Carrier-Mediated Tissue Distribution and Blood-Brain Barrier Transport of New Quinolones

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Animal and clinical investigations have shown that fluoroquinolones, new quinolone antibacterial agents (NQs), are well absorbed across the intestinal tract, with a bioavailability of 60-90% after oral administration. Although some types of carrier-mediated intestinal transport mechanisms have been reported for enoxacin (ENX) [1], ofloxacin (OFLX) [2] and sparfloxacin (SPFX) [3], recent results using a human intestinal epithelial cell line, Caco-2, indicated a passive or nonsaturable transport of SPFX, one of the most hydrophobic NQs [4]. The mechanism underlying the intestinal absorption of NQs is still largely unknown. The distribution of NQs into peripheral tissues including erythrocytes is very rapid and their tissue-to-plasma concentration ratios (Kp) are considerably larger than those of inulin (an extracellular fluid space marker), in spite of almost complete ionization of NQs at the physiological pH. Our findings suggest that OFLX and lomefloxacin (LFLX) are taken up by rat erythrocytes *via* a transport system common to that of a water-soluble vitamin, nicotinic acid [5].

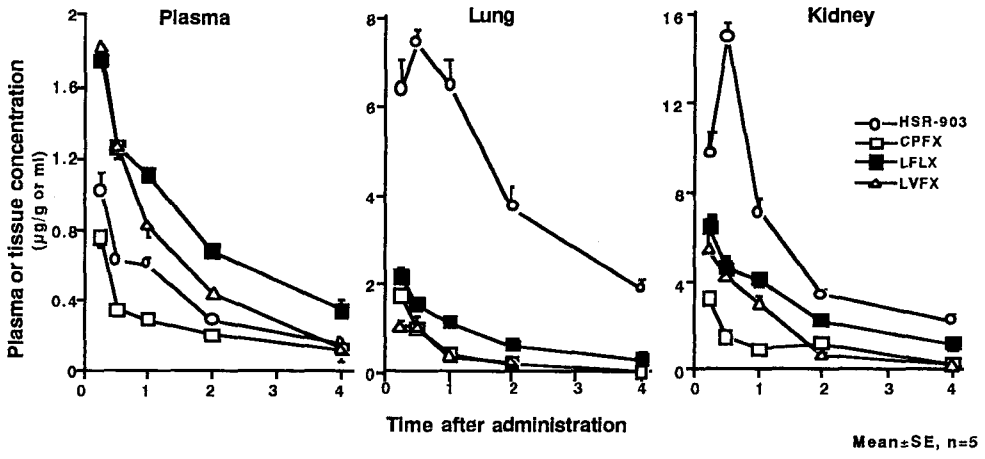

Fig. 1 Tissue concentrations of unchanged quinolones after a 5mg/kg oral dose to rats

The lung concentration of HSR-903, a synthesized NQ under clinical development, was about 9-times higher than in plasma after an oral administration in rats [6] (Fig. 1). The studies using isolated lung cells and isolated lung perfusion in rats indicated that [^{3}H]HSR-903 is taken up by the lung cells *via* a carrier-mediated transport mechanism (Fig. 2) which is Na^+ and Cl^- dependent, stereospecific, and common to the other lipophilic NQs such as grepafloxacin (GPFX) and SPFX [6], resulting in a concentrative distribution into lung. The lung accumulation of NQs is expected to be effective for pulmonary infections.

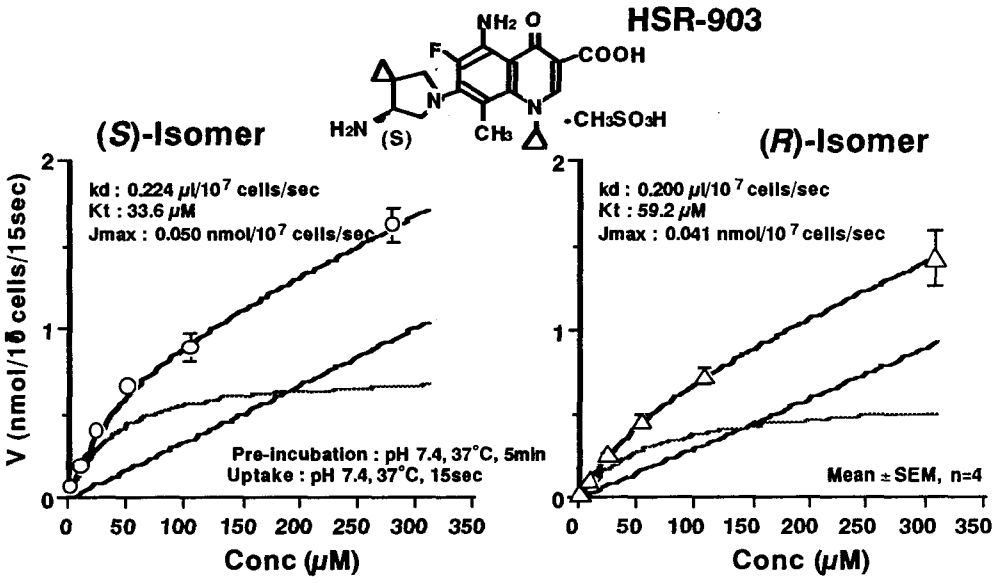

Fig. 2 Concentration Dependency on Uptake of HSR-903[(*S*)-Isomer] and (*R*)-Isomer by Lung Cells

In contract, the distribution into the central nervous system (CNS) is limited for NQs [7 - 11]. The knowledge of the disposition of antibacterial drugs in the cerebrospinal fluid (CSF) is of direct importance for the treatment of CNS infections. In addition, CSF and brain concentrations may be related to the incidence of CNS side effects. NQs cause severe CNS side effects such as convulsive seizures by themselves and when coadministered with nonsteroidal anti-inflammatory drugs. The convulsive seizures are attributed to, in a part, a competitive inhibition of NQs for binding of γ-aminobutyric acid (GABA) to GABA-benzodiazepine receptor, of which mechanism has been

proposed originally from our laboratory [12,13]. The distribution of NQs into CNS may, therefore, be an important factor in the occurrence of these neurotoxic side effects. In general, the distribution of compounds into the CNS is governed by lipophilicity, since penetration into the CNS from the circulating blood is restricted by the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB). In addition, cumulative evidence suggests the presence of several types of efflux transport systems at both barriers. Previous studies using primary cultured bovine brain capillary endothelial cells (BCECs) suggested that NQs are transported across the cell monolayer in a manner dependent on their lipophiliciy [14]. However, as shown in Fig. 3, there was no significant relationship between the logarithm of the apparent octanol-water (pH 7.4) partition coefficient, log(D), and the tissue concentration-to-plasma unbound concentration ratio, Kp,f -value in the brain after iv dose (5 mg/kg) of OFLX, SPFX, GPFX and HSR-903 in rats [6]. This result suggests that some transport systems efflux the lipophilic NQs from the BCECs into blood stream, resulting in the restricted penetration into brain across the BBB for GPFX and HSR-903. Most recently, we found that two efflux transporters result in the very limited BBB permeability of HSR-903, one of

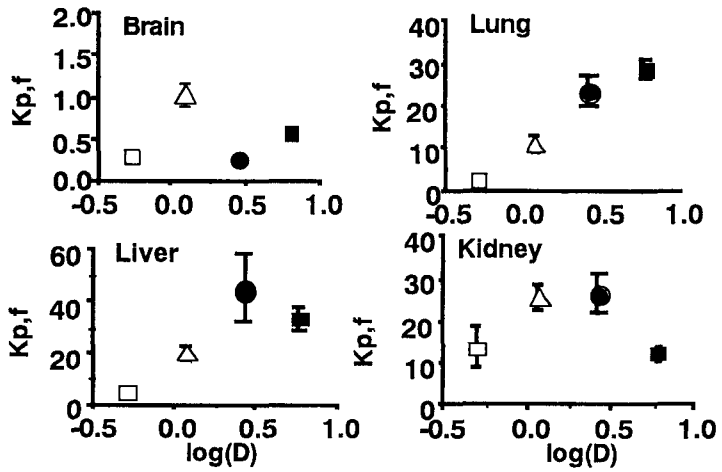

Fig. 3 Relationship between octanol-water partition coefficient and tissue-to-serum unbound concentration ratio for quinolone antibacterial agents

Data were represented by Kp,f values at 6 hr after iv dose of 5 mg/kg. Each symbol represents HSR-903 (closed circle), ofloxacin (open square), sparfloxacin (open triangle), and grepafloxacin (closed square).

which is an active efflux from the BCECs by P-glycoprotein (P-gp), as clarified recently for the restricted BBB permeability of anticancer agents [15, 16] and cyclosporin A [17, 18]. As shown in Fig. 4, the P-gp mediated efflux of HSR-903 from the brain was confirmed from the observation of about 8-fold increase of the brain Kp-value (2.85±0.25, mean ±S.E.M, n = 3) in mice which lack P-gp encoded by the *mdr*1a gene [19], when compared with that (0.38±0.06) in normal mice.

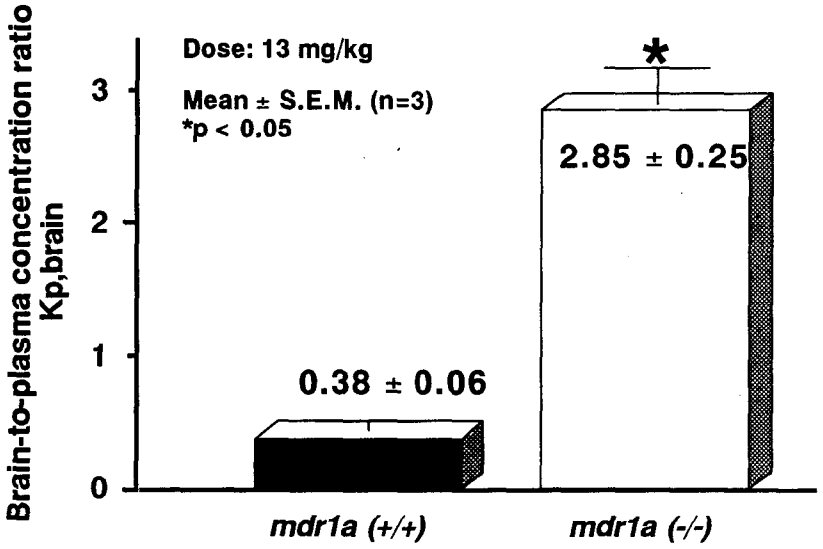

Fig. 4 Comparison of Brain Distribution of HSR-903 between Normal and *mdr1a* Gene Disrupted Mice

The other is an efflux by a bicarbonate exchanger at the BBB. As clearly seen in Fig. 5, the uptake (expressed by cell/medium ratio) of [^{14}C]HSR-903 by primary cultured bovine BCECs at the steady state was significantly decreased in the presence of 25 mM HCO_3^-. This bicarbonate-ion dependent- uptake was remarkably increased in the presence of unlabeled 5 mM HSR-903, whereas unlabeled HSR-903 produced no significantly increased uptake of [^{14}C]HSR-903 in the absence of HCO_3^-. The reduced uptake of [^{14}C]HSR-903 by bicarbonate was significantly increased in the presence of 10 mM probenecid, 1 mM sulfobromophthalein, 1 mM DIDS, an exchange transport inhibitor, and 5 mM several NQs. These results suggest that some NQs as well as HSR-903 are effluxed by utilizing bicarbonate-ion gradient from blood to BCECs *via*

organic anion exchange system at the BBB. Both efflux transports by P-gp and bicarbonate exchanger may result in the restricted transport across the BBB into brain interstitial fluid.

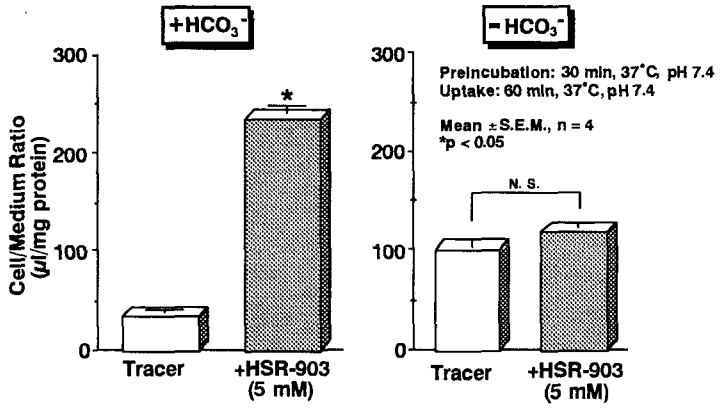

Fig. 5 Effect of HCO_3^- on [^{14}C]HSR-903 Uptake by Primary Cultured Monolayers of Bovine BCECs

The steady-state CSF to serum unbound concentration ratios, Kp,u_{CSF}, exhibit about 10-fold difference among sex NQs (norfloxacin (NFLX), AM-1155, fleroxacin (FLRX), OFLX, SPFX, pefloxacin (PFLX)) and were in the range of 0.109 - 1.0 in dogs and 0.048 - 0.378, being positively correlated with the lipophilicities of NQs (20). This suggests that the lipophilicity of these NQs is important for their CSF distribution. In both species, steady-state CSF concentrations were lower than unbound serum unbound concentration, which suggests the presence of a unidirectional efflux system of NQs from CSF to blood (20, 21). In vitro study using isolated choroid plexus demonstrates that these six NQs are effluxed by an active transport system common to organic anions at the choroid plexus to lower the concentration in cerebrospinal fluid [22].

The difference in distribution into the brain interstitial fluid and CSF among NQs may be caused either by the influx permeability at the BBB and BCSFB and by the active efflux ability at the BBB and the BCSFB.

Although the primary route of excretion of NQs is the kidney, significant non-renal excretion of NQs has also been reported in humans, 10% to 25% of the dose being eliminated in the bile and/or intestine. The mechanism which differentiates the extent of renal, biliary and intestinal secretion of NQs is still unknown. NQ is a zwitterionic drug with carboxylic acid and cationic amine

groups dissociated at physiological pH. Levofloxacin (LVFX), a racemate of OFLX, has been reported to be recognized as a cationic compound by a transporter at the apical and basolateral membrane of a kidney epithelial cell line, LLC-PK1 (23). It is also known that renal elimination of NQs is inhibited by probenecid (24). These data indicate that NQs are excreted into urine via both anionic and cationic transporters in tubular epithelial cells. Active transepithelial secretion from the basolateral-to-apical side by human intestinal Caco-2 cells has been observed for CPFX (25) and SPFX (26). The transepithelial secretion of both NQs was inhibited by verapamil, a multidrug resistant reversing (MDR) agent and was correlated with intracellular ATP levels, suggesting the involvement of P-gp in the secretion of NQs from blood into the intestinal lumen (25,26). The higher hapatobiliary excretion of GPFX, SPFX and HSR-903 among NQs is attributed to a carrier-mediated uptake of NQs from blood into hepatocytes and to an active secretion of the glucuronides of these NQs *via* multispecific organic anion transport system (cMOAT) across the bile canalicular membrane.

The difference in the affinity to the transporters responsible for NQs in renal tubular cells, hepatocytes and enterocytes may determine the excretion route *via* kideny, liver and intestine.

Acknowledgments

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References

[1] Hirano, T. *et al. J. Pharm. Pharamcol.* **46**: 676 - 679 (1994).
[2] Prieto, J. G. *et al. J. Pharm. Pharmacol.* **40**: 211 - 212 (1988).
[3] Yamagushi, T. *et al. Xenobiot. Metab. Dispos.* **6**: 53 - 59 (1991).
[4] Cormet, E. *et al. J. Pharm. Sci.* **86**:33 - 36 (1997).
[5] Simanjuntak, M. T. et al. *J. Pharmacobio-Dyn.* **14**: 475 - 481 (1991).
[6] Murata, M. *et al.* Abstract of 35th ICAAC, San Francisco, F203 (1995).
[7] Okezaki, E. *et al. Drug Metab. Dispos.* **16**: 865 - 874 (1988).
[8] Sto, H. *et al. J. Pharmacobio-Dyn.* **11**: 386 - 394 (1988).
[9] Sorgel, F. *et al. Clin. Pharmacokinet.* **16**: suppl. 1, 5 - 24 (1989).
[10] Jehde, U. *et al. J. Pharmacol. Exp. Ther.* **263**: 1140 - 1146 (1992).

[11] Ichikawa, N. *et al. Biol. Pharm. Bull.* **17**: 152 - 155 (1994).
[12] Tsuji, A. *et al. Animicrob. Agents Chemother.* **32**: 190 - 194 (1988).
[13] Tsuji, A. *et al. Biochem. Pharmacol.* **37**: 4408 - 4411 (1988).
[14] Jaehde, U. *et al. Eur. J. Pharmacol. Sci.* **1**: 49 - 55 (1993).
[15] Tsuji, A. *et al. Life Sci.* **51**:1427-1437 (1992).
[16] Ohnishi, T. *et al. Biochem. Pharmacol.* **49**:1541 - 1544 (1955).
[17] Tsuji, A. *et al. Biochem. Pharmacol.* **46**:1096 - 1099 (1993).
[18] Sakata, A. *et al.Biochem. Pharmacol.* **48**:1989 - 1992 (1994).
[19] Schinkel, A.H. *et al. Cell* **77**: 491 -
[20] Ooie, T, *et al. J. Pharmacol. Exp. Therap.* **278**: 590 - 596 (1996).
[21] Sato, H. *et al. J. Pharmacobio-Dyn.* **11**: 386 - 394 (1988).
[22] Ooie, T. *et al. Pharm. Res.* **13**: 523 - 527 (1996).
[23] Ohtomo, T. *et al. J. Pharmacol. Exp. Ther.* **276**: 1143 - 1148 (1996).
[24] Shimada, J. *et al. Antimicrob. Agents Chemother.* **23**: 15 - 18 (1982).
[25] Griffi. M. *et al. J. Pharmacol. Exp. Ther.* **269**: 496 - 502 (1994).
[26] Cormet, E. *et al. Drugs* **49**: 307 - 309 (1995).