Stereoselective Permeation of New Fluorinated Quinolone Derivatives across LLC-PK₁, Cell Monolayers

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We examined the stereoselective membrane permeation of new fluorinated quinolone derivatives (NQs) across LLC-PK₁, cell monolayers, using levofloxacin (LVFX) and its R(+)-isomer. LVFX permeation was 1.6-fold greater in the basal-to-apical direction than that in the apical-to-basal direction, suggesting that LVFX permeated LLC-PK₁, cell monolayers in a secretory-oriented manner. In contrast to LVFX, the permeation of the R(+)-isomer was almost identical in both directions. LVFX permeation in the basal-to-apical direction was significantly reduced in the presence of guanidine, enoxacin, and l-arginine, whereas tetracyclammonium, d-arginine, d- and l-lysine had no effect on the basal-to-apical permeation of LVFX. Basal-to-apical permeation of the R(-)-isomer was not affected by these compounds. Cellular accumulation of LVFX was inversely increased when guanidine suppressed the appearance of LVFX in the apical medium in a concentration-dependent manner. These results imply that the inhibitory effect of guanidine on the basal-to-apical permeation of LVFX involves the permeation process across the apical membrane. Guanidine trans-stimulated the efflux of LVFX from LLC-PK₁, cells but did not affect cimetidine efflux. These results suggest that some NQs, like LVFX and its R(+) isomer, are stereoselectively secreted across LLC-PK₁, cell monolayers and that an organic cation transport system, which favors guanidine as a typical substrate, may be involved in the secretory-oriented permeation of some NQs.

Key words stereoselectivity; levofloxacin; LLC-PK₁; permeation; secretion

Many new fluorinated quinolone derivatives (NQs), such as enoxacin (ENX), ciprofloxacin (CPFX), and levofloxacin (LVFX), are now being used clinically to treat a variety of infectious diseases. A well-known feature of these antibacterial agents is that most of them are extensively excreted in the urine without being metabolized. Therefore, many studies have been undertaken to clarify the renal handling of NQs. Recent studies have indicated that NQs are transported in part by organic cation transport systems, in spite of their zwiterionic dissociation at physiological pH.3-5 By using LLC-PK₁, cell monolayers, a well-established experimental model to characterize the renal organic cation transport system,1 we previously reported that ENX and CPFX permeate cell monolayers in a secretory-oriented manner. The basal-to-apical permeation of these NQs was saturable and significantly inhibited by guanidine, but not by tetracyclammonium (TEA), suggesting that an organic cation transport system, distinct from that governing TEA secretion, is involved in the secretory permeation of these NQs.6-7

Most of the transporters in the body exhibit substrate stereoselectively, as in the case of the carrier-mediated absorption of monosaccharides and amino acids. Organic cation transporters, located on the membrane of renal proximal tubules, are actively involved in the secretion of many endo- and exogenous organic cations.6 Since many organic cations are chiral and administered as racemic mixtures, a variety of studies have been performed to investigate the possible stereoselectivity of renal organic cation transporters. In vivo and in vitro evidence of stereoselective renal handling via organic cation transporters has been reported for a number of enantiomeric compounds.9-14 Some NQs, like ofloxacin and lomefloxacin, are also administered as racemates. In the case of ofloxacin, it has become obvious that the S(-) isomer (LVFX) is pharmacologically active whereas the R(+) isomer is inactive. However, little information is currently available on stereoselectivity in the renal handling of NQs. Recently, it was demonstrated in an in vivo model that there was no significant difference in the urinary excretion rates of R(+) and S(-)-grepafloxacin.15 However, the main part of the study focused on the hepatobiliary transport of grepafloxacin and its glucuronide in the rat. Therefore, the result provided insufficient evidence to conclude that there was no stereoselectivity in the renal handling of NQs.

In this study, we compared the permeation of LVFX and its R(+) isomer across LLC-PK₁, cell monolayers. The results indicate that LVFX permeates cell monolayers in a much more secretory-oriented manner compared with its R(+) isomer, suggesting that a stereoselective secretory process actually is involved in the renal handling of some NQs.

MATERIALS AND METHODS

Materials LVFX and its R(+) isomer were kindly supplied by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). ENX, tetracyclammonium chloride (TEA), guanidine hydrochloride, D- and L-lysine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Cimetidine (CMD) was obtained from Tokyo Chemical Ind. (Tokyo, Japan). D- and L-Arginine hydrochloride were obtained from Wako Pure Chemical Ind. (Osaka, Japan). All other chemicals used were of the highest purity available. α-[3H] Mannitol (832.5 GBq/mmol) was purchased from Du Pont New England Nuclear Research Products (Boston, MA, U.S.A.).

Cell Culture LLC-PK₁ cells, at passage 191, were obtained from the HSRRB cell bank (Osaka, Japan). At passage 208-234, the cells were seeded on 60 mm plastic culture dishes coated with rat tail collagen type I (Becton Dickinson, Bedford, MA, U.S.A.) at a density of 4×10⁶ cells in 5 ml cul-

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ture medium. The culture medium used was Medium 199 (Gibco, Grand Island, NY, U.S.A.) supplemented with 5% fetal calf serum. The medium was replaced with fresh medium every 2–3 d after inoculation, and the cells developed to confluence after 6–7 d of culture in an atmosphere of 5% CO₂ in air at 37 °C. Subcultures were carried out every 5–6 d using 0.02% EDTA and 0.25% trypsin.

**Permeation of LVFX and Its R(+)-Isomer across LLC-PK₁, Cell Monolayers** The permeation of LVFX and its R(+)-isomer was measured using LLC-PK₁ cell monolayers cultured on Transwell chambers with a surface area of 4.2 cm² (Becton Dickinson, Bedford, MA, U.S.A.). LLC-PK₁ cells were seeded at a density of 4×10⁵ cells/cm² on polycarbonate membrane filters (pore size, 0.45 μm) of Transwell chambers and the chambers were placed in a six-well cluster. The volume of medium applied to the apical and basal side was 2.0 and 2.5 ml, respectively. The medium was replaced every 2–3 d and the cell monolayers were used for permeation experiments between 7–8 d. Usually, the transepithelial electrical resistance (TEER) across the monolayers was over 1800 Ω·cm². The permeation experiments were started by adding Dulbecco’s phosphate-buffered saline (PBS, 137 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 1 mM CaCl₂, 0.5 mM MgCl₂, and 5 mM d-glucose, pH 7.4) containing 25 μM LVFX or R(+)-isomer, either to the apical or basal side of the monolayers (the medium volume on the apical and basal side was 1.5 and 2.2 ml, respectively). After incubation for specified periods, the medium in the opposite side was sampled. To measure cellular accumulation, the medium was immediately aspirated and both sides of the cell monolayers were rinsed three times with 2 ml ice-cold PBS. The filters with the cell monolayers were detached from the chambers, and the cells on the filters were immersed in 0.5 ml extraction medium [30 mM phosphate buffer (pH 7.0)–MeOH (1:1)] for 1 h at room temperature. The concentrations of LVFX or R(+)-isomer in the extracts were determined. In the assay of the permeation samples of LVFX and its R(+)-isomer, 20 μl pipemic acid (100 μM) was used as an internal standard and added to 100 μl sample solution, then, an aliquot of the mixture was applied to HPLC. In the assay of the cellular accumulation, 100 μl pipemic acid (100 μM) was added to 0.5 ml extract, the sample was centrifuged at 2750×g for 20 min, and the supernatant was applied to HPLC.

In order to evaluate the paracellular permeability in the LLC-PK₁ cell monolayers used in this study, the permeation of d-[³H]mannitol (4 μM) was investigated in separate experiments.

**Trans-stimulation Effect of Guanidine on Efflux of LVFX and CMD from LLC-PK₁ Cells** LLC-PK₁ cells were grown on 60-mm plastic culture dishes coated with rat tail collagen type I and LLC-PK₁ cells were seeded at a density of 4×10⁵ cells/cm². The medium was replaced every 2–3 d and the cell monolayers were used for permeation experiments between 6–7 d. After removing the culture medium, the cells were washed three times with 4 ml PBS and preincubated with drug-free PBS at 37 °C for 10 min. After removing the medium, cells were incubated with 2 ml PBS containing 0.2 mM LVFX or 1 mM CMD at 37 °C for 10 min in order to preload the LLC-PK₁ cells with these test compounds. The medium was removed after incubation and the dishes were rapidly rinsed three times with 4 ml PBS. Two milliliters PBS, with or without 0.1 mM guanidine, was added to the apical side and cells were incubated for 30 s. The medium was immediately aspirated at the end of the incubation. The cells were rinsed three times with 4 ml ice-cold PBS and scraped with a cell scraper into 2 ml ice-cold PBS and homogenized in a polytom homogenizer (Kinematica, Kriens-Luzen, Switzerland). One milliliter methanol was added to 1 ml homogenate and, after centrifugation, the supernatant was applied to HPLC.

**Analytical Methods** The HPLC analytical methods for LVFX, its R(+)-isomer, and CMD were developed in our laboratory. An HPLC system, LC-6A (Shimadzu, Kyoto, Japan), equipped with a UV detector, SPD-6A (Shimadzu), was used. The assay conditions were as follows: column, Inertsil C8 (5 μm, 5×250 mm, GL Sciences Inc., Tokyo, Japan); mobile phase, acetonitrile–30 mM citric acid containing 0.2% triethylamine (1:6) for NQs and acetonitrile–0.01 M KH₂PO₄ (2:9) for CMD; flow rate, 1 ml/min; wavelength, 293 nm for NQs and 228 nm for CMD; column temperature, 50 °C; injection volume, 20–50 μl. The internal standard for the CMD assay was fomotidine. Protein concentrations were determined by the method of Bradford[60] using a Bio-Rad Protein Assay Kit, with bovine serum albumin as a standard.

**Statistical Analysis** Statistical significance between mean values was analyzed by the non-paired Student's t-test. Multiple comparisons were done using one-way ANOVA followed by Tukey's test. The level of significance was p<0.05.

**RESULTS**

**Permeation of d-[³H] Mannitol, LVFX, and Its R(+)-Isomer across LLC-PK₁, Cell Monolayers** As shown in Table 1, permeation of d-[³H]mannitol (4 μM), a marker compound of paracellular permeation, across the LLC-PK₁ cell monolayers used in this study was slightly greater in the apical-to-basal direction than in the basal-to-apical direction. However, there was no statistical difference between them, indicating that paracellular permeation was substantially equal in both directions. The amount of d-[³H]mannitol adhering to the cell surface was negligible. Figure 1 demonstrates the permeation of 25 μM LVFX and its R(+)-isomer across LLC-PK₁ cell monolayers. Although the concentration of 25 μM used in this study was high, in terms of the therapeutic plasma concentration of LVFX, it was used to compare the permeation characteristics of LVFX with those of ENX and CFPX. LVFX permeation was much greater in the basal-to-apical direction than in the apical-to-basal direction (Fig. 1). Although the data in Fig. 1 involve transcellular and

<table>
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<tr>
<th>Direction</th>
<th>Permeation (nmol/cm²)</th>
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<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Apical-to-Basal</td>
<td>0.033±0.003</td>
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<tr>
<td>Basal-to-Apical</td>
<td>0.028±0.001</td>
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</table>

Table 1. Permeation of d-[³H] Mannitol across LLC-PK₁, Cell Monolayers. LLC-PK₁ cell monolayers were incubated at 37 °C with 4 μM d-[³H]mannitol added either to the apical or basal side of the monolayers. The appearance of d-[³H]mannitol in the opposite side was determined. Each piece of data represents the mean±S.E. of 3 determinations.
paracellular fluxes, it can be said that the secretory-oriented permeation of LVFX is involved in the transcellular transport process according to the results for d-[3H]mannitol (Table 1). The flux ratio, expressed as basal-to-apical permeation/apical-to-basal permeation, was 1.6 at 60 min. The apical-to-basal permeation of the R-(+)-isomer was almost identical to that of LVFX, and the basal-to-apical permeation of the R-(+)-isomer was significantly less than that of LVFX. Accordingly, the flux ratio of the R-(+)-isomer was, at most, 1.2 at 60 min, indicating very weak secretory-oriented movement of the R-(+)-isomer.

**Effect of Various Organic Cations on Basal-to-Apical Permeation of LVFX and Its R-(+)-Isomer across LLC-PK1, Cell Monolayers**

In order to clarify the mechanism underlying the secretory-oriented permeation of LVFX, the inhibitory effects of various compounds were examined on the basal-to-apical permeation of LVFX and its R-(+)-isomer. As shown in Table 2, of the compounds tested, guanidine, ENX, and l-arginine significantly inhibited the basal-to-apical permeation of LVFX. The inhibitory effect of these three inhibitors was almost identical and not very marked. On the other hand, TEA, d-arginine, p-, and l-lysine had no inhibitory effect on LVFX permeation in the same direction. A significant change in the cellular accumulation of LVFX was observed only in the presence of ENX, which reduced the cellular accumulation of LVFX. In addition, the basal-to-apical permeation of the R-(+)-isomer was unchanged in the presence of any of the compounds tested in this study. However, TEA increased the cellular accumulation of the R-(+)-isomer significantly.

**Concentration-Dependence of the Inhibitory Effect of Guanidine on Basal-to-Apical Permeation of LVFX and Its R-(+)-Isomer**

The relationship between guanidine concentration and its inhibitory effect on the basal-to-apical permeation and cellular accumulation of LVFX and its R-(+)-isomer was examined using 3 different concentrations of guanidine. As demonstrated in Fig. 2, the basal-to-apical permeation of LVFX was reduced and its cellular accumulation was increased when a higher guanidine concentration was used. It appeared that the inhibitory effect of guanidine on LVFX permeation reached a maximum at around 1 mm, al-

![Fig. 1. Permeation of LVFX and Its R-(+)
Isomer across LLC-PK1, Cell Monolayers](image1)

![Fig. 2. Concentration-Dependence of the Guanidine Effect on the Basal-to-Apical Permeation and Cellular Accumulation of LVFX and Its R-(+)
Isomer in LLC-PK1, Cell Monolayers](image2)

**Table 2. Effect of Various Compounds on Basal-to-Apical Permeation and Cellular Accumulation of LVFX and Its R-(+)
Isomer in LLC-PK1, Cells**

| Additives   | LVFX                     | R-(+)
<table>
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<tr>
<td></td>
<td>Permeation (nmol/cm²)</td>
<td>Accumulation (nmol/mg protein)</td>
</tr>
<tr>
<td>Control</td>
<td>0.350±0.012</td>
<td>0.353±0.018</td>
</tr>
<tr>
<td>+ Gua</td>
<td>0.292±0.005**</td>
<td>0.394±0.013</td>
</tr>
<tr>
<td>+ TEA</td>
<td>0.352±0.008</td>
<td>0.389±0.025</td>
</tr>
<tr>
<td>+ ENX</td>
<td>0.292±0.014*</td>
<td>0.232±0.018*</td>
</tr>
<tr>
<td>+ l-Arginine</td>
<td>0.311±0.007*</td>
<td>0.341±0.024</td>
</tr>
<tr>
<td>+ d-Arginine</td>
<td>0.338±0.006</td>
<td>0.345±0.023</td>
</tr>
<tr>
<td>+ l-Lysine</td>
<td>0.356±0.006</td>
<td>0.283±0.051</td>
</tr>
<tr>
<td>+ d-Lysine</td>
<td>0.365±0.010</td>
<td>0.365±0.038</td>
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LVFX and its R-(+)-isomer (25 μm) were added to the basal side of the cell monolayers, with or without 0.25 mm additive. The monolayers were incubated at 37°C for 60 min and then the concentration in the apical medium was determined. After incubation, the cell monolayers were immersed in an extraction medium to assay cellular accumulation. Each piece of data represents the mean±S.E. of 4 to 6 determinations. *p<0.05, **p<0.01, significantly different from control.
Table 3. Effect of Guanidine on Efflux of LVFX and CMD from LLC-PK₁ Cell Monolayers

<table>
<thead>
<tr>
<th></th>
<th>Amount in LLC-PK₁, cell monolayers (nmol/mg protein)</th>
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<tr>
<td></td>
<td>LVFX</td>
</tr>
<tr>
<td>Control</td>
<td>7.98±0.21</td>
</tr>
<tr>
<td>+0.1 mM Guanidine</td>
<td>6.80±0.33*</td>
</tr>
</tbody>
</table>

The cell monolayers were grown on plastic culture dishes. The cell monolayers were preincubated with 0.2 mM LVFX or 1 mM CMD for 10 min. After removal of the medium, the cells were rinsed with drug-free PBS. Efflux of LVFX and CMD was initiated by adding drug-free PBS or 0.1 mM guanidine to the cell monolayers. After 30 s, the amount remaining in the cells was determined. Each value represents the mean±S.E. of 5 determinations. * p<0.05, significantly different from control.

though 77% of the basal-to-apical permeation of LVFX was maintained. On the other hand, significant modification in the basal-to-apical permeation and intracellular accumulation of the R(-) isomer was observed only in the presence of 1 mM guanidine. However, the reduction in the basal-to-apical permeation of the R(-) isomer was very small, less than 10%.

**Effect of Guanidine on Efflux of LVFX and CMD from LLC-PK₁ Cell Monolayers** In order to determine if the reduction in the basal-to-apical permeation of LVFX in the presence of guanidine implies that these 2 compounds are common substrates of a specific transport system present in the LLC-PK₁ cell membrane, the trans-stimulation effect of guanidine on the efflux of LVFX and CMD across apical membrane was compared. As shown in Table 3, when 0.1 mM guanidine was added to the apical medium, the amount of LVFX remaining in LLC-PK₁ cell monolayers after 30 s was significantly less than that following addition of drug-free PBS. On the other hand, the amount of CMD remaining in the monolayers after 30 s was not altered by the presence or absence of guanidine in the apical medium.

**DISCUSSION**

In this study, we investigated the permeation of LVFX and its R(+)-isomer across LLC-PK₁ cell monolayers, focusing especially on whether there is stereoselective transport of NQs in this cell line. The results can be summarized as follows: 1) LVFX movement across the cell monolayers was secretory-oriented, compared with its R(-) isomer (Fig. 1), 2) the basal-to-apical permeation of LVFX was significantly inhibited by guanidine, ENX, and l-arginine, but not by TEA, d-arginine, D, and L-lysine (Table 2), 3) cellular accumulation of LVFX was inversely increased when its appearance in the apical medium was reduced in the presence of guanidine (Fig. 2), and 4) apically-added guanidine enhanced the efflux of LVFX from LLC-PK₁ cells, but not efflux of CMD (Table 3). According to these observations, it is very likely that a specialized transport system is involved in the basal-to-apical permeation of LVFX, as reported previously. The result that the basal-to-apical permeation of LVFX is affected by guanidine but not by TEA is consistent with our previous observations with ENX and CPFX. Using renal brush-border membrane vesicles from rabbit and human, several investigators have demonstrated that guanidine, an endogenous organic cation, is transported by a mechanism distinct from that involved in the transport of TEA and N¹-methylnicotinamide in the kidney. Therefore, the current belief is that the transport of organic cations in the kidney is mediated by several carrier systems. So far, however, there has been no direct evidence showing that a similar guanidine transport system is expressed on the apical membrane of LLC-PK₁ cells. In this study, the cellular accumulation of LVFX was inversely increased when guanidine suppressed the appearance of LVFX in apical medium in a concentration-dependent manner (Fig. 2). These results indicate that an interaction between LVFX and guanidine takes place during secretory permeation across the apical membrane of LLC-PK₁ cells. However, it is often pointed out that cis-inhibition is not always direct evidence of competition between substrates of a specialized transporter. In contrast, it is generally accepted that a trans-stimulation effect is a convincing demonstration of carrier-mediated transport. Based on such trans-stimulation effects, Saitoh *et al.* for example, clearly characterized the substrate specificity of choline transporter in the rat intestinal brush-border membrane. Very recently, we developed a simple method to evaluate the trans-stimulation effect between possible substrates of organic cation transport systems in LLC-PK₁ cells and we showed that TEA and guanidine are transported in a secretory direction by distinct transport systems (unpublished data). This suggests the existence of a so-called guanidine-selective organic cation transport system in this cell line. In this study, guanidine trans-stimulated LVFX efflux across the apical membrane, strongly indicating that these two compounds are common substrates of a transport system expressed on the apical membrane of LLC-PK₁ cells. It is likely that some NQs are transported via a common transport system. l-Arginine, a basic amino acid having a guanidyl group, also inhibited the basal-to-apical permeation of LVFX. This result seems reasonable assuming the presence of a guanidine-selective transport system in LLC-PK₁ cells. We are now examining the trans-stimulation effect of l-arginine on the efflux of some NQs. As shown in Table 2, ENX reduced not only the appearance of LVFX in the apical medium but also its cellular accumulation. This result suggests an additional interaction between NQs during permeation of the basal membrane.

The permeation of the R(+) isomer was rather different from that of LVFX, i.e., this NQ exhibited very weak secretory-oriented movement across LLC-PK₁ cell monolayers and its basal-to-apical permeation was inhibited only slightly in the presence of high concentrations of guanidine. Neither ENX nor l-arginine inhibited basal-to-apical permeation of R(+) isomer. These findings strongly suggest that the R(+) isomer is a rather poor substrate for the transport system compared with LVFX. Therefore, it can be concluded that some NQs are stereoselectively secreted into urine via an organic cation transport system. TEA significantly increased cellular accumulation of the R(+) isomer without changing its basal-to-apical permeation (Table 2). Although this result is indicative of an interaction between TEA and the R(+) isomer in the secretory process, the exact mechanism for this is currently unclear.

Recently, using a P-glycoprotein transfectant derivative cell line of LLC-PK₁ (LLC-GAS-COL150), Ito *et al.* have shown that P-glycoprotein present on the brush-border membrane of the renal epithelium acts as a potent efflux pump to
secrete LVFX from cytosol into urine. In that study, they showed that cyclosporin A (Cys A), a potent modulator of P-glycoprotein, markedly interfered with the P-glycoprotein-mediated transport of LVFX in LLC-GA5-COL150 cells and that this modulator had no effect on the secretary-oriented permeation of LVFX in LLC-PK1 cells. We have also confirmed that Cys A does not have any significant effect on the basal-to-apical permeation of ENX (data not shown). Accordingly, the transport system involved in the secretary-oriented permeation of LVFX in LLC-PK1 cells is not due to P-glycoprotein expressed on the apical membrane of LLC-PK1 to a very small degree. It is, therefore, likely that at least two systems, P-glycoprotein and a specialized organic cation transporter, are involved in the renal handling of LVFX. Besides kidney, P-glycoproteins are expressed in various other tissues such as liver, brain, and intestine. We have recently observed that LVFX absorption from the rat intestine is strongly restricted by intestinal P-glycoprotein which explains the low oral bioavailability of LVFX in the rat. We also found that there is no stereoselectivity in the P-glycoprotein-mediated transport because the R(+) isomer is also transported by P-glycoprotein to similar extent (unpublished data). However, it is not yet clear if most NQs are commonly transported by P-glycoprotein in the kidney.

Comparing the chemical structures of ENX, CPFX, and LVFX (Fig. 3), an essential component to be a substrate of a carrier-mediated transport system in LLC-PK1 seems to be the piperazine ring because only this can be protonated. It has been reported that there is no stereoselectivity in the urinary excretion of R(+) and S(-)-grepafloxacin. The chiral centers of grepafloxacin and lomefloxacin involve the piperazine ring, whereas those of LVFX and its R(+)-isomer are located away from the piperazine ring (Fig. 3). Therefore, the location of the chiral center in the NQ structure could be an important factor in determining the stereoselective renal handling of these antibacterial agents. It is conceivable that the chiral moiety located away from the piperazine ring could modify significantly the affinity for the transporter. If so, stereoselective secretary permeation would occur in only a limited number of NQs, like LVFX and its R(+) isomer. Further studies are required in order to investigate this possibility.

Among the basic amino acids tested in this study, only l-arginine inhibited the basal-to-apical permeation of LVFX. d-Arginine, l- and l-lysine had no inhibitory effect on LVFX permeation. These results suggest that the transporter which carries LVFX as well as guanidine exhibit clear arginine stereoselectivity, preferring the l-isomer to the d-isomer. The fact that the l-isomer of lysine had no effect on LVFX transport suggests that the stereoselectivity of the transporter might be a secondary factor in the recognition of substrate molecules. Hence, the role of the organic cation transport system in interacting with some particular amino acids should be examined in more detail.

In conclusion, the present results suggest that some NQs are stereoselectively secreted across LLC-PK1 cell monolayers, possibly via an organic cation transport system that favors guanidine as a typical substrate. The transporter favors the S(-) isomer (LVFX) much more than the R(+) isomer.

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