Intestinal transporters: enhanced absorption through P-glycoprotein-related drug interactions

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Introduction: There are many factors that can affect the absorption process of orally administered drugs. Intestinal transporters play an important role in drug absorption. These transporters are divided into two major classes: the solute carriers and the ATP-binding cassette (ABC) transporters. P-glycoprotein (P-gp), belonging to the ABC transporter superfamily, flushes out the substrate drugs from a cell, thus regulating the intestinal absorption of drugs.

Areas covered: This review gives a brief overview of uptake and efflux transporters localized in the intestine. However, because P-gp has been identified as an important underlying mechanism of drug interactions in humans, the review is strongly focused on summarizing the currently available data on the impact of P-gp for absorption of drugs.

Expert opinion: The concomitant use of P-gp substrates and inhibitors (preferably in a single nanocarrier formulation) could be an effective and safe way to improve the bioavailability of drugs. It seems the study of P-gp and modulating its activity may be an interesting therapeutic goal to be considered in future research.

Keywords: drug interaction, inhibitor, intestinal absorption, P-glycoprotein, transporter

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1. Introduction

Although oral drug delivery systems have many advantages over systems intended for other routes of drug administration, many drugs cannot be administered orally because of poor and/or highly variable uptake in the systemic circulation. Therefore, nowadays huge amount of research is directed toward modifying physicochemical properties of drugs to increase their gastrointestinal absorption [1]. In summary, there are three categories of factors that affect the rate and extent of oral absorption of drugs. The first category is physicochemical properties of a drug, including solubility, lipophilicity, intestinal permeability, pKa, particle size and so on. Physiological factors, such as gastrointestinal pH, gastric emptying, small intestinal transit time and absorption mechanism, fall into the second category, and finally, the third category comprises dosage form factors, such as solution, capsule, tablet, suspension and so on [2]. Among the physiological factors that impact the intestinal absorption, membrane drug transporter proteins are of great importance in drug disposition in the body. Intestinal transporters are membrane proteins that regulate the transport of molecules into and out of enterocytes. There are numerous classes of transporters, each with different substrate specificity, affinity and capacity, as well as specific tissue and cellular expression patterns. This review aims to represent the role of some important intestinal transporters probably involved in intestinal
bioavailability of oral drugs with focus on P-glycoprotein (P-gp) drug efflux transporter localized at the intestinal barrier. The relevance of possible interactions of drugs and excipients with P-gp and their effect on intestinal absorption of pharmaceutical compounds are presented.

## 2. Solute carrier transporters

According to the Human Genome Organization Gene Nomenclature Committee, two major transporter superfamilies, the ATP-binding cassette (ABC) and solute carrier (SLC) family, are present in the intestinal tissues. SLC transporter genes consist of 52 distinct subfamilies, including proton-dependent oligopeptide transporters (POT, SLC15A), organic anion transporters (OAT, SLC family 21A [SLC21A]), organic cation transporters (OCT, SLC22A), nucleoside transporters (CNT, SLC28A; ENT, SLC29A) and the monocarboxylate transporters (MCT, SLC16A) (Table 1).

<table>
<thead>
<tr>
<th>Article highlights.</th>
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<tr>
<td>Membrane drug transporter proteins are of great importance in drug disposition in the body.</td>
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<tr>
<td>Two major transporter superfamilies, the ATP-binding cassette and solute carrier family, are present in the intestinal tissues.</td>
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<tr>
<td>Recently, fourth-generation P-glycoprotein (P-gp) inhibitors with less toxicity and more potential were introduced.</td>
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<tr>
<td>In the light of numerous evidences indicating the effects of P-gp inhibitors on the pharmacokinetics of substrate drugs, clinicians should be aware of the hazards and advantages of concomitant administration of these two.</td>
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<tr>
<td>Because of their reported P-gp inhibition activities, excipient selection is an important factor to consider in rational formulation design for P-gp substrates.</td>
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This box summarizes the key points contained in the article.

### 2.1 POT family

Nowadays, a wide variety of peptidomimetic drugs is produced on commercial scale and used as therapeutic agents for the treatment of various diseases, including hypertension, AIDS and cancer. An uptake system for di- and tripeptides is present at brush border membrane of intestinal enterocytes, which is also capable of transporting the structurally related drugs. This subfamily, also known as SLC15A, includes peptide transporters 1 and 2, PepT1 (SLC15A1) and PepT2 (SLC15A2); peptide/histidine transporters 1 and 2, PHT1 (SLC15A4) and PHT2 (SLC15A3). However, PepT2 has not been shown to be expressed in GI tract [3]. Among these, PepT1 has captured the most attention in mucosal drug delivery. Its density increases from the duodenum to the ileum and is most abundant at the villus tip cells [4].

### 2.2 Organic anion transporters

According to pH-partition theory, only unionized form of drugs can traverse the intestinal epithelium by passive diffusion. Therefore, transporter systems exist for ionic agents, which could mediate their intestinal absorption. Involvement of specific anion transporters in the intestinal absorption of anionic drugs has been suggested. These transporters are categorized into two main classes: OATs and organic anion transporting polypeptides (OATPs), each having several structurally related isoforms. OATs have a broad range of clinically important substrates such as β-lactam antibiotics, NSAIDs, antiviral drugs and so on. However, due to the distribution pattern of this transporter family, which limits its expression to kidney and liver, and, to a lesser extent, to brain, muscle, eye and placenta, the role of the OAT family in the intestinal absorption of substrate drugs seems to be negligible. In contrast, there are evidences showing that several OATP isoforms are expressed in human small intestine. These genes were previously classified within the SLC21A, but currently, based on their putative phylogenetic relationships and the chronology of identification, they are classified within the OATP/SLCO superfamily [5]. Among them, OATP-B (2B1), OATP-D (3A1), OATP-E (4A1) and OATP1A2 isoforms were identified to be expressed in human intestine [6,7]. Several pharmaceutical compounds such as methotrexate, fexofenadine, digoxin, pravastatin and NSAIDs are substrates of OATP isoforms [6-10]. It was shown that grapefruit juice (GJ) acts as an inhibitor of intestinal OATP2B1. In a study by Tapaninen et al., 61 and 81% reduction in AUC and $C_{\text{max}}$ of aliskiren (an OATP2B1 substrate) were observed with concomitant administration of GJ, respectively [11]. The same reduced oral plasma exposure was shown for fexofenadine in the presence of grapefruit, orange and apple juices [12]. This effect is likely mediated by inhibition of intestinal absorption via OATP1A2. However, a recent study by Imanaga et al. reports that in some conditions, OATP2B1 may also mediate the intestinal uptake of fexofenadine [13]. The similar effects of grapefruit and/or orange juice were also reported for other substrates of OATP1A2 such as celiprolol [14], atenolol [15], talinolol [16] and ciprofloxacin [17].

### 2.3 Organic cation transporters

OCTs are required to enhance the intestinal uptake and absorption of a significant number of cationic drugs, including β blockers, antihistamines, endogenous bioactive amine (e.g., epinephrine, choline, dopamine and guanidine), skeletal muscle relaxants and so on. However, several anionic and uncharged compounds are also substrates of these transporters. For example, transport of anionic prostaglandins $E_2$ and $F_2$ is mediated by human OCT1 and OCT2 [18]. Although the other member of this family, OCT3, shows overlap in substrate and inhibitor specificity with OCT1-2 isoforms, their affinity and maximum transport rate are distinctly different [19,20]. Moreover, three more novel OCTs were identified,
the brush border membrane consists of three Na+-dependent CNTs (ENT, SLC29). The human SLC28 family located in concentrative CNT, SLC28 and the low-affinity equilibrative intestinal absorption of nucleoside analogues: the high-affinity transporters.

### 2.3 Organic cation transporters (OCT)

Two distinct families of transporter proteins mediate the intestinal absorption of organic cations: the high-affinity OCTs (OCT1, OCT2, OCT3, OCTN1-3). The members of this subfamily have different abilities to interact with organic cationic drugs and carnitine. Human OCTN1 work as H+/organic cation antiporter, whereas OCTN2 is a Na+/carnitine cotransporter with a high affinity to carnitine. OCTN2 maintains the carnitine homeostasis. Carnitine is essential for entry of long-chain fatty acids into mitochondria, which turns fat into energy. It also transports the generated toxic and waste compounds out of mitochondria to prevent their accumulation. Both OCTN1 and OCTN2 proteins are expressed in human small intestinal enterocytes. Human OCTN2 can also operate as polyspecific, Na+-independent OCT, which transports cations in both directions across the plasma membrane [21].

### 2.4 Nucleoside transporters (SLC28A, CNT; SLC29A, ENT)

Two distinct families of transporter proteins mediate the intestinal absorption of nucleoside analogues: the high-affinity concentrative CNT, SLC28 and the low-affinity equilibrative CNTs (ENT, SLC29). The human SLC28 family located in the brush border membrane consists of three Na+-dependent isoforms, SLC28A1 (CNT1), SLC28A2 (CNT2) and SLC28A3 (CNT3). On the other hand, in the basolateral membrane of absorptive epithelia, an Na+-independent family of CNTs is located, which contains four members, ENT1 (SLC29A1), ENT2 (SLC29A2), ENT3 (SLC29A3) and ENT4 (SLC29A4). This family was distinguished from the ENTs by their facilitated diffusion transport mechanism [19]. Nucleosides are glycosylamines consisting of a nucleobase bound to a ribose or deoxyribose sugar via a β-glycosidic linkage. In addition to their biological importance, their importance to cellular physiology and function is fundamental. In medicine, several nucleoside analogues are used as antiviral or anticancer agents. Zidovudin, lamivudine, cytarabine, gemcitabine, ribavirin and 5-flourouracil are examples of drugs for which interactions with CNTs have been extensively documented [22]. A novel member of the ENT family (SLC29) has been identified. Unlike the other ENT members, the novel transporter, plasma membrane monoamine transporter (PMAT), specifically transports monoamines, such as serotonin, dopamine and norepinephrine. However, its substrate and inhibitor specificity extensively overlaps with those of OCTs. PMAT is also known to play a role in drug transport at the intestine [18,20].

### 2.5 Monocarboxylate transporters (SLC16A: MCT)

The members of this family are proton-dependent transporter proteins responsible for symport of monocarboxylates. To date, 14 members (MCT1-MCT14) of this family with distinct transport properties and tissue distribution have been identified [23,24]. However, only the first four isoforms (MCT1-4) have been demonstrated to facilitate proton-linked transport of metabolically important monocarboxylates such as lactate, pyruvate and ketone bodies [25,26]. A wide range of endogenous and exogenous compounds, including butyrate, acetate, propionate, 3-hydroxybutyric acid [25], pravastatin, simvastatin, atorvastatin, XP13512, carindacillin, some β lactam antibiotics, penicillins, NSAIDs, valproic acid and nateglinide, are also substrates for MCTs [27-32]. Two isoforms in the MCT family, MCT8 and MCT10, exhibit proton-independent transport of substrates. MCT10 (SLC16A10; TAT1) transports aromatic amino acids, whereas MCT8 functions as thyroid hormones, T3 and T4 transporter. However, the role of the other MCT family members remains unknown [33,34]. Previous studies have demonstrated the expression and membrane localization of MCTs along the length of the human GI tract [35,36]. According to the results of those experiments, MCT1, 4 and 5 are the

### Table 1. Major SLC transporters expressed in human small intestine.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Protein</th>
<th>Mechanism</th>
<th>Cellular localization</th>
<th>Examples of drug substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC15A1 (SLC15 family)</td>
<td>PEPT1</td>
<td>H⁺/peptide symporter</td>
<td>A</td>
<td>Cefadroxil, ampicillin, amoxicillin, bestatin, cefaclor, emocaprilat, cefixime, enalapril, temocapril, midodrine, valganciclovir, valacyclovir</td>
</tr>
<tr>
<td>SLC22A1 (SLC22 family)</td>
<td>OCT1</td>
<td>OC uniporter</td>
<td>B</td>
<td>Quinidine, acyclovir, ganciclovir, metformin, quinine, cimetidine, zidovudine</td>
</tr>
<tr>
<td>SLC22A4 (SLC22 family)</td>
<td>OCTN1</td>
<td>H⁺ or OC antiporter</td>
<td>A</td>
<td>Ergothioneine, mepyramine, quinidine, gabapentin, verapamil</td>
</tr>
<tr>
<td>SLC22A5 (SLC22 family)</td>
<td>OCTN2</td>
<td>OC antiporter Na⁺ symporter (carnitine)</td>
<td>B</td>
<td>Verapamil, mepyramine, emetine, quinidine, valproate, cephaloridine</td>
</tr>
<tr>
<td>SLC01A2 (SLCO family)</td>
<td>OATP1A2</td>
<td>OA antiporter</td>
<td>A</td>
<td>Enalapril, fexofenadine, indomethacin, levofloxacin, ouabain, rocuronium, temocapril, rosuvastatin, pitavastatin, methotrexate, imatinib, saquinavir</td>
</tr>
<tr>
<td>SLC02B1 (SLCO family)</td>
<td>OATP2B1</td>
<td>OA antiporter</td>
<td>A</td>
<td>Pravastatin, benzylpenicillin, atorvastatin, pitavastatin, fluvastatin, bosentan, glibenclamide, rosuvastatin</td>
</tr>
</tbody>
</table>

A: Apical; B: Basolateral; OAT: Organic anion transporters; OCT: Organic cation transporters; SLC: Solute carriers.
predominant isoforms expressed in intestine with maximal expression in distal colonic regions. It was also shown that there are differences in preferential localization of these transporters in such a way that MCT1 is confined to apical membranes, whereas MCT4 and 5 are restricted to basolateral membranes of colonocytes. In contrast to previous data suggesting that MCT3 expression is restricted to retinal epithelium [37,38], nowadays there are data supporting its expression in human GI tract at a very low level. Expressions of MCT2 and 6 were not observed in the human intestine [35].

3. The ATP-binding cassette transporter

The ABC genes represent the largest family of transmembrane proteins. This family is one of the five distinct families of membrane protein characterized by Saier et al. [39]. The four others are multidrug and toxic compound efflux transporters, major facilitator superfamily transporters, resistance nodulation division transporters and small multidrug resistance transporters [40]. ABC proteins bind ATP and use the energy to drive transport of a wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids and steroids, inorganic anions, metal ions, peptides, amino acids, sugars and drugs [41,42]. It is present in all prokaryotes, as well as plants, fungi, yeast and animals. The human genome contains 51 known human ABC transporters arranged in seven distinct subfamilies of proteins [43-45]. A list of all known human ABC genes subfamilies is displayed in Table 2 [41,42].

<table>
<thead>
<tr>
<th>Name/symbol</th>
<th>No. of members</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subfamily A (ABC1)</td>
<td>14</td>
</tr>
<tr>
<td>Subfamily B (MDR/TAP)</td>
<td>11</td>
</tr>
<tr>
<td>Subfamily C (CFTR/MRP)</td>
<td>13</td>
</tr>
<tr>
<td>Subfamily D (ALD)</td>
<td>4</td>
</tr>
<tr>
<td>Subfamily E (OABP)</td>
<td>1</td>
</tr>
<tr>
<td>Subfamily F (GCFN20)</td>
<td>3</td>
</tr>
<tr>
<td>Subfamily G (white)</td>
<td>5</td>
</tr>
</tbody>
</table>

ABC: ATP-binding cassette; MRP: Multidrug resistance associated proteins.

P-gp (ABCB1)

3.1 P-glycoprotein (ABCB1)

The ATP-binding cassette transporter

This protein is responsible for encoding P-gp (commonly known as MDR1) belongs to the ABCB family of ABC transporters [48]. This gene is expressed in membrane of many cells, including cells in the kidney, liver, lungs, adrenal gland, colon, brain, placental syncytiotrophoblasts and intestine. Some mononuclear cells in peripheral blood such as neutral killer cells and T lymphocytes and also human hematopoietic stem cells express P-gp as well. Additionally, a low-level of P-gp expression was detected in prostate, skeletal muscle, heart, spleen, stomach, skin and ovary [49]. P-gp detoxifies cells by removing hundreds of chemically unrelated toxins and metabolites from the cells. But this gate keeper protein has been demonstrated to possess broader substrate specificity. It preferentially transports hydrophobic, amphipathic molecules ranging in size from 100 to 4000 Da. However, some neutral compounds such as cyclosporine A and digoxin, negatively charged molecules such as atorvastine and fexofenadine, and some hydrophilic drugs such as methotrexate are also substrates of P-gp. This substrate promiscuity is a distinctive feature of P-gp describing the importance of polyspecific drug binding for logical drug design. However, the CYP3A subfamily, the most abundant cytochrome P450 enzymes expressed in the intestine, may be functionally linked to P-gp [50,51]. It is possible that P-gp may influence first-pass metabolism in a complex cooperative manner. They have overlapping substrate specificity of inhibitors and inducers. It appears that P-gp prevents the saturation of CYP3A4 in the enterocytes by limiting the total drug transport across the membrane. This could lead to increased duration of exposure of the drug to the enzyme, providing greater extent of...
metabolism. Moreover, the generated metabolites are substrates for P-gp and are actively transported out of the enterocytes by P-gp. Therefore, the metabolites do not compete with the metabolism of parent drug [52].

3.1.2 P-gp mechanism of action

A conceptual model proposed for P-gp–drug interaction is the hydrophobic vacuum cleaner model. Based on this model, drugs partition into the inner leaflet of the membrane and are then flipped to the outer leaflet in a nonspecific manner [40]. From the molecular point of view, this transport process involves a number of key steps comprising substrate binding, binding site reorientation, energy supplying for transport, coupling substrate binding and energy supplying, substrate dissociation, and resetting the membrane transport pump [53]. However, the unifying mechanism of these steps remains unclear. On the other hand, there are difficulties in attaining complete structural information of human P-gp. Because human and mouse P-gp share nearly 87% protein sequence identity, the reported X-ray structure of mouse P-gp could also help to understand its conformational changes during transport process in human. For instance, previous studies based on crystal structure and crosslinking analysis have suggested that membrane P-gp is in the closed conformation in the presence or absence of ATP. Additionally, the closed conformation has a high affinity for drug substrates. Indeed, it is improbable that under normal physiological conditions P-gp adopt open conformation because the ATP concentration inside the cell (3–5 mM) is much more than the P-gp affinity for ATP (km = 0.5 mM) [54]. It seems the ‘ATP-driven switch’ mechanism, in which nucleotide-driven interaction of the nucleotide-binding domains leads to resetting of the membrane-bound domains and reduces drug affinity, may be reflected in the inward- and outward-facing structure of P-gp [48]. The interior of P-gp ‘binding pocket’ is lined with a variety of amino acids, including hydrophobic amino acids and those containing aromatic compounds. This variation of residues inside the pocket explains the ability of mammalian P-gp to accommodate a wide range of substrates.

Understanding P-gp structure and knowing where substrates bind may help scientists design more effective drugs. That means only redesigning of existing drugs and modifying portions of the drugs (instead of designing new drugs) could make them work better. However, predicting whether a ligand will be substrate or inhibitor remains challenging. Bearing in mind the potential for co-administration of pharmaceutical compounds of both kinds in clinical practice, it is of great importance to elucidate the possibility of P-gp-mediated drug–drug interaction. Given that the majority of drugs are developed for oral delivery, the following sections, therefore, will focus to a large degree on the role of P-gp in intestinal drug absorption. In human intestine, P-gp is highly expressed on the brush-border membrane of superficial columnar epithelial cells of the ileum and colon, and its expression gradually decreases proximally into the jejunum, duodenum, and stomach [55–57]. Generally, P-gp inhibition occurs by three mechanisms: i) blocking drug-binding site either competitively, noncompetitively or allosterically; ii) interfering with ATP hydrolysis; and iii) altering integrity of cell membrane lipids [58,59]. For example, cyclosporin A and verapamil are competitive inhibitors of P-gp, whereas cis-(Z)-flupentixol, a thioxanthene derivative, inhibits the P-gp-mediated drug transport through an allosteric mechanism. That means it prevents substrate translocation and dissociation, leading to stable but reversible P-gp-substrate complex [60]. For anthranilic acid derivative, tariquidar (XR9576), an allosteric effect on substrate recognition or ATP hydrolysis was demonstrated [61]. Inhibition of ATP hydrolysis was also reported for vanadate. In this case, a stable transition complex of P-gp/vanadate/ADP is formed. However, XR9576 binds to the TMDs of P-gp. Finally, P-gp inhibitor surfactants are considered to act on secondary and tertiary structure, resulting in altering integrity of membrane lipids [58]. However, for many others, the inhibitory mechanisms are yet to be fully understood.

**Figure 1. Structure of P-gp efflux pump.**

P-gp: P-glycoprotein; TMDs: Transmembrane domains.
design of peptidomimetics and dual-activity ligands emerged. Using natural products and their derivatives and the fluconazole, with high affinity to P-gp compared with previous generations were introduced. The potency of this generation appeared to be about 10-fold more than the first- and second-generation inhibitors. In a study by Novak et al., digoxin intestinal absorption was assessed when co-administered with acetaminophen. They reported > 200% increase in digoxin concentration in portal vein compared with digoxin alone. It was demonstrated that apical-to-basolateral transport of acetaminophen was not affected by verapamil, a typical P-gp inhibitor [66]. On the other hand, acetaminophen accumulation was the same in P-gp knock-down and wild-type HepG2 cells [66]. These findings suggested that acetaminophen was not a P-gp substrate and there was no possibility of competitive P-gp inhibition. An in situ single-pass intestinal perfusion study in rats indicated phenytoin to be a P-gp substrate [67]. To complete the study, phenytoin was administered orally to male rats in the presence and absence of verapamil. The obtained results showed an elevated mean AUC and mean maximum plasma concentration of drug when co-administered with classical P-gp inhibitor, verapamil [67]. In a similar study using single-pass intestinal perfusion technique in rats, the elevated effective intestinal permeability of cyclosporine was reported in the presence of clarithromycin and erythromycin as efflux pump inhibitors [68,69]. In another study involving the same inhibitors, the effective intestinal permeability of digoxin as a P-gp substrate was explored in rats [70,71]. Again the macrolides enhanced intestinal transport of digoxin significantly as a function of P-gp efflux transporter inhibition. In another study, applicability of rhamnolipids as absorption enhancers in Caco-2 cells was investigated. Rhamnolipids were demonstrated to inhibit P-gp activity and reduce efflux ratio (basolateral-to-apical/apical-to-basolateral) of rhodamine 123, a P-gp substrate [72]. Herbal medicines are more and more often used in recent years to enhance well-being and treat disease, including anxiety, arthritis, depression, high blood pressure, insomnia, hormonal imbalances, migraines and so on. Although so much is still unknown and speculative, nowadays several studies are centered on the potential interaction of plant-derived products with concomitantly used drugs. For instance, Oga et al. have shown that digoxin absorptive transport in Caco-2 cells was significantly enhanced in concurrent administration with Vernonia amygdalina, Carica papaya and Vernonia amygdalina, phytomedicines commonly used during malaria therapy [73]. Another well-known example is GJ. Wide consumption of GJ is attributed to its effects in reducing atherosclerotic plaque formation and inhibiting breast cancer cell proliferation and mammary cell tumorigenesis. In 1989, researchers found that GJ can significantly increase oral bioavailability of drugs. This was achieved from an interaction study of felodipine and alcohol in which GJ was used as a flavor to mask the taste of the ethanol [74]. GJ was also reported to be a potent inhibitor of P-gp-mediated colchicine [75], talinolol [76], and digoxin [77] transport in in vitro systems. In another study, the effects of furanocoumarin derivatives in GJ on the uptake of vinblastine by Caco-2 cells were examined. All GJ constituents were able to decrease the P-gp-mediated vinblastine efflux at concentrations ranging from 0.1 to 20 μM [77,78].

**Table 3. List of some P-gp inhibitors belonging to three known generations.**

<table>
<thead>
<tr>
<th>First generation</th>
<th>Second generation</th>
<th>Third generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>Dexverapamil</td>
<td>Tarquidar (XR9576)</td>
</tr>
<tr>
<td>Reserpine</td>
<td>Gallopamil</td>
<td>KR30031</td>
</tr>
<tr>
<td>Progesterone</td>
<td>PSC 833</td>
<td>Cyclopropylidenosuberen</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>(Valsaopodar)</td>
<td>Zosuquidar (LY335979)</td>
</tr>
<tr>
<td>Felodipine</td>
<td>VX-710</td>
<td>Laniquidar (R101933)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>(Biricodar)</td>
<td>Substituted diarylimidazole</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>MS-209</td>
<td>ONT-093</td>
</tr>
<tr>
<td>Flufenazine</td>
<td>GF 120918</td>
<td></td>
</tr>
<tr>
<td>Diltazem</td>
<td>(Elacridar)</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Reversin 121</td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>Reversin 125</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin A</td>
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</tbody>
</table>

3.1.3 P-gp inhibitors and their pharmacokinetic advantages

Based on the specificity and affinity, there are three main generations of P-gp inhibitors. The first-generation inhibitors are pharmacologically active substances with clinical use for other indications, which have been shown to inhibit P-gp as well. Verapamil, a calcium channel blocker, is a typical example of this class [62]. Using first-generation P-gp inhibitors could cause pharmacological side effects and toxicity. Moreover, designing and developing such combination pharmaceutical products requires long time. Therefore, the second-generation modulators were presented, which are more specific with less side effect inhibitors, such as dexverapamil or dextepidipine. However, complicated unfavorable drug–drug interactions as a result of inhibition of two or more ABC transporters by second-generation inhibitors limit their clinical use. A third generation of P-gp inhibitors comprised compounds such as tarquidar, with high affinity to P-gp at nanomolar concentrations. The potency of this generation appeared to be about 10-fold more than the first- and second-generation inhibitors. Recently, fourth-generation P-gp inhibitors with less toxicity and more potential at concentrations that can modulate P-gp compared with previous generations were introduced. Using natural products and their derivatives and the design of peptidomimetics and dual-activity ligands emerged as the fourth generation [63,64]. Table 3 shows some examples of P-gp efflux pumps inhibitors belonging to three known generations.

Various studies on the regulation of P-gp by its inhibitors have been reported. For example, in an attempt to improve the oral bioavailability of paclitaxel, Woo et al. have reported that apical-to-basolateral permeation of paclitaxel in Caco-2 monolayers was increased in the presence of KR-30031, a verapamil analogue with fewer cardiovascular effects. The ability of KR-30031 to reduce this efflux transport is equal to that of verapamil, a well-known P-gp inhibitor [65].
Cyclosporine is also a well-known P-gp substrate. It is also a substrate of CYP3A4. However, based on the study conducted by Lown et al., P-gp may be a more important determinant of cyclosporine absorption than enteric CYP3A [79]. On the other hand, there is considerable evidence indicating that GJ has minimal effect on P-gp in vivo [77,80-83]. Because GJ phytochemicals can also alter the activity of enzymes (esterases, sulfotransferases) and transporters other than P-gp (OATPs) in the body, therefore when the levels of a P-gp substrate drug are increased with GJ administration, it is difficult to explain the reason. For clopidogrel, an antiplatelet agent with poor aqueous solubility, which is a P-gp efflux pump substrate, the natural compound naringin showed more than threefold absorption enhancement in everted gut sac model [84]. In that study, quinidine was also proved to increase clopidogrel absorption by P-gp inhibition. It should be noted, however, that long-term use of GJ may cause P-gp induction, leading to reduction in therapeutic plasma levels of certain drugs. The probable reason is the interaction of GJ components with aryl hydrocarbon receptor that upregulate the P-gp expression [85].

It was demonstrated that the activity of P-gp in Caco-2 cells could also be affected by the extracts of bitter melon, soybean, dokudami and Welsh onion, bitter melon being the most potent inhibitor [86].

1-Monopalmitin, a simple monoglyceride with a great amount of fatty acid chain, was found to be the major inhibitor of P-gp in bitter melon [86,87]. This study was followed by Barta et al., who analyzed the effects of two common monoglyceride components of various lipid excipients, 1-monoolein and 1-monostearin, on the activity and expression of P-gp in Caco-2 cells. The results suggested that these two monoglycerides are inhibitors of P-gp [1]. Herbal products mistletoe, Natto K2, Agaricus and green tea also showed inhibitory effect on P-gp in vitro. These products enhanced digoxin transport in differentiated and polarized Caco-2 cells [88]. St John’s wort (Hypericum perforatum) is one of the most commonly used herbal antidepressants. It was recognized as a potent P-gp inducer, and according to the available human data it was able to decrease the blood concentrations of amitriptyline, cyclosporine, digoxin, fexofenadine, indinavir, methadone, midazolam, nevirapine, phenprocoumon, simvastatin, tacrolimus, theophylline and warfarin. Therefore, co-administration of drugs with St John’s wort and their possible interaction would be a major safety concern [89,90].

A study by Fortuna and colleagues demonstrated the possible active efflux mediated by P-gp of a series of dibenz (b,f) azepine-5-carboxamide derivatives. They found that after addition of verapamil the mucosal-to-serosal permeability of oxcarbazepine, R-licarbazepine, BIA 2-024 and carbamazepine-10,11-epoxide, lowered, whereas serosal-to-mucosal permeability increased, suggesting the involvement of P-gp on the transport of those compounds. Carbamazepine itself was not shown to be a P-gp substrate. This study was performed using freshly excised mouse jejunal segments mounted in Ussing chambers [91].

Pharmacoresistance of antiepileptic drugs in the treatment of epilepsy and psychiatric disorders and their transport by P-gp were also investigated by Zhang et al. using polarized cell lines MDCKII and LLC transfected with the human MDR1 gene [92].

Many factors can alter P-gp-based drug interactions. One of these factors is genetic differences of P-gp. Polymorphism at exon 26 (C3435) has been shown to influence the level of intestinal P-gp and consequently the amount of substrate drug absorption. It was also suggested that the effect of genetic variants might be substrate specific [93]. However, more research is needed to confirm this finding. Because P-gp kinetic is saturable, the relative contribution of intestinal P-gp to overall drug absorption is important only when a very small oral dose is given (e.g., digoxin, cyclosporine A, talinolol), or the dissolution and diffusion rates of the drug are very slow [94-97]. Typically for poor soluble drugs, solubilizing agents or excipients are included in the experiment medium. This leads to better solubility and concentration gradient, which could promote drug absorption. On the other hand, nano-sized formulations providing larger surface area for better dissolution and permeation across biological membranes are considered as promising tools for drug delivery. Recently, micro/nano-sized self-emulsifying systems were recognized as effective approach for improvement of drug pharmacokinetic behavior. These systems are isotropic mixtures of oil, surfactant, co-surfactant and drug capable of forming fine o/w nanoemulsion when introduced into gastrointestinal medium. The drug is delivered in the dissolved state inside the emulsion droplets, which can be easily absorbed. On the other hand, some of these lipids and surfactants have established P-gp modulation activity. Hence, self-emulsifying systems provide a good opportunity for enhanced oral bioavailability of the substrate molecules by P-gp modulation as well. β-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS or vitamin E TPGS), a novel nonionic surfactant, has been extensively used in drug delivery systems, including self-emulsifying systems [98,99]. There are several studies reporting its P-gp inhibition and absorption enhancement effects [100-102]. This effect was found to appear below TPGS critical micellization concentration of 0.20% (w/w) [103]. The micellar property for TPGS is provided by hydrophilic head of PEG 1000, which shows the most P-gp inhibition effect. The ability of P-gp inhibition was also seen in other nonionic surfactants such as Tween 80, Pluronic E8100, Pluronic E6100 and Cremophor EL. However, TPGS was the most effective inhibitor [98]. Recently, the effects of Tween excipients on the activity and expression of P-gp were investigated in our laboratory. The results indicated that Tween 20 at concentration of 0.01% (w/v) and Tween 40 at concentration of 0.05% (w/v) were able to block P-gp efflux pump significantly [104]. In another study by Yu et al., Brij35, another pharmaceutical excipient, was introduced as a P-gp...
inhibitor and potential permeation enhancer of bis(12)-hupyrndone (105). This effect was seen at concentrations lower than its critical micelle concentration. It seems hydrophilic-lipophilic balance (HLB values) of excipients could play a role in their multidrug-resistance-reversing effects. This issue was examined for a series of surfactant excipients in both Caco-2 cell line and the everted gut sacs of rat intestine (106).

The optimal transport enhancement effect on the epirubicin uptake (as a P-gp substrate drug) was characteristic of surfactants with intermediate HLB values ranging from 10 to 17. Furthermore, the effects of sodium deoxycholate and sodium caprate on the transport of epirubicin were examined using the same protocol (107). The study suggested that these two absorption enhancers could be used as P-gp modulators in drug formulations. For tacrolimus self-microemulsifying drug delivery system, developed by Wang et al., TPGS and Cremophor EL40 were chosen as P-gp inhibitors. This immunosuppressant drug exhibited sevenfold and eightfold increase in drug absorption compared with drug simple solution administered to the rats (108). TPGS was also used in novel multifunctional TPGS/PLGA nanoparticles (109). The novel nanoparticles improved the uptake of the loaded drug (SN-38) by novel mechanisms, including clathrin-mediated endocytosis of unbroken nanoparticles and at the same time escaping from the recognition of P-gp. The enhancing effects of Pluronic F68 and Labrasol on the intestinal absorption and pharmacokinetics of rifampicin were also investigated by an in situ single-pass perfusion method (110). The results of a study conducted by Dünnhaupt and colleagues on Caco-2 cells approved that S-protected thiolated chitosan is promising excipient for drug delivery systems providing improved permeation-enhancing and P-gp inhibition effects (111).

Phospholipids (e.g., phosphatidylcholine) are extensively used as excipients for poorly soluble drugs formulations. According to Simon et al., they were proven to be able to significantly inhibit P-gp in vitro (112). However, this capability needs to be further elucidated in vivo.

Irinotecan, a potent anticancer drug, has no oral formulation available so far. The reason is its high P-gp induced efflux, which results in very low oral bioavailability. Although there have been some studies addressing the issue, most recently its SMEDDS formulation was developed with excipients having P-gp modulation activity and examined further by in vitro, ex vivo and in vivo methods (113). The optimized formulation was found to be beneficial in enhancing the oral accessibility of P-gp substrate drug, irinotecan. Moreover, the inhibitory effects of polyethylene glycols for the intestinal P-gp function were assessed in previous studies (114,115). The findings revealed that glycols with different molecular weights and their derivatives are useful excipients to inhibit the function of P-gp in the intestine. Finally, as a new insight into the world of pharmaceutical excipients, acrylic copolymers (Eudragits®) have also been discovered to have potential P-gp inhibition effects when tested on Caco-2 cells (116). However, further work is required regarding a better understanding of the role of Eudragits® in drug absorption in intestine.

4. Conclusion

In the light of numerous evidences indicating the effects of P-gp inhibitors on the pharmacokinetics of substrate drugs, especially their intestinal absorption, which was reviewed in this paper, clinicians should be aware of the potential for these interactions and patients need to be educated about the hazards and advantages of concomitant administration of these two. Pharmacists must also take responsibility for monitoring drug interactions and notifying the physician and patient about potential problems. For the purpose of extrapolating the in vitro results to the usual clinical setting, substrate and inhibitor exposure manner must be consistent with the real amounts that are administered by the patients.

5. Expert opinion

SLC and ABC transporters expressed in the intestine are increasingly being recognized as significant determinants of drug absorption and drug–drug interaction. There are many examples in literature indicating the effect of the structure and function of transporter proteins, much remains undetermined regarding their molecular mechanisms, regulation, and structure-function relationships. On the other hand, there are also extensive data supporting the modulation of drug bioavailability through P-gp regulation. This regulation could occur by co-administered drugs or by components in food groups. Using P-gp substrates, prodrugs could be considered as a new strategy to bypass the efflux process. However, most of the available data have derived from in vitro systems, studying P-gp inhibition in isolation. Although these studies are valuable, they may not indicate the clinically important improvements in in vivo bioavailability of substrate drugs. On the other hand, P-gp expression and activity is dependent on individual’s genetic makeup, age, disease state, and so on. Therefore, to obtain a valid conclusions and better understanding of the role of P-gp in drug interactions, more work is required. Moreover, for the purpose of extrapolating in vitro results to the human situation in vivo, the drug substrate and also inhibitor concentrations have to be selected carefully. If the P-gp inhibition occurs at doses larger than what is used normally, in this case intolerable side effects by the P-gp inhibitor can occur. We should also consider the nonspecific action of P-gp inhibitors on P-gp and their nonselective distribution to nontarget organs. This may result in complication in their clinical use. Therefore, continued development of more specific inhibiting agents.
may be of great interest in future works. More studies on the exact mechanism of the suppressing effect of P-gp modulators are also needed. The probable mechanisms are alteration of plasma membrane fluidity, ATPase activity, changing surface P-gp level and intracellular ATP level.

A detailed study of any conformational changes in inhibition process will reveal important data on the molecular events responsible for this communication. This kind of knowledge will benefit the rational design of P-gp inhibitors with higher efficacy and lower toxicity.

Nanocarriers (such as liposomes, niosomes, polymeric nanoparticles, dendrimers and polymer–drug conjugates) are promising drug delivery systems capable of P-gp inhibition. Although some research articles have been published about nanomedicines' P-gp inhibition effect, many challenges remain. Future works should focus on the biological mechanisms by which these nanomedicines overcome P-gp. It seems performing in vivo studies is crucial to the development of more efficacious nanoparticles drug delivery systems with the purpose of P-gp inhibition. In addition, because of the lack of enough high-quality three-dimensional structural information about P-gp, designing molecules that circumvent P-gp efflux is still hampered. Therefore, only a small number of structure-based prediction models have been developed in recent years.

**Declaration of interest**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

**Bibliography**

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.


**This article provides excellent background information on the subject.**


23. Halestrap AP, Meredith D. The SLC16 gene family from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch 2004;447(5):619-28


27. Morris ME, Felmlee MA. Overview of the proton-coupled MCT (SLC16A) family of transporters: characterization, function and role in the transport of the drug of abuse gamma-hydroxybutyric acid. AAPS J 2008;10(2):311-21


45. Sharam FJ. ABC multidrug transporters: structure, function and role in chemoresistance. Pharmacogenomics 2008;9(1):105-27


Intestinal transporters


54. Leo TW, Bartlett MC, Clarke DM. Human P-glycoprotein is active when the two halves are clamped together in the closed conformation. Biochem Biophys Res Commun 2010;395(3):436-40


58. Amin ML. P-glycoprotein Inhibition for P-gp inhibition. Drug Target Insights 2013;7:27-34


** An important study providing the usefulness of lipid-based formulations for P-gp inhibition.**


85. Lim GE, Li T,Buttar HS. Interactions of grapefruit juice and cardiovascular
88. Engdal S, Nilsen OG. Inhibition of P-glycoprotein in Caco-2 cells: effects of herbal remedies frequently used by cancer patients. Xenobiota 2008;38(6):559-73
117. Very new work reporting the P-gp inhibitory effect of Eudragit®.
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