Drug Metab Drug Interact 2010;25(1-4):3–16 © 2010 by Walter de Gruyter • Berlin • New York. DOI 10.1515/DMDI.2010.006

Review

P-Glycoprotein- and cytochrome P-450-mediated herbal drug interactions

Yamsani Shravan Kumar, Devandla Adukondalu, Dharani Sathish, Yamsani Vamshi Vishnu, Gannu Ramesh, Athukuri Bharagavi Latha, Palem Chinna Reddy, Manda Sarangapani and Yamsani Madhusudan Rao*

Centre for Biopharmaceutics and Pharmacokinetics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, AP, India

Abstract

P-Glycoprotein (P-gp), the most extensively studied ATP-binding cassette transporter, functions as a biological barrier by extruding toxic substances and xenobiotics out of cells. Drug efflux pumps such as P-gp play a functional role in determining the pharmacokinetics of drugs administered by oral and parenteral routes. Determining the activity of drug efflux transport proteins has important implications in the identification of substrates and/or inhibitors. The significant role of the small intestine in reducing the oral bioavailability of drugs is due to metabolic enzymes and efflux transporters. The role of cytochrome P-450 3A (CYP3A) and P-gp in intestinal drug disposition has been highlighted. This review examines the structure, localisation and functional role of P-gp, the mechanism of drug efflux and drug-herb interactions.

Keywords: cytochrome P-450 3A; inhibitors; in vitro; in vivo; P-glycoprotein.

Introduction

Oral administration has been the most common route for drug delivery for decades. The obvious advantages of oral dosage forms are high patient compliance and a simple, cost-efficient manufacturing process. Besides the development of formulations for newly developed drugs, optimisation of dosage forms for well-established drugs is another goal in the design of new oral drug products. Such optimisation can include an improvement in bioavailability, or a decrease in side effects or administration frequency. In general, the design of new dosage forms should be based on the pharmacokinetic and pharmacodynamic properties of a drug (1). Information should be provided on the absorption mechanism, which can be responsible for insufficient bioavailability. Passive diffusion through biological membranes has been addressed as one important mechanism in drug absorption. Besides passive absorption, active transport processes mediated by transporters and pumps play an important role in the absorption of nutrients from the intestine into the circulation. This may also be applicable for the absorption of drugs that show affinity to intestinal transporters.

A considerable number of transporters may increase the absorption of drugs, in particular compounds for which passive diffusion is too low to be of relevance because of large molecular weight or a low partition coefficient. Moreover, a number of transporters facilitate transport in the opposite direction by secreting drug molecules from the interior of enterocytes back into the intestinal lumen (2). This phenomenon is referred to as intestinal drug efflux. The best-known efflux pump in the human intestine is P-glycoprotein (P-gp). This transporter was originally studied because of its high expression in cancer cells, where it acts as a mediator for multi-drug resistance (MDR). Two clinically relevant aspects should be considered for drugs that are substrates of P-gp. On the one hand, intestinal secretion may result in decreased bioavailability, and on the other hand, the transporters mediating intestinal drug efflux are saturable, which can result in dose-dependent, non-linear absorption. Such phenomena should be addressed in the design of oral dosage forms for P-gp substrates.

The concept of poor drug absorption due to intestinal metabolism was originally not considered clinically significant. Later, Watkins and co-workers were the first to report that a major cytochrome P-450 enzyme system, CYP3A, is relatively abundant in the intestinal mucosa (3, 4) and the substrates for this enzyme may have poor oral bioavailability owing to extensive first-pass metabolism in the intestine (5). The high levels of CYP3A in the intestine becomes of even greater importance when it is recognised that more than 50% of human drugs may be substrates for this enzyme.

It has been demonstrated that P-gp-mediated substance transport is modified not only by other P-gp substrates, but also by CYP3A-related compounds (6). Because there are some overlapping substrate specificities between CYP3A4 and P-gp. Benet and Cummins proposed that the synergistic effects of CYP3A4-mediated metabolism and P-gp-mediated efflux in gut epithelium may result in an unexpectedly high first-pass effect in the gut after oral administration (7). It is believed that a better understanding of the physiology and

*Corresponding author: Y. Madhusudan Rao, Centre for Biopharmaceutics and Pharmacokinetics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506 009, AP, India
Phone: +91-870-3091323, Fax: +91-870-2453508,
E-mail: ymrao123@yahoo.com
Received August 1, 2010; accepted December 1, 2010

biochemistry of the interactive nature of intestinal CYP3A and P-gp will be important in defining, controlling and improving the oral bioavailability of CYP3A and P-gp substrates.

The phenomenon of intestinal drug efflux

The cascade of events determining the systemic availability of drugs following oral administration has been extensively studied. Although many aspects are well-known, for some drugs the process leading to drug absorption and bioavailability requires further examination since it is relatively complex. Some of the mechanisms may involve poor compound solubility in gastrointestinal fluid, poor permeability across the gastrointestinal epithelium, and insufficient stability in some gastrointestinal segments owing to enzymatic and non-enzymatic degradation, complexation, and, in some cases, pronounced hepatic first-pass extraction (9).

The possible mechanisms involved in drug permeation across the intestinal epithelium are well-defined in many cases. These include paracellular and transcellular pathways of membrane permeation, although the contribution of the paracellular pathway to the total transmembrane (TM) drug flux is regarded as being of limited relevance in most cases. The transcellular pathway involves partitioning of drugs into the lipophilic epithelium and diffusion across the membrane, and it is well-recognised that forces generated by transport systems intrinsic to the membrane can drive the epithelial transport of several drugs (10). In these cases, a substance seems to permeate a biological membrane at a different rate than anticipated from its molecular size and hydrophobicity alone. Such stoichiometric transport systems are carriers and pumps. Carriers may be involved in three types of transport process: facilitated diffusion, co-transport (symport) and counter-transport (anti-port). Co-transporters and counter-transporters can perform secondary active transport using energy from the downhill transport of one substrate to drive the uphill transport of another. Pumps are distinguished from carriers by linkage of transport to an external energy source, provided by hydrolysis of a phosphate bond and leading to the generation of ADP from ATP. Pumps perform primary active transport.

Carriers and pumps in the intestinal epithelium may transport substrates from the intestinal lumen to the blood compartment (absorptive transport). Other transporters have been discovered that operate in the reverse direction, i.e., from the blood side to the luminal side (secretory transport). Both pathways are illustrated in Figure 1.

Carrier-mediated transport processes are saturable and inhibitable, and may be regulated by a variety of external and internal factors. Saturability of carrier-mediated transport may lead to dose-dependent pharmacokinetics of drugs that are substrates of carriers. This is frequently observed as a deviation from the linear relationship between drug dose and systemic exposure. Both induction and inhibition of carriers involved in drug transport may lead to diminished or enhanced absorption of drugs with affinity for these carriers.

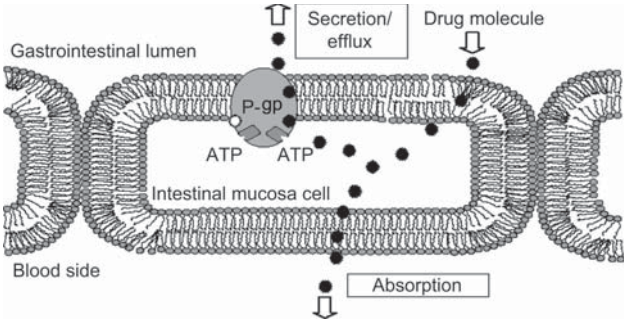

Figure 1 Absorption and secretion of a P-glycoprotein substrate in human enterocytes.

The intestinal efflux pump P-glycoprotein

The best characterised example of an efflux pump located in the intestine is P-gp. In the mid 1970s, Juliano and Ling (67) reported that overexpression of a membrane protein in colchicine-resistant Chinese hamster ovary cells conferred resistance to a wide range of amphiphilic drugs (11). P-gp is a glycosylated membrane protein consisting of 1280 amino acids with 12 hydrophobic, helical TM segments, two intracellular ATP-binding sites and a molecular weight of 170 Da (7, 13). Its structure is depicted in Figure 2.

A low-resolution structure for P-glycoprotein

The 25 Å resolution structure for P-gp was obtained by electron microscopy and single-particle image analysis of both detergent-solubilised and lipid-reconstituted P-gp (12). The structure was further refined by three-dimensional reconstruction of single-particle images and Fourier projection maps of small two-dimensional crystalline arrays. A diagrammatic representation of the structure is shown in Figure 3.

Location and function of P-glycoprotein

P-gp is physiologically expressed in the apical membrane of mucosal cells of the small and large intestine, as well as at the luminal membrane of proximal tubular cells in the kidney, the biliary canalicular membrane of hepatocytes, at the blood-brain barrier (BBB), in capillary endothelial cells of testis, the

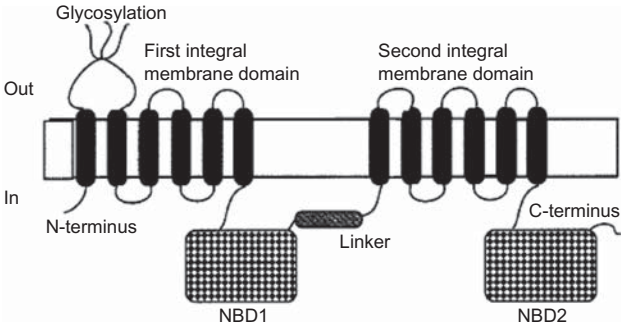

Figure 2 Topological map and domain organisation of P-glycoprotein as predicted from its primary sequence.

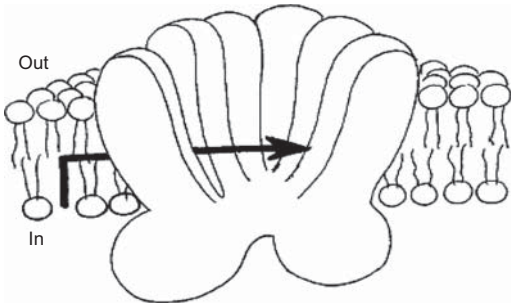

Figure 3 Three-dimensional structure of P-glycoprotein determined to a resolution of 25 Å by electron microscopy.

adrenal gland, and the endometrium of the pregnant uterus. P-gp plays a role in the excretion of toxic substances in the kidneys and in the liver. At the BBB, it prevents entrance of drugs to the central nervous system (CNS). In the intestine, P-gp-mediated efflux can reduce the bioavailability of drugs that are administered orally.

In addition, P-gp has been discussed in relation to interactions between different P-gp substrates due to displacement from the carrier. Besides drug-drug interactions with food components, such as ingredients of grapefruit juice (14) and apricot extract (18), have been discussed. A recent review of interactions mediated by inhibition and induction of P-gp has been published. Before the current focus on biopharmaceutical research into P-gp-related drug efflux was largely related to its involvement in the development of MDR in cancer chemotherapy. P-gp, like the MDR-associated proteins (MRPs), belongs to the family of ATP-binding cassette (ABC) transporters, members of which are also involved in *Plasmodium falciparum* resistance to chloroquine and the development of resistance to antibiotics in both prokaryotic and eukaryotic cells. The biochemistry, pharmacology and structure-activity relationships of P-gp and its substrates have been described in the literature (45, 46).

Mechanism of drug efflux

Drug transport across the cell membrane occur by passive diffusion, filtration or by specialised transport (8). The mechanism of drug transportation via P-gp involves the following steps:

Step 1: Drug or substrate recognition by P-gp;
Step 2: ATP binding and subsequent hydrolysis; and
Step 3: Efflux of substrate through the central pore.

Various models have been proposed to explain the mechanism of xenobiotic extrusion by P-gp; however, the exact site of substrate interaction with the protein is not well-resolved. The three prevalent models, the pore model, the flippase model and the hydrophobic vacuum cleaner (HVC) model, explain the efflux mechanism to a certain extent (Figure 4) (15).

1. In the pore model, drugs associate with P-gp in the cytosolic compartment and are transported out of the cell through a protein channel.
2. In the flippase model, drugs embed in the leaflet of the plasma membrane, bind to P-gp within the membrane plane and are translocated to the outer leaflet of the bilayer, from which they passively diffuse into the extracellular fluid.
3. The HVC model has gained wide acceptance. In this model, P-gp recognises substrates embedded in the inner leaflet of the plasma membrane and transports them through a protein channel.

Rosenberg et al. reported that the three-dimensional conformation of P-gp changes on nucleotide binding to the intracellular nucleotide-binding domain (12). In the absence of nucleotides, the two TM domains form a single barrel with a central pore that is open to the extracellular surface and spans much of the membrane depth. On nucleotide binding, the TM domains reorganise into three compact domains that open the central pore along its length in a manner that could allow access of hydrophobic drugs directly from the lipid bilayer to the central pore of the transporter (15).

Substrates of P-glycoprotein

The most striking property of ABCB1 P-gp includes its broad substrate specificity, so that it transports a large number of structurally diverse drugs used in a range of clinical

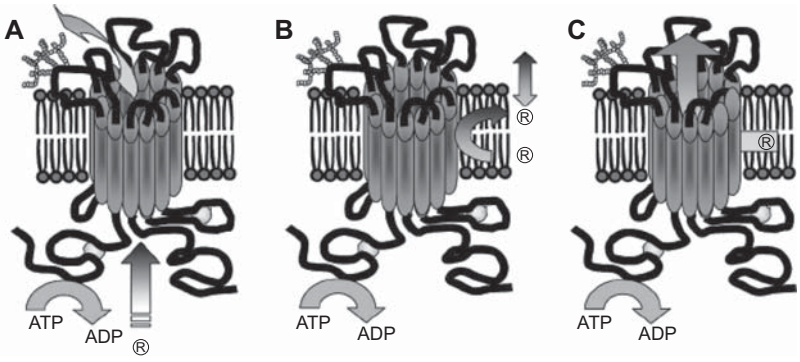

Figure 4 Models proposed to explain the mechanism of drug efflux by P-glycoprotein.
(A) Pore model; (B) flippase model; and (C) hydrophobic vacuum cleaner model.

applications (8). Early studies suggested that the minimum requirements were hydrophobicity, a basic nitrogen atom and two planar aromatic domains, but no highly conserved elements of recognition have been found. Pharmacophore models of P-gp substrate affinity have been proposed. These generally contain ring aromatic or hydrophobic functionalities, as well as hydrogen bond acceptor functional groups, that determine whether compounds act as substrates, modulators, or both. Therefore, the only common structural feature in all transported P-gp substrates is that they are amphipathic. Compounds range in size from 300 to 2000 Da, and can be basic, uncharged or acidic (11, 16, 17). The TM sequence of P-gp shows a high degree of side chains that are hydrogen bond donors, and these are thought to interact with hydrogen bond acceptors in substrate compounds. Gombar et al. developed a P-gp quantitative structure-activity relationship model that provides an in silico screen to aid in compound selection and prioritisation of in vitro efflux assays (21).

Clarke and colleagues used a variety of cysteine mutants of P-gp to demonstrate that, according to their reactivity with different thiol-reactive substrate analogues, the drug-binding site comprises multiple TM segments (19). This led to the hypothesis that P-gp binds substrates through an induced fit mechanism, in which the size and shape of the substrate change the packing of TM segments. The ability of a substrate to change the crosslinking pattern is consistent with the ability of TM segment to change their shape to accommodate structurally different compounds; each substrate can cause specific shifts in the different TM segments responsible for its binding (substrate-induced fit), allowing common residues to be involved in the binding of diverse substrates (19).

Intestinal P-glycoprotein

Intestinal drug efflux mediated by P-gp is widely accepted as a reason for low and variable oral absorption of drugs (20). The distribution of P-gp is not uniform among cells along the intestinal epithelial villi. Immunohistological studies of human jejunum and colon using antibody MRK 16 revealed that high levels of P-gp were only observed in the apical surface of columnar epithelial cells, but not in crypt cells (22). The distribution of P-gp is also not uniform along the length of the intestine. Fojo et al. measured MDR1 mRNA expression over the total length of the human gastrointestinal tract. The levels of mRNA seemed to increase progressively from the stomach to the colon, with low levels in the stomach (5 arbitrary units, a.u.), intermediate levels in the jejunum (20 a.u.) and high levels in the colon (30 a.u.). Consistent with these observations, Mdr1a mRNA levels in rat intestine also increase progressively from the stomach to the colon (stomach one, duodenum 20, jejunum 36 and ileum 100 a.u.) (20, 22, 23).

The uneven distribution of intestinal P-gp and interindividual variability in P-gp expression are major factors contributing to variations in drug absorption. Although interindividual variability in drug-metabolising enzymes is well-documented, only a few papers deal with the issue of interindividual P-gp variability. Lown et al. observed more than eight-fold differences in P-gp expression in a small population of 25 patients (24).

P-Glycoprotein inhibitors

Screening studies for P-gp-drug interactions identified a number of clinically important drugs as P-gp substrates (Table 1). A few of them were identified as inhibitors of P-gp, which opened an opportunity for MDR reversal. Improved clinical efficacy of various drugs observed after P-gp inhibition, especially drugs subject to MDR, led to the design and development of modulators. P-gp inhibitors are attracting attention in attempts to improve drug bioavailability by inhibiting P-gp in intestine, brain, liver and kidneys.

Table 1 Agents that interact with P-glycoprotein.

Category	Agents
Anti-arrhythmics	Amiodarone, lidocaine, propranolol, quinidine
Antibiotics and antifungals	Cefoperazone, ceftriaxone, erythromycin, itraconazole
Anticoagulants	Dipiridamole
Antimalarials and antiparasitics	Chloroquine, emetine, hydroxyl chloroquine, quinacrine, quinine
Calcium channel blockers	Bepridil, diltiazam, felodipine, nifedipine, nisoldipine, nitrendipine, tiapamil, verapamil
Cancer chemotherapeutics	Actinomycin D, colchicine, daunorubicin, etoposide, mitomycin C, mitramycin, podophyllotoxin, puromycin, taxol, topotecan, trimetrixate, vinblastin
Immunosuppressants	Cyclosporine A, cyclosporine H, SDZ PSC 833
DNA intercalators	Ethidium bromide
Fluorescent dyes	BCECF-AM, Fluo-3, Fura-2, rhodamine 123
Hormones	Aldosterone, clomiphene, cortisol, deoxycorticosterone, dexamethasone, prednisone, progesterone, tamoxifen
Indole alkaloids	Reserpine, yohimbine
Local anaesthetics	Bupivacaine
Surfactants and solvents	Cremophor-EL, Triton X-100, Tween 80
Peptides	N-Acetyl-leucyl-leucinal (ALLN), gramicidin D, valinomycin, leupeptin, nonactin, pepstatin A, beauvericin
Miscellaneous	Grapefruit juice, liposomes, quercetin, terfenadine, tumour necrosis factor, vitamin A, LY335979

Based on their specificity and affinity, P-gp inhibitors are classified as follows:

- **First-generation inhibitors:** These agents are drugs in clinical use for other indications that inhibit P-gp efflux in in vitro experiments. Owing to their relatively low binding affinity for P-gp and the need to increase doses to toxic levels, few of these agents have been further studied for clinical modulation of P-gp. However, early trials with these drugs provided valuable information regarding the consequences of P-gp inhibition. The inhibitors include calcium channel blockers (e.g., verapamil), immunosuppressants (e.g., cyclosporine) and antihypertensives (e.g., reserpine, quinidine and yohimbine).
- **Second-generation inhibitors:** These are agents that lack the pharmacological activity of first-generation compounds and usually posses higher P-gp affinity. They include dexniguldipine (B8509-035), dexverapamil (R-verapamil) and S 9788.
- **Third-generation inhibitors:** Like the second-generation modulators, these compounds were identified from efforts to produce agents whose primary activity involves inhibition of P-gp-mediated efflux with lower toxic effects. Many of these compounds have low nanomolar potency as P-gp inhibitors in vitro. They include GF120918 (GW918), valspodar (PSC833), and CGP41251.

In general P-gp can be inhibited by: i) blocking of the drug-binding site competitively, non-competitive or allosterically; ii) interfering with ATP hydrolysis (13); or iii) altering the integrity of cell-membrane lipids.

Although most drugs inhibit P-gp function by blocking drug-binding sites, the presence of multiple binding sites complicates the development of a true structure-activity relationship for substrates or inhibitors. However, if protein transport and/or inhibition is mediated only through binding sites, the mode of handling of substrates or inhibitors by P-gp should be the same. Thus, the issue to be addressed is how substrates and inhibitors are discriminated at the molecular level. In this regard, a plausible explanation is that the modulator or inhibitor flipped by P-gp can flop back into the inner leaflet of the membrane for further transport, which is very rapid and creates a large difference between the rates of efflux of substrates and inhibitors. Thus, the P-gp modulator is cycled repeatedly, preventing substrate efflux, which depends on the hydrophobicity of the compound (15).

Commonly used pharmaceutical surfactants are emerging as different P-gp inhibitors that act by altering the integrity of membrane lipids. Changes in secondary and tertiary structure were identified as the reason for loss of P-gp function due to surfactants disruption of the hydrophobic environment in a series of studies by Hugger and co-workers (25, 26).

Evidence of intestinal P-glycoprotein involvement in drug absorption

Evidence of the involvement of intestinal P-gp in drug absorption was first demonstrated in vitro using Caco-2 cells, in which P-gp is highly expressed. Transport of cyclosporine in the basolateral-apical (B-A) direction was much greater than A-B transport, which is indicative of active efflux. When the cells were treated with a P-gp inhibitor, progesterone, and chlorpromazine, A-B cyclosporine transport increased and B-A transport decreased (27).

Direct evidence of the role of intestinal P-gp in drug absorption was obtained in in vivo studies with *mdr1a*$^{-/-}$ knockout mice. Oral absorption of paclitaxel was studied in *mdr1a*$^{-/-}$ and *mdr1a*$^{+/+}$ mice (29). According to the plasma AUC after intravenous and oral administration, the bioavailability of paclitaxel was 11% and 35% for *mdr1a*$^{+/+}$ and *mdr1a*$^{-/-}$ mice, respectively. It is thus clear that P-gp limits the oral bioavailability of drugs by effluxing them back into the lumen.

Indirect evidence of P-gp involvement in drug absorption in humans has come from correlations between drug absorption profiles and intestinal P-gp levels. In one clinical study of 25 kidney transplant recipients, a strong negative correlation between intestinal P-gp and cyclosporine absorption was observed (24).

Saturable efflux transport of intestinal P-glycoprotein

There is a widespread misconception that the extent of oral absorption of a drug is always markedly limited by intestinal P-gp when the drug is a P-gp substrate. This has led to the notion that if a compound is a P-gp substrate, it must exhibit poor bioavailability. Unfortunately, this generalisation is not always true. Like drug-metabolising enzymes, P-gp has functional activity that is saturable. Many drugs are good P-gp substrates and yet have reasonably good oral bioavailability. Digoxin is a good example: although it is a good substrate, it has reasonable bioavailability, which ranges from 50% to 85%.

For P-gp substrates, the net amount of drugs absorbed into the mesenteric blood circulation is the difference between the amount absorbed via influx processes (passive diffusion and/or active uptake) and the sum of the amounts extruded via efflux transport and metabolised by enzymes. It is possible that the influx process of a drug is significantly greater than the P-gp efflux process (plus metabolism), even if it is a P-gp substrate. Therefore, the impact of P-gp efflux on drug absorption is generally less important than the impact on drug distribution, such as brain penetration.

P-Glycoprotein-mediated drug-drug interactions

Aside from metabolic interactions, it is increasingly recognised that drugs that are not subject to metabolism may have substantial potential for drug interactions. Drug-drug interactions mediated by inhibition and induction of P-gp in animals and humans have been reported. The pharmacokinetic consequences of P-gp induction and inhibition are similar to those observed for induction and inhibition of CYP enzymes. In other words, P-gp inhibition results in an increase in the systemic exposure and tissue distribution of drugs that are P-gp substrates, while the opposite is true for P-gp induction. Because of substrate overlap between CYP3A4 and P-gp, many inhibitors can affect both CYP3A4 and P-gp, and many

drug interactions may involve both the enzyme and transporter systems.

The most compelling clinical evidence of P-gp-mediated drug interaction in humans is the interaction of digoxin with other cardiac drugs, such as verapamil and quinidine (28, 29). Salphati and Benet reported that ketoconazole increased the plasma concentration, rate of absorption and bioavailability of digoxin (30). The effects of ketoconazole on AUC could be explained by inhibition of both CYP3A and P-gp, but the decrease in mean absorption time can only be explained by inhibition of P-gp in the intestine (30). There are several reports of P-gp-mediated drug-drug interactions, such as interactions between talinolol and verapamil (31), ketoconazole, ritonovir and saquinavir (32), and digoxin and rifampicin (33).

The interaction between P-gp substrates does not always follow simple kinetics. The pattern of P-gp interaction can be classified into at least three major categories: competitive inhibition, non-competitive inhibition and cooperative stimulation. The situation can be even more complicated if allosteric effects are involved in the interaction between the substrate and the inhibitor. The complexity of the molecular mechanism for P-gp inhibition limits our ability to predict, either quantitatively or qualitatively, the potential of P-gp-mediated drug-drug interactions.

CYP3A and oral bioavailability

CYPs localised in the smooth endoplasmic reticulum of numerous tissues are haem-containing membrane proteins that belong to a superfamily of mixed function oxidases. This diversity of enzymes has necessitated a systematic nomenclature (34). The root name given to all cytochrome P-450 enzymes is CYP (*CYP* for the gene). Enzymes with >40% amino acid sequence homology are placed in the same family, designated by an Arabic numeral. When two or more subfamilies are known to exist within the family, then enzymes with >60% homology are placed in the same subfamily, designated with a letter. Finally, an Arabic number representing the individual enzyme is assigned on an incremental basis. As of February 1999, there were approximately 650 P450 enzymes, organised into 96 families, identified in species from alfalfa to zebra finch; even the humble nematode *Caenorhabditis elegans* has over 60 CYP enzymes (35). Only the 35 CYP enzymes described in man (almost certainly an underestimate) are likely to be of any clinical relevance, although only the CYPs in families 1, 2 and 3 seem to be responsible for the metabolism of drugs and therefore are potential sites for drug interactions.

A survey of the elimination pathways of over 400 drugs marketed in Europe and the US revealed that CYP-mediated metabolism accounts for 55% of the total elimination of these drugs (36).

Intestinal CYP3A

Among adults, CYP3A levels in the small intestine are generally 10%–50% of those found in the liver. CYP3A protein levels and catalytic activity decrease longitudinally along the small intestine, with protein levels and midazolam 1′-hydroxylation increasing slightly from the duodenum to jejunum and then decreasing in the ileum and colon. Hepatic and intestinal CYP3A4 forms seem to be the same enzyme, with identical cDNAs. Despite this, CYP3A forms in the liver and small intestine are not coordinately regulated (37, 38).

CYP3A enzymes can be induced by a number of structurally unrelated compounds, including steroids (synthetic glucocorticoids and antiglucocorticoids), phenobarbital-type inducers, macrolide antibiotics and antifungal compounds. The molecular basis of CYP3A induction is not yet fully understood. An orphan nuclear receptor designated as the pregnane X receptor (PXR) has been identified as a mediator of hepatic CYP3A enzyme induction by pregnenolone 16α-carbonitrile and rifampicin.

Interindividual differences in the oral bioavailability and systemic clearance of CYP3A substrates can be attributed, in large part, to variable CYP3A expression in the mucosal epithelium of the small intestine and in the liver. This variation is manifest by differences of >10-fold in the in vivo metabolism of drugs that are substrates for CYP3A (17).

Substrates of CYP3A4

CYP 3A4 has very broad substrate specificity. CYP3A catalyses the metabolism of an increasing number of structurally diverse and clinically important drugs covering a wide therapeutic range, including anti-arrhythmics, antifungals, calcium channel antagonists, cancer chemotherapeutic agents, hormones, immunosuppressants and HIV protease inhibitors. The most common characteristic of CYP3A4 substrates is hydrophobicity, which was confirmed by a model of the active site of CYP3A4 built on the basis of sequence homology with CYP cam, a soluble CYP from *Pseudomonas putida*. Another unique characteristic of CYP3A is its ability to be activated by certain compounds such as 7,8-benzoflavone, which is itself a CYP3A4 substrate and can activate CYP3A4-catalyzed phenanthrene metabolism. A number of drugs from a broad range of therapeutic categories are CYP3A4 substrates (Table 2). For orally administered drugs, factors determining the bioavailability of these substrates can facilitate prediction of changes in pharmacokinetics that could occur on administration of a CYP3A inhibitor.

Inhibitors of CYP3A4

Numerous substances in various categories have been identified as inhibitors of CYP3A activity. The most clinically relevant of these are listed in Table 3. Specific inhibitors of CYP3A4 that deserve attention because of their potency and their association with clinically relevant interactions include azole antifungals, macrolide antibiotics, nefazodone, HIV protease inhibitors and grapefruit juice. Azole antifungal agents and first-generation HIV protease inhibitors seem to act via competitive inhibition by rapid, reversible binding of the inhibitor or its metabolite to CYP3A4. Macrolide antibiotics exhibit slowly reversible, non-competitive inhibition. The

Table 2 Pre-systemic metabolism of CYP3A and/or P-gp substrates and inhibitors.

Drugs	Pre-systemic metabolism	Oral bioavailability
Alfetanil, fentanyl, astemizole, buspirone, ergotamine, lovastatin, nisoldipine, saquinavir, daunorubicin, simvastatin, clotrimazole, miconazole, terfenadine, doxorubicin, paclitaxel, tamoxifen, vinblastine, vincristine, loratadine, rapamycin, flutamide, testosterone	Very high	<10
Erythromycin, oestradiol, atorvastatin, felodipine, indinavir, isradipine, nicardipine, nitrendipine, nimodipine, propafenone, lidocaine, pravastatin, dronabinol, nefazodone, sertraline, pimozide, tacrolimus, ethosuccimide, rifabutin	High	10–30
Azithromycin, amiodarone, amprenavir, carbamazepine, carvedilol, cisapride, cyclosporin, diltiazem, ethinyloestradiol, etoposide, losartan, midazolam, nifedipine, nelfinavir, ondansetron, pimozide, sildenafil, triazolam, fluconazole, zolpidem, finasteride, itraconazole, rifampicin, clarithromycin, zidovudine, cyclophosphamide, quinine, fluvastatin, verapamil	Intermediate	30–70
Alprazolam, amlodipine, dapsone, dexamethasone, donepezil, quinidine, ritonavir, disopyramide, diazepam, methyl prednisone, prednisone, clindamycin, ifosfamide, digoxin, temazepam	Low	>70

Table 3 Clinically relevant inhibitors of CYP3A4.

Inhibitors	Mechanism
Amprenavir, clarithromycin, cyclosporine, diltiazem, erythromycin, itraconazole, indinavir, ketoconazole, mebefradil, nefazodone, nelfinavir and ritonavir	Reversible inhibition
Bergamottin and dihydroxybergamottin	Suicide-based inhibition

furanocoumarins in grapefruit juice, dihydroxybergamottin and bergamottin, causes irreversible mechanism-based (suicide) inhibition. This presumably involves formation of a reactive metabolite (CYP3A4-mediated) that covalently binds to the enzyme, leading to its inactivation. Most potent orally administered inhibitors act at the level of the small bowel and liver. However, grapefruit juice is an example of an inhibitor that seems to be clinically active against only enteric CYP3A4, and may be useful as a probe of enteric CYP3A4 activity. There are numerous examples of drug interactions mediated through CYP3A4 (Table 4).

Erythromycin Although the rate of elimination of this CYP3A substrate can be determined from plasma pharmacokinetics, the erythromycin breath test (ERMBT) is less invasive and involves intravenous administration of a trace amount of ^{14}C-N-methyl erythromycin. At specified time points, the subject breathes through a one-way valve into a CO_2-trapping solution, and ^{14}C-CO_2 is subsequently measured by liquid scintillation counting. This test shows fairly good correlation with trough cyclosporine concentrations and clearly demonstrates the inductive effect of rifampin. However, there was poor correlation between ERMBT and clearance of the CYP3A4 substrate alfentanil. The test is still somewhat invasive (intravenous administration) and does not assess pre-systemic effects; a further limitation is the need to administer a radioactive compound.

Midazolam Midazolam doses in humans are eliminated renally (98%), with 1-hydroxy midazolam (the product of CYP3A metabolism) accounting for half of the renal elimination. Midazolam clearance provides a good estimate of CYP3A activity and correlates with CYP3A concentrations in liver biopsies, cyclosporine clearance, and ERMBT results. Midazolam clearance is increased in patients taking phenytoin and decreased in patients taking erythromycin or itraconazole, and thus has wide utility for drug-drug interaction studies.

Nifedipine Nifedipine was one of the first CYP3A4 substrates to be identified and it has been the subject of a huge number of drug-drug interaction studies both in vitro and in vivo. Pharmacokinetic studies of nifedipine have clearly identified inhibitors, such as itraconazole and grapefruit juice, and inducers, such as barbiturates and rifampin.

Regulation of ABC transporters and CYP via nuclear receptor pathways

Many drugs in clinical use directly or indirectly regulate ABC transporters and CYP enzymes through nuclear receptor pathways. Importantly, the orphan nuclear PXR co-regulates genes for CYP and ABC transporters (e.g., ABCB1) in the intestine. Furthermore, the constitutive androstane receptor (CAR), the glucocorticoid receptor and the vitamin D receptor are important transcriptional regulators and co-regulators of ABC transporter and CYP gene expression. Interestingly, the orphan nuclear receptor hepatocyte nuclear factor 4α seems to be directly involved in PXR- and CAR-mediated transcription of CYP3A4, and may also participate in cell-type-dependent upregulation of CYP3A4 (12).

CYP3A and P-gp: a synergistic role in oral bioavailability

It is believed that intestinal CYP3A and P-gp act in a concerted manner to control the absorption of their substrates (7, 17, 39). This suggestion is based on a considerable overlap in

Table 4 Drug interaction involving CYP3A4 isoenzymes.

	Drugs affected (substrates)
Inhibitors	
Azole antifungals	Midazolam, tacrolimus, terfenadine, riazolam
Itraconazole	Cisapride, quinidine, astemizole, buspirone, methyl prednisone, felodipine, vincristine
Ketoconazole	Terfenadine, astemizole, cyclosporine, triazolam
Fluconazole	Terfenadine, triazolam, alprazolam
Macrolide antibiotics	Tacrolimus, astemizole
Erythromycin	Carbamazepine, triazolam, buspirone, terfenadine, simvastatin
Clarithromycin	Pimozide, cyclosporin, midazolam
Serotonin re-uptake inhibitors	Midazolam, cisapride
Fluoxetine	Diazepam, alprazolam, midazolam, terfenadine
Paroxetine	Alprazolam
Calcium channel blockers	Tacrolimus
Verapamil	Simvastatin, carbamazepine, cyclosporine
Diltiazem	Triazolam, carbamazepine, cyclosporine, quinidine, simvastatin, midazolam, alfentanil
Nifedipine	Midazolam
Protease inhibitors	Terfenadine, astemizole, cisapride, midazolam
Grapefruit juice	Felodipine, nifedipine, nimodipine, simvastatin, nitrendipine, terfenadine, cyclosporine, midazolam, carbamazepine, verapamil, prednisone, artemether
Ciprofloxacin	Tacrolimus
Cimetidine	Carbamazepine, quinidine, cyclosporine, benzodiazepine
Propofol	Midazolam
Nafimidone, omeprazole	Carbamazepine
Inducers	
Rifampicin	Protease inhibitors, diazepam, triazolam, midazolam, oestradiol, norgesterol, lidocaine, zopiclone, zolpidem, ondansetron
Rifabutin	Protease inhibitors, oestradiol, norgesterol
Carbamazepine	Protease inhibitors, midazolam, itraconazole, vincristine
Phenytoin, phenobarbitone	Midazolam, vincristine, carbamazepine

the substrate specificity of the two proteins and the proximity of their expression in the intestine. Furthermore, it was demonstrated that modulators and substrates of P-gp and CYP3A upregulate these proteins in human colon carcinoma cells in a co-regulatory manner (40). When a drug is absorbed by passive process into enterocytes, where it may be metabolised by the enzyme, it is subject to active back transport into the intestine allowing further access to the enzyme on subsequent absorption (Figure 5).

A drug D that enters the epithelium may be effluxed via P-gp and/or metabolised by CYP3A to D*, with the residual native drug D absorbed across the epithelium. Recirculation of the drug out of the cell via P-gp and subsequent reabsorption allow greater access to a limited amount of CYP3A, and hence a greater proportion will be metabolised and reduced absorption of the drug will ensue.

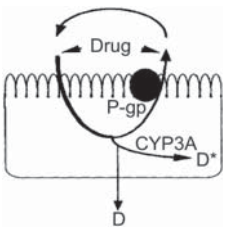

Figure 5 Model of drug absorption in the presence of both P-glycoprotein and cytochrome P-450 3A.

MDR- and CYP3A4-mediated drug-herb interactions

Many drug substances, along with a variety of naturally occurring dietary and herbal components, are capable of interacting with the CYP enzyme system and the P-gp efflux pump in several ways:

1. A herbal component can be a substrate of one or several CYP isoforms and/or efflux systems (P-gp, MRP and BCRP). Therefore, one substrate can compete for another substrate for metabolism by the same CYP isozyme and/or efflux system, resulting in higher plasma concentrations due to competitive inhibition.
2. A herbal constituent can also be an inducer of one or several CYP isoforms and/or efflux systems, thereby lowering plasma concentrations due to either higher metabolism and/or higher efflux. Such interactions may lead to subtherapeutic plasma drug concentrations.
3. A compound can also be an inhibitor of CYP enzymes, resulting in reduced activity of one or several CYP isoforms. If a compound is an inhibitor of the efflux system,

it will reduce drug efflux and result in improved absorption. However, induction is a slow process that depends on the rate of protein synthesis. Expression of specific mRNA may be possible within a few hours, but functional expression and maturation of such proteins may require a longer duration. By contrast, inhibition is more rapid and can produce results within a very short period of time, particularly if the inhibition is competitive in nature.

Role of CYP450 in drug-herb interactions

The most versatile enzyme system involved in the metabolism of xenobiotics is CYP. The CYP3A family of enzymes comprises the most predominant phase I drug-metabolising enzymes and accounts for approximately 30% of hepatic CYP and more than 70% of intestinal CYP activity. Moreover, CYP3A is estimated to metabolise between 50% and 70% of currently administered drugs (3). CYP3A4 is the most abundant form in the CYP family (4) and is present primarily in hepatocytes and enterocytes (41, 42). It is now well-established that naturally occurring dietary supplements can modulate hepatic and enterocytic CYP activity. Perhaps the best-documented clinically relevant drug interaction is observed with grapefruit juice. Simultaneous consumption of grapefruit juice and a number of therapeutic agents that are subject to first-pass intestinal or hepatic metabolism results in higher plasma levels with subsequent adverse effects (43, 44). Grapefruit juice acts through inhibition of intestinal CYP3A4, which regulates pre-systemic metabolism (45). Although hepatic biotransformation can make a major contribution to systemic drug elimination, a combination of hepatic and intestinal drug metabolism may cause significant pre-systemic or first-pass drug loss. Preliminary studies that investigated St. John's wort (SJW) interactions indicate that it may modulate CYP, particularly the 3A4 isoform. Several in vitro studies revealed that crude SYW extract inhibits CYP3A4 in a competitive manner (46, 47). These studies also identified hyperforin, hypericin, quercetin and 13,118-biapigenin as components primarily responsible for such inhibitory interactions. HIV protease inhibitors, macrolide antibiotics and azole antifungals, along with many herbal agents, are substrates of CYP3A4, so they can affect oral bioavailability of therapeutic agents used in the treatment of immunosuppression, cancer, AIDS and other opportunistic infections. Depending on the interaction mechanisms, these substances could lower blood levels of anti-HIV drugs and could thus put patients at risk of developing resistance. Although many clinical studies have reported that SJW has an inductive effect on CYP3A4, one study suggested that SJW had no statistically significant effect on CYP3A4 induction (48). This is consistent with another study describing CYP3A4 metabolic interaction (49). This study reported that 4-day treatment of mice with SJW extract or its constituents, hypericin and hyperforin, did not result in any CYP3A4 induction. By contrast, an inhibitory effect of the major constituents of SJW was observed for CYP-transfected cells (46). In line with this observation, quercetin, one of the major constituents of SJW, had an inhibitory effect on CYP3A4 (50). The discrepancy among results remains unresolved.

Herbal products can competitively inhibit CYP, so concomitant intake of these agents may increase blood levels of therapeutic agents, thus exposing patients to a greater risk of serious side effects. A recent study in our laboratory revealed that pure herbal constituents (quercetin, hypericin and kaempferol) inhibit CYP3A4-mediated cortisol metabolism (47). However, silibinin did not inhibit cortisol metabolism.

Pomegranate juice also inhibits intestinal CYP3A4 in vivo and has similar inhibitory potency to that of grapefruit juice (51, 52). Pomegranate juice has been touted in the popular press for its putative health benefits (53), but a case report suggests that pomegranate juice, like grapefruit juice, may increase the risk of rhabdomyolysis during statin therapy. Grapefruit and pomegranate juices contain an irreversible competitive inhibitor of intestinal CYP (54). In earlier studies, pomegranate juice influenced the pharmacokinetics of carbamazepine in rats, particularly in comparison with the effect of water. The AUC of carbamazepine increased approximately 1.5-fold in rats on exposure to pomegranate juice 1 h before administration of the drug. Thus, components of pomegranate inhibit the CYP3A-mediated metabolism of carbamazepine. Furthermore, pomegranate juice has an influence on the pharmacokinetics of drugs in rats (52).

Shravan et al. observed a significant difference ($p<0.05$) in buspirone transport from intestinal sacs pre-treated with silymarin and pomegranate juice compared to control. This suggests that both silymarin and pomegranate juice might act by inhibiting the transporters and enzymes responsible for transport and metabolism of buspirone (55).

Garlic preparations are commonly taken by HIV patients because they exert a cholesterol-lowering effect and alleviate hypercholesterolaemia caused by long-term highly active antiretroviral therapy (HAART). The bioavailability and therapeutic outcome of treatment with HIV protease inhibitors depend on intestinal and hepatic transporter-enzyme interplay. However, case reports and clinical studies of concomitant consumption of HIV protease inhibitors and garlic supplements have revealed significant pharmacokinetic changes and serious adverse reactions. In a patient taking a garlic supplement, severe gastrointestinal toxicity was observed after initiation of ritonavir therapy (56). Furthermore, concomitant saquinavir and garlic administration in 10 healthy male volunteers resulted in a >50% decrease in saquinavir AUC and c_{max}. Although CYP3A4 inhibition (short-term use) and induction (long-term use) by garlic phytochemicals are recognised as reasons for the changes in saquinavir pharmacokinetic parameters observed, the exact mechanism behind this interaction is still unclear. Thus, either the metabolites of garlic phytochemical(s) or the saquinavir metabolite responsible for the CYP3A4 inhibition and induction observed remain to be identified (57).

Katja et al. assessed the liver transport of HIV protease inhibitors (saquinavir, darunavir) in the presence of aged garlic extract because HIV patients often consume garlic supplements

together with prescribed therapy. The in vitro uptake of both drugs into HepG2 cells and precision-cut rat liver slices significantly increased in the presence of P-gp and the MRP-2 inhibitor ritonavir. Incubation medium containing aged garlic extract caused significant inhibition of saquinavir efflux from HepG2 cells and precision-cut liver slices, while the activity of darunavir efflux transporters in both liver models significantly increased. The opposite in vitro interactions observed for aged garlic extract and HIV protease inhibitors indicate that darunavir and saquinavir most probably bind to different binding sites on one or both efflux transporters. According to the study results, co-administration of saquinavir or darunavir with garlic supplements could result in significant in vivo modification of hepatic transport-enzyme interplay, possibly leading to further bioavailability changes (58).

It has been shown that garlic extracts decrease drug exposure for saquinavir, a P-gp and CYP3A4 substrate. To explore the underlying mechanisms and to study the effects of garlic on pre-systemic drug elimination, healthy volunteers were given a garlic extract for 21 days. Protein expression of duodenal P-gp and CYP3A4 was measured before and after the trial and was normalised to villin expression. Hepatic CYP3A4 function and simvastatin, pravastatin and saquinavir pharmacokinetics were also evaluated. Ingestion of garlic extract increased expression of duodenal P-gp to 131% (95% CI 105%–163%) without increasing CYP3A4 expression (87%, 95% CI 67%–112%) relative to baseline. The average ERMBT result was 96% (95% CI 83%–112%). Ingestion of garlic extract had no effect on drug and metabolite AUCs following a single dose of simvastatin or pravastatin, although the average saquinavir AUC decreased to 85% (95% CI 66%–109%), and changes in intestinal P-gp expression negatively correlated with this change. In conclusion, garlic extract induces intestinal expression of P-gp independent of cytochrome CYP3A4 in human intestine and liver (59).

Wana et al. studied the effects of schisandrol A (SCH) and gomisin A (GOM), two of the main bioactive components of *Fructus schisandrae chinensis*, on CYP3A4 activity and cellular glutathione (GSH) levels. In a cell-free system, both SCH and GOM inhibited CYP3A4 activity, with IC_{50} values of 32.02 and 1.39 mM, respectively. SCH or GOM at concentrations up to 100 mM did not alter cellular GSH levels in regular HepG2 cells and P-gp-overexpressing HepG2-DR cells. Since SCH and GOM may reverse MDR by impeding the activity of P-gp, they could affect cellular drug metabolism in addition to drug uptake (60).

Approaches to assessing drug-herb interactions

Overlapping substrate specificities of these proteins result in complex and sometimes perplexing pharmacokinetic profiles for multidrug regimens. Saquinavir undergoes extensive first-pass metabolism by the major metabolising isozyme CYP3A4.

Ketoconazole (a selective CYP3A4 inhibitor) inhibited the formation of all saquinavir metabolites. Saquinavir inhibits the metabolism of terfenadine and causes formation of 6-β-hydroxylation products of testosterone, indicating its specificity towards CYP3A4 (61). Ritonavir metabolism involves both CYP3A4 and CYP2D6 and it significantly inhibits the metabolism of CYP3A4 and CYP2D6 substrates such as nifedipine and dextromethorphan, respectively, when administered concomitantly (62). The major isozyme responsible for indinavir metabolism is CYP3A4. However, nelfinavir metabolism involves several isozymes, including CYP3A4, CYP2C19 and CYP2D6, and possibly CYP2C9 and CYP2E1 (63–65).

Role of efflux proteins in drug-herb interactions

MDR proteins play an important role in protecting cells against cytotoxic drugs (66). It has become apparent that MDR gene products also need to be considered in drug absorption. The MDR phenotype in tumours is associated with overexpression of ABC efflux pumps termed MDR proteins. P-gp (MDR1, ABCB1) is considered a versatile xenobiotic pump. The substrate specificity and tissue distribution of MDR proteins vary widely (67, 68). MRP1 is almost ubiquitously expressed, whereas P-gp expression is more restricted to tissues involved in absorption and secretion. Although P-gp was initially discovered in cancer cells, it was later observed that a number of normal tissues, such as intestine, liver, kidney, pancreas and adrenal glands, constitutively express P-gp. High levels of P-gp are expressed in BBB and the choroid plexus (69–71). All multidrug transporters are localised predominantly in the plasma membrane. In polarised cells, P-gp is localised on the apical (luminal) membrane surface (e.g., in intestinal epithelial cells, proximal tubules in kidney, and the biliary canalicular membrane of hepatocytes).

P-Glycoprotein-mediated drug-herb interactions

In the last 5 years, SJW has been among the most popular herbal remedies. Indinavir and saquinavir concentrations were reduced to 57% and 51% by SJW and garlic, respectively (72) and it is now clear that such interactions can alter the outcome of anti-HIV therapy. Such reductions in indinavir and saquinavir exposure may lead to the development of drug-resistant strains and may cause treatment failure in HIV patients. The reductions were attributed to induction of P-gp and CYP3A4 expression by SJW (73). Several flavanoids, which are one of the primary classes of active constituents in most herbs, seem to be capable of modulating P-gp. SJW induces P-gp (74, 75). Quercetin and kaempferol also induce P-gp (76–78). Ten-day treatment with pure herbal constituents (hypericin, kaempferol, quercetin and silibinin) can cause a significant increase in P-gp mRNA expression (47). Quercetin and kaempferol have also been reported to induce P-gp (76–78). Also, four- to seven-fold elevation in P-gp expression in LS180 intestinal carcinoma cells was caused by hypericin or SJW treatment (75). In vivo studies also indicated that long-term (14 days) exposure to SJW led to higher Mdr1 expression in rat intestine (74). SJW taken at 900 mg/day for 14 days resulted in a 1.4-fold increase in P-gp expression in healthy volunteers (74). In another clinical study, a 4.2-fold increase in P-gp expression

was observed in human peripheral blood lymphocytes after treatment with SJW for 16 days.

Since HIV protease inhibitors, macrolide antibiotics, azole antifungals and herbals are substrates of same the metabolising enzymes and transporters, herbal agents can adversely affect the course of treatment of HIV and other opportunistic infections. In vitro laboratory studies revealed that concomitant administration of erythromycin and SJW and/or ketoconazole can enhance erythromycin oral absorption. Depending on the mechanism by which herbal compounds interact with CYP and efflux proteins, these agents can decrease plasma levels of anti-HIV drugs, thus possibly reducing efficacy and enhancing the risk of developing drug resistance. Conversely, such compounds can increase blood levels of antiretroviral drugs, thus placing patients at greater risk of serious side effects. Herbs with reported effects on CYP3A4 and P-gp include SJW, garlic, ginseng, milk thistle and skullcap.

Uthai et al. studied the P-gp-modulating effects of extracts prepared from rhizomes of *Curcuma longa* and *Curcuma* sp. Khamin-oi (79). The roles of major curcuminoids, including curcumin, demethoxycurcumin and bisdemethoxycurcumin, in reversing P-gp function were also evaluated. Caco-2 was used as an in vitro intestinal model to study the effects on efflux transporters. LLC-PK1 and LLC-GA5-COL 300 (LLC-PK1 cells overexpressing human P-gp) cell lines were used to confirm the role in P-gp function. The results revealed that curcumin and demethoxycurcumin can inhibit P-gp, but bisdemethoxycurcumin may modulate the function of other efflux transporters such as MRP. Taken together, the data provide information on the impact of *Curcuma longa* and *Curcuma* sp. Khamin-oi on the pharmacokinetics of orally administered drugs that are P-gp substrates (79).

Using MDR1-transfected MDCK cells expressing high amounts P-gp, Patel et al. demonstrated that ritonavir uptake was enhanced five- to eight-fold in the presence of 100 μM pure herbal constituents (allicin, kaempferol, quercetin and hypericin) (47). In other in vitro studies, we observed a several-fold increase in erythromycin and ritonavir uptake in the presence of SJW extract. An inhibitory effect of SJW on MRP-mediated ritonavir efflux was also noted. These results demonstrate that simultaneous administration of SJW and drugs such as erythromycin, saquinavir and ritonavir can enhance drug absorption, primarily because of competitive inhibition of P-gp- and MRP-mediated efflux. These in vitro results demonstrate the inhibitory properties of herbs on P-gp-mediated efflux after short-term exposure. Thus, all these in vitro and in vivo studies have demonstrated that SJW, upon chronic exposure induces intestinal P-gp resulting in reduced intestinal absorption possibly through enhanced drug efflux.

El-Readi et al. investigated nine naturally occurring compounds isolated from *Citrus jambhiri* Lush and *Citrus pyriformis* Hassk (Rutaceae) for their potential to modulate P-gp activity in the MDR human leukaemia cell line CEM/ADR5000 (80). Limonin, deacetylnomilin, hesperidin, neohesperidin, stigmasterol and β-sitosterol-O-glucoside inhibited efflux of the P-gp substrate rhodamine 123 in a concentration-dependent manner. Some of these compounds were more active than verapamil, which was used as a positive control. Treatment of drug-resistant Caco-2 cells with the most active *C. jambhiri* and *C. pyriformis* compounds increased their sensitivity to doxorubicin and completely reversed doxorubicin resistance, in agreement with the decrease in P-gp activity. Limonin was the most potent P-gp inhibitor; when applied at a non-toxic concentration of 20 μM, it significantly enhanced doxorubicin cytotoxicity 2.98-fold ($p<0.001$) and 2.2-fold ($p<0.001$) in Caco-2 and CEM/ADR5000 cells, respectively. These isolated *Citrus* compounds could be considered good candidates for the development of novel P-gp reversal agents to enhance the accumulation and efficacy of chemotherapy agents (80).

Coordinated functions of efflux and metabolism

In addition to oxidative metabolism, conjugation reactions may play an important role in the detoxification of xenobiotics in the small intestine. Several drug molecules are effluxed into the intestinal lumen after conjugation to a glucuronide or sulfate moiety. The transport system responsible for cellular extrusion of conjugated metabolites and organic anions has recently been characterised (81). Herbs can pharmacokinetically act as inhibitors or inducers when anti-HIV medication or another conventional therapeutic is taken simultaneously. An understanding of the increased or decreased bioavailability of one drug in the presence of herbal products might greatly aid in the design of appropriate drug regimens.

Co-administration of herbal and therapeutic drugs can lead to increased absorption due to inhibition of P-gp-mediated efflux and CYP-mediated metabolism, with potential toxic effects. By contrast, chronic administration of certain herbal products (SJW, garlic, etc.) can enhance the production of MDR proteins (P-gp) and CYP enzymes, resulting in lower bioavailability and subtherapeutic plasma concentrations of drugs. Finally, this process can lead to the emergence of drug resistance. P-gp is regarded as one of the major factors in the development of cellular drug resistance. Upregulation of CYP enzymes in response to herbal products may also play an important role in lowering drug concentrations. The close chromosomal location of P-gp and CYP3A4 genes, their expression in mature enterocytes and similar substrate specificities suggest that the function of these two proteins may be complementary in nature and may form a coordinated intestinal barrier (82).

This view was further validated by the stimulatory effect of SJW on PXR, which regulates many CYP isoforms in rats (83). A recent study also demonstrated that SJW induces CYP3A4 by negatively acting on interleukin-6, which is known to inhibit PXR, which may in turn be involved in the expression of the CYP class of enzymes (84). Molecular mechanisms of induction of P-gp and drug-metabolising enzymes are still being investigated. The roles of two members of the nuclear receptor superfamily of transcription factors, PXR and CAR, have recently been discovered.

References

1. Agoram B, Woltosz WS, Bolger MB. Predicting the impact of physiological and biochemical processes on oral drug bioavailability. Adv Drug Deliv Rev 2001;50:S41–67.
2. Zhang Y, Benet LZ. The gut as a barrier to drug absorption; combined role of cytochrome P450 3A and P-glycoprotein. Clin Pharmacokinet 2001;40:159–63.
3. Watkins PB, Wrighton SA, Schuetz EG, Molowa DT, Guzelian PS. Identification of glucocorticoid inducible cytochrome P-450 in intestinal mucosa of rats and man. J Clin Invest 1987;80: 1029–36.
4. Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, Watkins PB. Identification of rifampin-inducible P450 3A4 in human small bowel enterocytes. J Clin Invest 1992;90:1871–8.
5. Kolars JC, Awni WM, Watkins PB, Merion RM. First pass metabolism of cyclosporin by gut. Lancet 1991;338:1488–90.
6. Zhang Y, Guo X, Lin ET, Benet LZ. Overlapping substrate specificities of cytochrome P450 3A and P-glycoprotein for a novel cysteine protease inhibitor. Drug Metab Dispos 1998;26:360–6.
7. Benet LZ, Cummins CL. The drug efflux-metabolism alliance: biochemical aspects. Adv Drug Del Rev 2001;50:S3–11.
8. Tandon VR, Kapoor B, Bano G, Guptha S, Gillani Z, Kour D. P-Glycoprotein: pharmacological relevance. Indian J Pharmacol 2006;38:13–24.
9. Wagner D, Spahn-Langguth H, Hanafy A, Koggel A, Langguth P. Intestinal drug efflux: formulation and food effects. Adv Drug Del Rev 2001;50:S13–31.
10. Tsuji A, Tamai I. Carrier-mediated intestinal transport of drugs. Pharm Res 1996;13:963–77.
11. Chan LM, Lowes S, Hirst BH. The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. Eur J Pharm Sci 2004;21:25–51.
12. Higgins CF, Callaghan R, Linton KJ, Rosenberg MF, Ford RC. Structure of the multidrug resistance P-glycoprotein. Sem Cancer Biol 1997;8:135–42.
13. Udeta K, Taguchi H, Morishima M. How does P-glycoprotein recognize its substrates? Sem Cancer Biol 1997;8:151–15.
14. Dresser GK, Bailey DG. The effects of fruit juices on drug disposition: a new model for drug interactions. Eur J Clin Invest 2003;33:10–6.
15. Varma MV, Ashokraj Y, Dey CS, Panchagula R. P-Glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. Pharmacol Res 2003;48:347–59.
16. Udeta K, Taguchi H, Morishima M. How does P-glycoprotein recognize its substrates? Sem Cancer Biol 1997;8:151–9.
17. Engman H. Intestinal barriers to oral drug absorption: cytochrome P450 3A and ABC-transport proteins. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 2003:296.
18. Deferme S, Van Gelder J, Augustijns P. Inhibitory effect of fruit extracts on p-glycoprotein related efflux carriers: an in vitro screening. J Pharm Pharmacol 2002;54:1213–9.
19. Loo TW, Bartlett C, Clarke DM. Substrate-induced conformational changes in the transmembrane segments of human P-glycoprotein. Direct evidence for the substrate induced mechanism for drug binding. J Biol Chem 2003;274:13603–6.
20. Stephens RH, O'Neill CA, Warhurst A, Carlson GL, Rowland M. Kinetic profiling of P-glycoprotein-mediated drug efflux in rat and human intestinal epithelia. J Pharmacol Exp Ther 2001;296:584–91.
21. Gombar VK, Polli JW, Humphreys JE, Wring SA, Serabjit-Singh CS. Predicting P-glycoprotein substrates by a quantitative structure activity relationship model. J Pharm Sci 2004;93: 957–68.
22. Lin JH. Drug-drug interactions mediated by inhibition and induction of P-glycoprotein. Adv Drug Del Rev 2003;55:53–81.
23. Anderle P, Huang Y, Sadee W. Intestinal membrane transport of drugs and nutrients: genomics of membrane transporters using expression microarrays. Eur J Pharm Sci 2004;21:17–24.
24. Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, Schmiedlen-Ren P, et al. Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporin. Clin Pharmacol Ther 1997;62:248–60.
25. Johnson BM, Charman WN, Porter CJ. An in vitro examination of the impact of polyethylene glycol 400, Pluronic P85, and vitamin E D-alpha-tocopheryl polyethylene glycol 1000 succinate on P-glycoprotein efflux and enterocyte-based metabolism in excised rat intestine. AAPS PharmSci 2002;4:1–11.
26. Doppenschmitt S, Spahn-Langguth H, Regardh CG, Langguth P. Role of P-glycoprotein-mediated secretion in absorptive drug permeability: an approach using passive membrane permeability and affinity to P glycoprotein. J Pharm Sci 1999;88:1067–72.
27. Lin JH. How significant is the role of P-glycoprotein in drug absorption and brain uptake? Drugs Today 2004;40:5–22.
28. Su SF, Huang JD. Inhibition of the intestinal digoxin absorption and exorption by quinidine. Drug Metab Dispos 1995;24: 142–7.
29. Verschraagen M, Koks CH, Schellens JH, Beijnen JH. P-Glycoprotein system as a determinant of drug interactions: the case of digoxin-verapamil. Pharmacol Res 1999;40:301–6.
30. Salphati L, Benet LZ. Effect of ketoconazole on digoxin absorption and disposition in rat. Pharmacology 1998;56:308–13.
31. Gramatte T, Oertel R. Intestinal secretion of intravenous talinolol is inhibited by luminal R-verapamil. Clin Pharmacol Ther 1999;66:239–45.
32. Khaliq Y, Gallicano K, Venance S, Kravcik S, Cameron DW. Effect of ketoconazole on ritonavir and saquinavir concentrations in plasma and CSF from patients infected with HIV. Clin Pharmacol Ther 2000;68:637–46.
33. Eichelbaum M, Greiner B, Fritz P, Kreichgauer H-P, von Richter O, Zundler J, et al. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. J Clin Invest 1999;104: 147–52.
34. Hunter J, Hirst BH. Intestinal secretion of drugs. The role of P-glycoprotein and related drug efflux systems in limiting oral drug absorption. Adv Drug Del Rev 1997;25:129–57.
35. Watkins PB, Wrighton SA, Schuetz EG, Molowa DT, Guzelian PS. Identification of glucocorticoid-inducible cytochromes P-450 in intestinal mucosa of rats and man. J Clin Invest 1987;80: 1029–36.
36. Clarke SJ. Human cytochromes P450 and their role in metabolism-based drug-drug interactions. In: Rodrigues AD, editor. Drug-drug interactions. New York: Marcel Dekker, 2002:55–88.
37. de Groot MJ, Ekins S. Pharmacore modeling of cytochromes P450. Adv Drug Del Rev 2002;54:367–83.
38. Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contributions to variable human CYP3A-mediated metabolism. Adv Drug Del Rev 2002;54:1271–94.
39. Wacher VJ, Silverman JA, Zhang Y, Benet LZ. Role of P glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. J Pharm Sci 1998;87:1322–30.
40. Schuetz EG, Beck WT, Schuetz JD. Modulators and substrates of P-glycoprotein and cytochrome P450 3A coordinately upregulate these proteins in human colon carcinoma cells. Mol Pharmacol 1996;49:311–8.

41. Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T, Waxman DJ. Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. J Biol Chem 1986;261:5051–60.
42. Parkinson A. An overview of current cytochrome P450 technology for assessing the safety and efficacy of new materials. Toxicol Pathol 1996;24:48–57.
43. Bailey DG, Malcolm J, Arnold O, Spence JD. Grapefruit juice-drug interactions. Br J Clin Pharmacol 1998;46:101–10.
44. Fuhr U. Drug interactions with grapefruit juice. Extent, probable mechanism and clinical relevance. Drug Saf 1998;18:251–72.
45. Guo LQ, Taniguchi M, Xiao YQ, Baba K, Ohta T, Yamazoe Y. Inhibitory effect of natural furanocoumarins on human microsomal cytochrome P450 3A activity. Jpn J Pharmacol 2000; 82:122–9.
46. Obach RS. Inhibition of human cytochrome P450 enzymes by constituents of St. John's wort, an herbal preparation used in the treatment of depression. J Pharmacol Exp Ther 2000;294:88–95.
47. Patel J, Buddha B, Dey S, Pal D, Mitra AK. In vitro interaction of the HIV protease inhibitor ritonavir with herbal constituents: changes in Pgp and CYP3A4 activity. Am J Ther 2004;11:262–77.
48. Markowitz JS, DeVane CL, Boulton DW, Carson SW, Nahas Z, Risch SC. Effect of St. John's wort (Hypericum perforatum) on cytochrome P-450 2D6 and 3A4 activity in healthy volunteers. Life Sci 2000;66:133–9.
49. Bray BJ, Perry NB, Menkes DB, Rosengren RJ. St. John's wort extract induces CYP3A and CYP2E1 in the Swiss Webster mouse. Toxicol Sci 2002;66:27–33.
50. Zou L, Harkey MR, Henderson GL. Effects of herbal components on cDNA-expressed cytochrome P450 enzyme catalytic activity. Life Sci 2002;71:1579–89.
51. Karch AM. The grapefruit challenge: the juice inhibits a crucial enzyme, with possibly fatal consequences. Am J Nurs 2004;104:33–5.
52. Hidaka M, Okumura M, Fujita K, Ogikubo T, Yamasaki K, Iwakiri T, et al. Effects of pomegranate juice on human cytochrome P450 3a (cyp3a) and carbamazepine pharmacokinetics in rats. Drug Metab Dispos 2005;33:644–8.
53. Pom: the new juice in town. Consumer Reports 2005;July:1–4.
54. Bailey DG, Malcolm J, Arnold O, Spence JD. Grapefruit juice-drug interactions. Br J Clin Pharmacol 1998;46:101–10.
55. Shravan KY, Ramesh G, Adukondalu D, Vamshi VY, Chinna RP, Shiva KR, et al. Effect of silymarin and pomegranate pretreatment on intestinal transport of buspirone across rat intestine. Acta Pharm Sci 2009;51:289–96.
56. Fugh BA, Ernst E. Herb-drug interactions: review and assessment of report reliability. Br J Clin Pharmacol 2001;52:587–95.
57. Piscitelli SC, Burstein AH, Welden N, Gallicano KD, Falloon J. The effect of garlic supplements on the pharmacokinetics of saquinavir. Clin Infect Dis 2002;34:234–8.
58. Berginc K, Trontelj J, Kristl A. The influence of aged garlic extract on the uptake of saquinavir and darunavir into HepG2 cells and rat liver slices. Drug Metab Pharmacokinet 2010;25:307–13.
59. Hajda J, Rentsch KM, Gubler C, Steinert H, Steiger B, Fattinger K. Garlic extract induces intestinal P-glycoprotein, but exhibits no effect on intestinal and hepatic CYP3A4 in humans. Eur J Pharm Sci 2010;41:729–35.
60. Wana CK, Tse AK, Yu ZL, Zhu GY, Wang H, Fong DW. Inhibition of cytochrome P4503A4 activity by schisandrol A and gomisin A isolated from Fructus schisandrae chinensis. Phytomedicine 2010;17:702–5.
61. Vella S, Floridia M. Saquinavir. Clinical pharmacology and efficacy. Clin Pharmacokinet 1998;34:189–201.
62. Hsu A, Granneman GR, Bertz RJ. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet 1998;35:275–91.
63. Li X, Chan WK. Transport, metabolism and elimination mechanisms of anti-HIV agents. Adv Drug Deliv Rev 1999;39:81–103.
64. Malaty LI, Kuper JJ. Drug interactions of HIV protease inhibitors. Drug Saf 1999;20:147–69.
65. Williams GC, Sinko PJ. Oral absorption of the HIV protease inhibitors: a current update. Adv Drug Deliv Rev 1999;39:211–38.
66. Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 2000;92:1295–302.
67. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976;455:152–62.
68. Ueda K, Cardarelli C, Gottesman MM, Pastan I. Expression of a full-length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicin, and vinblastine. Proc Natl Acad Sci USA 1987;84:3004–8.
69. Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular and pharmacological aspects of multidrug transporter. Annu Rev Pharmacol Toxicol 1999;39:361–98.
70. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 1993; 62:385–427.
71. Lin JH, Yamazaki M. Role of P-glycoprotein in pharmacokinetics: clinical implications. Clin Pharmacokinet 2003;42:59–98.
72. Piscitelli SC, Burstein AH, Chaitt D, Alfaro RM, Falloon J. Indinavir concentrations and St. John's wort. Lancet 2000;355:547–8.
73. Roby CA, Anderson GD, Kantor E, Dryer DA, Burstein AH. St. John's wort: effect on CYP3A4 activity. Clin Pharmacol Ther 2000;67:451–7.
74. Durr D, Stieger B, Kullak-Ublick GA, Rentsch KM, Steinert HC, Meier PJ, et al. St. John's wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. Clin Pharmacol Ther 2000;68:598–604.
75. Perloff MD, Von Moltke LL, Stormer E, Shader RI, Greenblatt DJ. Saint John's wort: an in vitro analysis of P-glycoprotein induction due to extended exposure. Br J Pharmacol 2001;134:1601–8.
76. Chieli E, Romiti N, Cervelli F, Tongiani R. Effects of flavonols on P-glycoprotein activity in cultured rat hepatocytes. Life Sci 1995;57:1741–51.
77. Scambia G, Ranelletti FO, Panici PB, De Vincenzo R, Bonanno G, Ferrandina G, et al. Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast-cancer cell line: P-glycoprotein as a possible target. Cancer Chemother Pharmacol 1994;34:459–64.
78. Shapiro AB, Ling V. Effect of quercetin on Hoechst 33342 transport by purified and reconstituted P-glycoprotein. Biochem Pharmacol 1997;53:587–96.
79. Ampasavate C, Sotanaphun U, Phattanawasin P, Piyapolrungroj N. Effects of Curcuma spp. on P-glycoprotein function. Phytomedicine 2010;17:506–12.
80. El-Readi MZ, Hamdan D, Farrag N, El-Shazly A, Wink M. Inhibition of P-glycoprotein activity by limonin and other secondary metabolites from Citrus species in human colon and leukaemia cell lines. Eur J Pharm Sci 2010;626:139–45.
81. Leslie EM, Deeley RG, Cole SP. Toxicological relevance of the multidrug resistance protein 1 MRP1 (ABCC1) and related transporters. Toxicology 2001;167:3–23.

82. Mitra AK, Patel J. Strategies to overcome simultaneous P-glycoprotein mediated efflux and CYP3A4 mediated metabolism of drugs. Pharmacogenomics 2001;2:401–15.
83. Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, et al. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. Proc Natl Acad Sci USA 2000;97:7500–2.
84. Fiebich BL, Holli A, Lieb K. Inhibition of substance P-induced cytokine synthesis by St. John's wort extracts. Pharmacopsychiatry 2001;34:S26–8.