Pharmacological properties of FPL 63547, a novel inhibitor of angiotensin-converting enzyme

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1 FPL 63547, in its active diacid form, was a potent inhibitor of rabbit lung angiotensin converting enzyme (ACE) *in vitro* (IC₅₀ 0.51 nM).

2 In conscious normotensive dogs, FPL 63547 (10–300 µg kg⁻¹ i.v.) produced prolonged, dose-related inhibition of plasma ACE activity and angiotensin I pressor responses, without affecting basal blood pressure, heart rate or pressor responses to angiotensin II.

3 In anaesthetized dogs, FPL 63547 diacid (3–300 µg kg⁻¹ i.v. cumulatively) produced dose-related increases in cardiac output accompanied by falls in total peripheral resistance indicative of vasodilatation. Mild stimulation of cardiac rate and contractility was also observed. Enalapril diacid had a similar profile.

4 FPL 63547 was a highly effective antihypertensive agent after oral administration to spontaneously hypertensive rats (SHR) pretreated with a diuretic. It lowered systolic blood pressure (SBP) on acute administration over the range 3×10^{-7} – 10^{-5} mol kg⁻¹ p.o. (≈0.13–4.5 mg kg⁻¹ p.o.). FPL 63547 was more potent than other ACE inhibitors tested, threshold active doses for lisinopril, enalapril and captopril being 10^{-6} , 10^{-6} and 3×10^{-5} mol kg⁻¹ p.o., respectively. The antihypertensive effects of FPL 63547, unlike those of enalapril and captopril, were of long duration.

5 The antihypertensive efficacy of FPL 63547 was also observed following chronic oral administration. A dose of 0.5 mg kg⁻¹ day⁻¹ once daily for 23 days produced a sustained reduction of SBP. By the end of the treatment period, SBP was significantly lowered both pre- and post-dose, i.e. effective 24 h control had been achieved.

6 The profile of FPL 63547 is consistent with it being a potent, selective and long-acting ACE inhibitor. As an antihypertensive agent in SHR it compared favourably with other members of this class with respect to potency and duration of action.

Introduction

The biological rationale for inhibition of angiotensin-converting enzyme (ACE: E.C.3.4.15.1) is well known (Cushman & Ondetti 1980). Agents acting through this mechanism are now established in the treatment of heart failure and hypertension, and recent appreciation of the possible pathophysiological roles of tissue renin-angiotensin systems (Dzau, 1988) suggests that further extensions of therapeutic utility are likely. Clinical demonstrations that ACE inhibitors can reduce mortality in congestive heart failure patients (CONSENSUS Trial Study Group, 1987) and improve quality of life (Croog *et al.*, 1986) add considerably to the attractiveness of this class of compound. Overall, ACE inhibitors appear destined to form one of the major drug classes in future cardiovascular therapy, possibly becoming first line treatment for certain conditions (Brunner *et al.*, 1987).

FPL 63547 (Figure 1) is the ester prodrug of a novel thiazolidine ACE inhibitor. This paper describes some of the biological properties of FPL 63547 and its active diacid form (FPL 63547 diacid) in comparison with captopril, enalapril, enalapril diacid and lisinopril. A preliminary account of this work has been presented to the British Pharmacological Society (Carr *et al.*, 1988).

The ACE inhibitors described to date are generally considered to be similar, differing mainly in potency or duration of action. This has led to recognition (Lancet, 1988) that newer agents of this class should demonstrate biological properties which distinguish them from the first generation ACE inhibitors. In the accompanying paper (Carr *et al.*, 1990) we describe the marked preference of FPL 63547 for the biliary route of elimination, which is a novel feature of this compound.

Methods

Angiotensin-converting enzyme inhibition *in vitro*

Inhibition of rabbit lung ACE was assessed by a method based on that described by Cushman & Cheung (1971). Enzyme activity was determined by measuring release of radiolabelled hippurate from the synthetic enzyme substrate hippuryl-histidyl-L-leucine (HHL).

Rabbit lung ACE was purified by lisinopril-Sepharose CL-4B affinity chromatography, as described by Bull *et al.* (1985) with minor modifications. This scaled-down version, suitable for 35 g wet weight of lung tissue, used 0.5% Nonidet P-40 (NP-40) in 50 mM potassium phosphate, 300 mM NaCl, 20 µM ZnCl₂, pH 8.3 for solubilisation of enzyme, and washing the column (volume 10 ml) to remove unbound protein (five column volumes at 15 ml h⁻¹). NP-40 was then

Figure 1 Structures of FPL 63547 and its active diacid.

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omitted and the column was washed with a further five volumes of buffer. ACE was eluted with 100 μM captopril in buffer, pooled and dialysed against 50 mM potassium phosphate, 100 μM ethylenediaminetetraacetic acid (EDTA) pH 8.3 (seven changes of 60 volumes), followed by dialysis against 50 mM potassium phosphate pH 8.3 (two changes of 60 volumes). The enzyme was divided into aliquots and stored at -70°C .

[^{14}C]-HHL was used as substrate (2 mM, containing 10 nCi [^{14}C]-HHL per tube). ACE was incubated with the ACE inhibitor/substrate mixture (final volume 0.25 ml) for 30 min at 37°C in 100 mM potassium phosphate, 300 mM NaCl, 20 μM ZnCl_2 at pH 8.3. Reactions were terminated by adding 0.25 ml 1 M HCl and [^{14}C]-hippurate extracted into 1.5 ml ethyl acetate and counted. The enzyme concentration was adjusted so that cleavage of the substrate did not exceed 10% in uninhibited controls.

Angiotensin-converting enzyme inhibition in vivo

Adult male Beagle dogs (10–17 kg) underwent aseptic surgery under general anaesthesia (pentobarbitone 30 mg kg^{-1} i.v.) to implant catheters into the femoral artery to record blood pressure and into the femoral vein for drug administration. Catheters were exteriorised in the nape of the neck and terminated on two-way titanium valves attached to skin buttons. Titanium electrodes were implanted subcutaneously in the neck and on the rump to record ECG.

Experiments were initiated 2–3 weeks following the operation at a time when the animals were fully recovered and had been trained to sit quietly in harnesses. Systolic (SBP) and diastolic (DBP) blood pressures were measured and mean blood pressure (MBP) derived from the pressure transducer signal. This signal also triggered a ratemeter to give a record of heart rate (HR). All variables, including ECG, were displayed on a Lectromed recorder. On each experimental day, dogs received a single i.v. dose of FPL 63547 (10–300 $\mu\text{g kg}^{-1}$) or vehicle (sterile citric acid–phosphate buffer). SBP, DBP, HR and ECG were measured before drug administration and at various times in the 24 h period afterwards. Plasma ACE activity, measured by a spectrophotometric method (Holmquist *et al.*, 1979), was determined in arterial blood samples taken at -0.5 , $+1$, $+6$ and $+24$ h. Angiotensin I (150 ng kg^{-1} i.v.) and angiotensin II (150 ng kg^{-1} i.v.) were administered sequentially at -0.5 , $+0.5$, $+1$, $+4$, $+5$, $+23$, $+24$ and $+25$ h approximately and the pressor responses produced measured as changes in MBP. Drug-induced effects on all variables were compared for statistical significance with the corresponding changes produced by vehicle in the same dogs ($P < 0.05$, paired *t* test).

Haemodynamic effects in anaesthetized dogs

Haemodynamic profiles of FPL 63547 diacid and enalapril diacid were compared in anaesthetized adult male Beagles (10–15 kg). The methodology has been described in detail previously (Humphries & O'Connor, 1988). Briefly, anaesthesia was induced with thiopentone and maintained with chloralose. The animals were artificially ventilated and surgically prepared to record mean blood pressure (BP), mean cardiac output (CO) using an electromagnetic flow probe positioned around the ascending aorta, and cardiac contractility (dP/dt P^{-1}) derived from measurement of left ventricular pressure. Total peripheral resistance (TPR) was obtained from continuous division of BP by CO and heart rate (HR) was triggered from the left ventricular pressure signal.

FPL 63547 diacid (1–300 $\mu\text{g kg}^{-1}$ i.v. $n = 4$), enalapril diacid (1–300 $\mu\text{g kg}^{-1}$ i.v. $n = 4$) or vehicle (saline) was administered as a series of cumulative i.v. bolus doses. Each dog received vehicle, followed by either FPL 63547 diacid cumulatively or enalapril diacid cumulatively. Percentage changes

from predose levels for each variable were calculated and analysed statistically to test for differences between drug and vehicle treatments ($P < 0.05$, unpaired *t* test).

Antihypertensive effects in spontaneously hypertensive rats

Conscious adult male spontaneously hypertensive rats (SHR), weight range 300–500 g, underwent a training period to accustom them to experimental procedures before use. Only rats with pretreatment systolic blood pressures > 180 mmHg and which responded normally to a standard antihypertensive agent (nifedipine 10^{-5} mol kg^{-1} p.o.) were included in the study.

Systolic blood pressure (SBP) was recorded indirectly by the tail-cuff technique. This involved restraining the animals and placing them in an incubator ($32 \pm 2^\circ\text{C}$) for 20 min to establish a satisfactory arterial tail pulse. SBP was measured by automatic inflation of a sphygmomanometer cuff (Narco Bio Systems) around the proximal portion of the tail. Each SBP value was the mean of at least three readings. Oral pretreatment with hydrochlorothiazide (HCTZ) was used in all experiments to increase the dependence of blood pressure on the renin-angiotensin system, thus producing a model which is more sensitive to the antihypertensive effects of ACE inhibitors (Natoff *et al.*, 1985).

Acute dosing studies The antihypertensive properties of FPL 63547 have been compared with those of other ACE inhibitors after acute oral dosing. For each experiment, rats were allocated into two groups of six with approximately equal basal mean SBP. HCTZ (2×10^{-5} mol kg^{-1} p.o.) was administered at -0.5 h. One group of animals received a single oral dose of ACE inhibitor at time zero in PEG 300 (1 ml kg^{-1}) and the other group received vehicle alone. Compounds tested in this fashion were FPL 63547 (10^{-7} – 10^{-5} mol kg^{-1} p.o.), captopril (10^{-5} – 10^{-4} mol kg^{-1} p.o.), lisinopril (3×10^{-7} – 10^{-5} mol kg^{-1} p.o.) and enalapril (3×10^{-7} – 10^{-5} mol kg^{-1} p.o.). To provide a composite picture of drug-induced changes in SBP over the 24 h period after dosing, each dose of compound was tested twice, on separate occasions. Initially in a group where SBP readings were taken 1 h before (-1 h) and then at 1, 3 and 5 h after dosing (morning-dosed), and secondly when readings were taken at -1 , 15, 18, 21 and 24 h (evening-dosed). Percentage changes in SBP at each time interval were calculated relative to the pre-dose (-1 h) reading. Post-drug treatment SBP values were compared against pre-dose readings and time-matched control group readings and were considered to have changed significantly only if $P < 0.05$ (unpaired *t* test) for both comparisons.

Chronic dosing study Three groups of SHR were used to investigate antihypertensive properties after chronic oral dosing. One received FPL 63547 treatment, one received enalapril for comparison and the third functioned as a control (vehicle). The rats were monitored and dosed daily over a continuous seven week period divided up as follows. A 14 day lead-in period (Days -14 to -1) during which the animals received HCTZ (5 mg kg^{-1} p.o.) and vehicle (PEG 300) each day. A 23 day treatment period during which the animals received HCTZ and the substance under test daily, i.e. either FPL 63547 0.5 mg kg^{-1} day $^{-1}$ p.o., enalapril 5 mg kg^{-1} day $^{-1}$ p.o. or PEG 300 1 ml kg^{-1} day $^{-1}$ p.o. Finally, a 13 day recovery period where the treatment was as for the lead-in period. SBP was measured 1 h pre-dose (-1 h) and 5 h post-dose ($+5$ h) at 2–3 day intervals throughout the study. Time-matched SBP values were compared between drug-treated and control groups to determine statistical significance ($P < 0.05$, unpaired *t* test).

Materials

FPL 63547, enalapril and their diacids were synthesized in the Department of Medicinal Chemistry, Fisons plc; lisinopril was a gift from Merck and captopril from Squibb; HCTZ, HHL, NP-40, EDTA were obtained from Sigma; Sepharose CL-4B from Pharmacia; [^{14}C]-HHL from DuPont; thiopentone from May and Baker and α -chloralose from Koch Light.

Results

Angiotensin-converting enzyme inhibition in vitro

Results from this study are shown in Table 1. FPL 63547 diacid was a highly potent inhibitor of rabbit lung ACE, active at subnanomolar concentrations. On the basis of mean IC_{50} values it was slightly more potent than lisinopril and enalapril and approximately four times more potent than captopril. The data for FPL 63547 indicate that the ester prodrug has no significant intrinsic ACE inhibitory properties.

Angiotensin-converting enzyme inhibition in vivo

FPL 63547 was a potent inhibitor of plasma ACE and angiotensin I pressor responses in the conscious dog after i.v. bolus dosing. These inhibitory effects were rapid in onset and long-lasting. They are summarized in Figure 2 which shows data generated 1 h after dosing, approximately the time of

Table 1 Inhibition of rabbit lung angiotensin-converting enzyme *in vitro*

	IC_{50} (nM)	95% CL	n
FPL 63547 diacid	0.51	0.26–1.02	5
Lisinopril	0.63	0.42–0.95	6
Enalapril diacid	0.95	0.70–1.30	6
Captopril	2.27	1.46–3.54	6
FPL 63547	> 100	—	2

Figure 2 Effects of a series of bolus i.v. doses of FPL 63547 on plasma angiotensin-converting enzyme (ACE) activity ($\circ$), angiotensin I pressor responses ($\bullet$) and angiotensin II pressor responses ($\square$) in conscious dogs. Results are from drug-treated animals 1 h after dosing expressed as percentage inhibition (mean with vertical lines indicating s.e., $n = 4$) when compared with pre-dose (-0.5 h) readings. * $P < 0.05$ compared with corresponding changes produced by vehicle.

peak inhibition. At this time point FPL 63547 (10 – $100 \mu\text{g kg}^{-1}$) produced dose-related inhibition of plasma ACE, reaching a peak of $97 \pm 1\%$. Angiotensin I pressor responses were also inhibited by FPL 63547 in a dose-related fashion over the range 10 – $300 \mu\text{g kg}^{-1}$, but were a less sensitive index. Mean ID_{50} values calculated with individual animal data were $12.3 \pm 1.3 \mu\text{g kg}^{-1}$ against plasma ACE and $60 \pm 28 \mu\text{g kg}^{-1}$ against angiotensin I pressor responses. Even at the highest dose used in the study ($300 \mu\text{g kg}^{-1}$) FPL 63547 did not affect pressor responses to angiotensin II (Figure 2). The time course of inhibition of plasma ACE by FPL 63547 is shown in Figure 3. Peak inhibitory effect was achieved at $+1$ h for all doses and declined slowly thereafter, still achieving statistical significance 24 h after dosing for each of the three highest doses tested.

Table 2 shows a comparison of haemodynamic variables in FPL 63547-treated and vehicle-treated dogs 1 h after dosing, the time point approximating to peak ACE inhibitory effect. No statistically significant changes in SBP, DBP, HR or ECG were observed throughout the dose range used (10 – $300 \mu\text{g kg}^{-1}$ i.v.).

Haemodynamic effects in anaesthetized dogs

The effects of cumulative i.v. administration of FPL 63547 diacid on the haemodynamics of the anaesthetized dog are illustrated in Figure 4. Vehicle (not shown) produced negligible changes. A dose of $1 \mu\text{g kg}^{-1}$ of FPL 63547 diacid

Figure 3 Time course of inhibition of plasma angiotensin converting-enzyme (ACE) activity in conscious dogs by various i.v. doses of FPL 63547. Results shown are percentage inhibition compared with pre-dose (-0.5 h) readings (mean with vertical lines indicating s.e., $n = 4$). FPL 63547 10 ($\blacksquare$), 30 ($\square$), 100 ($\bullet$) and 300 ($\circ$) $\mu\text{g kg}^{-1}$ i.v., vehicle (Δ). * $P < 0.05$, versus vehicle group.

Table 2 Effects of FPL 63547 on systolic (SBP) and diastolic (DBP) blood pressures and heart rate (HR) of conscious dogs ($n = 4$)

	SBP (mmHg)	DBP (mmHg)	HR (bpm)
Vehicle	169 ± 12	88 ± 14	75 ± 5
FPL 63547:			
$10 \mu\text{g kg}^{-1}$ i.v.	171 ± 11	88 ± 6	90 ± 8
$30 \mu\text{g kg}^{-1}$ i.v.	159 ± 11	83 ± 10	79 ± 7
$100 \mu\text{g kg}^{-1}$ i.v.	155 ± 2	79 ± 2	68 ± 6
$300 \mu\text{g kg}^{-1}$ i.v.	171 ± 11	87 ± 4	81 ± 11

All readings taken 1 h following dosing ($+1$ h).

Figure 4 Haemodynamic effects of (a) FPL 63547 diacid and (b) enalapril diacid in anaesthetized dogs ($1\text{--}300\text{ }\mu\text{g kg}^{-1}$ i.v.). Results are expressed as a percentage change from pre-dose (mean with vertical lines indicating s.e., $n = 4$). Cardiac output (○), $dP/dt P^{-1}$ (●), heart rate (□), mean blood pressure (■) and total peripheral resistance (△). * $P < 0.05$ versus vehicle.

appeared to be sub-therapeutic. At $3\text{--}300\text{ }\mu\text{g kg}^{-1}$ it produced dose-related increases in CO (maximum $+22\%$) accompanied by falls in TPR (maximum -29%), the latter indicative of vasodilatation. These changes were statistically significant (versus vehicle) for all doses above $3\text{ }\mu\text{g kg}^{-1}$. Mild dose-related increases in HR and $dP/dt P^{-1}$ were observed over the same dose range. FPL 63547 diacid also produced small falls in BP which did not reach statistical significance. No alterations in ECG waveform or heart rhythm were noted at any dose.

In a separate group of animals, enalapril diacid ($1\text{--}300\text{ }\mu\text{g kg}^{-1}$ i.v.) exhibited a qualitatively similar profile of haemodynamic changes which tended to be slightly more marked than those observed in the FPL 63547 diacid-treated group (Figure 4).

Antihypertensive effects in spontaneously hypertensive rats

On acute oral dosing, FPL 63547 produced significant falls in SBP in HCTZ-pretreated SHR over the dose range $3 \times 10^{-7}\text{--}10^{-5}\text{ mol kg}^{-1}$ ($\approx 0.13\text{--}4.5\text{ mg kg}^{-1}$). Antihypertensive effects of the minimum-active and maximum doses are shown in full in Figure 5. Each graph is a composite of morning-dosed and evening-dose experiments, providing the complete time course of drug- and vehicle-induced changes in SBP. A dose of $10^{-5}\text{ mol kg}^{-1}$ produced marked antihypertensive effects (SBP -32% at 5 h) which persisted throughout the 24 h

Figure 5 Antihypertensive effects of FPL 63547 (a) $3 \times 10^{-7}\text{ mol kg}^{-1}$ p.o., (b) $10^{-5}\text{ mol kg}^{-1}$ p.o., in diuretic-pretreated spontaneously hypertensive rats. Two panels shown on each graph represent results from morning-dosed ($-1\text{--}5\text{ h}$) and evening-dosed ($-1\text{--}24\text{ h}$) experiments respectively, drug-treated groups (●), vehicle-treated groups (○) (mean with vertical lines indicating s.e., $n = 5\text{--}6$). * $P < 0.05$ versus both pre-dose and vehicle group readings.

period following dosing. Long duration of action was a feature of FPL 63547 in this model. At all active doses antihypertensive effects were regularly observed in the evening-dosed animals, i.e. between 15–21 h after dosing.

Comparison of the acute antihypertensive properties of FPL 63547 with those of enalapril, captopril and lisinopril is summarised in Table 3. FPL 63547 was the only agent to reduce SBP significantly at $3 \times 10^{-7}\text{ mol kg}^{-1}$ p.o., the potency order being FPL 63547 $>$ lisinopril = enalapril $\gg$ captopril. Lisinopril, like FPL 63547, was long-acting, whereas enalapril and captopril were relatively short-acting, even at high doses.

Figure 6 shows the effects of chronic oral dosing with FPL 63547 ($0.5\text{ mg kg}^{-1}\text{ day}^{-1}$ for 23 days). During the lead-in period pre- and post-dose SBP in the two groups were stable and well-matched. Dosing with FPL 63547 produced a marked, consistent ($\approx 20\%$) and statistically significant fall in SBP 5 h post-dose throughout the study. A trend towards lowering of pre-dose (-1 h) SBP was also evident which became statistically significant from Day 11 onwards and for the remainder of the dosing period. By Day 21 of treatment pre- and post-dose SBP had fallen to similar levels ($\approx 180\text{ mmHg}$). SBP readings in vehicle-treated animals remained stable at $\approx 220\text{ mmHg}$ throughout the study. On termination of dosing, SBP in the drug-treated group recovered progressively towards, but did not exceed, control levels.

Chronic dosing with enalapril at a higher dose ($5\text{ mg kg}^{-1}\text{ day}^{-1}$ for 23 days) produced similar changes (data

Table 3 Antihypertensive effects of FPL 63547 in conscious diuretic-pretreated SHR—comparison with other angiotensin-converting enzyme inhibitors

<i>Dose</i> (mol kg ⁻¹ p.o.)	<i>Percentage fall in systolic pressure</i>						
	<i>1 h</i>	<i>3 h</i>	<i>5 h</i>	<i>15 h</i>	<i>18 h</i>	<i>21 h</i>	<i>24 h</i>
FPL 63547							
10 ⁻⁵	13%	25%	32%	19%	19%	20%	26%
3 × 10 ⁻⁶	NS	11%	15%	13%	10%	20%	NS
10 ⁻⁶	NS	10%	19%	21%	NS	16%	NS
3 × 10 ⁻⁷	NS	NS	NS	12%	13%	11%	NS
10 ⁻⁷	NS	NS	NS	NS	NS	NS	NS
Enalapril							
10 ⁻⁵	NS	17%	26%	NS	NS	NS	NS
3 × 10 ⁻⁶	NS	20%	22%	—	—	—	—
10 ⁻⁶	NS	10%	NS	—	—	—	—
3 × 10 ⁻⁷	NS	NS	NS	—	—	—	—
Captopril							
10 ⁻⁴	NS	19%	21%	NS	NS	NS	NS
3 × 10 ⁻⁵	NS	14%	NS	—	—	—	—
10 ⁻⁵	NS	NS	NS	—	—	—	—
Lisinopril							
10 ⁻⁵	NS	28%	24%	21%	19%	17%	18%
3 × 10 ⁻⁶	NS	NS	NS	NS	17%	18%	NS
10 ⁻⁶	NS	NS	25%	NS	NS	16%	17%
3 × 10 ⁻⁷	NS	NS	NS	NS	NS	NS	NS

Percentage changes calculated relative to pre-dose reading (−1 h). Values shown ($n = 5-6$) were significant ($P < 0.05$ unpaired t test) against both pre-dose reading and time-matched control group reading; NS = not significant.

not shown), suggesting that enalapril was approximately 10 times less potent than FPL 63547. Post-dose SBP was lowered consistently throughout the treatment period to a similar level to that achieved in the group treated with FPL 63547. Falls in pre-dose SBP did occur but were less marked, e.g. Day 21, SBP 196 mmHg, and statistically significant on Days 11, 16 and 21 only.

Discussion

In its active diacid form, FPL 63547 proved to be a highly potent inhibitor of rabbit lung ACE *in vitro*. Comparisons

with lisinopril, enalapril diacid and captopril presented above, together with unpublished observations on cilazapril and ramipril diacids in the same test system (IC_{50} 0.45, 0.59 nM respectively), illustrate that FPL 63547 diacid is at least equivalent in potency to the most active first and second generation ACE inhibitors. With such a potent inhibitor, the IC_{50} value is at best only an approximation of affinity, and is probably an underestimate. Based on its similar potency to lisinopril we would predict that FPL 63547 diacid would be lisinopril-like in its kinetic properties, i.e. a 'tight binding' inhibitor with a slow rate of dissociation from the enzyme (Bull *et al.*, 1985). If so, this may contribute to the long duration of action of FPL 63547 *in vivo*.

The ester prodrug FPL 63547 possessed no significant ACE inhibitory activity *in vitro*. Efficacy of this form *in vivo* was demonstrated by its ability to inhibit plasma ACE activity and angiotensin I pressor responses after i.v. administration to conscious dogs. Inhibition was evident within 10 min of dosing and peaked at approximately one hour. This relatively rapid onset suggests ready de-esterification to the active diacid, probably by the combined action of plasma and liver esterases. Persistence of plasma ACE inhibition 24 h after a single i.v. bolus dose of FPL 63547 is a clear demonstration of its intrinsically long duration of action. Of the two indicators measured, plasma ACE was five fold more sensitive than angiotensin I pressor responses to inhibition by FPL 63547. Other investigators have made similar observations (Shaller *et al.*, 1985), and suggested that 80–90% inhibition of plasma ACE was necessary to obtain significant inhibition of pressor responses to angiotensin I. Presumably *in vivo*, there is considerable spare enzymic capacity which has to be inhibited before conversion of i.v. angiotensin I is impaired, i.e. some reduction in rate of angiotensin II formation may occur without the total product concentration being significantly affected. Another possible explanation for the differential sensitivity is that plasma ACE may not be solely responsible for conversion of i.v. angiotensin I to angiotensin II in this situation, pulmonary vascular tissue ACE might also be expected to be involved. Pressor responses to angiotensin II were unaffected by FPL 63547 at doses $> 200 \times$ the ID_{50} for inhibition of plasma ACE.

In chloralose-anaesthetized dogs, FPL 63547 diacid produced falls in TPR accompanied by increases in CO and mild

Figure 6 Antihypertensive effects of FPL 63547 in diuretic-pretreated spontaneously hypertensive rats after chronic oral dosing. Open symbols—vehicle group, solid symbols—FPL 63547 treated group (0.5 mg kg⁻¹ day⁻¹, between Day 0–Day 22). Systolic blood pressure was measured both pre-dose (−1 h, ○, ●) and post-dose (+5 h, △, ▲). Values are means and vertical lines indicate s.e., $n = 5-6$. * $P < 0.05$ versus vehicle group.

stimulation of cardiac rate and contractility, over a wide dose-range. This profile is consistent with vasodilatation. We have observed qualitatively similar effects in the same model with the calcium channel blocker arteriolar dilator, nifedipine (Humphries & O'Connor, 1988). The haemodynamic properties of FPL 63547 diacid in the anaesthetized dog would appear to be adequately explained by ACE inhibition alone since enalapril gave an essentially similar profile. Further, the dose range over which vasodilatation was observed corresponds well with the dose-response relationships for inhibition of plasma ACE and angiotensin I pressor responses in the conscious dog. The observed vasodilatation may be due to a lowering of endogenous angiotensin II levels with subsequent lessening of its direct or indirect (i.e. mediated by facilitation of sympathetic nerve activity) vasoconstrictor properties, and/or elevation of bradykinin and prostaglandin levels. In conscious dogs, FPL 63547 produced no haemodynamic changes (SBP, DBP, HR) even at doses which caused maximal inhibition of plasma ACE. The latter finding is not uncommon for ACE inhibitors; spirapril, for example, was similarly ineffective in conscious normotensive dogs (Baum *et al.*, 1987), and probably reflects a relatively low dependence of BP on the renin-angiotensin system in this model.

In the anaesthetized dog both FPL 63547 and enalapril diacids produced mild but significant cardiac stimulation which was presumably reflex in origin. This deserves comment since ACE inhibitors as a class are considered to differ from other types of vasodilator by not causing reflex tachycardia, although the exact mechanism is unclear (Imai *et al.*, 1982), and may relate to the choice of chloralose as anaesthetic in our study. Unlike pentobarbitone, chloralose will preserve or even exaggerate the baroreceptor reflex (Brown & Hilton, 1956) and we have previously demonstrated that chloralose-anaesthetized dogs will respond with reflex tachycardia to a vasodilator (dipropyldopamine) (O'Connor *et al.*, 1982) which, under other circumstances, will lower heart rate by sympatho-inhibition. FPL 63547 and enalapril diacids consistently increased CO over a wide dose range. This contrasts with the fall in this variable obtained after administration of cilazapril to anaesthetized dogs (Holck *et al.*, 1986). This difference is probably model-dependent and may again relate to choice of anaesthetic or to the level of activation of the renin-angiotensin system. For example, Hof and colleagues (1987) have shown that spirapril lowers CO and HR in salt-depleted anaesthetized rabbits but has opposite effects in salt replete animals.

FPL 63547 was a very effective antihypertensive agent after acute oral dosing to diuretic-pretreated SHR. On the basis of

these studies, it shows high potency and, unlike captopril and enalapril, a duration of action compatible with once-daily treatment of hypertension. Since FPL 63547 diacid, enalapril diacid and lisinopril had similar *in vitro* ACE inhibitory potency and the former two were comparable as vasodilators after i.v. administration, other factors may have contributed to the antihypertensive effectiveness of FPL 63547 in the SHR. Oral bioavailability of the compound is high (B Mead—unpublished observation). In addition, FPL 63547 is a very potent inhibitor of local tissue ACE, producing significant effects at doses as low as $3 \mu\text{g kg}^{-1}$ p.o. in the rat (Carr *et al.*, 1989). In this respect it was between 3 and 20 fold more potent than enalapril, depending on the tissue in question. Previous investigators have demonstrated the importance of inhibition of tissue rather than plasma ACE in determining the magnitude and duration of blood pressure reduction with these compounds (Unger *et al.*, 1984; 1985).

The antihypertensive efficacy of FPL 63547 was retained on chronic once-daily dosing. No tolerance was observed as judged from post-dose SBP readings and by the end of the study effective 24 h control of this variable had been achieved. As with other ACE inhibitors, but unlike certain other antihypertensives (Parker & Atkinson, 1982), treatment withdrawal did not result in any rebound hypertensive effect. Chronic dosing with enalapril produce a similar lowering of SBP although a 10 fold higher dose was required. The gradual decline observed in the pre-dose SBP may simply reflect the long plasma half-life of FPL 63547. Alternatively, it may indicate that the mechanisms responsible for chronic reduction in blood pressure can differ from those involved in the acute antihypertensive response (Lever, 1986). Tissue ACE inhibition becomes particularly important during chronic dosing (Unger *et al.*, 1984). Furthermore, ACE inhibitor-induced reversal of adaptive structural changes, i.e. vascular or cardiac hypertrophy (Antonaccio *et al.*, 1979; Freslon & Guidicelli 1983) may influence the chronic antihypertensive response, although it is doubtful that such factors could exert a significant effect within the time-scale of our study.

In summary, FPL 63547, a novel thiadiazoline, is a potent and long-acting ACE inhibitor which by virtue of vasodilator and antihypertensive properties should prove useful in the treatment of chronic heart failure and hypertension. The accompanying paper (Carr *et al.*, 1990) describes the unusual elimination profile of FPL 63547, a feature which distinguishes it from other members of this class of drugs.

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