1} were $2.1 \pm 0.4\%$ and $97.8 \pm$

Figure 2 Effect of cilazapril and placebo on plasma ACE activity and angiotensin pressor response ratio (APRR) of conscious cats. Plasma ACE activity is expressed as % predose activity. APRR, calculated as AI pressor response/AII pressor response, is shown as % change from predose. All data points represent a mean of four animals.

a. ●, placebo plasma ACE; ○, placebo APRR; ▲, cilazapril 0.1 mg kg^{-1} plasma ACE; △, cilazapril 0.1 mg kg^{-1} APRR.

b. ●, ○ as for 2a; ■, cilazapril 0.3 mg kg^{-1} plasma ACE; □, cilazapril 0.3 mg kg^{-1} APRR.

All plasma ACE values up to 48 h significantly different from placebo, $P < 0.05$ (Student's unpaired *t*-test). * APRR values significantly different from placebo, $P < 0.05$ (Student's unpaired *t*-test).

2.3%. These also occurred at 2 h after dosing (Figure 2b). Plasma ACE activity was inhibited by $\geq 50\%$ for up to 18 h after dosing at both dose levels; APRR was inhibited by $\geq 50\%$ for 6 h after 0.1 mg kg^{-1} and 12 h after 0.3 mg kg^{-1} . Mean arterial pressure was reduced dose dependently, maximum decreases of 21 ± 10 (4 h) and 31 ± 4 (3 h) mm Hg being recorded at 0.1 and 0.3 mg kg^{-1} respectively (Burden & Holmes, unpublished observations). These data further exemplify the rapid onset and prolonged duration of action of cilazapril and the study demonstrates a good correlation between enzyme inhibition and pharmacodynamic effect.

Antihypertensive activity

Spontaneously hypertensive rats Blood pressure was recorded from the tail of SHR (Gerold & Tschirky, 1968) to assess the antihypertensive effect of cilazapril given daily (30 mg kg^{-1} p.o.) for 3 weeks. The results are shown in Figure 3. Cilazapril had a more pronounced antihypertensive effect after seven consecutive daily doses than after the first dose. No further increase in the response was obtained at 14 and 21 days although the predose systolic blood pressure values tended to decline with increasing cumulative doses showing that once daily dosing was adequate. The maximum decrease in blood pressure relative to the control group was 110

mm Hg on day 21. Similar results were obtained for the same dose of enalapril, but the maximum decrease detected was < 80 mm Hg (Natoff *et al.*, 1985).

Volume depleted renal hypertensive dogs The left renal artery of female mongrel dogs was partially occluded by a rigid plastic ring and the contralateral kidney encapsulated in cellophane (Holck *et al.*, 1986). Systolic blood pressure was monitored over 12 weeks using a sphygmomanometer, during which time the hypertension stabilised. The effects of cilazapril or enalapril ($2 \times 10 \text{ mg kg}^{-1} \text{ day}^{-1}$ p.o.) were evaluated in dogs pretreated with frusemide (40 mg day^{-1} i.v.) 16 h before the first daily dose of the ACE inhibitor. Blood pressure and heart rate were measured every 30 min for 6 h and then at 24 h after dosing.

Systolic blood pressure did not change significantly with frusemide alone but there were small increases in heart rate on days 3 and 4 (peak $+ 9 \pm 2 \text{ beats min}^{-1}$). Cilazapril evoked an immediate ($< 30 \text{ min}$) and significant ($P < 0.05$) fall in blood pressure which was still present at the end of the 6 h observation period (Figure 4a). Second and subsequent administrations of cilazapril led to progressively greater falls in blood pressure. The maximum recorded decrease was 39 ± 6 mm Hg on day 3. Enalapril showed a similar time course for its antihypertensive effect

Figure 3 Effect of cilazapril (□) and enalapril (○) on systolic blood pressure of spontaneously hypertensive rats. A dose of 30 mg kg^{-1} p.o. daily was given for 3 weeks. Control rats (●) received vehicle alone. Each point is the mean of six observations $\pm$ s.e. mean. ** $P < 0.01$; *** $P < 0.001$ from control values.

Figure 4 Effects of cilazapril and enalapril (both at $2 \times 10 \text{ mg kg}^{-1} \text{ day}^{-1} \text{ p.o.}$) on the time course of systolic arterial blood pressure (SAP, panel a) and heart rate (panel b) of conscious renal hypertensive, volume depleted dogs. Three groups of five dogs are represented: a frusemide-pretreated saline group (○); a frusemide-pretreated enalapril group (▲); and a frusemide-pretreated cilazapril group (●). Arrows indicate when ACE inhibitor or saline was administered. Both enalapril and cilazapril caused a significant ($P < 0.05$, analysis of variance) reduction in SAP, but no significant change in heart rate.

(Figure 4b). The maximum effect was slightly lower, but overall there were no statistically significant differences (analysis of variance) between the actions of the two drugs (Holck *et al.*, 1986). Neither compound caused significant tachycardia, but there was an upward trend in heart rate in both treatment groups and in the control group (Figure 4b). The results show that ACE inhibitors are effective antihypertensive agents in renal hypertension where renin release has been further provoked by volume depletion brought about by co-administration of a diuretic (Keeton & Campbell, 1980).

Haemodynamic actions Holck *et al.* (1986) reported the haemodynamic actions of intravenously administered doses of cilazapril (0.03,

0.1, 0.3 and 1 mg kg^{-1}) in groups of four anaesthetised open chest normotensive mongrel dogs prepared by the method of Gerold *et al.* (1982). At the lowest dose tested (0.03 mg kg^{-1}) cilazapril exerted no statistically significant effect on the 17 haemodynamic parameters measured. Cilazapril ($0.1\text{--}1 \text{ mg kg}^{-1}$) evoked a dose dependent decrease in mean arterial pressure (18–27%) and left ventricular pressure (12–17%). Left ventricular end diastolic pressure (LVEDP) remained unchanged except for a 15% decrease at 1 mg kg^{-1} . Total peripheral resistance (TPR) decreased significantly (14%) at 0.1 mg kg^{-1} , but no further decrease was apparent at 0.3 and 1 mg kg^{-1} . Cardiac output (CO) was significantly reduced at 0.3 and 1 mg kg^{-1} (15 and 16% respectively), but heart

rate (HR) was unchanged. Left ventricular minute work (LVMW) and stroke work were significantly reduced in a dose dependent manner. For example, at the 0.3 mg kg^{-1} dose level decreases of 31 and 40% were calculated. The rate of rise of the left ventricular pressure curve (dLVP/dt), was significantly decreased (18%) at the highest dose level only. Coronary blood flow (CBF) decreased by 12 and 21% at 0.3 and 1 mg kg^{-1} , but there were no significant changes in coronary vascular resistance (CVR). The peak effects of cilazapril occurred 15–30 min after dosing and were maintained for at least 2 h.

Thus the hypotensive response to cilazapril was accompanied by a decrease in TPR and, at higher doses, by small decreases in CO and LVMW. The fall in CO in the absence of a change in HR suggests a decrease in stroke volume. The small decrease in LVEDP suggests a reduced cardiac filling pressure implying that cilazapril may have an action on both afterload and preload. The dose range evoking a vasodilator effect in this model is in good agreement with that reported to inhibit AI responses in the anaesthetised dog. The small reductions in CO at higher doses were surprising, but similar findings have been reported for captopril in the rat (Smits & Struyker-Boudier, 1984) and for ramipril in the dog (Schölkens *et al.*, 1984) and may be related to the conditions used, namely anaesthesia and thoracotomy. The decrease in CBF in the absence of a change in CVR indicates that the flow reduction was a consequence of a decrease in systemic blood pressure rather than a direct action on the coronary vasculature.

Effects on the baroreceptor reflex

Spontaneously hypertensive rats with indwelling cannulae in the left femoral artery and vein were used to determine the effects of cilazapril, lisinopril, enalapril, prazosin and vehicle on the reflex tachycardia evoked by graded bolus injections ($1\text{--}100 \text{ } \mu\text{g kg}^{-1} \text{ i.v.}$) of sodium nitroprusside (Cowlrick, 1987). The slopes of the regression lines (maximum change in heart rate to maximum change in mean arterial pressure) were compared before and after dosing. Table 4 shows that baroreflex sensitivity was unchanged by cilazapril, lisinopril, enalapril and saline vehicle, but was significantly reduced by prazosin (Cowlrick, 1987). The findings for prazosin are consistent with those of Pierce and Shepperson (1984). The results for the ACE inhibitors are in agreement with those of Pierce & Shepperson (1984). The Reid (1986) who could find no clinical evidence for an action on the baroreflex. In contrast, other workers have suggested that the absence

Table 4 Effects of antihypertensive agents on mean arterial pressure, heart rate and baroreflex in conscious rats

Treatment (i.v.)	n	Pre-dose		Post-dose	
		MAP (mm Hg)	HR (beats min^{-1})	MAP (mm Hg)	HR (beats min^{-1})
Saline (1 ml kg^{-1})	5	188 $\pm$ 12	378 $\pm$ 9	188 $\pm$ 12	380 $\pm$ 8
Cilazapril (1 mg kg^{-1})	5	188 $\pm$ 12	372 $\pm$ 8	186 $\pm$ 11	388 $\pm$ 6
Lisinopril (1 mg kg^{-1})	6	183 $\pm$ 5	403 $\pm$ 13	178 $\pm$ 10	412 $\pm$ 9
Enalapril (1 mg kg^{-1})	5	196 $\pm$ 9	384 $\pm$ 18	184 $\pm$ 11*	408 $\pm$ 12
Prazosin ($3 \text{ } \mu\text{g kg}^{-1}$)	6	196 $\pm$ 13	398 $\pm$ 8	184 $\pm$ 12*	407 $\pm$ 8
					Baroreflex (beats $\text{min}^{-1} \text{ mm Hg}^{-1}$)
					1.6 $\pm$ 0.3
					1.1 $\pm$ 0.2
					1.3 $\pm$ 0.3
					1.2 $\pm$ 0.1
					0.5 $\pm$ 0.1**

* $P < 0.05$; ** $P < 0.01$ (paired *t*-test).
Data are mean $\pm$ s.e. mean.

of reflex tachycardia with ACE inhibitors in animals and man might be due to interference with the baroreflex (Takeda *et al.*, 1986; Giudicelli *et al.*, 1985).

Effects on cardiovascular responses to exercise

The effects of cilazapril, lisinopril, enalapril, propranolol, prazosin and phenoxybenzamine on the tachycardia and vasopressor responses to exercise were evaluated in conscious cats trained to exercise in a metal drum for 30 s at 20 rev min⁻¹ (Blaber & Burden, 1982). Mean arterial pressure and heart rate were recorded via exteriorised catheters. Mean resting heart rate was 196 ± 10 beats min⁻¹ and blood pressure was 126 ± 10 mm Hg. Exercise induced a tachycardia of 86 ± 13 beats min⁻¹ and a pressor response of 38 ± 5 mm Hg. The results of drug treatment are shown in Figure 5. Propranolol (10 mg kg⁻¹ p.o.) markedly reduced the heart rate response to exercise but had no effect on the pressor response, consistent with reported observations from dogs (Staib *et al.*, 1980) and man (Leenen *et al.*, 1983). Phenoxybenzamine (20 mg kg⁻¹ p.o.) reduced both the pressor response and the exercise induced tachycardia; the reason for the latter effect is not clear. In contrast cilazapril, captopril, enalapril, lisinopril and ramipril had minimal effects on the cardiovascular responses

to exercise (Burden *et al.*, 1988). These findings are in agreement with the work of Becker *et al.* (1985) who showed that ramipril did not impair exercise performance in dogs.

Oral absorption

Rats were dosed orally with cilazapril, cilazaprilat or enalapril (1 mg kg⁻¹) and 1 week later they received the same dose by the intravenous route. Urine voided over the first 24 h after dosing was collected, diluted to a fixed volume and assayed after serial dilution for ACE inhibitory activity using [¹⁴C]Hip-His-Leu as substrate and rabbit lung ACE. The dilution factor for 50% inhibition was obtained by regression analysis of the percent uninhibited control against log dilution factor. Oral absorption was calculated as 100 × (ID₅₀ p.o./ID₅₀ i.v.) (Natoff *et al.*, 1985). The results are shown in Figure 6. From the oral/intravenous 50% dilution factors for the parent drug doses, the oral absorption was calculated to be 98% for cilazapril and 46% for enalapril. Tocco *et al.* (1982) reported a lower figure (34%) for enalapril. Pretreatment of cilazapril urines with rat plasma to induce hydrolysis of any unchanged parent drug before serial dilution did not significantly alter the results showing that little unchanged parent drug was excreted via the kidney. Oral absorption of the biologically

Figure 5 Effects of ACE inhibitors, propranolol, phenoxybenzamine and placebo on exercise induced changes in heart rate and blood pressure in conscious cats. Data are shown as peak changes (± s.e. mean) from the initial (pre-drug) responses. Hatched bars represent changes in heart rate; solid bars show blood pressure changes. Abbreviations are as follows: PLAC = Lactose placebo; CIL = cilazapril; CAP = captopril; ENAL = enalapril; LIS = lisinopril; RAM = ramipril; PROP = propranolol; PHEN = phenoxybenzamine; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Student's unpaired *t*-test).

Figure 6 Inhibition of rabbit lung angiotensin-converting enzyme activity against Hip-His-Leu substrate by various dilutions of 24 h urine samples collected from rats treated orally (open symbols) and intravenously (closed symbols) with enalapril, cilazapril and cilazaprilat at dose levels of 1 mg kg^{-1} . Each point is the mean of observations from two rats, each rat being used for both routes.

active di-acid cilazaprilat was relatively poor at 10%, a value close to the 7% reported for enalaprilat (Tocco *et al.*, 1982), confirming the requirement for ester prodrugs for oral administration.

The higher level of urinary ACE inhibitory activity after intravenous cilazaprilat implies

that a significant proportion of intravenously administered cilazapril may be cleared by non-renal pathways. Alternative clearance pathways for the unchanged prodrug could also account for the relatively high intravenous ED_{50} value for inhibition of the APRR in the rat for cilazapril compared with cilazaprilat (Table 3).

Thus the oral absorption of cilazapril was substantially greater than that of enalapril confirming the tentative conclusions drawn from the *ex vivo* studies on plasma ACE inhibitory activity in rats and baboons.

Conclusion

In vitro studies show that cilazapril is one of the most potent ACE inhibitors currently available. Studies on a number of enzymes indicate a high degree of specificity for ACE. *Ex vivo* and *in vivo* experiments in rats, dogs, cats and baboons show that the onset of enzyme inhibition is rapid and the duration of action prolonged. The duration of the antihypertensive action in spontaneously hypertensive rats and volume depleted renal hypertensive dogs indicates that once daily dosing with cilazapril is a realistic clinical target.

Blood pressure decreases in open chest anaesthetised dogs could be accounted for by a reduction in total peripheral resistance, small decreases in cardiac function occurring at high doses only.

Cilazapril, as with other ACE inhibitors did not impair the baroreflex or exercise mediated increases in sympathetic drive. Oral absorption of cilazapril in the rat was double that of enalapril, being close to 100%. This factor, combined with the greater potency of cilazapril is reflected in a number of comparative studies *in vivo* as a more rapid onset of action, longer duration and improved efficacy.

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