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Intestinal Elimination of Ofloxacin Enantiomers in the Rat: Evidence of a Carrier-Mediated Process

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The aim of this work was to examine the mechanism involved in intestinal elimination of the two optical isomers of ofloxacin in the rat. An intestinal segment was isolated in situ and perfused with saline, while drug solution was administered via the carotid artery. Blood samples and intestinal effluents were collected and analyzed by a high-performance liquid chromatography method. We observed saturable and stereoselective intestinal elimination of the ofloxacin enantiomers. The elimination process favored the *R*-(+) form of the molecule. After a parenteral dose of 20 mg of racemic ofloxacin per kg of body weight, intestinal clearances were 0.23 ± 0.03 versus 0.30 ± 0.03 ml/min for *S*-(−)- and *R*-(+)-ofloxacin, respectively. Ciprofloxacin and pefloxacin interfered with ofloxacin elimination and significantly reduced the intestinal clearance of *S*-(−)- and *R*-(+)-ofloxacin. With concomitant ciprofloxacin, intestinal clearances became 0.13 ± 0.02 versus 0.17 ± 0.03 ml/min and 0.14 ± 0.01 versus 0.19 ± 0.05 ml/min with pefloxacin for *S*-(−)- and *R*-(+)-ofloxacin, respectively. Those findings argue for the presence of a common transport system in the rat intestine with variable affinities for fluoroquinolones. In addition, verapamil and quinidine, two P-glycoprotein blockers, significantly reduced the intestinal elimination of both ofloxacin isomers (with concomitant verapamil, intestinal clearances were 0.12 ± 0.02 versus 0.18 ± 0.03 ml/min for *S*-(−)- and *R*-(+)-ofloxacin, respectively, while with concomitant quinidine, values were 0.18 ± 0.01 versus 0.23 ± 0.01 ml/min without modifying their areas under the concentration-time curve in serum. Similar results were found with another fluoroquinolone, ciprofloxacin, in previous work. P-glycoprotein appears to be involved in the intestinal elimination of fluoroquinolones in rats. The characterization of fluoroquinolone intestinal elimination has significant clinical relevance for the better evaluation of the influence of this secretory pathway on antibiotic efficacy and selection of resistant bacteria within the intestinal flora.

In recent years, there has been considerable interest in the development and clinical use of new fluoroquinolone agents (6, 18). Relative to earlier compounds (nalidixic acid and oxolinic acid), the newer agents are more potent, have broader spectra of activity in vitro, and are less prone to selection of resistant strains (19). From a pharmacokinetic point of view, fluoroquinolones can be classified by their predominant clearance pathway, which is renal (ofloxacin, temafloxacin, and lomefloxacin), hepatic (pefloxacin), or both (norfloxacin, ciprofloxacin, enoxacin, fleroxacin, etc.). However, the gastrointestinal tract plays a significant role in overall elimination of fluoroquinolones (35, 36).

Fluoroquinolone elimination by the human gut can be studied with the charcoal model, which prevents gastrointestinal reabsorption of drug eliminated via the transintestinal route. This model provided evidence that nonrenal clearance of fluoroquinolones increases in the presence of charcoal (34).

The mechanisms of intestinal fluoroquinolone clearance are unclear. Griffith et al. (13) demonstrated that ciprofloxacin was secreted by Caco-2 cell layers and that basal-to-apical flux was mediated by an active transport system. Verapamil and other fluoroquinolones reduced ciprofloxacin secretion to various degrees (13). Vergin et al. (38) observed passive elimination of lomefloxacin by the rat intestine, but this was based only on the lack of effect of probenecid (a ligand of the renal dianionic transporter) on lomefloxacin elimination. Huneau et al. (20) observed a net secretory flux of sparfloxacin in the rabbit ileum.

The aim of this study was to elucidate the intestinal elimination of fluoroquinolones by using a simple in vivo model of perfused rat intestine in which intestinal elimination can be measured directly. The model does not require extensive modeling, contrary to the charcoal model applied to humans. The use of an open continuous perfusion system with collection of bile and intestinal efflux provides direct quantification of biliary and intestinal elimination.

Ofloxacin, the compound chosen for this study, has a methyl group at the C-3 position of the pyridobenzoxazine group, resulting in an asymmetric carbon and two optical isomers. Our purpose was to examine the transintestinal elimination of both enantiomers in the rat. Our animal model was adapted from that of Shanker et al. (31). It allows direct quantification of transintestinal elimination and, contrary to in vitro models, has the advantage of presenting intact intestinal vascularization and peristaltism as well as a complete and intact intestinal membrane. This work has significant clinical relevance for the better evaluation of the influence of the fluoroquinolone intestinal secretory pathway on antibiotic efficacy and selection of resistant bacteria within the intestinal flora.

We first examined the stereoselectivity of ofloxacin elimination, its saturability with increasing doses, and competition with other fluoroquinolones. We then investigated the possible involvement of P-glycoprotein in the intestinal elimination of

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ofloxacin enantiomers. Three substrates of this ATP pump (7) were used in vivo: verapamil, quinidine, and cefoperazone. Concentrations of *S*-(−)- and *R*-(+)-ofloxacin in serum and intestinal effluents were measured separately with a sensitive and stereoselective high-performance liquid chromatography (HPLC) assay.

MATERIALS AND METHODS

Drugs and chemicals. Ofloxacin was supplied by Diamant, Puteaux, France; ciprofloxacin was supplied by Bayer, Puteaux, France; and pefloxacin was supplied by Roger Bellon, Neuilly s/Seine, France. Verapamil, quinidine, and cefoperazone were from Sigma, St Quentin Fallavier, France. The reagents for HPLC analysis were of analytical grade and were as follows: potassium dihydrogen phosphate (Prolabo, Paris, France), dichloromethane (Merck, Nogent s/Marne, France), and *N-N*-dimethyloctylamine (Sigma).

Perfusion technique. For perfusion, we used a technique described in reference 31. We used male Sprague-Dawley rats weighing 240 to 300 g. After an overnight fast with free access to water, animals were anesthetized with urethane (1.5 g/kg of body weight intraperitoneally) and were placed on a heating pad at 37°C. A catheter was inserted into the carotid artery and was flushed frequently with a dilute heparin solution (500 IU/ml) to prevent clotting. A midline abdominal incision was made, and the common bile duct was ligated.

A 30-cm intestinal segment starting 2 cm distal to the pylorus was isolated in situ with its blood supply intact. A polyethylene catheter with an internal diameter of 0.8 mm was placed in the proximal end of the segment to deliver the perfusate at a rate of 0.6 ml/min (Minipuls 2; Gilson), determined as optimal by Savina et al. (29). A polyethylene cannula with an internal diameter of 2.15 mm was fixed to the distal end to collect effluents. In some experiments, a second adjacent segment of distal jejunum (30 cm also) was isolated in the same way. The lumen of the segment was washed with saline at 37°C until the efflux was clear. 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, during which the segments were continuously perfused with saline, was used to rule out leakage from the intestinal segments, to allow the intestinal temperature to return to 37°C, and to determine if the animals tolerated the procedure well. The animals were then injected rapidly (1 min) with racemic ofloxacin at various doses (20, 40, 80, and 100 mg/kg) diluted in 1 ml of saline. In competition experiments, pefloxacin, ciprofloxacin, or P-glycoprotein substrates were administered via the carotid artery 5 min before ofloxacin injection. Blood samples from the carotid artery (0.35 ml) were obtained at 5, 15, 30, 45, and 60 min. Intestinal effluxes were collected at 15-min intervals for 60 min. Volumes were measured, and aliquots were stored at −20°C. Blood samples were immediately centrifuged, and serum was kept at −20°C until assayed.

At the end of each experiment, the animals were killed with urethane. The intestinal segments were excised and measured. Six animals were used for each type of experiment.

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]polyethylene glycol (Amersham International plc, Buckinghamshire, United Kingdom), and intestinal effluxes were collected over a 60-min period to determine radioactivity in a 1209 Rackbeta counter (LKB, Uppsala, Sweden).

Analytical assay. Ofloxacin enantiomers were separated on a chiral stationary phase consisting of a bovine serum albumin column (Macherey & Nagel, Düren, Germany). The mobile phase consisted of 0.2 M potassium dihydrogen phosphate and 5 mM *N-N*-dimethyloctylamine. The final pH was adjusted to 8 with KOH. The flow rate was 1 ml/min, and detection was performed by spectrofluorimetry (excitation wavelength, 298 nm; emission, 458 nm). Intestinal effluxes were centrifuged before analysis, and serum was extracted with dichloromethane (22). Bile (100 μl) was mixed with phosphate buffer (pH 7 [1:10]), and 500 μl of methanol was then added. The mixture was shaken for 1 min and centrifuged at 1,000 × *g* for 5 min. The supernatant was used for HPLC.

Retention times were 6 min for *S*-(−)-ofloxacin and 7.8 min for *R*-(+)-ofloxacin. The detection limit was 0.1 μg/ml for each enantiomer. The linearity and reproducibility of the method were checked, and coefficients of variation ranged from 1.5 to 5%.

Pharmacokinetic and statistical analysis. Pharmacokinetic calculations were based on an open two-compartment model (24). The areas under the concentration-time curves (AUCs [micrograms · minute per milliliter]) were calculated from 0 to 60 min by the trapezoidal rule (Siphar program; SIMED, Creteil, France).

Student's paired *t* test was used to assess the statistical significance of the differences in pharmacokinetic parameters between the enantiomers. To compare different animal groups, an analysis of variance was used. Intestinal elimination is expressed as the amount (*A* [micrograms]), of ofloxacin enantiomer eliminated in 60 min by the intestinal segment, and also as follows: intestinal clearance (CL_i [milliliter per minute]) = *A* (0 to 60 min)/AUC in serum (serum AUC) (0 to 60 min) of the enantiomer.

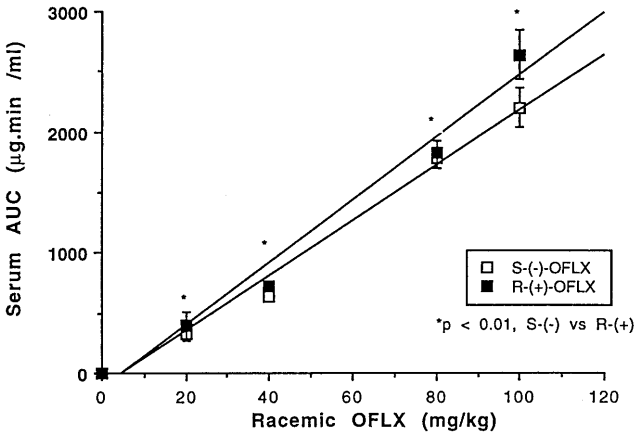

FIG. 1. Relationship between serum AUCs (mean ± standard deviation) of *S*-(−) and *R*-(+) isomers and parenteral dose of racemic ofloxacin (OFLX) administered [$r = 0.993$, $P < 0.001$ for *S*-(−)-ofloxacin and $r = 0.989$, $P < 0.001$ for *R*-(+)-ofloxacin].

RESULTS

No radioactivity was detected in any of the intestinal effluxes (perfusion medium, 0.9% NaCl) of the animals receiving [^{3}H]polyethylene glycol.

Serum AUCs of *S*-(−)- and *R*-(+)-ofloxacin increased linearly over the concentration range tested (from 20 to 100 mg of racemic ofloxacin per kg) (Fig. 1). The *R*-(+)-ofloxacin AUC was significantly larger than that of *S*-(−)-ofloxacin at all doses ($P < 0.001$).

Ofloxacin biliary elimination was stereoselective. With the parenterally administered dose of 20 mg of racemic ofloxacin per kg [10-mg/kg *S*-(−)-ofloxacin plus 10-mg/kg *R*-(+)-ofloxacin], 3.92% ± 0.59% and 1.56% ± 0.17% of *S*-(−)- and *R*-(+)-ofloxacin, respectively, were eliminated unchanged in bile over 60 min. Enantiomer bile excretion increased linearly with the dose (Fig. 2).

At 20 mg/kg, ofloxacin intestinal elimination was maximum in the segment isolated from the upper gut (duodenum-proximal jejunum) (189 ± 27 versus 139 ± 25 μg in the distal segment).

Our results and those of Rubinstein et al. (27) confirm that

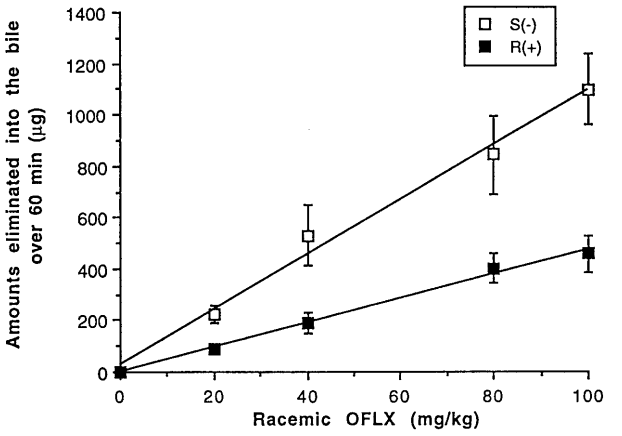

FIG. 2. Amounts (mean ± standard deviation) of *S*-(−)- and *R*-(+)-ofloxacin (OFLX) eliminated into the bile over 60 min after different parenteral doses of racemic ofloxacin [$r = 0.934$, $P < 0.01$ for *S*-(−)-ofloxacin and $r = 0.951$, $P < 0.01$ for *R*-(+)-ofloxacin].

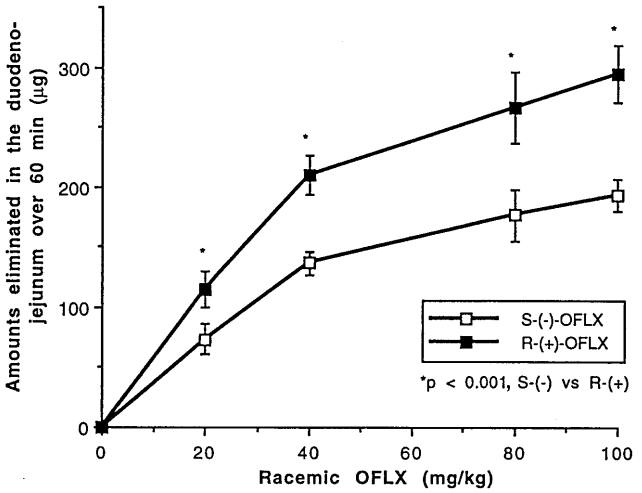

FIG. 3. Amounts (mean ± standard deviation) of *S*-(−)-ofloxacin (OFLX) and *R*-(+)-ofloxacin eliminated in the duodeno-jejunal segment (30 ± 3 cm) after different parenteral doses of racemic ofloxacin.

intestinal elimination of fluoroquinolones is more important in the upper portion of the rat intestine. For this reason, the following experiments were performed with the duodenum-proximal jejunum.

The amounts of enantiomers excreted by the intestinal segment were significantly different. Elimination of *R*-(+)-ofloxacin was predominant in the effluxes at all doses, and a saturable elimination process was observed when the dose was increased (Fig. 3). The CL_i of each enantiomer was not constant over the 60-min period and was reduced at doses superior to 40 mg of racemic ofloxacin per kg (Fig. 4). At all doses investigated, *R*-(+)-ofloxacin CL_i was higher than that of *S*-(−)-ofloxacin ($P < 0.0001$).

Parenteral administration of pefloxacin (80 mg/kg) and ciprofloxacin (80 mg/kg) was associated with an increase in biliary excretion of unchanged ofloxacin enantiomers [pefloxacin, 5.45% ± 0.54% ($P < 0.005$) and 2.23% ± 0.31% ($P < 0.001$) for *S*-(−)-ofloxacin and *R*-(+)-ofloxacin, respectively]. With ciprofloxacin, the corresponding values were 5.12% ± 0.36% ($P < 0.005$) and 2.16% ± 0.36% ($P < 0.005$).

Ciprofloxacin and pefloxacin significantly reduced the CL_i of both ofloxacin enantiomers, with no significant change in serum AUCs (Table 1). The extents of inhibition were 43% for each isomer with ciprofloxacin and 39% for the *S*-(−) isomer and 41% for the *R*-(+) isomer with pefloxacin. P-glycoprotein inhibitors (verapamil, quinidine, and cefoperazone) did not modify the enantiomer serum AUCs. However, *S*-(−)- and *R*-(+)-ofloxacin CL_i was clearly reduced by verapamil and quinidine. The extents of inhibition were 48 and 40% for the *S*-(−) and *R*-(+) enantiomers, respectively, with verapamil and 22 and 23%, respectively, with quinidine. Conversely, cefoperazone (12 mg/kg) failed to modify the CL_i of ofloxacin isomers.

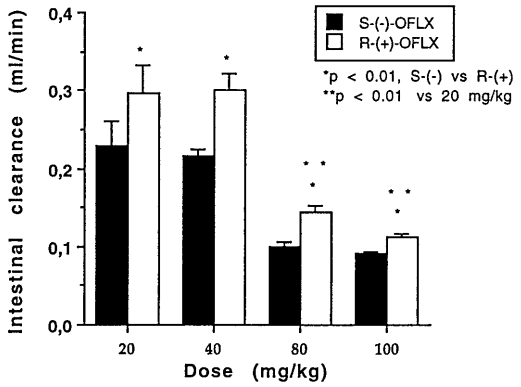

FIG. 4. CL_i (mean ± standard deviation) of ofloxacin (OFLX) enantiomers over 60 min after four parenteral doses of racemic ofloxacin.

DISCUSSION

Ofloxacin is a chiral fluoroquinolone, and its spatial orientation affects antibacterial activity as well as pharmacokinetic properties in rats and humans (9, 25, 26). After oral administration to rats, Okazaki et al. reported a marked difference in the biliary excretion of the two enantiomers (26). Stereoselective ester glucuronidation occurs: *S*-(−)-ofloxacin is preferentially metabolized to the glucuronide ester, which is rapidly excreted into the bile. This stereoselective glucuronidation may account for the different AUC values of the two enantiomers [*R*-(+)-ofloxacin AUC > *S*-(−)-ofloxacin AUC]. Our results support those of Okazaki et al., because *S*-(−)-ofloxacin was more strongly secreted into the rat bile: 3.92% ± 0.59% of *S*-(−)-ofloxacin and 1.56% ± 0.17% of *R*-(+)-ofloxacin were recovered in the bile over 60 min (20-mg/kg dose of racemic ofloxacin).

Biliary excretion was linear up to 100 mg/kg and was not influenced by verapamil or quinidine, contrasting with the effect of the latter on CL_i. This suggests that both verapamil and quinidine exert their action solely on the intestinal membrane. Pefloxacin and ciprofloxacin increased the biliary excretion of the unchanged forms of both ofloxacin enantiomers. This could be secondary to an inhibition of the metabolism of both isomers, resulting in a compensatory increase in the unchanged forms. Further studies are necessary to better comprehend this interaction between fluoroquinolones.

Intestinal ofloxacin elimination appeared to be greater in the upper portion of the intestine (duodenum-proximal jejunum), confirming previous reports with other fluoroquinolones (27).

As shown in Fig. 3 and 4, we observed a clear stereoselectivity in the intestinal elimination of ofloxacin.

The more rapid glucuronide conjugation of the *S*-(−) enantiomer could be responsible for the difference in AUC values between the two enantiomers. However, the *R*-(+)-ofloxacin/*S*-(−)-ofloxacin concentration ratio remained lower in the serum than in the intestinal effluxes (1.02 versus 1.41 at 5 min and 1.16 versus 1.70 at 60 min). It can thus be considered that passive diffusion is not the sole mechanism responsible for intestinal secretion of ofloxacin, because enantiomeric ratios would otherwise be identical on the two sides of the intestinal barrier (serum and intestinal lumen). Differences in enantiomer plasma protein binding are probably not responsible for the stereoselectivity in intestinal elimination, because the level of ofloxacin protein binding is relatively low (25% in human plasma) (23) and is not stereoselective in vitro (25).

Enantiomer clearance values over 60 min were significantly different. CL_i of *R*-(+)-ofloxacin was greater than that of *S*-(−)-ofloxacin ($P < 0.001$) (Fig. 4).

Because there is no difference between the two enantiomers in their aqueous or lipid solubility, drug elimination would not be stereoselective if it involved only passive processes. We therefore assume that the intestinal secretion of ofloxacin is carrier mediated, with the two optical isomers having different affinities for receptor sites, like many chiral drugs (1, 39).

TABLE 1. Serum AUCs and CL_is of *S*-(−)- and *R*-(+)-ofloxacin[a]

Drug	*S*-(−)-Ofloxacin		*R*-(+)-Ofloxacin	
	Mean (SD) AUC ($\mu g \cdot min \cdot ml^{-1}$)	Mean (SD) CL_i ($ml \cdot min^{-1}$)	Mean (SD) AUC ($\mu g \cdot min \cdot ml^{-1}$)	Mean (SD) CL_i
Control	323.6 (42.8)	0.23 (0.03)	389.2 (53.6)	0.30 (0.03)
Ciprofloxacin	361.8 (20.1)	0.13 (0.02)[b]	384.1 (20.7)	0.17 (0.03)[b]
Pefloxacin	391.2 (31.1)	0.14 (0.01)[c]	406.6 (24.7)	0.19 (0.05)[b]
Quinidine	313.0 (25.9)	0.18 (0.01)[c]	343.6 (17.3)	0.23 (0.01)[c]
Verapamil	298.0 (16.3)	0.12 (0.02)[b]	345.2 (21.5)	0.18 (0.03)[b]
Cefoperazone	299.7 (29.4)	0.24 (0.03)	357.4 (45.7)	0.31 (0.03)

[a] Results were determined over 60 min after a parenteral dose of 20 mg of racemic ofloxacin per kg preceeded or not by the parenteral administration of one of the drugs mentioned above.
[b] $P < 0.001$ versus control.
[c] $P < 0.05$ versus control.

Enantiomer serum AUC values increased linearly (Fig. 1). In contrast, CL_i was saturable (Fig. 4), with a net decrease in both enantiomers above 40 mg/kg. Saturable intestinal elimination has been described for various fluoroquinolones in vivo and in vitro (13, 14, 27) and supports the existence of a saturable transporter mediating (at least partially) intestinal fluoroquinolone secretion. However, passive diffusion remains the predominant mechanism of intestinal ofloxacin elimination, given the chemicophysical properties of this molecule. The relatively low level of protein binding and the small size of fluoroquinolone molecules in general and ofloxacin in particular are important for good diffusion across membranes (4). Furthermore, the lipophilicity of fluoroquinolones is greatest at neutral pH (when zwitterionic species predominate), suggesting that a blood pH of 7.4 would maximize drug passage through biological membranes (15). Ofloxacin is an amphoteric drug with two protonation sites. Its pK_as are 6.05 for the carboxylic group and 8.22 for the piperazine nitrogen (10). The log P value (octanol/water partition coefficient) is 0.33 (16). At blood pH, 87% of the drug is in the zwitterionic neutral form $HQ^{+/-}$ (10). This form of the molecule is the most hydrophobic and can thus readily diffuse through membrane lipids (15).

Competition seems to occur between fluoroquinolones at the transporter sites. Ciprofloxacin and pefloxacin decreased the intestinal secretion of both ofloxacin enantiomers (Table 1). The extent of inhibition was slightly higher with ciprofloxacin. Serum AUCs remained similar for both enantiomers in the presence of ciprofloxacin or pefloxacin over the 60-min experimental period.

All new fluoroquinolones are significantly eliminated by nonrenal mechanisms. In humans, temafloxacin, sparfloxacin, and fleroxacin show significant fecal elimination (12, 33). In rats, lomefloxacin is significantly eliminated by the intestine, and this is not influenced by probenecid, a substrate of the anionic transporter (8, 38). In rats, 7% of a parenterally administered ciprofloxacin dose is eliminated via the intestine, mostly the jejunum (28). In humans, this fraction is 17.8% (3). Oral administration of activated charcoal, which binds fluoroquinolones, decreases renal secretion and increases the total and nonrenal clearances of ciprofloxacin and other fluoroquinolones in humans, suggesting that the transintestinal route is an important secretory pathway for fluoroquinolones (30, 40).

Intestinal ciprofloxacin secretion has been extensively studied. Uptake and/or release by gastrointestinal cells was assumed to be active (36). Griffith et al. (14) studied the intestinal transport of fluoroquinolones by Caco-2 cell monolayers and observed a net secretory flux with ciprofloxacin, which was inhibited to various extents by other fluoroquinolones. According to the same authors, basal-to-apical flux of norfloxacin and pefloxacin by Caco-2 cells was saturable. Position I of the 4-quinolone nucleus seems to be critical in determining exit permeability at the apical membrane. Ciprofloxacin secretion by Caco-2 cells was also saturable and was abolished when intracellular ATP levels were compromised (13). It was inhibited by verapamil (38%) but not by indomethacin, a drug which blocks an ATP-dependent export mechanism distinct from that of P-glycoprotein and which is probably responsible for the efflux of the anionic fluorochrome bis-carboxyethyl-carboxyfluorescein (BCECF) across the apical membrane of Caco-2 cells (2).

Intestinal fluoroquinolone elimination seems to occur via complex mechanisms combining passive diffusion and active transport. Fluoroquinolones could have a common transporter in the intestine and, according to their affinities, compete with each other for binding when coadministered.

We also investigated the effect of P-glycoprotein blockers on intestinal ofloxacin elimination in rats. P-glycoprotein is an energy-dependent drug-efflux system located at several sites, particularly at the plasma apical membrane of intestinal cells (5, 37). This transport molecule seems to protect intestinal cells from plant alkaloids and other cytotoxic hydrophobic compounds (21). It is also capable of transporting peptides (32). The clinical use of verapamil as a P-glycoprotein blocker is limited because of its cardiovascular activity. Therapeutic doses of quinidine (800 to 2,400 mg/day) result in plasma drug levels within the range of its anti-P-glycoprotein activity (7). Cefoperazone, used at therapeutic doses of 3,000 mg/day, is a less-potent inhibitor (7).

We tested verapamil (10 mg/kg), quinidine (20 mg/kg), and cefoperazone (12 mg/kg). Cefoperazone failed to affect intestinal ofloxacin secretion, probably because of insufficient concentrations in plasma. Verapamil and quinidine reduced *S*-(−)- and *R*-(+)-ofloxacin CL_i (Table 1) without modifying their serum AUCs.

Previous work in our laboratory showed that intestinal elimination of ciprofloxacin was inhibited by verapamil and quinidine: ciprofloxacin CL_i (over 60 min) was reduced by 51% with verapamil and by 67% with quinidine. Verapamil and quinidine levels in serum achieved in our studies altered intestinal fluoroquinolone elimination without modifying serum profiles. This mechanism cannot be explained by a modification of splanchnic blood flow, because verapamil does not modify it (11), and no evidence for such an effect was found with quinidine. This strongly suggests an in vivo action of these two compounds on a P-glycoprotein-mediated intestinal secretion of ofloxacin and ciprofloxacin.

The study demonstrates the stereoselectivity and saturability

of intestinal ofloxacin secretion in vivo, together with the competition between fluoroquinolone antibiotics and the involvement of both a P-glycoprotein-mediated process and passive diffusion. The existence of an additional secretory mechanism is possible, because enoxacin interacts with a cationic transporter in rat intestinal brush-border membranes (17). We are now investigating the involvement of other transport systems present in the rat intestine in intestinal fluoroquinolone transport processes (absorption and secretion).

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