Transport of Levofloxacin in the OK Kidney Epithelial Cell Line: Interaction with *p*-Aminohippurate Transport

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Purpose. To evaluate the mechanism of renal transport of quinolone antibacterial drugs, we examined the interaction of levofloxacin with *p*-aminohippurate (PAH) transport systems and the transport of levofloxacin in renal epithelial cells.

Methods. Transport of [^{14}C]PAH or [^{14}C]levofloxacin was measured using OK cell monolayers grown on microporous membrane filters.

Results. Transcellular transport from the basolateral to the apical side and cellular accumulation of [^{14}C]PAH were inhibited by levofloxacin. Both the initial uptake of [^{14}C]PAH from the basolateral side and the efflux to the apical side were inhibited by levofloxacin. The basolateral-to-apical transcellular transport of [^{14}C]levofloxacin was greater than that in the opposite direction. [^{14}C]Levofloxacin efflux to the apical side was greater than that to the basolateral side. Unlabeled levofloxacin and grepafloxacin inhibited the transcellular transport of [^{14}C]levofloxacin, accompanied by an increase of cellular accumulation. However, neither PAH nor an anion transport inhibitor 4-4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) affected the basolateral-to-apical transport of [^{14}C]levofloxacin nor its uptake from the basolateral side.

Conclusions. These results indicated that levofloxacin inhibits PAH transport across both the basolateral and apical membranes of OK cells, but are not transported via the systems for PAH transport. The existence of a specific transport system for quinolones was indicated in OK cells.

KEY WORDS: *p*-aminohippurate; quinolone antibacterial drugs; transcellular transport; OK cells; renal secretion; organic anion transport systems.

INTRODUCTION

A number of quinolone antibacterial drugs have been developed and used clinically. Most of these drugs are zwitterions at physiological pH and excreted into the urine. The urinary excretion of a wide variety of endogenous organic ions and xenobiotics is mediated via organic cation or anion transport systems expressed in the renal proximal tubular cells. Both cimetidine and probenecid have been shown to decrease the renal clearance of several quinolone antibacterial drugs in humans (1–5). These findings suggest that organic cation or anion transport system may contribute to the renal secretion of quinolone antibacterial drugs.

Levofloxacin, a widely used quinolone antibacterial drug, is well absorbed from the intestine and mainly excreted into the urine in humans (6). The renal excretion of levofloxacin has been reported to involve tubular secretion in addition to glomerular filtration (7). Renal secretion is a transcellular transport event across the proximal tubular epithelium, and cultured epithelial cells derived from the kidney offer advantages for the study of mechanisms responsible for renal secretion of solutes. We reported previously that levofloxacin potently inhibits the function of organic cation transport systems in the pig kidney epithelial cell line LLC-PK$_1$ (8). Recently, we also demonstrated that levofloxacin is transported by specific mechanisms distinct from the organic cation transport systems in LLC-PK$_1$ cells (9). On the other hand, since LLC-PK$_1$ cells do not have the organic anion transport activity (10), the interactions of quinolone antibacterial drugs with the organic anion transport systems in renal epithelial cells and whether these transport systems contribute to the quinolone transport remain to be elucidated.

The American opossum kidney epithelial cell line OK (11) was established as a model for the functional analysis of epithelial cells. Hori *et al.* (10) found that *p*-aminohippurate (PAH), a typical substrate of organic anion transport systems, was transported principally from the basolateral to apical side of OK cells, which corresponds to renal secretion. In addition, this vectorial transport was shown to be a specifically mediated process on both apical and basolateral membranes (12). We also showed that the basolateral PAH transport system has a similar substrate specificity to that in renal proximal tubules (13). Based on these findings, OK cells appear useful to investigate the organic anion transport systems at the cellular level. To evaluate the renal transport mechanism of quinolone antibacterial drugs, we examined the effect of levofloxacin on organic anion transport systems using PAH in OK cells. In addition, the transport of levofloxacin was also investigated. The present results suggested that levofloxacin has a potent inhibitory effect on PAH transport systems in both basolateral and apical membranes. However, we found that transport system distinct from those for PAH is involved in the transport of levofloxacin in OK cells.

MATERIALS AND METHODS

Materials

p-[Glycyl-1-^{14}C]Aminohippuric acid (1.6–2.0 GBq/mmol), D-[^{3}H]mannitol (728.9–828.8 GBq/mmol) and [^{3}C]sucrose (455.1 GBq/mmol) were purchased from Du Pont-New England Nuclear Research Products (Boston, MA). [^{14}C]Levofloxacin (1.07 GBq/mmol) and unlabeled levofloxacin were kindly supplied by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Unlabeled grepafloxacin was a gift from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Enoxacin was kindly supplied by Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). Tetraethylammonium was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). *p*-Aminohippuric acid, probenecid and 4-4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) were purchased from Sigma Chemical (St. Louis, MO). All other chemicals used were of the highest purity available.

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Cell Culture

OK cells were cultured as described previously (10). In the present study, OK cells were used between passages 77 and 101.

Transport Measurements

The transcellular transport, cellular uptake and efflux of PAH and levofloxacin were measured at 37°C in OK cell monolayers cultured in Transwell chambers (Costar, Cambridge, MA) as described (13). Dulbecco's phosphate-buffered saline (PBS; containing 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1 mM $CaCl_2$, and 0.5 mM $MgCl_2$) supplemented with 5 mM D-glucose was used in the transport experiment. After removal of the culture medium, cell monolayers were washed twice with PBS containing 5 mM D-glucose and preincubated for 10 minutes.

The transcellular transport, cellular accumulation, cellular uptake and efflux of [^{14}C]PAH (15 μM) and [^{14}C]levofloxacin (15 μM) was measured as described previously (10,12). D-[^{3}H]Mannitol (15 μM) and [^{3}H]sucrose (15 μM) were used to correct for the paracellular flux, extracellular trapping and/or nonspecific uptake of PAH and levofloxacin, respectively. In the transport measurements, the volume of PBS containing D-glucose in both the apical and basolateral compartments was 2 ml. The radioactivity of the collected medium and the solubilized cell monolayers was determined in 5 ml of ACS II (Amersham International, Buckinghamshire, UK) by liquid scintillation counting. The protein contents of the cells solubilized in 0.1 N sodium hydroxide were determined by the method of Bradford (14) using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA) with bovine γ-globulin as the standard.

Statistical Analysis

Statistical significance of differences between mean values was calculated using the non-paired *t* -test. Multiple comparisons were performed using Dunnett's test after analysis of variance. $P < 0.05$ was considered significant.

RESULTS

Effect of Levofloxacin on the Transport of PAH in OK Cells

We first examined the effects of levofloxacin on transcellular transport from the basolateral-to-apical side and cellular accumulation of [^{14}C]PAH (15 μM) by OK cell monolayers. As shown in Figure 1, the transcellular transport of [^{14}C]PAH was completely inhibited and cellular accumulation was moderately decreased in the presence of 1 mM levofloxacin added to the basolateral side. These results indicated that levofloxacin completely inhibited PAH transport on the apical membrane and also interacted with its transport on the basolateral membrane of OK cell monolayers.

The effects of 500 μM levofloxacin on the kinetics of basolateral [^{14}C]PAH uptake were examined to analyze the type of inhibition (Fig. 2). Eadie-Hofstee analysis (Fig. 2, inset) showed that the Michaelis constants (K_m) in the absence and presence of levofloxacin were 66 ± 10 and 165 ± 19 μM, and the maximum uptake rates (V_{max}) were 229 ± 43 and 195 ± 35 pmol/mg protein/min (mean ± S.E. of three independent experiments), respectively. Thus, levofloxacin caused a significant increase in the K_m ($P < 0.05$), but did not affect the V_{max}. The apparent inhibition constant (K_i) of levofloxacin against PAH uptake was calculated as 333 μM, assuming the inhibition was a competitive type.

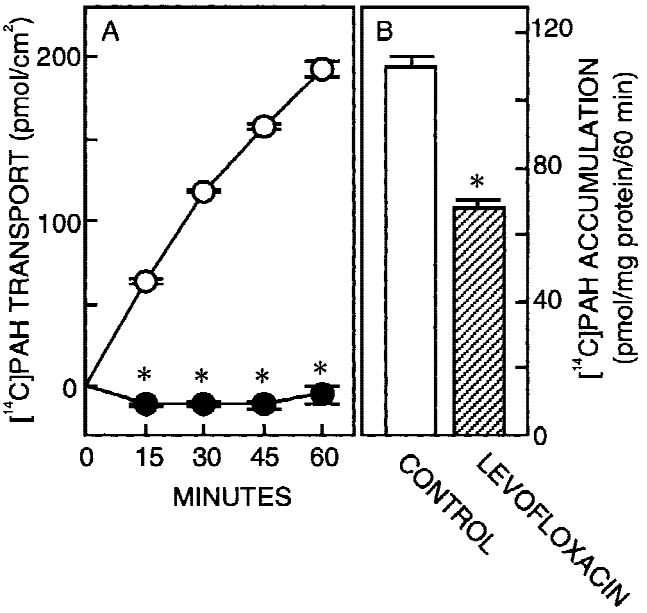

Fig. 1. Effect of levofloxacin on the basolateral-to-apical transcellular transport (A) and cellular accumulation (B) of PAH by OK cell monolayers. The monolayers were incubated for 60 min at 37°C with [^{14}C]PAH (15 μM) added to the basolateral side in the absence (○) or presence (●) of 1 mM levofloxacin. After incubation, the appearance of radioactivity on the opposite side was measured. After 60 min, accumulation was determined in the absence or presence of 1 mM levofloxacin. Each point or column represents the mean ± S.E. of three monolayers. *$P < 0.05$, significantly different from corresponding open symbol.

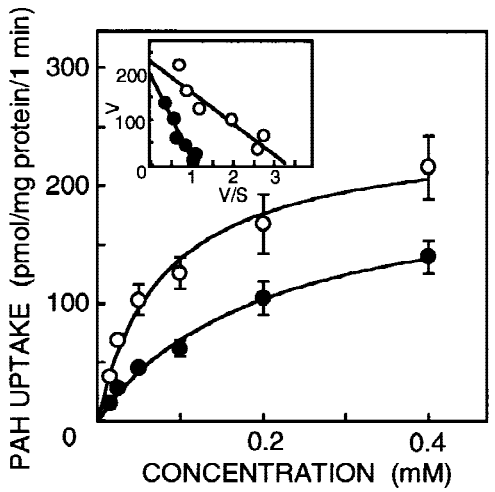

Fig. 2. Effect of levofloxacin on kinetics of PAH uptake from the basolateral side of OK cell monolayers. The basolateral uptake of various concentrations of [^{14}C]PAH for 1 min was measured in the absence or presence of 500 μM levofloxacin added to the basolateral side. The inset shows Eadie–Hofstee plots of the data. Each point represents the mean ± S.E. of three separate experiments.

To investigate the interaction of other quinolones with the basolateral transport of PAH, we examined the effects of various quinolones on [^{14}C]PAH uptake from the basolateral side (Fig. 3). The basolateral uptake of [^{14}C]PAH was markedly inhibited by the presence of all quinolones examined (1 mM). On the other hand, tetraethylammonium, a cationic compound, did not influence [^{14}C]PAH uptake.

Next, to confirm that levofloxacin also interacts with PAH transport across the apical membrane, we measured the efflux of [^{14}C]PAH to the apical side in the presence of levofloxacin. As shown in Figure 4, the efflux of [^{14}C]PAH was decreased and the amount of [^{14}C]PAH remaining in the cell was increased by levofloxacin as well as probenecid, a typical anion transport inhibitor.

Transport of Levofloxacin in OK Cells

To clarify the mechanisms of quinolone transport by OK cell monolayers and whether organic anion transport systems contribute to the transport of quinolones, we examined the transcellular transport and cellular accumulation of [^{14}C]levofloxacin (15 μM). The basolateral-to-apical transport of [^{14}C]levofloxacin was much greater than the apical-to-basolateral transport at each time point (Fig. 5A). The transcellular transport of [^{14}C]levofloxacin from the basolateral-to-apical side was significantly decreased and that in the opposite direction was significantly increased by 1 mM unlabeled levofloxacin added to the same compartment as [^{14}C]levofloxacin. The cellular accumulation of [^{14}C]levofloxacin from both sides was significantly increased in the presence of 1 mM unlabeled levofloxacin (Fig. 5B). These results indicated that [^{14}C]levofloxacin was transported in the outward direction on the apical membrane and unlabeled levofloxacin inhibited this transport.

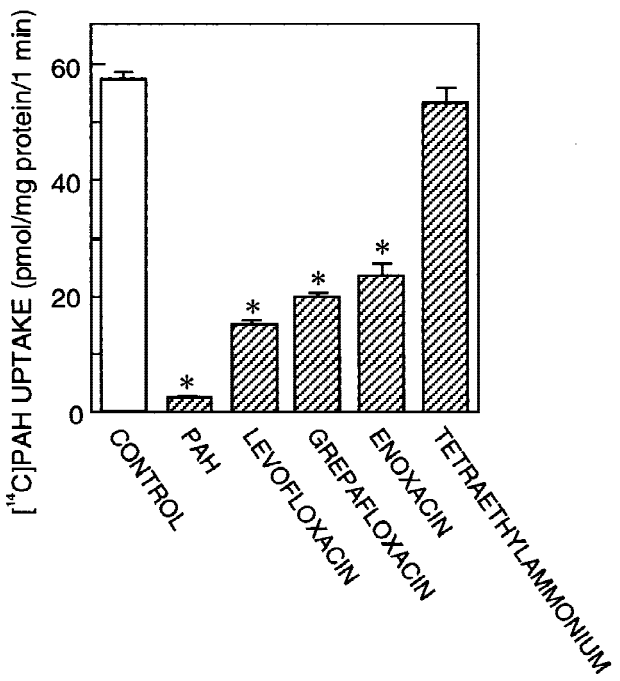

Fig. 3. Effect of various compounds on PAH uptake from the basolateral side of OK cell monolayers. [^{14}C]PAH (15 μM) uptake for 1 min was measured in the absence (control) or presence of 1 mM various compounds added to the basolateral side. Each column represents the mean ± S.E. of three monolayers. *P <0.05, significantly different from control.

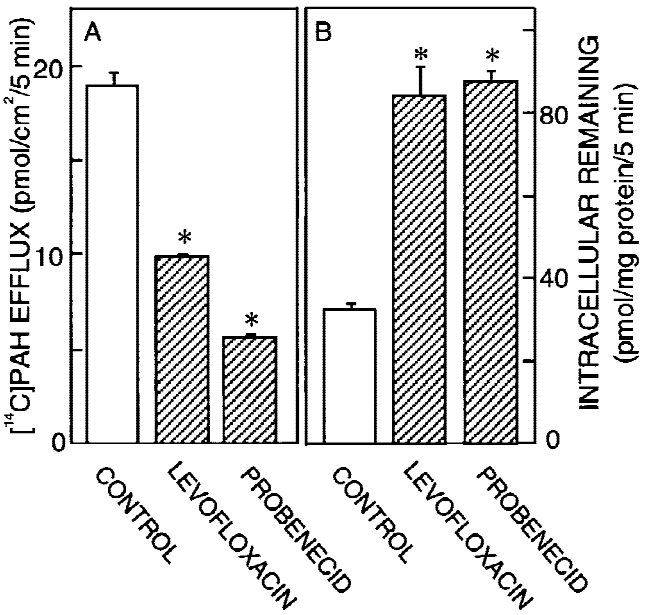

Fig. 4. Effect of levofloxacin and probenecid on PAH efflux to the apical side of OK cell monolayers (A) and residual intracellular PAH (B). [^{14}C]PAH (15 μM) was added to the basolateral side of the monolayers. After incubation for 30 min at 37°C, the monolayers were washed and [^{14}C]PAH efflux to the apical side was measured at 5 min in the absence (control) or presence of 2 mM levofloxacin or probenecid added to both sides. Intracellular remaining [^{14}C]PAH during the efflux experiment was measured at 5 min. Each column represents the mean ± S.E. of three monolayers. *P <0.05, significantly different from control.

To ascertain that a specific transport mechanism contributes to the transport of quinolones on the apical membrane, we examined the efflux of [^{14}C]levofloxacin to the apical and basolateral sides of OK cell monolayers. The efflux of [^{14}C]levofloxacin to the apical side was greater than that to the basolateral side (Fig. 6A). The amount of [^{14}C]levofloxacin remaining in the cell was almost zero at 5 min due to efflux (Fig. 6B).

Because levofloxacin interacted with the PAH transport in the basolateral membrane, we examined the effects of PAH (1 mM) and the anion transport inhibitors probenecid (1 mM) and DIDS (0.1 mM) on [^{14}C]levofloxacin uptake from the basolateral side. The basolateral uptake of [^{14}C]levofloxacin was not influenced by PAH or probenecid (control, 56.1 ± 2.6; with PAH, 63.7 ± 2.8; with probenecid, 60.3 ± 2.4 pmol/mg protein/1 min, mean ± S.E. of three monolayers) or DIDS, although unlabeled levofloxacin (30 mM) significantly inhibited the basolateral uptake of [^{14}C]levofloxacin (control, 72.1 ± 0.7; with DIDS, 67.7 ± 4.0; with unlabeled levofloxacin 53.1 ± 2.3 pmol/mg protein/1 min, mean ± S.E. of three monolayers).

Next, to characterize the transport of levofloxacin and to evaluate the contribution of the organic anion transport systems to the transport of levofloxacin at the apical membrane, we examined the effects of quinolones, PAH and anion transport inhibitors on the basolateral-to-apical transcellular transport and cellular accumulation of [^{14}C]levofloxacin by OK cell monolayers. The transcellular transport of [^{14}C]levofloxa-

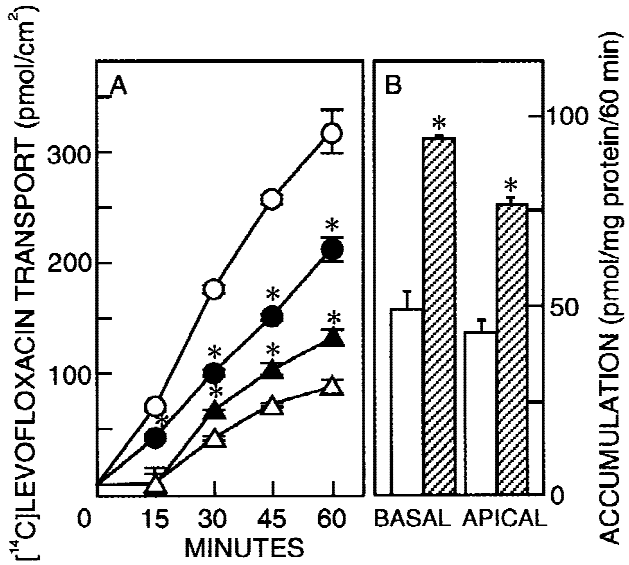

Fig. 5. Effect of unlabeled levofloxacin on the transcellular transport (A) and cellular accumulation (B) of [^{14}C]levofloxacin by OK cell monolayers. The monolayers were incubated for 60 min at 37°C with [^{14}C]levofloxacin (15 μM) added to either the basolateral (○,●) or apical side (△,▲) of monolayers in the absence (open symbols) or presence (solid symbols) of 1 mM unlabeled levofloxacin. After incubation, the appearance of radioactivity on the opposite side was measured. After 60 min, accumulation was determined in the absence (open columns) or presence (hatched columns) of 1 mM unlabeled levofloxacin. Each point or column represents the mean ± S.E. of three monolayers. *P <0.05, significantly different from corresponding open symbol.

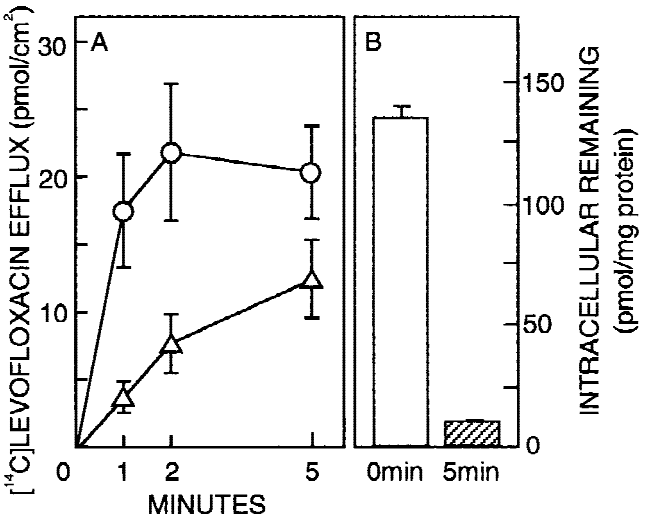

Fig. 6. Efflux of levofloxacin from OK cell monolayers (A), and residual intracellular levofloxacin (B). [^{14}C]Levofloxacin (15 μM) was added to both sides of the monolayers. After incubation for 30 min at 37°C, the monolayers were washed and [^{14}C]levofloxacin efflux to the apical (○) or the basolateral (△) side was measured at 1, 2, and 5 min. Intracellular remaining [^{14}C]levofloxacin during the efflux experiment was measured at 0 and 5 min. Each point and column represents the mean ± S.E. of six monolayers from two separate experiments.

cin was inhibited by unlabeled levofloxacin and grepafloxacin added to the basolateral side, whereas cellular accumulation was increased (Fig. 7). The anionic compound probenecid showed similar effects, although the inhibition of the transcellular transport was not statistically significant. In another experiment, the transcellular transport of [^{14}C]levofloxacin at 60 min was also inhibited by unlabeled levofloxacin and grepafloxacin (1 mM), accompanied by increased cellular accumulation (transcellular transport: control, 302 ± 10; with unlabeled levofloxacin, 144 ± 2; with grepafloxacin, 146 ± 4 pmol/cm^2/60 min, mean ± S.E. of three monolayers) (accu-

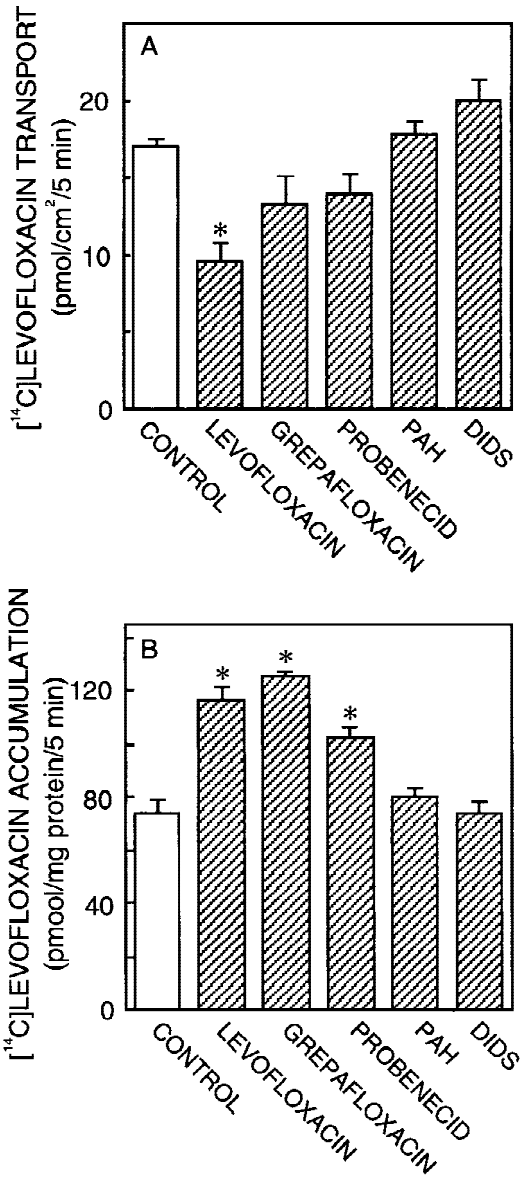

Fig. 7. Effect of various compounds on the transcellular transport (A) and cellular accumulation (B) of levofloxacin by OK cell monolayers. The monolayers were incubated for 5 min at 37°C with [^{14}C]levofloxacin (15 μM) added to the basolateral side in the absence (control) or presence of various compounds (1 mM or 0.1 mM for DIDS) added to the same compartment as [^{14}C]levofloxacin. After incubation, the appearance of radioactivity on the opposite side and accumulation were measured. Each column represents the mean ± S.E. of three monolayers. * P< 0.05, significantly different from control.

mulation: control, 58.8 ± 1.7; with unlabeled levofloxacin, 86.2 ± 3.1; with grepafloxacin 129 ± 9 pmol/mg protein/60 min, mean ± S.E. of three monolayers). These results indicated that quinolones and probenecid inhibited the apical efflux of levofloxacin out of the cell monolayers. However, PAH and DIDS did not affect either transcellular transport or accumulation of [^{14}C]levofloxacin.

DISCUSSION

The urinary excretion of a wide variety of endogenous organic ions and xenobiotics is mediated via organic cation or anion transport systems expressed in the renal proximal tubular cells. Since quinolone antibacterial drugs are zwitterions at physiological pH and most of them are excreted into the urine by tubular secretion, the contribution of organic cation or anion transport system to the renal secretion of quinolone antibacterial drugs is suggested. In this study, to evaluate the transport characteristics of quinolones, we examined the effect of levofloxacin on PAH transport and the transport of levofloxacin by OK cell monolayers.

Inhibition studies on PAH uptake from the basolateral side and efflux to the apical side revealed that levofloxacin inhibited both basolateral and apical transport of PAH. That is, levofloxacin decreased the basolateral uptake of PAH, and completely inhibited the apical efflux after entering the cell monolayers. These two continuous inhibitory processes could cause the complete inhibition of transcellular transport of PAH from the basolateral to apical side, with moderate inhibition of cellular accumulation. In OK cells, the organic anion transport system on the basolateral membrane has been well characterized (10,12,13). Therefore, we further examined the interaction of quinolones with the basolateral transport of PAH. Levofloxacin inhibited PAH uptake from the basolateral side in a competitive fashion with an apparent K_i of 333 μM. Grepafloxacin and enoxacin also significantly inhibited PAH uptake across the basolateral membrane. Using the stopflow peritubular capillary perfusion method, Ullrich *et al.* (15) demonstrated that quinolones inhibited the transport of PAH through the basolateral membrane, consistent with the present findings. Recently, organic anion transporter (OAT1) was isolated from rat kidney (16,17) and the characteristics of PAH uptake across basolateral membrane in OK cells were shown to be similar to those of OAT1 (18). Therefore, quinolones were considered to interact with PAH transport via OAT1.

To determine whether organic anion transport systems contribute to levofloxacin transport, we examined the transport of levofloxacin by OK cell monolayers. Levofloxacin was transported unidirectionally from the basolateral to apical side by OK cell monolayers, corresponding to renal secretion. This unidirectional transport of [^{14}C]levofloxacin was inhibited by unlabeled levofloxacin. Another quinolone, grepafloxacin, was also transported unidirectionally from the basolateral to apical side (data not shown). These results suggested that the unidirectional transport of quinolones is mediated by specific transport systems in OK cell monolayers. However, DIDS (0.1 mM) and PAH (1 mM) did not affect the basolateral uptake or transcellular transport of levofloxacin. Therefore, we considered that PAH transport systems on both the basolateral and apical membranes did not contribute to the transport of levofloxacin. On the other hand, probenecid, another organic anion transport inhibitor, showed a moderate inhibitory effect on the apical transport of levofloxacin. Because probenecid also inhibited other transport systems such as the organic cation transport systems (19), we speculated that probenecid inhibited transport of levofloxacin via transport mechanisms other than the PAH transport system. Previous studies showed that probenecid decreased the renal clearance of several quinolones and suggested the contribution of the organic anion transport systems to the excretion of quinolones (3,5). The present results suggested that this inhibition would be caused by the interaction of probenecid with the transport systems other than the organic anion transport systems for PAH.

In a previous study using LLC-PK$_1$ cell monolayers, we demonstrated that levofloxacin was transported via carrier-mediated processes distinct from the organic cation transport systems on both apical and basolateral membranes, and that levofloxacin had higher affinity for the transport system on the apical membrane compared with that on the basolateral membrane (9). In the present study using OK cell monolayers, the efflux of levofloxacin to the apical side was greater than that to the basolateral side. In addition, quinolones (1 mM) inhibited the transport of levofloxacin across the apical membrane. These results indicated that the specific transport mechanism for quinolone antibacterial drugs is also present on the apical membrane of OK cell monolayers. The coincidence of the results obtained in these two different kidney epithelial cell lines emphasized the possibility that the specific transport system that contributes to renal secretion of quinolone antibacterial drugs may exist on the apical membrane of renal proximal epithelial cells. In addition, the finding that LLC-PK$_1$ cells do not have the PAH transport activity (10) also supports our speculation that PAH transport systems in OK cells did not contribute to the transport of levofloxacin. We previously showed that levofloxacin was transported by human P-glycoprotein, an ATP-dependent efflux pump, in cells transfected with *MDR1,* with an apparent K_m of 3.0 mM (20). Because 1 mM unlabeled levofloxacin significantly inhibited the vectorial transport of [^{14}C]levofloxacin, P-glycoprotein was not considered to contribute to the transport of levofloxacin across the apical membrane of OK cell monolayers. Although 1 mM unlabeled levofloxacin did not inhibit the basolateral transport of [^{14}C]levofloxacin (data not shown), 30 mM unlabeled levofloxacin showed a significant inhibitory effect in OK cell monolayers. Therefore, we speculated that the specific transport system is also involved in the basolateral transport of levofloxacin, and that levofloxacin might have a low affinity for the basolateral transport system in OK cells, as well as in LLC-PK$_1$ cells. We recently demonstrated that basolateral uptakes of levofloxacin and grepafloxacin were mediated by the same specific transport system using rat renal cortical slices (21), which supported the present speculations.

In conclusion, levofloxacin inhibits PAH transport on both the basolateral and apical membranes of OK cells, but are not transported via the transport systems for PAH. The existence of a specific transport system for quinolones was indicated in OK cells.

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