Evaluation of Renal Tubular Secretion and Reabsorption of Levofloxacin in Rats

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Purpose. Levofloxacin, a quinolone antibacterial drug, is a zwitterion at physiological pH. We examined the effect of cationic and anionic drugs on renal excretion of levofloxacin by means of *in vivo* clearance to characterize the mechanisms of renal excretion of this drug.

Methods. *In vivo* clearance was studied in male Wistar albino rats. A bolus dose of 2.85 mg/kg of levofloxacin was administered, followed by a constant infusion of 7.08 μg/min. Cimetidine, tetraethylammonium, or p-aminohippurate was administered as a bolus and incorporated into the infusion solution. After reaching steady state, urine and blood concentrations were measured, and pharmacokinetic parameters were calculated.

Results. Renal clearance was 2.56 ± 0.42 ml/min in control, which accounted for 34% of the total body clearance. Renal clearance was significantly decreased to 0.83 ± 0.25 ml/min by cimetidine ($p<.05$), corresponding to 32% of the control value. The cationic drug, tetraethylammonium also reduced the renal clearance of levofloxacin, but the effect of the anionic drug, p-aminohippurate, was slight. The clearance ratio of levofloxacin, which was calculated by renal clearance divided by the plasma unbound fraction and the glomerular filtration rate, was 1.60 ± 0.38 in the control and it was decreased to 0.68 ± 0.17 and 1.11 ± 0.22 by cimetidine and tetraethylammonium, respectively.

Conclusions. The present results suggest that the renal excretion of levofloxacin in rats involves tubular secretion and reabsorption, in addition to glomerular filtration, and that tubular secretion is inhibited by cimetidine.

KEY WORDS: levofloxacin; quinolone antibacterial drugs; renal secretion; organic cation transport.

INTRODUCTION

Levofloxacin is a fluorinated quinolone that is frequently used to treat various bacterial infections. Levofloxacin is well absorbed from the intestine and distributed to tissues, and is mainly eliminated by renal excretion in humans (1). The mechanisms for the renal excretion of levofloxacin in humans are glomerular filtration, tubular secretion, and tubular reabsorption (2). Several pharmacokinetic studies have suggested that some quinolone antibacterial drugs interact with probenecid or cimetidine and undergo tubular secretion as either acids or bases (3). However, little information has been reported concerning the mechanisms of tubular secretion of levofloxacin.

Levofloxacin possesses both a carboxylic group (pKa_1, 5.5) and a piperazinyl group containing a nitrogen atom in position 4 (pKa_2, 8.0), and the drug is a zwitterion at physiological pH. Organic ions are actively secreted into urine via the organic anion or/and cation transport systems present in the proximal tubule (4). We reported that levofloxacin potently inhibits the apical H^+/organic cation antiporter expressed in the kidney epithelial cell line, LLC-PK_1, and that transcellular transport of levofloxacin would be mediated by transport systems which are distinct from the systems for tetraethylammonium (5). These were consistent with our previous findings that ofloxacin potently inhibits the uptake of tetraethylammonium in renal brush-border membrane vesicles (6).

In this study, we examined the effect of cationic and anionic drugs on the renal excretion of levofloxacin by means of *in vivo* clearance to characterize the mechanisms of the renal excretion of this drug.

MATERIALS AND METHODS

Materials

Levofloxacin was supplied by Daiichi Seiyaku Co. (Tokyo, Japan). Cimetidine, tetraethylammonium chloride, p-aminohippuric acid sodium salt monohydrate, and inulin were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals used were of the highest purity available.

In Vivo Clearance Method

Male Wistar albino rats weighing 230–290 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The femoral artery and vein were cannulated with polyethylene tubing (Intramedic PE-50, Becton Dickinson and Co., Parsippany, NJ) 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. After stabilizing for 30 min, blank urine was collected for 10 min and blank blood was sampled at 5 min. Thereafter, bolus doses of 2.85 mg/kg of levofloxacin, 146 mg/kg of mannitol, and 73.4 mg/kg of inulin were administered, followed by a constant infusion of levofloxacin (0.193 mg/ml), 4% mannitol, and 2% inulin at a rate of 2.2 ml/hr using an automatic infusion pump (Natsume Seisakusho, Tokyo, Japan). This dosage was selected to obtain a 1 μg/ml (2.7 μM) plateau concentration of plasma levofloxacin based on kinetic parameters obtained in a preliminary bolus injection study. This concentration is in the therapeutic range for humans. Mannitol was administered to maintain a sufficient and constant urine flow rate, and the glomerular filtration rate was determined by inulin clearance. The plasma levofloxacin concentration reached a plateau at 40 min of equilibration.

Thereafter, the effect of cimetidine, tetraethylammonium, or p-aminohippurate was investigated as follows. One of these drugs was administered as a bolus via the femoral vein and incorporated into the infusion solution. The respective loading and maintenance doses of the drugs were: 317 μmol/kg and 21.6 μmol/ml for cimetidine, 61.6 μmol/kg and 104 μmol/ml for tetraethylammonium, 30 μmol/kg and 70 μmol/ml for p-aminohippurate. The infusion rate was 2.2 ml/hr. The dosages of the drugs were calculated to obtain concentrations of 160 μM, 1 mM, and 0.4 mM for cimetidine, tetraethylammonium, and p-aminohippurate, respectively, based on previous reports (7,8). After a 30 min equilibration, urine samples were collected twice at 10 min intervals and blood samples were obtained at

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Table I. Effect of Ionic Drugs on Various Parameters of Levofloxacin Clearance Method in Rats

	GFR (ml/min)	Urinary excretion rate (μg/min)	$C_{p,ss}$ (μg/ml)	fu (%)
Control (n = 4)	2.29 ± 0.22	2.39 ± 0.22	0.98 ± 0.10	74.0 ± 6.5
Cimetidine (n = 4)	1.92 ± 0.28	1.03 ± 0.26**	1.31 ± 0.13	66.1 ± 9.2
TEA (n = 3)	2.06 ± 0.05	1.56 ± 0.09	1.06 ± 0.06	68.9 ± 11.6
PAH (n = 3)	2.48 ± 0.22	2.85 ± 0.11	1.23 ± 0.12	83.2 ± 6.4
Cimetidine and PAH (n = 4)	2.42 ± 0.15	1.45 ± 0.14*	1.28 ± 0.06	70.1 ± 3.1

Note: Each value represents the mean ± S.E. of three or four animals. Significant difference from control, *p < .05, **p < .005. TEA, tetraethylammonium; PAH, p-aminohippurate; GFR, glomerular filtration rate; $C_{p,ss}$, plateau plasma concentration; fu, plasma unboumd fraction.

the midpoint of urine collection. The plasma was immediately separated from erythrocytes by centrifugation.

The animal experiments were performed in accordance with the *Guideline for Animal Experiments of Kyoto University.*

Plasma Protein Binding

The plasma unbound fraction of levofloxacin was determined by ultrafiltration using a Micropartition System (MPS-1, Amicon, Inc., Beverly, MA). Briefly, an adequate volume of blood was taken from rats at the end of the clearance method and centrifuged. Plasma (100 μl) was placed in an ultrafiltration device equipped with a YMT ultrafiltration membrane (Amicon, Inc.) and centrifuged at 1,500 g for 5 min at 25°C to filter the plasma. The cup containing the ultrafiltrate was replaced and another plasma sample (400 μl) was placed in the same device and centrifuged at 900 g for 2 min at 25°C. This procedure was necessary to prevent the adsorption of levofloxacin to the membranes.

Tissue Concentration

After the clearance method was performed, rats were killed and excised tissues were placed into ice-cold saline. The kidneys were removed, blotted onto filter paper and decapsulated, then thin slices were cut using a Stadie-Riggs microtome. The slices were separated into cortex and medulla, weighed and homogenized with 9-volumes of saline. Other tissues, such as brain, heart, muscle, and liver were also weighed and homogenized in the same manner as the kidney.

Analytical Methods

The concentration of levofloxacin in plasma, urine, and tissue homogenate was measured by high-performance liquid chromatography (HPLC) with a slight modification of the reported procedure (5). The lower limit of the assay was 0.01 μg/ml. The concentration of inulin in plasma, urine, and tissue homogenate was determined spectrophotometrically (9).

Pharmacokinetic Analyses

Total body and renal clearances of levofloxacin were calculated by dividing the infusion and urinary excretion rates by the plasma concentration at the midpoint of urine collection. Nonrenal clearance of levofloxacin was calculated by subtracting renal clearance from total body clearance. The clearance ratio was calculated by dividing the renal clearance by the plasma unbound fraction and the glomerular filtration rate.

Statistical Analysis

Data are shown as means ± S.E. Statistical analysis was performed by the Scheffé test. Differences were considered significant when p < .05.

RESULTS

Various parameters of levofloxacin clearance method in rats are shown in Table I. Although the glomerular filtration rate and plasma unbound fraction were not significantly changed, the urinary excretion rate of levofloxacin was significantly decreased by cimetidine (p < .005) and also by the coadministration of cimetidine and p-aminohippurate (p < .05). The plateau plasma concentration was increased a little by these drugs, but did not reach statistical significance. Tetraethylammonium tended to reduce the urinary excretion rate of levofloxacin, but with no significance. These drugs did not influence plasma or urine pH (data not shown).

Figure 1 shows the calculated total body, renal, and nonrenal clearances of levofloxacin. In the control, total body and renal clearances of levofloxacin were 7.53 ± 0.79 and 2.56 ± 0.42 ml/min, respectively, and renal clearance accounted for 34% of total body clearance. Renal clearance with cimetidine

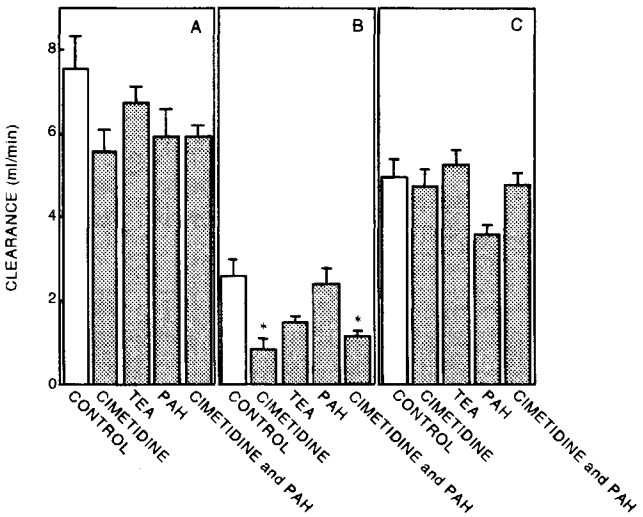

Fig. 1. Effect of ionic drugs on total body (A), renal (B), and nonrenal clearances (C) of levofloxacin in rats. The ionic drugs were cimetidine, tetraethylammonium (TEA), and p-aminohippurate (PAH). Each column represents the mean ± S.E. of three or four animals as shown in Table I. *P < .05, significant difference from control.

was significantly decreased to 0.83 ± 0.25 ml/min ($p < .05$), corresponding to 32% of the control value. Tetraethylammonium also reduced the renal clearance of levofloxacin, but the effect of the anionic drug, p-aminohippurate, was slight. Coadministration of cimetidine and p-aminohippurate also decreased the renal clearance of levofloxacin (1.13 ± 0.11 ml/min, $p < .05$). These drugs did not significantly affect the nonrenal clearance of levofloxacin, although p-aminohippurate tended to reduce the value.

Figure 2 shows the clearance ratio of levofloxacin in the absence or presence of ionic drugs. In the control, the clearance ratio of levofloxacin was 1.60 ± 0.38, indicating at the least a tubular secretion of levofloxacin in rats. The clearance ratio was decreased to 0.68 ± 0.17 and 0.67 ± 0.05 by cimetidine and coadministered cimetidine plus p-aminohippurate, respectively, which represents the presence of tubular reabsorption of levofloxacin as well as tubular secretion. The clearance ratio was 1.11 ± 0.22 and 1.17 ± 0.14 with tetraethylammonium and p-aminohippurate, respectively.

Figure 3 shows the tissue/plasma concentration ratio in the kidney. The values in the kidney cortex and medulla were 3.25 ± 0.29 and 4.44 ± 0.31 in the control, and were not changed by cimetidine, tetraethylammonium, or p-aminohippurate. We also measured the levofloxacin concentrations in other tissues to examine the selective tissue distribution. Figure 4 shows the tissue distribution of levofloxacin in rats given a constant intravenous infusion. The tissue/plasma concentration ratios in the kidney cortex and medulla were higher than those in other tissues such as heart, muscle, and liver. The ratio in the brain was 0.15 ± 0.01, indicating that the distribution of levofloxacin to the brain is restricted.

DISCUSSION

As levofloxacin is a zwitterion at physiological pH, we have studied the roles of organic cation or anion transporter systems in the renal excretion of this drug. Previously, we reported that levofloxacin potently inhibits the apical H^+/organic cation antiporter expressed in the kidney epithelial cell line, LLC-PK_1 (5). In this study, we examined the effect of cationic and anionic drugs on the renal excretion of levofloxacin by *in vivo* clearance method.

The present results show that cimetidine decreased the renal clearance of levofloxacin (Fig. 1). The cationic drug, tetraethylammonium also reduced the renal clearance of levofloxacin, but the anionic drug, p-aminohippurate had little effect. We also examined the effect of coadministered cimetidine and p-aminohippurate and found that the effect of cimetidine plus p-aminohippurate was the same as that of cimetidine alone and that p-aminohippurate had little effect on the renal excretion of levofloxacin. These inhibitory effects were probably not caused by impaired renal function, because the glomerular filtration rate did not change regardless of these drugs. Cimetidine coadministration reduced enoxacin renal clearance by 26% in healthy subjects (10). The decreased renal clearance

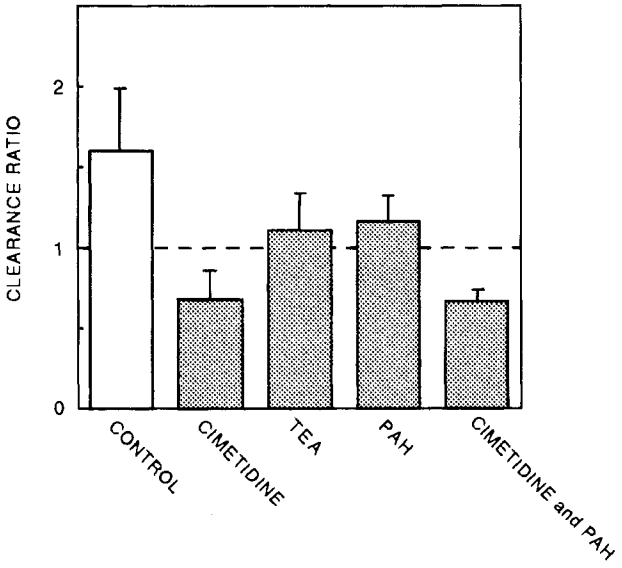

Fig. 2. Effect of ionic drugs on the clearance ratio of levofloxacin in rats. The clearance ratio was calculated as renal clearance divided by the plasma unbound fraction and the glomerular filtration rate. The ionic drugs were cimetidine, tetraethylammonium (TEA), and p-aminohippurate (PAH). Each column represents the mean ± S.E. of three or four animals as shown in Table I.

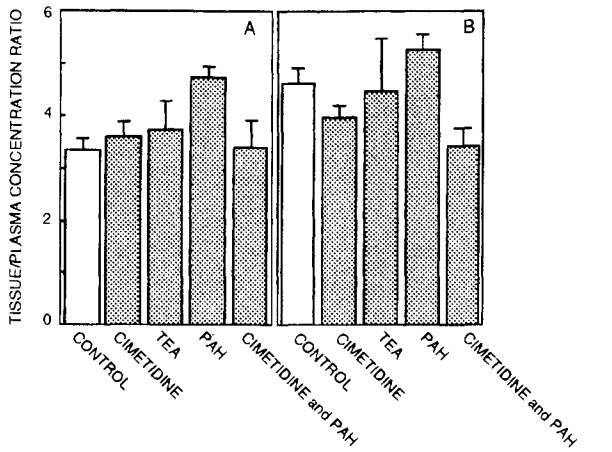

Fig. 3. Effect of ionic drugs on the tissue/plasma concentration ratio of levofloxacin in the kidney cortex (A) and medulla (B) in rats. The ionic drugs were cimetidine, tetraethylammonium (TEA), and p-aminohippurate (PAH). Each column represents the mean ± S.E. of three or four animals as shown in Table I.

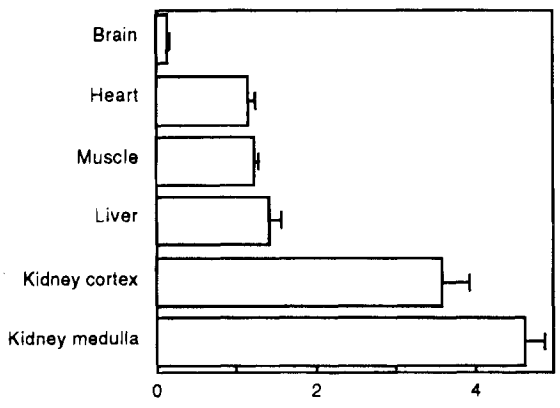

Fig. 4. Tissue distribution of levofloxacin in rats given a constant intravenous infusion. Each column represents the tissue/plasma concentration ratio and the mean ± S.E. of four animals.

could produce significant increase in the plasma concentrations of levofloxacin for humans, as over 85% of the administered dose was excreted unchanged into urine (1).

We found that the net renal excretion of levofloxacin in rats is determined by tubular secretion and reabsorption, in addition to glomerular filtration as it is in humans (Fig. 2). Renal clearance (CL_r) of levofloxacin can be described by the following equation: $CL_r = (CL_{rf} + CL_{rs})(1\text{-}R)$, where CL_{rf} is renal filtration clearance calculated by glomerular filtration rate multiplied by the plasma unbound fraction, CL_{rs} is renal secretion clearance, and R is the reabsorption fraction of drug filtered and secreted (11). Here, we propose the following. 1) Renal secretion clearance of levofloxacin is completely inhibited by cimetidine and, 2) the reabsorption fraction of levofloxacin is not affected by cimetidine, tetraethylammonium, nor p-aminohippurate. Based on the first notion, the calculated reabsorption fraction was 0.312, which is close to the reported value in humans (2). The calculated mean values of renal filtration and secretion clearances were 1.71 and 2.02 ml/min, respectively, in the control. The calculated renal secretion clearance of levofloxacin coadministered with cimetidine and p-aminohippurate was zero, which supported our proposals. The calculated renal secretion clearance with tetraethylammonium and p-aminohippurate were 0.36 and 1.44 ml/min, respectively, and we assumed that levofloxacin is secreted by the organic cation transport mechanism in the renal proximal tubules.

The tissue/plasma concentration ratio in the kidney was higher than those in other tissues (Fig. 4). As the kidney tissue includes urine in which the concentration of levofloxacin is over 200-fold than the concentration in plasma, the interpretation of the tissue concentration in the kidney is more complex than that in non-eliminating tissues. We found that the tissue/plasma concentration ratios of inulin, which is eliminated from the kidney only by glomerular filtration and does not enter the renal tubular cells, were 3.97 ± 0.34 and 3.17 ± 0.30 (n = 4) for the kidney cortex and medulla, respectively. Taking plasma protein binding into consideration, the mean values of the tissue/plasma unbound concentration ratio of levofloxacin were 4.61 and 6.31 for the kidney cortex and medulla, respectively, indicating the uptake of levofloxacin into the renal tubular cells and its active secretion.

Tubular secretion is a transcellular transport process consisting of entry into the cell across the basolateral membranes, movement in the cytosol, and expulsion across the apical membranes. We considered that the transport of levofloxacin from the cell to urine, that is, across the apical membranes, might be completely inhibited by cimetidine, as the renal secretion of levofloxacin was considered to be almost zero with cimetidine. The tissue/plasma concentration ratio in the kidney was not affected by cimetidine (Fig. 3), although the urine concentration of levofloxacin was reduced to less than one-half of the control. We presumed that the transport of levofloxacin across the basolateral membranes is unaffected by cimetidine and that the increased concentration in the renal tubular cells would cancel out the decreased urine concentration. The tissue concentration in the kidney would be significantly decreased, if the basolateral uptake of levofloxacin from blood was also inhibited. Ullrich et al. (12) reported, by use of the stopflow peritubular capillary perfusion method, that the basolateral uptake of ofloxacin was not inhibited by tetraethylammonium or probenecid, consistent with the present results.

In our previous study using LLC-PK_1 cells, the basal-to-apical transport and cellular accumulation of levofloxacin were inhibited by neither cimetidine nor tetraethylammonium (5). These findings are not consistent with the present results. Renal secretion of levofloxacin in rats might be mediated by transporters which are not expressed in LLC-PK_1 cells.

As shown in Figure 4, the brain/plasma concentration ratio was 0.15 ± 0.01, which may be caused either by the relatively low influx permeability at the blood-brain barrier and blood-cerebrospinal fluid barrier and/or by the active efflux transport systems at both barriers (13). We found that levofloxacin is transported by human P-glycoprotein expressed in LLC-PK_1 cells (unpublished data). As P-glycoprotein, a member of the ATP-binding cassette family of transporter proteins, is localized in the normal brain and kidney (14,15), this transporter might partly mediate the efflux of levofloxacin from the brain and/or the renal secretion of this drug.

In conclusion, the present results suggest that the renal excretion of levofloxacin in rats involves tubular secretion and reabsorption, in addition to glomerular filtration, as it does in humans and that tubular secretion is inhibited by cimetidine.

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