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P-Glycoprotein-Based Drug-Drug Interactions: Preclinical Methods and Relevance to Clinical Observations

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Multiple drug administration is common in elderly, HIV, and cancer patients. Such treatments may result in drug-drug interactions due to interference at the metabolic enzyme level, and due to modulation of transporter protein functions. Both kinds of interference may result in altered drug distribution and toxicity in the human body. In this review, we have dealt with drug-drug interactions related to the most studied human transporter, P-glycoprotein. This transporter is constitutively expressed in several sites in the human body. Its function can be studied *in vitro* with different cell lines expressing P-glycoprotein in experiments using methods and equipment such as flow cytometry, cell proliferation, cell-free ATP as activity determination and Transwell culture equipment. *In vivo* experiments can be carried out by mdr1a(-/-) animals and by noninvasive methods such as NMR spectrometry. Some examples are also given for determination of possible drug-drug interactions using the above-mentioned cell lines and methods. Such preclinical studies may influence decisions concerning the fate of new drug candidates and their possible dosages. Some examples of toxicities obtained in clinics and summarized in this review indicate careful consideration in cases of polypharmacy and the requirement of preclinical studies in drug development activities.

Key words: Drug-drug interactions, P-Glycoprotein, Polypharmacy, Preclinical studies, Clinical studies

INTRODUCTION

Transmembrane proteins, which transport molecules across plasma membranes, may significantly affect the pharmacokinetics and toxicity of drugs. A description and the general function of known transporters in humans are detailed in previous publications. One of the most significant transporters in humans is the 170 kDa P-glycoprotein (Pgp). This transporter has perhaps been studied more than any other transporter, especially in terms of its so-called drug-drug interactions. Other transporters, such as Multidrug Resistance Protein 1 (MRP1) and Multidrug Resistance Protein 2 (MRP2) or MOAT also contribute to drug distribution and pharmacokinetics considerably. These transporters are present in many tissues and therefore their contribution to drug-drug interactions cannot be studied very effectively in detail. However, they play a significant role in the treatment of resistant tumors. Their drug-substrates include mostly organic anions such as nonsteroidal anti-inflammatory drugs besides sulfinpyrazone and probenecid. Other transporters known to transport drugs are ABCB11 (BSEP, sPgp) and ABCG2 (MXR1), but again, these have not been studied in detail for possible drug-drug interactions. For these reasons, this review will concentrate on drug-drug interactions, based on the function and location of Pgp in humans.

The significance of Pgp in terms of drug-drug interactions is several-fold. Pgp, which is a nonspecific transport protein, is expressed constitutively at the blood-brain barrier, in the intestines, kidney, liver and by some activated T cells (Gottesman *et al.*, 2002). Drug-drug interactions at the blood brain barrier may occur if Pgp is blocked by a Pgp-substrate-drug and a concomitantly administered second drug, which would not penetrate the brain if administered singly, can then penetrate the brain freely. Also, if Pgp is blocked by a coadministered drug in the kidney, the excretion of unwanted metabolites may be blocked, causing toxic symptoms. Other unwanted drug-drug interactions may occur at other sites-listed above-in

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human bodies causing unwanted pharmacologic and toxic events. In this paper, we present preclinical studies to assess potential drug-drug interactions in humans concerning clinical applications of drugs. At the end of this review, we list some documented adverse effects in patients which are attributed to Pgp-based drug-drug interactions.

PHYSIOLOGICAL ROLE OF Pgp IN HUMANS

The physiological distribution of this transporter is important from the point of view of understanding drug-drug interactions. Pgp is constitutively expressed in several tissues in humans. It is expressed at the canalicular membrane of the liver, in epithelial cells of the kidney, in brain capillary endothelial cells, intestinal epithelial cells, the choriod plexus and in some circulating blood cells.

Several experiments led to the conclusion that Pgp is expressed at the blood brain barrier. For example, Tsuji *et al.* (1992) showed that vincristine was effluxed from bovine endothelial cells expressing Pgp at the luminal side. Similar observations were made with murine and porcine endothelial cells (Hegmann *et al.*, 1992; Tatsura *et al.*, 1992). The *in vivo* experiments of Schinkel *et al.* (Schinkel *et al.*, 1994) using homozygous mice deficient for the *mdr1a* (*mdr3*) gene were very enlightening. They observed that these mice have 100-fold increased sensitivity to the pesticide invermectin and to up to 3-fold more sensitivity to vinblastine. Concomitantly, they showed that the blood brain barrier contains mdr1a P-glycoprotein and that the brains of the treated animals contained more invermectin and vinblastine than that of the mdr1a (+/+) mice. It is generally accepted that the role of Pgp at the bood brain barrier is to protect the brain from unwanted molecules. Breaking the blood brain barrier with a Pgp substrate or modulator may cause severe, adverse central nervous system (CNS) effects to animals as well as humans.

A high level of Pgp and multi-drug resistance associated protein (MRP) was shown to be present at the choroid plexus (Rao *et al.*, 1999). The blood-cerebrospinal-fluid drug-permeability barrier is located at the epithelium of the choroid plexus.

It was shown that Pgp is expressed at the brush border membrane of mammalian proximal renal cells. In one of the earliest studies, Horio *et al.* (1989) demonstrated that the canine kidney cell line (MDCK) carrying the human *mdr1* gene and expressing Pgp, transport anticancer drugs in a polarized fashion. Drugs were transported from the basal side to the apical side and not visa-versa. Tanigawara *et al.* (1992) demonstrated that in the porcine kidney epithelial cell line, LLC-PK1, transfected with MDR1 cDNA, several Pgp-substrate drugs inhibited the transport of digoxin from the basal side to the apical side. In animal models, using dogs, De Lannou *et al.* (1994) showed that cyclosporin inhibited the renal secretion of two Pgp substrate drugs, vincristine and vinblastine. These results suggested a role of Pgp in oriented transport in urinary secretion. Inhibition of the function of Pgp in renal cells may cause nephrotoxicity.

The first study of the involvement of Pgp in biliary excretion was in canalicular membrane vesicles, by Kaminoto *et al.* (1989). These vesicles were shown to express Pgp and transport daunorubicin, implying that Pgp can secrete drugs into the bile. Studies in rats confirmed the *in vitro* results when it was shown that secretion of colchicines was decreased when rats were coadministered the cyclosporin analog, valspodar (PSC833), a Pgp inhibitor (Speeg and Maldonodo, 1994).

Some blood cells were found to express Pgp. Gupta *et al.* (1992) found that Pgp is expressed at a low level in CD4+ and at some higher level in CD8+ cells in freshly isolated normal human blood. Klimecki *et al.* (1994) extended the study into the blood cell lineage-related expression of Pgp. They found that the order of expression is CD8+>CD4+=CD19+>CD14+ in normal human peripheral blood cells, but the expression of Pgp in myeloma cells is much higher than in the CD8+ cells. The low-level expression of Pgp at the plasma membrane in blood cells is changed by HIV infection of these cells, according to Malorni *et al.* (1988). After HIV infection, intracellular Pgp relocalizes to the plasma membrane. The presence of Pgp in blood cells contributes to the ineffectiveness of HIV protease inhibitors because these drugs are Pgp substrates.

P-glycoprotein was shown to be present in the intestines by the early work of Mayer *et al.* (1996). These authors compared excretion of digoxin in wild-type and Mdr1a(-/-) mice and found that the urinary excretion of digoxin was significantly more in the Mdr1a(-/-) mice. Using the same Mdr1a(-/-) mice, Sparreboom *et al.* (1997) found that valspodar, a modifier of Pgp function, reduced the fecal excretion of orally administered paclitaxel from 87% in wild-type to less than 3% in Pgp knock-out mice. Inhibition of wild type mice with valspodar resulted in a substantial increase in the systemic availability of orally administered taxol (Van Asperen *et al.*, 1997). One reason for this systemic increase of taxol is the blocking of Pgp in the intestines by valspodar. These findings stimulated efforts to make drugs which are substrates of Pgp orally administrable by blocking Pgp activity with known, otherwise pharmacologically inert Pgp-blockers.

Absorption of a drug through the intestines may vary in individuals due to Pgp polymorphism. Also, mutation in TT homozygotes results in a definite decrease of Pgp expression (Hoffmeyer *et al.*, 2000). Such persons absorb drugs through the gut more easily. In addition to the above variations, some drugs induce Pgp expression. For example, rifamycin administration was found to reduce verapamil

absorption through the intestines of rats, presumably due to increased expression of Pgp in the intestines (Borst and Elferik, 2002). This example may indicate that drug-drug interactions occur by induction of higher levels of Pgp at all of the sites discussed above. Well-characterized endothelial cells were also isolated from human full-term placentas (Schutz and Friedel, 1996). At this site, Pgp protects the fetus from unwanted molecules.

PRE-CLINICAL METHODS USED TO STUDY DRUG-DRUG INTERACTIONS AT THE Pgp LEVEL

In vitro and animal models for studying drug-drug interactions greatly facilitate drug candidate selection and animal and human pharmacological studies. The following three sections give short summaries of some past studies in this field.

Cells used in *in vitro* studies for drug-drug interactions

For modeling the blood brain barrier *in vitro*, several immortalized and primary cells were used in the past. These cells could mimic the physical barrier between blood and the brain, preventing the non-controlled passage of most polar substances. Some investigators used primary cells, but these preparations were costly and time consuming and subject to cell differentiation *in vitro*. Some primary cells, isolated from microvascular endothelium derived from different animals, were immortalized.

Teifel and Friedel established a permanent microvascular endothelial cell line, PBMEC/C1-2, from a porcine brain (Teifel and Friedel, 1996). The isolated primary cells were transfected with pRNA-1 encoding for the T-antigens of SV40. After appropriate selections, these cells showed the typical morphology and markers of capillary endothelial cells. Cells modeling the blood brain barrier were also isolated from bovine brain microvessel endothelial cells (Weber *et al.*, 1993). These cells form tight junctions and attenuate pinocytosis. Typical markers, such as gamma-glutamyl transpeptidase, alkaline phosphatase and angiotensin-converting enzyme are present in these cells. They grow to monolayers best in astrocyte-conditioned media to avoid extracellular leaks.

Immortal human brain microvascular endothelial cells were produced from capillaries and microvascular endothelial cells by Muruganandam *et al.* (1997). The isolated cells were transfected with plasmid pSV3-neo coding for the SV40 large T antigen and the neomicin gene. After cell selection, these cells grow with accelerated proliferation and express all of the normal phenotypic characteristics of endothelial cells. Similarly, Dorovini-Zis *et al.* isolated endothelial cells from cortical fragments removed by surgery for seizure disorders and from brain autopsy specimens (Dorovini-Zis *et al.*, 1991). The cultured cells grew to confluent monolayers, and exhibited factor VM/Von Willebrand antigen and other characteristics of human endothelium.

The most commonly used cell lines for studying drug-drug interactions at the Pgp level are the human colon adenocarcinoma CaCo-2 and the canine kidney (MDCK) cell lines. Studies with the CaCo-2 cell line relate well to *in vivo* absorption of drugs in the human intestines (Karlson *et al.*, 1993). However, CaCo-2 cells have the disadvantage that they grow slowly to form monolayers and express a relatively small number of Pgp molecules on the apical side (Ibrahim *et al.*, 2001). The MDCK/MDR1 cells were produced by retrovirus infection of the kidney cells, resulting in polarized expression of a good number of Pgp molecules on the apical side (Ibrahim *et al.*, 2001; Pastan *et al.*, 1988).

P-Glycoprotein reference substrate molecules, drugs used for studying drug-drug interactions using the above mentioned cell lines, are those which have low IC_{50} values or have already been used extensively for this purpose. These drugs are cyclosporinA, valspodar, erythromycin, ketoconazole, quinidine, verapamil, nelfinavir and ritonavir. For flow cytometric studies the fluorescence molecules Rhodamine 123, calceine, doxorubicine and daunorubicin are frequently used. The effect of a new drug candidate on the modulation of Pgp, as evaluated using the above mentioned cell lines, could influence the advancement of the drug candidate towards clinical trials.

Methods used to study drug-drug interactions *in vitro*

There are several ways to study drug-drug interactions *in vitro*. One of the most commonly used pieces of equipment is the Transwell culture plate available from Corning Costar, Inc. and Millipore, Inc., shown in Fig. 1. The insert of the Transwell plate could be made, for example, from a collagen-coated polycarbonate membrane. Cells are introduced to the top of the insert of the plate and cultured in medium placed in the lower and upper chambers. When cells reach confluence, the monolayer is checked for possible intercellular leaks by electrical resistance (TER) measurements, passage of Evans Blue-albumin and sucrose. After these checks, an appropriate amount of the drug to be studied is introduced in either the upper or the lower chamber. Samples are taken from the opposite chamber periodically and the concentration of the drug is determined with an appropriate assay. Introduction of a second drug, perhaps a Pgp inhibitor, may alter the time course of the passage of the first drug and can indicate interference with the passage at the Pgp level. Naturally, variations in this assay can give different

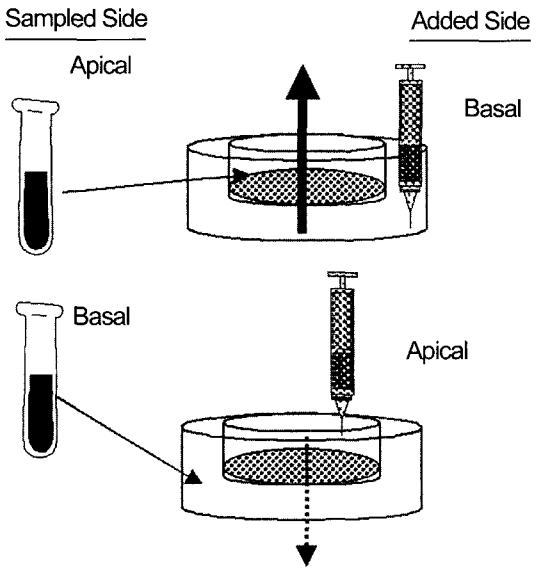

Fig. 1. Schematic representation of the function of Transwell culture plates. Upper: drug is added to the lower compartment (basal) with a syringe and samples are taken, periodically, from the upper compartment (apical). Lower: opposite drug addition and sampling to upper part of figure. Difference in the rate of diffusion of a Pgp drug substrate through the confluent cell layer (bottom of the upper chamber) depends on the directional expression of Pgp on the plasma membranes of the cells.

information about drug-drug interactions at the Pgp level. Different cell lines, some of them mentioned above, could be used in Transwell experiments.

Another method used to study drug-drug interactions at the Pgp level is flow cytometry. This method is based on detection of modulation of the fluorescence intensity of substrates of Pgp, such as rhodamine 123 or doxorubicin by the drug to be studied. Cells are treated with the drug in suspension of Pgp expressing cells (10^4 cells/mL) for a short time followed by rhodamine 123 or doxorubicin for about 1-3 min. After this short incubation, the fluorescence emission is detected for rhodamine 123 (525 nm) or doxorubicine (580 nm) using standard flow cytometry procedures. The obtained fluorescence intensity is compared with that of the cells treated only with the fluorescent substrate. If a difference exists, it indicates that the tested drug modulates the function of the Pgp in the cells. It is important that the drug to be tested should not emit fluorescence at the emission wavelength of the fluorescence substrate. This is determined prior to the above-described test, using parental, non-Pgp expressing cells. Calculations can be made for % inhibition of Pgp by the drug to be tested by the equation:

$$\% \text{ Inhibition} = \frac{FI_{\text{MDR cells with drug}} - FI_{\text{MDR cells without drug}}}{FI_{\text{par cells without drug}} - FI_{\text{MDR cell without drug}}}$$

where FI = fluorescence intensity obtained by flow cytometry of the cells

A third way to study *in vitro* drug-drug interactions is by determination of cell proliferation in the presence of a Pgp drug-substrate affecting cell growth and of the drug to be tested. The advantages of using MDCK/MDR1 cells, including their short generation time (16 h) and the ability to use them in the monolayer studies described above were emphasized earlier (Ibrahim *et al.*, 2001). A cytotoxic drug such as doxorubicine can be used as a cell proliferation inhibitor. Modulation of the effect of this cytotoxic drug by the drug to be studied can be measured by cell count or by a proliferation indicator dye, such as Alamar Blue. Percent of cell growth can be calculated by the equation:

$$\% \text{ Cell growth} = \frac{(\text{No. of drug+Dox treated cells})-(\text{No. of drug treated cells})}{\text{No. of untreated cells}} \times 100$$

where Dox = doxorubicine or any cytotoxic drug
(The number of cells can be replaced by fluorescence intensity numbers in this equation.)

Drug-drug interactions can also be studied in a cell-free system. Preparations of crude membranes of Pgp expressing cells, such as NIH3T3 MDR1 cells, could be used for this purpose (Ambudkar, 1998). Such preparations are incubated in the appropriate buffer with 0.3 mM sodium orthovanadate, which inhibits ATP hydrolysis, but allows the measurement of vanadate-specific activity of Pgp. The substrate or drug to be tested is introduced and after 3 min incubation at 37°C, the ATP hydrolysis is initiated by addition of disodium ATP. The reaction is terminated by sodium dodecyl sulphate at a desired time and the liberated inorganic phosphate is measured calorimetrically with ammonium molybdate in a spectrophotometer tuned to 880 nm wavelength. To determine drug-drug interactions by this method, kinetic measurements have to be done. The rate of hydrolysis of ATP by a known substrate, such as verapamil, is compared to the rate of hydrolysis by the mixture of the known substrate and the drug to be tested. Alteration in kinetics by the mixture is indicative of interaction at the Pgp level.

Preclinical *in vivo* methods used to study drug-drug interaction and P-glycoprotein related drug distribution

Kim *et al.* (1998) correlated the function of Pgp on drug distribution *in vitro* and *in vivo* models. They used the above-mentioned mdr1a(+/+) and mdr1a(-/-) mice and determined distribution of HIV protease inhibitors in the two mice colonies. Indinavir, nelfinavir and saquinavir were present 2.5-fold higher in the plasma of mdr1a(-/-) mice after oral administration of these drugs. The same drugs were found in up to 36-fold higher quantities in the brains of mdr1a(-/-) mice than in the mdr1a(+/+) mice after

i.v. administration. These results indicate the usefulness of genetically altered mice for studying drug-drug interactions in the intestines and at the blood brain barrier.

In vivo studies can be done with non-invasive methods. One such study was done by Wang *et al.* using the Nuclear Magnetic Resonance (NMR) technique and a drug containing fluorine atoms (Wang *et al.*, 2000). Using this method, the amount of fluorinated drug can be assessed in the brain of an animal, such as a rat, by placing a volume coil on the head of the animal. The volume coil, for a rat, is a curved, rectangular-shaped wire 22 mm × 17 mm made of copper. A reference sample was placed on the head, near the volume coil and a second coil was placed on the abdomen of the animal to monitor the success of administration of the drug. Trifuoroperazine was used as the fluorine-containing drug, which is a substrate of Pgp and, at clinical concentration, does not penetrate the CNS. The drug can be administered to a rat through the tail-vain or through other suitable veins. The animal is placed into the NMR instrument, and the attached coil outlet is led to the electronic hardware of that instrument. The appearance of a fluorine signal can then be monitored. When a suitable small dose of trifuoroperazine was administered, no signal could be detected from the head coil. However, when a second Pgp-modifier drug was also administered to the rat, preferably before trifluoroperazine, a fluorine signal could be detected by the volume coil placed on the head, indicating penetration of trifuoroperazine into the CNS compartment. Cyclosporin, ketaconasole and valspodar were tested as second Pgp modifier drugs. Drug-drug interaction can be monitored this way, and the effect can be correlated to clinical dose levels. Following the NMR experiment, assessment of the tested drug directly from tissue preparation can confirm the results of the NMR technique. In fact, invasive drug-drug interactions were performed frequently in many other laboratories by assessing drug concentrations in different organs of animals.

Other animal studies are aimed at finding ways to absorb Pgp substrate drugs through the intestine. Such studies assess the blood concentration of the studied drug with or without an additional, preferably pharmacologically inert, Pgp modulator. These studies are invasive animal studies. In one such study, albeit not with an inert Pgp modulator, cyclosporinA is in clinical trials to make taxol orally administrable. In another, similar study, an inert Pgp modulator, valspodar, also was shown to increase oral availability of taxol in mice (Van Asperen *et al.*, 1997).

EXAMPLES OF DRUG-DRUG INTERACTIONS STUDIES

Preclinical studies

We will now present several examples to illustrate drug-drug interactions at the Pgp level. The methods for studying these interactions are presented above. While many studies have been done, only a few examples of the different types of studies can be presented here.

One study by Ibrahim *et al.* (2001) used flow cytometry in conjunction with transmembrane diffusion (Transwell) and with cell proliferation experiments to detect possible interactions with different classes of drugs. These classes included beta-adrenergic antagonists, H1-receptor antagonists, analgesics, quinolone antibiotics and diuretics. Daunorubicin was used as a Pgp reference substrate molecule. In their studies, they correlated the clinically used plasma concentration of the tested drug with that concentration which inhibited the efflux of daunorubicin by Pgp in the NIH3T3 Pgp expressing cells. They showed that the quinolone antibiotics, norfloxacin, ciprofloxacin, ofloxacin and lomefloxacin and most of the diuretic drugs, including furosemide, bumetanide and indapamide do not affect the accumulation of daunorubicin in the cells. These drugs then are not likely to cause drug-drug interactions at the Pgp level, even at higher than clinical blood levels. In contrast, the H1-receptor antagonists, astemisole and terfenadine, the beta-adrenergic antagonists, propranolol and atenolol block Pgp to some extent at clinical dose levels. Among the antiemetic drugs, prochlorperazine, droperidol, and trifluprомazine block Pgp extensively at clinical dose levels, as shown in Fig. 2. Similar observations were made with analgestic-antipyretic drugs such as propoxyphene, which inhibited Pgp function by 10% at the clinical dose level.

When combinations of drugs were used in the above system, synergistic effects were obtained. For example, propranolol and astemisole and the antifungal drug ketoconasole, at clinical or close to clinical levels, and added simultaneously, increased the blocking of Pgp 25% higher than the additive effect would be. The significance of this finding is that in the case of polipharmacy, used in elderly, HIV and cancer patients, unwanted drug-drug interactions could be observed. These adverse effects could be manifested in relation to organ sites, such as the kidney, blood-brain barrier and intestines, where Pgp is constitutively expressed. Ibrahim *et al.* supported the above flow cytometric studies with cell proliferation and monolayer studies. In their monolayer studies, kidney MDCK/MDR1 cells were used to establish the monolayer, and daunorubicin was used as a Pgp reference substrate.

In a similar study, Ibrahim *et al.* (2000) showed that of the antiemetic drugs, trifupromazine, droperidol and prochlorperazine inhibit Pgp extensively at clinical blood levels.

It is important to establish potential drug-drug interactions with anti-HIV drugs, since several of these drugs are used simultaneously in clinical therapy. Glynn and Yazdanian

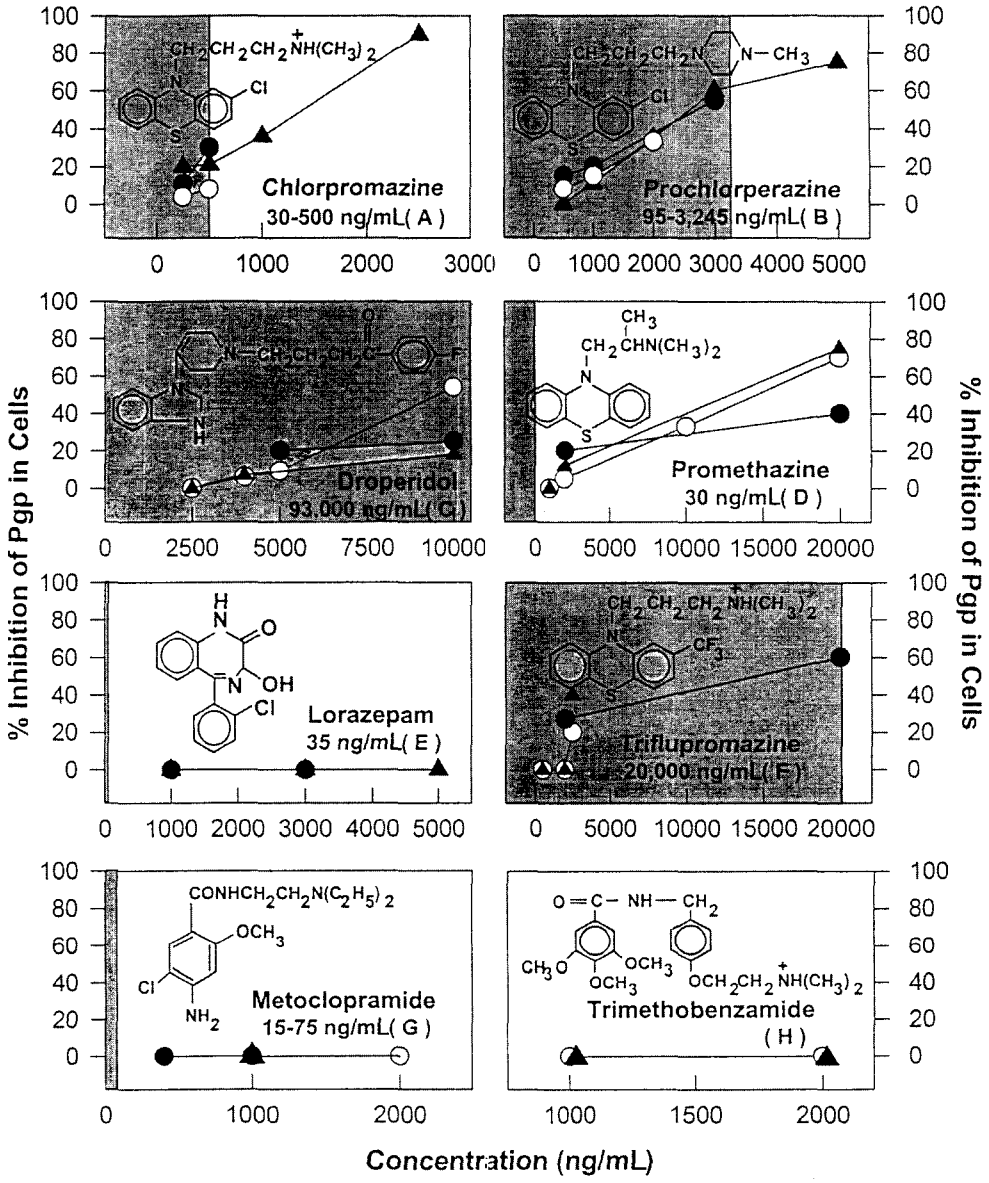

Fig. 2. Extent of inhibition of Pgp function by antiemetic drugs in NIH 3T3/MDR1 cells: ▲--▲, in human blood brain barrier endothelial/MDR1 cells: ○–○, and in CaCo-2/MDR1 cells: ●–●. Determination was done with rhodamin 123 as a Pgp substrate and by flow cytometry. Inhibition of Pgp function was calculated on the basis of rhodamin 123 fluorescence retention in cells in the presence and absence of the studied drug. *Shaded areas:* clinical blood level of drugs.

(1998) studied the permeability of several nucleoside and nonnucleoside reverse transcriptase inhibitors and protease inhibitors through blood brain barrier cells. The blood brain barrier cells were isolated from capillaries of bovine brain gray matter and grown on porous filters. For their experiments, Transwell plates with polycarbonate filters, described above, were used. From the drugs tested, the protease inhibitor saquinavir showed marked low permeability, attributed to the fact that it is a good substrate of Pgp. None of the nucleoside or nonnucleoside reverse transcriptase inhibitors proved to be substrates of Pgp, and the cell monolayer did not inhibit their passage. This experiment was carried out in the presence and absence of known Pgp inhibitors, such as verapamil or probenecid. From concentrations of the drug in both chambers in time, permeability factors were determined and compared. One can conclude from this study that a high dose of saquinavir could facilitate the entry of other simultaneously used drugs, such as some antifungal or cardiac drugs, into the CNS.

Caco-2 cells were frequently used in several laboratories to study drug-drug interactions. For example, Fromm *et al.* (1999) studied the interaction of quinidine and digoxin using Caco-2 cell monolayers grown in Transwell

plates. First, they established that the transport of quinidine was markedly greater in the direction of basal to epical than in the direction of epical to basal, indicating that quinidine is a substrate of Pgp expressed in these cells in a polarized fashion. Separately, they established that the transport of [3*H*]-digoxin (5 μM) was about 8 times greater in the direction of basal to epical than in the direction of epical to basal after 4 h. When 5 μM quinidine was introduced together with 5 μM digoxin, the transport rate of digoxin was only 5 times greater in the direction of basal to apical than in the direction of apical to basal. A 100 μM quinidine reduced the difference to zero. Fromm et al. concluded from these experiments that blocking the function of Pgp by a drug can increase digoxin concentration in normal animals and in humans. In fact, this conclusion was verified in animals, and such drug-drug interactions have been seen in human patients also (Sachs *et al.*, 1993).

One preclinical study was aimed at measuring drug-drug interactions in perfused rat livers by studying the hepatobiliary distribution of doxorubicin (Booth *et al.*, 1998). For this purpose, livers were isolated and perfused 24 h at 37°C with rat blood. The Pgp modulators GF120918 or quinidine were administered to the perfusate 5 min prior to addition of doxorubicin. The concentrations of doxorubicin and its metabolite, doxorubicinol, were measured in the perfusate, liver and biliary excretion. No change was observed in the perfusate or in the liver. However, significant changes, reductions, were seen in the biliary doxorubicin concentration when the two Pgp-function modifiers were administered. From the data obtained, a pharmacokinetic model was calculated based on goodness-of-fit criteria. The model indicated that the rate-limiting process for doxorubicin elimination was the biliary excretion. It was concluded that the reason for this reduced biliary excretion was the interaction of the two Pgp-function modifiers with Pgp with the canalicular egress. This type of preclinical drug-drug interactions study is very useful to determine elimination of drugs and their metabolites when multiple Pgp modulator drugs are administered to patients.

Drug-drug interactions observed in clinics and attributed to Pgp

Polypharmacy, the simultaneous use of several drugs on one patient, is very common today for elderly, cancer and HIV patients. As reported to the U.S. Food and Drug Administration (FDA) and by Lazarou *et al.* (1998), about 2 million hospitalized patients in the U.S. suffer serious adverse drug reactions each year, attributable to polipharmacy. Some of these adverse effects could relate to metabolic interaction at the Pgp level. For these reasons, the FDA is preparing to issue guidelines for the clinical use of a combination of Pgp modulator drugs. Other clinical adverse effects may relate to interference with metabolism at the cytochrome system level. There are existing guidelines issued by FDA for this purpose. Following are some documented clinical cases where adverse effects were attributed to modulation of Pgp.

A 69-year-old patient received digoxin, a Pgp substrate, 0.125 mg/day and quinidine 325 mg three times a day. After coronary bypass surgery, the patient developed sternal osteomyelitis due to an aspergillus infection. In addition to the previous drugs, the patient received the antifungal drug itaconazole, also a Pgp substrate. The ninth day after itaconazole therapy started, the patient complained of nausea and vomiting and after 35 days started to see a yellow halo around objects (Sachs et al., 1993). It was suggested that the three Pgp substrate drugs, administered simultaneously, opened the blood brain barrier and the drugs entered the CNS, causing the symptoms.

Another antifungal agent, ketaconazole, was found to modulate the bioavailability of the orally administered, immunosuppressor drug tacrolimus. The study, conducted in normal patients, showed that the bioavailability of tacrolimus increased to 30% from 14% when no ketaconazole was added (Floren *et al.*, 1997). The conclusion was reached that the two administered drugs acted simultaneously on Pgp expressed in the intestines.

Increased tacrolimus concentration can cause toxicity. A 68-year-old man who had a liver transplant received 8 mg tacrolimus twice daily, besides 5 other drugs to control his atrial fibrillation. His blood tacrolimus concentration was 12.9 ng/mL. Diltiazem was also later administered, and after three days with this additional drug the patient developed confusion and delirium. His tacrolimus concentration increased to 55 ng/mL. After stopping the diltiazem administration, the blood concentration of tacrolimus was adjusted to 10 ng/mL again and the adverse symptoms ceased. It was concluded that diltiazem, tacrolimus and the other drugs synergized, opened the blood brain barrier and caused the above syndromes (Hebert and Lam, 1999).

In a study with eight humans, the pharmacokinetics of a single dose of oral or intravenous administration of digoxin (1 mg each) was studied, with or without coadministration of rifamycin (600 mg/day for 10 days). The area under the curve (AUC) parameter was lower with oral rifamycin administration, but no significant change occurred with intravenous administration. The reason for this change in AUC could be correlated with increased expression of Pgp in the intestines of the patient (Greiner *et al.*, 1999).

Drug-drug related adverse symptoms must be reported to the FDA through the MEDWATCH reporting system. One of the reports indicated drug-drug interaction-based adverse symptoms due to modulation of the Pgp system.

A 48-year-old female patient who had cancer reported somnolence, hallucination and ataxia during the fifth cycle of chemotherapy. Her chemotherapy included doxorubicine, decarbazine, ondansetron, dexamethasone, lorazepam, diltiazem and enarapril. Since doxorubicin, diltiazem, dexamethasone and ondansetron all modulate Pgp and can synergize, as discussed above, the syndromes of this patient could be attributed to penetration of some of these drugs to the CNS. This conclusion is strengthened by the fact that the liver and renal functions of the patient were normal during the course of the therapy (UF/Dist. Rep. # 1426509).

CONCLUSIONS

The intent of this review article was to demonstrate how important it is to study drug-drug interactions in case of polypharmacy. Polypharmacy, multiple drug administration, is quite frequent, especially for elderly, AIDS and cancer patients. Drug-drug interactions could be for several reasons; we have mentioned related subjects only when interference with constitutively expressed Pgp would occur. Recognition of these drug-drug interactions has prompted many preclinical cell culture-based, organ, animal and clinical studies. By now, cell culture and knock-out animal models are widely available for this purpose. We have mentioned several drug-drug interaction studies using *in vitro* and animal methods. We have also listed clinical observations attributed to drug-drug interactions at the Pgp level. Because of the availability of proven methods and because of the significant clinically observed adverse effects, the FDA formulated guidelines for new drug development and for clinical protocols in polypharmacy. These guidelines and certain required actions, such as drug labeling indicating possible adverse effects, could and hopefully will prevent many adverse clinical events.

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