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Transporter-mediated drug delivery: recent progress and experimental approaches

Yoshimichi Sai and Akira Tsuji

A comprehensive list of drug transporters has recently become available as a result of extensive genome analysis, as well as membrane physiology and molecular biology studies. This review covers recent progress in identification and characterization of drug transporters, illustrative cases of successful drug delivery to, or exclusion from, target organs via transporters, and novel experimental approaches to therapeutics using heterologously transduced transporters in tissues. We aim to provide clues that could lead to efficient strategies for the use of transporters to deliver drugs and/or to optimize lead compounds.

Yoshimichi Sai
Department of Pharmaceutics
Kyoritsu University of Pharmacy
Tokyo, 105-8512, Japan
Akira Tsuji
Division of Pharmaceutical Sciences
Graduate School of Natural Science and Technology
Kanazawa University
Kakuma-machi
Kanazawa 920-1192, Japan
e-mail: tsuji@kenroku.kanazawa-u.ac.jp

▼ In the past decade, a comprehensive list of membrane transporters has become available owing to the progress in genome analysis, as well as extensive membrane physiology and molecular biology studies on membrane transporters. Indeed, not only are sequence data available, but also cDNA clones can be obtained easily through researchers, public resources or commercial providers. 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 (http://www.gene.ucl.ac.uk/nomenclature/).

Typical transporter candidates for drug delivery

The SLC family consists of 43 gene subfamilies and a total of ~300 family members, including ion-coupled transporters, facilitated transporters and exchangers (http://www.bioparadigms.org/slc/). In the ABC transporter family, 56 genes have been identified and classified into seven subfamilies (http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html). Some of these transporters accept not only physiological or endogenous substrates, but also xenobiotics, including drugs, and are therefore referred to as drug transporters. Typical drug transporters are members of the families of organic solute transporters (OCTs, OCTNs and OATs) (Table 1), organic anion transporting polypeptides (OATPs) (Table 2), oligopeptide transporters (Table 3) and ABC transporters (Table 4). Such drug transporters are expressed in specific cell membranes of various tissues, where they have pivotal roles in determining the pharmacokinetic profiles of particular drugs and thereby determine the overall pharmacological effects; for example, they influence drug absorption, distribution, elimination and concentration at the target sites (Figure 1) [1]. For example, the organic solute transporter OCT1 (Table 1) is expressed in the basolateral membrane of hepatocytes and renal proximal tubular epithelial cells, and mediates the uptake of various organic cation xenobiotics, including tetraethylammonium, 1-methyl-4-phenylpyridinium, tributylmethylammonium, *N*-methylquinine, *N*-methylquinidine, acyclovir and ganciclovir (Table 1). This review aims to provide clues that might enable us to establish efficient strategies to use transporters for target and lead discovery and/or lead optimization. Recent successes in delivery of drugs to target organs via transporters are illustrated in Figure 2 and discussed in the subsequent sections.

Successful drug delivery to or exclusion from various target organs

Liver-selective distribution of an HMG-CoA reductase inhibitor

Pravastatin, an HMG-CoA reductase inhibitor that undergoes enterohepatic circulation, which prolongs the exposure of the target organ – the

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Table 1. Members of the organic solute transporter family (SLC22A)

Transporter names (gene symbols)	Endogenous substrates	Xenobiotics/drugs
Organic cation transporters[a]		
OCT1 (SLC22A1)	Prostaglandin E2, prostaglandin F2,	Tetraethylammonium, 1-methyl-4-phenylpyridinium, tributylmethylammonium, *N*-methylquinine, *N*-methylquinidine, acyclovir, ganciclovir
OCT2 (SLC22A2)	Choline, histamine, dopamine, serotonin, noradrenaline, agmatine, prostaglandin E2, prostaglandin F2,	Tetraethylammonium, 1-methyl-4-phenylpyridinium, *N*-methylnicotinamide, amantadine, memantine,
OCT3 (SLC22A3)	Serotonin, adrenaline, noradrenaline, agmatine	1-methyl-4-phenylpyridinium, cimetidine
Organic cation/carnitine transporters[b]		
OCTN1 (SLC22A4)	L-carnitine	Tetraethylammonium, quinidine, pyrilamine, verapamil
OCTN2 (SLC22A5)	Acetyl-L-carnitine, L-carnitine, D-carnitine	Tetraethylammonium, quinidine, pyrilamine, verapamil
Organic anion transporters[c]		
OAT1 (SLC22A6)		*p*-aminohippurate, adefovir, cidofovir, acyclovir, ganciclovir, zidovudine, methotrexate
OAT2 (SLC22A7)	Prostaglandin F2a	Zidovudine, tetracycline, salicylate
OAT3 (SLC22A8)	Estrone-3-sulfate,	*p*-aminohippurate, valacyclovir, zidovudine, methotrexate
OAT4 (SLC22A11)	Estrone-3-sulfate, DHEAS	Zidovudine, methotrexate

[a]Organic cation transporters have important roles in the tissue distribution and elimination of various cationic drugs, some weak bases, non-charged compounds and anions in the liver, kidney and other organs [66,67].
[b]Organic cation/carnitine transporters are expressed in the apical membranes of renal proximal tubular epithelial cells and other tissues. They are thought to have a role in the secretion of organic cations from the cells as well as re-absorption of carnitine [68,69].
[c]These transporters mediate uptake of a wide variety of organic anions that are relatively hydrophilic, and are involved in the elimination of such drugs from the kidney, brain and liver [67,70].

liver – and minimizes adverse effects in other tissues. Hisang *et al.* first described a liver-specific transporter, OATP-C/OATP2/LST1 (SLC21A6) (Table 2) that accepts pravastatin as a substrate [2]. Nakai *et al.* confirmed that the active and Na^+-independent pravastatin uptake in the liver is mostly mediated by OATP-C in human hepatocytes [3]. After exerting its pharmacological action in the hepatocytes, pravastatin is excreted into the bile via MRP2 [4]. Regarding transport across the small intestinal brush-border membrane, a proton-gradient-dependent uptake mechanism has been shown to be involved [5]. Recently, Kobayashi *et al.* [6] and Nozawa *et al.* [7] revealed that HEK293 cells transfected with OATP-B take up pravastatin at acidic pH and suggested that OATP-B has a role in the (re)absorption of pravastatin at the small intestine, at least in part. OATP-B (SLC21A9) is also a member of the family of human organic anion transporting polypeptides (Table 2), and is expressed in the small intestine, as well as the liver and other organs [8]. Although OATP-B can transport various substrates including estrone-3-sulfate, dehydroepiandrosterone-sulfate and bromosulfophthalein at neutral pH [8,9], uptake of pravastatin by OATP-B at neutral pH was negligible [6,7]. OATP-B expressed in other tissues might have a role in the distribution of other substrates at neutral pH, however, there could be a different situation in the small intestine, where the physiological microclimate is acidic. As a consequence of the expression profile of the transporter, its substrate selectivity and transport characteristics, pravastatin is preferentially distributed to the liver, and thereby liver-specific inhibition of cholesterol synthesis is accomplished.

Carrier-mediated lung distribution of antimicrobial agents

New quinolone antibacterial agents have a high intrinsic antibacterial activity with a wide spectrum of action and have been used in the treatment of a variety of infections. Among them, HSR-903 [(*S*)-(-)-5-amino-7-(7-amino-5-azaspiro[2.4]hept-5-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid methanesulfonate] is well distributed into tissues [10]. The distribution volume of HSR-903 (4.9 l kg^{-1}) is comparable to that of sparfloxacin (~6.0 l kg^{-1}) and is much higher than that of other new quinolones, such as lomefloxacin or ofloxacin

Table 2. Members of the organic anion transporting polypeptide family (SLC21A/SLCO)

Transporter names (gene symbols)	Endogenous substrates	Xenobiotics/drugs (typical)
OATP-A (SLC21A3, SLCO1A2)	Bile salts, DHEAS, T3, T4	Fexofenadine, ouabain, BQ-123, DPDPE,
OATP-B (SLC21A9, SLCO2B1)	Prostaglandin E2	Estrone sulfate, pravastatin, fexofenadine, BSP
OATP-C (SLC21A6, SLCO1B1)	Bile salts, bilirubin conjugates, estradiol-17β-glucuronide, estrone-3-sulfate, dehydroepiandrosterone-3-sulfate, prostaglandin E2	Benzylpenicillin, methotrexate, pravastatin, rifampicin, DEDPE, DADLE, bromosulfophthalein
OATP-D (SLC21A11, SLCO3A1)	Prostaglandin E2, estrone-3-sulfate	Benzylpenicillin
OATP-E (SLC21A12, SLCO4A1)	Taurocholate, T3, rT3, T4, prostaglandins, estrone-3-sulfate	
OATP-F (SLC21A14, SLCO1C1)	Estrone-3-sulfate, E2–17β-glucuronide, T3, rT3, T4,	Bromosulfophthalein
OATP-8 (SLC21A8, SLCO1B3)	Bile salts, T3, rT3, T4, monoglucuronosyl bilirubin, E2–17β-glucuronide	Digoxin, methotrexate, ouabain, rifampicin, bromosulfophthalein
PGT (SLC21A2, SLCO2A1)	Prostaglandins, thromboxane B2	

These transporters accept many relatively hydrophobic organic cationic drugs, neutral peptides, bile salts and conjugated and unconjugated steroid hormones ([2,3,6–9,20]; mainly human data).

(1.46 and 1.54 l kg^{-1}, respectively) [11]. HSR-903 exhibits a particularly high concentration in the lung after oral administration, even higher than that of sparfloxacin [12]. The lung concentration of unchanged HSR-903 was about nine times higher than that in plasma after oral administration (5 mg kg^{-1}), whereas other quinolones gave values close to unity. Murata *et al.* studied the uptake of HSR-903 into the lung using perfused lung and isolated lung cells of rats [13]. Initial uptake of HSR-903 by isolated rat lung cells was temperature-dependent, saturable, stereospecific, and Na^+ and Cl—dependent. The uptake of HSR-903 was inhibited by other quinolone antibacterial agents, including sparfloxacin and grepafloxacin. The extraction ratio of HSR-903 in isolated lung perfusion was temperature-dependent and saturable. These findings suggest that HSR-903 is taken up by lung cells via a carrier-mediated transport mechanism, resulting in a high distribution to the lung. Identification of the responsible transporter remains to be accomplished, and could provide a specific route for drug delivery to the lung.

Groneberg *et al.* demonstrated the presence of a high-affinity peptide transporter PEPT2 (Table 3) in alveolar type II pneumocytes, bronchial epithelium and endothelium of small vessels in the lung by measuring uptake of peptides instilled in the trachea, in combination with northern blotting and immunohistochemical staining of rat and human lungs [14,15]. PEPT2 accepts many kinds of drugs, including β-lactam antibiotics [16], ACE (angiotensin-converting enzyme) inhibitors [17], antiviral drugs (valacyclovir) [18] and δ-aminolevulinic acid [19], so it is of major pharmaceutical interest as a route for drug delivery.

Restriction of brain entry of non-sedating H1-antagonists by transporters at the BBB

H1 histamine receptor antagonists (H1-antagonists) are used to treat several allergic disorders. To date, two generations

Table 3. Members of the oligopeptide transporter family (SLC15A)

Transporter names (gene symbols)	Endogenous substrates	Xenobiotics/drugs
PEPT1 (SLC15A1)	Di- and tripeptides, carnosine	Glycylsarcosine, cefaclor, cefadroxil, cefdinir, cefixime, FK089, ceftibuten, temocapril, enalapril, valacyclovir, bestatin, methyldopa-L-phenylalanine, L-dopa-L-phenylalanine
PEPT2 (SLC15A2)	Di- and tripeptides,	Cephalexin, bestatin, valacyclovir, zidovudine, ∂-aminolevulinic acid

Oligopeptide transporters mediate electrogenic uphill transport of the substrates by using an H^+ gradient as the driving force. [1,16,18,19,30,31,36–43,46,47]

Table 4. Members of the ABC transporter family (ABC)

Transporter names (gene symbols)	Endogenous substrates	Xenobiotics/drugs
MDR1 (ABCB1)		Vincristine, daunorubicin, etoposide, methotrexate, paclitaxel, digoxin, tacrolimus, indinavir, grepafloxacin, fexofenadine, cetirizine, carebastine, taninolol, cyclosporin A, cimetidine, azasetron, quinidine, rhodamine-123, fluo-3
MRP1 (ABCC1)		Vincristine, daunorubicin, etoposide, methotrexate, grepafloxacin, rhodamine-123, fluo-3
MRP2 (ABCC2)		Vincristine, etoposide, cisplatin, indinavir, grepafloxacin, fluo-3
MRP3 (ABCC3)		Vincristine, etoposide, methotrexate
BCRP (ABCG2)	Estrone-3-sulfate	Daunorubicin, etoposide, methotrexate, rhodamine-123, mitoxantrone

ATP-binding cassette (ABC) transporters are primary active transporters. They utilize ATP hydrolysis as the driving force and work as drug efflux pumps at the BBB, small and large intestine, kidney, liver and other various barrier tissues. [23,32,22]

of H1-antagonists have been developed. Classical antihistamines, such as diphenhydramine, chlorpheniramine and cyproheptadine, are cationic drugs and are distributed well to the brain, thereby causing sedation, which limits their usefulness. By contrast, the second-generation H1-antagonists, such as fexofenadine and cetirizine, do not cross the blood–brain barrier (BBB) and are frequently referred to as non-sedating antihistamines. The underlying mechanisms limiting brain penetration have been studied, and fexofenadine is the first non-sedating H1-antagonist that has been shown to be a P-glycoprotein (Pgp) substrate both *in vitro* (i.e. transfected cells) and *in vivo* [i.e. mdr1a (–/–) mice] [20,21]. Pgp functions as an active efflux transporter at the BBB and in other normal tissues, as well as in multi-drug resistant tumor cells [22] and restricts the entry of various anti-tumor drugs, immunosuppressants and other drugs to the tissues (Table 4). Pgp-mediated efflux transport contributes to the restriction of the brain entry of fexofenadine at least in part, although an additional mechanism other than Pgp has also been postulated [20].

The transport mechanism of another non-sedating H1-antagonist, ebastine, has been studied [23]. Interestingly, the brain uptake index of [^{14}C]-ebastine measured by intra-carotid artery injection to rats was high, suggesting involvement of a carrier-mediated uptake mechanism similar to that characterized using a classical model H1-antagonist, mepyramine [24]. The mepyramine transporter identified in brain capillary endothelial cells recognized various lipophilic cationic drugs,

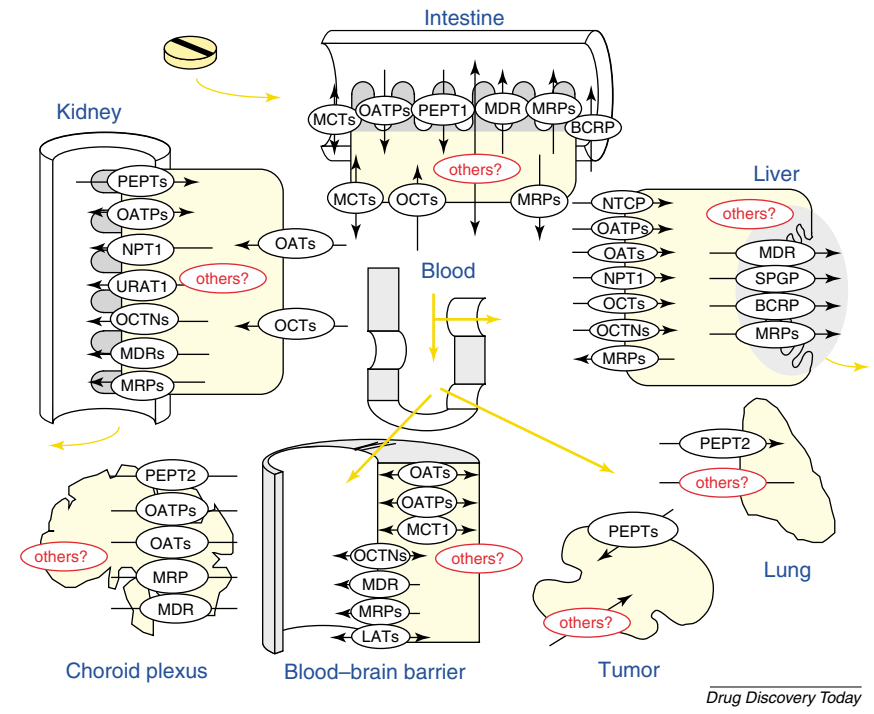

Figure 1. A schematic of transporter-mediated oral delivery and disposition of drugs. Although several drug transporters can be used as novel delivery systems (shown in black), transport mechanisms for the majority of drugs are still unknown (shown in red). To establish the potential use of transporter-mediated drug delivery, all participants have to be identified. Abbreviations: PEPTs, oligopeptide transporters; OATPs, organic anion transporting polypeptides; NPT1, Na^+-dependent phosphate transporter 1; URAT1, uric acid transporter 1; OCTNs, organic cation/carnitine transporters; MDRs, multidrug resistance proteins; MRPs, multidrug resistance-associated proteins; OATs, organic anion transporters; MCT1, monocarboxylate transporter 1; LATs, L-type amino acid transporter 1; NTCP, Na^+/taurocholate cotransporting peptide; OCTs, organic cation transporters; SPGP, sister of P-glycoprotein; BCRP, breast cancer resistance protein.

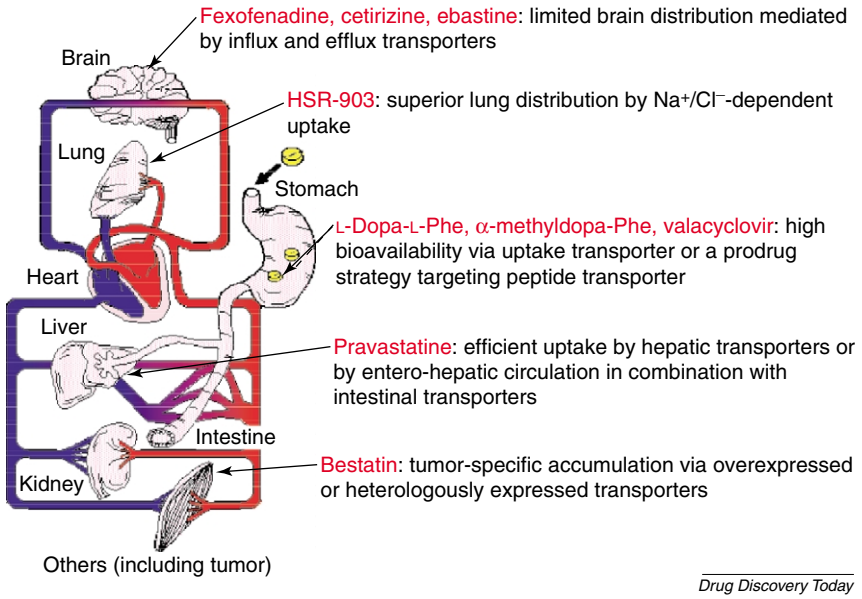

Figure 2. Drugs that have been successfully distributed to, or excluded from, target organs. Some successes in the delivery of drugs to target organs via transporters are illustrated. By knowing the substrate specificity and the expression profile of each transporter, efficient drug delivery to, or exclusion from, the target organs can be accomplished by appropriately modifying the structure of the drug.

such as imipramine, propranolol and lidocaine, as well as many H1-antagonists that are structurally related [24]. These results seem inconsistent with the fact that ebastine is a non-sedating H1-antagonist: it is, however, is rapidly metabolized to an active carboxylic acid metabolite, carebastine, in both experimental animals and humans. Carebastine also has a high and selective H1-antagonist activity [25], and is considered to be a major contributor to the activity of the parent drug. Tamai *et al*. examined the transport mechanisms of carebastine in the brain using *in vitro* and *in vivo* experimental methods and showed that carebastine is not a good substrate for the mepyramine transporter, but is a substrate for Pgp-mediated efflux from the brain at the BBB [23]. Therefore, it was concluded that the brain distribution of ebastine is restricted owing to rapid metabolism to zwitterionic carebastine, which has a lower affinity to the uptake transporter for lipophilic cationic compounds and undergoes active efflux from the brain. All these factors might account for the minimal sedative effect of the drug in the brain.

Other zwitterionic H1-antagonists, such as fexofenadine and cetirizine, also have low brain distribution [26–29]. The affinity of the mepyramine transporter for zwitterionic derivatives is low, therefore it is likely that these H1-antagonists have only a weak sedative effect because of lower transport into the brain at the BBB, at least in part. Thus, the brain distribution of H1-antagonists is influenced by both influx and efflux transporters. When the characteristics of these transport systems and the factors determining the affinity between substrate and transporters have been clarified, it should be possible to develop drugs that have no CNS side-effects by rational modification of classical structures. For example, introducing a zwitterionic moiety into the drug molecule could help to restrict its entry into the brain. In addition, efficient *in vitro* screening systems should be used to access whether candidates are Pgp substrates or not.

Transporter-mediated oral drug absorption

The oral route is preferable for the treatment of chronic diseases. For the rational design of orally active drugs, it is important to understand the mechanisms underlying intestinal absorption. Many factors influence absorption of drugs, including gastric emptying, pH, food, dissolution, lipophilicity, particle size, passive or active membrane permeation and active exclusion.

Recently, transporter-mediated processes have attracted great interest. Certain organic solutes, such as amino acid-mimetic or peptide-mimetic drugs, monocarboxylic acid drugs, phosphonic acid drugs, bile acids, choline and water-soluble vitamins, are suggested to be absorbed from the gastrointestinal tract through transporter-mediated mechanisms including proton-coupled transport, ion exchange transport, membrane potential-dependent transport and others [30,31]. By contrast, absorption of many lipophilic drugs is limited by Pgp or other ATP-dependent active secretory mechanisms (Table 4) at the brush border membranes of intestinal epithelial cells [32]. Although expression of a variety of transporter transcripts and proteins has been detected, the absorption mechanisms of most drugs are poorly understood, with a few exceptions. Some examples are described in subsequent sections; for details, see other reviews [30,31,33].

Oligopeptide transporters

Numerous studies over the past two decades have shown that an H^+/oligopeptide co-transport activity is expressed in the apical membranes of the small intestinal absorptive epithelial cells [34–44] (Table 3). Since the first success in cloning of PEPT1 cDNA as a gene encoding an oligopeptide transporter [39], it has been one of the most extensively

studied drug transporters [34–38]. The pharmacological role of PEPT1 lies in the absorption of orally active cephalosporins, aminopenicillins, ACE inhibitors, renin inhibitors and bestatin, as well as di- and tripeptides derived from digestion of dietary proteins [34,37–39]. These compounds possess peptide-like chemical structures with a peptide bond, an *N*-terminal α-amino group, and a *C*-terminal carboxyl group. However, these groups do not appear to be critical requirements, although their modification generally diminishes the affinity of the substrate to the transporter [41–43]. The broad substrate specificity of this transporter makes it an attractive candidate to improve intestinal absorption of drugs that can be chemically converted to di- or tripeptide type prodrugs. Indeed, it was reported that the absorption of L-dopa and α-methyldopa could be improved by dipeptidyl derivation [44,45]. Furthermore, the prodrug strategy targeting peptide transporter has been extended to nonpeptidyl-type prodrugs, such as the amino acid ester prodrug valacyclovir [46,47].

Organic anion transporting polypeptide

Members of the organic anion transporting polypeptide (OATP) family are involved in the transport of various endogenous xenobiotic compounds, such as bile salts, conjugated metabolites of steroid hormones, thyroid hormones, anionic oligopeptides, drugs and toxins [48] (Table 2). In the small intestine, OATP-B (SLC21A9) is localized at the apical membranes of enterocytes [6]. Expression of other OATPs, such as OATP-D (SLC21A11) and OATP-E (21A12), has also been detected at the mRNA level [8]. Furthermore, it has been reported recently that fruit juices decreased the absorption of an H1-antagonist, fexofenadine, in human volunteers and it was suggested that fexofenadine might be absorbed via intestinal OATP transporters [49]. Recently, Nozawa *et al.* [7] demonstrated that fexofenadine is a substrate of OATP-B. As described previously, an HMG-CoA reductase inhibitor, pravastatin, is absorbed to the extent of ~30% after oral administration in healthy volunteers, despite its hydrophilicity [50]. Involvement of a pH-dependent transporter in the intestinal apical membrane transport had been suggested on the basis of studies with brush-border membrane vesicles [5], and recently, pravastatin was demonstrated to be taken up by OATP-B in a pH-dependent manner [6,7].

Amino acid transporters

In the large intestine, Hatanaka *et al.* have examined the potential of the Na^+ and Cl^--coupled amino acid transporter $ATB^{0,+}$ as a delivery route for amino acid-based prodrugs [51]. $ATB^{0,+}$ recognizes neutral and cationic amino acids in their L- and D- isomeric forms, nitric oxide synthase inhibitors and carnitines [52–54]. It is expressed abundantly on the luminal surface of cells lining the lumen of the large intestine, as well as the airways of the lung, and in various ocular tissues [51]. They screened various β-carboxyl and γ-carboxyl derivatives of aspartate and glutamate, respectively, and found that the substrate specificity of $ATB^{0,+}$ was very broad; it transported many derivatives that have a neutral or cationic side chain. The large intestine is a suitable route for drug delivery in the form of colon-directed oral pills. This transporter might complement PEPT1 for delivery of amino acid-mimetic drugs from the large intestine [51].

Although it has long been believed that all drugs are absorbed through the gastrointestinal epithelium by a simple diffusion mechanism depending on their lipophilic nature, direct and indirect evidence for participation of transporter-mediated mechanisms has accumulated. Studies on the responsible transporters or other processes underlying transport are of great importance.

Tumor-targeted delivery of drugs using an oligopeptide transporter

An oligopeptide transport activity is expressed in some tumor cells, including fibrosarcoma HT-1080 and two pancreatic tumor cell lines – AsPc-1 and Capan-2 – but not in a normal diploid cell line, IMR-90 [55,56]. These findings open up a means for specific delivery of drugs to the tumor cells, because oligopeptide transporters, namely PEPT1 and PEPT2, are expressed mainly in the small intestinal epithelial cells and the renal proximal cells among non-tumor cells. To pursue the possibility of selective delivery of anti-tumor drugs by an oligopeptide transporter, Nakanishi *et al.* have examined the *in vivo* accumulation of bestatin in tumor tissues and also the effect on tumor growth using nude mice bearing PEPT1-overexpressing HeLa cell tumors [57]. After oral administration, bestatin was accumulated in the tumor tissues that overexpressed PEPT1. Furthermore, repeated oral administration of bestatin to the mice for 28 days specifically suppressed the growth of PEPT1-overexpressing tumors, although it was less effective in tumor cells that express an endogenous oligopeptide transporter, such as HT-1080 cells. This strategy can be attractive if the expression level of the oligopeptide transporter in tumor cells is high enough. An anti-tumor compound that is a substrate for an oligopeptide transporter should be absorbed effectively from the small intestine via PEPT1 and be distributed specifically to the tumor cells. By contrast, the compound might be reabsorbed from the kidney, where PEPT1 and PEPT2 are present, therefore the possibility of nephrotoxicity should be considered. To avoid this, the efflux mechanism of peptide drugs from the basolateral

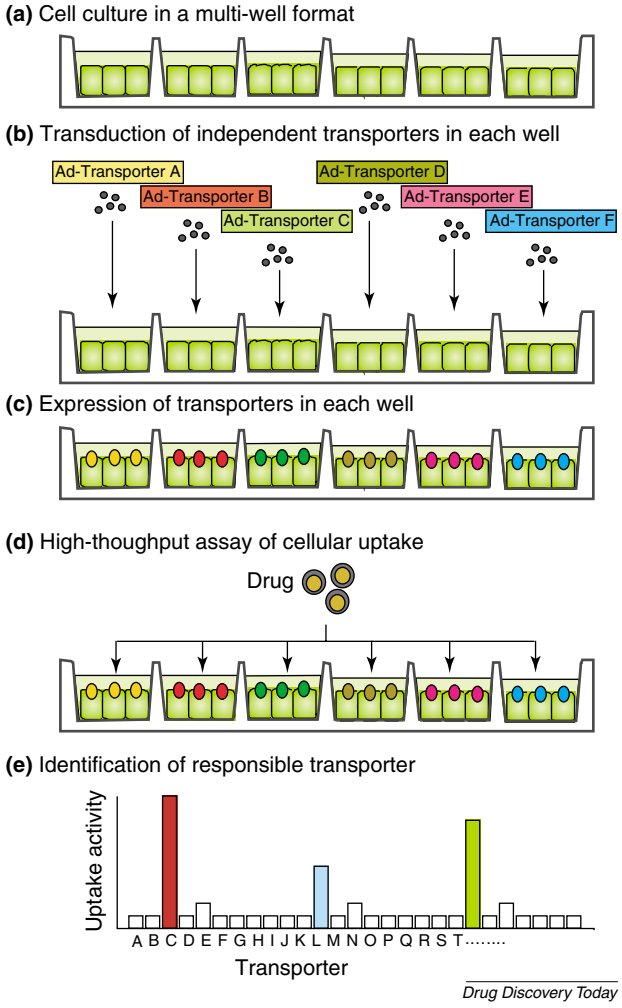

Figure 3. A schematic of a proposed transporter activity protein array. **(a)** Host cells are cultivated in a multi-well format culture plate. **(b,c)** Each well is transduced with adenovirus expressing a transporter, and **(d)** the uptake activities are examined by incubating each well with a test compound, **(e)** followed by quantitation using an LC–MS–MS or liquid scintillation counter. **(e)** The responsible transporter(s) can then be identified.

membranes of the intestinal and renal tubular epithelial cells should be clarified: several reports have characterized oligopeptide transport activity [58–60].

Novel approaches to experimental therapeutics

Brain delivery of drugs by heterologous expression of a transporter in the BBB

Toyobuku *et al.* [61] examined the feasibility of heterologous transduction of a transporter gene to the BBB. Peptides have multiple biological actions in the brain, therefore, they are potentially valuable as neuropharmaceuticals in the treatment of various disorders, such as Alzheimer's disease and depression. Delivery of peptide drugs to the brain, however, is a big challenge because distribution of peptides to the brain is generally very low owing to the BBB. Toyobuku *et al.* constructed a recombinant adenovirus vector encoding PEPT1 and transduced the transporter into the BBB of rats by carotid artery injection of the vector. Heterologous expression of PEPT1 at the BBB successfully increased the brain distribution of a model substrate for the PEPT1 (cefadroxil) [61]. Malignant gliomas are the most common primary neoplasms of the CNS. The prognosis for high-grade malignant gliomas remains bleak; survival is generally less than 1 year. As bestatin, the substrate for PEPT1, was reported to induce apoptosis in glioma cells [62], this system might be applicable to the treatment of malignant glioma.

Summary and perspectives

With the help of great progress in key pharmaceutical technologies, we are now entering a new era of drug delivery. A comprehensive list of drug transporters has become available and now information on the tissue distributions and the mechanisms of these transporters is accumulating. However, as the transport mechanisms for the majority of drugs still remain to be clarified, much work remains to be done. In general, most SLC transporters and ABC transporters mediate influx and efflux transport, respectively. Therefore, by knowing the substrate specificity and expression profile of each transporter, efficient drug delivery to, or exclusion from, target organs should be feasible by appropriately modifying the structure of the drug [30,31]. For instance, substrate SARs have been studied for PEPT1 and MDR1 [34,36,63].

To date, substrate preferences and other functional characteristics of transporters have been studied by using *in vitro* expression systems, for example, transporter cDNA-transfected cells or *Xenopus laevis* oocytes microinjected with transporter cRNAs. Now, drug screening can be accomplished by using cell lines that stably express a transporter(s) in combination with multi-well format cell culture to achieve high-throughput quantification. The next step might be to establish an efficient screening system to pick appropriate transporter(s) that can be used for delivery and also for target and/or lead discovery. We consider that this could be accomplished by arraying drug transporters in a multi-well format system: the concept is illustrated in Figure 3.

Another aspect that demands careful consideration is drug–drug interaction, because the pharmacokinetics of drugs that are transporter substrates might be influenced by co-administered drugs that work as inhibitors or enhancers of the transporter function (see review [64]). For the development of personalized medicine, single-nucleotide polymorphisms (SNPs) of drug transporters would also

need to be considered. Although information regarding the impact of the SNPs is thus far limited, a standard method for the evaluation of the effect of polymorphisms on transporter function was recently presented [65].

References

1 Tsuji, A. (1999) Tissue selective drug delivery utilizing carrier-mediated transport systems. *J. Control. Release* 62, 239–244
2 Hisang, B. *et al.* (1999) A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *J. Biol. Chem.* 274, 37161–37168
3 Nakai, D. *et al.* (2001) Human liver-specific organic anion transporter, LST-1, mediates uptake of pravastatin by human hepatocytes. *J. Pharmacol. Exp. Ther.* 297, 861–867
4 Yamazaki, M. *et al.* (1997) Biliary excretion of pravastatin in rats: contribution of the excretion pathway mediated by canalicular multispecific organic anion transporter. *Drug Metab. Dispos.* 25, 1123–1129
5 Tamai, I. *et al.* (1995) Proton-cotransport of pravastatin across intestinal brush-border membrane. *Pharm. Res.* 12, 1727–1732
6 Kobayashi, D. *et al.* (2003) Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane. *J. Pharmacol. Exp. Ther.* 306, 703–708
7 Nozawa, T. *et al.* (2004) Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. *J. Pharmacol. Exp. Ther.* 308, 438–445
8 Tamai, I. *et al.* (2000) Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem. Biophys. Res. Commun.* 273, 251–260
9 Kullak-Ublick, G.A. *et al.* (2001) Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* 120, 525–533
10 Murata, M. *et al.* (1995) Carrier-mediated tissue distribution and pharmacokinetics of HSR-903, a new quinolone, *The 35th Interscience Conference on Antimicrobial Agents and Chemotherapy,* American Association for Microbiology, Washington, DC, US (Abstract F203)
11 Okezaki, E. *et al.* (1988) Structure-tissue distribution relationship based on physiological pharmacokinetics for NY-198, a new antimicrobial agent, and the related pyridonecarboxylic acids. *Drug Metab. Dispos.* 16, 865–874
12 Yoshizumi, S. *et al.* (1998) *In vivo* activity of HSR-903, a new fluoroquinolone, against respiratory pathogens. *Antimicrob. Agents Chemother.* 42, 785–788
13 Murata, M. *et al.* (1999) Carrier-mediated lung distribution of HSR-903, a new quinolone antibacterial agent. *J. Pharmacol. Exp. Ther.* 289, 79–84
14 Groneberg, D.A. *et al.* (2001) Localization of the peptide transporter PEPT2 in the lung: implications for pulmonary oligopeptide uptake. *Am. J. Pathol.* 158, 707–714
15 Groneberg, D.A. *et al.* (2002) Distribution and function of the peptide transporter PEPT2 in normal and cystic fibrosis human lung. *Thorax* 57, 55–60
16 Ganapathy, M.E. *et al.* (1995) Differential recognition of beta-lactam antibiotics by intestinal and renal peptide transporters, PEPT 1 and PEPT 2. *J. Biol. Chem.* 270, 25672–25677
17 Zhu, T. *et al.* (2000) Differential recognition of ACE inhibitors in *Xenopus laevis* oocytes expressing rat PEPT1 and PEPT2. *Pharm. Res.* 17, 526–532
18 Ganapathy, M.E. *et al.* (1998) Valacyclovir: a substrate for the intestinal and renal peptide transporters PEPT1 and PEPT2. *Biochem. Biophys. Res. Commun.* 246, 470–475
19 Doring, F. *et al.* (1998) Delta-aminolevulinic acid transport by intestinal and renal peptide transporters and its physiological and clinical implications. *J. Clin. Invest.* 101, 2761–2767
20 Cvetkovic, M. *et al.* (1999) OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab. Dispos.* 27, 866–871
21 Soldner, A. *et al.* (1999) Grapefruit juice activates P-glycoprotein-mediated drug transport. *Pharm. Res.* 16, 478–485
22 Tamai, I. and Tsuji, A. (2000) Transporter-mediated permeation of drugs across the blood-brain barrier. *J. Pharm. Sci.* 89, 1371–1388
23 Tamai, I. *et al.* (1999) Blood-brain barrier transport of H1-antagonist ebastine and its metabolite carebastine. *J. Drug Target.* 8, 383–393
24 Yamazaki, M. *et al.* (1994) Carrier-mediated transport of H1-antagonist at the blood-brain barrier: a common transport system of H1-antagonists and lipophilic basic drugs. *Pharm. Res.* 11, 1516–1518
25 Yakuo, I. *et al.* (1994) Pharmacological study of ebastine, a novel histamine H1-receptor antagonist. *Nippon Yakurigaku Zasshi* 103, 121–135
26 Gonzalez, M.A. and Estes, K.S. (1998) Pharmacokinetic overview of oral second-generation H1 antihistamines. *Int. J. Clin. Pharmacol. Ther.* 36, 292–300
27 Yanai, K. *et al.* (1999) New findings in pharmacological effects induced by antihistamines: from PET studies to knock-out mice. *Clin. Exp. Allergy* 29, 29–36
28 Chen, C. *et al.* (2003) P-Glycoprotein limits the brain penetration of nonsedating but not sedating H1-antagonists. *Drug Metab. Dispos.* 31, 312–318
29 Polli, J.W. *et al.* (2003) P-Glycoprotein influences the brain concentrations of cetirizine (Zyrtec), a second-generation non-sedating antihistamine. *J. Pharm. Sci.* 92, 2082–2089
30 Tsuji, A. and Tamai, I. (1996) Carrier-mediated intestinal transport of drugs. *Pharm. Res.* 13, 963–977
31 Steffansen, B. *et al.* (2004) Intestinal solute carriers: an overview of trends and strategies for improving oral drug absorption. *Eur. J. Pharm. Sci.* 21, 3–16
32 Chan, L.M. *et al.* (2004) The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. *Eur. J. Pharm. Sci.* 21, 25–51
33 Mizuno, N. *et al.* (2003) Impact of drug transporter studies on drug discovery and development. *Pharmacol. Rev.* 55, 425–461
34 Brandsch, M. *et al.* (2004) The intestinal H(+)/peptide symporter PEPT1: structure-affinity relationships. *Eur. J. Pharm. Sci.* 21, 53–60
35 Nielsen, C.U. and Bordin, B. (2003) Di/tri-peptide transporters as drug delivery targets: Regulation of transport under physiological and patho-physiological conditions. *Curr. Drug Targets* 4, 373–388
36 Vaabeno, J. *et al.* (2004) Phe-Gly dipeptidomimetics designed for the di-/tripeptide transporters PEPT1 and PEPT2: Synthesis and biological investigations. *J. Med. Chem.* 47, 1060–1069
37 Daniel, H. (1996) Function and molecular structure of brush border membrane peptide/H^+ symporters. *J. Membr. Biol.* 154, 197–203
38 Sai, Y. *et al.* (1996) Immunolocalization and pharmacological relevance of oligopeptide transporter PepT1 in intestinal absroption of beta-lactam antibiotics. *FEBS Lett.* 392, 25–29
39 Fei, Y.J. *et al.* (1994) Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature* 368, 563–566
40 Terada, T. *et al.* (1998) Interaction of beta-lactam antibiotics with histidine residue of rat H^+/peptide cotransporters, PEPT1 and PEPT2. *J. Biol. Chem.* 273, 5582–5585
41 Temple, C.S. *et al.* (1998) Peptide mimics as substrates for the intestinal peptide transporter. *J. Biol. Chem.* 273, 20–22
42 Doring, F. *et al.* (1998) Minimal molecular determinants of substrates for recognition by the intestinal peptide transporter. *J. Biol. Chem.* 273, 23211–23218
43 Doring, F. *et al.* (1998) Delta-aminolevulinic acid transport by intestinal and renal peptide transporters and its physiological and clinical implications. *J. Clin. Invest.* 101, 2761–2767
44 Tamai, I. *et al.* (1998) Improvement of L-dopa absorption by dipeptidyl derivation, utilizing peptide transporter PepT1. *J. Pharm. Sci.* 87, 1542–1546

45 Tsuji, A. *et al.* (1990) Mechanism of absorption of the dipeptide alpha-methyldopa-phe in intestinal brush-border membrane vesicles. *Pharm. Res.* 7, 308–309
46 Balimane, P.V. *et al.* (1998) Direct evidence for peptide transporter (PepT1)-mediated uptake of a nonpeptide prodrug, valacyclovir. *Biochem. Biophys. Res. Commun.* 250, 246–251
47 Han, H.K. and Amidon, G.L. (2000) Targeted prodrug design to optimize drug delivery. *AAPS PharmSci* 2, E6
48 Hagenbuch, B. and Meier, P.J. (2003) The superfamily of organic anion transporting polypeptides. *Biochim. Biophys. Acta* 1609, 1–18
49 Dresser, G.K. (2002) Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin. Pharmacol. Ther.* 71, 11–20
50 Pan, H.Y. (1991) Clinical pharmacology of pravastatin, a selective inhibitor of HMG-CoA reductase. *Eur. J. Clin. Pharmacol.* 40, S15–S18
51 Hatanaka, T. *et al.* (2004) Transport of amino acid-based prodrugs by the Na^+- and Cl— coupled amino acid transporter $ATB^{0,+}$ and expression of the transporter in tissues amenable for drug delivery. *J. Pharmacol. Exp. Ther.* 308, 1138–1147
52 Hatanaka, T. *et al.* (2002) Transport of D-serine via the amino acid transporter ATB(0,+) expressed in the colon. *Biochem. Biophys. Res. Commun.* 291, 291–295
53 Hatanaka, T. *et al.* (2001) Na^+- and Cl—coupled active transport of nitric oxide synthase inhibitors via amino acid transport system B(0,+). *J. Clin. Invest.* 107, 1035–1043
54 Nakanishi, T. *et al.* (2001) Na^+- and Cl—coupled active transport of carnitine by the amino acid transporter ATB(0,+) from mouse colon expressed in HRPE cells and *Xenopus* oocytes. *J. Physiol.* 532, 297–304
55 Nakanishi, T. *et al.* (1997) Carrier-mediated transport of oligopeptides in the human fibrosarcoma cell line HT1080. *Cancer Res.* 57, 4118–4122
56 Gonzalez, D.E. (1998) An oligopeptide transporter is expressed at high levels in the pancreatic carcinoma cell lines AsPc-1 and Capan-2. *Cancer Res.* 58, 519–525
57 Nakanishi, T. *et al.* (2000) Cancer cell-targeted drug delivery utilizing oligopeptide transport activity. *Int. J. Cancer* 88, 274–280
58 Dyer, J. *et al.* (1990) Glycyl-L-proline transport in rabbit enterocyte basolateral-membrane vesicles. *Biochem. J.* 269, 565–571
59 Terada, T. *et al.* (2000) Functional expression of novel peptide transporter in renal basolateral membranes. *Am. J. Physiol. Renal Phsyiol.* 279, F851–F857
60 Sugawara, M. *et al.* (2003) Uptake of dipeptide and beta-lactam antibiotics by the basolateral membrane vesicles prepared from rat kidney. *Biochim. Biophys. Acta* 1609, 39–44
61 Toyobuku, H. *et al.* (2003) Delivery of peptide drugs to the brain by adenovirus-mediated heterologous expression of human oligopeptide transporter at the blood-brain barrier. *J. Pharmacol. Exp. Ther.* 305, 40–47
62 Schlapbach, R. and Fontana, A. (1997) Differential activity of bcl-2 and ICE enzyme family protease inhibitors on Fas and puromycin-induced apoptosis of glioma cells. *Biochim. Biophys. Acta* 1359, 174–180
63 Seelig, A. and Landwojtowicz, E. (2000) Structure-activity relationship of P-glycoprotein substrates and modifiers. *Eur. J. Pharm. Sci.* 12, 31–40
64 Tsuji, A. (2002) Transporter-mediated drug interactions. *Drug Metabol. Pharmacokin.* 17, 253–274
65 Ishikawa, T. *et al.* (2004) The genetic polymorphism of drug transporters: functional analysis approaches. *Pharmacogenomics* 5, 67–99
66 Jonker, J.W. and Schinkel, A.H. (2004) Pharmacological and physiological function of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). *J. Pharmacol. Exp. Ther.* 308, 2–9
67 Dresser, M.J. *et al.* (2001) Transporters involved in the elimination of drugs in the kidney: organic anion transporters and organic cation transporters. *J. Pharm. Sci.* 90, 397–421
68 Yabuuchi, H. *et al.* (1999) Novel membrane transporter OCTN1 mediates multispecific, bidirectional, and pH-dependent transport of organic cations. *J. Pharmacol. Exp. Ther.* 289, 768–773
69 Ohashi, R. *et al.* (1999) Na(+)-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. *J. Pharmacol. Exp. Ther.* 291, 778–784
70 Hosoyamada, M. *et al.* (1999) Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am. J. Physiol.* 276, F122–F128