Preferential biliary elimination of FPL 63547, a novel inhibitor of angiotensin-converting enzyme, in the rat

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1 The route of elimination of FPL 63547, a novel inhibitor of angiotensin-converting enzyme (ACE), has been investigated in the anaesthetized rat. Comparisons have been made with other ACE inhibitors.
2 Bile and urine samples were collected over a 5 hour period following a single i.v. dose of ACE inhibitor (2 μmol kg⁻¹). Samples were bioassayed for ACE inhibitory activity using affinity-purified rabbit lung ACE and the amounts of the active form of inhibitor present in each sample were calculated by comparison with a standard curve.
3 FPL 63547 was rapidly and extensively excreted as the diacid in the bile but appeared in the urine in negligible amounts. The bile:urine ratio was 21.4:1 indicating a marked preference for the biliary route. A similar elimination profile was observed when the compound was dosed in its active form (FPL 63547 diacid), 87.9% of which was found in the bile over the 5h collection period, with a bile: urine ratio of 14.6:1.
4 The marked preference of FPL 63547 for biliary elimination was not shared by the other ACE inhibitors tested in this study. Lisinopril demonstrated the opposite pattern, being excreted almost exclusively by the kidney (bile:urine ratio 0.06:1). Enalapril was eliminated in approximately equal amounts in bile and urine (ratio 0.7:1) while spirapril diacid showed a slight preference for the bile (ratio 2.6:1).
5 The physical chemical properties of FPL 63547 diacid may be responsible for its unusual preference for biliary elimination. In particular, the amphipathic character and strong acid functionality of the compound are thought to favour transport into the bile.
6 Elimination by the biliary route will be preferred in patients whose renal function is impaired as a result of disease or age. In such patients the elimination of renally-excreted ACE inhibitors is known to be compromised, resulting in compound accumulation and the need for closer monitoring. Therefore, the elimination profile of FPL 63547, if confirmed in man, may prove to be clinically advantageous.

Introduction

Inhibitors of angiotensin-converting enzyme (ACE) are proving to be very effective in the treatment of cardiovascular disease, particularly heart failure and hypertension. A number of compounds of this class are already approved (e.g. captopril, enalapril, and lisinopril) or in the later stages of development (e.g. ramipril, cilazapril). However, to date, those ACE inhibitors for which pharmacokinetic data are available in man use the kidney as the primary organ of excretion. Since impairment of renal function is a relatively common accompani-}

ment of chronic heart failure and hypertension, the route of elimination of therapeutic agents is a significant consideration in the clinical management of these diseases. There have been a number of studies (e.g. Kelly et al., 1986; van Schaik et al., 1987; Shionoiri et al., 1987) showing that plasma levels of ACE inhibitors in current clinical use are elevated in patients with renal impairment, necessitating closer monitoring and possible dose reduction. Thus, renal excretion of ACE inhibitors is potentially disadvantageous.

FPL 63547, a novel thiazidolone, is a potent and long acting ACE inhibitor with antihypertensive properties in spontaneously hypertensive rats (Carr et al., 1990). Mackaness (1985) first suggested that ACE inhibitors may be developed which are suited to biliary transport, thus reducing the clinical problems associated with their use in patients with impaired kidney function. In the light of this, the route of elimination of FPL 63547 has been examined in the anaesthetized rat, a preliminary account of which has been presented to the British Pharmacological Society (Carr et al., 1988).

Route of elimination has been investigated by measuring levels of compound in the bile and urine by bioassay of the samples for ACE inhibitory activity. The technique therefore detects ACE inhibitors in the form in which they are biological active. Since FPL 63547 is a mono-ester prodrug, active only after de-esterification, it has been administered in both its mono-ester and active diacid forms. Route of elimina-}

tion comparisons have been made with lisinopril, enalapril (mono-ester) and spirapril (diacid).

Methods

Surgery and protocol

Male Sprague-Dawley rats (250 g) were anaesthetized with pentobarbitone, 54 mg kg⁻¹ i.p. The trachea was exposed and catheterised. The jugular vein was catheterised and pentobarbitone infused at a rate of 250 μg kg⁻¹ min⁻¹ to maintain anaesthesia. The abdomen was opened to reveal the liver and bile duct. An incision was made in the bile duct and a 0.6 mm cannula inserted and tied in place close to the point of inser-}

tion. Once adequate bile flow was achieved, pre-dose control samples were collected. After the penis has been ligated, the bladder was exposed and sampled, prior to dosing, by inser-}

tion of a fine needle and withdrawal of the urinary contents into a syringe. The bladder was kept moist with saline swabs.

The ACE inhibitor (2 μmol kg⁻¹ in 10% ethanol:saline) was administered via the femoral vein by a fine needle. Over the following 5 h bile was collected continuously (sample tube changed at hourly intervals) and urine was sampled as

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Table 1 Comparison of route of elimination profiles for angiotensin-converting enzyme (ACE) inhibitors in the anaesthetized rat

<table>
<thead>
<tr>
<th>Active ACE inhibitor</th>
<th>% of total dose eliminated</th>
<th>Bile</th>
<th>Urine</th>
<th>Bile: urine ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>*FPL 63547 diacid</td>
<td>60.9 ± 6.8</td>
<td>2.8 ± 0.3</td>
<td>21.7:1</td>
<td></td>
</tr>
<tr>
<td>FPL 63547 diacid</td>
<td>87.9 ± 1.9</td>
<td>6.0 ± 2.0</td>
<td>14.6:1</td>
<td></td>
</tr>
<tr>
<td>Spirapril diacid</td>
<td>52.4 ± 11.6</td>
<td>20.2 ± 3.4</td>
<td>2.6:1</td>
<td></td>
</tr>
<tr>
<td>†Enalapril diacid</td>
<td>19.6 ± 3.1</td>
<td>28.0 ± 3.0</td>
<td>0.7:1</td>
<td></td>
</tr>
<tr>
<td>Lisinopril</td>
<td>4.1 ± 1.5</td>
<td>64.3 ± 5.5</td>
<td>0.06:1</td>
<td></td>
</tr>
</tbody>
</table>

*† Dosed as the mono-ester (*FPL 63547, †enalapril). Values shown are means ± s.e. for five animals. Statistical analysis of the individual animal bile:urine ratios shows that the FPL 63547 diacid groups differed significantly from spirapril diacid, enalapril diacid and lisinopril groups (P < 0.01, Wilcoxon rank test) but not from each other.

Biliary elimination of FPL 63547

Figure 1 Time-dependence of biliary elimination of (a) FPL 63547 diacid, (b) FPL 63547 diacid (dosed as mono-ester), (c) spirapril diacid, (d) enalapril diacid (dosed as mono-ester), (e) lisinopril, in the anaesthetized rat. Bile samples were taken at hourly intervals after a single i.v. dose of angiotensin-converting enzyme inhibitor (2 μmol kg⁻¹) and the amount of active form of compound present in each sample was determined by bioassay and expressed as a percentage of total dose. Results shown are means with bars indicating s.e., n = 5.

Figure 2 Total urinary elimination of (a) FPL 63547 diacid, (b) FPL 63547 diacid (dosed as mono-ester), (c) spirapril diacid, (d) enalapril diacid (dosed as mono-ester), (e) lisinopril, in anaesthetized rats over the 5 h collection period following dosing with angiotensin-converting enzyme inhibitor (2 μmol kg⁻¹ i.v.). The amount of active form of compound present in the urine sample was determined by bioassay and expressed as a percentage of total dose. Results shown are means with bars indicating s.e., n = 5.

described above. After the volumes had been noted, the samples were frozen (−20°C) before analysis.

Measurement of angiotensin-converting enzyme inhibitor levels in bile and urine

A bioassay method was employed which involved the determination of ACE inhibitory activity in bile and urine samples by use of a rabbit lung ACE assay. Enzyme activity was evaluated with a method based on that described by Cushman & Cheung (1971) which involved measurement of radiolabelled hippurate release from the synthetic enzyme substrate, hippuryl-histidyl-L-leucine (HHL). Levels of enzyme inhibitor present in samples were therefore quantified according to the degree of reduction in hippurate released in the assay.

Preparation of enzyme  Rabbit lung ACE was affinity purified on lisinopril-Sepharose according to the method of Bull et al. (1985), modified as described in the accompanying paper (Carr et al., 1990).

Preparation of substrate  HHL (42.9 mg base) was suspended in 150 mM phosphate, 600 mM sodium chloride, 20 μM zinc chloride, pH 8.3 (buffer A). Phosphate buffer (50 mM, 20 μM zinc chloride, pH 8.3) containing potassium hydroxide (100 mM) was added dropwise and stirred vigorously until the substrate had dissolved (approx. 30 min). The solution was then adjusted to pH 8.3 with potassium dihydrogen phosphate (50 mM, 20 μM zinc chloride) and made up to 10 ml with phosphate buffer (50 mM, 20 μM zinc chloride, pH 8.3) to give a final concentration of 10 mM (stored at 4°C). ¹⁴C-labelled HHL (10 μCi ml⁻¹) was diluted 1:25 with this stock solution prior to use in the ACE assay.

Assay of angiotensin-converting enzyme inhibition  Standards and samples were diluted in buffer A. The level of dilution varied depending on the type of sample and was determined in preliminary experiments. A standard curve was assayed with each experiment and duplicates of all samples were analysed. Aliquots of sample or blank (100 μl) were pre-incubated (shaken) with 100 μl enzyme solution (from a working stock activity of 4.29 μm u⁻¹) for five minutes at 37°C in a water bath. The reaction was started by the addition of substrate (50 μl, final concentration 2 mM) and after 30 min stopped by addition of hydrochloric acid (1 M, 0.25 ml). Radiolabelled hippurate was extracted with ethyl acetate (1.5 ml) and the sample centrifuged for 10 min at 3000 r.p.m. at 4°C (Damon Centra-7R bench top centrifuge). A 1 ml aliquot of the ethyl acetate layer was counted in Optiphase (3 ml) on a scintillation counter (C-14 programme, four minutes, Packard Tri-Carb 460).
By constructing a standard curve of inhibition of ACE-mediated hydrolysis (14C-count versus log inhibitory concentration) the concentration of ACE inhibitor in the 'unknown' samples was determined.

**Materials**

FPL 63547 (2,3-dihydro-3-[N-(1S)-1-ethoxycarbonyl-3-phenylpropyl]-1-alanyl]-5-(1,1-dimethyl ethyl)-1,3,4-thiadiazole-2-(S)-carboxylic acid), enalapril and their active diacids were synthesized in the Department of Medicinal Chemistry, Fisons plc; lisinopril with a gift from Merck; captopril a gift from Squibb and spirapril diacid a gift from Schering. BSA, HHL and affinity column materials (Sepharose CL-4B, 1,4 butanedioi diglycidyl ether, sodium borohydride) were purchased from Sigma. Zinc chloride was obtained from JMC Ltd, and [14C]-HHL from NEN Research Products. All other materials used were supplied by Fisons.

**Results**

Total recovery of compound over the 5h period following i.v. administration to the anaesthetized rat, derived from individual animal data by combining the total amounts eliminated in bile and urine, was as follows: FPL 63547 diacid (93.9 ± 2.4%), spirapril diacid (72.6 ± 12.4%), lisinopril (68.4 ± 5.6%). FPL 63547 (63.7 ± 7.0%) and enalapril (47.6 ± 5.5%). Possible explanations for these variations in recovery are discussed later.

Compound content in the bile was determined hourly, whereas data from urine samples were pooled over the total 5h period because hourly samples of a viable size could not always be obtained.

The time-dependence of biliary excretion is shown for each compound in turn in Figure 1. FPL 63547 diacid (Figure 1a) was extensively and rapidly excreted in the bile with 72.4 ± 6.4% of total dose eliminated by this route in the first hour after dosing, with the rate of elimination declining rapidly thereafter. When dosed in its mono-ester form (FPL 63547) the elimination of FPL 63547 diacid in the bile showed a qualitatively similar profile (Figure 1b). There was significant biliary excretion of spirapril diacid (Figure 1c) and, to a lesser extent, enalapril diacid (dosed as the mono-ester, Figure 1d). In each case, the maximum levels of compound were found in the first samples after dosing. The biliary elimination of lisinopril was very slight (Figure 1e), 1.2 ± 0.3% and 1.3 ± 0.5% in the first and second hours after dosing respectively, declining thereafter.

Figure 2 shows the total elimination of each compound in the urine over the 5h sampling period. Lisinopril demonstrated extensive elimination by this route with 64.3 ± 5.5% of total dose recovered from the urine. Enalapril diacid and spirapril diacid were also present in the urine in significant amounts. By contrast, the urinary excretion of FPL 63547 diacid was at a very low level irrespective of whether diacid (6.0 ± 2.0%) or mono-ester (2.8 ± 0.3%) was administered.

Table 1 shows a summary of the biliary and urinary excretion data and the calculated bile:urine ratio which describes the overall elimination profile of each compound. FPL 63547 diacid showed a strong preference for the biliary route with similarly high bile:urine ratios achieved after dosing of diacid and mono-ester. Spirapril diacid showed some preference for the bile, while enalapril was excreted approximately equally in the bile and in the urine. Lisinopril markedly favoured the renal route of elimination. On statistical analysis of the individual animal bile:urine ratios the FPL 63547 diacid groups differed significantly from the spirapril diacid, enalapril diacid and lisinopril groups (P < 0.01, Wilcoxon rank test) but not from each other.

**Discussion**

This examination of the relative use of biliary and renal routes of elimination by different ACE inhibitors in the anaesthetized rat has uncovered a wide spectrum of elimination profiles. The two extremes are represented by FPL 63547, on the one hand, whose active form is excreted almost exclusively in the bile and by lisinopril, on the other, which strongly favours the renal route. Spirapril diacid and enalapril had intermediate elimination properties.

Total recovery levels approximated to 70%, but varied between compounds, with the recoveries of FPL 63547 diacid and enalapril being most and least complete, respectively. There are a number of possible explanations for incomplete recovery. Bile and urine samples were taken for 5h following dosing but all the compounds tested are long-acting probably as a result of tight binding to ACE, and pharmacological and pharmacokinetic evidence supports their continued presence in the body beyond 5h. For example, in conscious dogs the inhibition of plasma ACE produced by a single i.v. dose of FPL 63547 peaks at 0.5–1h and is still evident 24h after certain doses (Carr et al., 1990). In all probability a proportion of each compound is taken up into tissues sites from which it is released and eliminated at a very slow rate. There are reasons to believe that the elimination profiles determined over the 5h study period are representative. Peak biliary elimination occurred early in this period, declining steeply thereafter and, where data are available (not shown), a similar decay was evident in the urinary elimination. Longer study periods and correspondingly greater compound recovery would not therefore be expected to have influenced the profiles.

The bioassay technique used to measure compound levels in the bile and urine in this study does differ in some significant respects from other methods for evaluating elimination, e.g. detection of radiolabelled compound. In this situation it can be argued that bioassay is the more appropriate method, because it measures elimination of the biologically relevant active form only rather than total compound. Correspondingly, bioassay will not detect elimination of either inactive prodrug or inactive metabolites and non-detection of these inactive species could, theoretically, have contributed to less than complete compound recovery. There is little evidence that the ACE inhibitors used in this study undergo significant further metabolism from the diacid form, although a minor metabolite of spirapril diacid has been described (Drummer & Kourtis, 1987). Some elimination of FPL 63547 mono-ester may explain the difference in recovery observed when FPL 63547 was dosed in its mono-ester and diacid forms. However, even if this occurred, it is noteworthy that the elimination profile was not influenced by the form in which the compound was dosed, favouring the bile strongly in both cases. Studies in which the route of elimination of 14C-labelled FPL 63547 was measured in conscious rats have essentially confirmed the results presented here (B. Mead—unpublished observation).

The elimination of lisinopril, enalapril and spirapril diacid as determined in this study agrees well with published data on these compounds. In man, lisinopril has been shown to be eliminated primarily in the urine (Ulm et al., 1982). Tocce et al. (1982) have shown that i.v. enalapril undergoes both urinary and biliary excretion in rat and dog with a preference for the renal route. Oral administration to man has produced similar results (Ulm et al., 1982). Captopril was also investigated in our model and appeared to be eliminated predominantly by the kidney in accordance with other studies using labelled compound (Krippelani et al., 1980). The data have not been included in this paper because recovery of active thiol was very poor, suggesting rapid autoxidation to inactive disulphide. This instability in biological fluids has been noted previously (Krippalani et al., 1980). Spirapril was the first biliary-selective ACE inhibitor to be described quantitatively with a bile:urine ratio of ≈3:1 obtained after i.v. adminis-
BILIARY ELIMINATION OF FPL 63547

Our results with spirapril diacid confirm this and also demonstrate that FPL 63547 is substantially more selective than spirapril for the biliary route in this species. Unlike spirapril diacid which also appeared in the urine in significant quantities, the renal excretion of FPL 63547 diacid was so low as to be considered negligible.

Compounds pass from the blood into the bile for elimination by means of specialised transport processes linking liver parenchymal cells and the bile canaliculi. In general, the extent to which any given compound undergoes biliary elimination is influenced primarily by its physical chemical characteristics, particularly molecular weight, polarity and other special structural features (Smith, 1973). Recently, Ondetti (1988) has attempted to relate the physical chemical properties of a series of captorpril and enalapril analogues to their routes of elimination, and formed the conclusion that increased molecular weight and lipophilicity are probably responsible for preferential biliary elimination. Contrary to the conclusion of Ondetti (1988), the results of this study suggest that molecular weight is not a key determinant of the extent of biliary elimination. FPL 63547 diacid (mol.wt. 421) and lisinopril (mol.wt. 405) are of similar molecular weights but widely different elimination properties. In addition FPL 63547 diacid was considerably more biliary-selective than spirapril diacid (mol.wt. 438) despite having a lower molecular weight.

According to Smith (1973) the presence of a highly polar group in the molecule is a requirement for extensive biliary excretion. The 'carboxy-terminus' carboxyl group of FPL 63547 diacid is highly ionised (pKa 1.79; unpublished observation) and therefore comfortably fulfils this criterion. Furthermore, the strong acidity of this group distinguishes FPL 63547 diacid from the other compounds tested. However, since enalapril diacid, lisinopril and spirapril diacid each show a pKa which falls within the range 3-4 (unpublished observation) described as being compatible with biliary elimination (Smith 1973), it is by no means certain that the strong acid functionality of FPL 63547 diacid, alone, is responsible for its biliary selectivity. FPL 63547 is relatively lipophilic (unpublished observation) and, although Smith (1973) has expressed the view that there appears to be no simple relationship between lipid solubility and biliary elimination, this factor may be significant in the context of our study. One striking characteristic of many compounds excreted extensively in the bile is their amphipathic character, i.e. the presence of both strongly polar and essentially non-polar groups within their molecular structure (Smith, 1973; Gregus and Klassen, 1987). In this respect they resemble the bile salts, e.g. taurocholic acid. The balance between polar and non-polar aspects may be critical for interaction with the carrier molecule responsible for transport into the bile. FPL 63547 can be considered to have amphipathic characteristics by virtue of its highly ionised carboxylic acid residue and the hydrophobic tertiary butyl substitution. Perhaps this, rather than any single property, explains its preference for biliary elimination.

The extent to which compounds are excreted in the bile can vary very significantly with species (Smith, 1973). Although the reasons for this variation are not fully understood, it is thought that the molecular weight threshold for biliary transpot alters, being lowest in the rat (≈ 350) and highest in the rabbit (≈ 475). FPL 63547 undergoes extensive biliary excretion (measured as faecal content after i.v. administration) in conscious dogs (B. Mead—unpublished observation) as well as in the rat. As the molecular weight threshold for biliary elimination in man is thought to resemble rabbit more than rat or dog these data may not be the best guide. However, information on biliary elimination of drugs in man is very scarce and since factors other than molecular weight appear to be the predominant influence responsible for the biliary selectivity of FPL 63547 in this rat study, historical concepts of species variation may not be applicable. Studies of the route of elimination of FPL 63547 in man will clarify this issue.

Renal dysfunction of varying severity, resulting from cardiovascular disease and/or advanced age (Reid, 1987), is a fairly common finding in the types of patients for whom ACE inhibitors are likely to be prescribed. In renal dysfunction, the clearance of renally-exceted ACE inhibitors is compromised, resulting in undesirable elevation of plasma levels (Kelly et al., 1986; van Schaik et al., 1987), supra-optimal inhibition of ACE and increased potential for adverse reactions. Hence the need for careful monitoring when these compounds are used and, usually, some reduction in dosage or dose interval in patients whose renal function is impaired. The development of biliary-eliminated ACE inhibitors would provide the clinician with a new treatment option which, by avoiding the problem of compound accumulation, may be of value in the management of such patients.

One potentially important consideration when dealing with compounds which are excreted in the bile is the possibility of enterohepatic recirculation. This reabsorption of compounds from the intestine subsequent to biliary excretion provides an additional clinical variable, usually with undesirable consequences. The phenomenon can result in significant elevation in plasma levels, thus prolonging pharmacological (e.g. digitoxin) or toxic (e.g. indomethacin) effects (Gregus & Klaassen, 1987). FPL 63547 will not be subject to enterohepatic recycling since the diacid form in which it is eliminated, although biochemically active, would not be absorbed from the gut. Only the mono-ester prodrug form possesses significant oral bioavailability.

In summary, in a rat model the novel ACE inhibitor, FPL 63547, was extensively and preferentially eliminated by the biliary route. In this respect it differed from other compounds of this class tested and may be unique amongst ACE inhibitors in the level of its selectivity for the biliary route. This mode of elimination, if confirmed in man, is likely to be clinically advantageous. FPL 63547 has accordingly been selected for further development for the treatment of hypertension and heart failure.

We wish to thank Brian Mead for permission to quote route of elimination studies using labelled FPL 63547, Dave Payling and Carol Manners for physical chemical data and Merck, Squibb and Schering for their gifts of lisinopril, captorpril and spirapril diacid, respectively.

References


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