Renal Handling of Fleroxacin in Rabbits, Dogs, and Humans

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The renal handling of fleroxacin was studied by renal clearance and stop-flow techniques in rabbits and dogs and by analyzing the pharmacokinetics with and without probenecid in humans. In rabbits the excretion ratios (fleroxacin intrinsic renal clearance/glomerular filtration rate) were greater than unity (2.01) without probenecid and were decreased to a value below unity (0.680) with probenecid. In dogs, on the other hand, the excretion ratios were less than unity (0.608 and 0.456) both without and with probenecid, and so were not affected by probenecid. This fact suggested that fleroxacin was excreted into urine by both glomerular filtration and renal tubular secretion in rabbits, but only by glomerular filtration in dogs, accompanied by partial renal tubular reabsorption in both species; these mechanisms were also supported by stop-flow experiments. In humans probenecid treatment induced increases in the elimination half-life and area under the serum concentration-time curve and decreases in apparent serum clearance, renal clearance, and urinary recovery of fleroxacin. The excretion ratio without probenecid was 1.13, which was significantly decreased to 0.750 with probenecid. These results indicated that both renal tubular secretion and reabsorption contributed to renal excretion of fleroxacin in humans. The contribution of tubular secretion was species dependent and was extensive in rabbits, minimal in dogs, and moderate in humans. Renal tubular reabsorption was commonly found in every species. The long elimination half-life of fleroxacin in humans might be explained by its small total serum clearance and renal clearance, which are attributed to less tubular secretion and more tubular reabsorption.

Fleroxacin (AM-833; Ro 23-6240), a new trifluorinated quinolone, has potent and broad in vitro activity against gram-positive and gram-negative bacteria and also exhibits significant in vivo activity against various experimental infections after oral administration (5, 9, 16). Pharmacokinetic studies in laboratory animals (14, 15) demonstrated that fleroxacin is rapidly and completely absorbed from the gastrointestinal tract and is well distributed to various tissues, except the brain. Animals with larger body weights tend to show a longer elimination half-life of fleroxacin, except rabbits, which show a short half-life. Pharmacokinetic studies in healthy volunteers revealed that fleroxacin is metabolized to a small extent and is characterized pharmacokinetically by a long elimination half-life and high concentration in plasma (17, 24, 26). This drug was principally excreted into urine by laboratory animals and humans (15, 17, 24, 26). Little is known, however, on the mechanism of renal excretion of fleroxacin. Many reports that have been published on the renal handling of quinolones showed different mechanisms, depending on the animal species and drugs (1, 10–13, 19, 20, 22, 25). Shimada et al. (22) found species differences in the renal handling of norfloxacin in rabbits, dogs, and humans.

The purpose of the present study was to examine the mechanism of renal excretion of fleroxacin in rabbits, dogs, and humans. Renal clearance and stop-flow experiments were carried out in rabbits and dogs. In humans pharmacokinetic analysis was conducted after single oral administration of fleroxacin with and without probenecid.

MATERIALS AND METHODS

Chemicals. The fleroxacin (lot no. 830530), its metabolites demethyl fleroxacin and fleroxacin N-oxide (17), and an internal standard (pipemidic acid) to be analyzed were synthesized at the Central Research Laboratory of Kyorin Pharmaceutical Co., Ltd. (Tochigi, Japan). Fleroxacin tablets (100 mg; lot no. S710880) were prepared by Kyorin Pharmaceutical Co., Ltd. (Tokyo, Japan). Probenecid tablets (Probenemid; 250 mg) were purchased from Japan Merck Banyo Co., Ltd. (Tokyo, Japan). All other reagents were of analytical grade.

Animals. Eighteen male Japanese White rabbits (body weight, 2.4 to 2.9 kg) and six male beagle dogs (body weight, 8.7 to 12.5 kg) were used after they were fasted overnight.

Volunteers. Six male volunteers participated after giving written informed consent. They were aged 20 to 21 years (mean ± standard deviation, 20.2 ± 0.4 years) and weighed 60.0 to 74.4 kg (mean ± standard deviation, 67.3 ± 5.2 kg). Pre- and poststudy physical examination and clinical laboratory parameters were normal. All volunteers fasted overnight before doses were administered. The first meal was allowed 4 h after dosing. They took the same meal at the same time after dosing throughout the urine collection period. No other drugs were permitted to be taken during the study. Subjects had normal access to water but did not ingest any beverage or food containing alcohol or caffeine.

Renal clearance experiments in rabbits and dogs. Fifteen rabbits (weight, 2.4 to 2.9 kg) and three dogs (weight, 8.7 to 8.9 kg) were used in the renal clearance experiments. The experiments were performed by a previously described method (22), with minor modifications. Briefly, animals were anesthetized with pentobarbital sodium and were intrave-
nously infused with a solution of 10% mannitol in isotonic saline (solution 1). When urine flow was stabilized, urine and blood samples were collected. Then, inulin (40 mg/kg) and fleroxacin were injected intravenously as priming doses, followed by a sustaining infusion of solution 1 containing inulin (24 mg/kg per h) and fleroxacin (solution 2). At 30 min after the start of the infusion, urine samples were collected from both ureters during three successive 5-min intervals. Blood samples were taken at the midpoint of urine collections. This procedure was repeated for each of the three doses in the experiment in dogs. Then, probenecid was administered intravenously at a dose of 30 mg/kg, followed by a sustaining infusion of solution 2 containing probenecid (6 mg/kg per h). When 30 min had passed, the experimental maneuvers described above were performed. Rabbies were injected at priming doses of fleroxacin of 2.5, 12.5, and 25 mg/kg and then infused with fleroxacin at the respective rates of 1.1, 5.5, and 11 mg/kg per h. Dogs were injected at successive initial fleroxacin doses of 1.4, 5.5, and 6.8 mg/kg and infused with fleroxacin at the respective rates of 0.10, 0.50, and 1.0 mg/kg per h. The infusion rates were 1.0 ml/min in rabbits and 3.0 ml/min in dogs throughout the experiments.

Stop-flow analysis in rabbits and dogs. Three rabbits (weight, 2.6 to 2.8 kg) and three dogs (weight, 10.3 to 12.5 kg) were used in the stop-flow analysis. The procedure was almost the same as that described previously (22). Briefly, sodium p-aminohippurate (PAH; 20 mg/kg) and creatinine (100 mg/kg) were injected intravenously into dogs as a priming dose. A sustaining solution (15% mannitol, 0.9% NaCl, 0.25% creatinine, and 0.2% PAH [solution 3]) was then infused. After the urine flow rate became constant, urine and blood samples were collected. Then, fleroxacin was injected intravenously as a priming dose, followed by injection of the sustaining infusion of solution 3 containing fleroxacin (solution 4). About 1 h after starting the infusion of solution 4, urine samples were collected from the right ureter twice at 3-min intervals for the determination of free-flow clearance. Blood samples were taken at the same time. The urine flow was then stopped by applying a hemostat clamp to the ureter; the clamp was removed 6 min later. After removal of the clamp, the urine was collected serially in 20 and 30 0.5-ml test tubes for rabbits and dogs, respectively. One minute before removal of the clamp, inulin was administered intravenously at a dose of 25 mg/kg. Free-flow clearance was determined as described above. After completion of the control experiment, probenecid was administered intravenously at a priming dose of 30 mg/kg, followed by a sustaining infusion of solution 4 containing probenecid (6 mg/kg per h). After 30 min, the experimental maneuvers described above were performed. Fleroxacin was given to rabbits at a priming dose of 7.5 mg/kg and infused at a rate of 3.3 mg/kg per h and was given to dogs at a priming dose of 13.7 mg/kg and infused at a rate of 1.0 mg/kg per h. The infusion rates were 1.5 ml/min in rabbits and 3.0 ml/min in dogs throughout the experiments.

Human study. The human subjects received a single 200-mg oral dose of fleroxacin with a glass of water. Blood samples (6 ml) were taken at 0.5, 1, 2, 3, 5, 7, 9, 11, 24, and 48 h after drug administration. Urine samples were collected just before, 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 24, and 24 to 48 h after dosing. Urine pH was determined with pH test paper (Pehanon; Macherey-Nagel). One week later, the same volunteers received the same dose of fleroxacin and were given 500 mg of probenecid 0.5 h before and 12, 24, and 36 h after they received the dose of fleroxacin. Blood and urine samples were collected as described above.

**Assay procedure.** Serum and urine samples were stored at -20°C until they were assayed. Fleroxacin in serum and fleroxacin and its metabolites in urine were determined by high-performance liquid chromatography with fluorescence detection as described previously (17). Serum (0.5 ml) and ultrafiltrate samples (0.1 ml) were mixed with 0.1 ml of internal standard solution, 0.4 ml of 0.5 M phosphate buffer (pH 7.0), and 6 ml of chloroform. Urine samples (0.2 ml) were mixed with 0.1 ml of internal standard solution, 0.5 ml of 1 M acetic acid, 0.5 ml of 5 mM sodium dodecyl sulfate, and 7 ml of chloroform-isopropanol (7:3). The internal standard solution was prepared by dissolving pipemidic acid in 0.5 M phosphate buffer (pH 7.0) for the serum and ultrafilterate (0.1 mg/ml) and in 0.01 M sodium hydroxide for urine (0.4 mg/ml). After the extraction, 5 ml of the organic layer was evaporated. The resulting residue was dissolved in 0.1 or 0.2 ml of acetonitrile -0.04 M phosphoric acid (3:7 or 1:1). A portion of the solution was injected into the high-performance liquid chromatographic system (model 655; Hitachi) equipped with a TSK gel ODS-120T column (particle size, 5 μm; 4.6 by 250 nm; Tosoh Corp.). The eluting mobile phase was a mixture of methanol and tetra-n-butylammonium hydrogen sulfate (28:72). The flow rate was 0.75 or 0.80 ml/min. The excitation and emission wavelengths of the fluorescence detector (model 650-10LC or F-1000; Hitachi) were set at 290 and 450 nm, respectively. For serum, standard curves were linear from 0.01 to 10 μg/ml. Overall recovery was 84% for fleroxacin. The intraday variability (coefficient of variation) was less than 4%. The detection limits were 0.01 μg/ml for serum and 0.05 μg/ml for ultrafiltrate. For urine, standard curves were linear from 0.2 to 200 μg/ml for fleroxacin and from 0.2 to 50 μg/ml for its metabolites. Overall recoveries were greater than 94% for flerox-

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**TABLE 1. Renal clearance of fleroxacin in rabbits**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>Urine pH</th>
<th>Urine flow (ml/min)</th>
<th>Fleroxacin concn in serum (μg/ml)</th>
<th>GFR (ml/min)</th>
<th>Intrinsic Cl- of fleroxacin (ml/min)</th>
<th>Excretion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td></td>
<td>Unbound</td>
<td></td>
</tr>
<tr>
<td>Without probenecid</td>
<td>1</td>
<td>7.8 ± 0.2</td>
<td>1.61 ± 0.24</td>
<td>1.31 ± 0.13</td>
<td>0.87 ± 0.11</td>
<td>10.8 ± 1.8</td>
<td>28.8 ± 8.0</td>
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<tr>
<td></td>
<td>2</td>
<td>7.5 ± 0.2</td>
<td>1.76 ± 0.18</td>
<td>7.77 ± 0.74</td>
<td>5.04 ± 0.53</td>
<td>14.6 ± 1.2</td>
<td>24.7 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.6 ± 0.4</td>
<td>2.12 ± 0.20</td>
<td>15.2 ± 1.8</td>
<td>10.8 ± 1.4</td>
<td>12.4 ± 2.6</td>
<td>20.4 ± 5.5</td>
</tr>
<tr>
<td>With probenecid</td>
<td>1</td>
<td>7.9 ± 0.2</td>
<td>1.11 ± 0.26</td>
<td>1.20 ± 0.03</td>
<td>0.99 ± 0.09</td>
<td>5.84 ± 1.31</td>
<td>4.38 ± 1.04</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.5 ± 0.2</td>
<td>1.52 ± 0.09</td>
<td>7.25 ± 0.83</td>
<td>5.39 ± 0.75</td>
<td>9.94 ± 0.91</td>
<td>6.73 ± 1.01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.7 ± 0.3</td>
<td>1.41 ± 0.33</td>
<td>14.0 ± 1.9</td>
<td>11.2 ± 1.7</td>
<td>8.75 ± 2.61</td>
<td>5.17 ± 1.28</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± standard deviation of five animals.

Statistically significant (P < 0.01).

Statistically significant (P < 0.05).
Fleroxacin and its metabolites. The intraday variabilities were almost less than 4%. The detection limits were 0.2 µg/ml for fleroxacin and its metabolites. PAH and probenecid did not interfere with the assay of fleroxacin or its metabolites.

For inulin determination, the method of Dische and Borenfreund (7) was used. PAH concentrations were determined by the method of Bratton and Marshall (3). Creatinine was measured with an autoanalyzer (model 706D or 705; Hitachi) by the improved Jaffe method (2). Sodium ion was determined by flame photometry (model 775-A; Hitachi).

**Serum protein binding.** The in vivo serum protein binding of fleroxacin was determined by the centrifugal ultrafiltration method by using all samples from the renal clearance experiments in rabbits and dogs and the samples taken at 3, 5, 7, 9, and 11 h after dosing in human. Portions (1 ml) of serum were loaded onto a membrane (MPS-1; Amicon Corp., Lexington, Mass.). The concentrations of fleroxacin in serum and filtrate were determined by high-performance liquid chromatography for the total and unbound concentrations, respectively. The extent of adsorption of fleroxacin to the membrane was negligible.

**Data analysis.** Results are expressed as means ± standard deviations. The significance of the data was evaluated by the Student unpaired t test. A value of P < 0.05 was considered to be significant.

In renal clearance experiments the glomerular filtration rate (GFR) was obtained from the concentrations of inulin in serum and urine and the urine flow. The intrinsic renal clearance of fleroxacin was calculated by the concentration in urine, the unbound drug concentration in serum, and the urine flow. The excretion ratio was obtained by dividing the intrinsic renal clearance of fleroxacin by GFR. In stop-flow analysis, the locations of secretion from the proximal renal tubules and reabsorption through the distal renal tubules were determined by using PAH and sodium, respectively. Inulin was administered as a marker for glomerular urine. The urine-to-serum concentration ratio of creatinine (U/P Cr) was used as a parameter of the concentration of urine. The ratios of the urine-to-serum concentration ratio of each component to the corresponding U/P Cr were plotted on the ordinate of the stop-flow pattern.

In the human studies, the serum concentration-time profiles were analyzed by a one-compartment open model. Pharmacokinetic parameters were obtained as follows. T_{max} was the time at which the peak concentration in serum was achieved. C_{max} was the peak concentration that was observed in serum, t_{1/2} was the elimination half-life in serum calculated from the elimination rate constant determined by linear regression analysis. AUC_{0-∞} was the area under the serum concentration-time curve from time zero to infinity, which was calculated as the sum of the AUC obtained from time zero to 48 h (AUC_{0-48}) by the trapezoidal rule and the ratio of the last concentration measured to the elimination rate constant. V/F, which was calculated by dividing the dose by the elimination rate constant and AUC_{0-∞}, was the volume of distribution (V) divided by bioavailability (F). CL_{app} was the apparent serum clearance of fleroxacin calculated by dividing the dose by AUC_{0-48}. CL_{R} was the renal clearance of fleroxacin calculated by dividing the urinary excretion of fleroxacin for 48 h by AUC_{0-48}. Creatinine clearance, fleroxacin intrinsic renal clearance, and the ratio of fleroxacin intrinsic renal clearance to creatinine clearance for each 2-h period were calculated.

**RESULTS**

**Results in rabbits.** Results of renal clearance experiments in rabbits are given in Table 1. Urine pH remained almost constant throughout the experiments. Serum protein binding of fleroxacin was 29 to 35% in the wide range of total drug

### TABLE 2. Renal clearance of fleroxacin in dogs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sustaining fleroxacin dose (mg/kg per h)</th>
<th>Urine pH</th>
<th>Urine flow (ml/min)</th>
<th>Sustaining fleroxacin concn in serum (µg/ml)</th>
<th>GFR (ml/min)</th>
<th>Intrinsic Cl_R of fleroxacin (ml/min)</th>
<th>Excretion ratio</th>
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<td></td>
</tr>
<tr>
<td>Without probenecid</td>
<td>0.10</td>
<td>7.4 ± 0.4</td>
<td>3.82 ± 0.51</td>
<td>1.08 ± 0.12</td>
<td>0.64 ± 0.06</td>
<td>34.7 ± 2.0</td>
<td>22.4 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>7.1 ± 0.3</td>
<td>5.14 ± 0.20</td>
<td>5.79 ± 0.64</td>
<td>3.40 ± 0.19</td>
<td>30.9 ± 3.8</td>
<td>20.3 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>7.0 ± 0.4</td>
<td>4.02 ± 0.32</td>
<td>10.6 ± 1.0</td>
<td>6.73 ± 0.33</td>
<td>25.9 ± 3.2</td>
<td>13.1 ± 1.2</td>
</tr>
<tr>
<td>With probenecid</td>
<td>1.0</td>
<td>7.1 ± 0.4</td>
<td>3.49 ± 0.46</td>
<td>8.65 ± 0.56</td>
<td>5.93 ± 0.15</td>
<td>22.7 ± 3.9</td>
<td>10.3 ± 2.1</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± standard deviation of three animals.
concentrations in serum (1.31 to 15.2 μg/ml) without probenecid. A little reduction in binding was observed with probenecid (17 to 26%). Probenecid induced a decrease in GFR with a decrease in urine flow. The excretion ratios of fleroxacin were greater than unity (1.66 to 2.66) without probenecid. These ratios tended to decrease with an increase in concentrations in serum. Probenecid induced a marked reduction in the excretion ratios to values below unity (0.607 to 0.752).

Results of stop-flow experiments in rabbits are shown in Fig. 1. A trough of fleroxacin was observed at the trough of sodium, while a broad peak of fleroxacin was found at the peak of PAH. The peaks of fleroxacin and PAH disappeared with the administration of probenecid.

Results in dogs. Results of renal clearance experiments in dogs are given in Table 2. Urine pH and flow rate remained almost constant throughout the experiments. Without probenecid serum protein binding of fleroxacin was 36 to 41% in the wide range of total drug concentrations in serum (1.08 to 10.6 μg/ml). A slight reduction in binding was observed with probenecid (32%). The excretion ratios were always less than unity (0.509 to 0.661), independent of the concentrations in serum. Probenecid had little effect on the renal excretion of fleroxacin. The mean excretion ratio with probenecid was 0.456.

Results of stop-flow experiments in dogs are shown in Fig. 2. A trough of fleroxacin was observed corresponding to the distal tubules. No definite peak of fleroxacin was found corresponding to the proximal tubules. With the administration of probenecid, the peak of PAH disappeared, but the stop-flow pattern of fleroxacin showed no significant change.

**Results in humans.** Table 3 gives the concentrations of fleroxacin in serum following 200-mg single oral doses with and without probenecid in humans. Without probenecid the concentrations of fleroxacin in serum reached a peak (2.37 μg/ml) within 1 h and declined according to first-order kinetics. With the administration of probenecid the concentrations of fleroxacin in serum reached a peak (2.54 μg/ml) within 1 or 2 h and remained at higher levels than those without probenecid over 48 h. The pharmacokinetic parameters of fleroxacin are given in Table 4. With the administration of probenecid, the $t_{1/2}$ increased from 10.9 to 15.0 h. AUC$_{0-\infty}$ also increased from 32.6 to 44.7 μg · h/ml, whereas CL$_{app}$ decreased from 104 to 75.8 ml/min and CL$_{R}$ decreased from 73.4 to 44.4 ml/min ($P < 0.01$). Probenecid had no effect on $T_{max}$, $C_{max}$, or V/F. The serum protein bindings were 42 and 38% without and with probenecid, respectively.

The urinary recoveries of fleroxacin, demethyl fleroxacin, and fleroxacin N-oxide for 48 h were 67.2, 5.9, and 4.5% of the dose without probenecid and 52.1, 6.0, and 5.1% of the dose with probenecid, respectively (Table 4). Treatment with probenecid significantly reduced the urinary recovery of fleroxacin by 15.1% of the dose ($P < 0.01$). Urine pH and flow rate remained almost constant throughout the experiment.

Creatinine clearance, fleroxacin intrinsic renal clearance, and the ratio of one to the other for each time period are given in Table 5. The fleroxacin intrinsic renal clearance was a little greater than the creatinine clearance without probenecid, whereas the intrinsic renal clearance during probenecid treatment was less than the creatinine clearance. When the subjects received fleroxacin alone, the excretion ratios were 1.06 to 1.16 (mean, 1.13), but they significantly declined to 0.703 to 0.860 (mean, 0.750) with the administration of probenecid ($P < 0.01$ or 0.05).

**DISCUSSION**

Tubular secretion has been observed with nalidixic acid (10), piromidic acid (10), miloxacin (10), and norfloxacin (22) in rabbits; pipemidic acid (12) in dogs; and cinoxacin (11, 20), norfloxacin (22), and ciprofloxacin (25) in humans. Tubular reabsorption has been found with nalidixic acid (10), piromidic acid (10), and miloxacin (10) in rabbits in renal clearance experiments. It has been suggested that the tubular reabsorption might contribute to the renal excretion of cinoxacin in rats (13), dogs (19), and humans (1) because of the remarkable influence of alkalotic and acidotic urine on serum elimination and urinary excretion. On the contrary, enoxacin was excreted only by glomerular filtration in dogs (23) and humans (21). Shimada et al. (22) noted that the renal excretion of norfloxacin differed with the animal species being tested. Fleroxacin was principally excreted into urine in laboratory animals and humans (15, 17, 24, 26). It might be interesting to know the mechanism of renal excretion of fleroxacin because of its longer $t_{1/2}$ than other quinolones in humans (17, 24, 26). Probenecid is known to be actively secreted through the renal tubules and to block the active secretion of many organic acids by its potent affinity for the carrier of the anion transport mechanism (6). The mechanism of renal excretion was thus examined in rabbits, dogs, and humans in the absence and presence of probenecid.

In rabbits the marked reduction in GFR with probenecid was noted with a decrease in urine flow, which might be attributed to a decrease in renal blood flow, because a significant decrease in blood pressure was observed (data not shown). The excretion ratios were greater than unity.
TABLE 4. Pharmacokinetic parameters of fleroxacin after oral administration of 200 mg of fleroxacin with and without probenecid in humansa

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Without probenecid</th>
<th>With probenecid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td></td>
<td>0.75 ± 0.27</td>
<td>1.33 ± 0.52</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td></td>
<td>2.37 ± 0.38</td>
<td>2.54 ± 0.60</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td></td>
<td>10.9 ± 1.4</td>
<td>15.0 ± 1.6b</td>
</tr>
<tr>
<td>V/F (liter/kg)</td>
<td></td>
<td>1.44 ± 0.08</td>
<td>1.45 ± 0.08</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (µg · h/ml)</td>
<td></td>
<td>32.6 ± 4.6</td>
<td>44.7 ± 6.6b</td>
</tr>
<tr>
<td>CL$_{\text{app}}$ (ml/min)</td>
<td></td>
<td>104 ± 13.4</td>
<td>75.8 ± 10.3b</td>
</tr>
<tr>
<td>CL$_{R}$ (ml/min)</td>
<td></td>
<td>73.4 ± 11.3</td>
<td>44.4 ± 6.6b</td>
</tr>
<tr>
<td>Urinary excretion (0 to 48 h) (% of dose)</td>
<td></td>
<td>67.2 ± 4.1</td>
<td>52.1 ± 2.8b</td>
</tr>
<tr>
<td>Fleroxacin</td>
<td></td>
<td>5.9 ± 0.6</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>Demethyl fleroxacin</td>
<td></td>
<td>4.5 ± 0.8</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>Fleroxacin N-oxide</td>
<td></td>
<td>6.6b ± 1.33</td>
<td>5.1b ± 1.2</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± standard deviation of six volunteers.

b Statistically significant ($P < 0.01$).

(mean, 2.01) and had a decreasing trend with an increase in fleroxacin concentrations in serum. These results suggest that carrier-mediated active secretion contributes to a part of the renal excretion of fleroxacin. The administration of probenecid caused a marked reduction in the excretion ratios, resulting in a value below unity (mean, 0.680). This fact suggests the contribution of renal tubular secretion and reabsorption to the renal excretion of fleroxacin. This proposed mechanism in rabbits was confirmed by the stop-flow experiments.

In dogs the excretion ratios of fleroxacin were less than unity (mean, 0.608), independent of the concentrations of fleroxacin in serum, and were altered little by the administration of probenecid (mean, 0.456). These results indicate that the renal excretion of fleroxacin in dogs takes place mostly through glomerular filtration and tubular reabsorption and that there is little or no contribution of renal tubular secretion. The stop-flow analysis also supported the proposed mechanism described above.

In humans probenecid had no effect on the $C_{\text{max}}$ of fleroxacin in serum, but prolonged the $t_{1/2}$ of fleroxacin from 10.9 to 15.0 h. Furthermore, probenecid induced an increase in AUC$_{0-\infty}$ and decreases in CL$_{\text{app}}$, CL$_{R}$, and urinary recovery of fleroxacin. Other pharmacokinetic parameters remained unchanged. This fact suggested that probenecid has no effect on gastrointestinal absorption or on the distribution of fleroxacin. The intrinsic CL$_{R}$ of fleroxacin was somewhat greater than the GFR (creatinine clearance), and the excretion ratios were 1.06 to 1.16 (mean, 1.13). Therefore, it can be assumed that in humans, fleroxacin is excreted by both glomerular filtration and tubular secretion. Probenecid reduced the excretion ratios to values which were constant below unity (0.703 to 0.860; mean, 0.750), which suggested the contribution of the renal tubular reabsorption to the renal excretion of fleroxacin. The inhibition of secretion with probenecid could explain the pharmacokinetic alterations of fleroxacin. Weidekamm et al. (24) reported that the intrinsic CL$_{R}$ of fleroxacin in humans is approximately 137 ml/min, which was close to the GFR, and that probenecid has no effect on serum elimination of fleroxacin. They concluded that fleroxacin is excreted into urine mainly by glomerular filtration and that tubular secretion plays an insignificant role in humans. In their study, however, volunteers received two 500-mg oral doses of probenecid 10 min
prior to and 4 h after the administration of fleroxacin. Hence, the inhibition of the tubular secretion by probenecid might have been incomplete at the terminal elimination phase because of the relatively short $t_{1/2}$ of probenecid (6). In our study, the tubular secretion might have been more completely inhibited during the experiment because volunteers took four 500-mg oral doses of probenecid 0.5 h prior to and 12, 24, and 36 h after the administration of fleroxacin. This discrepancy in conclusions between the two studies must be attributed to the different time schedule of probenecid doses.

In this study, the contribution of the renal tubular secretion of fleroxacin was clarified to be species dependent: extensive in rabbits, minimal in dogs, and moderate in humans. The same trend was found for norfloxacin (22). Inhibition of the urinary excretion by probenecid suggests that fleroxacin is actively secreted into the tubules by an anion transport mechanism. On the other hand, among the quinolone antimicrobial agents, fleroxacin is the first agent that has been proved to be reabsorbed through the renal tubules in some animal species. It is generally known that the lipophilic compound is well reabsorbed from renal tubules (8). Fleroxacin is more lipophilic than norfloxacin. The apparent partition coefficients between chloroform and 0.1 M phosphate buffer (pH 7.4) are +0.69 and −0.54 for fleroxacin and norfloxacin, respectively (H. Kusajima, M. Machida, N. Ishikawa, H. Uchida, and K. Masuzawa, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 432, 1986). Both drugs are dipolar ions over the usual urinary pH region because of the $pK_a$ s of fleroxacin (5.03 and 8.65) and norfloxacin (6.34 and 8.75) determined by potentiometry. Thus, the contribution of reabsorption of fleroxacin to renal excretion might be explained by its more lipophilic property.

The elimination of fleroxacin from serum was found to be exceptionally fast in rabbits, although animal species smaller in body weight have a tendency to show a shorter $t_{1/2}$ (14). In a previous study (15) it was demonstrated that this phenomenon might be associated with the extensive metabolism of fleroxacin in rabbits. The present study also showed that the extensive tubular secretion of fleroxacin might be another reason for the fast serum elimination in rabbits.

The $t_{1/2}$ of fleroxacin was two or three times longer than those of ciprofloxacin, norfloxacin, ofloxacin, and enoxacin (4, 17, 18, 24, 26). The $t_{1/2}$ of drug is expressed as a mixed function of total serum clearance ($CL_{total}$) and $V$ ($t_{1/2} = 0.693 V/CL_{total}$). The $V$ of fleroxacin was similar to or smaller than those of other quinolones. The $CL_{total}$ of fleroxacin was at least two to three times smaller than those of other quinolones. Therefore, the long $t_{1/2}$ of fleroxacin in humans might be due to the smaller $CL_{total}$ and the smaller $CL_{R}$, which were attributed to less tubular secretion and more tubular reabsorption.

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**LITERATURE CITED**


