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REVIEW ARTICLE

Biological Transporters as Targets for New Drug Design

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The biological system, which forms the basis for drug-host, drug-food, and drug-drug interactions, is full of mechanisms designed to manipulate drug action. These mechanisms, namely absorption, distribution, metabolism, and excretion (ADME), thus become targets in pharmaceutical research for optimizing the pharmacodynamic/pharmacokinetic profiles of drugs. The body system is also full of resources, such as transporters, for designing new chemical entities as therapeutic agents. This review highlights current research in using biological transporters as a proactive approach in drug delivery to optimize efficacy in the early stages of drug development, and as a target for rational design of prodrugs as novel therapeutic agents. The structural design of prodrugs using delivery moieties such as D-phenylglycine for improving intestinal absorption of levodopa is presented as an example.

1. Introduction

Mechanism-based drug design, based on the interaction of drugs and specific target proteins (pharmacodynamics, PD) is the mainstream in conventional drug discovery and development. Pharmacokinetic (PK) profile, the descriptor of the drug-host interaction, is usually conducted in the later stages of drug discovery. However the disposition of biologically active substances (xenobiotics by nature) by the body system determines the success of these chemical entities in becoming therapeutic agents.[1] As a consequence, the success rate of bringing chemical entities with potent pharmacological activity from discovery to clinic is rather low, estimated to be 1 in 2000.[2] In most cases, the failure is due to unsatisfactory PK after the chemical entities have entered the biological system.[3] Therefore, integration of PK and PD for optimizing drug efficacy (PD/PK optimization) and thus increasing the success rate during the early stages of drug discovery is a common practice in modern drug research (Figure 1).[4,5]

2. Drug Design Based on Drug-Host Interaction

Drug delivery systems such as biodegradable polymers and liposomes are common formulation approaches to PD/PK optimization in pharmaceutical research. However, most of the materials used as drug delivery systems are xenobiotics to the biological system. This system, forming the basis of drug-host interaction, is full of mechanisms for drug delivery and biotransformation.[6] Some examples associated with drug-host interactions are changes of drug absorption and renal excretion via transporters, multiple drug resistance due to efflux transporters, or alteration in metabolic enzyme activity. The drug-host interaction mechanisms, namely absorption, distribution, metabolism, and excretion (ADME), thus become wonderful resources in manipulating and optimizing drug action.[7] Moreover, the application of ADME mechanism and PK theory in the rational design of new drug entities during the early discovery stages has become a modern pharmaceutical approach.[8]

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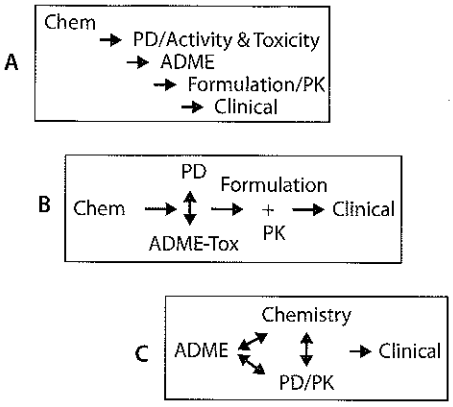

Figure 1 Evolution of drug research and development from: (A) Conventional sequential involvement of chemistry, pharmacodynamics (PD), absorption, distribution, metabolism and excretion (ADME), then pharmacokinetics (PK); (B) PD/ADME-Tox abreast; and (C) ADME-based strategy for new drug design.

3. Transporters in Biological Systems

It is well-documented that transporters in the intestine, liver, kidney, and brain are involved in the uptake and efflux of chemical substances like food and drugs.[9–12] Thus, the function of transports located in specific tissues highly influences drug disposition and pharmacological effects.[13–18] Evidence also supports the involvement of transporters in human PK variability and drug use.[19,20] The success of using transporters for specific drug delivery to target tissues is thus an important consideration in drug design.[5,21–23] A predictable ADME-toxicity modulation is also important in the drug development process.[24] As a result of pharmacovigilance concern, transporters associated with drug-drug and drug-food interactions have also attracted great attention from regulatory authorities.[25]

4. Transporters as Drug Delivery Systems

Drug delivery across cellular barriers is a challenging task.[26,27] Oral absorption ratio has limited the development of many potential therapeutic agents from becoming drugs. Therefore, targetable design by utilizing endogenous transporters is an opportunity for effective transmembrane drug delivery.[28] As the GI tract is the primary site for oral absorption of drugs, region-specific drug delivery using GI tract absorption and efflux systems could determine drug bioavailability post-oral administration.[29–31] There has been intensive research on the design of delivery systems for effective enhancement of transmembrane permeation of poorly absorbed drugs across biological barriers.[32,33] Current approaches to enhance transmembrane drug delivery include the formulation design of parent drugs, chemical modification of parent drugs to prodrugs, and use of biological transporters as targets for designing chemical drug delivery systems.[34,35]

5. Transporters and Drug Absorption

The intestinal peptide transporter system for drug absorption, previously recognized as the proton-coupled oligopeptide transporter (POT) family, is classified into peptide transporter 1 (PEPT1), peptide/histidine transporter 1 (PHT1), and peptide/histidine transporter 2 (PHT2).[36] PEPT1, the main mammalian POT regulating intestinal peptide absorption, has been under intensive investigation not only with regard to disclosure of transporting profile from a molecular and pharmacogenetic aspect, but also for its usefulness in new drug discovery.[37–41] The orally absorbable amino-β-lactams such as the tripeptide mimetics cephalexin and cephradine,[42–47] and angiotensin converting enzyme inhibitors such as the dipeptide mimetics lisinopril and fosinopril,[48,49] are substrates of intestinal PEPT1 transporter (Figure 2).[50] Recent studies have explored the structural features of substrates for PEPT1,[51,52] from structural biology and structure-absorption relationship analyses to the application of PEPT1 in drug discovery and clinical evaluation.[53–56]

6. Strategies of Using Transporters for Oral Absorption of Drugs

The prodrug approach is an effective way to improve absorption and oral bioavailability of drugs.[35,57–59] Among these, the use of intestinal transporter systems for designing oral prodrugs that facilitate the transport of drugs is a growing field in pharmaceutical research.[60–62]

Most amino acid drugs, such as α-methyldopa and levodopa, demonstrate inter- and intrasubject variation of drug availability, to which the absorption level is affected by dietary food.[63] To improve bioavailability by alternative absorption route, we chose α-methyldopa as a model compound for intestinal absorption via PEPT1 by virtue of *in vitro* brush-border membrane vesicles (BBMV) uptake studies. We analyzed the structure-absorption relationship of amino-β-lactams and realized that D-phenylglycine and structurally similar moieties are common core structures in the molecules of these orally absorbable β-lactam drugs (Figure 2).

Either directly or via an amino acid spacer, D-phenylglycine was chemically attached to α-methyldopa in order to form a series of di- and tripeptide derivatives of α-methyldopa. The transport of these peptides via PEPT1 was determined by measuring the uptake in BBMV prepared from rat intestine. As a result, both carrier-mediated uptake (V_{max}/K_m) and passive diffusion (Kd) were involved in the uptake of the di- and tripeptides.

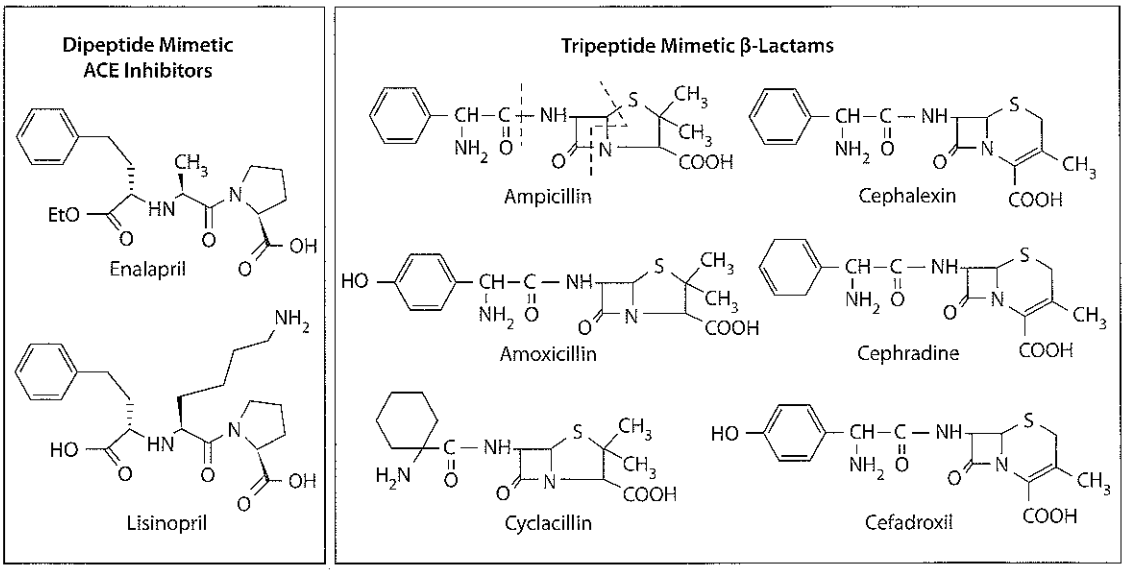

Figure 2 Angiotensin converting enzyme (ACE) inhibitors and amino-β-lactams are substrates of intestinal peptide transporter 1.

Table 1 Kinetic parameters of brush-border membrane vesicles uptake of α-methyldopa derivatives ($n=6$)

$$v = \frac{V_{max}[S]}{K_m + [S]} + K_d[s]$$

Compound	K_m (mM)	V_{max}	V_{max}/K_m	K_d
PhG-Md (a)	0.06±0.13	2.18±0.28	36.4	0.14±0.02
HOPhG-Md (b)	3.52±0.60	3.12±0.19	0.89	0.19±0.01
PhG-ALa-Md (c)	2.24±0.31	0.41±0.08	0.18	0.58±0.02
HOPhG-Pro-Md (d)	2.97±0.65	3.84±0.28	1.29	0
Phe-Md*	–	–	1.9	–

*Data of Phe-Md retrieved from Reference 65. PhG = D-phenylglycine; HOPhG = D-hydroxyphenylglycine; Ala = L-alanine; Pro = L-proline; Phe = L-Phenylalanine-α-methyldopa; Md = α-methyldopa.

Unlike the complicated and inconsistent uptake of α-methyldopa (Figure 3E), D-phenylglycine-α-methyldopa demonstrated a carrier-mediated consistent uptake profile (Figure 3A). The high value of V_{max}/K_m (36.4, representing carrier-mediated uptake) and the low value of Kd (0.14±0.02, representing uptake via passive diffusion) calculated with the Michalis-Menton equation indicated that the majority of D-phenylglycine-α-methyldopa is absorbed via the transporter-mediated process (Table 1).[64,65] Other D-phenylglycine-containing tripeptides also exhibited PEPT1-mediated BBMV uptake, with lower efficacy than dipeptide D-phenylglycine-α-methyldopa (Figures 3B–3D). Moreover, the uptake of D-phenylglycine-α-methyldopa via PEPT1 was more efficient than that of D-phenylalanine-α-methyldopa, an α-methyldopa derivative that utilizes essential amino acids as the delivery tool.[65]

We further synthesized a series of D-phenylglycine-containing di- and tripeptides of L-dopa as dopamine prodrugs with the expectation of improving the inconsistent oral bioavailability associated with L-dopa. In BBMV uptake studies, the dipeptides Gly-Pro, Gly-Phe and the amino-β-lactam cephradine inhibited the uptake of D-phenylglycine-L-dopa, while the amino acids L-phenylalanine and L-dopa did not (Figure 4). D-phenylglycine was successfully proven to be an absorption enhancer for guiding amino acid drugs like α-methyldopa and L-dopa to penetrate through intestinal epithelium via PEPT1 transporter.[66,67] Proof-of-concept PK studies in rabbits indicated that the fraction of absorption of D-phenylglycine-L-dopa reached 83±18%, which is 14 times higher than that of L-dopa.[68] Additionally, the anti-Parkinsonism effect of this dipeptide was significantly higher than that of L-dopa.[69]

7. Transporters and Drug-Drug Interaction

Transporters are important in manipulating drug delivery in biological systems and consequently affecting

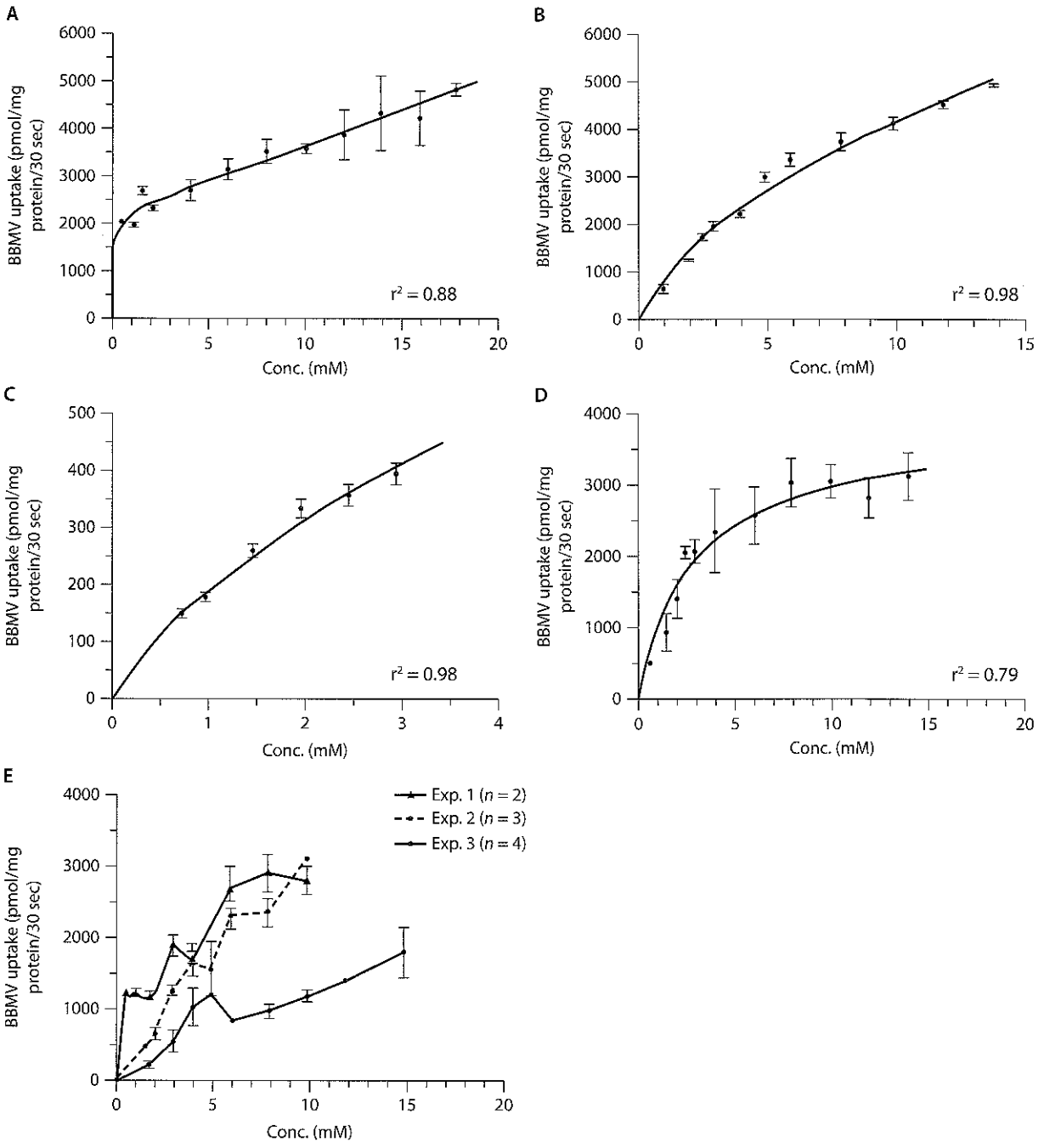

Figure 3 Brush-border membrane vesicles uptake of: (A) D-phenylglycine-α-methyldopa; (B) α-methyldopa-D-phenylglycine; (C) D-phenylglycine-L-serine-α-methyldopa; (D) D-phenylglycine-L-proline-α-methyldopa; (E) α-methyldopa.

their PK profile. The pitfalls of transporter-mediated drug-drug interactions are an important issue with regard to safety concerns.[70–75] For example, renal excretion, primarily driven by transporters, is important in the elimination of xenobiotics such as drugs, food supplements, and herbal medicine.[76] Thus, competition for renal transporters is one of the important factors causing drug-drug interaction. Table 2 summarizes cases of drug-drug interaction resulting from competition for renal transporters.[77–90] Cimetidine and probenecid, for example, were reported to exhibit drug-drug interaction via competition toward renal organic anion transporters (OAT, inhibited by probenecid) and organic cation transporters (OCT, inhibited by cimetidine).[77,78] In addition to previously known OCTs, cimetidine was reported to inhibit MATE-1 (multidrug and toxin extrusion 1).[80,85]

Complicated drug-drug interactions due to alterations in the genetic expression level of transporter proteins have also been reported (Table 3).[91–94] For example, drug-drug interactions resulting from genetic regulation were not observed in acute treatment with amiodarone. Only via repeated administration and longer onset

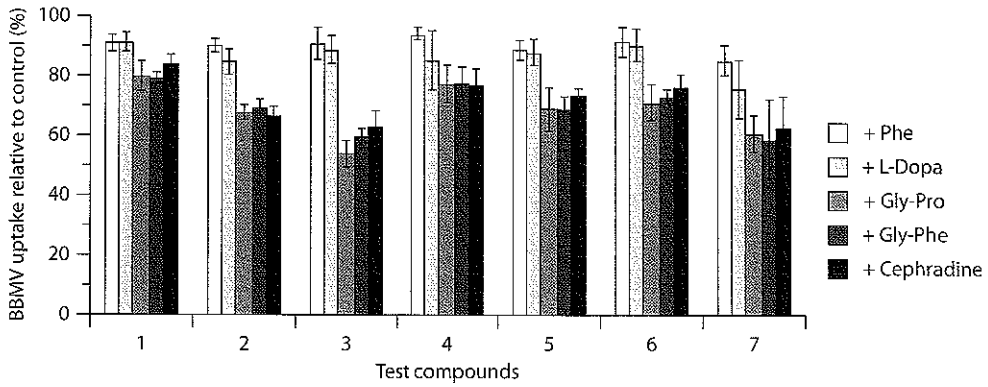

Figure 4 Inhibition of brush-border membrane vesicles uptake of L-Dopa derivatives by amino acids (L-phenylalanine and L-dopa), dipeptides (Gly-Pro, Gly-Phe), and the amino-β-lactam cephradine.

Table 2 Recent cases of drug-drug interaction resulting from competition for renal transporters

Regulator	Drug affected	Transporter involved	Effect	Ref.
Beta-blocker	Metformin	OCT2	Metformin reabsorption inhibited	79
Probenecid	Fexofenadine	OAT3	Renal secretion of fexofenadine inhibited	80
Gemcabene and its glucuronidated metabolite	Quinaprilat	OAT3	Quinaprilat reabsorption inhibited	81
Gemfibrozil and its glucuronide metabolite	Pravastatin	hOAT3	Renal clearance of pravastatin inhibited	82
Ciprofloxacin and gatifloxacin	Carboxyfluoroquinolone	hOAT3	Renal secretion of carboxyfluoroquinolone inhibited	83
Mycophenolate mofetil	Substrate of hOAT1 and hOAT3	hOAT1 and hOAT3	Renal secretion of compounds inhibited by mycophenolate mofetil	84
Cimetidine	Cationic compounds	MATE-1	Secretion of compounds inhibited	85
Cimetidine	Fexofenadine	MATE-1	Secretion of fexofenadine inhibited	80
Proton pump inhibitor	Methotrexate	BCRP	Renal clearance of methotrexate inhibited	86
Luteolin	γ-Hydroxybutyrate	MCT1	Reabsorption of γ-hydroxybutyrate inhibited	87
Clarithromycin	Sirolimus	P-gp	Renal clearance of sirolimus inhibited	88
Probenecid	Gemifloxacin	Unknown	Gemifloxacin secretion inhibited	89
Piperacillin	Flucloxacillin	Unknown	Renal clearance of flucloxacillin inhibited	90

OCT = organic cation transporter isoform 2; (h)OAT3 = (human) organic anion transporter isoform 3; hOAT1 = human organic anion transporter isoform 1; MATE-1 = multidrug and toxin extrusion transporter; BCRP = breast cancer resistance protein; MCT1 = monocarboxylate transporter 1; P-gp = P-glycoprotein.

Table 3 Examples of drug-drug interaction resulting from alterations in genetic expression of renal transporters

Regulator	Transporters	Drug affected	Mechanism	Effect	Ref.
Amiodarone	P-gp and MRP2	–	Expression of P-gp (MDR1) and MRP2 increased	Renal excretion enhanced	91
Insulin	Na^+ ion channel	–	Insulin/dopamine receptor D5 interaction alters Na^+ ion channel expression	Renal reabsorption driven by Na^+ gradient in renal proximal tubule altered	92
Estrogen and progesterone	Amiloride-sensitive epithelial sodium channel	–	Estrogen and progesterone change expression of Na^+ ion channel	Active transport driven by Na^+ gradient in renal proximal tubule altered	93
Curcumin	P-gp	Peroral celiprolol, midazolam	Curcumin decreases P-gp expression	Renal secretion of peroral celiprolol and midazolam decreases	94

P-gp = P-glycoprotein; MRP2 = multidrug resistance-associated protein.

time and duration did drug-drug interaction result with amiodarone, highlighting a potential risk of certain drugs used in chronic disease management.[91] Cloned renal drug transporters have been useful tools for studies on these interactions.[95–98] Transporter-associated evidence of drug-drug interactions also forms the basis for pharmacovigilance evaluation by regulatory agencies such as the US Food and Drug Administration.[99]

8. Conclusion

The biological system is full of mechanisms that manipulate drug behavior. ADME, the mechanisms of drug-host interaction, are thus important and useful sites to optimize drug PD/PK profile as well as to minimize drug toxicity. Additionally, they are wonderful resources for designing new chemical entities as therapeutic agents during the early stages of drug discovery. This review highlights current research in using ADME as a proactive approach for PD/PK optimization. Use of D-phenylglycine as a delivery moiety for guiding α-methyldopa and L-dopa to transport via intestinal PEPT1 transporter was presented as improving the oral bioavailability. This indicates that the ADME mechanism is an interesting target for new drug design.

Acknowledgments

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