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Identification of Influx Transporter for the Quinolone Antibacterial Agent Levofloxacin

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Abstract: Quinolone antibacterial agents exhibit high intestinal absorption, selective tissue distribution, and renal and biliary excretion. Several ATP-binding cassette transporters are involved in efflux transport of these agents, but no influx transporters have yet been molecularly identified. In the present study, we aimed to identify the influx transporter(s) of quinolone antibiotics using levofloxacin as a model compound. Several candidate transporter genes were selected based on differential expression of mRNAs among Caco-2 cell subclones that exhibited differential uptake activities for levofloxacin. Based on a functional analysis of each transporter gene for which a good correlation was found between expression level and levofloxacin transport activity in the Caco-2 subclones, organic anion transporting polypeptide 1A2 (OATP1A2 (OATP-A), SLCO1A2) was concluded to transport levofloxacin. When OATP1A2 was expressed in *Xenopus* oocytes, levofloxacin transport was essentially pH-independent and was not stereoselective. OATP1A2-mediated uptake of levofloxacin showed a K_m value of 136 μM. Apparent uptake of levofloxacin by Caco-2 cells showed high- and low-affinity components with K_m values of 0.489 and 14.6 mM, respectively. Accordingly, plural transporters are functional for the transport of levofloxacin in Caco-2 cells, and OATP1A2 is likely to function as a high-affinity transporter. The inhibitory effects and the expression of transport activity of other quinolone antibacterial agents suggested that OATP1A2 commonly transports all the agents tested. In conclusion, this is the first identification of an influx transporter for fluoroquinolones, and the results suggest that active influx transport at least partially explains the high membrane permeability of the quinolone agents in various tissues.

Keywords: Fluoroquinolone; transporter; OATP; absorption; intestine; levofloxacin; Caco-2 cell

Introduction

Quinolone antibacterial agents such as levofloxacin are generally well absorbed from the small intestine, showing high values of bioavailability ranging from 70% to almost 100% in humans.[1,2] The volume of distribution of the agents is significantly higher than the total body water, so they appear to cross the cellular membrane efficiently and be accumulated in tissues.[1] In addition, many of the agents are excreted into urine via both glomerular filtration and active secretion, while some of them are selectively recovered in bile in the intact and/or metabolized forms.[1,3,4] Since these

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(1) Wolfson, J. S.; Hooper, D. C. Fluoroquinolone antimicrobial agents. *Clin. Microbiol. Rev.* **1989**, *2*, 378−424.

(2) Fish, D. N.; Chow, A. T. The clinical pharmacokinetics of levofloxacin. *Clin. Pharmacokinet.* **1997**, *32*, 101−119.

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antibacterial agents are zwitterionic compounds, a passive diffusion mechanism may not fully explain their high intestinal absorption, selective tissue distribution, and selective excretion. Accordingly, involvement of membrane transporters has been proposed. For intestinal absorption, although absence of carrier-mediated transport was suggested for sparfloxacin,[5,6] others have concluded that transporters are involved in the intestinal absorption process. They include peptide transporter for sparfloxacin,[7] amino acid transporter for ofloxacin,[8] and unknown transporters for levofloxacin, greapafloxacin, and ofloxacin.[9,10] As regards tissue distribution, grepafloxacin and HSR-903 were taken up by lung cells via a partially sodium-dependent transporter, though their transport characteristics could not be fully explained in terms of known transporters.[11,12] Hepatic uptake of grepafloxacin and HSR903 was mediated by a sodium-independent transporter, which also exhibited different characteristics from those of known transporters.[4,13] Renal basolateral uptake of levofloxacin was also mediated by an unknown transporter, which was inhibited by cimetidine and quinidine, but not by *p*-aminohippuric acid.[14−16] Thus, there is evidence that quinolone antibacterial agents are taken up by cells via specialized transporters, but the molecular identification of these transporters remains to be achieved. On the other hand, involvement of transporters such as multidrug resistance P-glycoprotein (MDR1, ABCB1), multidrug resistance associated proteins (MRPs, ABCCs), and breast cancer resistance protein (BCRP/ABCG2) in efflux of quinolone antibacterial agents has been established. We suggested an involvement of P-glycoprotein and MRP2 in the intestinal luminal secretion of grepafloxacin using small intestinal tissues from Sprague−Dawley and Eisai hyperbilirubinemic rats (EHBRs) and Caco-2 cells.[17,18] Furthermore, direct evidence of levofloxacin and grepafloxacin transport by P-glycoprotein was obtained using MDR1-gene transfected cultured cells and *mdr1a/1b* gene-deficient mice.[19,20] Similarly, we observed an increased brain distribution of sparfloxacin, as well as levofloxacin and grepafloxacin, in *mar1a/1b* gene-deficient mice.[21] Transport of fluoroquinolones by MRP1 and BCRP was also indicated by *mrp1*- and bcrp-deficient mice, respectively.[22,23] These observations in transporter gene-transfected cells and transporter gene-deficient animals clearly demonstrated that several ATP-binding

(3) Sasabe, H.; Tsuji, A.; Sugiyama, Y. Carrier-mediated mechanism for the biliary excretion of the quinolone antibiotic grepafloxacin and its glucuronide in rats. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 1033−1039.

(4) Murata, M.; Tamai, I.; Sai, Y.; Nagata, O.; Kato, H.; Sugiyama, Y.; Tsuji, A. Hepatobiliary transport kinetics of HSR-903, a new quinolone antibacterial agent. *Drug Metab. Dispos.* **1998**, *26*, 1113−1119.

(5) Cormet, E.; Huneau, J. F.; Bouras, M.; Carbon, C.; Rubinstein, E.; Tome, D. Evidence for a passive diffusion mechanism for sparfloxacin uptake at the brush-border membrane of the human intestinal cell-line Caco-2. *J. Pharm. Sci.* **1997**, *86*, 33−36.

(6) Iseki, K.; Hirano, T.; Tsuji, K.; Miyazaki, S.; Takada, M.; Kobayashi, M.; Sugawara, M.; Miyazaki, K. Ionic-diffusion potential-dependent transporter of anew quinolone, spafloxacin, across rat intestinal brush-border membrane. *J. Pharm. Pharmacol.* **1998**, *50*, 627−634.

(7) Yamaguchui, T.; Yokogawa, M.; Sekine, Y.; Hashimoto, M. Intestinal absorption characteristics of sparfloxacin. *Xenobiot. Metab. Dispos*. **1991**, *6*, 53−59.

(8) Rabbaa, L.; Dautrey, S.; Colas-Linhart, N.; Carbon, C.; Farinotti, R. Absorption of ofloxacin isomers in the rat small intestine. *Antimicrob. Agents Chemother.* **1997**, *41*, 2274−2277.

(9) Prieto, J. G.; Barrio, J. P.; Alvarez, A. I.; Gomez, G. Kinetic mechanism for the intestinal absorption of ofloxacin. *J. Pharm. Pharmacol.* **1988**, *40*, 211−212.

(10) Yamaguchi, H.; Yano, I.; Saito, H.; Inui, K. Transport characteristics of grepafloxacin and levofloxacin in the human intestinal cell line Caco-2. *Eur. J. Pharmacol*. **2001**,*431*, 297−303.

(11) Sasabe, H.; Kato, Y.; Suzuki, T.; Itose, M.; Miyamoto, G.; Sugiyama, Y. Carrier-mediated uptake of grepafloxacin, a fluoroquinolone antibiotic, by the isolated rat lung cells. *Drug Metab. Pharmacokinet.* **2005**, *20*, 491−495.

(12) Murata, M.; Tamai, I.; Sai, Y.; Nagata, O.; Kato, H.; Tsuji, A. Carrier-mediated lung distribution of HSR-903, a new quinolone antibacterial agent. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 79−84.

(13) Sasabe, H.; Terasaki, T.; Tsuji, A.; Sugiyama, Y. Carrier-mediated hepatic uptake of quinolone antibiotics in the rat. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 162−171.

(14) Yano, I.; Ito, T.; Takano, M.; Inui, K. Evaluation of renal tubular secretion and reabsorption of levofloxacin in rats. *Pharm. Res.* **1997**, *14*, 508−511.

(15) Ito, T.; Yano, I.; Masuda, S.; Hashimoto, Y.; Inui, K. Distribution characteristics of levofloxacin and grepafloxacin in rat kidney. *Pharm. Res.* **1999**, *16*, 534−539.

(16) Matsuo, Y.; Yano, I.; Habu, Y.; Katsura, T.; Hashimoto, Y.; Inui, K. Transport of levofloxacin in the OK kidney epithelial cell line: interaction with p-aminohippurate transport. *Pharm. Res.* **2001**, *18*, 573−578.

(17) Naruhashi, K.; Tamai, I.; Inoue, N.; Muraoka, H.; Sai, Y.; Suzuki, N.; Tsuji, A. Involvement of multidrug-associated protein 2 in intestinal secretion of grepafloxacin in rats. *Antimicrob. Agents Chemother.* **2002**, *46*, 344−349.

(18) Naruhashi, K.; Tamai, I.; Li, Q.; Sai, Y.; Tsuji, A. Experimental demonstration of the unstirred water layer effect on drug transport in Caco-2 cells. *J. Pharm. Sci.* **2003**, *92*, 1502−1508.

(19) Ito, T.; Yano, I.; Tanaka, K.; Inui, K. Transport of quinolone antibacterial drugs by human P-glycoprotein expressed in a kidney epithelial cell line, LLC-PK1. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 955−960.

(20) Yamaguchi, H.; Yano, I.; Saito, H.; Inui, K. Pharmacokinetic role of P-glycoprotein in oral bioavailability and intestinal secretion of grepafloxacin in vivo. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 1063−1069.

(21) Tamai, I.; Yamashita, J.; Kido, Y.; Ohnari, A.; Sai, Y.; Shima, Y.; Naruhashi. K.; Koizumi, S.; Tsuji, A. Limited distribution of new quinolone antibacterial agents into brain caused by multiple efflux transporters at the blood-brain barrier. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 146−152.

(22) Sasabe, H.; Kato, Y.; Suzuki, T.; Itose, M.; Miyamoto, G.; Sugiyama, Y. Differential Involvement of multidrug resistance-associated protein 1 and P-glycoprotein in tissue distribution and excretion of grepafloxacin in mice. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 648−655.

(23) Merino, G.; Alvarez, A. I.; Pulido, M. M.; Molina, A. J.; Schinkel, A. H.; Prieto, J. G. Breast cancer resistance protein (BCRP/ABCG2) transports fluoroquinolone antibiotics and affects their oral availability, pharmacokinetics and milk secretion. *Drug Metab. Dispos.* **2006**, *34*, 690−695.

cassette (ABC) transporter molecules contribute to the active efflux of the drugs from cells and significantly affect the pharmacokinetic characteristics of the drugs.

Since both influx and efflux transporters should affect the pharmacokinetic characteristics of the agents, it is important to molecularly identify all the relevant transporters. However, no influx transporter of quinolone antibacterial agents has been identified. Accordingly, the purpose of the present study is to identify the influx transporter(s) for these agents using levofloxacin, which exhibits good bioavailability, tissue distribution, and urinary excretion, as a model compound.

For this purpose, we analyzed differentially expressed transporter genes among cells that exhibited different levels of transport activity for the agent. We used Caco-2 cells as a human-derived cell model, because this cell line exhibits carrier-mediated influx transport of levofloxacin, though the responsible transporter molecule has not been identified.[10] In addition, Caco-2 cells are heterogeneous in usual cultivation, and include cell clones that express different amounts of transporters and enzymes.[24] So, it is probable that the apparent uptake activity of different subclones would be correlated with the expression level of the responsible transporter molecule. Therefore, we first selected subclones of Caco-2 cells that showed various levels of uptake activity of levofloxacin and then analyzed the expressed transporter gene profiles with DNA microarrays to select candidate transporter molecules functioning for the influx transport of levofloxacine.

Experimental Section

Materials. [^{14}C]Levofloxacin (899.9 MBq/mmol) was synthesized by Daiichi Pure Chemical Co., Ltd. (Ibaraki, Japan). [^{14}C]Inulin (92.5 GBq/g) was from American Radiochemicals (St. Louis, MO). Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD). Fetal bovine serum (FBS) was obtained from Invitrogen (Carlsbad, CA). Unlabeled levofloxacin, DR-3354, DR-8493, and grepafloxacin were synthesized by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Other antibacterial agents, lomefloxacin and norfloxacin, were from Sigma-Aldrich (St. Louis), and ciprofloxacin, enoxacin, gatifloxacin, and moxifloxacin were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were purchased from Sigma-Aldrich (St. Louis) and Wako Pure Chemical Industries (Osaka).

Cell Culture. Caco-2 cells were grown in a humidified incubator at 37 °C, under 5% CO_2 in air, in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1% nonessential amino acids, 1% L-glutamine, 100 units/mL penicillin G, and 100 μg/mL streptomycin.

Isolation of Caco-2 Subclones. Parental Caco-2 cells were plated at 100 cells/100 mm dish, and the cells were allowed to grow. After cell adhesion had occurred, single colonies were marked and cultivated for 1−2 weeks with no change of culture medium at least for 10 days. Individual clones were transferred to 24-well tissue culture plates. These individual clones were stepwise expanded into 6-well tissue culture plates and then into 100 mm dishes for further functional assay. For routine cultivation of the subcloned cells by 100 mm dishes, it took about 3 days to be confluent when 2.5×10^5 cells/dish were seeded.

Uptake Experiments with Caco-2 Cells. Uptake of levofloxacin by cultured monolayers of Caco-2 cells was examined using the method reported previously.[25] Caco-2 cells (1×10^5 cells/well) were grown on 4-well plates coated with collagen (Nitta Gelatin Inc., Osaka, Japan) for 2 weeks. During the cultivation, the medium was changed every other day, starting after 4 days. For uptake studies, initially the growth medium was aspirated and the cells were washed twice with 400 μL of transport buffer (125 mM NaCl, 4.8 mM KCl, 5.6 mM glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM HEPES, pH 7.4) at 37 °C. Uptake was initiated by adding 200 μL of transport buffer containing [^{14}C]levofloxacin to the cells. At a designated time, the cells were washed three times with 400 μL of ice-cold transport buffer to terminate the uptake. To solubilize the cells, 200 μL of 1 M NaOH was added to each well and the plate was left at room temperature for 6 h. After neutralization with 40 μL of 5 M HCl, the cells were transferred to a tube and mixed with liquid scintillation cocktail (Cleasol 1, Nacalai tesque, Kyoto, Japan), and then the radioactivity was quantified with a liquid scintillation counter (Aloka, Tokyo, Japan). The concentration of [^{14}C]-levofloxacin was usually 10 μM or 20 μM. The value of apparent uptake of [^{14}C]inulin was used to correct for surface-adhering water volume. Cellular protein content was determined according to the method of Lowry with bovine serum albumin as the standard.[26]

Uptake Experiments with *Xenopus* Oocytes Expressing OATP1A2. Uptake experiments were conducted with *Xenopus laevis* oocytes that had been microinjected with complementary RNA (cRNA) of OATP1A2 (OATP-A), SLCO2A1, synthesized in vitro using T7 RNA polymerase (Ambion, Austin, TX), using the methods reported previously.[27] Briefly, defoliculated oocytes were injected with 50 nL of water containing 25 ng of cRNA, cultured 3 days in modified Barth's solution (MBS, 88 mM NaCl, 1 mM KCl_2, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO)_3$, 0.41 mM $CaCl_2$ and 10 mM HEPES adjusted to pH 7.4 with

(24) Horie, K.; Tang, F.; Borchardt, R. T. Isolation and characterization of Caco-2 subclones expressing high levels of multidrug resistance protein efflux transporter. *Pharm. Res.* **2003**, *2*, 161−168.

(25) Tsuji, A.; Takanaga, H.; Tamai, I.; Terasaki, T. Transcellular transport of benzoic acid across Caco-2 cells by a pH-dependent and carrier-mediated transport mechanism. *Pharm. Res.* **1994**, *11*, 30−37.

(26) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−267.

(27) Ishiguro, N.; Saito, A.; Yokoyama, K.; Morikawa, M.; Igarashi, T.; Tamai, I. Transport of the dopamine D2 agonist pramipexole by rat organic cation transporters OCT1 and OCT2 in kidney. *Drug Metab. Dispos.* **2005**, *33*, 495−499.

NaOH), and used for uptake experiments. Uptake of quinolone antibacterial agents was measured at room temperature in the MBS buffer. Uptake was terminated by the addition of ice-cold MBS buffer. Oocytes were washed three times by the addition of ice-cold MBS buffer. For the uptake of [^{14}C]levofloxacin, oocytes were solubilized in 5% sodium dodecyl sulfate solution, and the radioactivity was measured with a liquid scintillation counter after addition of liquid scintillation cocktail (Nacalai tesque). As the control, the same volume of water was injected into oocytes, which were then cultured for the same number of days, and the uptake was measured in the same manner as described above. For the other nonlabeled quinolone compounds, the oocytes incubated with each quinolone compound were washed by ice-cold MBS buffer and the adhered water was blotted by filter paper. Then, each oocyte was placed in 500 μL of mixture of 10 mM ammonium fromate/acetonitrile (5/95, v/v) containing DL-8493 (an analogous compound) as the internal standard, and homogenized by sonication. Insoluble materials in the samples were removed by membrane filter, and filtrate was applied for LC/MS assay.

Microarray Analysis. Caco-2 cells were homogenized in Isogen (Wako Pure Chemical Industries), and the RNA phase was separated by using chloroform. The total RNA was precipitated with isopropyl alcohol, and the resultant pellet was washed with 70% ethanol for subsequent DNA microarray analysis. The expression profiles of transporter genes in the selected subclones were analyzed by Kurabo Co. (Osaka, Japan) using CodeLink Bioarray (GE Healthcare Bio-Sciences KK, Piscataway, NJ). Briefly, first-strand cDNA was transcribed from total RNA using T7 oligo primer and reverse transcriptase at 42 °C. The second-strand cDNA was synthesized from first-strand cDNA using DNA polymerase mix at 16 °C, then cleaned up with a QIAquick PCR purification kit (QIAGEN, Valencia, CA). Biotin-labeled cRNA was synthesized from the double-stranded cDNA using T7 RNA polymerase-catalyzed in vitro transcription in the presence of biotin-labeled NTP, then fragmented at 94 °C. Biotin-labeled cRNA was heated at 90 °C for 5 min and was hybridized with CodeLink Bioarray at 37 °C for 20 h. The CodeLink Bioarray was washed with buffer, stained with streptavidin-Cy5 solution, washed again, and scanned with a laser scanner (Applied Precision Inc., Pittsburgh, PA).

Quantitation of Nonlabeled Quinolone Compounds by LC−MS. LC−MS/MS analysis was carried out using a high-performance liquid chromatograph system consisting of an Agilent 1100 binary pump, a vacuum degasser, and an autosampler with a 25 μL loop interfaced to an API 4000 SCIEX triple-quadrupole tandem mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Canada). DR-3354 and the internal standard (DL-8493, analogous compound) were separated on an Atlantis dC18 2.1 × 150 mm column (Waters, MA). The mobile phase consisted of 10 mM ammonium formate (pH 3.5):acetonitrile (7:3 v/v for DR-3354 and 6.5/3.5 v/v for the other quinolones). The sample was delivered at a flow rate of 0.2 mL/min for DR-3354 and 0.3 mL/min for the other quinolones. The mass spectrometer was operated in the turbo ion spray mode with positive ion detection. The detection and quantification of analytes were performed using the multiple reaction monitoring mode with the transitions *m*/*z* 362.2 to 344.1 for DR-3354, *m*/*z* 332.0 to 314.1 for ciprofloxacin, *m*/*z* 321 to 303.2 for enoxacin, *m*/*z* 320.4 to 302.0 for norfloxacin, *m*/*z* 352.1 to 265.1 for lomefloxacin, *m*/*z* 376.1 to 358.0 for gatifloxacin, and *m*/*z* 388.3 to 370.1 for DL-8493. All calibration samples were made by adding each of the quinolone compounds to drug-free oocyte solution made as described above. The correlation coefficients were more than 0.998. There was no background interference from the oocytes. All raw data were processed with MDS SCIEX Analyst software, version 1.2.

Data Analysis. All data were expressed as mean ± SEM, and statistical analysis was performed by the use of Student's *t*-test with $p < 0.05$ as the criterion of significance. Cell-to-medium ratio was obtained by dividing the cellular uptake amount by the concentration of test compound in the uptake medium. To estimate kinetic parameters for saturable transport, the uptake rate (v) was fitted to eq 1 or 2 by means of nonlinear least-squares regression analysis using MULTI.[28]

For the case of a single saturable component,

$$v = V_{max}s/(K_m + s) \quad (1)$$

and for the case of two saturable components, i.e., a high-affinity component with K_{m1} and V_{max1} and a low-affinity component with K_{m2} and V_{max2}, as well as a nonsaturable component,

$$v = V_{max1}s/(K_{m1} + s) + V_{max2}s/(K_{m2} + s) + k_d s \quad (2)$$

where v and s are the uptake rate and concentration of substrate, respectively, K_m and V_{max} represent the half-saturation concentration (Michaelis constant) and the maximum transport rate for the saturable component, and k_d is the first-order rate constant for the nonsaturable transport.

To calculate the half-inhibitory concentration (IC_{50}) values of DR-3354 in levofloxacin uptake, the eq 3 was used,

$$\%\ \text{of control} = IC_{50}/(IC_{50} + I) \quad (3)$$

where % of control, IC_{50}, and I are observed % of control uptake, the 50% inhibitory concentration, and the inhibitor concentration used, respectively.

Results

Screening of Caco-2 Cell Subclones with Differential Transport Activity for Levofloxacin. Forty-nine Caco-2 subclones were isolated from the parental Caco-2 cells by means of a dilution cloning technique. Among these subclones, 36 subclones formed colonies and were examined for levofloxacin uptake activity. The uptake activity was

(28) Yamaoka, K.; Tanigawara, Y.; Nakagawa, T.; Uno, T. A pharmacokinetic analysis program (multi) for microcomputer. *J. Pharmacobio-Dyn.* **1981**, *4*, 879−885.

evaluated in terms of the extent of decrease of the initial uptake of [^{14}C]levofloxacin (20 μM) at 1 min in the presence of an excess (20 mM) of unlabeled levofloxacin. Subclones that exhibited a high percentage decrease were considered as having high transport activity. By repeated transport activity measurements, we finally obtained cell subclones that stably exhibited differential activities, and finally selected two subclones with high uptake activity (clones #9 and #21) and one clone with negligible uptake activity (clone #19).

Microarray Analysis. From the subcloned Caco-2 cells, mRNAs were obtained and analyzed for gene expression by Kurabo Biomedical (Osaka, Japan) using the CodeLink Bioarray, which carries 54 359 records on slide glass, of which 380 encode fragments of solute carrier (SLC) transporter genes. The scatter plots of normalized fluorescence intensities of these 380 records for the high-activity subclones #9 and #21 vs the low-activity subclone #19 are shown in Figure 1, panels A and B, respectively. Candidate SLC transporter genes, for which the expression level is apparently correlated with levofloxacin uptake activity, were chosen based on the criterion of 2-fold or higher expression level in high-activity subclones (#9 and #21, *y*-axis) versus the low-activity subclone (#19, *x*-axis). The numbers of genes which showed an expression ratio higher than 2 were 31 and 59 in #9 vs #19 and #21 vs #19, respectively. Moreover, the number of genes that showed higher expression levels in both of the high-activity subclones was 19. Therefore, these 19 transporter genes were considered as candidate influx transporters for levofloxacin. Since the clones were from Caco-2 cells, and the major purpose of the present study was to find the human intestinal transporter(s), the candidate 19 transporter genes were further evaluated for expression in human small intestinal tissues. Based on these results, 7 transporter genes were selected and cDNAs of these transporters were cloned and transferred into expression plasmid vectors, which were used to transfect *Xenopus* oocytes or mammalian cultured cells, depending on the transporters. Examination of the transport activity for levofloxacin revealed that *Xenopus* oocytes expressing OATP1A2 exhibited significant activity. Fold increase of expression of OATP1A2 in high activity clones (#9) and (#21) relative to low expression clone (#19) were 2.29 and 5.53, respectively. Furthermore, the transporter genes that exhibited the expression of 2-fold or higher commonly in both the two high activity Caco-2 clones in the order of higher increase of expressions were *SLC26A3, 10A2, 2A1, 40A1, 2A5, 5A10, 19A1, 7A6, 39A14, 16A4, 22A18, 12A6, 20A2, 35A5, 26A6, 16A10, 1A1,* and *SLCO2B1* in addition to *SLCO1A2*. We did not detect any transport activity for levofloxacin by any transporters other than SLCO1A2 in our experimental system (data not shown).

Characterization of Levofloxacin Transport by OATP1A2. The time course of the uptake of [^{14}C]levofloxacin by *Xenopus* oocytes expressing OATP1A2 is shown in Figure 2A. Uptake of levofloxacin by the oocytes expressing OATP1A2 was significantly higher than that by water-injected control oocytes. Since the uptake increased

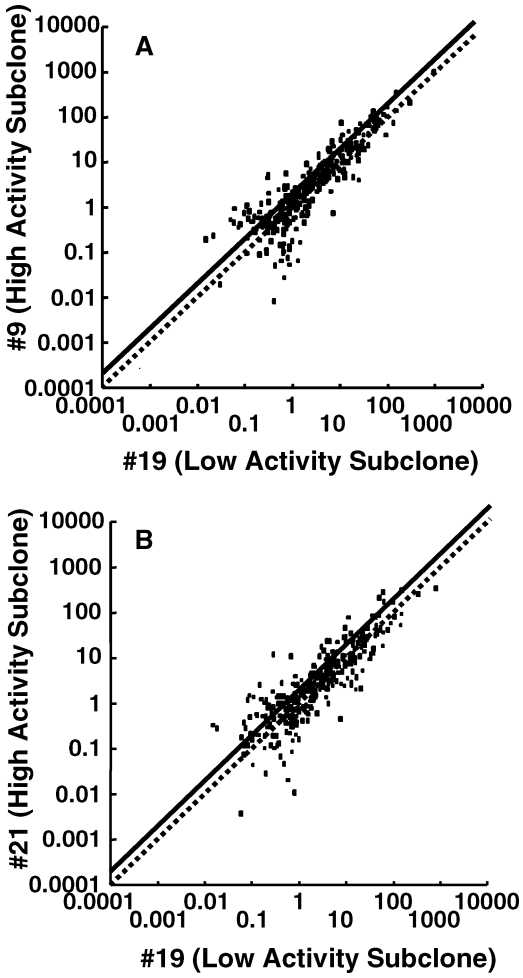

Figure 1. Comparison of normalized fluorescence intensities of SLC transporter genes expressed in high- (#9 (A) and #21 (B)) and low-activity (#19) Caco-2 cell subclones. Scatter analysis of the expression of SLC transporter genes in high-activity subclones (#9 (A) and #21 (B)) and a low-activity subclone (#19) are shown. The *x*- and *y*-axes represent the expression levels of 380 genes encoding SLC transporters after normalization of the fluorescence intensity of (A) #9 vs #19 and (B) #21 vs #19. The solid and dotted lines represent relative expression ratios of 1.0 and 2.0 between high- and low-activity subclones, respectively.

linearly for over 2 h, the uptake by OATP1A2 was characterized by measuring the uptake at 60 min. Initial uptake rate of levofloxacin by OATP1A2, obtained after subtraction of the uptake by water-injected oocytes from that by OATP1A2-expressing ooytes, was saturable in the concentration range from 10 μM to 1000 μM (Figure 2B). The half-saturation concentration (K_m) and maximum uptake rate (V_{max}) of levofloxacin by OATP1A2 were 136 $\pm$ 48.0 μM and 33.0 $\pm$ 3.90 pmol/oocyte/60 min, respectively. Figure 3 shows the pH dependence of the initial uptake rates of levofloxacin at pHs from 5.0 to 8.0. OATP1A2-specific uptake showed slight pH dependence, with lower activity at acidic pH, but this was not considered to be significant. Stereoselectivity of OATP-mediated transport of levofloxacin was examined by measuring the saturation kinetics of uptake

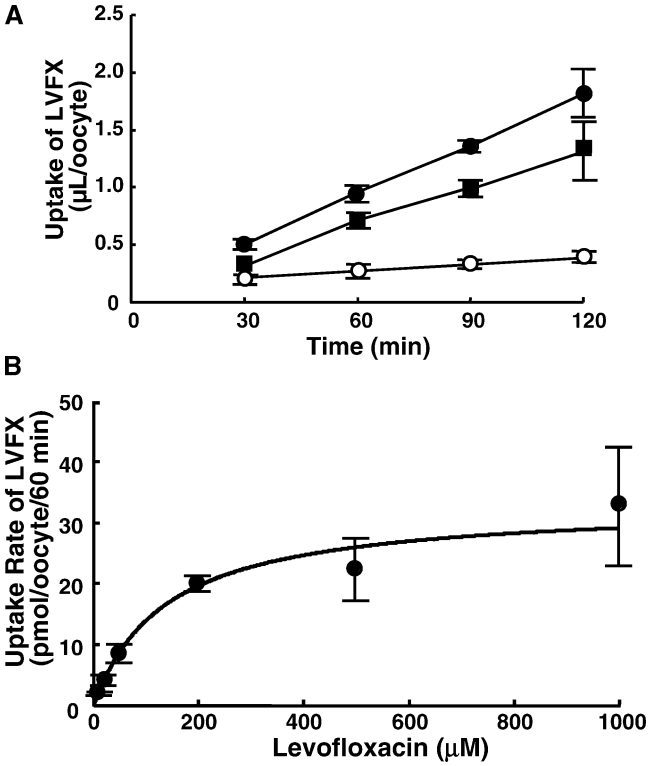

Figure 2. (A) Time course of [^{14}C]levofloxacin uptake by *Xenopus* oocytes expressing OATP1A2. Uptake of [^{14}C]-levofloxacin (10 μM) by *Xenopus* oocytes injected with cRNA of OATP1A2 (closed circles) or water (open circles) was measured over 120 min at room temperature and pH 7.4. Square symbols represent OATP1A2-specific uptake of [^{14}C]-levofloxacin, obtained by subtracting the uptake by water-injected oocytes from that by OATP1A2 cRNA-injected oocytes. Each result represents the mean ± SEM (n = 9 or 10). (B) Concentration dependence of levofloxacin uptake by *Xenopus* oocytes expressing OATP1A2. OATP1A2-specific uptake of [^{14}C]levofloxacin obtained by subtracting the uptake by water-injected oocytes from that by OATP1A2 cRNA-injected oocytes was measured for 60 min at various concentrations from 10 μM to 1000 μM at pH 7.4. Each result represents the mean ± SEM (n = 3−9).

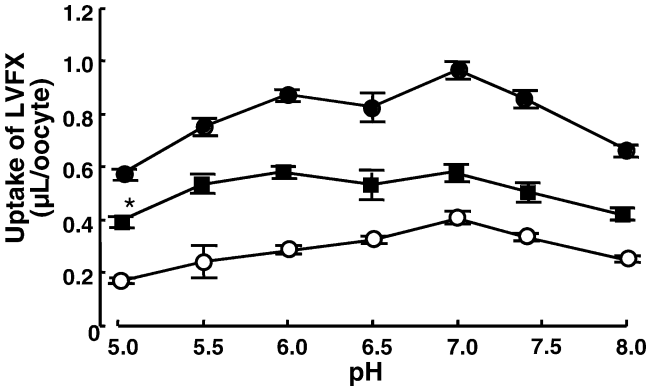

Figure 3. pH dependence of [^{14}C]levofloxacin uptake by *Xenopus* oocytes expressing OATP1A2. Uptake of [^{14}C]-levofloxacin (20 μM) by *Xenopus* oocytes injected with cRNA of OATP1A2 (closed circles) or water (open circles) at 60 min was measured at various extracellular pH values in the range from 5.0 to 8.0 at room temperature. Square symbols represent OATP1A2-specific uptake of [^{14}C]levofloxacin, obtained by subtracting the uptake by water-injected oocytes from that by OATP1A2 cRNA-injected oocytes. Each result represents the mean ± SEM (n = 6−10), and * indicates a significant difference from the uptake at pH 7.4. (p < 0.05).

of the *R*-isomer of levofloxacin (DR-3354) at 60 min in the concentration range from 10 μM to 500 μM (Figure 4A). The evaluated K_m and V_{max} values of DR-3354 were 157 ± 74.4 μM and 7.80 ± 1.50 pmol/oocyte/60 min, respectively. Figure 4B shows the concentration dependence of the inhibitory effect of DR-3354 on the uptake of [^{14}C]levofloxacin (20 μM). The uptake of levofloxacin decreased with increasing concentration of DR-3354, and IC_{50} of DR-3354 was 159 ± 39.0 μM. The IC_{50} value of DR-3354 was very close to the K_m value of DR-3354 itself, suggesting that DR-3354 and levofloxacin share a common affinity site for uptake by OATP1A2. In order to evaluate the contribution of OATP1A2 to uptake of other quinolone antibacterial agents, the transports of those compounds and their inhibitory effects on the uptake of [^{14}C]levofloxacin by OATP1A2 were examined. The results are shown in Tables 1 and 2, respectively. All of the agents used as inhibitors, including ciprofloxacin, enoxacin, grepafloxacin, and moxifloxacin, at 500 μM significantly reduced the uptake of [^{14}C]levofloxacin (20 μM) by OATP1A2 (Table 1). Furthermore, uptake of ciprofloxacin, enoxacin, gatifloxacin, lomefloxacin, and nodfloxacin by the oocytes injected with OATP1A2 was significantly higher than that by water-injected oocytes (Table 2). So, these quinolone antibacterial agents are shown to be transported by OATP1A2 with a common activity site on OATP1A2.

Characterization of Levofloxacin Transport by Subcloned Caco-2 Cells. To evaluate the contribution of OATP1A2 to the apparent uptake of levofloxacin by Caco-2 cells, the uptake was evaluated using subclone #9, which has high transport activity. Uptake of [^{14}C]levofloxacin (20 μM) increased linearly for over 2 min and attained a steady state by 15 min (Figure 5). The initial uptake was evaluated in terms of the uptake at 1 min. Initial uptake rates of levofloxacin exhibited a slight pH dependence (pH 5.0−8.0), with lower activity at pH 5.0, but the pH dependence was considered minimal (Figure 6A). Replacement of sodium ions with other cations (choline$^+$, *N*-methylglucamine$^+$, potassium$^+$, lithium$^+$) did not affect the uptake of levofloxacin (Figure 6B). The initial uptake rate of levofloxacin at concentrations from 10 μM to 20 mM was saturable (Figure 7A). The Eadie−Hofstee plot showed the involvement of two saturable components (Figure 7B). The kinetic parameters obtained by nonlinear least-squares regression analysis based on eq 2, which includes two saturable components and a nonsaturable component, were as follows: K_{m1} and V_{max1} (high-affinity component) were 0.498 ± 1.07 mM and 1.16 ± 1.59 nmol/mg/min, K_{m2} and V_{max2} (low-affinity component) were 14.6 ± 4.10 mM and 20.6 ± 4.30 nmol/

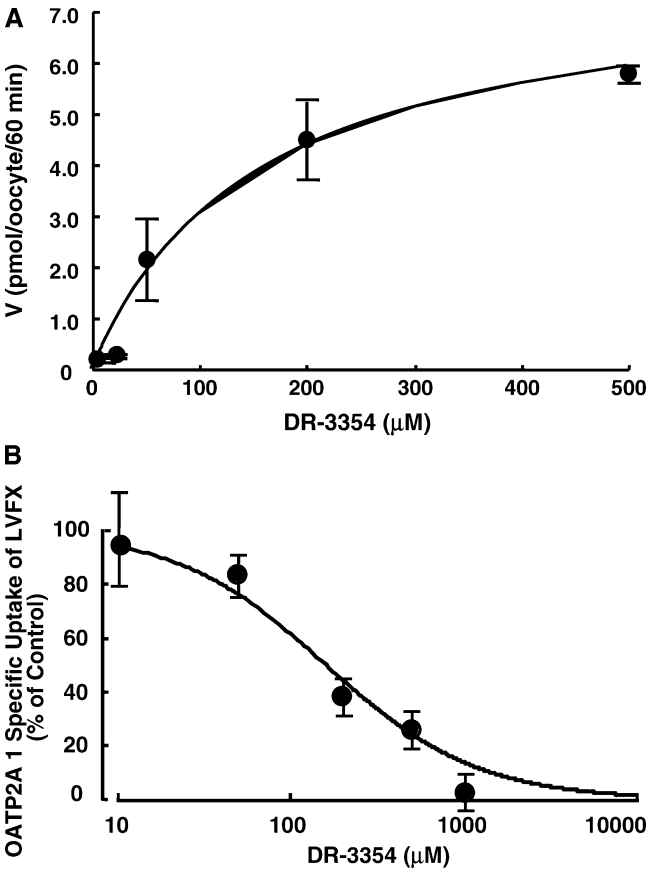

Figure 4. Concentration dependence of uptake of DR-3354 (A) and inhibitory effect of DR-3354 on uptake [^{14}C]levofloxacin by *Xenopus* oocytes expressing OATP1A2. (A) OATP1A2-specific uptake of DR-3354, obtained by subtracting the uptake by water-injected oocytes from that by OATP1A2 cRNA-injected oocytes, was measured for 60 min at various concentrations from 10 μM to 500 μM at pH 7.4. (B) Uptake of [^{14}C]levofloxacin (20 μM) was measured for 60 min at room temperature and pH 7.4. The results are shown as percentage of control uptake measured in the absence of inhibitor after subtracting the uptake by water-injected oocytes from that by OATP1A2 cRNA-injected oocytes. Each result represents the mean ± SEM (n = 7−10).

mg/min, respectively, and k_d (nonsaturable component) was 2.43 ± 0.18 μL/mg/min.

Discussion

Several studies have shown the involvement of carrier-mediated transport in the intestinal absorption of quinolone antibacterial agents such as levofloxacin, sparfloxacin, and grepafloxacin. Although P-glycoprotein, MRPs, and BCRP act as efflux transporters, no transporter that provides intestinal epithelial apical uptake of quinolone antibacterial agents has been molecularly identified. In addition, although these agents exhibit specific tissue distribution and urinary and biliary excretions via carrier-mediated transport mechanisms, no influx transporter has been molecularly identified in any tissue. A knowledge of the mechanism of the efficient membrane permeability of these agents would be very useful for the development of drugs with superior membrane permeability and for the prediction of clinically significant drug interactions involving the membrane transport process.

Table 1. Inhibitory Effect of Various Quinolone Antibacterial Agents on [^{14}C]Levofloxacin Uptake by *Xenopus* Oocyte Expressed with OATP1A2[a]

inhibitor	percentage of control
control	100.0 ± 9.5
levofloxacin	8.6 ± 4.3* [b]
DR-3354	4.7 ± 3.6*
ciprofloxacin	22.3 ± 8.3*
enoxacin	13.3 ± 3.3*
grepafloxacin	3.9 ± 1.5*
moxifloxacin	5.3 ± 2.4*

[a] *Xenopus* oocytes expressed with OATP1A2 were incubated at room temperature for 60 min in medium containing [^{14}C]levofloxacin (20 μM) with or without (control) those agents at 500 μM final concentration. Uptake was expressed as percentage of the uptake. Each value represents the mean ± SEM (n = 7−10). [b] The asterisk (*) designates $p < 0.05$, compared with control (Student's t test).

Table 2. Uptake of Various Quinolone Antibacterial Agents by *Xenopus* Oocytes Expressed with OATP1A2[a]

	uptake (μL/oocyte)	
	water	OATP1A2
ciprofloxacin	0.023 ± 0.002	0.173 ± 0.020
enoxacin	0.109 ± 0.014	0.224 ± 0.019
gatifloxacin	0.131 ± 0.002	0.575 ± 0.061
lomefloxacin	0.150 ± 0.007	0.475 ± 0.030
norfloxacin	0.027 ± 0.005	0.119 ± 0.010

[a] Uptake of quinolone antibacterial agents (20 μM) by *Xenopus* oocytes injected with OATP1A2-cRNA or water alone was measured for 60 min at pH 7.4. Uptake shows the cell-to-medium ratio and mean ± SEM (n = 9 or 10). Uptake of all compounds by OATP1A2-expressed oocytes was significantly higher than that by water-injected oocytes.

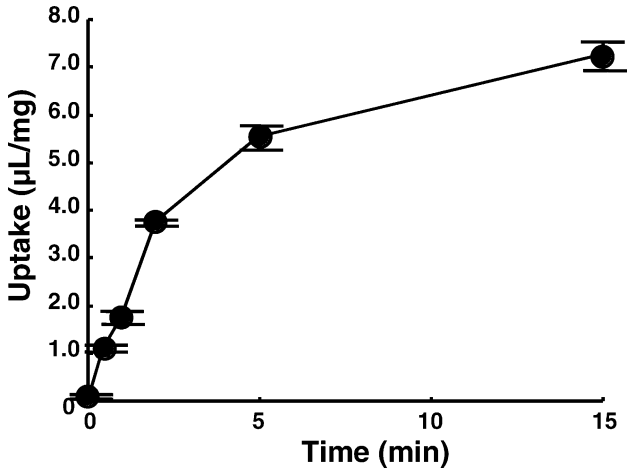

Figure 5. Time course of [^{14}C]levofloxacin uptake by subcloned Caco-2 cells. Uptake of [^{14}C]levofloxacin (10 μM) by subcloned Caco-2 cells (subclone #9) over 15 min was measured at 37 °C and pH 7.4. Values were corrected for extracellularly adhering radioactivity based on the apparent uptake of [^{14}C]inulin as a cell membrane-impermeable marker. Each result represents the mean ± SEM (n = 3 or 4).

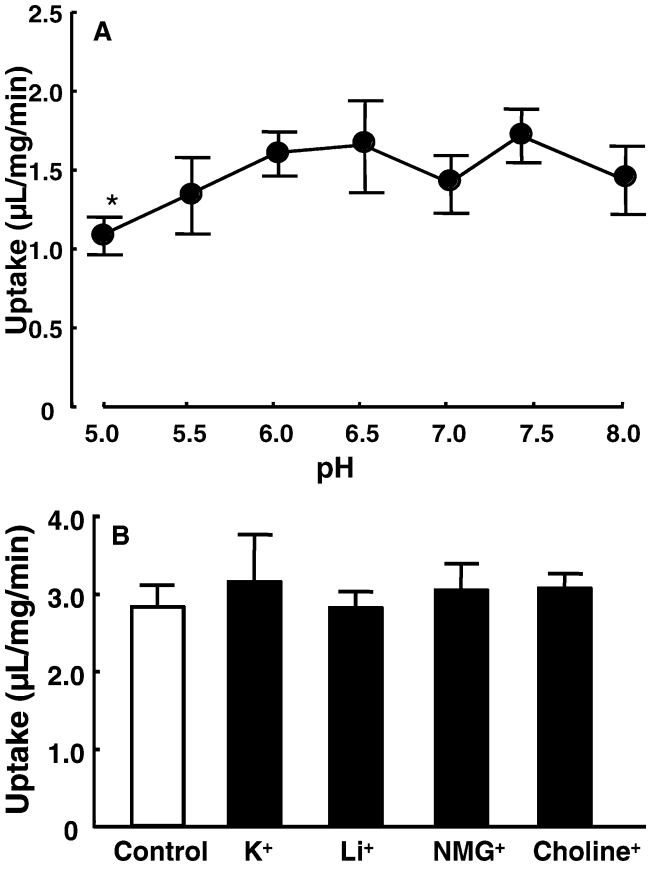

Figure 6. (A) pH- and (B) sodium-ion dependence of [^{14}C]-levofloxacin uptake by subcloned Caco-2 cells. Uptake of [^{14}C]-levofloxacin (20 μM) by subcloned Caco-2 cells at 1 min was measured at various extracellular pH values in the range from 5.0 to 8.0 at 37 °C. The total uptake values were corrected for extracellularly adhering radioactivity based on the apparent uptake of [^{14}C]inulin as a cell membrane-impermeable marker. In the experiments on sodium dependence, sodium ions in the standard buffer were replaced with choline, *N*-methylglucamine, potassium, or lithium. Each result represents the mean SEM ($n = 3$ or 4), and * indicates a significant difference ($p < 0.05$) from the uptake at pH 7.4.

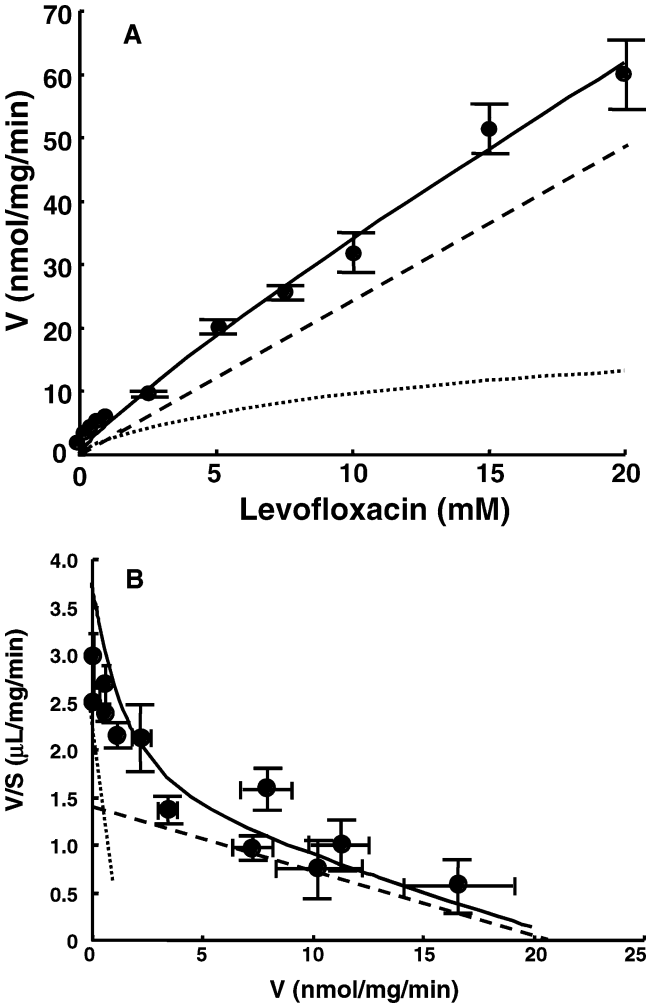

Figure 7. Concentration dependence of [^{14}C]levofloxacin uptake by subcloned Caco-2 cells. (A) Total (solid line), saturable (dotted line), and nonsaturable (dashed line) uptake of [^{14}C]levofloxacin was measured at various concentrations ranging from 20 μM to 20 mM at 37 °C for 1 min at pH 7.4. The total uptake values were corrected for extracellularly adhering radioactivity based on the apparent uptake of [^{14}C]-inulin as a cell membrane-impermeable marker. (B) Eadie−Hofstee plot of saturable uptake of levofloxacin is shown after correction for nonsaturable uptake evaluated from the first-order rate constant obtained by nonlinear least-squares regression analysis, as described in Results. Solid, dotted, and dashed lines represent apparently saturable, high-affinity, and low-affinity uptake components, respectively. Each result represents the mean ± SEM ($n = 4$).

Accordingly, the purpose of the present study was to identify the influx transporter(s) responsible for the cellular uptake of quinolone antibacterial agents.

To find the transporter molecule, we used a series of Caco-2 cell subclones and a DNA microarray technique to screen for transporter genes that are more highly expressed in cell subclones with higher uptake activity. After cultivation of the original Caco-2 cells in culture dishes for 1 to 2 weeks at low cell concentrations, we obtained 36 Caco-2 cell subclones. As an index of carrier-mediated uptake, we used the extent of decrease of the initial uptake activity of radiolabeled levofloxacin in the presence of an excess amount of unlabeled levofloxacin. Several subclones that exhibited high and low uptake activities were selected by the initial screening, and the selected subclones were further examined to confirm their uptake activities. Finally, we obtained two and one subclones with high and low uptake activities, respectively, for DNA microarray analysis. We used a DNA microarray carrying 54 359 records; several records corresponding to different regions of the same target genes are therefore present (the length of each probe is 30 nucleotides). Transporters are classified to ABC and SLC transporters. The ABC transporters are primary active and often work for the efflux of their substrates from cells. The SLC transporters include many influx transporters. Therefore, in the present study, we focused on screening of SLC transporters. The number of records of SLC transporters in the DNA microarray was 380. As candidate SLC transporter genes expressed

more strongly in the subclones with higher uptake activities, we selected those with at least 2-fold greater expression in a high-activity subclone than in the low-activity subclone, and identified 31 and 59 SLC transporter genes from the high-activity subclones #9 and #21, respectively. Among them 19 SLC transporter genes exhibited higher expression in both subclones. Of these, we selected the genes that are expressed in human small intestinal tissues, and we isolated cDNAs of these 7 transporter genes. Finally, we found that expression of the transporter OATP1A2 in *Xenopus* oocytes imparts levofloxacin uptake activity. Thus, OATP1A2 is the first human gene encoding an influx transporter for quinolone antibacterial agents to have been found.

When OATP1A2 was expressed in *Xenopus* oocytes, levofloxacin uptake was significantly increased (Figure 2A) and the K_m value was 136 μM. Yamaguchi et al. reported a K_m value of 9.3 mM for levofloxacin uptake in Caco-2 cells, which is significantly higher. However, in the present study, the Caco-2 subclone used for the identification of OATP1A2 exhibited high- and low-affinity components with K_m values of 0.498 and 14.6 mM, respectively. It is possible that the low-affinity component observed in our Caco-2 subclone may correspond to that reported by Yamaguchi et al. (2001),[10] since the concentration range examined in their study might not have been broad enough to detect the high-affinity component. So, the high-affinity component with a K_m value of 0.498 mM may correspond to the OATP1A2-mediated uptake. The relative contributions of the high- and low-affinity components and the nonsaturable component in our Caco-2 subclone evaluated from the kinetic parameters were 38%, 23%, and 39%, respectively. It is not clear what the nonsaturable component represents: it may include adsorption and binding on the membrane surface and simple diffusion. Accordingly, since the apparent nonsaturable uptake may overestimate true uptake, it is likely that the high-affinity component, which may correspond to OATP1A2, contributes predominantly to the apparent permeation of levofloxacin across Caco-2 cell membranes.

Sodium independence and trivial pH dependence of levofloxacin uptake by OATP1A2 (Figure 3) were similar to those of our Caco-2 subclones (Figure 6A) and previously reported Caco-2 cells.[10] However, uptake transport of grepafloxacin and HSR903 exhibited partial sodium ion dependence in lung cells.[11,12] Accordingly, influx transporters of quinolone antibacterial agents may be different in lung and Caco-2 cells.

Levofloxacin is the *S*-isomer of ofloxacin, and it was reported that levofloxacin and its *R*-isomer (DR3354) have similar pharmacokinetic properties, including intestinal absorption, tissue distribution, and elimination, in humans.[29] In the present study, the K_m of levofloxacin and DR3354 for OATP1A2 were comparable (136 μM and 157 μM, respectively (Figures 2B and 4A)), so the absence of stereospecificity in the intestinal absorption of ofloxacin is consistent with the characteristics of OATP1A2-mediated transport.

In humans, OATP1A2 is expressed in several tissues, including liver, lung, kidney, brain, and testis.[30] It was also reported that OATP1A2 protein is expressed in human small intestine,[31] and apical membrane localization of OATP1A2 in human small intestinal epithelial cells was suggested.[32] Accordingly, it is possible that OATP1A2 may contribute to the intestinal absorption of quinolone antibacterial agents. However, we have to be careful about the expression of OATP1A2 in human small intestine, since Abe et al. and we failed to detect the expression of OATP1A2 in the tissue by Northern blot and RT-PCR analysis.[33,3434] However, we previously reported that OATP2B1 (OATP-B/SLCO2B1) is expressed at the apical membrane of human small intestinal epithelial cells and transports several anionic compounds, including fexofenadine and pravastatin, in a sodium-independent, but pH-dependent, manner.[35,36] We did not observe significant transport of levofloxacin by OATP2B1, OATP1B1 (OATP-C/SLCO1B1), or OATP1B3 (OATP8/SLCO1B3) in our system (data not shown). Since fexofenadine is a substrate of OATP1A2, as well as OATP2B1,[37] OATP1A2 may contribute to the intestinal absorption of drugs more generally than OATP2B1.

A previous kinetic study on the intestinal absorption of ofloxacin in the concentration range from 0.34 to 13.5 mM,

(29) Okazaki, O.; Kojima, C.; Hakusui, H.; Nakashima, M. Enantioselective disposition of ofloxacin in humans. *Antimicrob. Agents Chemother.* **1991**, *35*, 2106−2109.

(30) Kullak-Ublick, G. A.; Hagenbuch, B.; Stieger, B.; Schteingart, C. D.; Hofmann, A. F.; Wolkoff, A. W.; Meier, P. J. Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. *Gastroenterology* **1995**, *109*, 1274−1282.

(31) Su, Y.; Zhang, X.; Sinko, P. J. Human organic anion-transporting polypeptide OATP-A (SLC21A3) acts in concert with P-glycoprotein and multidrug resistance protein 2 in the vectorial transport of Saquinavir in Hep G2 cells. *Mol. Pharm.* **2004**, *1*, 49−56.

(32) Glaeser, H.; Bailey, D. G.; Lee, W.; Dresser, G. K.; Gregor, J. C.; McGrath, J. S.; Jolicoeur, E.; Roberts, R.; Smith, L. H.; Kim, R. B. *Drug Metab. Rev.* **2004**, *36*, 260.

(33) Abe, T.; Kakyo, M.; Tokui, T.; Nakagomi, R.; Nishio, T.; Nakai, D.; Nomura, H.; Unno, M.; Suzuki, M.; Naitoh, T.; Matsuno, S.; Yawo, H. Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *J. Biol. Chem.* **1999**, *274*, 17159−17163.

(34) Tamai, I.; Nezu, J.; Uchino, H.; Sai, Y.; Oku, A.; Shimane, M.; Tsuji, A. Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem. Biophys. Res. Commun.* **2000**, *273*, 251−260.

(35) Kobayashi, D.; Nozawa, T.; Imai, K.; Nezu, J.; Tsuji, A.; Tamai, I. Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 703−708.

(36) Nozawa, T.; Imai, K.; Nezu, J.; Tsuji, A.; Tamai, I. Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 438−445.

(37) Cvetkovic, M.; Leake, B.; Fromm, M. F.; Wilkinson, G. R.; Kim, R. B. OATP-A and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab. Dispos.* **1999**, *27*, 866−871.

using an intestinal perfusion method, gave a K_m value between 7 and 10 mM.[9] Since racemic ofloxacin should have comparable K_m values to levofloxacin or DR3354, as described above, the observed K_m value is thought to correspond to the low-affinity component. Their examined concentration range may not have been sufficient to allow clear detection of a high-affinity component with a K_m value of less than 1 mM. Accordingly, we speculate that high-affinity OATP1A2 as well as an unknown lower-affinity transporter may be involved, at least in part, in uptake of quinolone antibacterial agents at the intestinal membrane.

Hepatic uptake of grepafloxacin in rats exhibited sodium independence with a K_m value of 173 μM.[13] Since OATP1A2 is also expressed in liver, sodium-independent uptake of levofloxacin with K_m 136 μM by OATP1A2 may correspond to the hepatic uptake of quinolone antibacterial agents.[30] OATP1A2 in brain exhibits localized expression at the luminal surface of the blood−brain barrier,[38,39] and quinolone antibacterial agents are distributed well to the central nervous system when P-glycoprotein is not functional.[21] Although there is no report on the mechanism of brain distribution of these agents, their distribution to the brain might be mediated by influx transporters such as OATP1A2 that are expressed at the luminal membrane of the blood−brain barrier.

The IC_{50} of DR3354 (159 μM, Figure 4B) for levofloxacin uptake is close to the K_m value of DR3354 itself (Figure 4A). In addition, several fluoroquinolones such as ciprofloxacin, enoxacin, grepafloxacin, and moxifloxacin inhibited the OATP1A2-mediated uptake of levofloxacin (Table 1). Furthermore, all of the fluoroquinolones examined exhibited transport activity mediated by OATP1A2 (Table 2). Accordingly, fluoroquinolones are likely to be transported commonly by OATP1A2.

In conclusion, the present study has yielded the first identification at the molecular level of an influx transporter involved in the membrane transport of quinolone antibacterial agents. Since the identified transporter molecule, OATP1A2, is expressed in various tissues, including small intestine, blood−brain barrier, liver, lung, and testis, it may play a role in regulating the intestinal absorption, tissue distribution, and hepatic elimination of these agents. In addition, it was demonstrated that multiple transporters are involved in the uptake of levofloxacin in Caco-2 cells, so further studies will be needed to identify the other influx transporters involved, in order to fully understand the factors that determine the membrane permeability of quinolone antibacterial agents.

Abbreviations Used

ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; IC_{50}, the half-inhibitory concentration; MDR, multidrug resistance; MRP, multidrug resistance associated protein; OATP, organic anion transporting polypeptide; SLC, solute carrier.

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(38) Gao, B.; Hagenbuch, B.; Kullak-Ublick, G. A.; Benke, D.; Aguzzi, A.; Meier, P. J. Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 73−79.

(39) Bronger, H.; Konig, J.; Kopplow, K.; Steiner, H.-H.; Ahmadi, R.; Herold-Mende, C.; Keppler, D.; Nies, A. T. ABCC drug efflux pumps and organic anion uptake transporters in human gliomas and the blood-tumor barrier. *Cancer. Res.* **2005**, *65*, 11419−11428.