Impact of transporter-mediated drug absorption, distribution, elimination and drug interactions in antimicrobial chemotherapy

著者	Tsuji Akira
journal or publication title	Journal of Infection and Chemotherapy
volume	12
number	5
page range	241-250
year	2006-10-01
URL	http://hdl.handle.net/2297/3234

doi: <https://doi.org/10.1007/s10156-006-0478-3>

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Impact of Transporter-Mediated Absorption, Distribution, Elimination and Drug Interactions in Antimicrobial Chemotherapy

Abstract

A comprehensive list of drug transporters has recently become available as a result of extensive genome analysis. Membrane transporters play important roles in determining the pharmacokinetic aspects of intestinal absorption, tissue distribution and urinary and biliary excretions of a wide variety of therapeutic drugs. Identification and characterization of transporters responsible for transfer of nutrients and xenobiotics, including drugs, is expected to provide a scientific basis for understanding drug disposition, as well as the molecular mechanisms of drug-drug/drug-food/drug-protein interactions and inter-individual/inter-species differences. This review focuses on the influence of transporters on the pharmacokinetics of β -lactam antibiotics, new quinolones, and other antimicrobial agents, as well as on drug-drug/drug-food interactions associated with transporter-mediated uptake from the small intestine and transporter-mediated elimination from the kidney and liver.

Key words Pharmacokinetics • β -lactam antibiotics • New quinolones • Influx transporter • Efflux transporter • Blood-brain barrier

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Introduction

Metabolic biotransformations, especially those mediated by the cytochrome P-450 family of monooxygenases, have clinically significant effects on the disposition of many drugs. Although membrane transport of synthetic drugs had long been believed to proceed by simple diffusion, depending on the lipophilic character of the drug molecules, it has recently been well established that drug transporters play pivotal roles in determining the pharmacokinetic profiles of particular drugs and thereby determine the overall pharmacological effects, i.e., drug absorption, distribution, elimination and concentration at the target sites.¹ In the past decade, a comprehensive list of membrane transporters has become available owing to the progress in genome analysis. Major membrane transporters have been classified into the solute carrier (SLC) transporter family and the ATP-binding cassette (ABC) transporter family by the HUGO Gene Nomenclature Committee. The human SLC family consists of 319 gene family members, including ion-coupled transporters, facilitated transporters and exchangers (SLC1 to SLC43, URL: <http://www.gene.ucl.ac.uk/nomenclature/genefamily/slc.php>). In the human ABC transporter superfamily, 49 genes have been identified and classified into seven subfamilies (ABCA to ABCG, URL: <http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html>). Some of these transporters accept not only physiological or endogenous substrates, but also drugs.¹

Modulation of the functions of such membrane transporters may potentially alter the disposition of certain drugs, as well as modifying drug-drug interactions. This review describes several examples of altered pharmacokinetics of antimicrobial drugs. Although the participation of an individual transporter in the observed pharmacokinetics of drugs can be difficult to confirm in humans, the identification of mutations resulting in deficient transport function may provide valuable information about the role of the transporter as a pharmacokinetic determinant. Since studies on polymorphisms of human drug transporters

have only recently been initiated, the present review is limited to β -lactam antibiotics and new quinolones whose pharmacokinetics can be clearly judged as transporter-dependent. In this review, the transporter prefixes are indicated as m: mouse, r: rat, h: human.

Pharmacokinetics of β -Lactam Antibiotics

The β -lactam antibiotics display a broad spectrum of antibacterial activity with a relatively low risk of allergic and toxic reactions. After intravenous or oral administration, despite the similarity in their chemical structures, various β -lactam antibiotics exhibit marked differences in their oral bioavailability, tissue distribution and elimination from the body, which cannot be explained in terms of their physicochemical properties.

1) Intestinal absorption

Although it has long been believed that most drugs are absorbed through the gastrointestinal epithelium by a simple diffusion mechanism depending on their lipophilicity, much direct and indirect evidence for the participation of transporter-mediated absorption has been accumulated.

Figure 1 illustrates transporters expressed on human intestinal epithelial cells. Some transporters are located at the brush-border membrane (luminal side), and the others are located on the basolateral side (portal vein side). These transporters play important roles in intestinal absorption and/or secretion of drugs.

Fig. 1

The mammalian SCL transporter, PEPT1 (SLC15A1) transports nutritional oligopeptides across the luminal membrane into small-intestinal epithelial cells at a microclimate pH of 6.1 to 6.8. This transporter is driven by an inwardly directed proton gradient and catalyzes the cotransport of its substrates with H^+ . Although the natural substrates are di- and tripeptides, PEPT1 has a very broad substrate specificity and transports orally effective peptide-mimetic drugs, such as β -lactam antibiotics, ACE inhibitors, the antineoplastic agent bestatin and the

antiviral val-acyclovir.²⁻⁴ The plasma peak time and dose-normalized AUC after oral administration of 5 mg/kg cefadroxil in human were significantly delayed and decreased, respectively, upon co-administration of 15 mg/kg cephalixin, presumably due to the competitive inhibition of the intestinal PEPT1-mediated transport of cefadroxil by cephalixin.⁵ Bretschneider et al.⁶ demonstrated that the transepithelial fluxes of 23 β -lactam antibiotics in Caco-2 cells (widely used as a model of human intestinal cells) and the bioavailability (BA) in humans both correlate well with the affinity constants (K_i) of the drugs for PEPT1 expressed in Caco-2 cells. The K_i values of ceftibuten and cyclacillin were comparable with those of natural dipeptides ($K_i = 0.3$ and 0.5 mM, respectively). Cefadroxil, cefamandole, cephradine, cefaclor, cefuroxime-axetil, cefixime, cephalothin, cephalixin and ampicillin also interact with PEPT1 ($K_i = 7 - 14$ mM). In contrast, cefapirin, cefodizime, cefuroxime, cefmetazole, ceftazime, benzylpenicillin, ceftriaxone, cefpirom, cefotaxime, cefepime, cephaloridine and cefsulodin display no affinity for this transport system ($K_i > 20$ mM). These results suggest that the oral BA of these drugs is mainly determined by their affinity for PEPT1, parenteral cefamandole (BA <1%) being the only exception.⁶ Using the *Xenopus laevis* expression system, Tamai et al. also demonstrated a predominant role of PEPT1 in the carrier-mediated intestinal absorption of β -lactam antibiotics.⁷

Nozawa et al.⁸ demonstrated increased BA of cefixime upon co-administration of a proton-releasing polymer, Eudragit L100-55, in rats. This enhanced intestinal absorption of cefixime was achieved by increasing the transport activity of proton-coupled PEPT1 through an increase in the concentration of protons, supplied by the co-administered acidic polymer, as the driving force at the apical surface of epithelial cells. In a very similar way, the 70% increase in absorption rate and 25% increase in the BA of amoxicillin (1 g) upon co-administration with nifedipine (20 mg) in humans are considered to be attributable to the increase of the proton concentration owing to calcium channel blockade by nifedipine, leading to increased amoxicillin transport via PEPT1.⁹

The basolateral peptide transporter mediates the efflux of substrates from the intracellular space to the portal vein and enables unidirectional transport through intestinal epithelial cells. Irie et al.¹⁰ suggested that reducing the affinity of drugs for the basolateral peptide transporter on the intracellular surface in human intestinal epithelial Caco-2 cells would lead to more efficient absorption of small peptides and peptide-mimetic drugs taken up via PEPT1.

2) Elimination from the kidney

Numerous organic anions, including endogenous metabolites, drugs and xenobiotics, are excreted into the urine through the renal proximal tubules and/or via glomerular filtration. The active excretion process, except filtration, is achieved via unidirectional transcellular transport. This involves the uptake of drugs into the tubular cells from the blood across the basolateral membrane, followed by extrusion across the brush-border membrane into urine. **Figure 1** shows transporters expressed in the human kidney.¹ Some are located on the basolateral membrane (blood side), while the others are located on the brush-border membrane side. These transporters play important roles in the tubular secretion and/or reabsorption of drugs.¹

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Most β -lactam antibiotics (penicillins and cephalosporins, and carbapenems) are excreted into urine in nonmetabolized form, and in addition to the glomerular filtration of the antibiotics unbound with plasma albumin, their renal tubular secretion via the para-aminohippuric acid (PAH) transporter and probenecid-sensitive system is thought to be an important pathway of renal clearance.^{12,13} Some cephalosporins, such as cephaloridine, have severe nephrotoxicity, and it is suggested that their toxic effect is related to the transport systems in the renal proximal tubules.¹⁴

Transport of β -lactam antibiotics mediated by renal organic anion transporter has an important influence on their pharmacokinetics and toxicokinetics. Among human organic anion transporters, OAT1 (SLC22A6) and OAT3 (SLC22A8) are PAH/dicarboxylate

exchangers, and are localized on the basolateral side of the proximal tubules, whereas OAT4 (SLC22A11) is localized on the apical side of the proximal tubules.¹⁵⁻¹⁷ Jariyamato et al.¹⁸ demonstrated that benzylpenicillin and cephaloridine are taken up by rOAT1 (Slc22A6) expressed in *Xenopus laevis* oocytes. They also demonstrated that PAH uptake by rOAT1 was inhibited by most β -lactam antibiotics in a competitive manner, whereas other antibiotics, such as gentamicin, streptomycin and vancomycin, which do not contain anionic moieties, did not interact with rOAT1. Cells expressing rOAT1 were most susceptible to cephaloridine cytotoxicity.¹⁸ Uwai et al.¹⁹ demonstrated that rOAT1 transports urinary-excreted cefazolin, cefotiam and cephalexin, and these antibiotics inhibit PAH uptake by rOAT1, whereas biliary-excreted cefoperazone does not, and they also showed that rOAT1 plays an important role in transport of cephalosporins and PAH in the rat kidney. These results suggest that rOAT1 is the major organic anion transporter in the kidney, and is responsible for the renal excretion of β -lactam antibiotics in rats.

A recent report²⁰ indicated that the OAT3 mRNA level was well correlated with the elimination rate of the free fraction of cefazolin in patients with mesangial proliferative glomerulonephritis, which is most common form of primary renal disease. In contrast, the correlation with the level of OAT1 mRNA was lower. Ueo et al.²¹ demonstrated that the uptakes of cephaloridine, cefdinir and cefotiam by OAT3 stably transfected into HEK293 cells were 35- to 50-fold greater than those by control cells. In contrast, the uptake of these antibiotics by HEK-OAT1 was within two-fold of that by control cells.²¹ These results indicate that OAT3 plays a much more important role than OAT1 in the renal basolateral transport of cephalosporins in humans.

With respect to the substrate recognition and transport activity of OAT family members, there are some species differences.

In humans, β -lactam-exporting transporters localized in the apical membrane of the proximal tubules include OAT4, P-gp (MDR1, ABCB1), multidrug resistance-associated

proteins 2 (MRP2, ABCC2) and 4 (MRP4, ABCC4), sodium-dependent inorganic phosphate transporter (NPT1, SLC17A1), and OAT_v1.²²⁻³⁰

OAT4 is an apical organic anion/dicarboxylate exchanger, which mainly functions under physiological conditions as an apical pathway for the reabsorption of some organic anions from urine into tubular cells, driven by an outwardly directed dicarboxylate gradient, probably with coupling to apical organic efflux transporters, such as MRP2, NPT1 and, possibly, the human homologue of OAT_v1.²⁵

NPT1 expressed in *Xenopus laevis* oocytes transports estradiol-17 β -glucuronide, PAH, benzylpenicillin, faropenem, indomethacin and uric acid and has affinity for several organic anions, including 2-ketoglutaric acid, β -lactam antibiotics (ampicillin, cefazolin, cephalixin), benzoic acid, lactic acid, probenecid and phenol red.^{27, 28} Since the uptake of faropenem by mNPT1 is reduced in the presence of high concentrations of chloride,²⁷ NPT1 is suggested to participate in the secretion of β -lactam antibiotics from tubular cells to urine. Therefore, NPT1 acts as a multifunctional transporter for the sodium-dependent reabsorption of phosphate from urine into tubular cells and simultaneously for sodium-independent secretion of organic anions, including β -lactam antibiotics, from tubular cells to urine.²⁸

ATP-driven drug efflux pumps, such as P-gp and MRP2, are localized on the apical membrane of tubular cells and have been thought to handle the excretion of a wide range of substrates into urine.^{29,30} However, the renal clearance of cefpiramide, which is mainly excreted into bile, was reported to be increased 2-fold in Eisai hyperbilirubinemic rats (EHBR) with a deficiency of rMRP2 (Abcc2) compared with that in normal rats, after intravenous administration.³¹ Renal clearance of cefoperazone and cephalixin showed no significant difference between normal rats and EHBR (unpublished observation). These results suggest that rMRP2 makes no significant contribution to the renal excretion of β -lactam antibiotics.

The human peptide transporters PEPT1 and PEPT2 (SLC15A2), which exhibit only about

50% homology in amino acid sequence, are localized on the brush-border membrane of the proximal tubule. PEPT1 is expressed in the early part of the proximal tubule (pars convolute), whereas PEPT2 is expressed further along the proximal tubule (pars recta).³² These transporters may play roles in the reabsorption of di- and tri-peptides and peptide-mimetics, such as β -lactam antibiotics, from urine into tubular epithelial cells.³² The affinities of β -lactam antibiotics are higher for PEPT2 and lower for PEPT1.³³ Orally effective β -lactam antibiotics, such as cefaclor, cefadroxil, cephalixin, cephadrine, ceftibuten, amoxicillin and cyclacillin have affinity constants of 0.003 – 0.4 mM for PEPT2 and 0.3 – 25 mM for PEPT1. However, only a few cephalosporins (cephalexin and cefadroxil) have been reported to be substrates of PEPT2.³⁴

Therapeutic use of cephaloridine in humans has been found to cause nephrotoxicity. Because cephaloridine contains a quaternary nitrogen and exists as a zwitterion under physiological conditions, and has significant structural similarity with carnitine, Nezu et al. postulated a possible interaction of cephaloridine with the sodium-dependent organic cation/carnitine transporter OCTN2 (SLC22A5), of which gene mutation causes primary carnitine deficiency.³⁵ Ganapathy et al.³⁶ demonstrated that many β -lactam antibiotics that are not recognized by OCTN2 are good substrates for the H^+ -coupled PEPT1 and PEPT2, and found that cephaloridine, cefoselis, cefepime and ceftuprenam significantly inhibited OCTN2-mediated transport of carnitine. These antibiotics possess a quaternary nitrogen, as does carnitine. Since cephaloridine is taken up by OCTN2-transfected cells in a sodium-dependent manner, it is suggested that OCTN2 plays a crucial role in the pharmacokinetics and therapeutic efficacy of certain β -lactam antibiotics, and that cephaloridine-induced carnitine deficiency is likely to be due to inhibition of carnitine reabsorption from urine.³⁶

2) Elimination from the liver

Most β -lactam antibiotics are eliminated into urine. However, some of them, such as nafcillin, cefpiramide, cefoperazone, ceftriaxone and cefodizime, are excreted into the bile duct. The influences of lipophilicity and molecular weight on the biliary excretion of β -lactam antibiotics have been examined,³⁷ but the critical factors determining the excretion route remain to be fully established. In the process of biliary excretion, the antibiotics must cross both the hepatic sinusoidal and canalicular membranes.

Figure 1 shows transporters expressed in the liver. Uptake transporters are located on the sinusoidal membrane (blood side), whereas efflux transporters are located on the bile canalicular membrane, although some efflux transporters are on the sinusoidal membrane and take part in secretion into the blood.

Organic anion transporters such as OAT2 (SLC22A7) and OATPs (SLC21A) expressed on the sinusoidal membrane of the liver are thought to be responsible for the uptake of β -lactam antibiotics from blood into hepatocytes.³⁸ Khamdang et al.³⁹ demonstrated that hOAT2 and rOAT2 (Slc22A7) interact with various cephalosporins and mediate the uptake of biliary-excreted cefoperazone and ceftriaxone. Among organic anion transporting polypeptides (OATPs), OATP-B (SLC21A9, SLCBO2B1), -C (SLC21A6, SLCO1B1) and -8 (SLC21A8, SLCO1B3) mainly accept bulky and amphipathic organic anions as substrates, although they also accept neutral compounds, such as digoxin. Since several β -lactam antibiotics have reported to have affinity for OATPs, OATPs may transport these cephalosporins across the sinusoidal membrane of liver.⁴⁰ hNPT1 and mNPT1 are located on the apical membrane of the proximal tubule, as well as on the sinusoidal membrane of the liver, and mNPT1 has affinity for most β -lactam antibiotics.⁴¹ Because sodium-independent uptake of benzylpenicillin by mNPT1 expressed in *Xenopus laevis* oocytes is reduced in the presence of high concentration of chloride, this transporter has been suggested to participate in hepatic sinusoidal efflux transport of β -lactam antibiotics, as well as other organic anions, from hepatocytes –to blood under physiological conditions.⁴¹

Several β -lactam antibiotics are known to induce choleresis. Verkade et al. reported that intravenous administration of ampicillin at the dose of 180 μ mol/kg to rats induced choleresis, but this was not observed in rMRP2-deficient TR⁻.⁴² Ito et al.⁴³ demonstrated that β -lactam antibiotics which are rMRP2 substrates induce choleresis via the stimulation of rMRP2-mediated GSH excretion into bile. Indeed, biliary excretion of β -lactam antibiotics such as cefpiramide,³¹ benzylpenicillin⁴³, cefodizime⁴⁴ and as well as indocynine green and dibromosulfophthalein,⁴⁴ has been reported to be dramatically reduced in rMRP2-deficient EHBR, indicating that rMRP2 plays an important role in the biliary excretion of organic anions. Although P-gp and one of the ABC-transporters, breast cancer resistance protein, BCRP (ABCG2), in addition to MRP2, are located on the bile canalicular membrane,⁴⁵ β -lactam antibiotics appear not to be substrates for P-gp and BCRP. Therefore, ATP-dependent MRP2 is likely to be primarily responsible for the excretion of β -lactam antibiotics from hepatocytes into bile.

Human MRP3 (ABCC3), a MRP family transporter, is expressed on the sinusoidal membrane and considered to be involved in transport to the blood from hepatocytes. It accepts many glucuronides, glutathione conjugates, methotrexate, etc.⁴⁶

3) Tissue distribution

The values of steady-state volume of distribution (V_{dss}) of β -lactam antibiotics have been reported to be around 0.3 L/kg in animals and humans, suggesting that tissue distribution of these antibiotics is limited to the interstitial fluid space of tissues.^{47, 48} This is due to little activity of uptake transporters in non-eliminating tissues (except lung), in contrast to intestine, kidney and liver described above, resulting in poor antibacterial efficacy for intracellular infection.

Groneberg et al.⁴⁹ demonstrated the presence of a high-affinity peptide transporter PEPT2 in alveolar type II pneumocytes, bronchial epithelium and endothelium of small

vessels in the lung by measuring the uptake of peptides instilled in the trachea. Bahadduri et al.⁵⁰ also demonstrated the functional expression of the high-affinity peptide transporter PEPT2 in primary cultured human lung cells obtained from multiple donor subjects. The PEPT2-mediated uptake of H⁺-GlySar in human lung epithelial cells was inhibited by penicillins, cephalosporins and other peptidomimetics. Among these β -lactam antibiotics, ampicillin and cefadroxil had high affinity, and captopril and enalapril showed very low or no affinity, consistent with the previous reports concerning substrates for PEPT2.⁵¹

An oligopeptide transport activity is expressed in some tumor cells, including fibrosarcoma HT-1080⁵² and two pancreatic tumor cell lines, AsPc-1 and Capan-2, but not in a normal diploid cell line, IMR-90.⁵³ Nakanishi et al. demonstrated over-expression of PEPT1 and/or PEPT2 in a majority of tumor cell lines.⁵⁴ Antiviral and antineoplastic drugs, such as valaciclovir and bestatin, which are substrates of PEPT1 and PEPT2, can thus be effective in the treatment of viral pneumonia or pulmonary carcinoma, respectively.

3-1) Blood-brain and blood-cerebrospinal barriers

Matsushita et al. demonstrated that the concentration of cefodizime in the intracellular fluid of the brain is less than the unbound plasma concentration, although the participation of an uptake system from blood to brain was observed.⁵⁵ Kikuchi et al. found that benzylpenicillin, as well as PAH, is effluxed from the rat brain.⁵⁶ These results suggest participation of some kind of efflux system in the BBB for β -lactam antibiotics. The limited distribution into the central nervous system (CNS) of β -lactam antibiotics after administration to humans, as well as animals, is due to (a) the limited, possibly molecular-size-dependent, ability to cross the barrier of CNS, the degree of plasma protein binding and ionization,⁵⁷ and/or (b) the efflux from CNS to blood via an active transport system.⁵⁸

As shown in Fig. 1, transporters expressed at the brain capillary endothelial cells mediate uptake from the bloodstream to the brain for essential nutrients, especially hydrophilic

nutrients and some types of nutrient-mimetic drugs, and efflux from brain to blood for endogenous and/or exogenous compounds, i.e., they form a part of the blood-brain barrier (BBB).^{55, 56, 58} The discovery of the brain-to-blood efflux transporter, P-gp, encoded by multidrug resistance gene (MDR1), in 1992^{58,59} completely changed the concept of the BBB from a static lipoidal membrane barrier to a dynamic interface that regulates movement of compounds between brain and blood by means of active transport mechanisms. The most convincing evidence of P-gp-mediated efflux is the demonstration of significantly increased brain distribution of numerous drugs in *mdr1a* and/or *mdr1b* gene knockout mice.^{60,61} Many studies on the significance of P-gp at the BBB using *mdr1a/b* knockout mice have been performed for many drugs. However, it has been reported that β -lactam antibiotics are not transported by P-gp, suggesting that the limited entry of β -lactam antibiotics from blood into brain across the BBB may be due to participation of another efflux transporter(s).

In addition to P-gp, various transporters, including SLC family members, such as OATs, and ABC family members, such as BCRP and MRPs, have been found to mediate efflux of endogenous and/or exogenous compounds from the brain to the blood stream.⁶²

Among OAT family members, rOAT3 has been reported to be localized at the abluminal membrane of brain capillary endothelial cells.^{56, 63, 64} Almost all β -lactam antibiotics, as well as benzylpenicillin and PAH, are substrates of rOAT3 and therefore are likely to be actively transported by OAT3 from brain to plasma across the BBB. Regarding transport at the luminal membrane, it is likely that ATP-driven active transporters (MRPs) other than P-gp remove anionic drugs, including β -lactam antibiotics, from endothelial cells, transporting them to the circulating blood. MRP1, MRP2, MRP4, MRP5 and MRP6, which mediate transport in this direction, are expressed at the BBB.^{65, 66}

Low concentrations of β -lactam antibiotics in cerebrospinal fluid (CSF) are often presumed to be the result of poor BBB and blood-CSF barrier (BCSFB) penetration. Fishman⁶⁷ suggested that a saturable and probenecid-sensitive system transports penicillin

from CSF. Later, Spector and Lorenzo⁶⁸ demonstrated that the active efflux pump was located in the choroid plexus and was saturable at high penicillin concentrations. This saturable efflux mechanism was later confirmed to operate for the efflux of other β -lactam antibiotics.

OAT3 and PEPT2 are expressed in the choroid plexus, which forms the BCSFB. Kuroda et al.⁶⁹ demonstrated that cefaclor is cleared from CSF more rapidly than cephalexin after intracerebroventricular administration in rats. They suggested that cefaclor and cephalexin are eliminated from the CSF by different transporters, although both are substrates of rOAT3 and rPEPT2, and that rapid elimination of cefaclor from the CSF occurs via a benzylpenicillin-sensitive mechanism distinct from rOAT3, whereas cephalexin is removed by a GlySar-sensitive transporter.⁶⁹ PEPT2 also functions as an efflux pump for removal of endogenous or exogenous peptide-like chemicals, such as β -lactam antibiotics.⁷⁰ Neither PEPT2 mRNA nor PEPT2 protein is expressed in the brain capillary endothelial cells that form the rat BBB.⁷¹ Recently, Shen et al. demonstrated, using mPEPT2-deficient mice, that PEPT2 acts as an efflux transporter in the removal of cefadroxil from CSF, and that the process is unidirectional from CSF to blood.⁷² These findings by Kuroda et al.⁶⁹ and Shen et al.⁷² suggest that cefaclor and cephalexin do not share the same efflux transport mechanism in choroid plexus and that OAT3 and PEPT2 both play important roles in regulating the CSF concentration of β -lactam antibiotics.

Therefore, brain capillary endothelial cells and choroid plexus selectively regulate the uptake of compounds essential for brain function from blood to brain and CSF by means of influx transporters, and prevent toxic or non-essential compounds from entering the brain from blood, or eliminate them from the brain, by means of efflux transporters.

3-2) Blood-placenta barrier

In the placenta, there is thought to be a tissue-barrier system but, little is known as to the mechanism by which the placenta carries out this barrier function. Fetal blood is separated

from the maternal blood circulation by polarized cells, i.e. syncytiotrophoblasts, which possesses carrier-mediated transport systems similar to those in the renal proximal tubules and intestinal epithelial cells. The placenta probably plays the primary role in the elimination of toxic compounds from the fetus. Cha et al. found high levels of mRNA expression of OAT4 in the placenta and the kidney, whereas rOAT2 and hOAT3 are not expressed in the placenta²² The expression of OAT1 in the placenta is extremely weak or absent, and OAT4 is predominantly expressed in the placenta among OAT isoforms.²² Since OAT4 is a tertiary active organic anion/dicarboxylate exchanger under physiological conditions, it might be responsible for the elimination and detoxication of estrone sulfate and other organic anions, such as NSAIDs, diuretics, sulfobromophthalein, benzylpenicillin and bile acids, which interact with this transporter.²² Two other excretory pathways have been identified in the placenta. The xenobiotics transporters ABCP,⁷³ a member of the ABC superfamily, and OCT3⁷⁴ may also contribute to the placenta barrier.

Pharmacokinetics of New Quinolones

New furoroquinolone antibacterial agents (new quinolones) are well absorbed from the gastrointestinal tract and distributed to various tissues with high distribution volume. The distribution volumes of sparfloxacin, lomefloxacin and ofloxacin are about 6.0, 1.46 and 1.54 L/kg, respectively,⁷⁵ being higher than those of β -lactam antibiotics (about 0.3 L/kg).

Grepafloxacin and olamufloxacin exhibit particularly high concentrations in the lung after oral administration.⁷⁶ The lung concentration of unchanged olamufloxacin is about nine times higher than that in plasma after oral administration (5 mg/kg), whereas other quinolones showed values close to unity.⁷⁶ Sasabe et al.⁷⁷ and Murata et al.⁷⁶ demonstrated that the uptake of grepafloxacin and olamufloxacin by isolated rat lung cells was temperature-dependent, saturable, stereospecific, and at least Na^+ -dependent. The uptake of both quinolones was inhibited by other quinolones, including sparfloxacin. It should be noted that clarithromycin

has been reported to be taken up by carrier-mediated active transport into isolated rat lung cells.⁷⁸ Although identification of the transporter(s) of quinolones and clarithromycin remains to be done, carrier-mediated system(s) may at least partially account for the efficient distribution of grepafloxacin, olamufloxacin and clarithromycin, and could provide a specific route for drug delivery to the lung.

Some quinolones exhibit very low distribution into the brain compared with that into other peripheral tissues.⁷⁹ The efflux across the blood-brain barrier (BBB) may be an important determinant for quinolones, rather than the transport across the BCSFB.⁸⁰ Tamai et al.⁸¹ demonstrated that the permeability coefficient of [¹⁴C]grepafloxacin measured by the brain perfusion technique was increased by an excess of unlabeled grepafloxacin, suggesting participation of a saturable BBB efflux system. Murata et al.⁸² demonstrated that the uptake coefficients of [¹⁴C]grepafloxacin, [¹⁴C]sparfloxacin, and [¹⁴C]levofloxacin by immortalized rat cultured brain capillary endothelial cells (RBEC1) in the steady state were increased in the presence of the unlabeled quinolones, and the steady-state uptake of [¹⁴C]grepafloxacin was increased in the presence of various quinolones. As shown in Fig.2, brain distributions of [¹⁴C]grepafloxacin, [¹⁴C]sparfloxacin, [¹⁴C]olamufloxacin, and [¹⁴C]levofloxacin evaluated in terms of the brain-to-plasma free concentration ratio in *mdr1a/1b* gene-deficient mice, were significantly higher than those in wild-type mice, demonstrating an involvement of P-glycoprotein as the efflux transporter. Anionic compounds, including 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) and genistein, increased the steady-state uptake of [¹⁴C]grepafloxacin by RBEC1 cell.⁸² Because [¹⁴C]grepafloxacin is transported by multidrug resistance-associated protein (MRP) in MRP1-overexpressing cells, and because RBEC1 and primary-cultured brain capillary endothelial cells express MRP1, this protein may be an additional efflux transporter for quinolones. Furthermore, the permeability coefficient of [¹⁴C]grepafloxacin across the BBB was increased by DIDS or in the absence of bicarbonate ions as determined by the brain perfusion method. DIDS or

bicarbonate ion did not affect MRP1 function.⁸¹ Very similar efflux transport by P-gp at the BBB was demonstrated for olamufloxacin⁸² and a new prodrug-type quinolone, prulifloxacin,⁸³ the brain-to-plasma concentration ratio (Kp) in *mdr1a/1b* gene-deficient mice being significantly higher for prulifloxacin than that in the normal mice.

Accordingly, the very low brain distribution of most quinolone antibacterial agents is a consequence of the action of multiple efflux transporters, including P-glycoprotein, MRP1, and other unknown anion exchange transporter(s).

Most quinolones are mainly excreted into urine after oral administration, though grepafloxacin and olamufloxacin exhibit greater hepatobiliary transport than other quinolones.⁸⁴⁻⁸⁷ The biliary excretion of the unchanged forms of grepafloxacin⁸⁴⁻⁸⁶ and olamufloxacin⁸⁷ in EHBR amounted to less than 30% of that in normal rats, whereas the 3-glucuronide (a main metabolite of both quinolones) was hardly excreted into the bile in EHBR. ATP dependence was observed in the uptake of grepafloxacin by bile canalicular membrane vesicles (CMV), although the extent was not very marked, whereas no ATP-dependent uptake was observed by CMV prepared from EHBR. The uptake of grepafloxacin glucuronide by CMV from normal rats showed a marked ATP dependence, whereas there was little ATP-dependent uptake in EHBR.⁸⁴⁻⁸⁶ These results indicate that at least a part of the transport of quinolones and a major part of the glucuronide transport across the bile canalicular membrane in rats involve a primary active transport mechanism mediated by rMRP2.

Griffiths et al.⁸⁸ demonstrated that in the transepithelial transport of three fluoroquinolones, norfloxacin, ciprofloxacin and pefloxacin, across cultured human intestinal Caco-2 cell layers, the absorptive (apical-basal) fluxes of ciprofloxacin and norfloxacin are small relative to the basal-to-apical fluxes, both quinolones being subject to active transepithelial secretion. A number of fluoroquinolones are capable of inhibition of both net secretion of ciprofloxacin and cellular accumulation across the basal-lateral cell surface, suggesting that

fluoroquinolones may compete for a common carrier at the basolateral membrane. It is likely that the mechanism of transepithelial secretion involves a common accumulative transport at the basolateral membrane, followed by facilitated exit across the apical membrane. Pefloxacin may interact with a brush-border carrier for which norfloxacin and ciprofloxacin are poor substrates, enhancing the absorptive flux of this fluoroquinolone.

Similar studies by Naruhashi et al.⁸⁹ demonstrated that grepafloxacin showed secretory-directed transport which was decreased by cyclosporin A, an inhibitor of P-glycoprotein, and probenecid, an inhibitor of anion transport systems. This suggested contributions of P-glycoprotein and anion-sensitive transporter(s). In *mdr1a(-/-)/1b(-/-)* mice, the intestinal secretory clearance was smaller than that in wild-type mice after intravenous administration of grepafloxacin. Moreover, the absorption from an intestinal loop in *mdr1a(-/-)/1b(-/-)* mice was larger than that in wild-type mice.⁸⁹ Accordingly, it appears that some quinolones are transported by secretory transporters, including P-glycoprotein. The involved transporters function in vivo not only to transport grepafloxacin from blood to intestine, but also to limit its intestinal absorption.

Oral bioavailability of fluoroquinolones is relatively good; indeed, some of them are completely absorbed, even though they are secreted into the intestine by efflux transporters such as P-gp and MRPs, as indicated above. This suggests that uptake transport system(s) participate in the intestinal absorption of fluoroquinolones. This hypothesis remains to be tested.

Drug interactions after treatment with rifampin

A number of clinically important drug interactions with rifampin have been reported to result from its powerful induction of intestinal cytochrome P450 3A4.^{90, 91} It was recently reported that concomitant administration of rifampin at a dose of 600 mg/day for 10 days in healthy volunteers reduced digoxin plasma concentrations substantially after an oral

single-dose (1 mg) administration compared with the plasma-time profile without rifampin treatment.⁹² The effect was less pronounced after intravenous administration of digoxin. The oral bioavailability of digoxin decreased by 30% during rifampin therapy. When duodenal biopsies obtained before and after administration of rifampin were analyzed, it was found that rifampin treatment increased the content of intestinal P-gp 3.5+/- 2.1-fold, which correlated well with the change in the area under the curve (AUC) after oral digoxin, but not after intravenous digoxin. Since rifampin is a known inducer of cytochrome P450 enzymes (e.g. CYP3A4), as well as P-gp, the induction of intestinal CYP3A4 protein would be expected to increase intestinal metabolism, so that a decrease of plasma digoxin level after rifampin treatment might be predicted. Although CYP3A in duodenal samples was enhanced 4.4-fold, there was no correlation between the AUC of digoxin and CYP3A4 expression of individual volunteers.⁹² These data strongly indicate that P-gp modulates intestinal digoxin absorption, leading to low drug concentrations in individuals with high P-gp expression and high concentrations in those with low P-gp expression.⁹² Accordingly, rifampin-mediated P-gp induction is associated with a reduction of plasma digoxin.

Conclusions

With the help of great progress in key pharmaceutical technologies, we are now entering a new era in drug delivery. Although several drug transporters have been utilized as novel delivery systems, transport mechanisms for the majority of drugs are still unknown (Fig. 2). Therefore, we have to identify all the participating transporters in order to establish the potential utility of this approach to drug delivery. We require an efficient screening system to pick appropriate transporter(s) that can be used for delivery and also for target and/or lead discovery. Another aspect that demands careful consideration is pharmacokinetic drug-drug interaction at the transporter and the effects of single-nucleotide polymorphisms (SNPs) of drug transporters. Recent progress in these areas has been described elsewhere.

There are surprisingly many transporter-mediated drug-drug interactions associated with the transfer of drugs across the cell membranes in pharmacokinetically important tissues, such as intestine, kidney, liver, and brain. Molecular cloning and the functional characterization of drug transporters expressed in various tissues, as well as the utilization of transporter-gene knockout or deficient animals will be of great help in identifying therapeutically significant drug-drug interactions involving drug transport processes.

Acknowledgment

The author thanks Ms. Sayaka Kato for preparation of Figure 1.

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Fig. 1 Human Membrane Transporters Expressed in Intestine, Kidney, Liver, Lung, Blood-Brain Barrier, Choroid Plexus, and Tumor.

BCRP, breast cancer resistance protein.. LATs, L-type amino acid transporter; MCT1, monocarboxylate transporter 1; MDRs, multidrug resistance proteins; MRPs, multidrug resistance-associated proteins; NTCP, Na⁺/taurocholate cotransporting peptide; NPT1, Na⁺-dependent phosphate transporter 1; OATs, organic anion transporters; OATPs, organic anion transporting polypeptides; URAT1, uric acid transporter 1; OCTs, organic cation transporters; OCTNs, organic cation/carnitine transporters; PEPTs, oligopeptide transporters; SPGP, sister of P-glycoprotein;

Fig. 2 Brain-to-Plasma Free Concentration Ratio ($K_{p,r}$) of New Quinolones, Grepafloxacin (GPFX), Olumefloxacin (HSR-903), Spalfloxacin (SPFX) and Levofloxacin (LVFX), after Intravenous Administration to Wild-type Mice (*mdr1a,1b* (+/+)) and *mdr1a,1b* Gene Knockout Mice (*mdr1a,1b*(-/-)).

Dose: [¹⁴C]GPFX, 34 nmol/head; [¹⁴C]HSR-903, 0.36 μmol/head; [¹⁴C]SPFX, 110 nmol/head; [¹⁴C]LVFX, 19 nmol/head. Each column represents the mean ± S.E. of three to six experiments. * Significantly different compared with *mdr1a,1b* (+/+) mice (p < 0.05).

Fig. 1

Fig. 2