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Involvement of Multiple Transport Systems in the Disposition of an Active Metabolite of a Prodrug-type New Quinolone Antibiotic, Prulifloxacin

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Summary: Prulifloxacin is a prodrug-type new quinolone. The purpose of this study is to clarify the mechanism of biliary excretion and brain distribution of its active metabolite, UFX. UFX was efficiently excreted into the bile in rats, with its concentration in the bile being 30–60 times higher than that in plasma. The *in vivo* disposition study revealed that multidrug resistance-associated protein 2 (MRP2) was involved in the biliary excretion of glucuronide metabolite, but not of the unchanged UFX. A transport study using a P-glycoprotein (P-gp) overexpressing cell line, LLC-GA5-COL150, showed that UFX was a substrate of P-gp. Nevertheless, the biliary clearance (CLbile) of UFX in P-gp-gene-deficient mice was not different from that in the normal mice, although the concentration in the liver was slightly higher than that in the normal mice. These observations suggest that multiple transport systems are involved in the biliary excretion of UFX, with minor contribution of P-gp. The distribution of UFX in the rat brain was quite low, and its tissue to plasma concentration ratio (Kp) in the brain was much less than the unity and was increased by cyclosporin A. The Kp in the brain of mdr1a/1b(-/-) mice was higher than that in the normal mice, suggesting that efflux by P-gp played a major role in the limited brain distribution of UFX.

Key words: prulifloxacin; pharmacokinetics; biliary excretion; brain; p-glycoprotein

Introduction

Prulifloxacin (PUFX) is a prodrug-type new quinolone antibiotic. It is quickly converted to the active metabolite, UFX ((±)-6-fluoro-1-methyl-4-oxo-7-(1-piperazinyl)-4*H*-[1,3] thiazeto [3,2-a] quinoline-3-carboxylic acid), during the absorption process (**Fig. 1**). UFX is a zwitter ionic compound. UFX has strong antimicrobial activities against gram positive and negative bacteria and shows good efficacy in infections in the various tissues. In humans, 30.6–46.0% of the dose was recovered in the urine as UFX after oral administration of PUFX. Thus, urinary excretion is the major elimination route of this drug.[1)] On the other hand, the concentration of UFX in the bile was reported to be higher than that in the plasma, and that could be one of the reasons why UFX is most effective for the bile duct infection among new quionolones.[2)]

UFX interacts with $GABA_A$ receptors like other new quinolones. The oral administration of PUFX at a dose of 1000 mg/kg did not show any convulsant effect in mice, but coadministration with fenbufen led to convulsion at PUFX doses of 300 mg/kg or higher. Despite the stronger inhibition potency of UFX to $GABA_A$ receptor binding *in vitro* than enoxacin, such convulsant effect of PUFX was weaker *in vivo* than enoxacin.[3,4)] Autoradiogram of the rat brain after oral administration of [^{14}C]-PUFX suggested the limited distribution of radioactivity in the brain. Thus, the low concentrations of the drug and its active metabolites in the brain seem to account for the low toxicological effect *in vivo*.

The purpose of this study is to clarify the mechanism for the above beneficial pharmacokinetic behavior of PUFX. We focused on the mechanism of the biliary excretion and brain distribution of UFX.

Materials and Methods

Chemicals: UFX (**Fig. 1**) was synthesized at Nippon Shinyaku Co., Ltd. (Kyoto, Japan). Enoxacin and norfloxacin were purchased from Sigma-Aldrich Japan

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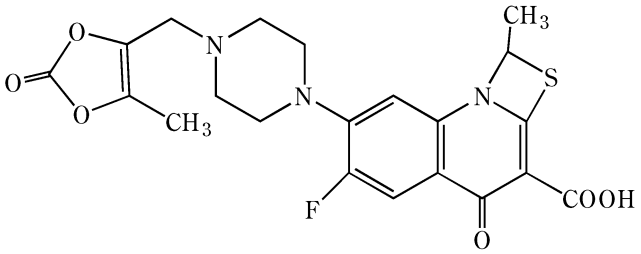
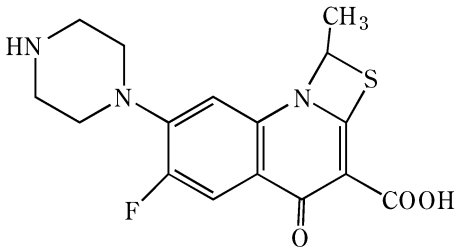


Prulifloxacin (PUFX)



UFX

Fig. 1. Chemical structures of prulifloxacin and UFX.

(Tokyo, Japan), and cyclosporin A (Sandimmun® injection, 50 mg/mL) from Novartis Pharma KK (Tokyo, Japan). All the other chemicals were of the analytical grade.

Animals: Male Sprague-Dawley (SD) rats and Eisai hyperbilirubinemic rats (EHBR) which have an inherited deficiency in their multidrug resistance-associated protein 2 (MRP2), aged 7 weeks, were supplied by Japan SLC Co. (Shizuoka, Japan). FVB mice were purchased from Taconic Farms, Inc. (Germantown, NY, USA) and Clea Japan Inc. (Tokyo, Japan), and mdr1a/1b(-/-) mice were purchased from Taconic Farms, Inc. They were housed and handled according to the ''Principles of Laboratory Animal Care'' (NIH publication #85–23, revised 1985) and the ''Guidance for the Care and Use of Laboratory Animals'' (Pharmaceutical Research Center, Meiji Seika Kaisha, LTD.). The rats were kept in the temperature- and light-controlled environment with the standard food and tap water provided ad libitum.

Biliary clearance study in rats: Under anesthesia with diethylether, the femoral artery and bile duct were cannulated with polyethylene tubings, PE50 and PE10, for blood sampling and bile collection, respectively. The femoral vein was cannulated with SP31 for injection of cyclosporin A. UFX was injected from the tail vein at a dose of 5 mg/kg. Cyclosporin A (30 mg/kg) was injected 5 minutes prior to the administration of UFX. At designated time points, blood samples were collected and the plasma was prepared by centrifugation. The bile and urine were collected in the pre-weighed test tubes.

Bile excretion study in mice: The abdominal cavity was opened and the common bile duct was ligated. The gallbladder was then cannulated with PE10 for bile collection. UFX was injected from the tail vein at a dose of 10 mg/kg.

Brain, CSF and liver distribution in rats: After loading dose of 3.3 mg/kg, constant infusion of UFX through SP31 placed in the femoral vein was started at a dose rate of 2.2 mg/hr/kg. The cyclosporin A-treated group received an injection of cyclosporine A at a dose of 30 mg/kg 5 minutes prior to the loading dose of UFX. At 1, 1.5, 2 and 2.5 hr, blood was drawn through PE50 placed in the femoral artery and the plasma was separated by centrifugation. At 2.5 hr, cerebrospinal fluid (CSF) specimens were obtained by cisternal puncture with a 25-gauge needle and then the whole brain and a lobule of liver were isolated. During the experiments, the rats were kept anesthetized with pentobarbital.

Brain distribution in mice: UFX was injected from the tail vein at a dose of 10 mg/kg. At designated time points, blood was drawn from the vena cava under anesthesia with diethylether. The brain, liver and content of urinary bladder were collected.

Cell culture: The transport study was performed with LLC-PK1/LLC-GA5-COL150 cells[5,6] obtained from Riken Cell Bank (Tsukuba, Japan) and grown in M199 medium containing 10% calf serum, 14.3 mM $NaHCO_3$ and 3% L-glutamine, without or with 150 colchicine. For the transport experiments, the cells were grown on Transwells (Costar #3402, pore size 3.0 μm, polycarbonate membrane, surface area 1 cm^2). The confluent cells were washed with Hanks' balanced salt solution (HBSS, composition (mM): 0.952 $CaCl_2$, 5.36 KCl, 0.441 KH_2PO_4, 0.812 $MgSO_4$, 136.7 NaCl, 0.385 Na_2HPO_4, 25 D-glucose and 10 HEPES, pH 7.4; the osmolality of 315 mOsm kg^{-1}). HBSS (0.5 and 1.5 mL, respectively) was then added on the apical and basolateral sides of a cell insert. To measure apical-to-basolateral (A-to-B) or basolateral-to-apical (B-to-A) flux, 20 μM of UFX was added to the apical or basolateral side, respectively, and incubated at 37°C. At the designated time points, samples (0.5 mL of basolateral- or 0.2 mL of apical-side solution) were withdrawn from the acceptor compartment and replaced with an equal volume of HBSS. In some cases of incubation, 10 μM of cyclosporin A was added to both sides of the cell.

Drug analysis: Concentrations of UFX in plasma, bile and urine samples were determined by the HPLC method after liquid-liquid extraction of the sample specimens. Briefly, aliquots of the samples added with internal standard (I.S., enoxacin) were extracted into

dichloromethane containing 1% of ethylchlorocarbonate.[7] The dichloromethane layer was evaporated to dryness under a nitrogen gas stream. Limit of quantitation (LOQ) was 0.1 μg/mL for plasma, 0.05 μg/mL for bile and urine and 0.025 μg/mL for CSF. For the assay of bile and liver samples obtained from mice, and liver samples from rats, the samples were extracted using OASIS HLB® Extraction Cartridge (Nihon Waters, Tokyo). UFX and I.S. (enoxacin) were eluted with acetonitrile and evaporated to dryness. The residue was dissolved in methanol to prepare an HPLC sample. The LOQ was 1 μg/mL for bile and 0.5 μg/g for mouse and rat liver. The samples from the transport study were diluted with HBSS, if necessary, and added with I.S. (enoxacin). Prepared HPLC samples were injected onto the Capcell pak® C18 (4.6 mm I.D. × 35 mm–4.6 mm I.D. × 250 mm, Shiseido, Tokyo, Japan). The mobile phase was composed of 0.05 M phosphate buffer (pH 2.0)-methanol-acetonitrile (5:2:3 for plasma, urine and CSF or 4:2:3 for bile). The flow rate and column temperature were 1.0 mL/min and 35°C, respectively. The elution was monitored at 275 nm. The LOQ was 2.5 ng/mL. Drug concentrations in the mouse plasma and brain, and rat brain were assayed by the LC-MS/MS method after solid phase extraction. Briefly, plasma and brain homogenate, added with I.S. (norfloxacin for rat brain or ofloxacin for mouse plasma and brain), were deproteinized with 7%-perchloric acid. The supernatant was diluted with water and applied to OASIS HLB solid phase extraction cartridge. After washing the cartridge with water, analyte and I.S. were eluted with methanol. The eluate was dried under the nitrogen stream, and reconstituted in the mobile phase. The samples were injected on Atlantis dC18 column (2.1 × 50 mm, 3 μm, Waters), and eluted using 0.1% formic acid/acetonitrile linear gradient condition solvent system from 95:5 to 80:20 of 0.1% formic acid, with a flow rate of 0.4 mL/min, at 40°C. The run time was 5 min. The eluate was introduced to a tandem mass spectrometer equipped with Turbo Ion spray probe (API-3000, PE Sciex), and data was obtained by multiple reaction monitoring (MRM), positive scan mode. For quantification, m/z 350.2→248.1 or 306.4 was monitored for UFX in the rat brain or mouse plasma and brain, respectively. For norfloxacin and ofloxacin, m/z 320.1→275.6 or 361.9→317.5 were monitored, respectively. LOQ was 10 ng/mL for plasma and 10 ng/g for brain.

Data analysis: PK parameters were calculated using the software, WinNonlin (Ver.3.1, Pharsight, CA, USA). The biliary clearance (CLbile) was obtained by dividing the amount excreted into the bile by the area under the concentration-time curve from time zero to time infinity ($AUC_{0-\infty}$).

Statistics: All data are expressed as mean ± S.D. unless otherwise stated. The statistical test used for comparison between groups was Student's t-test with $p < 0.05$ taken as a significant difference.

Table 1. Concentration of unchanged UFX in the bile and plasma after intravenous administration of UFX in SD rats

Concentration (μg/mL)	Time		
	0–4 hr	4–8 hr	8–24 hr
Bile	23.6 ± 6.2	4.47 ± 2.29	0.90 ± 0.54
Plasma	0.43 ± 0.11	0.12 ± 0.08	
	(2 hr)	(6 hr)	—
Ratio (Bile/Plasma)	59.3 ± 25.8	32.8 ± 12.5	—

Mean ± SD of 4 rats.
SD rats received intravenous administration of UFX at a dose of 5 mg /kg.

Table 2. Pharmacokinetic parameters of UFX in SD rats and EHBR after intravenous administration at a dose of 5 mg/kg

Parameter	SD	EHBR
CLtot (mL/min/kg)	23.1 ± 1.9	23.4 ± 2.9
Vss (L/kg)	4.87 ± 0.81	3.21 ± 0.07*
Excretion in urine (% of dose)	60.6 ± 3.1	65.9 ± 4.3
Excretion in bile (% of dose)	9.1 ± 2.0	9.0 ± 1.9

*Significantly different from SD rats ($p < 0.05$).
Mean ± S.D. of 3–5 rats.

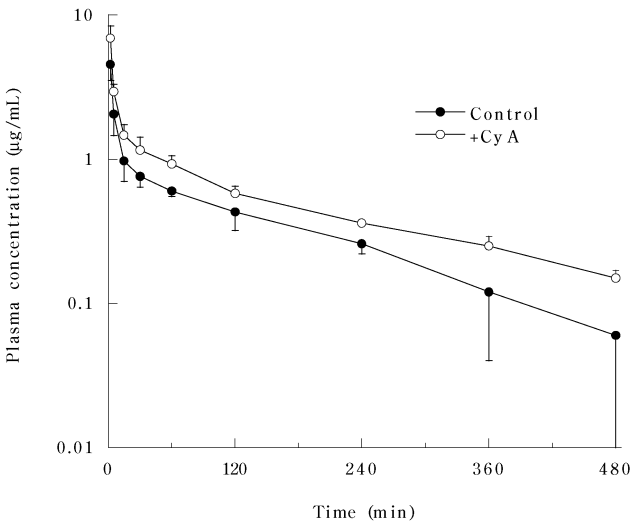


Fig. 2. Plasma concentration profile of UFX after intravenous administration at a dose of 5 mg/kg to rats with or without pre-treatment with cyclosporin A.

Results

Biliary excretion of UFX in rats: To evaluate the involvement of MRP2 in the biliary excretion of UFX, bolus injection of UFX was given to SD rats and EHBR, and pharmacokinetic parameters were obtained. The

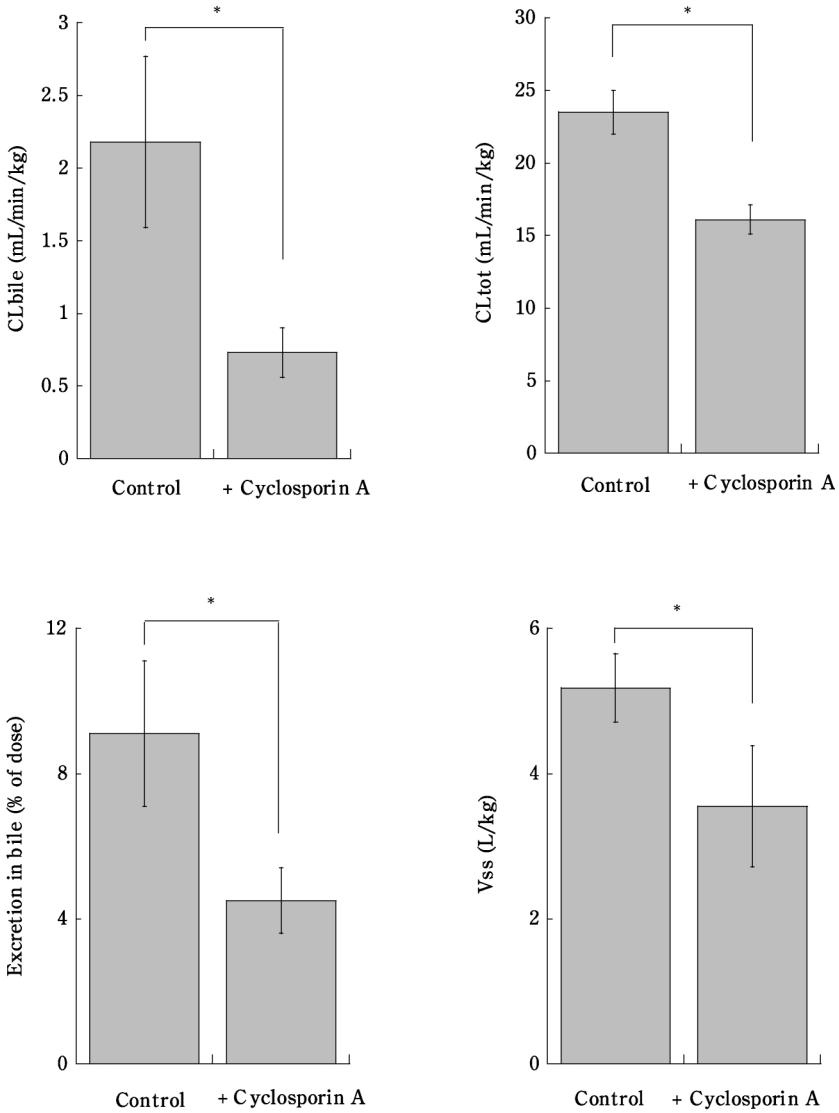


Fig. 3. Effect of cyclosporin A on the pharmacokinetic parameters of UFX in rats
Rats with the bile duct cannulation received 30 mg/kg of cyclosporine A 5 min before intravenous administration of UFX. Mean ± S.D. of 3–4 rats. *Significantly different from the control ($p < 0.05$).

concentration of UFX in the bile was approximately 30–60 times higher than that in the plasma at the midpoint of each collection period (**Table 1**). **Table 2** shows the comparison of pharmacokinetic parameters of UFX between SD rats and EHBR. The total body clearance (CLtot) was similar in both strains (**Table 2**). The biliary excretion of the unchanged UFX was approximately 9% in both SD rats and EHBR (**Table 2**). The biliary excretion of the glucuonide metabolite of UFX was markedly decreased to the negligible level in EHBR, compared to that (8.4% of dose) observed in SD rats. There was a minimal difference observed in the urinary excretion of unchanged UFX between SD rats and EHBR, with the excretion being approximately 61–66% of dose (**Table 2**). The volume of distribution at steady state (Vss) was lower in EHBR than in SD rats (**Table 2**).

To investigate the involvement of P-gp, SD rats were pretreated with cyclosporin A. The plasma concentrations in the treated rats were higher than those in the control rats (**Fig. 2**). The CLtot and Vss in the treated rats decreased to 67.9% and 68.6% of the control, respectively (**Fig. 3**). The excretion into the bile and CLbile decreased to 49.5% and 33.5% of the control, respectively (**Fig. 3**).

Biliary excretion in the mdr1a/1b(-/-) mice: UFX was administered intravenously to wild-type and mdr1a /1b(-/-) mice and the bile was collected over 2 hours. There was no difference observed in the plasma concentrations 2 hours after administration between wild-type

Table 3. Biliary excretion of UFX in wild-type and mdr1a/1b(-/-) mice

	Plasma concentration (2 hr)	Bile excretion (% of Dose)
Wild-type	567 ± 130	14.3 ± 0.8
mdr1a/1b(-/-)	600 ± 250	15.8 ± 0.7

UFX was intravenously administered to mice with ligated common bile duct and a cannulated gallbladder. Bile was collected for 2 hours under anesthesia with pentobarbital. (Mean ± S.D. of 3 mice.)

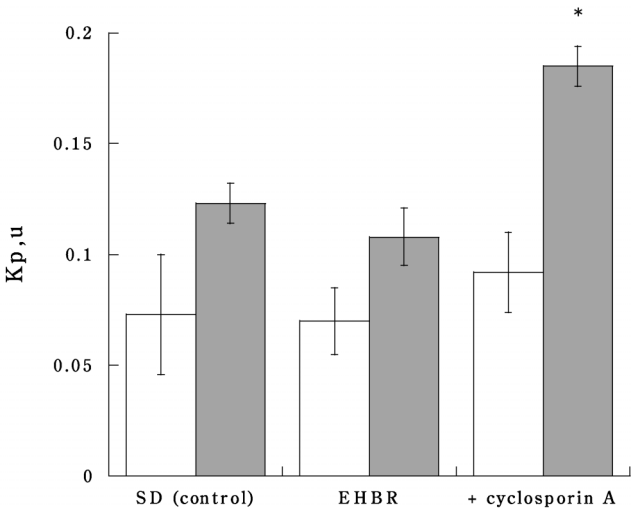


Fig. 4. Distribution of UFX into the brain in rats
Rats were given a loading dose and then a constant rate infusion. At 2.5 hr after the initial dosing, the CSF and brain were isolated from rats. The Kp,u was defined as the ratio of CSF (open bar) and brain (closed bar) concentration to steady-state plasma concentration divided by the serum unbound fraction (0.505). Data are the mean ± S.D. of 3 rats.
*Significantly different from the control ($p < 0.05$)

and mdr1a/1b(-/-) mice (**Table 3**). The biliary excretion was approximately 15% of the dose in both wild-type and knockout mice. Thus, there was no obvious difference in hepatobiliary transport between strains (**Table 3**).

Brain and liver distribution in rats: The rats were given constant rate infusion of UFX after the initial loading dose. The plasma concentration of UFX was kept constant at and after 1.5 hr. **Figure 4** illustrates the distribution of UFX in the CSF and brain at 2.5 hr. In the control SD rats, the averages of CSF-to-unbound plasma concentration ratio (Kp,u_{CSF}) and brain-to-unbound plasma concentration ratio (Kp,u_{brain}) were 0.073 and 0.123, respectively (**Fig. 4**), and both values were much less than unity. To evaluate the contribution of MRP2 and other transporters in the CNS distribution, Kp,u's in EHBR and SD rats pretreated with cyclosporin A were determined. The Kp,u values for the CSF and brain in EHBR were not different from those

Table 4. Concentrations of UFX in the plasma and brain of wild-type and mdr1a/1b(-/-) mice 2 hours after intravenous administration

	Plasma (ng/mL)	Brain (ng/g)	Kp_{brain} Brain/plasma
Wild-type	321 ± 76	36.6 ± 5.4	0.116 ± 0.019
Mdr1a/1b(-/-)	273 ± 101	72.3 ± 7.5	0.292 ± 0.109*

Wild-type and mdr1a/1b(-/-) mice received 10 mg/kg of UFX intravenously.
* Significantly different from wild-type mice ($p < 0.05$).
Mean ± S.D. of 3–4 mice.

in the control SD rats (**Fig. 4**). The Kp,u for the brain in SD rats treated with cyclosporin A was 1.5-fold that in the control (**Fig. 4**). The liver concentration of UFX in SD rats was 12.9 ± 1.5 μg/g, which was 9.7 ± 1.5 fold of the total plasma concentration.

Brain distribution in the mdr1a/1b(-/-) mice: The brain distribution of UFX was examined in the wild-type and knockout mice lacking the mdr1a/1b gene encoded P-gp. The plasma concentration of UFX 2 hours after intravenous bolus administration of UFX was similar to that in the normal and mdr1a/1b(-/-) mice (**Table 4**). The brain-to-plasma concentration ratio in mdr1a/1b(-/-) mice was 2.5-fold that in the normal mice (**Table 4**).

UFX transport across LLC-GA5-COL150 cell layer: To determine if UFX is the substrate of P-gp, transcellular transport and cellular accumulation of UFX by the monolayer of the cells overexpressing P-gp on the apical membrane (LLC-GA5-COL150 cell) were evaluated and compared with those in the parental LLC-PK1 cells. The transport of UFX across LLC-GA5-COL150 cell layer was directional with greater flux in the B-to-A direction (**Fig. 5**). In the presence of 10 μM cyclosporin A, such directionality was diminished. The difference in B-to-A and A-to-B permeability across the parental LLC-PK1 cells and the effect of cyclsoporin A on it were not obvious (**Fig. 5**). Accumulation of UFX was evaluated by the amount of UFX in the cell after the period of transport study. In the absence of cyclosporin A, the amount of UFX in the LLC-PK1 cells was larger than that in the LLC-GA5-COL150 cells (**Fig. 6**). In the presence of cyclosporin A, the amount in the LLC-PK1 cells increased to 114% and 167% of the control when the substrate was added to the apical and basal sides of the cell layer, respectively (**Fig. 6**). The accumulation in the LLC-GA5-COL150 cell increased more prominently to 181% and 312% of the control, respectively (**Fig. 6**). In the presence of cyclosporin A, the amount of UFX in the LLC-GA5-COL150 cells was close to that in the LLC-PK1 cells (**Fig. 6**).

Discussion

In the present study, the mechanism of biliary excre-

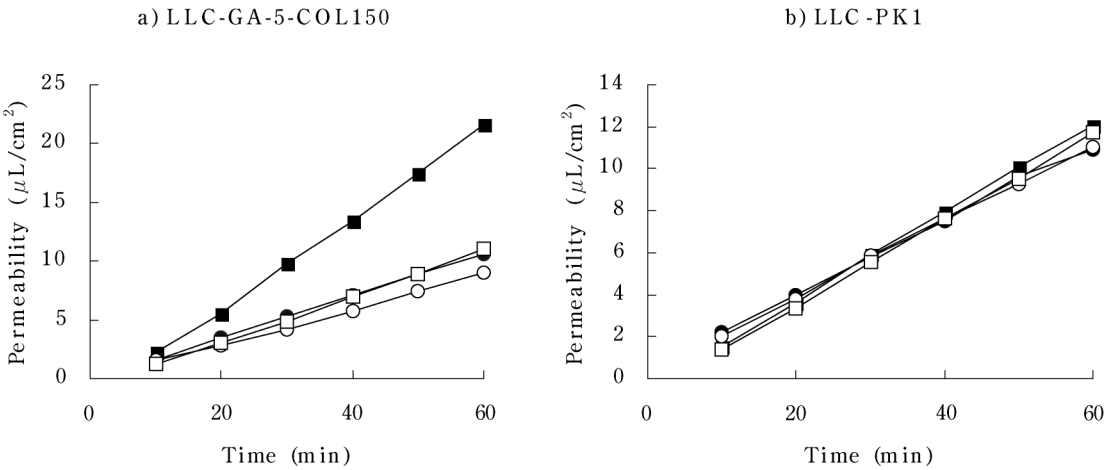


Fig. 5. Permeation of UFX across LLC-GA-5-COL150 (a) cell and LLC-PK1 (b) cell monolayers
Transport of UFX was measured in the apical-to-basolateral (circles) and in the basal-to-apical (squares) direction in the presence (open symbols) and absence (closed symbols) of cyclosporin A. Concentrations of UFX and cyclosporin A were 20 μM and 10 μM, respectively. Each point represents the average of results in 3 experiments.

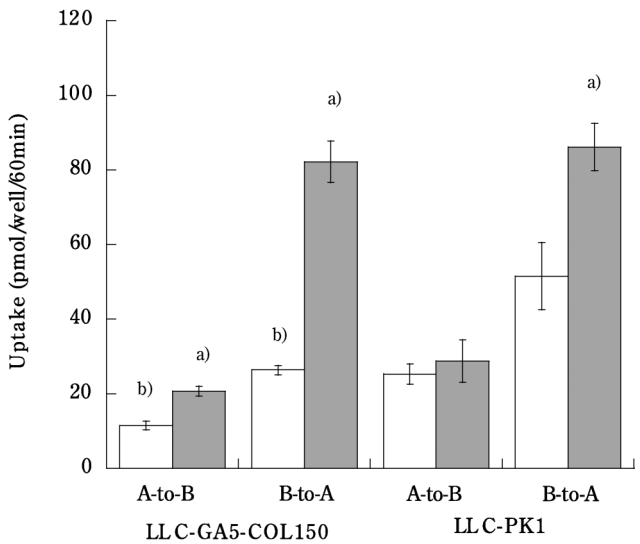


Fig. 6 Effect of cyclosporin A on the uptake of UFX into LLC-GA5-COL150 cells and LLC-PK1 cells during the transport study
Open and closed bars represent the amounts of UFX in the cells incubated in the absence and presence of cyclosporin A, respectively. Mean ± S.D. of 3 experiments.
a) Significantly different from the control. ($P < 0.05$)
b) Significantly different from LLC-PK1 cells. ($P < 0.05$)

tion and brain distribution of UFX, an active metabolite of a new quinolone PUFX, were studied. We found that multiple transporters were involved in delivery of UFX to the bile, which is one of the pharmacological target sites. In addition, Mdr1 is, at least partially, involved in the limited distribution of UFX to the brain.

The higher drug concentration could generally lead to good efficacy in the bile duct infection. Therefore, it is important to clarify the mechanism of hepatobiliary transport of UFX. In humans, the concentration of UFX was shown to be much higher than that in the plasma, although renal excretion is the major elimination route for UFX.[1,2] In this study, UFX was given to rats and mice to achieve the plasma concentration of UFX comparable to that in healty male volunteers given clinical dose of PUFX (Cmax = 1–2 μg/mL). Such concentrative excretion into the bile was also observed in rats (**Table 1**). Considering the plasma unbound fraction of UFX (0.505), the average concentration ratio of bile to plasma at 4 hr after intravenous administration was approximately 65, being greater than that of liver to plasma (19). Therefore, we first examined the biliary excretion mechanism of UFX to know if the transporter(s) are involved in this process. As shown in **Table 2** and **Fig. 3**, the systemic clearance and biliary excretion of unchanged UFX in EHBR were not different from those in SD rats, indicating that MRP2 was not the major factor governing the biliary excretion. On the other hand, biliary excretion of gluculonide metabolites was negligible in EHBR, whereas approximately 8.4% of the dose was excreted in SD rats. The plasma concentration of UFX in EHBR increased after enzymatic hydrolysis of glucuronides. Thus, MRP2 was demonstrated to predominantly mediate the biliary excretion of glucuronide of UFX, but not UFX itself. With regard to the biliary excretion of quinolone antibiotics, kinetic analysis after administration of HSR-903 to normal rats and EHBR showed that it was efficiently taken up into hepatocytes *via* a carrier-mediated active transport process and then excreted into the bile via MRP2.[8] In EHBR, the biliary excretion of unchanged drug and glucuronide was significantly lower than that in normal SD rats, and such difference was especially obvious for glucuronide.[8] The biliary clearance of unchanged and

glucuronide metabolites of grepafloxacin was also lower in EHBR than in normal rats.[9] Thus, biliary excretion mechanism of UFX is different from that of these quinolones, and possible involvement of transporters other than MRP2 should be considered.

Another candidate transporter is P-gp, which is expressed on canalicular membranes of hepatocytes and recognizes various quionolones. Involvement of P-gp was then examined using the mice with a defect in both mdr1a and 1b genes encoding P-gp.[10] The biliary excretion did not decrease in mdr1a/1b(-/-) mice (**Table 3**). The plasma concentration 2 hours after intravenous administration in mdr1a/1b(-/-) mice was also similar to that in normal mice (**Table 3**). Thus, it was considered that P-gp was not a major transporter for the biliary excretion of UFX. However, preliminary analysis suggested that the liver concentration of UFX was higher in the mdr1a/1b(-/-) mice compared to that in the wild-type mice. Therefore, the possibility of minor contribution of P-gp to the excretion cannot be excluded. In fact, the transport study using LLC-GA5-COL150 cells and its parental LLC-PK1, whose P-gp expression was negligible,[5,6] showed (i) B-to-A permeability was higher than A-to-B across the LLC-GA5-COL150 cells over-express human P-gp, whereas the directionality was not clear in LLC-PK1 cells. (ii) B-to-A permeability across LLC-GA5-COL150 cells was decreased and the directionality was diminished in the presence of 10 μM of cyclosporin A. (iii) Cellular uptake of UFX increased significantly in the presence of cyclsoporin A (**Fig. 6**). Therefore, UFX was indicated to be a substrate of P-gp.

Interestingly, increased uptake in the presence of cyclosporin A was also observed in LLC-PK1, which was, however, less remarkable compared to that in LLC-GA5-COL150 cells (**Fig. 6**). It indicates efflux transporter(s) that are expressed in the parental LLC-PK1 cells also contribute to the transcelluar transport of UFX. Furthermore, an increase in cellular uptake in the presence of cyclosporine A was greater when UFX was added to the basolateral medium compared to the case when it was added to the apical medium (**Fig. 6**). Inhibition of efflux processes alone does not explain this difference. This phenomenon can be explained by a hypothesis that cyclosporine A inhibits influx across the apical membrane to compensate increased accumulation resulting from inhibition of the efflux. This hypothesis does not contradict with the fact that A-to-B permeability of UFX was not increased in the presence of cyclosporine A, whereas B-to-A permeability was decreased remarkably. Further studies are required to examine the validity of this hypothesis.

The involvement of multiple transport systems in the biliary excretion of UFX was also suggested from the study in rats treated with cyclosporin A, which is an inhibitor of P-gp. The CLbile was decreased markedly by pretreatment with cyclosporin A (**Fig. 3**). Nevertheless, as discussed above, major contribution of P-gp to the excretion was excluded based on the present data using gene knockout mice. Cyclsoporin A has been reported to have inhibitory effect not only on P-gp, but also on other transporters including MRP2.[11,12] Yamaguchi *et al*. reported that biliary excretion of grepafloxacin was comparable between wild-type and mdr1a/1b(-/-) mice, and was inhibited by cyclosporin A in both mice,[13] suggesting the involvement of cyclosporin A-sensitive transporter(s) other than p-gp.

Recently, Shitara *et al*. has reported that cyclosporine A inhibited the transporter-mediated uptake of cerivastatin, a substrate of OATP2, in the liver.[14] It has not been reported that uptake of any quinolone antibiotics is mediated by a known transporter. However, decreased biliary excretion clearance with decreased Vss (**Fig. 3**) suggests that cyclosporine A-sensitive transport systems mediate the uptake of UFX into the liver. Further studies are needed to clarify the molecular mechanism for the excretion of these quinolones.

Second, we have examined the distribution of UFX in the brain. The quinolone antibiotics have been reported to have a potential of CNS side effect resulting from the inhibition of $GABA_A$ receptor binding.[15,16] However, systemic administration of PUFX rarely causes convulsant effect despite its relatively high affinity to $GABA_A$ receptor.[4] The distribution of various quinolone antibiotics in the brain has been reported to be low owing to the efflux transporter existing on BBB and/or BCSFB.[17–21] Kp,u_{CSF} and Kp,u_{brain} of UFX in SD rats were much less than the unity at plasma concentration comparable to the Cmax of UFX in human after oral administration of PUFX (**Fig. 4**). The Kp,u values for UFX were as low as the predicted value from its lipophilicity (octanol-water coefficient at pH 6.8, log P $= -1.06$) based on the reported relationship between log P (octanol) and steady-state Kp,u values of quinolone derivatives (norfloxacin, gatifloxacin, fleroxacin, sparfloxacin and pefloxain).[18] Therefore, there could be some transport mechanism for such limited distribution of UFX to the brain. Kp,u_{brain} for UFX significantly increased in rats pretreated with cyclosporin A at the dose level which had been reported to inhibit efflux transport of grepafloxacin by P-gp on BBB.[13] In addition, the higher brain-to-plasma concentration ratio (Kp_{brain}) was observed in mdr1a/1b(-/-) mice than in the wild-type mice (**Table 4**). These results suggested the involvement of P-gp in the limited distribution of UFX to the brain. On the other hand, Kp,u_{CSF} did not change significantly by cyclosporin A (**Fig. 4**). The possible explanation is that the concentration in CSF was kept lower than that in the plasma mainly by the efflux

mechanism functioning as BCSFB, which could not be inhibited by cyclosporin A. The possibility of MRP2 as a major transporter for both brain and CSF distribution of UFX was excluded from the observation that the Kp,u_{brain} and Kp,u_{CSF} in EHBR were not different from those in SD rats (**Fig. 4**).

As already mentioned in relation to the biliary excretion mechanism of UFX, Vss was decreased by the treatment with cyclosporin A in rats (**Fig. 3**). Thus, cyclosporin A inhibits the overall distribution of UFX in the body. The similar decrease in Vss was also observed in EHBR compared with SD rats (**Table 2**). Since the distribution of radioactivity is highest in the liver and kidney, after oral administration of [^{14}C]-PUFX in rats,[22] further studies are required to clarify the possible involvement of influx and/or efflux transport systems in these organs which may affect the distribution of UFX.

In conclusion, UFX is a substrate of P-gp. The biliary excretion of UFX is a transporter-mediated concentrative process that can be inhibited by cyclosporin A. Neither MRP2 nor P-gp is a major transporter governing the biliary elimination. In addition to the low lipophilicity, efflux transport by P-gp plays a role in the limited brain distribution of this compound.

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