ELSEVIER

Drug–drug interactions affected by the transporter protein, P-glycoprotein (ABCB1, MDR1) I. Preclinical aspects

Adorjan Aszalos

Laboratory of Cell Biology, 37 Convent Drive, Room 2108, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, United States

When certain coadministered drugs interfere with the transport of one another at the P-glycoprotein (Pgp) level, we observe the phenomenon of ''drug–drug interactions''. We describe here the physical and biochemical ways that drugs react with Pgp, the induction of Pgp by xenobiotics and the polymorphisms of Pgp related to drug–drug interactions. We also describe methods that can be used to detect potential drug–drug interactions. Among the widely used methods are flow cytometry, cell culture with polarized expression of Pgp, liposome and inside-out membrane preparations, *ex vivo* studies, *in silico* calculations, cDNA microarrays, *in vivo* mouse models, positron emission tomography (PET), and nuclear magnetic resonance (NMR) methods with live animals.

Introduction

Transport proteins, especially Pgp, have well-defined transport functions in the metabolism of humans. Externally introduced xenobiotics can also be transported by Pgp and other transporters. The modulation of the function of Pgp by xenobiotics can upset the natural metabolism of humans. In addition, when several drugs are coadministered, they may cause unexpected outcomes in the modulation of Pgp. Interactions at the Pgp level by several chemotherapeutics (drug–drug interactions) may be defined as modulation of the pharmacology of a Pgp-modifier drug by another coadministered Pgp-modifier drug. These drug–drug interactions have been categorized before as intentional modulation of the function of Pgp, resulting in drug–drug interaction and unintentional modulation of Pgp, resulting in unexpected toxic drug–drug interactions [1]. Pgp is intentionally modulated when a drug cannot be delivered to a site in a patient because the drug is a substrate of Pgp. A pharmacologically unrelated compound may be introduced, in order to suppress the function of Pgp, thereby permitting the entry of the chemotherapeutically active drug to the desired site. Such intentional modulations are practiced in cancer chemotherapy to facilitate entry of a drug into Pgp-expressing tumor cells, to allow orally administered Pgp substrate drugs to be absorbed through the intestines, and sometimes to allow drugs to enter the CNS compartment through the blood–brain barrier. Unintentional modulation of Pgp can occur when a coadministered drug alters the function of Pgp and the pharmacokinetics and/or the pharmacodynamics of the first drug are changed, causing unwanted side effects.

In vivo assessment of drug–drug interactions can be complicated in humans, especially if the administered drug(s) or metabolite(s) are substrates of several of the ABC-type cassette pumps (efflux pumps, transporters) located in many organs. A description of the function of each of these pumps is beyond the scope of this review. For summary reviews, one may consult the publications of Szakacs *et al.* [2], Mizuno *et al.* [3], Schinkel and Jonker [4], and Dean *et al.* [5]. Testing of a candidate drug for modulation of an efflux pump alone, or in combination with another, intended for possible simultaneous use, is possible only if appropriate *in vitro* or *in vivo* models and methods exist. For many efflux pumps, those are nonexistent. Fortunately they do exist for Pgp, as we elaborate below. The primary structure of Pgp as embedded in the plasma membrane of cells is shown in Figure 1. Figure 2 is a diagram depicting the commonly understood efflux action of Pgp.

Influence of molecular, biochemical and biophysical characteristics of Pgp on drug–drug interactions

The Pgp (ABCB1, MDR1) gene is located on chromosome 7 in humans and encodes 1280 amino acids [6]. It functions as an ATP-driven efflux transporter and transports its substrates from intracellular to extracellular domains. It has 12 transmembrane

Corresponding author: Aszalos, A. (aszalosa@mail.nih.gov)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2007.07.022

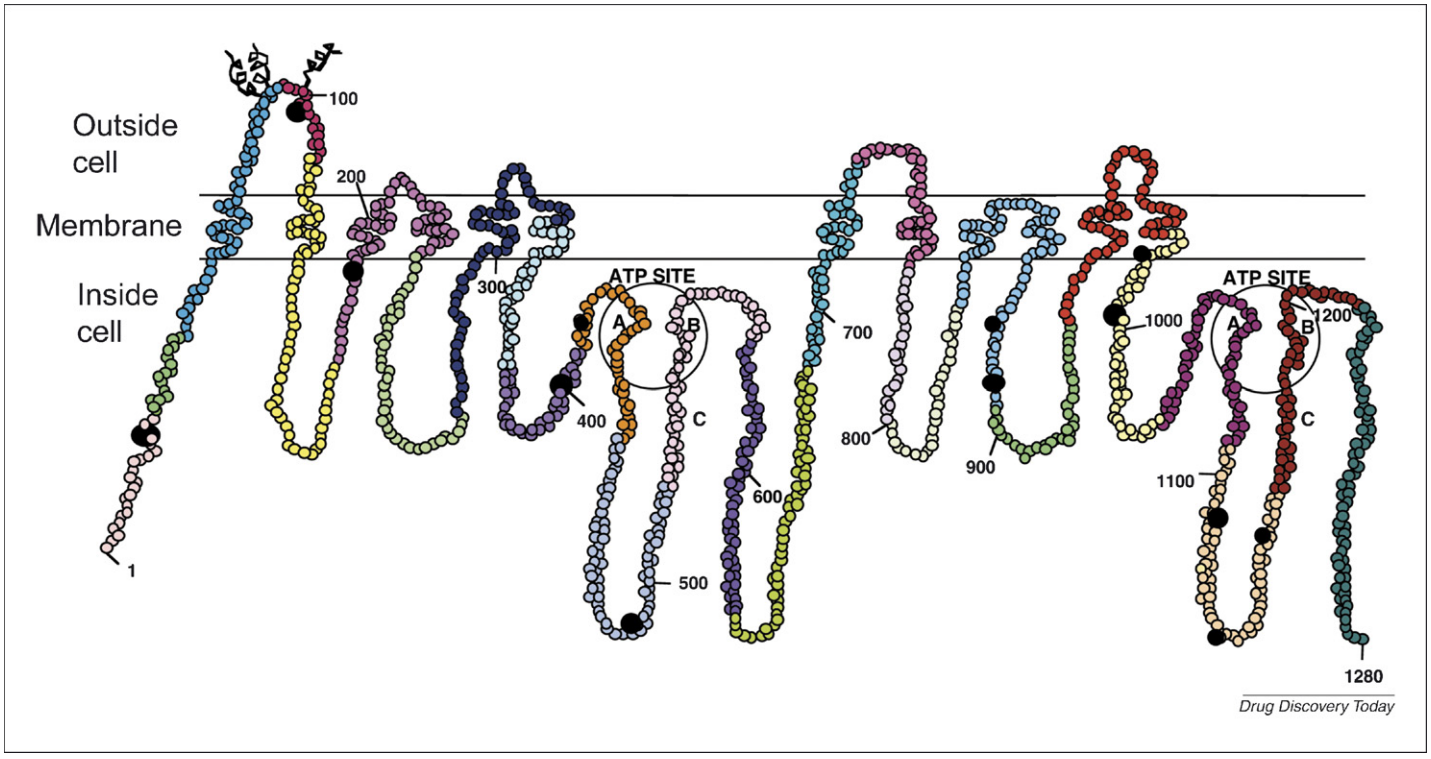

FIGURE 1

Schematic representation of the primary structure of Pgp as embedded in the plasma membrane. The molecule contains 1280 amino acids spanning 28 exons (each exon sequence shown in a different color). Black dots show the location of some of the identified SNPs (modified from: Ambudkar, S.V. *et al.* (2003) *Oncogene* 22, 7468–7485; reprinted with permission of author).

sequences, of which the 5, 6, 11 and 12 sequences seem to be responsible for its transport function. Pgp is mainly responsible for limiting entry of orally administered substrates (drugs) into the body, for excretion of metabolites into the bile and through the kidney and limits entry of drugs into the CNS, testis, blood cells, and through the placenta. Informative reviews on the distribution of Pgp in humans can be found in the articles of Lin [7] and Mizuno *et al.* [3].

The binding of substrates that are modulators of Pgp involves a complex mechanism. This complexity is due to the fact that Pgp has more than one binding site, and as a result of conformational changes caused by the binding of ATP to the molecule. It is possible

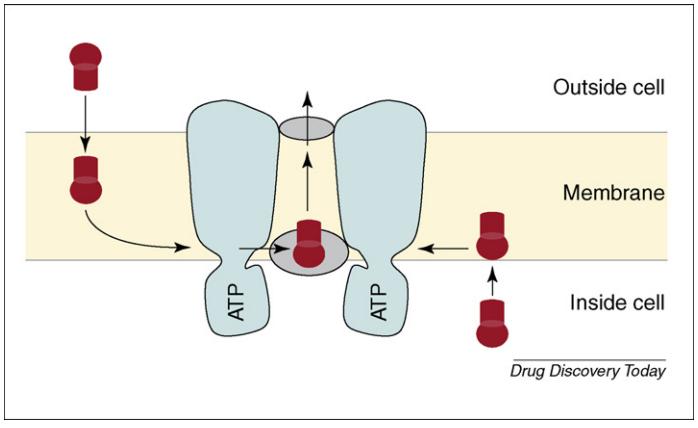

FIGURE 2

Schematic representation of the function of Pgp. Red objects represent substrates (adapted from: Gottesman, M.M. and Pastan, I. (1993) *Annu. Rev. Biochem.* 62, 385–427).

to block transport of a substrate by another substrate (a modulator), but the reverse is not necessarily true. For example, transport of digoxin by Pgp in LLC-PK cells was inhibited by cyclosporine, but digoxin did not block cyclosporine transport [8]. Besides blocking transport due to drug–drug interactions, stimulation of transport is also possible due to drug–drug interactions. For example, verapamil-induced ATP activity was stimulated by progesterone, propranolol, diltiazem, and amitriptyline, all substrates of Pgp [9].

The above studies *inter alia* indicate that competitive, noncompetitive, and cooperative binding is possible for substrates of Pgp. With competitive modulation of Pgp activity, substrates bind at the same location, with noncompetitive modulation the substrates bind at different locations on Pgp and with cooperative modulation, one substrate binding to Pgp induces conformational change, enhancing the affinity of a second substrate to Pgp [10].

It is also possible to categorize Pgp substrates and modulators by the physical way they interact with Pgp. Hwang *et al.* [11] have shown by electron spin resonance studies that cremophor modulates the activity of Pgp by altering the biophysical conditions of the surrounding plasma membrane. Another type of modulator, cyclosporine, is a substrate of Pgp, but also alters the biophysical status of the plasma membrane. The third type of modulator, which includes drugs such as verapamil, modulates the activity of Pgp, but has no effect on the plasma membranes of cells.

Another phenomenon that complicates the assessment of drug–drug interactions is the fact that induction of Pgp by different drugs, and also by St John's Wort extract, can occur in many cell lines and animal and human tissues [12]; in this respect, we will limit our comments here to human physiology only. It was shown that the ligand-binding site of the pregnane X receptor, SXR, is

responsible for induction of CYP3A metabolizing enzyme, as well as of Pgp [13]. Another study demonstrated that oral administration of St John's Wort extract, to healthy volunteers for 14 days, elevated Pgp expression 1.4 times and CYP3A4 expression 1.5 times in the duodenum. Results of this study suggested that these two proteins are co-regulated in humans by SXR. It could also be shown that dexamethasone, a known CYP3A4 inducer, besides this enzyme also induces Pgp in cultured human hepatoma cells [14]. These studies correlate well with other cell culture and animal studies indicating that CYP3A4 and Pgp are coordinately and dose dependently induced through SXR [15]. It is also interesting to note that the MDR gene is mapped to chromosome locus 7q21.1 and the CYP3A4 gene is at 7q22.1 [16,17]. However, no coordinated expression of Pgp and CVP3A4 was found in the human small intestine [18]. As an extension of studying the induction of Pgp via SXR, Ekin and Erickson [19] calculated ligand binding to this receptor *in silico*. This method could differentiate between potent and poor activators, and may be used for drug designs and possibly to study drug–drug interactions.

About 50 single nucleotide polymorphisms have been discovered in Pgp (www.ncbi.nlm.nih.gov/SNP/GeneGt.cgi?geneID=5243). These polymorphisms can influence, somewhat, the expression of Pgp in cells and its substrate specificity; however, nonfunctional Pgp polymorphisms (due to an as yet undiscovered polymorphism) may influence the extent of drug–drug interactions in different polymorphic human populations. For some polymorphic Pgp, no difference of pharmacokinetics has been measured (see below) but interactions between new drug candidates and other human polymorphic populations have not been well characterized yet. In this respect, one of the significant problems is to predict brain-drug levels. For this reason, it is advisable to test the pharmacological parameters of new candidate drugs in combination with drugs that may be used in the clinic.

The complex interactions of drugs with Pgp, the possible elevation of the expression of Pgp by certain drugs and food-additives and the existing polymorphisms of Pgp make it difficult to predict the possible drug–drug interactions of a newly developed drug. Therefore, rigorous testing is necessary, primarily *in vitro* tests and membrane preparations, and with some animal models. In clinical trials, polymorphisms of Pgp in various human populations should be taken into account.

Methods for detection of drug–drug interactions

We will now summarize some of the preclinical methods for detection of drug–drug interactions at the Pgp level.

Cell-based assays

Several review articles have been written on methods for *in vitro* evaluation of potential drug–drug interactions at the Pgp level. One review by Hochman *et al.* [20] discusses the use of cells expressing Pgp in a polarized fashion, such as Caco-2, MDCK, or LLC-PK1 cells. These cells express Pgp on only one side, the apical side. They are attached to specific membranes on Transwell devices and are grown to confluency. Transport of drug candidates can then be studied in both directions. If the potential drug cannot penetrate, or can penetrate to only a limited extent, from the Pgp-expressing side (the apical side) but can penetrate from the other side (the basal side) then it is a substrate or inhibitor of Pgp. Using two drugs in this system, the kinetics of modulation of transport and extent of drug–drug interactions at the Pgp level can be assessed.

Fast and reproducible results can be obtained by studying the modulation of the retention of a fluorescent Pgp substrate such as rhodamine 123, or a fluorescent drug-substrate, such as daunorubicin, by the drug to be tested, using flow cytometric methods [21,22]. If the drug to be tested causes more fluorescent molecules to be retained in cells, that is an indication that it is a substrate or modulator of Pgp. For both of the above methods, quantitative definitions or modulator category and values can be assigned, depending on the mode of interaction of the substrate with Pgp [23,24]. These methods can be used to assess modulation of the activity of Pgp by two or more drugs in combination.

Artificial membrane assays

An alternative to the cell-based flow cytometry method is Pgp-incorporated liposome preparations. Pgp is purified from cells and is incorporated into liposomes. Usually, ATP utilization is measured to assess Pgp activity. The advantage of this type of system is that it eliminates many variables. However, it does not necessarily reflect the actual function of Pgp embedded in the plasma membranes of cells [25].

An assay based on inside-out membrane vesicles, prepared from Pgp-expressing and nonexpressing cells, is a clinically relevant method [26]. Accumulation of drugs or interaction of drugs with Pgp can be deduced by a radiotracer assay or by blocking Pgp activity using a known substrate or by removing ATP from the assay system. ATP provides the driving energy for the function of Pgp. This method can predict the clinical behavior of drugs very realistically in some cases. However, nonspecific drug binding makes evaluations more difficult, especially when testing higher doses of a candidate drug [25,27].

Ex vivo cell-based assay

Another clinically relevant assay published by Szabo *et al.* [28] is based on human blood cells expressing Pgp, tested *ex vivo*. This assay is most relevant for drugs intended for treatment of hematopoietic diseases. Patients with acute myeloid leukemia (AML) have peripheral blood lymphocytes that express Pgp. These cells, prepared from the individual patients' blood, were incubated with antipsychotic drugs and uptake of the anticancer drug daunorubicin into the cells was assessed by flow cytometry. Among the tested antipsychotic drugs were amitriptyline, fluphenazine, maprotiline, desipramine, and haloperidol. All the tested antipsychotic drugs and daunorubicin are Pgp substrates or modifiers and, therefore, drug–drug interactions were predicted to occur when they were used in combination. Among these drugs, maprotilin blocked Pgp the most, resulting in almost double the uptake of daunorubicin into the lymphocytes. While the combined interaction of these drugs with the anticancer drug daunorubicin is advantageous for the effective treatment of the AML cells, more daunorubicin is retained in the lymphocytes. But the possible increase of penetration of all these drugs through the blood–brain barrier could be disadvantageous.

In silico testing

Several studies have assessed the interaction of substrates with Pgp *in silico* [29,30]. One of them was based on the ability of a molecule

to form H-bonds (two sites separated by 4.6 Å, or three sites separated by 2.5 Å). The conclusion of this study was that if two substrates are present, the substrate with higher potential to form H-bonds will be the inhibitor. They found that competition for interaction of substrates with Pgp is also influenced by the lipophilicity of the substrates. Another *in silico* model was advanced for the understanding of structure–activity relations by Stouch and Gudmousson [31]. These systems may eventually be used for prediction of drug–drug interactions, especially when Pgp is crystallized together with a substrate. Such efforts are in progress.

Analysis of gene expression in cancer cells may also contribute to the possible selection of the best combinations of drugs for treatment of a cancer patient. It was shown by Kudoh *et al.* [32] by cDNA microarray, that a drug treatment-induced gene expression profile could be analyzed. They were able to show that transient doxorubicin treatment of MCF-7 cells altered the gene expression intensity of 19 genes. When cancer treatment protocols call for multiple drug treatments, potentially altered gene expression profiles could be determined for individual drug treatments and for optimal treatment combinations. Such analyses may shed light on unwanted drug–drug interactions.

Testing for synergistic modulation of Pgp function

Increasing doses of candidate drugs are also tested in different systems for evaluation of their potential to modulate Pgp. It is possible that the clinically efficacious dose of the candidate drug affects the function of Pgp only very moderately, but at a higher dose there may be significant interaction with Pgp. Such a candidate drug may cause significant drug–drug interactions in clinical practice. This candidate drug may, synergistically and significantly, modulate Pgp when coadministered with other Pgp substrate drugs. The possibility of synergistic effects was shown by Aszalos and Ross [33] with the Pgp modulators verapamil, cremophor, and valspodar. These three modulators interact with Pgp by different mechanisms. They were able to show, by flow cytometry, that in Pgp-expressing L1210 and NIH3T3 cells the combination of 1/12th of a normal dose of verapamil (0.3 μg/ml), 1/50th a dose of cremophor (0.002% (v/v)) and 1/160th of a dose of valspodar (0.03 μg/ml) can block the function of Pgp synergistically, to the same degree or more than these Pgp modulators when used singly at full doses. The synergistic effects of these three Pgp modulators were also demonstrated in cell proliferation studies using NIH3T3 cells with daunorubicin as the cytotoxic, antitumor drug. Used in combination, these Pgp modulators suppressed cell growth synergistically, and significantly more than when they were used singly.

The same approach was taken by Hwang *et al.* [11]. They showed with *in vitro* experiments, using Pgp-expressing L1210 and NIH3T3 cells, that verapamil, cremophor, and cyclosporine can act synergistically to suppress Pgp activity. They found that 50–100-fold reduction of the normal therapeutic dose of each of the single modifiers added in combinations of three can result in the same blocking of the activity of Pgp as a single agent used at full effective dose.

In vivo testing

In vitro evaluations are usually followed by *in vivo* evaluations with mouse models being the most common. Drug candidates can be tested in regular mdr1 (+/+) and in gene knockout mice (mdr1a (−/−)) [34] or in mdr1a-deficient CF-1 mice. This model is best used for detection of compounds that have entered the CNS compartment. One has to be aware, however, that the affinities of drugs for Pgp (MDR1), and to mdr1a and mdr1b (the mouse transporters) are not necessarily equal. Yamazaki *et al.* [35] studied directional transport of several compounds with different transport ratios (apical to baso-lateral and reverse) in porcine LLC-PK1 cells transfected with MDR1 or mdr1a genes. They found that the data of the transport studies correlated with substrate accumulation data obtained with human epidermal carcinoma cells (KB-V1) expressing over a million Pgp molecules per cell [36]. They also compared the *in vitro* data obtained with mdr1a transfected LLC-PK1 cells, with data obtained with mdr1 (−/−)/(+/+) CF-1 mice. Their results indicated that drug accumulation ratios in mdr1 (−/−)/(+/+) mice brains up to 60 min correlated well with data of the transcellular transport ratios. They also found, in accordance with Tang-Wai [37], who studied transport ratios in Chinese hamster LR73 ovary cells stably transfected individually with the three Pgp isoforms, that the cells with the three isoforms can yield varied results. Tang-Wai found that these cells show differences in resistance to colchicine and actinomycin D, but not to vinblastine. In comparing *in vitro* and *in vivo* results, one should also keep in mind that *in vivo*, transporters other than Pgp can influence the observed pharmacological results, and that metabolism by cytochrome P4503A may also modulate pharmacology [23].

Positron emission tomography (PET) and nuclear magnetic resonance (NMR) tests

To predict the influence of drug–drug interactions on brain-drug levels, PET, or perhaps a sensitive NMR assay with an external probe, might be the most suitable methods. The *in vivo* suitability of the PET method was evaluated by Hendrikse *et al.* [38]. They tested the accumulation rate of radiolabeled daunorubicin and verapamil in Pgp-positive and Pgp-negative tumor bearing rats with or without coadministration of cyclosporin. After introducing cyclosporin, the concentration of daunorubicin and verapamil increased in Pgp-positive tumors to the level found in Pgp-negative tumors. Without cyclosporin, the concentration of these drugs was about half of that in the Pgp-negative tumors. The PET method was also used successfully to determine drug concentration in the CNS in humans with six different Pgp polymorphs [39]. For this purpose [^{11}C]-verapamil was administered to healthy volunteers followed by 16 min PET scans. Brain uptake clearance, one-tissue compartment model, and the AUC ratio between brain and plasma were calculated. The uptake clearance values indicated no difference between the haplotypes 1236TT, 2677TT, and 3435TT versus 1236CC, 2677GG, and 3435CC.

The suitability of the NMR method was shown by Wang *et al.* [40]. They devised an external probe to fit the head of rats and introduced trifuoroperazin with or without Pgp modifiers to anesthetized rats through the tail vain. The NMR method measured [^{19}F]-trifuoroperazin concentration in the brain. The Pgp modifiers, cyclosporine, valspodar, and erythromycin were used. Significantly more accumulation of [^{19}F] could be measured in the presence of the Pgp modifiers and they found this to be concentration-dependent. This method can utilize drugs containing carbon ([^{11}C]), phosphorous ([^{31}P]), and fluorine ([^{19}F]) isotopes or synthetically modified drugs with isotope labeling.

Conclusions

Pgp has an essential transport function in the biology of humans. Besides its natural substrates, many drugs and other xenobiotics are also its substrates or modulators. For this reason, candidate drugs are routinely evaluated for possible interactions with Pgp. Such interactions become more complex if several drugs, all substrates of Pgp, are simultaneously administered to patients. We describe here different ways that modulators bind to Pgp, its physical function, genetic expression, and its polymorphisms, in relation to drug–drug interactions. We also describe some of the most common and useful testing methods by which drug–drug interactions can be evaluated at the Pgp level. Results of these test methods can guide the safe use of a candidate drug, especially in combination with other drugs in cases of polypharmacy. Clinical aspects of drug–drug interactions at the Pgp level will be discussed in a following paper.

Acknowledgements

The author appreciates the encouragement and hospitality of Dr Michael Gottesman, chief of the Laboratory of Cell Biology, and the editorial assistance of Mr George Leiman.

References

1 Liang, X.-J. and Aszalos, A. (2006) Multidrug transporters as drug targets. *Curr. Drug Targets* 7, 911–921
2 Szakacs, G. *et al.* (2006) Targeting multidrug resistance in cancer. *Nat. Rev. Drug Discovery* 5, 219–229
3 Mizuno, N. *et al.* (2003) Impact of drug transporter studies on drug discovery and development. *Pharmacol. Rev.* 55, 261–425
4 Schinkel, A.H. and Jonker, J.W. (2003) Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv. Drug Deliv. Rev.* 55, 3–29
5 Dean, M. *et al.* (2001) The human ATP-binding cassette (ABC) transporters superfamily. *Genome Res.* 11, 1156–1166
6 Ambudkar, S.V. *et al.* (1999) Biochemical, cellular and pharmacological aspects of the multidrug transporter. *Annu. Rev. Pharmacol. Toxicol.* 34, 361–398
7 Lin, J.H. (2003) Drug–drug interaction mediated by inhibition and induction of P-glycoprotein. *Adv. Drug Deliv. Rev.* 55, 53–81
8 Okamura, N. *et al.* (1993) Digoxin–cyclosporin A interaction: modulation of the multidrug transporter P-glycoprotein in the kidney. *J. Pharmacol. Exp. Ther.* 266, 1614–1619
9 Litman, T. *et al.* (1997) Competitive, non competitive and cooperative interaction between substrates of P-glycoprotein measured by its ATPase activity. *Bichem. Biophys. Acta* 1361, 169–176
10 Ayash, S. *et al.* (1996) Co-operative, competitive and non-competitive interactions between modulators of P-glycoprotein. *Biochem. Biophys. Acta* 1316, 6–18
11 Hwang, M. *et al.* (1996) Effect of combination of P-glycoprotein blockers on the proliferation of MDR1 gene expressing cells. *Int. J. Cancer* 65, 389–397
12 Durr, D. *et al.* (2000) St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin. Pharmacol. Ther.* 68, 598–604
13 Synold, T.W. *et al.* (2001) The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat. Med.* 7, 584–590
14 Zhao, J.Y. *et al.* (1993) Modulation of multidrug resistance gene expression by dexamethason in cultured hepatoma cells. *Endocrinology* 133, 521–528
15 Geich, A. *et al.* (2001) Nucleus receptor response elements mediate induction of intestinal MDR1 by rifamycin. *J. Biol. Chem.* 276, 14581–14587
16 Callen, D.F. *et al.* (1987) Localization of the human multidrug resistance gene, MDR1, to 7q21.1. *Hum. Genet.* 77, 142–144
17 Inoue, K. *et al.* (1992) Assignment of the human cytochrome P450 nifedipin oxidase gene (CYP3A4) to chromosome 7 at band q22.1 by fluorescence in situ hybridisation. *Jpn. J. Hum. Genet.* 37, 133–138
18 von Richter, O. *et al.* (2004) Cytochrome P4503A4 and P-glycoprotein expression in human small intestinal enterocytes and hepatocytes: a comparative analysis in paired tissue specimens. *Clin. Pharmacol. Ther.* 75, 172–183
19 Ekin, S. and Erickson, J.A. (2002) A pharmacophore for human pregnane X receptor ligands. *Drug Metab. Dispos.* 30, 96–99
20 Hochman, J.H. *et al.* (2000) Influence of P-glycoprotein on the transport and metabolism of indinavir in Caco-2 cells expressing cytochrome P4503A4. *J. Pharmacol. Exp. Ther.* 292, 310–318
21 Aszalos, A. and Weaver, J.L. (1998) Estimation of drug resistance by flow cytometry. *Meth. Mol. Biol.* 91, 117–122
22 Ibrahim, S. *et al.* (2000) Influence of beta-adrenergic antagonists, H1-receptor blockers, analgesies, diuretics and quinolon antibiotics on the cellular accumulation of the anticancer drug, daunorubicin: P-glycoprotein modulation. *Anticancer Res.* 21, 847–856
23 Ueda, K. *et al.* (2001) Inhibition of the biliary excretion of methotrexate by probenecid in rats: quantitative prediction of the interaction from in vitro data. *J. Pharmacol. Exp. Ther.* 297, 1036–1043
24 Adachi, Y. *et al.* (2001) Comparative studies on in vitro methods for evaluating in vivo function of MDR1, P-glycoprotein. *Pharm. Res.* 18, 1660–1668
25 Sharom, F.J. *et al.* (1995) Interaction of the P-glycoprotein multidrug transporter with peptides and ionophores. *J. Biol. Chem.* 270, 10334–10341
26 Kharash, E.D. *et al.* (2005) Evaluation of first pass cytochrome P4503A (CYP3A) and P-glycoprotein activities using alfentinil and fexofenadine in combination. *J. Clin. Pharmacol.* 45, 79–88
27 Wheeler, R. *et al.* (2000) Use of membrane vesicles to investigate drug interactions with transporter P-glycoprotein and multidrug resistance-associated protein. *Int. J. Clin. Pharmacol. Ther.* 38, 122–129
28 Szabo, D. *et al.* (1999) Anti-psychotic drugs reverse multidrug resistance of tumor cell lines and human AML cells ex-vivo. *Cancer Lett.* 139, 115–119
29 Seelig, A. and Landwojtowicz, E. (2000) Structure–activity relationship of P-glycoprotein substrates and modifiers. *Eur. J. Pharm. Sci.* 12, 31–40
30 Ecker, G. *et al.* (1999) The importance of a nitrogen atom in modulators of multidrug resistance. *Mol. Pharmacol.* 56, 791–796
31 Stouch, R.T. and Gudmousson, O. (2002) Progress in understanding the structure–activity relationship of P-glycoprotein. *Adv. Drug Deliv. Rev.* 54, 315–328
32 Kudoh, K. *et al.* (2000) Monitoring the expression profiles of doxorubicin-induced and doxorubicin-resistant cancer cells by cDNA microarray. *Cancer Res.* 60, 4161–4166
33 Aszalos, A. and Ross, D.D. (1998) Biochemical and clinical aspects of efflux pump related resistance to anti-cancer drugs. *Anticancer Res.* 18, 2937–2944
34 Schinkel, A. *et al.* (1994) Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. *Cell* 7, 491–502
35 Yamazaki, M. *et al.* (2001) In vitro substrate identification studies for P-glycoprotein-mediated transport: species difference and predictability of in vivo results. *J. Pharmacol. Exp. Ther.* 296, 723–735
36 Aleman, C. *et al.* (2003) P-glycoprotein, expressed in multidrug resistant cells, is not responsible for alterations in membrane fluidity or membrane potential. *Cancer Res.* 63, 3084–3091
37 Tang-Wai, D.F. *et al.* (1995) Human (MDR1) and mouse (mdr1, mdr3) P-glycoprotein can be distinguished by their profiles and sensitivity to modulators. *Biochemistry* 34, 32–39
38 Hendrikse, W.H. *et al.* (1999) A new in vivo method to study P-glycoprotein transport in tumors and the blood–brain barrier. *Cancer Res.* 59, 2411–2416
39 Takano, A. *et al.* (2006) Evaluation in vivo P-glycoprotein function at the blood–brain barrier among MDR1 gene polymorphism by using ^{11}C-verapamil. *J. Nucl. Med.* 47, 1427–1433
40 Wang, P.C. *et al.* (2003) A pharmacokinetic study of trifluoroperazine crossing blood–brain barrier due to P-glycoprotein modulation. *Proc. Intl. Magn. Reson. Med.* 11, 125