Effect of Cisplatin-Induced Acute Renal Failure on Bioavailability and Intestinal Secretion of Quinolone Antibacterial Drugs in Rats

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Purpose. The aim of this study was to clarify the effects of renal failure on intestinal secretion of quinolone antibacterial drugs.
Methods. Pharmacokinetics of grepafloxacin, levofloxacin, and ciprofloxacin in cisplatin-induced acute renal failure (ARF) rats were evaluated, and intestinal and biliary clearance studies were examined. Transport experiments using culture cells were performed.
Results. The bioavailability of grepafloxacin in ARF rats was 1.2-fold higher than that in normal rats. On the other hand, the bioavailability of ciprofloxacin in ARF rats was markedly decreased to half of that in normal rats, and that of levofloxacin was not changed. Intestinal clearance of grepafloxacin in ARF rats was 75% of that in normal rats, whereas that of ciprofloxacin was 1.4-fold higher than in normal rats, and that of levofloxacin was comparable between normal and ARF rats. Transport experiments using P-glycoprotein-expressing LLC-GA5-COL150 cells and human intestinal Caco-2 cells suggested that grepafloxacin and levofloxacin were substrates of P-glycoprotein and that ciprofloxacin was not, and that intestinal secretion of ciprofloxacin was mediated by a specific transport system distinct from organic cation and anion transporters and multidrug resistance-associated protein 2.
Conclusions. Cisplatin-induced ARF differentially modulated the bioavailability and intestinal secretion of quinolones in rats.

KEY WORDS: quinolones; intestinal secretion; acute renal failure; transport; P-glycoprotein.

INTRODUCTION

Quinolone antibacterial drugs have been frequently used to treat various bacterial infections because of their broad spectrum of activity against gram-positive and gram-negative bacteria (1). The bioavailabilities of quinolones are relatively high, and those of grepafloxacin, levofloxacin, and ciprofloxacin in humans are reported as 72%, approximately 100%, and 50–80%, respectively (2–4). Although quinolones are mainly eliminated by hepatic metabolism and/or urinary excretion, some studies suggest the involvement of intestinal secretion in the mechanisms of their elimination (5,6). There have been some reports that intestinal secretion of quinolones is mediated by multiple active secretory mechanisms. In human intestinal epithelial Caco-2 cells, we reported that intestinal secretion of grepafloxacin and levofloxacin was mediated by P-glycoprotein and another transporter distinct from organic cation and anion transporters and multidrug resistance-associated protein (MRP) (7). Naruhashi *et al.* (8) demonstrated the transport of grepafloxacin via P-glycoprotein and anion-sensitive transport system(s) in Caco-2 cells. On the other hand, secretory transport of ciprofloxacin in Caco-2 cells was reported to be mediated by another transport system distinct from P-glycoprotein (9,10). In rats, Dautrey *et al.* (11) suggested that the pharmacokinetics of ciprofloxacin involved one or more active secretory mechanisms different from P-glycoprotein in the intestine. Thus, the mechanisms underlying the intestinal secretion might be different among quinolones.

Renal failure is known to modulate the pharmacokinetics of various drugs. The alteration of intestinal handling of drugs is one of the reasons for renal failure-related modification of pharmacokinetics of some drugs (12). The bioavailabilities of propranolol and tacrolimus were reported to be increased in experimental acute renal failure (ARF) rats (13,14). On the other hand, Tilstone and Fine (15) showed that the absorption of furosemide decreased in renal failure. Rohwedder *et al.* (16) reported that elimination of ciprofloxacin into feces was increased in renal failure patients. Moreover, Dautrey *et al.* (17) demonstrated that intestinal clearance of ciprofloxacin was increased in nephrectomized rats. However, little information is available about the effects of renal failure on intestinal secretion of other quinolones, which are mediated by different transport mechanisms.

In the present study, we examined the effects of cisplatin-induced ARF on bioavailability and intestinal secretion of grepafloxacin, levofloxacin, and ciprofloxacin in rats. We found that intestinal secretion of grepafloxacin was decreased, that of ciprofloxacin was increased, and that of levofloxacin was not changed in ARF rats. Therefore, intestinal secretion of these drugs appeared to be regulated differentially by cisplatin-induced ARF according to the mediated transporters.

MATERIALS AND METHODS

Materials

[^{14}C]Grepafloxacin (1.17 GBq/mmol) and grepafloxacin were kindly supplied by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). [^{14}C]Levofloxacin (1.07 GBq/mmol) and levofloxacin were gifts from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan), and [^{14}C]ciprofloxacin hydrochloride (2.55 GBq/mmol) and ciprofloxacin hydrochloride were from Bayer AG (Leverkusen, Germany). [^{3}H]Digoxin (629 GBq/mmol) was purchased from NEN™ Life Science Products, Inc. (Boston, MA). Cisplatin (Randa® injection, 0.5 mg/ml) was obtained from Nippon Kayaku Co., Ltd. (Tokyo, Japan), and cyclosporin A was from Novartis Pharma KK (Tokyo, Japan). Monoclonal antibody C219 was from CIS Bio International (Gif-sur-Yvette, France). All other chemicals used were of the highest purity available.

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ABBREVIATIONS: ARF, acute renal failure; AUC, area under the plasma concentration–time curve; BUN, blood urea nitrogen; C_{max}, peak plasma concentration; CL_{NR}, nonrenal clearance; CL_R, renal clearance; CL_T, total body clearance; F, bioavailability; Q, intercompartmental clearance; T_{max}, time to peak plasma concentration; V_1, central volume of distribution; V_{ss}, volume of distribution at steady state; MRP, multidrug resistance-associated protein.

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Animals

Male Wistar rats weighing 200–250 g were used. Acute renal failure was induced by intraperitoneal administration of 5 mg/kg of cisplatin 3 days before the experiments. Preceding the experiments, animals were fasted overnight but given free access to water. Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Supplemental doses of pentobarbital were administered as required. Body temperature was maintained with appropriate heating lamps. The animal experiments were performed in accordance with the *Guidelines for Animal Experiments of Kyoto University*. The experimental protocol was approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University (Med Kyo 01200).

Pharmacokinetic Studies in Rats

The femoral artery and vein were cannulated with polyethylene tubing (PE-50, BD Biosciences, San Jose, CA) filled with heparin solution (100 U/ml) for blood sampling and drug administration, respectively. The bladder was also cannulated with PE-50 tubing for urine collection. Grepafloxacin, levofloxacin, or ciprofloxacin was injected intravenously at a dose

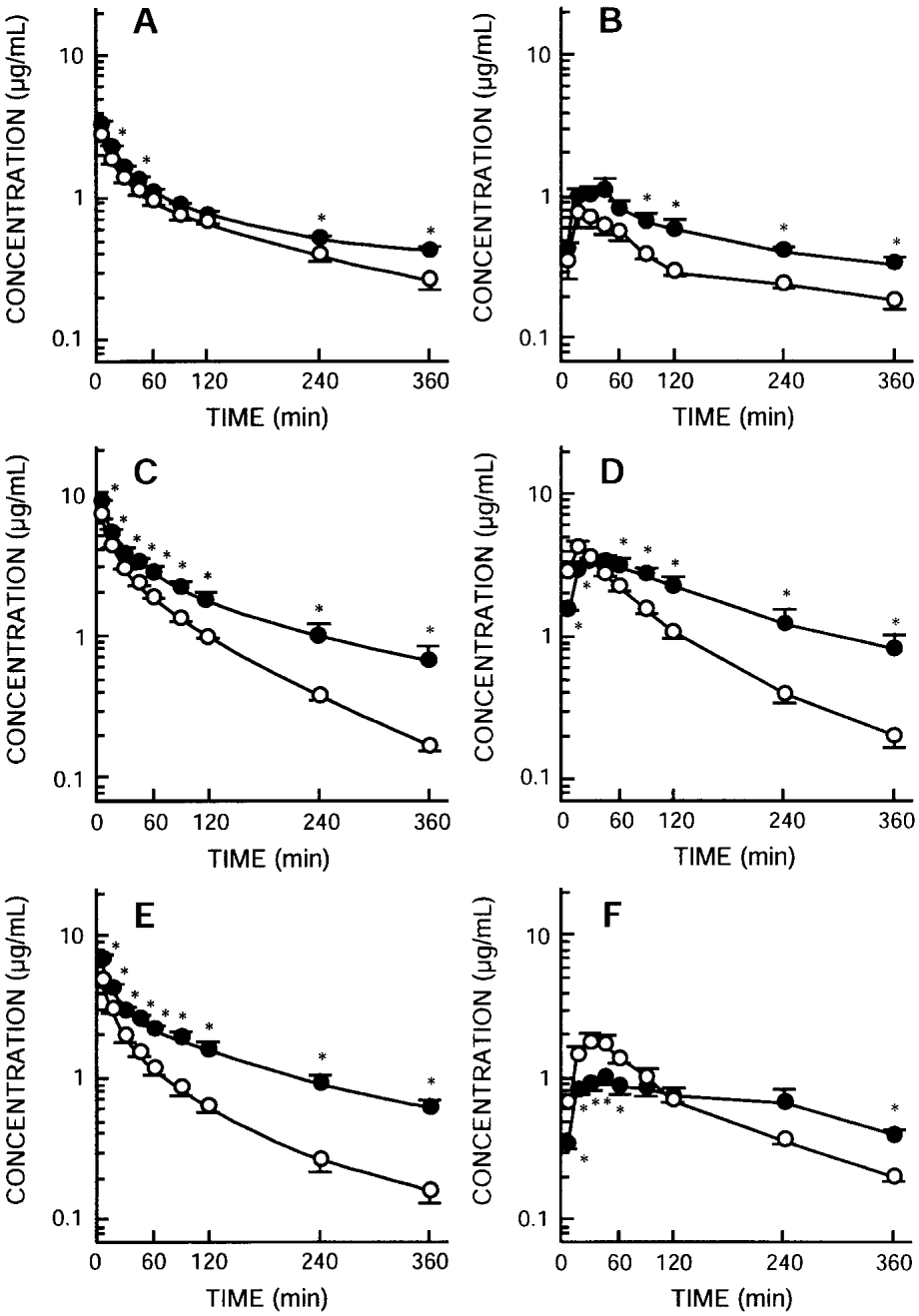


Fig. 1. Plasma concentrations of grepafloxacin (A, B), levofloxacin (C, D), and ciprofloxacin (E, F) after intravenous (A, C, E) and intraintestinal (B, D, F) administration. Grepafloxacin, levofloxacin, or ciprofloxacin was injected at a dose of 10 mg/kg into normal (○) and cisplatin-induced acute renal failure (●) rats. Blood samples were collected at specified times after injection. Each point represents the mean ± SE of five rats. *$p < 0.05$, significantly different from normal rats.

of 10 mg/kg via the catheterized right femoral vein for 1 min. In a separate experiment for intraintestinal administration of grepafloxacin, levofloxacin, or ciprofloxacin, the abdominal cavity of rats was opened via a midline incision, and the upper part of the duodenum was exposed to administer the drug. Each drug was injected into the lumen of the duodenum at a dose of 10 mg/kg. Blood samples were collected from the left femoral artery at 5, 15, 30, 45, 60, 90, 120, 240, and 360 min after the end of injection.

Intestinal and Biliary Clearance in Rats

The femoral artery and vein were cannulated as described in the pharmacokinetic studies. The abdominal cavity of rats was opened via a midline incision to gain access to the small intestine. The common bile duct was cannulated with polyethylene tubing (PE-10, BD Biosciences) for bile collection. The whole small intestine starting from the Treitz ligament was used to make an intestinal loop. After the loop had been washed with saline until the effluent was clear, 5 ml of saline was injected into the loop. Grepafloxacin, levofloxacin, or ciprofloxacin was injected intravenously at a dose of 10 mg/kg via the catheterized right femoral vein for 1 min. Blood samples were collected at 2, 5, 15, 30, 45, and 60 min after the end of injection from the left femoral artery. After 60 min, the content of the loop was withdrawn as completely as possible, and the lumen was washed with the saline to give a volume of 30 ml.

Assays

The concentrations of grepafloxacin, levofloxacin, and ciprofloxacin in plasma, intestinal fluid, urine, and bile were measured by high-performance liquid chromatography according to slight modifications of the reported procedures (18–20). Calibration curve correlation coefficients for the assays of these three drugs were above 0.999. The lower limit of the assay for each drug was 0.01 μg/ml. The plasma concentration of creatinine was measured using the Jaffé method with kits obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Blood urea nitrogen (BUN) was measured using a biochemical assay system (i-STAT® portable clinical analyser; i-STAT Co., Princeton, NJ).

Pharmacokinetic Analysis

A conventional two-compartment model was used to analyze the plasma concentration–time profiles of grepafloxacin, levofloxacin, and ciprofloxacin after intravenous administration in rats. The parameters total body clearance (CL_T), central volume of distribution (V_1), intercompartmental clearance (Q), and volume of distribution at steady state (V_{ss}), were calculated by the nonlinear least-squares method. Renal clearance (CL_R) was calculated by dividing the amount of grepafloxacin, levofloxacin, or ciprofloxacin eliminated into urine during 360 min by the area under plasma concentration–time curve (AUC) for 360 min, calculated using the linear trapezoidal rule. Nonrenal clearance (CL_{NR}) was obtained by subtracting the CL_R from the CL_T.

The apparent oral clearance (CL_T/F) expressed by the CL_T and bioavailability (F) after intraintestinal injection was calculated from dose divided by AUC. The AUC after intraintestinal injection was calculated using the linear trapezoidal rule and extrapolated to infinity by adding the ratio of the last measurable grepafloxacin, levofloxacin, or ciprofloxacin concentration to the mean terminal disposition rate constant after intravenous administration. The F value following intraintestinal injection was calculated from the CL_T and CL_T/F values. Intestinal and biliary clearances in rats were calculated by dividing the amount of grepafloxacin, levofloxacin, or ciprofloxacin eliminated into the intestinal loop and bile over 60 min by the AUC for 60 min, respectively.

Cell Culture

Caco-2 cells at passage 18 obtained from the American Type Culture Collection (ATCC HTB37) were maintained by serial passages in plastic culture dishes as described previously (21). For the transport studies, Caco-2 cells were seeded on polycarbonate membrane filters (3-μm pores, 4.71 cm^2 growth area) inside Transwell cell culture chambers (Costar, Cambridge, MA) at a density of 6.3×10^4 cells/cm^2. Transwell chambers were placed in 35-mm wells of tissue culture plates with 2.6 ml of outside (basolateral side) and 1.5 ml of inside (apical side) medium. The medium for Caco-2 cells consisted of Dulbecco's modified Eagle's medium (Invitrogen Life Technology Co., Tokyo, Japan) supplemented with 10% fetal

Table I. Pharmacokinetic Parameters of Grepafloxacin, Levofloxacin, and Ciprofloxacin After Intravenous and Intraintestinal Administration in Normal and Cisplatin-Induced Acute Renal Failure Rats

	Grepafloxacin		Levofloxacin		Ciprofloxacin	
	Normal	ARF	Normal	ARF	Normal	ARF
CL_T (ml/min/kg)	32.6 ± 2.7	22.0 ± 1.3[a]	23.8 ± 0.2	13.2 ± 1.8[a]	35.3 ± 2.9	14.4 ± 1.3[a]
V_1 (L/kg)	3.1 ± 0.2	2.7 ± 0.2	1.3 ± 0.1	1.0 ± 0.1[a]	2.0 ± 0.2	1.1 ± 0.1[a]
Q (ml/min/kg)	88.2 ± 10.2	64.0 ± 4.6	27.5 ± 2.9	36.1 ± 5.4	32.9 ± 2.5	51.3 ± 2.8[a]
V_{ss} (L/kg)	7.2 ± 0.5	7.4 ± 0.7	2.5 ± 0.1	2.4 ± 0.1	4.3 ± 0.4	3.0 ± 0.2[a]
CL_R (ml/min/kg)	4.6 ± 0.4	0.8 ± 0.2[a]	8.5 ± 0.5	1.9 ± 1.0[a]	21.7 ± 1.9	0.6 ± 0.3[a]
CL_{NR} (ml/min/kg)	28.0 ± 2.4	21.1 ± 1.4[a]	15.3 ± 0.6	11.4 ± 1.3[a]	13.6 ± 2.6	13.9 ± 1.2
CL_T/F (ml/min/kg)	61.2 ± 3.3	35.3 ± 0.8[a]	23.7 ± 1.1	13.4 ± 2.0[a]	38.8 ± 1.7	33.3 ± 6.1
T_{max} (min)	30.0 ± 8.2	30.0 ± 8.2	15.0 ± 0.0	33.0 ± 7.3[a]	36.0 ± 3.7	57.0 ± 16.0
C_{max} (μg/ml)	0.8 ± 0.2	1.1 ± 0.3	4.2 ± 0.3	3.5 ± 0.3	1.7 ± 0.2	1.0 ± 0.2
F (%)	53.3	62.2	100.0	98.6	90.9	43.3

Grepafloxacin, levofloxacin, or ciprofloxacin was intravenously or intraintestinally injected at a dose of 10 mg/kg to normal and cisplatin-induced acute renal failure rats. Each value represents the mean ± S.E. of five rats.

[a] Significantly different from the value for normal rats ($p < 0.05$).

calf serum (Microbiological Associates, Bethesda, MD) and 1% nonessential amino acids (Invitrogen Life Technology Co.) without antibiotics. The Caco-2 cells were grown in an atmosphere of 5% CO_2/95% air at 37°C and given fresh medium every 3 or 4 days.

LLC-GA5-COL150 cells, stably transfected with human MDR1 cDNA (22), were maintained by serial passage in plastic culture dishes as described previously (23). For the transport studies, LLC-GA5-COL150 cells were seeded on polycarbonate membrane filters (3 μm pores, 4.71 cm^2 growth area) inside Transwell cell culture chambers at a density of 5 × 10^5 cells/cm^2. The cell monolayers were fed fresh complete medium every 2 days and were used on the sixth day for the transport experiments.

Measurements of Transcellular Transport

Transcellular transport of [^{14}C]grepafloxacin (5 μM, 5.8 kBq/ml), [^{14}C]levofloxacin (5 μM, 5.4 kBq/ml), [^{14}C]ciprofloxacin (2 μM, 5.3 kBq/ml) and [^{3}H]digoxin (58.8 nM, 37 kBq/ml) was measured using monolayer cultures grown in Transwell chambers. The composition of incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, 5 mM HEPES (pH 7.4). The pH of the medium was adjusted with a solution of HCl or NaOH. After removal of the culture medium from both sides of the monolayers, the cell monolayers were preincubated with incubation medium (2 ml each side) at 37°C for 15 min. Then, 2 ml of incubation medium containing the radioactive substrate was added to either the basolateral or apical side, 2 ml of nonradioactive incubation medium was added to the opposite side, and the monolayers were incubated for specified periods at 37°C. For transport measurements, aliquots of the incubation medium on the other side were taken at specified times, and the radioactivity was counted. The radioactivity of the collected medium was determined in ACS II (Amersham International, Buckinghamshire, UK) by liquid scintillation counting.

Immunoblot Analysis

Crude plasma membrane fractions from the intestine were isolated as described previously (24). After blotting onto Immobilon-P membranes (Millipore Co., Bedford, MA), monoclonal antibody C219 (200 ng/ml) was used to detect the expression of P-glycoprotein. The relative density of the band in each lane was determined using NIH image 1.61 (National Institutes of Health, Bethesda, MD).

Statistical Analysis

Values are expressed as means ± SE. The statistical significance of difference between mean values was analyzed using the nonpaired *t* test. Multiple comparisons were performed using Scheffé's test following ANOVA. Differences were considered significant at $p < 0.05$.

RESULTS

Pharmacokinetics of Grepafloxacin, Levofloxacin, and Ciprofloxacin in Cisplatin-Induced ARF Rats

Body weight on 3 days after intraperitoneal administration of cisplatin was lower than that in normal rats (227 ± 3 g in normal rats; 201 ± 4 g in ARF rats, mean ± SE of 10 to 11 rats), and the concentrations of BUN and plasma creatinine in ARF rats were significantly higher than those in normal rats (BUN 9.5 ± 0.6 mg/dl in normal rats, 55.7 ± 4.5 mg/dl in ARF rats; plasma creatinine 0.53 ± 0.03 mg/dl in normal rats, 1.70 ± 0.09 mg/dl in ARF rats; mean ± SE of 10 to 11 rats), indicating the induction of renal failure.

Plasma concentrations of grepafloxacin, levofloxacin, and ciprofloxacin after both intravenous and intraintestinal administration in normal and ARF rats are shown in Fig. 1. In ARF rats, plasma concentrations of levofloxacin and ciprofloxacin after intravenous administration were much higher than those in normal rats, but that of grepafloxacin was slightly higher than that in normal rats. In intraintestinal administration studies, plasma concentration of grepafloxacin at the early stage after administration was elevated in ARF rats, whereas those of ciprofloxacin and levofloxacin were significantly decreased. Pharmacokinetic parameters of grepafloxacin, levofloxacin, and ciprofloxacin after intravenous and intraintestinal administration are summarized in Table I. The CL_T values of grepafloxacin, levofloxacin, and ciprofloxacin

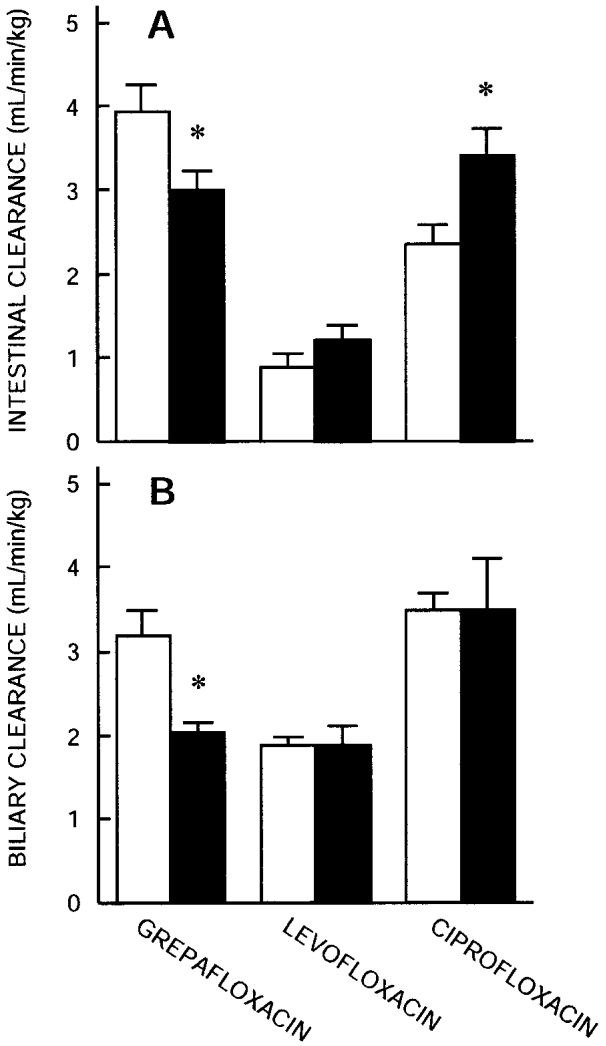


Fig. 2. Intestinal (A) and biliary clearance (B) of grepafloxacin, levofloxacin, and ciprofloxacin over 60 min after intravenous administration. Each drug was injected at a dose of 10 mg/kg into normal (open bars) or cisplatin-induced acute renal failure (solid bars) rats. Each bar represents the mean ± SE of four to six rats. *$p < 0.05$, significantly different from normal rats.

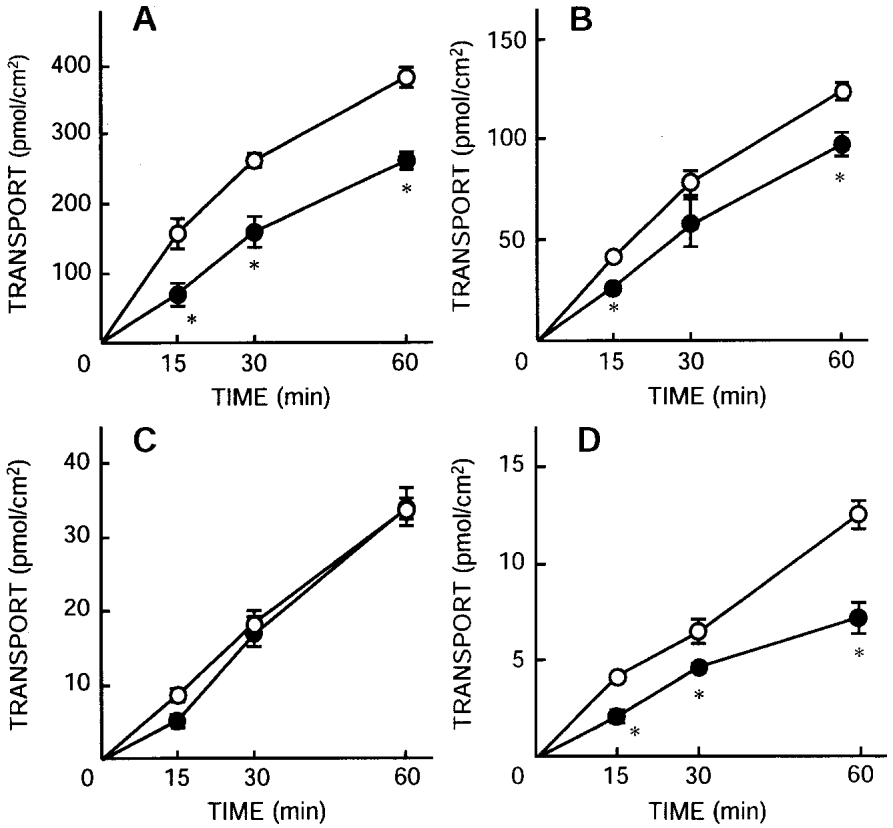


Fig. 3. Basolateral-to-apical transport of grepafloxacin (A), levofloxacin (B), ciprofloxacin (C), and digoxin (D) by LLC-GA5-COL150 cell monolayers. The monolayers were incubated at 37°C with 5 μM [^{14}C]grepafloxacin, 5 μM [^{14}C]levofloxacin, 2 μM [^{14}C]ciprofloxacin, or 58.8 nM [^{3}H]digoxin added to the basolateral side in the absence (○) or presence (●) of 10 μM cyclosporin A. After incubation, the radioactivity on the apical side was measured. Each point represents the mean ± SE of three to six monolayers. *$p < 0.05$, significantly different from control.

in ARF rats were significantly decreased to 68, 56, and 41% of the corresponding control values, respectively. The values of V_1 of levofloxacin and ciprofloxacin were decreased, and the V_{ss} of ciprofloxacin was decreased to 70% of the respective control values. The CL_R values of grepafloxacin, levofloxacin, and ciprofloxacin in ARF rats were markedly decreased. The CL_{NR} values of grepafloxacin and levofloxacin were significantly decreased, but that of ciprofloxacin was unchanged. The CL_T/F of grepafloxacin and levofloxacin after intraintestinal administration were also significantly decreased, but that of ciprofloxacin was not changed. The time to peak plasma concentration (T_{max}) of levofloxacin was significantly increased in ARF rats. Although the peak plasma concentration (C_{max}) of grepafloxacin was slightly increased, those of levofloxacin and ciprofloxacin were slightly decreased. The F of grepafloxacin in ARF rats was increased to 62% as compared with 53% in the control. On the other hand, the F of ciprofloxacin was markedly decreased to 43% from 91% in the control, and that of levofloxacin was not changed.

Intestinal and Biliary Clearance of Grepafloxacin, Levofloxacin, and Ciprofloxacin in Cisplatin-Induced ARF Rats

To elucidate the effects of renal failure on the elimination mechanisms, intestinal and biliary secretory clearances of grepafloxacin, levofloxacin, and ciprofloxacin were examined. Intestinal clearance of grepafloxacin in ARF rats was decreased to 76% of that in normal rats, and biliary clearance of grepafloxacin was also decreased to 64% (Fig. 2). Intestinal and biliary clearance of levofloxacin were comparable between normal and ARF rats. On the other hand, intestinal clearance of ciprofloxacin in ARF rats was significantly higher (1.4-fold) than that in normal rats.

Effects of Cyclosporin A on the Basolateral-to-Apical Transport of Grepafloxacin, Levofloxacin, and Ciprofloxacin by LLC-GA5-COL150 Cell Monolayers

To evaluate the affinity of grepafloxacin, levofloxacin, or ciprofloxacin for P-glycoprotein, we examined the inhibitory effects of cyclosporin A, a typical inhibitor of P-glycoprotein, on the basolateral-to-apical transport of these compounds using LLC-GA5-COL150 cells, which express human P-glycoprotein on the apical membrane (23). As shown in Fig. 3, basolateral-to-apical transport of grepafloxacin, levofloxacin, and a P-glycoprotein substrate digoxin was inhibited by cyclosporin A. The degree of inhibition for transport of grepafloxacin by cyclosporin A was greater than that of levofloxacin. However, transport of ciprofloxacin was not inhibited by cyclosporin A at any time point examined.

Transcellular Transport of Ciprofloxacin by Caco-2 Cell Monolayers

We reported that both grepafloxacin and levofloxacin showed greater basolateral-to-apical transport than that in

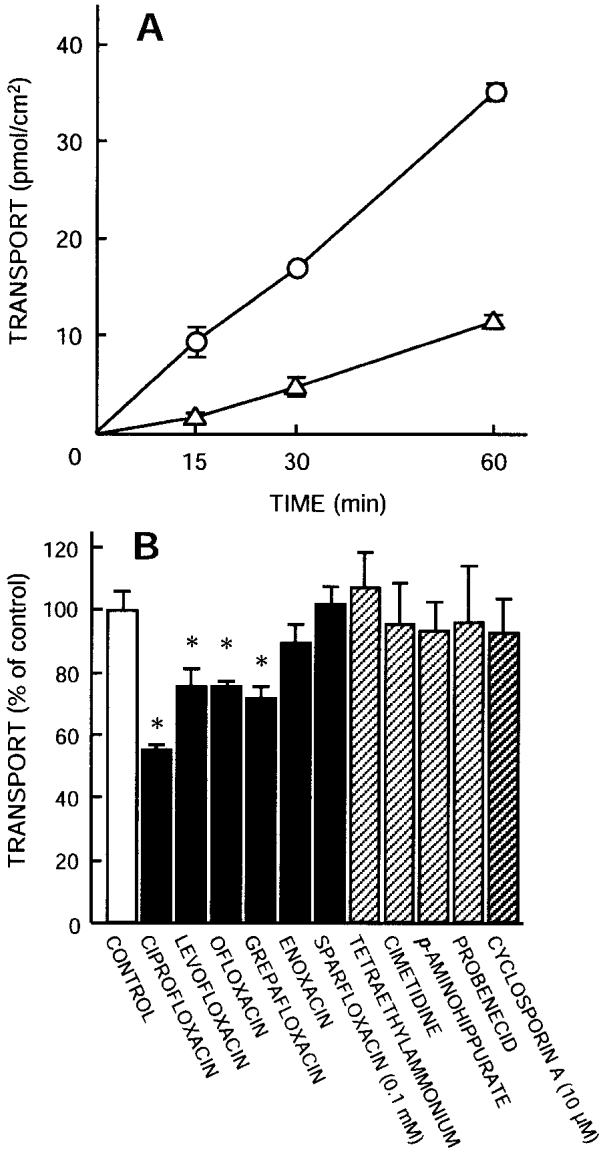


Fig. 4. Transcellular transport of ciprofloxacin by Caco-2 cell monolayers. (A) Time course of ciprofloxacin transport by Caco-2 cell monolayers. The monolayers were incubated at 37°C with 2 μM [^{14}C]ciprofloxacin added to either the basolateral (○) or apical (△) side. After incubation, the radioactivity on the opposite side was measured. Each point represents the mean ± SE of three monolayers. (B) Effects of various compounds on the basolateral-to-apical transport of ciprofloxacin by Caco-2 cell monolayers. The monolayers were incubated at 37°C for 15 min with 2 μM [^{14}C]ciprofloxacin added to the basolateral side in the absence or presence of inhibitors. Concentrations of inhibitors were 1 mM except for sparfloxacin (0.1 mM) and cyclosporin A (10 μM). After incubation, the radioactivity of the apical medium was measured. Each column represents the mean ± SE of three to six monolayers. *$p < 0.05$, significantly different from control.

the opposite direction and that intestinal secretion of grepafloxacin was mainly mediated by P-glycoprotein, whereas that of levofloxacin was mainly mediated by a specific transport system distinct from organic cation and anion transporters or MRP in Caco-2 cell monolayers (7). To confirm that ciprofloxacin is secreted via a specific transport system but not P-glycoprotein, we examined the transcellular transport and effects of several compounds on transport of ciprofloxacin using Caco-2 cell monolayers. Basolateral-to-apical transport of ciprofloxacin was much greater than apical-to-basolateral transport at each time point (Fig. 4A) and was inhibited by the presence of excesses of some quinolones (unlabeled ciprofloxacin, levofloxacin, ofloxacin, and grepafloxacin) (Fig. 4B). However, no significant inhibition was observed by the organic cations such as tetraethylammonium and cimetidine, organic anion *p*-aminohippurate, cyclosporin A, or the MRP2 inhibitor probenecid.

Western Blot Analysis of P-Glycoprotein Level in the Intestine

To examine whether the expression level of P-glycoprotein in the intestine is modulated in cisplatin-induced ARF rats, immunoblot analysis was performed. Figure 5 shows P-glycoprotein contents in the intestine of normal and ARF rats. The densities of the bands in ARF rats were identical to those in normal rats (1.0 ± 0.1 and 1.1 ± 0.2 for normal and ARF rats, respectively, mean ± SE of four rats).

DISCUSSION

The gastrointestinal tract plays important roles not only for absorption but also in elimination or as an absorption barrier. Quinolone antibacterial drugs such as ciprofloxacin and temafloxacin are reported to eliminate from the gastrointestinal tract in humans (5,6). Renal function modulates the pharmacokinetic behavior such as metabolic function and intestinal absorption of various compounds (12). In this study, we evaluated the effects of ARF on the bioavailability and gastrointestinal secretion of grepafloxacin, levofloxacin, and ciprofloxacin in rats.

Plasma concentrations of levofloxacin and ciprofloxacin after intravenous administration were significantly increased in ARF rats (Fig. 1). On the other hand, plasma concentration of grepafloxacin was modified only slightly because the ratio of CL_R to CL_T of grepafloxacin was relatively small. The CL_{NR} values of grepafloxacin and levofloxacin in ARF rats were decreased, whereas that of ciprofloxacin was comparable between normal and ARF rats. As shown in Fig. 2, both intestinal and biliary clearance of grepafloxacin were decreased, whereas those of levofloxacin were unchanged, and intestinal clearance of ciprofloxacin was increased in ARF rats. It was reported that renal failure decreased the metabolism of some drugs (12), which might be one of possible reasons for the decreased CL_{NR} of grepafloxacin and levofloxacin. On the other hand, the lack of change in CL_{NR} of ciprofloxacin might be explained by compensatory mechanisms such as increased intestinal secretory clearance. In ARF rats,

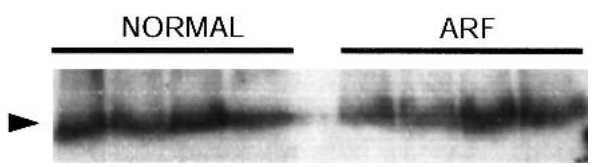
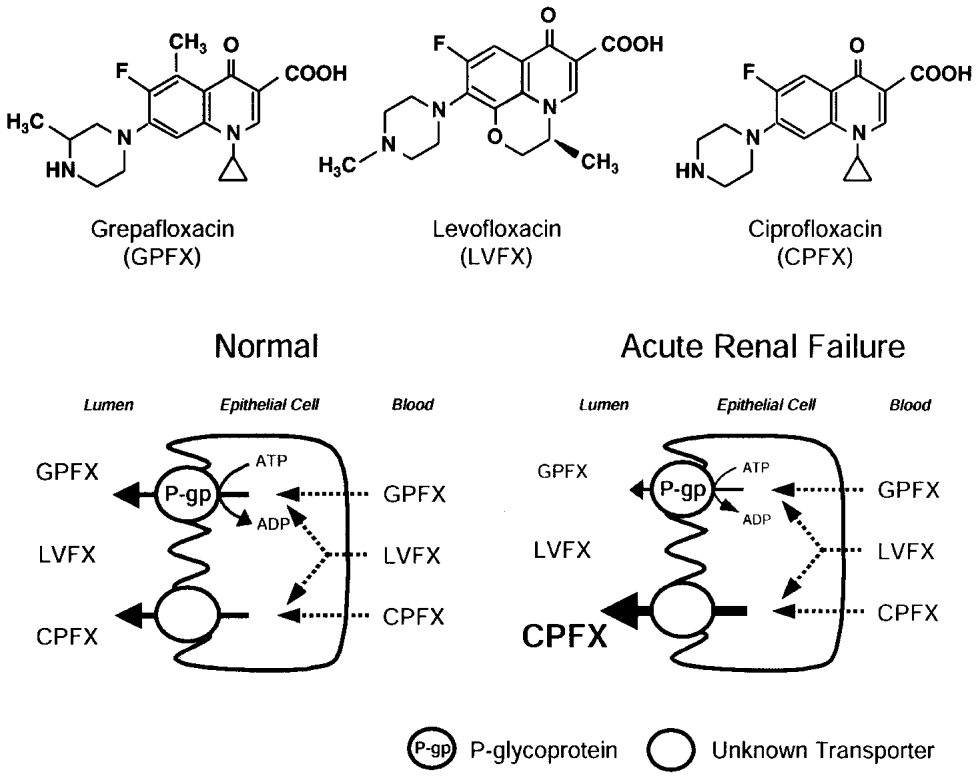


Fig. 5. Immunoblot analysis of P-glycoprotein contents isolated from the rat intestine. Crude membranes (10 μg) isolated from the intestine of normal and cisplatin-induced acute renal failure rats were separated on SDS-PAGE (7.5%). P-glycoprotein was identified using C219.



	Bioavailability	Intestinal Secretion
Grepafloxacin	Increased	Decreased
Levofloxacin	Unchanged	Unchanged
Ciprofloxacin	Decreased	Increased

Fig. 6. Chemical structures and mechanisms of intestinal secretion of quinolone antibacterial drugs in cisplatin-induced acute renal failure.

the F of grepafloxacin was increased by 1.2-fold. Conversely, the F of ciprofloxacin was decreased to half of that in normal rats, and C_{max} of the drug was slightly decreased in ARF rats. These results suggested that intestinal absorption of quinolones was differentially regulated in renal failure.

We reported previously that intestinal secretion of grepafloxacin was mainly mediated by P-glycoprotein using Caco-2 cells (7), and furthermore, we clarified that P-glycoprotein mediated the intestinal secretion of grepafloxacin and limited the bioavailability of this drug *in vivo* (25). Because intestinal clearance of grepafloxacin in ARF rats was decreased to 76% of that in normal rats (Fig. 2), we evaluated the protein level of P-glycoprotein in ARF rats and found that P-glycoprotein contents in the intestine of ARF rats were identical to those in normal rats (Fig. 5). Therefore, the decreased intestinal clearance of grepafloxacin was considered to be caused by the functional down-regulation of P-glycoprotein rather than by a change in its expression. Although a contribution of MRP2 to the secretory transport of grepafloxacin was reported, the contribution of MRP2 as an absorption barrier was negligible in contrast to P-glycoprotein *in vivo* (26). Therefore, there is a low possibility that the increased bioavailability of grepafloxacin could be explained by down-regulation of MRP2 function by ARF. Recently, Veau *et al.* (27) reported that P-glycoprotein-mediated intestinal elimination of rhodamine 123 was reduced in chronic renal failure rats and that chronic renal failure led to a change in the activity of P-glycoprotein but not to a decrease in its protein expression, consistent with our results. In addition, Laouari *et al.* (28) demonstrated that the expression level of MRP2 in the kidney and liver was induced by chronic renal failure, although that of P-glycoprotein was unchanged. Huang *et al.* (29) reported that P-glycoprotein function in the kidney and liver was suppressed in glycerol-induced ARF rats, and they discussed that some endogenous P-glycoprotein substrates/modulators might be accumulated in the plasma of ARF rats and inhibit the P-glycoprotein function. Precise mechanisms underlying the decreased P-glycoprotein-mediated intestinal secretion of compounds in ARF remain to be clarified.

We reported that intestinal secretion of grepafloxacin was mainly mediated by P-glycoprotein and that that of levofloxacin was mainly mediated by another transport system in Caco-2 cells (7). Cavet *et al.* (9) and Griffiths *et al.* (10) reported that secretory transport of ciprofloxacin in Caco-2 cells was not mediated by P-glycoprotein. Therefore, we hypothesized that the different effects of ARF on intestinal se-

cretion of quinolones were carried out by distinct transport mechanisms, and transport experiments using culture cells were performed. Using LLC-GA5-COL150 cell monolayers, which express human P-glycoprotein on the apical membrane, basolateral-to-apical transport of ciprofloxacin was shown not to be inhibited by the P-glycoprotein inhibitor cyclosporin A, although those of grepafloxacin and levofloxacin were significantly inhibited (Fig. 3). In addition, ciprofloxacin was preferentially transported in the secretory direction by Caco-2 cell monolayers, and from the results of inhibition studies, this secretory transport was mediated by a specific transport system distinct from P-glycoprotein, organic cation and anion transporters, and MRP2. The intestinal secretion of ciprofloxacin was increased by 1.4-fold in cisplatin-induced ARF rats as compared to normal rats (Fig. 2). Becausee Demeule *et al.* (30) demonstrated that renal expression levels of P-glycoprotein and MRP2 in rats were induced by cisplatin treatment, a specific transport system for quinolones might be regulated similarly. Furthermore, Dautrey *et al.* (17) reported that intestinal clearance of ciprofloxacin was increased in nephrectomized rats, suggesting that the intestinal secretion of ciprofloxacin might be generally increased in renal failure. Intestinal secretion of levofloxacin was reported to be mainly mediated by a specific transport system in Caco-2 cells, and P-glycoprotein appeared to contribute to its transport in rats (7,25). In the present study, intestinal secretion of levofloxacin was not changed in ARF rats. One possible explanation for this result was a combination of decreased P-glycoprotein-mediated transport and increased transport mediated by a specific transport system. Biliary secretory clearance of grepafloxacin in ARF rats was decreased to two-thirds of that in normal rats (Fig. 2). Because we previously showed that biliary secretion of grepafloxacin was mediated via a cyclosporin A–inhibitable transport system but not via P-glycoprotein (25), this transport system might also be down-regulated in ARF rats similarly to P-glycoprotein. Biliary clearances of levofloxacin and ciprofloxacin were unchanged. The transporters that contribute to the biliary secretion of these quinolones remain to be clarified.

In conclusion, we demonstrated the differential effects of cisplatin-induced ARF on the pharmacokinetics of quinolone antibacterial drugs, especially on bioavailability and intestinal secretion (Fig. 6). Transport experiments suggested the involvement of different mechanisms of quinolone transport in the intestine. Cisplatin-induced ARF may depress P-glycoprotein-mediated transport and activate a transporter that specifically recognizes quinolones in the intestine.

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