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Intestinal Elimination of Sparfloxacin, Fleroxacin, and Ciprofloxacin in Rats

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The intestinal transepithelial elimination of sparfloxacin and fleroxacin was compared with that of ciprofloxacin in a rat model following a single parenteral administration of 25 mg of each of the antibiotics per kg of body weight. All three fluoroquinolones were eliminated through the small intestine. Ciprofloxacin was eliminated in the proximal jejunum at a rate of 1.97 ± 0.70 μg/cm², while the elimination rates of fleroxacin and sparfloxacin were 0.64 ± 026 and 0.21 ± 0.10 μg/cm², respectively, over a 90-min collection period. In the ileum, the elimination rates of ciprofloxacin, fleroxacin, and sparfloxacin over the same period were 1.44 ± 0.77, 1.00 ± 0.33, and 0.41 ± 0.26 μg/cm², respectively. These data suggest that these fluoroquinolones undergo a transepithelial elimination process in the small intestine. This route of elimination may be important in the therapy of bacterial diarrhea.

Extrarenal routes have been considered important for the elimination of fluoroquinolones. In rats, some 7% of a parenterally administered ciprofloxacin dose is eliminated in the intestine, mostly in the jejunum (10). In rabbits, the fraction eliminated in the small intestine amounts to 19%, mostly in the ileum (9). In humans, this fraction is 17.8% (1). Oral administration of activated charcoal, which binds fluoroquinolones, was found to decrease renal secretion and to increase the total and nonrenal clearances of ciprofloxacin and other fluoroquinolones in humans, suggesting that the intestinal tract is an important excretory organ for fluoroquinolones (7, 11, 15). Since the fractions of ciprofloxacin, fleroxacin, and sparfloxacin excreted in the bile amount to only 1 to 3% of the administered dose (4, 12–14) and ciprofloxacin concentrations in feces reach 185 to 2,220 μg/g (2, 9), the transepithelial route is probably responsible for ciprofloxacin elimination in the intestine. For fleroxacin 10 to 15% of the dose is eliminated in the feces (6) and for sparfloxacin 60% of the dose is eliminated in the feces (8), suggesting that the same transintestinal route might be an important secretory pathway for these fluoroquinolones as well.

In the present investigation we compared the extent and anatomical site of the transepithelial intestinal elimination of fleroxacin and sparfloxacin with those of ciprofloxacin in a rat model.

MATERIALS AND METHODS

Antibiotics. Ciprofloxacin hydrochloride (Bayer AG, Leverkusen, Germany), fleroxacin (Hoffmann-La Roche, Basel, Switzerland), and sparfloxacin (Rhone-Poulenc Rorer, Antony, France) were provided by the indicated manufacturers.

Animals and procedures. Male Sprague-Dawley rats weighing ca. 300 g were used. Following an overnight fast, but with free access to water, animals were anesthetized with urethane (1.5 g/kg of body weight administered intraperitoneally) and were placed on a heating pad at 37°C. A catheter was inserted in the carotid artery and was flushed frequently with a diluted heparin solution (500 U/ml) to prevent clotting. A midline abdominal incision was made, and the common bile duct was identified and ligated. An isolated proximal jejunal segment, with its intact blood supply, measuring ca. 3 to 4 cm in length starting 2 cm distal to the ligament of Treitz was created by placing surgical ligatures that secured polyethylene catheters with an internal diameter of 0.8 mm in the proximal and distal ends of the segment. The content of the segment was copiously lavaged with phosphate-buffered saline (PBS) solution (pH 7.4) until the efflux was clear. Similar segments were created in the distal jejunum, ca. 75 cm away from the first segment, and in the distal ileum, ca. 1 cm proximal to the ileocecal valve, and the same procedure was repeated. The catheters from the proximal ends of the three segments were attached to a continuous pump (Minipuls 2, Gilson) and perfused with 0.15 M PBS (pH 7.4) at a temperature of 37°C at a rate of 0.2 ml/min. The segments were returned to the abdominal cavity, and the abdomen was covered with a cotton pad to avoid evaporation and heat loss. An equilibration period of 15 min (the segments were perfused with the same PBS) was used to ascertain that no leaks from the intestinal segments occurred, that the intestinal temperature returned to 37°C, and that the animals tolerated the procedure well. The animals were then infused parenterally with fleroxacin, sparfloxacin, or ciprofloxacin in a single dose of 25 mg/kg of body weight in a volume of 7 ml of saline over a 5-min period. Blood samples from the carotid artery (0.5 ml) were obtained at time zero and at 10-min intervals thereafter, and effluxes from the three intestinal segments were collected at 15-min intervals for 90 min. The blood was allowed to clot for 2 h, and the serum was separated and frozen at −80°C until it was assayed. Effluxes were weighed, frozen at −80°C, and lyophilized.

At the end of each experiment the animals were killed with pentothal. The intestinal segments were excised, and their mucosal surface areas were calculated by spreading the wet excised segment semistretched on a glass plate (serosal surface down) and measuring the length and width of the excised segment. For each antibiotic 10 animals were used. To determine the extent of nonspecific paracellular transport, animals were prepared as described above, four rats were injected parenterally with 15 μCi of [^{3}H]inulin (Amersham International plc, Buckinghamshire, United Kingdom) and their intestinal effluxes were collected over a 90-min period for the determination of radioactivity in a 1209 Rackbeta counter (LKB Uppsala, Sweden). In an additional group of four rats 15 μCi of [^{3}H]inulin was introduced into each of the three intestinal segments and blood was collected at 10-min intervals for 90 min to determine the radioactivity in serum.

For antibiotic assay the high-pressure liquid chromatography method was used. Before the assay all intestinal effluxes were reconstituted with 1 ml of a methanol-phosphoric acid (10^{-1} M) mixture (50/50; vol/vol) containing ofloxacin as an internal standard. Following centrifugation at 3,000 rpm for 10 min with a Sigma 3K-2 centrifuge, 20 μl of the supernatant was injected into the chromatograph. Separation of the fluoroquinolones was performed by ion-paired liquid chromatography at pH 3.0 with heptane sulfonate as a counterion. A Novapack C18 column (3.9 by 150 mm; Millipore) was used. The mobile phase was a mixture of two solvents: solvent A (acetonitrile or methanol) and solvent B (10^{-2} M potassium phosphate buffer, 2.5×10^{-2} M heptane sulfonate [PIC B7], 2×10^{-2} M triethylamine). The mixtures A (methanol) plus B at 25-75 (vol/vol) A + B at 35-65; (vol/vol), and A (acetronitrile) plus B at 16-84 (vol/vol) were used for the determination of ciprofloxacin, sparfloxacin, and fleroxacin concentrations, respectively. The flow rate was fixed at 1.5 ml/min, and detection was performed

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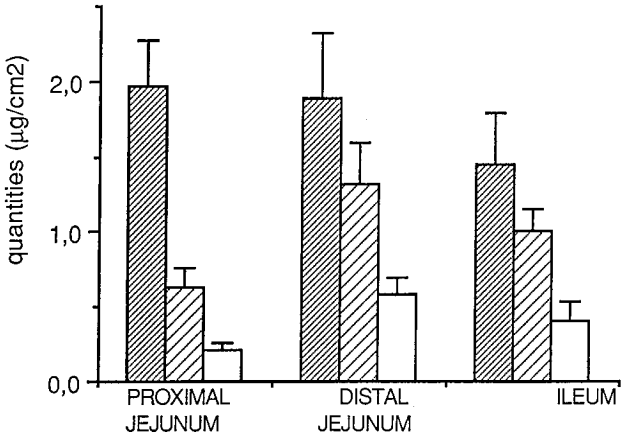

FIG. 1. Amounts of ciprofloxacin (▨), fleroxacin (▨), and sparfloxacin (□) eliminated in the proximal jejunum, distal jejunum, and ileum.

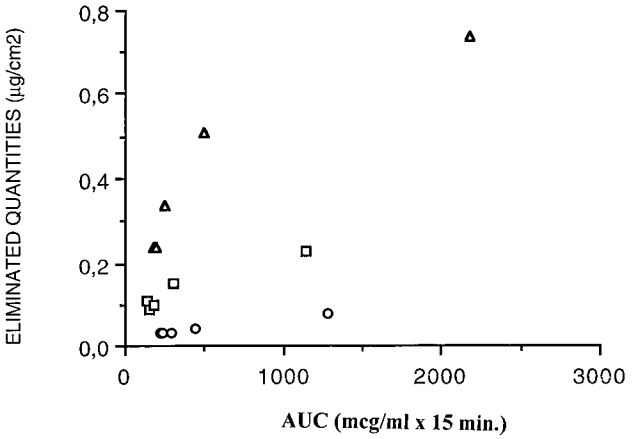

FIG. 2. Eliminated quantities of ciprofloxacin (△), fleroxacin (□), and sparfloxacin (○) in the proximal jejunum in relation to serum AUC.

by spectrofluorometry for ciprofloxacin (excitation of 330 nm and emission of 440 nm for ciprofloxacin and excitation of 290 nm and emission of 430 nm for fleroxacin) or by UV spectrophotometry (364 nm) for sparfloxacin. For determination of ciprofloxacin concentrations in serum, serum samples were deproteinized with methanol containing ofloxacin as an internal standard. To increase the sensitivity of the method, serum samples containing sparfloxacin and fleroxacin were extracted by dichloromethane at pH 7.5. Over the studied concentration range the methods were reproducible (intrarun and interrun coefficients of variation were <8%, and the interday variation was <1.7%). The limits of sensitivity were 10 µg/liter for ciprofloxacin and fleroxacin and 100 µg/liter for sparfloxacin.

Antibiotic concentrations in sera and intestinal effluxes were plotted, and the areas under the concentration-time curves (AUCs) calculated over the 0- to 90-min time period by the trapezoidal method.

For statistical analysis the one-factor analysis of variance was used.

RESULTS

No radioactivity related to [^{3}H]inulin could be detected in any of the serum samples of the animals into which [^{3}H]inulin was administered into the various intestinal segments. Similarly, in none of the animals that received parenteral [^{3}H]inulin could radioactivity be detected in any of the intestinal effluxes.

The concentrations of ciprofloxacin, sparfloxacin, and fleroxacin in serum were similar. At 10 min, the mean serum ciprofloxacin concentrations were 66.5 ± 9.7 µg/ml, and those of fleroxacin and sparfloxacin were 42.7 ± 11.0 and 53.8 ± 13.7 µg/ml, respectively.

In the proximal jejunal segment 17.0 ± 5.1 µg of ciprofloxacin was recovered during the 90 min following antibiotic administration; for sparfloxacin and fleroxacin the quantities recovered were 1.4 ± 0.6 and 5.1 ± 1.8 µg, respectively. In the distal jejunal segment the recovered quantities were 21.1 ± 11.2, 6.7 ± 3.1, and 14.4 ± 6.5 µg, respectively. In the ileal segment, the recovered quantities were 16.2 ± 8.6, 4.4 ± 2.9, and 11.0 ± 3.4 µg for ciprofloxacin, sparfloxacin, and fleroxacin, respectively.

In relation to the mucosal surface area of the segment, during the 90 min following administration the elimination of ciprofloxacin was 1.97 ± 0.70 µg/cm^2; those of sparfloxacin and fleroxacin were 0.21 ± 0.1 0.64 ± 0.26 µg/cm^2, respectively. In the distal jejunal segment the respective values were 1.88 ± 0.99, 0.58 ± 0.26, and 1.31 ± 0.63 µg/cm^2, and in the ileal segment the values were 1.44 ± 0.77, 0.41 ± 0.26, and 1.00 ± 0.33 µg/cm^2 for ciprofloxacin, sparfloxacin, and fleroxacin, respectively (Fig. 1).

While the elimination of ciprofloxacin (expressed in micrograms per square centimeter per minute) was not significantly different in the three intestinal segments, sparfloxacin and fleroxacin were both eliminated significantly faster in the distal jejunum and ileum than in the proximal jejunum ($P < 0.05$ according to the Scheffe F test in a one-factor analysis of variance for both compounds).

By using individual data, no linear relationship between the serum AUC and intestinal elimination (expressed in micrograms per square centimeter) of cirprofloxacin, sparfloxacin, and fleroxacin could be demonstrated in any of the segments. A tendency for a saturable elimination process (expressed in micrograms per square centimeter), however, was observed with rising serum AUCs measured over 15-min periods (Fig. 2 to 4).

When elimination in all three segments was added, the elimination rates of the three fluoroquinolones were significantly different from each other: ciprofloxacin > fleroxacin > sparfloxacin ($P < 0.05$).

The mean intestinal ciprofloxacin concentration ranged from 0.94 to 1.64 µg/ml in jejunal effluxes and from 1.26 to 1.72 µg/ml in the ileum. The respective mean concentrations of sparfloxacin were 0.084 ± 0.032, 0.87 ± 0.28, and 0.54 ± 0.14 µg/ml for the proximal, distal, and ileal effluxes. For fleroxacin the concentrations were 0.40 ± 0.21, 1.16 ± 1.10, and 0.84 ± 0.20 µg/ml for the three segments, respectively.

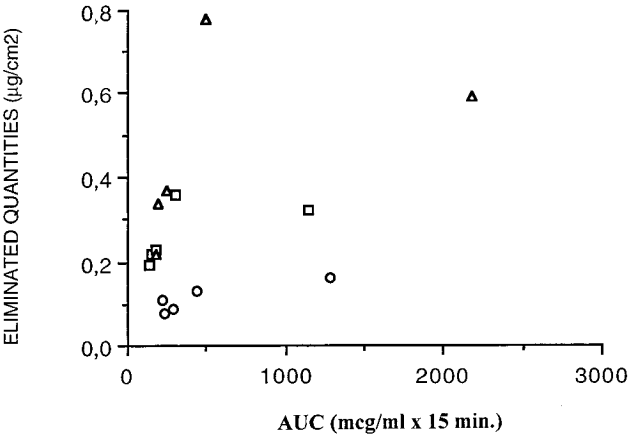

FIG. 3. Eliminated quantities of ciprofloxacin (△), fleroxacin (□), and sparfloxacin (○) in the distal jejunum in relation to serum AUC.

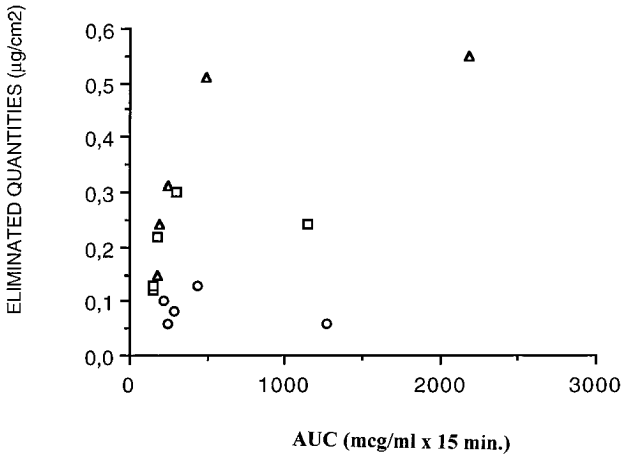

FIG. 4. Eliminated quantities of ciprofloxacin (△), fleroxacin (□), and sparfloxacin (○) in the ileum in relation to serum AUC.

DISCUSSION

In the present investigation we found that the three fluoroquinolones, ciprofloxacin, sparfloxacin, and fleroxacin, are eliminated in the small intestine of the rat.

Considering that the length of the jejunum in the rat is 90 to 135 cm and that the average diameter is 3 to 5 mm (5), the quantity of ciprofloxacin eliminated in the jejunum during the first 90 min following a single parenteral administration of 25 mg/kg would range from 270 to 390 μg and in the ileum (measuring 25 to 35 mm) an additional 67 μg would be eliminated yielding a fraction of 5 to 6% of the administered dose. For sparfloxacin the fraction would be 1.25 to 1.5%, and for fleroxacin it would be 3 to 4.5% of the parenteral dose. This is evidently a minimal estimate since the dose that was reabsorbed from the small intestine during the study period was not measured; thus, these estimates represent the net clearance of these antibiotics in the small intestine. Since the elimination of most fluoroquinolones in the bile is only minimal, amounting to 1 to 3% of the administered dose (4, 12–14), and since no reduction in the dose of fluoroquinolones has to be made until renal failure is far progressed, without evidence of drug accumulation it can be assumed that under these circumstances transepithelial intestinal elimination may serve as an alternative route for the clearance of some fluoroquinolones.

The difference in the rate of intestinal elimination of the three fluoroquinolones cannot be explained by the differences in the molecular weights of the three antibiotics or differences in the degree of their protein binding.

The pH of the intestinal perfusate was 7.4 and was aimed to be similar to that of blood and of the epithelial lining fluid which bathes the epithelial surfaces in order to facilitate possible diffusion. According to the diffusion theory the limiting factors for passage through the membrane are tonicity, the ionization state of the compound, and the degree of its lipophilicity (hydrophobicity). According to Furet et al. (3), at pH 7.4 the fractions of ciprofloxacin, sparfloxacin, and fleroxacin that are in a zwitterionic form are 95.6, 87.7, and 82.4%, respectively. The partition coefficients of the three fluoroquinolones in octanol-phosphate buffer (pH 7.4) are 0.2 for ciprofloxacin and fleroxacin and 1.2 for sparfloxacin. Thus, the same dose of the three fluoroquinolones would have theoretically produced the following rank of intestinal elimination: sparfloxacin > ciprofloxacin > fleroxacin. Our results, however, showed an inverse order of elimination, namely, ciprofloxacin > fleroxacin > sparfloxacin, suggesting that the diffusion process was not the sole mechanism responsible for elimination. In addition, the lack of the influence of the serum antibiotic concentrations measured as serum AUC over the first 90 min on the intestinal elimination of the three antibiotics favors the existence of a saturable active transport process.

The possibility of a paracellular diffusion of these antibiotics was also excluded by the fact that inulin, which uses the paracellular space for transport, was not recovered in the intestinal lumen following systemic administration.

Mean ciprofloxacin and fleroxacin concentrations in the small intestinal effluxes in the first 90 min ranged from 0.94 to 1.64 and 0.40 to 1.16 μg/ml, respectively. For sparfloxacin the concentrations were 0.084 to 0.87 μg/ml. These concentrations would be expected to inhibit the susceptible pathogens that cause bacterial diarrhea if there were no antibiotic inactivation by the intestinal contents or other unfavorable conditions.

We have previously shown that in rats neither reabsorption nor excretion of ciprofloxacin occurs distal to the ileocecal valve (10); thus, the antibiotic fraction reaching the large intestine would undergo a concentration process because of the reabsorption of water, producing very high fecal drug concentrations, as indeed have been measured in the feces of animals and humans.

In summary, we have demonstrated that ciprofloxacin, sparfloxacin, and fleroxacin are eliminated in the small intestines of rats through a transepithelial transport process which is not based entirely on diffusion. The impacts of the different elimination patterns of the three fluoroquinolones on their pharmacokinetics and their therapeutic effects on the intestinal tract deserve further study.

REFERENCES

1. **Beermann, D., H. Scholl, W. Wingender, D. Forster, E. Beutler, and W. R. Kukovetz.** 1986. Metabolism of ciprofloxacin in man, p. 141–149. *In* H. C. Neu and H. Weuta (ed.), Proceedings of the 1st International Ciprofloxacin Workshop. Excrepta Medica, Amsterdam.
2. **Brumfitt, W., I. Franklin, D. Gradey, J. M. T. Hamilton-Miller, and A. Eliffe.** 1984. Changes in the pharmacokinetics of ciprofloxacin and fecal flora during administration of a 7-day course to human volunteers. Antimicrob. Agents Chemother. **26:**757–761.
3. **Furet, Y. X., J. Deshusses, and J. C. Pechere.** 1992. Transport of pefloxacin across bacterial cytoplasmaic membrane in quinolone-susceptible *Staphylococcus aureus*. Antimicrob. Agents Chemother. **36:**2506–2511.
4. **Hayton, W., V. Vlahov, N. Bacrasheva, I. Viachki, R. Portman, G. Muirhead, K. Stoeckel, and E. Weidekamm.** 1990. Pharmacokinetics and biliary concentrations of fleroxacin in cholecystomized patients. Antimicrob. Agents Chemother. **34:**2375–2380.
5. **Hebel, R., and M. V. Stromberg.** 1986. Anatomy and embryology of the laboratory rat, p. 46–52. Biomed Verlag, Woerthsee, Germany.
6. **Kinzig, M., R. Seelmann, G. Mahr, F. Sorgel, K. G. Naber, E. Weidekamm, and K. Stockel.** 1991. Significant gastrointestinal secretion of fleroxacin in man, abstr. 388. *In* 17th International Congress on Chemotherapy. Futuramed Publishers, Munich.
7. **Mahr, G., F. Sörgel, K. G. Naber, P. Muth, M. Kinzig, and D. Weigel.** 1991. Principles of gastrointestinal secretion of quinolones in humans, abstr. 585, p. 196. *In* Program and abstracts of the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
8. **Montay, G., R. Bruno, J. J. Thebault, J. C. Vergniol, D. Chassard, M. Ebmeier, and J. Guillot.** 1990. Dose-dependent pharmacokinetic study of sparfloxacin (SPX) in healthy young volunteers, abstr. 1248, p. 294. *In* Program and abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
9. **Ramon, J., S. Dautery, R. Farinoti, C. Carbon, and E. Rubinstein.** 1994. Intestinal elimination of ciprofloxacin in rabbits. Antimicrob. Agents Chemother. **38:**757–780.
10. **Rubinstein, E., L. St. Julien, J. Ramon, S. Dautrey, R. Farinotti, J.-F. Huneau, and C. Carbon.** 1994. The intestinal elimination of ciprofloxacin in the rat. J. Infect. Dis. **169:**218–221.
11. **Segeti, J., L. J. Goodman, R. M. Petrak, R. L. Kaplan, G. W. Parkhurst, and**

G. M. Trenholme. 1988. Serum and faecal levels of ciprofloxacin and trimethoprim-sulfamethoxazole in adults with diarrhea. Rev. Infect. Dis. **10**(Suppl. 1)**:**S206–S207.
12. **Sorgel, F., K. G. Naber, U. Jaehde, A. Reiter, R. Seelmann, and G. Sigl.** 1989. Gastrointestinal secretion of ciprofloxacin. Evaluation of the charcoal model for investigation in healthy volunteers. Am. J. Med. **87**(Suppl. 5A)**:**S62–S65.
13. **Sorgel, F., K. G. Naber, M. Kintzig, G. Mahr, and P. Muth.** 1991. Comparative pharmacokinetics of ciprofloxacin and temafloxacin in humans: a review. Am. J. Med. **91**(Suppl. 6A)**:**S51–S66.
14. **Wittke, R. R., W. Fabian, and C. Leperlier.** 1992. Biliary concentrations of sparfloxacin and its glucoronide in man, abstr. 96. *In* 4th International Symposium on New Quinolones. Futuramed Publishers, Munich.
15. **Wolfson, J. S., and D. C. Hooper.** 1991. Pharmacokinetics of quinolones: newer aspects. Eur. J. Clin. Microbiol. Infect. Dis. **10**(Special Issue)**:**47–54.