Functional Characterization of Multidrug and Toxin Extrusion Protein 1 as a Facilitative Transporter for Fluoroquinolones

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ABSTRACT

Many fluoroquinolones are mainly eliminated by urinary excretion, in which tubular secretion by carrier-mediated transport systems has been suggested to be involved. In the present study, we examined the possibility that multidrug and toxin extrusion protein (MATE) 1, which is abundantly expressed in the kidney, might be involved in that, using rat MATE (rMATE) 1 expressed in MDCKII cells. It was found that rMATE1 can transport fluoroquinolones such as ciprofloxacin, enoxacin, gatifloxacinc, levofloxacin, norfloxacin (NFX), pefloxacin, and tosufloxacin. Although rMATE1 has been known as an apical organic cation/H^+ antiporter, detailed investigation of rMATE1-mediated uptake of NFX has revealed that it is not sensitive to intracellular acidification by treatments using NH_4Cl or nigericin, suggesting that the transmembrane proton gradient is not involved in its transport as a driving force. However, it was dependent on extracellular pH, being greatest at pH 7.0 and smaller at both acidic and basic pH in agreement with the profile of zwitterionization of NFX. The basal-to-apical transcellular transport of NFX in rMATE1-expressing MDCKII cells was greater than that in mock cells and insensitive to acidification of the apical medium, demonstrating proton gradient-independent functionality of rMATE1 in NFX efflux. Finally, rMATE1-mediated NFX uptake at pH 7.4 was saturable with the Michaelis constant of 55.3 µM and inhibited by cationic compounds, such as TEA and cimetidine. These results suggest that rMATE1 mediates the transport of NFX by a facilitative manner. MATE1 may play a key role in the renal tubular secretion of fluoroquinolones.

Fluoroquinolones are synthetic antibacterial agents with broad antimicrobial activity and widely used in the therapy of infections of urinary tract and respiratory tract and also systemic infections (Emmerson and Jones, 2003). Fluoroquinolones are usually administered orally because they are absorbed rapidly with relatively high bioavailability (Sörgel and Kinzig, 1993a). Many of them are eliminated mainly by renal excretion, although hepatic metabolism and biliary excretion may not be negligible. Earlier studies indicated that the renal excretion of fluoroquinolones involves tubular secretion in addition to glomerular filtration because the renal clearances of unbound fluoroquinolones were greater than the glomerular filtration rate (Sörgel and Kinzig, 1993b). It is likely that carrier-mediated transport mechanisms are involved in the tubular secretion of fluoroquinolones as that of various drugs and metabolic wastes.

It has been shown recently that breast cancer resistance protein (BCRP) has the ability to transport several fluoroquinolones, such as ciprofloxacin, norfloxacin (NFX), and ofloxacin (Merino et al., 2006). BCRP is an ATP-binding cassette (ABC) transporter, which can mediate efflux of a variety of exogenous and endogenous compounds (Sarkadi et al., 2006). It is expressed ubiquitously in the body, being most abundant in the epithelial cells of the intestine, kidney, and mammary gland, where it is localized at the apical membrane (Maliepaard et al., 2001; Huls et al., 2008). Thus, a possibility is that BCRP might be involved in the tubular secretion of fluoroquinolones. However, a recent pharmacokinetic study using Bcrp knockout mice has failed to demonstrate that (Ando et al., 2007). In the study, the total clearances of ciprofloxacin and ofloxacin, for which renal excretion...
involved significant tubular secretion has been suggested to be the major elimination pathway, were shown to be comparable between Bcrp knockout and wild-type mice. Therefore, BCRP does not seem to play a significant role in the tubular secretion of fluoroquinolones, and some other transporters might be responsible for that.

Multidrug and toxin extrusion protein (MATE) 1 is another type of efflux transporter identified quite recently (Otsuka et al., 2005). This transporter is primarily expressed in the kidney and liver, being localized at the apical membranes facing the lumen of the renal tubules and bile canaliculi, respectively, and can mediate the efflux of organic cations, such as TEA and cimetidine, using the transmembrane proton gradient as a driving force (Ohta et al., 2006; Tsuda et al., 2007). Although MATE1 has been characterized as an organic cation\textsuperscript{H\textsuperscript{+}} antiporter, it has recently been shown that human MATE (hMATE) 1 can also transport some organic anions and amphoteric compounds (Tanihara et al., 2007). Because fluoroquinolones are amphoteric and mostly exist as zwitterions at physiological pH because of the dissociation of a carboxyl group at 3-position of the quinolone ring and the protonation of a piperazine nitrogen in the side chain, we hypothesized that MATE1 may be able to mediate the transport of fluoroquinolones and play an important role in their renal tubular secretion.

Therefore, we examined that possibility, using rMATE1 expressed in MDCKII cells. Because we found that several fluoroquinolones are transported by rMATE1, we further investigated the transport mechanism, using NFX as a probe substrate.

Materials and Methods

Materials. \(^{14}\text{C}\text{-TEA} \) bromide (2.4 mCi/mmol) and \(^{3}\text{H}\text{-cimetidine} \) (20.0 Ci/mmol) were obtained from GE Healthcare (Chalfont St. Giles, UK). Ciprofloxacin, gatifloxacin, pazufloxacin, and tosufloxacin were obtained from LKT Labs (St. Paul, MN). Enoxacin, norfloxacin, and niflumic acid were obtained from Sigma-Aldrich (St. Louis, MO). Levofloxacin was synthesized by Daiichi Sankyo Co. Ltd. (Tokyo, Japan). All other reagents were of analytical grade and commercially obtained.

Cell Culture. MDCKII cells were maintained at 37°C and 5% CO\textsubscript{2} in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin.

Preparation of MDCKII Cells Stably Expressing rMATE1. cDNA of rMATE1 was cloned as described previously (Ohta et al., 2006). MDCKII cells were transfected with the plasmid carrying cDNA of rMATE1 by using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions, and cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and 800 µg/ml geneticin for 2 to 3 weeks. Antibiotic-resistant clones were selected and tested for the transport of \(^{3}\text{H}\text{-cimetidine.} \)

Cellular Uptake Study. MDCKII cells stably expressing rMATE1 (2.0 \times 10\textsuperscript{5} cells/well initially) were grown on 24-well plates for 3 days. The cells were preincubated in 1.0 ml of substrate-free K-based buffer (142 mM KCl, 0.8 mM KH\textsubscript{2}PO\textsubscript{4}, 0.8 mM MgSO\textsubscript{4}, 1.0 mM CaCl\textsubscript{2}, 25 mM glucose, and 10 mM HEPES, pH 7.4) for 5 min, and then transport assays were started by replacing the substrate-free K-based buffer with one containing a substrate (0.5 µM), unless otherwise indicated. When the effect of various compounds on substrate uptake was examined, test compounds were added only to the buffer for uptake period. All the procedures were conducted at 37°C.

Assays were stopped by addition of ice-cold substrate-free buffer (2 ml), and the cells were washed twice with 2 ml of the same buffer. The substrate accumulated in the cells was extracted with 300 µl of 0.1% formic acid/acetonitrile (70:30 \textsuperscript{(v/v)}) for 30 min, and niflumic acid (500 µM) was added as a reference compound. The extract was then centrifuged at 4°C and 15,000 g for 5 min. The supernatant was subjected to analysis by LC/MS/MS. Cellular protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. MDCKII cells transfected with empty pCRI-neo vector were used to determine the basal activity and are designated as mock cells.

Transcellular Transport Study. MDCKII cells stably expressing rMATE1 were seeded at a density of 1 \times 10\textsuperscript{5} cells on each polycarbonate membrane insert of Transwell (12-mm i.d., 3.0-µm pore size; Costar; Corning Life Science, Acton, MA) and grown to confluence for 7 days. The medium was replaced with substrate-free Na\textsuperscript{+}-based buffer (140 mM NaCl, 5 mM KCl, 0.4 mM KH\textsubscript{2}PO\textsubscript{4}, 0.8 mM MgSO\textsubscript{4}, 1.0 mM CaCl\textsubscript{2}, 25 mM glucose, and 10 mM HEPES, pH 7.4; 0.5 and 1.5 ml, respectively, for apical and basal compartments) for preincubation for 5 min. Transport assays were started by replacing the buffer in the basal compartment with one containing NFX (1 µM) with or without corticosterone (200 µM) as an rMATE1 inhibitor. Aliquots of medium (100 µl) were taken from the apical compartment at designated time points, and then the same volume of substrate-free Na\textsuperscript{+}-based buffer was added. The sample from the apical compartment was mixed with 100 µl of 0.3% ammonium formate/acetonitrile [90:10 (v/v)] and niflumic acid (500 µM) was added. It was then centrifuged at 4°C and 15,000 g for 5 min. The supernatant was subjected to analysis by LC/MS/MS.

LC/MS/MS Analysis. All samples were analyzed by using an LC/MS/MS system consisting of an Agilent series 1100 LC system (Agilent Technologies, Santa Clara, CA) and a tandem mass spectrometer (API 4000; Applied Biosystems, Foster City, CA). Chromatographic separation was performed with a Shim-pack XR-ODS column (2-mm i.d. \times 30 mm; Shimadzu, Kyoto, Japan). The mobile phase consisted of solvent A, 5 mM ammonium acetate/acetonitrile [5:95 (v/v), pH 3.0], and solvent B, 5 mM ammonium acetate/acetonitrile [95:5 (v/v), pH 3.0]. The mobile phase gradient was used for chromatographic separation: 50 to 100% B in 2 min (linear gradient) with a flow rate of 0.6 ml/min. The analysis was carried out in the multiple reaction monitoring mode with monitoring of precursor-product ion pairs of \textit{m/z} 320 \rightarrow 302 for norfloxacin and \textit{m/z} 283 \rightarrow 265 for niflumic acid as a reference compound, respectively. The precursor-product ion pairs for CFX, enoxacin, gatifloxacin, levofloxacin, pazufloxacin, and tosufloxacin were \textit{m/z} 322.1 \rightarrow 313.9, 321.0 \rightarrow 303.2, 376.0 \rightarrow 358.4, 363.1 \rightarrow 319.1, 319.0 \rightarrow 301.2, and 404.9 \rightarrow 387.0, respectively.

Data Analysis. To estimate the kinetic parameters of the Michaelis constant (\textit{K}_{m} \text{, half-saturation concentration}) and the maximum transport rate (\textit{V}_{max}) for the saturable transport, the following equation was fitted to the profile of uptake rate (\textit{v}) versus substrate concentration (\textit{s}) by means of nonlinear least-squares regression analysis using WinNonlin (Pharsight, Mountain View, CA): \textit{v} = \textit{V}_{max} \times s/(\textit{K}_{m} + s). Parameters are presented as computer-fitted ones with S.E. Experimental data are presented as the means ± S.E., and statistical analysis was performed using two-tailed, unpaired Student’s \textit{t} test or, when multiple comparisons were needed, analysis of variance followed by Dunn’s test, with \textit{p} < 0.05 considered significant.

Results

Transport of Fluoroquinolones by rMATE1. To examine whether fluoroquinolones are recognized by rMATE1 as substrates, we first determined the transport of several fluoroquinolones (ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, norfloxacin, pazufloxacin, and tosufloxacin) in MDCKII cells stably expressing rMATE1 cells and also for comparison in mock cells (Fig. 1). It was found that all the fluoroquinolones
tested by rMATE1, exhibiting 2- to 5-fold greater uptake in rMATE1 cells than in mock cells. Because NFX showed the greatest rMATE1-mediated transport relative to transport in mock cells, we chose it as a probe substrate for further investigation of the transport mechanism.

Effect of pH on rMATE1-Mediated NFX Transport. rMATE1 has been known as an organic cation/H\(^+\) antiporter, which requires an outwardly directed proton gradient to transport TEA and cimetidine into the cells efficiently (Ohta et al., 2006). The functionality of rMATE1 as an organic cation/H\(^+\) antiporter was confirmed by using TEA as a probe substrate in the present experimental setting. The uptake of TEA (10 \(\mu\)M) was approximately 3.5-fold enhanced by the procedure for intracellular acidification using NH\(_4\)Cl in rMATE1 cells (89.3 \(\pm\) 0.8 and 319.8 \(\pm\) 7.6 pmol/min/mg protein, respectively, as means \(\pm\) S.E. for \(n = 3\) in control and acidified cells), whereas it was minimally changed in mock cells (23.5 \(\pm\) 0.5 and 26.8 \(\pm\) 0.5 pmol/min/mg protein). Therefore, we examined the role of proton in NFX transport mediated by rMATE1. However, as shown in Fig. 2A, the uptake of NFX was not significantly altered in rMATE1 and mock cells by intracellular acidification, by the pretreatment with K\(^+\)-based buffer containing NH\(_4\)Cl, and subsequently with the K\(^+\)-based buffer (NH\(_4\)Cl free). Likewise, NFX uptake was not altered by intracellular acidification by the mannitol-based buffer containing nigericin (K\(^+\)/H\(^+\)-exchanging ionophore) or by intracellular alkalization by the K\(^+\)-based buffer containing NH\(_4\)Cl. These results indicate that an outwardly directed proton gradient is not required for rMATE1 to transport NFX. Thus, it is likely that rMATE1 mediates NFX transport by a facilitative manner.

When the extracellular pH was varied in a range from 5.0 to 8.5, the uptake of NFX changed dramatically in rMATE1 cells, whereas it remained minimal in mock cells (Fig. 2B). NFX uptake in rMATE1 cells was greatest at pH 7.0 and decreased significantly when pH became more alkaline or more acidic. These results indicate that rMATE1-mediated NFX transport is highly sensitive to extracellular pH, being maximal at around neutral pH. This pH-dependent profile of NFX transport was in agreement with that of the percentage of zwitterionic NFX molecule; hence, it could be the transported molecular species.

**Basal-to-Apical Transport of NFX across the Monolayer of rMATE1-Expressing Cells.** To examine the functionality of rMATE1 in the efflux of NFX across the apical membrane, rMATE1 cells were cultured on the permeable membrane filter, which was inserted in a well and separating the apical and basal compartments (Transwell), and then the basal-to-apical transcellular transport of NFX was evaluated. Experiments in this section were conducted under a more physiologically relevant condition using the Na\(^+\)-based buffer, whereas regular uptake experiments in the other sections were designed to examine the function of rMATE1 under the depolarized condition by the use of the K\(^+\)-based buffer, which could also suppress H\(^+\) transport by Na\(^+\)/H\(^+\) exchangers to avoid shifts in the membrane potential and pH, which might modulate rMATE1-mediated transport. As shown in Fig. 3A, the transcellular transport of NFX was much greater in rMATE1 cells than that in mock cells, and transcellular NFX transport in rMATE1 cells was inhibited to the level observed in mock cells by the addition of corticosterone (200 \(\mu\)M), a potent rMATE1 inhibitor, in the apical compartment. The apical localization of rMATE1 was con-
firmed by confocal laser scanning microscopic observation of green fluorescent protein (GFP)-tagged rMATE1 (GFP-rMATE1) introduced into MDCKII cells (Supplemental Fig. 1; Materials and Methods). We also confirmed that the characteristics of NFX transport in the cells expressing GFP-rMATE1 are in agreement with those in rMATE1 cells. These results suggest that rMATE1 is functional in mediating the efflux of intracellular NFX into the apical medium.

When extracellular pH in the apical medium was changed from 7.4 to 6.5, transcellular NFX transport was not altered significantly (Fig. 3B). This result is in agreement with the preceding finding that a transmembrane proton gradient generated by intracellular acidification did not alter NFX transport in the cellular uptake study (Fig. 2A). Thus, rMATE1 does not require a proton gradient for transporting NFX in the direction of cellular efflux, which is the transport direction expected in the physiological situation. On the other hand, it was found that alkalization of the apical medium from pH 7.4 to pH 8.5 caused a marked decrease in transcellular NFX transport.

Effect of Extracellular Ions on rMATE1-Mediated NFX Transport. Because it was found unexpectedly that rMATE1 does not require a proton gradient for transporting NFX, we further examined a possibility that some other ions might be involved. As shown in Fig. 4, the uptake of NFX in rMATE1 cells was found to be greater in K⁺-based buffer than in those in which K⁺ was replaced with Na⁺ or Li⁺, whereas NFX uptake in mock cells remained only minimal. When K⁺ was replaced with Rb⁺, NFX uptake was comparable with that in K⁺-based buffer, although somewhat lower. Because such high concentrations of extracellular K⁺ and Rb⁺ can depolarize the plasma membrane, a possibility is that NFX transport might be enhanced by the elevated membrane potential in the K⁺- and Rb⁺-rich mediums. Therefore, we examined the effect of Ba²⁺, a nonselective K⁺ channel inhibitor, in Na⁺-based buffer. However, depolarization of the plasma membrane by that did not alter NFX uptake. Therefore, K⁺ and Rb⁺ seem to be involved in enhancing rMATE1-mediated NFX transport by some other mechanism. Although it is unknown and would need to be investigated in the future, the K⁺-rich condition, which is normally maintained inside the cells, is at least in favor of the efflux transport of NFX, which is expected in the physiological situation.

Kinetic Features of rMATE1-Mediated NFX Transport. Figure 5A shows the time courses of NFX uptake in rMATE1 cells and mock cells. The uptake of NFX increased with time rapidly in rMATE1 cells, being in proportion to time in the initial 30 s. Therefore, we decided to evaluate the initial uptake for kinetic analysis at 20 s as a time point within that range. The uptake of NFX in mock cells was far slower, being only minimal even at 2 min. As shown in Fig. 5B, kinetic analysis indicated that NFX transport mediated by rMATE1 was saturable with a Kᵣ of 55.3 μM and a Vₛₘₐₓ of 0.99 nmol/min/mg protein.

Effect of Various Compounds on rMATE1-Mediated NFX Transport. As indicated above, it is likely that the mechanism of NFX transport by rMATE1 is different from the H⁺-antiport mechanism for organic cations, such as TEA and cimetidine. In this section, we examined whether various compounds, which have been identified as substrate or inhibitor of organic cation/H⁺ antiport by rMATE1, still inhibit NFX transport by rMATE1. As shown in Fig. 6, it was found that organic cations such as TEA, cimetidine, verapamil, and...
corticosterone are potent inhibitors, whereas organic anions such as estrone sulfate, methotrexate, probenecid, and taurocholate did not exhibit any inhibitory activity. We also examined the effect of \( p \)-chloromercuribenzene sulfonate (pCMBS), a sulfhydryl group-modifying reagent, with which the pretreatment was shown to extensively inhibit organic cation/\( K^+ \) antiport by rMATE1 in our previous study (Ohta et al., 2006). As shown in Fig. 7, pretreatment of cells with pCMBS significantly reduced NFX transport in rMATE1 cells but showed no effect in mock cells. All these results suggest that NFX and possibly also other fluoroquinolones may share the substrate recognition site with organic cations, despite the difference in the mode of transporting operation.

**Discussion**

Many fluoroquinolones are known to be mainly eliminated by excretion into urine. For those examined for transport by rMATE1 in the present study, the urinary excreted percentage of the intravenous dose reportedly ranges from 35 to 84% in humans and/or rats (Nakamura et al., 1983; Neuman, 1988; Maeda et al., 1989; Hayakawa et al., 1995; Nouaille-Degorce et al., 1998). Because their excretion rates into urine are much greater than those predicted for the glomerular filtration of unbound molecule, it has been postulated that tubular secretion by carrier-mediated transport systems is involved in their renal excretion. In the present study, we have demonstrated that rMATE1 can recognize such fluoroquinolones as substrates, suggesting the involvement of MATE1 in the renal excretion of fluoroquinolones. Although Tanihara et al. (2007) reported earlier that hMATE1 and also hMATE2-K, of which the ortholog is absent in the rat, did not transport levofloxacin and ciprofloxacin, it could be because they used a higher pH of 8.4 for transport assessments. As shown in Fig. 2B, NFX transport by rMATE1 is highly sensitive to the extracellular pH and almost negligible at pH 8.5. Also for levofloxacin, a similar trend with a very low transport at pH 8.5 was observed (data not shown). It is notable that rMATE1 is an ortholog of a bacterial multidrug efflux transporter, NorM, which is responsible for the efflux of NFX in *Vibrio parahaemolyticus* (Morita et al., 1998), and several other bacterial proteins homologous to NorM confer resistance to various fluoroquinolones (Miyamae et al., 2001; Rouquette-Loughlin et al., 2003). Thus, such ability to mediate...
the transport of fluoroquinolones seems to be common to MATE-type transporters.

We showed previously that the transport of TEA mediated by rMATE1 was stimulated in the presence of a transmembrane proton gradient across the membrane, which was generated by intracellular acidification in rMATE1-expressing HEK293 cells (Ohta et al., 2006). Tsuda et al. (2007) further demonstrated that TEA transport into the membrane vesicles prepared from rMATE1-expressing HEK293 cells was stimulated by an outwardly directed H\(^+\) gradient and reduced by the collapse of the H\(^+\) gradient. Thus, it is evident that rMATE1 mediates the transport of organic cations, such as TEA, by exchange with H\(^+\). In the present study, however, we found that the uptake transport of NFX mediated by rMATE1 is not sensitive to intracellular acidification (Fig. 2A). Likewise, the efflux transport of NFX mediated by rMATE1 was not sensitive to extracellular pH in the apical medium (Fig. 3B). These results suggest that rMATE1 does not require a transmembrane proton gradient for transporting NFX. It was found, however, that rMATE1-mediated NFX transport is highly sensitive to extracellular pH, with the optimal pH of 7.0. NFX is an amphoteric compound possessing a carboxyl group with a pKa of 6.3 and a piperazine nitrogen with a pKa of 8.4 and, hence, exists predominantly in the zwitterionic form at around neutral pH in the physiological condition (Wagenlehner and Naber, 2003). The percentage of zwitterionic NFX molecule decreases with a decrease in pH, accompanying an increase in the cationic form, or an increase in pH, accompanying an increase in the anionic form. Therefore, it is likely that rMATE1 mediates the transport of NFX and possibly also other fluoroquinolones by a facilitative manner, and the zwitterionic form of them is the transported molecular species. The mode of rMATE1 operation seems to depend on the electrochemical characteristics of the substrate molecule.

The K_m of rMATE1-mediated NFX transport (55.3 \(\mu\)M) is much higher than the peak plasma level (2–5 \(\mu\)M) of NFX achieved by therapeutic doses (Neuman, 1988; Turnidge, 1999). Although NFX could be accumulated in the renal tubular epithelial cells, it would not be much more than approximately five times, as reported in rats (1.3 and 6.4 \(\mu\)M, respectively, in plasma and renal tissue at peak for the oral dose of 50 mg/kg) (Nakamura et al., 1983). Thus, the intracellular concentration of NFX in vivo is expected to be lower than the K_m, suggesting that rMATE1 could efficiently operate for efflux transport of NFX. Taking also into account that the transport activity represented by V_{max}/K_m of rMATE1 for NFX (18 \(\mu\)l/min/mg protein) is high and comparable with those for such typical organic cations as TEA (21 \(\mu\)l/min/mg protein) and cimetidine (29 \(\mu\)l/min/mg protein) (Ohta et al., 2006), rMATE1 may play a key role in the renal tubular secretion of NFX and possibly also other fluoroquinolones.

Among secretory transporters identified at the brush-border membrane of the renal tubular epithelial cells, multidrug resistance protein (MDR) 1, multidrug resistance-associated protein (MRP) 2, and BCRP, which are all ABC transporters, are reported to be able to mediate the transport of fluoroquinolones, whereas MRP4, another ABC transporter, is not. However, their roles in the transport of fluoroquinolones seem to be only limited. In a study using human MDR1-expressing MDCKII cells, grepafloxacin was shown to be transported by MDR1, but ciprofloxacin was not (Lowes and Simmons, 2002). In another study in which the blood-brain barrier penetration of fluoroquinolones such as ciprofloxacin, fleroxacin, NFX, pefloxacin, and sparflxacin were examined using mdr1a knockout mice, only a modest permeation enhancement, compared with wild-type mice, was observed only for sparflxacin, indicating a minimal role of mdr1a in extruding fluoroquinolones from the brain (de Lange et al., 2000). MRP2 has been suggested to be involved in the biliary secretion and intestinal secretion of fluoroquinolones because those of grepafloxacin were smaller in Eisai-hyperbilirubinemic rats, which genetically lack functional MRP2, than in normal rats (Sasabe et al., 1998; Naruhashi et al., 2002). However, that is so far reported only for grepafloxacin. Thus, MDR1 and MRP2 seem to accept only limited fluoroquinolones as substrates. BCRP was shown more recently to transport several fluoroquinolones, such as ciprofloxacin, grepafloxacin, NFX, ofloxacin, and ulifloxacin. In Bcrp knockout mice, a reduction in the secretion of ciprofloxacin into milk, compared with that in wild-type mice, was shown to be extensive when it was administered intravenously, indicating a significant role of Bcrp in the secretion process (Merino et al., 2006). A similar reduction in biliary secretion in Bcrp knockout mice was shown for ciprofloxacin, grepafloxacin, ofloxacin, and ulifloxacin (Ando et al., 2007). However, increases in plasma concentrations of those fluoroquinolones in Bcrp knockout mice were only modest or insignificant, suggesting that Bcrp does not play a significant role in their renal tubular secretion, which is their major elimination pathway.

To achieve the efficient tubular secretion of fluoroquinolones, transporters are required to be present also at the basolateral membrane of tubular epithelial cells and coordinately operate with those at the brush-border membrane. The facilitative mode of transport, which has been suggested in the present study for the transport of fluoroquinolones by rMATE1, depends in principle only on the chemical gradient of the substrate. Therefore, assuming that MATE1 is the major transporter responsible for the efflux process of fluoroquinolones at the brush-border membrane, fluoroquinolones need to be efficiently taken up into the epithelial cells for some accumulation by transporters at the basolateral membrane. It has been reported already that the mouse ortholog of organic anion transporter 3, which is highly expressed at the basolateral membrane and involved in the transport of various organic anions into the tubular epithelial cells, has the ability to transport ciprofloxacin and also that the transport of estrone sulfate by Oat3 is inhibited by fluoroquinolones, such as ciprofloxacin, grepafloxacin, NFX, and ofloxacin. Furthermore, the plasma concentrations of ciprofloxacin after intravenous administration have been reported to be significantly higher in Oat3 knockout mice than in wild-type mice (Vanwert et al., 2008). Thus, it is likely that organic anion transporter 3 is involved in the basolateral uptake transport of fluoroquinolones.

In conclusion, we have demonstrated that rMATE1 can mediate the transport of fluoroquinolones. As demonstrated for NFX, it does not depend on transmembrane proton gradient across the plasma membrane, even though rMATE1 has been known as an organic cation/H\(^+\) antiporter. This finding suggests that rMAT1 operates in a facilitative mode, depending on the chemical gradient of the substrate. It is also notable that the transport activity of rMATE1 for NFX is high and comparable with those for typical organic cations,
such as TEA and cimetidine. rMATE1 is highly similar to hMATE1 in terms of expression profile and functional characteristics. Thus, MATE1 may play a key role in the renal tubular secretion of fluoroquinolones not only in rats but also in humans.

References


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