Single- and Multiple-Dose Pharmacokinetics of Fleroxacin, a Trifluorinated Quinolone, in Humans

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Fleroxacin (Ro 23-6240; AM-833) is a new trifluorinated quinolone exhibiting high activity against a broad spectrum of gram-negative and gram-positive bacteria. Healthy male volunteers received, according to a randomized scheme, oral doses of 200, 400, or 800 mg of fleroxacin in tablet form, an intravenous infusion of 100 mg, or 400 mg of fleroxacin orally together with 1,000 mg of probenecid. Fleroxacin is characterized pharmacokinetically by a long elimination half-life (9 to 10 h) and high concentrations in plasma (e.g., maximum concentration of 2.3 μg/ml after an oral dose of 200 mg). The volume of distribution clearly exceeds 1 liter/kg and suggests a good tissue penetration. Within 60 h, the cumulative urinary recovery of unchanged drug amounted to 50 to 60% of the dose. The renal clearance of unbound drug was 137 ml/min, and probenecid had no significant effect on renal elimination. A good linear correlation (r = 0.999) was found between doses from 100 to 800 mg and the resulting values of area under the concentration-time curve. The absolute bioavailability of the administered tablet was practically 100%. During oral multiple dosing of 800 or 1,200 mg of fleroxacin once a day over 10 consecutive days, the accumulation of the drug in plasma was close to the theoretically predicted value of 1.3 and reflected the persistence of linear pharmacokinetics.

Floroxacin [6,8-difluoro-1(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid; Ro 23-6240; AM-833] is a new trifluorinated quinolone under clinical investigation. Its chemical structure is shown in Fig. 1.

Early pharmacokinetic and tissue distribution studies in different animal species revealed that this new quinolone was rapidly and completely absorbed following oral administration and that drug levels in lungs, spleen, liver, and kidneys exceeded the corresponding levels in serum (9). Elimination half-lives (τ1/2) were species dependent and reached more than 9 h in dogs.

In this study the pharmacokinetics of fleroxacin in healthy volunteers were investigated after single and multiple dosing; factors studied included dose proportionality, absolute bioavailability of the used tablet, and the influence of probenecid on renal excretion.

MATERIALS AND METHODS

Subjects. The clinical part of this study was performed at the Institut für Klinische Pharmakologie, Grünstadt, Federal Republic of Germany, under the supervision of P. Lücker. Twelve healthy male volunteers, aged 20 to 32 years and within 10% of their ideal weights (J. R. Geigy, ed., Documenta Geigy Tables, vol. 4, p. 10, J. R. Geigy AG, Basel) were selected for the single-dose-administration studies. In addition, another six healthy male volunteers, aged 21 to 31 years participated in the multiple-dose investigation.

Drug administration. (i) Single dosing. Twelve volunteers each received fleroxacin tablets, each containing 200 mg of active substance. The administered doses corresponded to 200, 400, and 800 mg. An open four-way randomized Latin square was used as the randomization procedure. The tablets were taken with 100 ml of tap water after an overnight fast. During the administration all subjects were seated. Infusion solutions, containing 100 mg of fleroxacin dissolved in 50 ml of aqueous medium, were infused into the cubital vein of each of six volunteers, who were supine. The duration of infusion was 20 min. The remaining six volunteers each received an oral dose of 500 mg of probenecid (Benemid tablets from Merck Sharp & Dohme) 10 min prior to the administration of 400 mg of fleroxacin (corresponding to two tablets) and 500 mg of probenecid 4 h after the administration of fleroxacin. One subject had to be excluded from the evaluation of this part of the study owing to noncompliance with the protocol.

(ii) Multiple dosing. Six healthy volunteers received oral doses of 800 mg of fleroxacin once a day on 10 consecutive days. Then, after a washout period of 3 days, the same subjects received 10 daily doses of 1,200 mg of fleroxacin. During both regimens the dosing interval was exactly 24 h. All subjects remained seated for 2 h after dosing.

Sample collection. (i) Single-dose administration. In addition to the blank samples taken shortly before drug administration, serial blood samples (5 ml) were collected into VACUTAINERS containing a mixture of potassium oxalate and ammonium oxalate as anticoagulant (Becton Dickinson Vacutainer Systems. Within 30 min of collection, each blood sample was centrifuged at 1,000 × g for 15 min, and the plasma was carefully transferred to glass tubes and stored at −20°C until analysis. Total urine was collected in appropriate fractions up to 60 h after oral administration or the start of infusion. After complete voiding of the urine at the end of each sampling period, the exact volume and pH of the urine were recorded. A 20-ml portion was put into a polyethylene flask and frozen at −20°C until analysis.

(ii) Multiple-dose administration. Serial blood samples were collected on the first and last day of each dose regimen. In addition, predose samples were taken on days 1, 2, 4, 6, 8, 10, 14, 19, 21, and 23 (dosing of 800 mg, days 1 to 10; dose-free interval, days 11 to 13; dosing of 1,200 mg, days 14
to 23). Total urine was collected in fractions on days 1, 2, 10, 11, 14, 15, 23, and 24. Materials used and plasma separation are described above.

Since fleroxacin is light sensitive, appropriate precautions were taken during blood and urine collection and plasma separation. Unnecessary exposure to direct sunlight was avoided by using brown glassware, covering tubes with aluminum foil, and placing all samples in the deep-freezer as soon as possible.

Assay procedure. A reversed-phase ion pair high-pressure liquid chromatography method was used. Plasma or urine, to which pipemidic acid (internal standard), acetic acid, and sodium dodecyl sulfate had been added, was extracted with a mixture of chloroform and isopropl alcohol. After centrifugation and removal of the aqueous supernatant, a portion of the organic phase was transferred to a conical glass tube and evaporated to dryness under water pump vacuum. The drug residue was dissolved in the mobile phase, and a portion of this solution was chromatographed.

The high-pressure liquid chromatography system consisted of the following components: Toyo Soda ODS-120T 5-μm column, 250 mm by 4.6 mm; Kontron 410 LC pump, flow 0.8 ml/min; Spectroflow 773 UV detector, set at 290 nm (urine), or Kontron Spectrofluorimeter SFM23, excitation 290 nm, emission 450 nm (plasma); WISP 710B autoinjector; and Spectrophysics 4200 computing integrator. The mobile phase was a mixture of 5 mM tetrabutylammonium hydrogen sulfate (aqueous solution) and methanol (18:7, vol/vol). Under these conditions, the retention times for the parent drug, internal standard, N-demethyl metabolite, and N-oxide metabolite were 8.2, 6.3, 9.7, and 12.8 min, respectively. The interassay precision (relative standard deviation) was 5.6, 1.6, and 1.6% for the parent substance, N-demethyl metabolite, and N-oxide metabolite, respectively. The response was linear within the range 0.02 to 2 μg/ml of plasma (fluorescence) and 0.8 to 160 μg/ml of plasma (UV). The limit of quantification for unchanged drug was 20 and 500 ng/ml for plasma and urine, respectively.

Pharmacokinetic evaluation. The drug concentration-time data for each volunteer were fitted individually under the assumption of a two-compartment open model by using the nonlinear least-squares computer program NONLIN (11). A weighting factor of reciprocal measured concentration was chosen for curve fitting. The areas under the plasma concentration-time curve (AUC0–∞) were obtained by using the trapezoidal rule (4). These AUC values were used to calculate the total systemic clearance (CL\text{ISH}) by using the equation CL\text{ISH} = dose/AUC0–∞. The average renal clearance of total drug (CL\text{R}) was calculated by using the equation CL\text{R} = f_o \cdot CL\text{ISH}, where f_o is the fraction of dose which was ultimately excreted into the urine. The nonrenal clearance of total drug (CL\text{NR}) was determined by using the equation CL\text{NR} = CL\text{ISH} - CL\text{R}, and the renal clearance of free drug (CL\text{F}) was calculated by using the equation CL\text{F} = CL\text{R}/f_o, where f_o is the fraction of unbound drug in plasma. The apparent volume of distribution at steady state (Vss) was calculated by a model-independent method (12).

Absolute bioavailability. The amount of unchanged drug that reaches the systemic circulation is defined as bioavailability (i.e., extent of absorption). This parameter was measured by comparing the AUC0–∞ values following oral administration to those after intravenous infusion in the same individuals:

\[ F = \frac{AUC_{0–∞} \text{ (oral)}}{AUC_{0–∞} \text{ (i.v.)}} = \frac{t_{1/2} \text{ (i.v.)}}{t_{1/2} \text{ (oral)}} \cdot \text{dose (i.v.)} \text{ dose (oral)} \]

In this equation intrasubject variability in changes in area is corrected by the concomitantly measured elimination half-lives (t\text{1/2}). Appropriate corrections were made for equal doses (4). The theoretical accumulation of fleroxacin in plasma can be expressed by the accumulation factor (R):

\[ R = 1/(1 - e^{−β}) \]

where τ is the dosing interval and β is the elimination rate constant.

The plasma profiles following multiple dosing (see Fig. 5) were generated by using a computer program iterating over all measured datum points during each dosage regimen (7).

Statistical analysis. The influence of probenecid on t\text{1/2}, AUC, f_o, CL\text{ISH}, CL\text{F}, and Vss was determined by the paired t test. The influence of time or dose on CL\text{F} was examined by the rank test of Friedman (8, 13). Dose proportionality was evaluated by analysis of variance after normalizing the AUC values to a 200-mg dose.

RESULTS

Single-dose pharmacokinetics following intravenous infusion. A semilogarithmic plot of the mean drug concentrations in plasma versus time for six volunteers following a single-dose intravenous infusion of 100 mg of fleroxacin is shown in Fig. 2. The biphasic decline of the concentration in plasma clearly reflects the existence of a distinct distribution phase and justifies curve fitting based on a two-compartment model.

A mean maximum concentration in plasma (C_max) of 2.85 μg/ml was determined at the endpoint of infusion (T_max = 20 min). t\text{1/2}, which was determined in the postdistribution phase, reached 8.6 h, and the AUC0–∞ amounted to 10.2 μg · h/ml. Vss was 110 liters, which corresponded to approximately 1.5 liters/kg of body weight.

CL\text{ISH} was 168 ml/min, and CL\text{F} reached 105 ml/min. Since CL\text{ISH} is the sum of CL\text{F} and CL\text{NR}, approximately one-third of the drug is cleared by nonrenal pathways. The in vitro plasma protein binding of fleroxacin in humans was determined by equilibrium dialysis. In the concentration range 0.05 to 137 μg/ml, only 23% of the total drug was bound to plasma proteins (J. Meyer and R. Brandt, personal communication). The correction of CL\text{F} for plasma protein binding (f_o = 0.77) resulted in a CL\text{F} of 136.6 ml/min.

Within 60 h the cumulative urinary excretion of unchanged drug reached 62.4% of the infused dose. During the same collection period, the mean urinary excretion of the microbiologically active N-demethyl derivative and the inactive N-oxide amounted to 3.6 and 2.9% of the dose, respectively. Thus, the total amount of dose eliminated in the urine is approximately 79%.

In plasma, the concentrations of the two metabolites were
The pharmacokinetic parameters and concentration profiles in plasma evaluated after oral administration of 200, 400, and 800 mg are given in Table 1 and Fig. 3, respectively. C_max values of 2.33, 4.36, and 7.04 µg/ml of plasma appeared within 1 to 2 h of administration of these doses (T_max), and the t_{1/2P} values ranged between 8.9 and 10.3 h. The cumulative urinary recovery of unchanged drug (collection period, 0 to 60 h) amounted to 64.6, 49.8, and 51.8% of the dose after administration of 200, 400, and 800 mg, respectively; the corresponding recoveries for the N-demethyl derivative varied between 4.7 and 6.5%, and those for the N-oxide varied between 3.1 and 5.0%. CL_A was also determined for the different collection intervals up to 12 h. Owing to large variations of the individual values, it was not possible to establish a statistically significant dependency on time or dose.

The mean urinary concentrations determined in the 2-h interval fractions up to 8 h after administration ranged between 100 and 200 µg/ml. The concentration in the 24- to 36-h fraction was 25 µg/ml and exceeds the breakpoint of 16 µg/ml of most fleroxacin-susceptible pathogens in urine.

**Dose proportionality.** In general, dose proportionality is calculated by area comparison. In this study a good linear correlation was found between administered doses of 100,
200, 400, and 800 mg of fleroxacin and the resulting AUC (Fig. 4). The coefficient of correlation obtained from these values was 0.999. At a multiple significance level of 5%, the normalized AUC values after the 200-mg doses were significantly different from the AUC values after the 400 and 800 mg doses.

**Bioavailability.** The absolute bioavailability was measured by comparing the AUCs following the intravenous infusion of 100 mg of fleroxacin and the oral administration of the 200-mg tablet. The 200-mg dose was chosen for comparison because it was most closely related to the parenteral dose and required the smallest correction for area normalization. In this study an excellent mean bioavailability of 0.99 ± 0.14 was obtained. The individual bioavailability data ranged from 0.88 to 1.18 and reflect the small variation in the extent of absorption.

**Influence of probenecid.** To investigate the effect of probenecid on the pharmacokinetics of fleroxacin and to obtain more insight into the mechanism of CLNR, five volunteers each received an oral dose of 200 mg of fleroxacin without probenecid and an oral dose of 400 mg of fleroxacin together with 1,000 mg probenecid. The presence of probenecid had no significant influence (P > 0.05) on the t1/2β, AUC value, Vss, cumulative urinary excretion, and CLf or CLx of fleroxacin.

**Multiple dosing.** The pharmacokinetic data following oral administration of 800 or 1,200 mg of fleroxacin once a day over 10 days are listed in Table 2, and the corresponding mean profiles of concentration in plasma are shown in Fig. 5.

After the first 800-mg dose a mean Cmax of 9.4 μg/ml appeared generally in less than 2 h in the plasma. In the postdistributive phase a t1/2β of 9.2 h was measured and the AUC0-∞ reached 107.4 μg·h/ml. The Vss was 1.3 liters/kg, and a CLx of 128.5 ml/min was obtained. After the last (10th) dose of the 800-mg regimen, the mean Cmax value increased to 11.2 μg/ml and the t1/2β was slightly but not significantly prolonged (P > 0.01).

During multiple dosing of 1,200 mg, the Cmax values increased from 11.9 μg/ml on day 1 to 13.5 μg/ml on day 10. During the same dosing period the t1/2β increased from 9.7 to 11.8 h (P > 0.05).

For the administered fleroxacin doses of 1,200 and 800 mg, the ratio of the AUC values (AUC1,200 mg/AUC800 mg) should be 1.5 at corresponding time intervals on days 1 and 10. Owing to the lack of blood samples at 24 h on day 1, the ratio of the respective AUC values from 0 to 12 h had to be taken. This ratio was 1.58 on day 1 and 1.67 on day 10, respectively, and reflects the good dose proportionality before and after multiple dosing. The actual accumulation calculated by AUC comparison (AUCday10/AUCday1) reached 1.38 after multiple dosing of 800 mg and 1.46 after multiple dosing of 1,200 mg.

The mean predose plasma concentrations (Cmax) at steady state, which is reached after about 2 days, were 2.4 μg/ml during multiple dosing of 800 mg and 4.2 μg/ml during multiple dosing of 1,200 mg (Fig. 5).

Following the last dose of the 800- and the 1,200-mg dose regimens, total urine was collected over 48 h. Within that time approximately 60% of the dose was eliminated renally as unchanged drug. The fraction of the N-demethyl derivative amounted to ca. 11%, and that of the N-oxide amounted to 7 to 10%.

**DISCUSSION**

The applied dose regimens were well tolerated by all volunteers. Vital parameters, electrocardiogram, lung function tests, clinical chemistry, hematology, and urinalysis showed no changes attributable to the trial medication.

Curve fitting for concentration in plasma following administration of fleroxacin was best achieved by assuming an open linear two-compartment model. The marked biphasic decline of the concentrations in plasma after intravenous infusion was less pronounced after oral administration owing to the relatively small absorption rate constant. Intravenous and oral administration of this new quinolone resulted in high levels in plasma, and in the postdistributive phase t1/2β of approximately 10 h was determined. Both these parameters are favorable prerequisites for daily administration. The Vss clearly exceeded 1 liter/kg and reflects the good tissue penetration of this drug. This finding is in good agreement with the results of a skin blister study, which revealed a rapid penetration of fleroxacin into the inflammatory fluid, the percentage of penetration being close to 90% by area comparison (14). The value for the CLx of free drug was approximately 137 ml/min, which is close to the average glomerular filtration rate in humans (ca. 125 ml/min) and might indicate that renal elimination of fleroxacin is mainly by glomerular filtration and that tubular secretion plays an insignificant role. This assumption is also supported by the lack of influence of probenecid on the elimination of fleroxacin, since probenecid is known to inhibit competitively the renal tubular secretion of many drugs such as penicillins and other β-lactam antibiotics (6). In addition, probenecid has been shown to reduce biliary excretion and/or metabolic reactions to this process is not yet known and will be investigated by excretion balance and metabolic studies. The high urinary concentrations of unchanged drug were maintained over a long period and represent an important prerequisite for the treatment of urinary tract infections.

The linear correlation between administered doses and resulting AUC values points to dose concentration-independent linear pharmacokinetics over the range studied. However, the AUC values showed some tendency to increase disproportionately at higher doses. This effect, which was not observed during the multiple dosing of 800 and 1,200 mg, is
TABLE 2. Pharmacokinetic parameters following multiple dosing of 800 and 1,200 mg of fleroxacin on 10 consecutive days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value* for 800-mg dose on:</th>
<th>Values† for 1,200-mg dose on:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 10</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>9.35 ± 3.46</td>
<td>11.2 ± 1.60</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.7 ± 1.4</td>
<td>1.0 ± 0.8</td>
</tr>
<tr>
<td>t1/2B (h)</td>
<td>9.2 ± 1.0</td>
<td>11.1 ± 1.9</td>
</tr>
<tr>
<td>Vss/Fb (liters)</td>
<td>93.3 ± 8.7</td>
<td></td>
</tr>
<tr>
<td>Vss/Fb (liters/kg)</td>
<td>1.3 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>CL/Fb (ml/min)</td>
<td>128.5 ± 25.3</td>
<td></td>
</tr>
<tr>
<td>AUC (µg • h/ml)</td>
<td>107.4 ± 22.0</td>
<td></td>
</tr>
<tr>
<td>AUCO-12h</td>
<td>61.6 ± 10.7</td>
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</tr>
</tbody>
</table>

* Values are given as mean ± standard deviation (n = 6).
† F was taken as 1, since the absolute bioavailability of the used tablets is 99%.

without clinical significance, and discussions concerning saturation of metabolism are too speculative.

The high absolute bioavailability of the tablets used in this study exclude any absorption problems from the gastrointestinal tract and any significant first-pass metabolism.

Assuming an overall t1/2B of 10.5 h and a dosing interval of 24 h, equation 1 predicts a theoretical drug accumulation in plasma of 1.3. During multiple dosing of 800 and 1,200 mg once a day over 10 days, the measured accumulation of the drug in plasma was close to this theoretically predicted value, and thus time-dependent effects such as induction or inhibition of metabolizing or eliminating enzymes can be excluded. At steady-state conditions, the minimum concentrations in plasma were well above the MIC for 90% of strains tested for most fleroxacin-susceptible pathogens and support the foreseen daily dosage regimen (2, 10).

The higher urinary excretion of the metabolites during multiple dosing compared with that after single-dose administration does not seem to be related to the dose regimen, because similar high recoveries of both metabolites were found during previous single-dose tolerance studies.

In comparison with other fluorinated quinolones such as ciprofloxacin, ofloxacin, pefloxacin, enoxacin, and norfloxacin the trifluorinated fleroxacin exhibits favorable pharmacokinetics by combining the advantages of high concentrations in plasma (large AUC values), long t1/2, high urinary levels of unchanged drug, and good tissue penetration.

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LITERATURE CITED


