Pharmacokinetics of $[^{14}\text{C}]$FCE 22891, a Penem Antibiotic, Following Oral Administration to Healthy Volunteers

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FCE 22891 is a prodrug of the penem antibiotic FCE 22101 and is suitable for oral administration. The pharmacokinetics of FCE 22891 were investigated in four healthy male volunteers following the oral administration of 500 mg of $[^{14}\text{C}]$FCE 22891. Levels of radioactivity in plasma were always higher and persisted for longer than those of FCE 22101. The time to the maximum concentration of radioactivity in plasma generally coincided with that of FCE 22101. The respective values for the maximum concentrations of radioactivity in plasma were, on average, 8.57 ± 2.95 pg equivalent/ml and 2.97 ± 2.05 µg/ml. Over a 5-day period, mean urinary and fecal recovery of radioactivity accounted for 53.2 and 41.0% of the dose, respectively. The average amount of FCE 22101 excreted in urine and feces corresponded to 9.0 and 1.6% of the dose, respectively. The urinary recovery of the open-ring metabolite P1 and of its 5-S epimer P2 accounted for about 6.5 and 1.2% of the dose, respectively. Other chromatographic peaks corresponding to unidentified compounds accounted for about 14.0% (polar metabolite fraction; peak P), 3.7% (less polar fraction; peak X), and 15.4% (least polar fraction) of the dose. Elimination of radioactivity and FCE 22101 in urine was rapid. Intersubject variability in the kinetics of total radioactivity in plasma was far less than that observed for FCE 22101. The results of the present study support suggestions that presystemic metabolism of FCE 22101 and/or transformation of the prodrug to compounds other than FCE 22101 are the main cause of intersubject variability in the kinetics of FCE 22101 produced in plasma following oral administration of its prodrug.

FCE 22891 (Fig. 1) is a prodrug of FCE 22101, a penem antibiotic with a broad spectrum of activity against gram-positive and -negative bacteria, and is stable to most bacterial β-lactamases (4, 14). While FCE 22101 must be administered parenterally, FCE 22891 is absorbed from the gastrointestinal tract.

The pharmacokinetics of FCE 22891 in humans have been studied following different single doses ranging from 500 mg to 2 g (7, 9, 12, 13), following repeated administration of 500 mg to 1 g (1), and after food intake (9). Its absolute bioavailability as FCE 22101 is about 30 to 40% (7, 9, 13). FCE 22891 is rapidly hydrolyzed to FCE 22101 in vivo; no detectable unchanged FCE 22891 is found in human plasma or urine, even when samples are collected over potassium fluoride to inhibit ex vivo hydrolysis (12).

The aim of the present study was to determine the excretion balance and to investigate the pharmacokinetics of FCE 22891 following administration of the $[^{14}\text{C}]$-labeled compound. Furthermore, a comparative evaluation of total radioactivity and FCE 22101 data would help to elucidate previous findings on the extent of absorption and intersubject variability in the kinetics of FCE 22101 following oral administration of FCE 22891.

MATERIALS AND METHODS

The protocol for the study was approved by the Research Ethics Committee of the Royal Postgraduate Medical School and the Hammersmith Hospital Special Health Authority, London, United Kingdom. Permission to administer the radiolabeled compound was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC). All subjects gave written informed consent to participate in the study.

Subjects. Four healthy male volunteers, aged 25 to 57 years, with a body weight of 67 to 83 kg and a height of 178 to 194 cm participated in the study. Subjects were determined to be in good health by means of medical history, physical examination, electrocardiogram, vital signs, and routine laboratory examinations (hematology, blood chemistry, and urinalysis). Subjects had no history of alcohol or drug abuse or hypersensitivity to β-lactam antibiotics and had not received any antibacterial agents in the previous 2 weeks. No concomitant medications or alcohol-containing beverages were permitted in the 24-h period prior to and during the experimental period.

Treatment of subjects. $[^{14}\text{C}]$-labeled FCE 22891 was supplied by Farmitalia Carlo Erba, Milan, Italy, as a dry powder in individual glass bottles (one per subject). Its radiochemical purity was >96%, as verified by radio-thin-layer chromatography on silica gel Merck F254 plates. The specific activity was 0.211 µCi/mg. Each individual dose of FCE 22891 was nominally 500 mg (corresponding to 400 mg of the active moiety FCE 22101 as a free acid); the actual doses were 478 mg (subject 1), 478 mg (subject 2), 489 mg (subject 3), and 480 mg (subject 4). The contents of each bottle were suspended in 100 ml of a flavored water vehicle that was supplied by Farmitalia Carlo Erba and were administered by the oral route. Each bottle was then rinsed three times with 20 ml of tap water, which was also drunk by the volunteers; this was followed by ingestion of an additional 100 ml of tap water (the total volume ingested was 260 ml). The drug was administered after an overnight fast (from 2200 h), and food was not permitted for an additional 4 h, when a standard meal was given. Water was allowed ad libitum.

Blood, urine, and feces sampling. Blood samples (10 ml)
were collected from a peripheral forearm vein into heparinized tubes before drug administration (time zero) and at 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 12, 24, 33, and 48 h after dosing. The samples were centrifuged (average of 1,500 × g for 15 min at 4°C) to separate the plasma, which was divided into two aliquots and stored at −80°C until analysis.

Urine was collected before drug administration and over the periods 0 to 1, 1 to 2, 2 to 4, 4 to 6, 6 to 12, 12 to 24, 24 to 48, 48 to 96, and 96 to 120 h postdosing. The volume and pH of each sample were measured, and three 10-ml portions were stored at −80°C until analysis.

Feces were collected before drug administration (blank sample) and then every 24 h for up to 5 days. The samples were stored at −80°C until analysis.

Radioactivity assays. Radioactivity in plasma and urine samples was measured by direct liquid scintillation counting by using a Packard Tri-Carb liquid scintillation spectrometer with on-line quench correction. Radioactivity in feces was measured after homogenization in ca. 2 volumes of water, subsequent digestion with Soluene (Packard), and decolorization with hydrogen peroxide. Instagel (Packard) was used as the liquid scintillant. All samples were counted for 10 min, with two standard deviations (SDs) ≤15%. Results were corrected for background counts, which were determined in the corresponding blank samples. The limit of detection was set at twice the background (i.e., 0.1 μg equivalent [eq]/ml of 14C-labeled material).

Determination of FCE 22101 in plasma. Concentrations of FCE 22101 in plasma were determined by using a previously described reverse-phase high-performance liquid chromatographic (HPLC) method (2) with slight modifications. Prior to analysis, the samples (0.5 ml portion) were diluted with 0.5 ml of 0.05 M potassium phosphate buffer (pH 7.0) containing 20 μg of indole-3-acetic acid (internal standard) and were then centrifuged in a Centrifree micropartition system cartridge (Amicon) (average of 1,500 × g for at least 40 min at 4°C). A 100-μl aliquot of each sample was injected onto the HPLC column (Waters μBondapak C18, 300 by 4.1 mm), which was equipped with a Millipore Resolve 18 guard column, via a 100-μl loop (Rhodyne). The mobile phase was a mixture of 0.05 M potassium phosphate buffer (pH 7.0)–methanol (85:15; vol/vol), and the flow rate was 1.0 ml/min. The UV detector was operated at 300 nm. Standard curves ranged from 0.5 to 100 μg/ml with acceptable linearity (r ≥ 0.999). Peak areas were used for the quantification of FCE 22101; the detection limit was 0.5 μg/ml in this study. The intrasassay coefficient of variation was 3% at 1 μg/ml and 1.6% at 25 μg/ml. The intersassay coefficient of variation was 9% at 1 μg/ml and 4% at 25 μg/ml.

Urinary radioactivity profiles. The profile of radioactivity in urine samples was determined as described by Battaglia et al. (2). Briefly, 100-μl aliquots of the samples were injected onto a Merck Lichrosorb RP8 column (250 by 4 mm, 10 μm) equipped with a Waters Guardpak C18 precolumn. The mobile phase comprised a mixture of 0.02 M potassium phosphate buffer (pH 2.5) and acetonitrile at a flow rate of 1 ml/min. The composition of the phase was held at 0% acetonitrile for 2 min and then increased linearly over 5 min to 10% acetonitrile and was held at this composition for another 5 min; it was then increased linearly to 60% acetonitrile in 10 min, held at this composition for 8 min, and finally, decreased to 0% acetonitrile over 5 min. The A400 of the eluate was monitored. Fractions of eluate were collected manually every 0.5 min, and the radioactivity of each fraction was determined by liquid scintillation counting, as described above. Radioactivity levels were plotted against the elution time, and six main chromatographic peaks were reproducibly identified. On the basis of previous work (2, 10), the six peaks were assigned as follows (in order of decreasing polarity, with retention time ranges given in parentheses): most polar fraction, P (0 to 5.5 and 6.0 min); 5-S epimer of ring-opened metabolite of FCE 22101 (P1), P2 (5.5 and 6.0 to 10.0 and 11.0 min); ring-opened metabolite of FCE 22101, P1 (10.0 and 11.0 to 12.0 and 13.0 min); less polar unidentified metabolite fraction, X (12.0 and 13.0 to 15.5 and 17.0 min); FCE 22101 (15.5 and 17.5 to 17.0 and 20.0 min); and least polar metabolite fraction, LP (17.0 and 20.0 to 30 min). The levels of FCE 22101 in the urine samples were determined from the urinary radioactivity profiles, because endogenous interfering substances prevented quantification by UV detection. The sensitivity of this assay for FCE 22101 was 0.5 μg/ml. Assay linearity was good (r ≥ 0.999), and the coefficients of variation were acceptable (within assay, 1 μg/ml, 3%; 100 μg/ml, 1.6%; between assay, 1 μg/ml, 5%; 100 μg/ml, 2.3%).

Determination of FCE 22101 in feces. The concentration of FCE 22101 in fecal samples was determined by offline radiometric HPLC. Portions of the fecal homogenates, which were spiked with unlabeled FCE 22101, were subjected to HPLC as described above for the plasma samples. Fractions of eluate were collected in polyethylene scintillation vials, and the radioactivity of the fraction corresponding to authentic FCE 22101, determined from its UV absorption (retention time, 5.0 to 6.0 min), was determined by liquid scintillation spectrometry as described above. The extraction efficiency was 78 ± 2% (mean ± SD). The sensitivity of this assay for FCE 22101 was 0.5 μg/ml. The linearity was good (r > 0.995). The intraassay coefficient of variation was 4% at 1 μg/ml and 2% at 100 μg/ml. The interassay coefficient of variation was 7% at 1 μg/ml and 3% at 100 μg/ml.

Pharmacokinetic analysis. Maximum concentrations of radioactivity in plasma (Cmax) and the times to Cmax (Tmax) were determined by inspection of the data. Areas under the plasma radioactivity-time curve from time zero to infinity (AUC0∞) were calculated by the linear trapezoidal rule up to
FIG. 2. Levels of total radioactivity in the plasma of individual subjects after oral administration of 500 mg of [14C]FCE 22891 to four healthy male volunteers. ●, subject 1; *, subject 2; ▲, subject 3; ■, subject 4. The boxed area on the left is expanded in the inset.

the last measured concentration with extrapolation to infinity, the extrapolated area being calculated from $C_t/k_{el}$, where $C_t$ is the last measured concentration and $k_{el}$ is the elimination rate constant of total radioactivity, which was determined by nonlinear regression analysis of the terminal elimination phase. Areas under the plasma radioactivity-time curves to time $t$ were calculated by the linear trapezoidal rule. The total amounts of radioactivity eliminated in urine and feces and the corresponding percentages of the dose administered were also calculated.

For unchanged FCE 22101, $C_{max}$ and $T_{max}$ were obtained by inspection of the data. $k_{el}$ was determined by iterative nonlinear regression analysis of the terminal log-linear phase of the plasma concentration-time curve by using Siphar-Base release 4.0 software (Cimed, Creteil, France) (5). The half-life was calculated by conventional means, i.e., $0.693/k_{el}$. The finite (AUC$_{0-m}$) and total (AUC$_{0-\infty}$) areas under the plasma concentration-time curve were calculated by using the linear trapezoidal rule to the last measurable concentration (at time $t$) with extrapolation to infinity (for AUC$_{0-m}$), the extrapolated AUC being calculated from $C_t/k_{el}$. The total amount excreted in the urine and the corresponding percentage of the dose, the total amount excreted in the feces, and the corresponding percentage of the dose were determined by summation of the individual amounts excreted in the urine and feces, respectively. The renal clearance (Cl$_{r}$) was calculated as the total amount excreted in urine/AUC$_{0-\infty}$. Pharmacokinetic parameters were normalized to a dose of 500 mg of FCE 22891, as necessary.

Safety assessments. Vital signs and electrocardiograms were monitored for 4 h after dosing. Laboratory safety tests, as described above, were repeated 48 h after treatment. In addition to spontaneous reporting, possible adverse events were assessed by asking the subjects nonspecific questions.

FIG. 3. Levels of FCE 22101 in the plasma of individual subjects after oral administration of 500 mg of [14C]FCE 22891 to four healthy male volunteers. ●, subject 1; *, subject 2; ▲, subject 3; ■, subject 4.
TABLE 1. Pharmacokinetic parameters of total radioactivity after a single oral administration of 500 mg of [14C]FCE 22891 to healthy volunteers

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>C\text{max} (\mu g eq/ml)*</th>
<th>T\text{max} (h)</th>
<th>AUC\text{0–24} (\mu g eq \cdot h/ml)*</th>
<th>Cumulative amt (%) excreted in²:</th>
<th>Urine</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.04</td>
<td>0.50</td>
<td>46.34</td>
<td>56.38</td>
<td>37.04</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.52</td>
<td>0.50</td>
<td>83.05</td>
<td>49.74</td>
<td>45.96</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.45</td>
<td>0.50</td>
<td>53.73</td>
<td>51.85</td>
<td>43.84</td>
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</tr>
<tr>
<td>4</td>
<td>12.26</td>
<td>0.33</td>
<td>49.33</td>
<td>54.85</td>
<td>37.14</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD 8.57 ± 2.95 0.46 ± 0.09 58.86 ± 16.26 53.21 ± 2.98 41.00 ± 4.59

* Corrected to a dose of 500 mg.

b Expressed as percentage of the dose administered.

RESULTS

Safety. No adverse events were reported, and no clinically significant changes were observed in any of the laboratory tests.

Pharmacokinetics. The levels of radioactivity in the plasma of individual subjects are shown in Fig. 2, and those of FCE 22101 are shown in Fig. 3. The corresponding pharmacokinetic parameters are summarized in Tables 1 and 2, respectively.

Comparison of the profiles of total radioactivity and FCE 22101 in plasma indicated the presence of metabolites circulating in plasma for periods longer than FCE 22101 did. Furthermore, radioactivity levels were higher than those of FCE 22101 in all subjects. Absorption of radioactivity was rapid, with C\text{max} being reached within 0.5 h postdosing. The partial AUC of total radioactivity from 0 to 12 h (AUC\text{0–12}) corresponded to 35% of the AUC\text{0–24}, while the AUC\text{0–24} was equal to 44% of the AUC\text{0–24} and the AUC\text{0–48} was equal to 59% of the AUC\text{0–24}, suggesting that much of the radioactivity in plasma was eliminated rapidly. C\text{max}s of FCE 22101 were also reached rapidly and T\text{max}s generally, coincided with those for radioactivity levels. However, FCE 22101 C\text{max}s were up to fivefold lower than maximum radioactivity levels. The total AUC\text{0–24} of FCE 22101 represented a small percentage (mean, 7%; range, 3 to 11%) of the AUC\text{0–24} of total radioactivity. Wide intersubject variability was observed in C\text{max} and total AUC values of FCE 22101 (coefficients of variation, 70 and 40%, respectively). In contrast, variabilities in the corresponding values for total radioactivity were lower (34% for C\text{max} and 29% for AUC\text{0–24}). The half-life of FCE 22101 in plasma was only 1.09 ± 0.44 h and the CLR = 12.7 ± 5.2 liters/h.

Most of the radioactivity in urine (94% of the total) was recovered within 12 h postdosing, the major part (about 37% of the dose) being eliminated in the first 2 h following drug administration. The average amount of the dose excreted in urine as FCE 22101 corresponded to 9% of that administered; the major part (about 80% of the total) was eliminated in the first 2 h, which was in agreement with the short elimination half-life of FCE 22101. Radioactivity in feces accounted for about 40% of the dose, most of which (about 69% of the total) was recovered within 3 days after drug administration. FCE 22101 in feces accounted for only 1.5% of the administered dose. Attempts to quantify FCE 22891 in fecal homogenates were unsuccessful because of the variable recovery of the compound. FCE 22101 added to blank feces was not degraded to any significant extent following incubation with blank feces for 30 min at 37°C. In contrast, added FCE 22891 was rapidly and completely degraded within 30 min, largely to a derivative more polar than FCE 22101 (data not shown).

As shown in Table 3, the urinary excretion of metabolite P1 (Fig. 1) accounted for about 6.5% of the dose administered. Urinary excretion of metabolite P2, which is the S-epimer of P1 (8), accounted for about 1.2% of the dose. Peak P represented about 14% of the dose. Finally, about 15% of the dose was eliminated in urine in the least polar metabolite(s) fraction (LP), and less than 5% was eliminated as peak X.

DISCUSSION

The pharmacokinetic parameters of FCE 22101 obtained in the present study after oral administration of its prodrug FCE 22891 are in good agreement with those reported previously (7, 9, 13). Recovery of radioactivity in this study was almost complete, because about 90 to 95% of the dose...
TABLE 3. Recovery of metabolites in urine 120 h after a single oral administration of 500 mg of [14C]FCE 22891 to healthy volunteers

<table>
<thead>
<tr>
<th>Subject</th>
<th>% of the administered dose eliminated as metabolite*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td>14.24</td>
</tr>
<tr>
<td>2</td>
<td>14.59</td>
</tr>
<tr>
<td>3</td>
<td>15.72</td>
</tr>
<tr>
<td>4</td>
<td>11.33</td>
</tr>
</tbody>
</table>

Mean ± SD 13.97 ± 1.87 1.19 ± 0.61 6.53 ± 1.24 3.71 ± 0.77 15.43 ± 2.67

* P, most polar fraction; P1, open-ring metabolite of FCE 22101; P2, 5-S epimer of P1; X, less polar fraction; LP, least polar fraction.

was found in urine and feces over 5 days. It is likely that a small percentage of the dose was eliminated as CO₂ in the expired air, as has been observed after oral administration of [14C]-labeled FCE 22891 to rats (2).

FCE 22101 has been shown in vitro to be a substrate of the tubular dipeptidase dehydropeptidase I (DHP-I), yielding the open-ring metabolites P1 (major) and P2 (3). Furthermore, the urinary recovery of FCE 22101 increased from 17.5 to 53.0%, when it was given alone intravenously, to 73.2 to 91.9%, when it was coadministered with imipenem-cilastatin (7), suggesting that normally approximately 50% of FCE 22101 in the kidneys is metabolized by DHP-I. The data obtained in the present study are consistent with this, with similar amounts of FCE 22101 and metabolites P1 and P2 being excreted in the urine.

The difference between the Cmax,5 of total radioactivity and FCE 22101, which was associated with their short and almost identical T1/2, supports the hypothesis of presystemic biotransformation of FCE 22891 and/or FCE 22101. The biotransformation of FCE 22891 could be chemical or enzymatic. Fecal homogenate is capable of rapidly converting FCE 22891 to a product(s) that is more polar than FCE 22101. The stability of FCE 22891 at neutral pH is relatively good, while at acidic pH it degrades more rapidly to products that are not exclusively FCE 22101. For example, after 1 h at pH 1.2 and 37°C, only 75% of FCE 22891 was recovered as the parent compound, with FCE 22101 accounting for about 5% (3a). However, significant chemical degradation because of the acidic pH of the stomach is unlikely to have occurred in the present study because of the very rapid absorption of the drug (T1/2, < 30 min).

Presystemic biotransformation of FCE 22891 and/or FCE 22101 appears to be the main cause of the variability in the extent of absorption observed in the present and previous studies. This is in agreement with the limited intersubject variabilities in AUCs observed after intravenous administration of FCE 22101 (7, 9, 13). The variabilities in CLint and urinary excretion of FCE 22101 observed after intravenous administration reflect intersubject differences in the metabolic activity of renal DHP-I.

In contrast to FCE 22101, intersubject variabilities in the plasma AUC and urinary elimination of total radioactivity in the present study were relatively small; the AUC/CLint values varied by 29%, and the total amount of radioactivity eliminated in urine varied by only 6%. The identity of the radioactive species in plasma was not determined. However, significant levels of the metabolite P1 have been observed in plasma following oral administration of FCE 22891 to humans (6), whereas only traces of this metabolite have been detected after intravenous administration (7). The presence of this metabolite in plasma after oral administration might be due to the action of DHP-I in the human gut, the presence of which has recently been reported (11). The observed high intersubject variability of levels of FCE 22101 in plasma, if confirmed, may have important clinical implications. Actually, if one considers the lowest values obtained, it appears that such variability is unlikely to affect therapeutic efficacy in the case of infections caused by gram-positive organisms (MIC for 90% of organisms tested, ≤0.25 μg/ml), whereas it might become of critical importance in the case of infections caused by gram-negative organisms, particularly members of the family Enterobacteriaceae other than Escherichia coli (MIC for 90% of organisms tested, ≥1 μg/ml) (14). It is of interest to compare the urinary metabolic pattern for oral FCE 22891 observed in humans in the present study with that in other species (2). Elimination of radioactivity by the renal route in humans was slightly more important than that by the fecal route, as in the rat, while in monkeys renal elimination was even more important, whereas in dogs fecal elimination was relatively more important. As in dogs, but in contrast to rats and monkeys, FCE 22101 was not the major component of the radioactivity eliminated in the urine of humans. Another similarity between the urinary metabolic patterns of humans and dogs is that the amount corresponding to peak P was about twice that corresponding to FCE 22101. However, in contrast to dogs, the less polar fraction (LP) in humans represented a significant portion of urinary radioactivity. Peak X was only a minor component in humans, as it was in all other species studied. Finally, in the present study, intersubject variability in the amount of P, X, and LP was relatively small (about 10 to 20%), while for FCE 22101, P1 and P2 variability was much higher (about 50%). The origin of this difference as well as the identity of the component(s) of P and LP cannot be determined from presently available data.

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REFERENCES