Multidrug Transporters as Drug Targets

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Abstract: Transport molecules can significantly affect the pharmacodynamics and pharmacokinetics of drugs. An important transport molecule, the 170kDa P-glycoprotein (Pgp), is constitutively expressed at several organ sites in the human body. Pgp is expressed at the blood-brain barrier, in the kidneys, liver, intestines and in certain T cells. Other transporters such as the multidrug resistance protein 1 (MRP1) and MRP2 also contribute to drug distribution in the human body, although to a lesser extent than Pgp. These three transporters, and especially Pgp, are often targets of drugs. Pgp can be an intentional or unintentional target. It is directly targeted when one wants to block its function by a modifier drug so that another drug, also a substrate of Pgp, can penetrate the cell membrane, which would otherwise be impermeable. Unintentional targeting occurs when several drugs are administered to a patient and as a consequence, the physiological function of Pgp is blocked at different organ sites. Like Pgp, MRP1 also has the capacity to mediate transport of many drugs and other compounds. MRP1 has a protective role in preventing accumulation of toxic compounds and drugs in epithelial tissue covering the choroid plexus/cerebrospinal fluid compartment, oral epithelium, sertoli cells, intestinal tubules and urinary collecting duct cells. MRP2 primarily transports weakly basic drugs and bilirubin from the liver to bile. Most compounds that efficiently block Pgp have only low affinity for MRP1 and MRP2. There are only a few effective and specific MRP inhibitors available. Drug targeting of these transporters may play a role in cancer chemotherapy and in the pharmacokinetics of substrate drugs.

Key Words: Drug-drug interactions, P-glycoprotein, multidrug transporters, cancer chemotherapy, intentional and/or unintentional targets, drug resistance, polypharmacy.

INTRODUCTION TO P-GLYCOPROTEIN

Transport molecules expressed at the plasma membranes of cells play important physiological functions and also affect the pharmacokinetics of drugs in humans. The modulation of the normal function of these transporters can cause unwanted changes in drug pharmacodynamics, pharmacokinetics, and drug related toxicities and hinder their physiological role. The most studied transporter molecule is the 170 kDa P-glycoprotein (Pgp), in the nomenclature of ABC transporters: ABCB1. The general description and function of this transporter is detailed in the literature [1, 2]. In brief, Pgp is embedded in the plasma membranes of cells and its function requires hydrolysis of two ATP molecules to bind to a substrate and to release the substrate into the extracellular compartment. Although it is glycosylated, glycosylation is not required for Pgp function. Pgp has twelve trans-membrane segments and there is some evidence that trans-membrane segments 5, 6 and 11, 12 are involved in substrate transport.

P-glycoprotein is constitutively expressed at several organ sites in humans [3, 4]. A high level of Pgp expression has been found in the gastrointestinal tract, where it is expressed on the surface of mucosa. This expression of Pgp on cell surfaces prevents absorption of molecules, such as drugs which are substrates of Pgp [5]. In the kidney, Pgp is present on the brush border and its function is associated with excretion of metabolites into the urine [6]. It is also known that Pgp is expressed at the blood-brain barrier. The role of Pgp at this site is to protect the brain from xenobiotic molecules [7]. The brain is also protected by Pgp and MRP1 at the site of the choroid plexus [8]. Human blood cells also express a low level of Pgp [9]. In myeloma and HIV-infected cells the expression of Pgp increases. In humans, Pgp is found in the biliary canicular membranes of hepatocytes and on the apical surface of epithelial cells of the pancreas [10]. At all of these sites, intentional and unintentional modulation of the function of Pgp may occur.

INTENTIONAL MODULATION OF THE FUNCTION OF P-GLYCOPROTEIN RESULTING IN DRUG-DRUG INTERACTIONS

Intentional modulation of the function of Pgp occurs when one wants to deliver therapeutic drugs but the delivery is hindered by Pgp because the drug is a substrate of Pgp. The two main reasons for intentional modulation of the function of Pgp are to facilitate both oral drug delivery and the direct treatment of tumors where Pgp hinders the delivery of therapeutic drugs to the affected cells.

In some cases, the bioavailability of an orally administered drug is enhanced by another drug, when both of these molecules are substrates of Pgp. For example, the oral absorption of digoxin is restricted by Pgp which is expressed in the intestines. The cardiological drug talinolol, a beta-blocker, is also a substrate of Pgp. Pharmacokinetic studies conducted by Westphal et al. [11] in human volunteers showed that oral administration of both drugs resulted in an increase in the area under the concentration-time curve (AUC) and therefore enhanced the bioavailability of both drugs significantly. For this reason, when both drugs are administered simultaneously to humans, their doses can be reduced. From et al. [12] and his group [13] studied the bioavailability of digoxin when quinidine is simultaneously administered, in vitro and in vivo. The in vitro cell line studies were followed up in MDR1a (-/-) and wild-type animals and showed that quinidine administration increased both plasma and brain concentrations of digoxin by 73% in wild-type animals. The increase in digoxin concentration in plasma was not significant in MDR1a (-/-) mice when the doses of both drugs were the same as in the wild-type animals. These studies were followed by human studies which showed that perfusion of an isolated segment of human small intestine, in volunteers, with quinidine reduced intestinal digoxin elimination into the isolated intestine segment. In these studies, digoxin was administered intravenously, and quinidine accumulated in the isolated segment of the intestine.

The above studies indicate the possibility of improving drug delivery systems. There are several clinical trials aiming to improve drug administration, in which drugs taken orally are not efficiently absorbed through the gut, by suppressing the efflux function of Pgp.

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Paclitaxel is one such drug. In a clinical trial, cyclosporinA is being used to suppress Pgp in the gut to achieve this objective. Another reason to intentionally create drug-drug interactions is to block Pgp when the cells targeted for destruction express Pgp and the intended cell-toxic drugs are eliminated from those cells by Pgp-mediated efflux. The Pgp-blocker drug then modifies the pharmacokinetics and pharmacodynamics of the primary drug. One of the main reasons for intentionally blocking the function of Pgp is in the case of cancer chemotherapy. Some aspects of strategies for blocking Pgp in cancer chemotherapy were reviewed recently by Fojo and Bates [14]. Below, we provide several examples of studies aiming to use drug-drug interactions for this clinically important therapeutic reason.

Clinical trials have been performed with many Pgp blockers with the aim of suppressing Pgp in chemotherapy-resistant, Pgp-expressing tumors. The first generation of such agents did not end up in clinical practice, mostly because the necessary concentration required to block Pgp was so high that it resulted in host toxicity. One such agent, prochloperazine, was administered to non-small cell lung carcinoma patients with the cytotoxic agent doxorubicin in clinical trials. The concentration of doxorubicin was shown to increase in tumor cells with prochloperazine due to suppression of the activity of Pgp [15]. Verapamil was one of the first agents in clinical trials. The necessary concentration for blocking Pgp in tumors resulted in cardiac toxicity. The dextro-(d)-stereoisomer of verapamil proved to be less toxic and a dose of 120 mg/m² could be introduced orally. However, hypotension and bradycardia was sill associated with this treatment in patients with renal cell carcinoma [16]. Quinidin was also tried with the cytotoxic drug vinblastine [17]. In a cohort of 23 patients with metastatic renal cell carcinoma, quinidine doses of 100 to 400 were given with vinblastine intravenously as the cytotoxic drug. It was found that leukopenia occurred with the higher doses of quinidine. No clear advantage could be shown with the use of quinidine in terms of tumor regression. Similarly, tamoxifen, also a Pgp function modifier was tried with vinblastine in a phase I study [18]. The antiestrogen tamoxifen was administered orally to 53 patients with epithelial tumors at a maximum dose of 260 mg/m² twice a day with vinblastine intravenously. The dose limiting toxic effects of tamoxifen were tremor, hyperreflexia, dysmetria and dizziness. A dose of 150 mg/m² of tamoxifen was recommended for possible clinical application to suppress the function of Pgp. The immunosuppressor drug cyclosporinA was found to be an excellent Pgp blocker. It was administered in a high dose with the cytotoxic drug etoposide [19]. The clinical study showed that the major toxic effect of cyclosporinA, myelosuppression, is in a small part due to inhibition of Pgp in bone marrow precursor cells so that the intracellular concentration of etoposide increased in these cells. However, the major reason for the toxic effect of the 3 days infusion of 5 to 21 mg/kg/day cyclosporinA was another type of pharmacological drug-drug interaction, which resulted in an increase in unbound etoposide in the circulation. The clinically most successful Pgp inhibitor is vasopar (PSC833) which is a cyclosporinA type molecule, but it is not an immunosuppressor. It does not bind to cyclophillin, the intracellular molecule to which cyclosporinA has to bind in order to initiate immunosuppression. Since the USFDA approved its use for treatment for some tumors, several recent clinical trials have aimed to enlarge the scope of its use [20-22]. In these phase II studies, vaspodar was administered at up to 10 mg/kg/day as a continuous infusion for 3 or 4 days, after an initial loading dose or oral administration. The effect of this Pgp inhibitor was evaluated with daunorubicine and cytarabine in acute myeloid leukemia patients, with doxorubicine and cisplatin in patients with refractory ovarian cancer and with paclitaxel in patients with several types of advanced cancer. It was concluded that with the above doses of vaspodar the administered daunorubicine could be reduced to 35 mg/m² in myelogenous leukemia patients. In patients with ovarian carcinoma and non-small cell lung cancer, the dose of paclitaxel was reduced to 13.1 mg/m² intravenously, when the above dose of vaspodar was used. In a clinical trial in which doxorubicin with a fixed dose of cisplatin was used, vaspodar administration defined the maximum tolerated dose (MTD) of doxorubicin as 35 mg/m². These clinical trials have shown that the concentrations of the administered cytotoxic agents (daunorubicin, paclitaxel and doxorubicin) could be reduced, in some cases to one third of the doses when these agents were administered without vaspodar. This reduction could be achieved because the Pgp-associated efflux of the cytotoxic agents could be blocked by vaspodar, demonstrating an intentional modulation of the function of Pgp.

Interestingly, a plant extract was also tried recently to block the function of Pgp [23]. In the in vitro studies, using sensitive and resistant Pgp-expressing KB cells, it was shown that the leaf extract of the bitter melon (Momordica charantia L) inhibits the efflux of [³H]-vinblasin from the resistant cells and increased the sensitivity of these cells to vinblastine. The finding that a plant extract can modify the function of Pgp is an important observation. Namely, many plant extracts are consumed today as suggested health improving agents. If they block Pgp, they may cause unwanted pharmacological changes in other administered drugs, with potential toxic effects.

Webster et al. [24] studied the necessary concentration of cremophor, a drug solubilizing vehicle and Pgp function modifier, to increase the concentration of paclitaxel in Pgp-expressing tumor cells. By flow cytometric assay, using T-cell leukemia cells of patients infused with different concentrations of cremophor, they determined that for maximal retention of drugs in these cells a plasma concentration of 0.1% cremophor is necessary. In fact, one of the original formulations of paclitaxel contains cremophor.

Because some of the agents intended to block the function of Pgp were found to be too toxic in humans, combinations of these agents were tried in order to reduce toxicity. For example, Ross et al. [25] investigated the synergistic effect of two Pgp function modifiers, cyclosporinA and cremophor, to suppress Pgp function in acute myelogenous leukemia cells of patients. His studies were initiated because these modifiers, when used individually, require concentrations so high that they alone can cause toxicity in humans. They found that using a combination of cyclosporinA and cremophor permits the reduction of the concentration of cyclosporinA about 6-fold to obtain the same intracellular drug concentration when cyclosporinA is used alone. In a separate study, but with the same intent as indicated above, Hwang et al. [26] investigated the potential synergistic effects of different types of Pgp modifiers. They compared combinations of cyclosporinA, verapamil and cremophor using Pgp expressing L1210 and NIH3T3 cells. They showed that these three Pgp modifiers function in three different ways: cremophor acts through the modification of the physical status of cell membranes, verapamil directly blocks Pgp, and cyclosporinA does both. Using combinations of these Pgp modulators, one can achieve similar effects to the singly used modulators at more then 10-fold lower concentrations of the individual modulators as determined in cell toxicity assays, using daunorubicin as the cytotoxic agent. The clinical results of Ross et al. [25], as discussed above, have proven that these combinations of Pgp modifiers can be useful to avoid the associated toxicity of singly used Pgp modifiers. Actually, cremophor, a solubilizing agent, was found to be the most effective and safest Pgp modifier among the Pgp function modifier surfactant molecules [27].

Another way to intentionally block the function of Pgp may be by modifying the lipid composition of the plasma membrane in which Pgp is embedded. The feasibility of this type of approach was demonstrated by Callahan et al. [28], who observed that incorporation of heptadecanonic acid into the plasma membranes of Pgp-expressing cells modified the drug efflux functions of Pgp. It was
thought that the reason for this modification could be an induced alteration in the biophysical milieu of Pgp, embedded in the plasma membrane, due to the incorporation of this fatty acid. Furthermore, it was shown by Saeki et al. [29] that the function of Pgp is modulated by the cholesterol content of lipid bilayers. In relation to this approach, Wesolowska et al. [30] have shown that a Pgp modulator, a phenothiazine derivative, occupies a different location in lipid bilayers composed with differently charged lipid components. It is then postulated that molecules intercalating differently into plasma membranes of normal and Pgp-expressing malignant cells may facilitate drug delivery selectively to the malignant cells by modifying the function of Pgp. This postulation is based on the observation that there are differences between the lipid composition of normal cell membranes and that of the membranes of transformed malignant cells [31].

There are several ways to study drug-drug interactions in vitro and in vivo. Most of these methods have been described earlier [32, 26, 33, 34]. We would like to mention one unconventional but interesting method, based on positron emission tomography (PET), introduced by Hendrickse et al. [35]. They administered radiolabeled daunorubicin and verapamil to Pgp-negative and Pgp-positive tumor-bearing rats. They were able to show that after introduction of cyclosporin A, a Pgp modifier, the concentration of daunorubicin and verapamil increased in the Pgp-positive tumors to the level of the Pgp-negative tumors. Without cyclosporin A the concentration of these two drugs in Pgp-negative tumors was almost twice as much as in the Pgp-positive tumors. One other interesting in vivo assessment of the effectiveness of Pgp inhibitors is based on imaging. Tc-99m sestamibi, a Pgp substrate and imaging agent. When administered together with vaspodar, enhancement of tumor visualization and AUC time was achieved. This study indicates statistically significant alteration of sestamibi accumulation in the presence of vaspodar in a cohort of nine patients with metastatic renal carcinoma [36]. A similar effect was achieved when the Pgp inhibitor XR9576 was used instead of sestamibi [37].

UNINTENTIONAL MODULATION OF THE FUNCTION OF P-GLYCOPROTEIN RESULTING IN TOXIC DRUG-DRUG INTERACTIONS

Unintentional and unexpected toxic side effects have been noted many times in clinical practice when several drugs were administered simultaneously to patients. To avoid such unpredictable toxic side effects, some investigators have assessed the potential of many drugs to cause these effects, and specifically their tendency to interfere with the function of constitutively expressed Pgp. Such interference may cause, for example, the unwanted opening of the blood-brain barrier or hinder the elimination of metabolites through the kidney. With this background in mind, Ibrahim et al. [38, 39] studied the extent of the ability of different classes of drugs to block the function of Pgp. They have studied the extent of Pgp inhibition by drugs in human blood-brain barrier capillary endothelial cells, and in MDCKpHNaMDR kidney and in Caco-2 MDR intestinal cell lines. Experiments were carried out in monolayers and by flow cytometric assessments. Cells in the monolayers express Pgp in a polarized fashion and mimic drug transport in in vivo endothelial cells. They assessed the ability of several classes of drugs, such as beta-adrenergic antagonists, H1-receptor blockers, analgesics, diuretics, quinolone antibiotics, antipsychotic and antiemetic drugs and Cao2 channel blockers. The extent of the efflux of the anticancer drug daunorubicin was determined in the presence of these different drugs. They found that some drugs inhibit the efflux of daunorubicin completely and that many drugs do this to some extent at the clinical dose level, indicating potential unwanted drug–drug interactions. Additional studies resulted in an important observation that combinations of 2 or 3 drugs, which individually block Pgp only to some extent at clinical dose level, synergistically inhibited the efflux of daunorubicin. This observation is important for clinical practice when several drugs are administered to patients. Some of the results of the study indicating the extent of blocking Pgp at different doses are shown in Table 1.

A similar study extended the above assessments to clinical blood cells of 3 patients with acute myeloid leukemia, ex vivo [40]. The retention of daunorubicin was assessed in these cells after exposure to antipsychotic drugs. It was found that the studied antipsychotic drugs blocked the efflux of daunorubicin mediated by Pgp comparable to the extent with Cyclosporin A. It should be mentioned here that many cancer patients also receive antipsychotic drugs and that in such case the blood-brain barrier may be opened to anticancer drugs. In line with the above studies new antidepressant drugs were assessed also for their ability to inhibit Pgp in porcine kidney and brain capillary endothelial cells expressing Pgp [41].

Modification of the absorption of drugs through the intestine can also be influenced by certain drugs in a different way. Interestingly unintentional drug-drug interactions occur when certain drugs and some herbs targeting the function of Pgp in fact induce above normal expression of Pgp. One related study by Westphal et al. [42] showed that the Pgp substrate rifampin increases human intestinal secretion of talinol, another Pgp substrate drug. After intravenous coadministration of rifampin, the serum level of talinol, Cmax, was reduced by 20% and the systemic clearance increased by 28%. These differences increased further with the oral administration of rifampin. It could be shown that these differences are not due to changes in metabolic enzyme activity and that increased expression of Pgp in the gut wall resulted in the pharmacokinetical changes.

Not only drugs, but also herbal medicines can cause unintentional drug-drug interactions. A good example of this is St John's Wort, a herb that is widely used for treatment of mild depression. Durr et al. [43] investigated first the effect of St John's Wort extracts in rats. They found a 3.8-fold higher expression of Pgp in the intestines along with a 2.5-fold increase in hepatic CYP3A2 enzyme. Extension of this study in volunteer humans resulted in a 1.4-fold expression of Pgp along with a 1.5-fold increase in expression of CYP3A4 in the duodenum. This overexpression of Pgp resulted in an 18% decrease in bioavailability of digoxin in these humans. It should be mentioned that digoxin is not metabolized by CYP3A4 and therefore all the pharmacokinetical changes could be attributed to the increased expression of Pgp. A similar study was conducted by Hennessy et al. [44]. In a placebo-controlled human study, St John's Wort was administered for 16 days and its effect on the co-administered HIV drug and the immunosuppressor drug cyclosporin A were assessed in peripheral blood lymphocytes. The retention of rhodamin, a fluorescent Pgp substrate, was assessed in the peripheral lymphocytes of patients treated and not treated with St John's Wort, using the HIV drug and Pgp substrate ritonavir. After treatment with St John's Wort, the efflux of rhodamin from the lymphocytes increased from 24% to 75%. It was also found that the amount of expressed Pgp in the lymphocytes of patients treated with St John's Wort increased 4.2-fold, as compared to the patients not treated, as detected by Pgp-specific antibodies and flow cytometry.

Unintentional drug-drug interactions with severe toxic effects have been observed many times in clinics. Such cases are subject to MEDWACH reporting to the USFDA. One example of such a case was reported when diltiazem, a drug that inhibits Pgp at 500 µg/ml concentration, was introduced to a liver transplant patient to reach the normal clinical blood level of 200 µg/ml. Tacrolimus was also introduced at the normal clinical dose. However, the combination of these two drugs resulted in observed neurotoxicity to the patient. It was concluded that the synergistic combination of these two drugs led to modulation of Pgp expression at the blood-brain barrier, causing penetration of these drugs into the CNS compartment and the observed neurotoxicity [45]. In another case, a cancer patient
complained about somnolence, ataxia and hallucination. The chemotherapy included doxorubicine, ondansetron, dexamethason, lorazepam and diltiazem. Since these drugs can modulate Pgp to some extent, they most likely acted synergistically, causing CNS penetration of some of these drugs to a level which led to the observed toxic effects. This conclusion was supported by the fact that the liver function and the renal function of the patient were normal during the course of the therapy (UF/Dist.Rep. #1426509).

The importance of unintentional drug-drug interactions is underscored by the fact that their potential problems have been exclusively dealt with by several authors in the literature [46, 47]. It should be mentioned also that drug-drug interactions could be mediated by metabolizing liver enzymes, such as P-4503A enzymes. Pgp substrates, including drugs, could also be substrates of these metabolizing enzymes. However, there is no correlation between the inhibitory potencies of Pgp drug substrates and the liver metabolizing enzymes [48].

INTRODUCTION TO MRP1 AND MRP2

Studies using cell lines selected for MDR demonstrate an association between expression of MRP1 or MRP2 and MDR. Like Pgp, MRP (multidrug resistance protein) has the capacity to mediate transmembrane transport of many (conjugated) drugs and other compounds. Nine members have been identified in the MRP family. However, the study of MRP has mainly focused on MRP1 and MRP2. Several cell lines that display a multidrug resistance phenotype have been isolated without detectable Pgp expression, despite having undergone drug selection similar to that elevating expression of Pgp. MRP1 was discovered in these cells by a differential hybridization screen aimed at identifying mRNA species whose expression is associated with the gain or loss of the multidrug resistance phenotype [49]. An isoform of MRP1 has been cloned and localized predominantly to the hepatocyte canalicular membrane. This apical conjugate-transporting ATPase has been termed as multidrug resistance protein 2 (MRP2) because there is some similarity in substrate specificity and sequence with the multidrug resistance protein (MRP1). MRP2 is also known as canalicular MRP (cMRP) or canalicular multispecific organic anion transporter (cMOAT). It is important to understand the mechanism of MRP1 and MRP2 in the disposition of endogenous compounds, drugs and other xenobiotics in many organs in order to selectively target these transporters. Since MRP1 and MRP2 are not as well known as Pgp, some characterization of them will follow.

Characteristics of the MRP1 and MRP2 Transporters

Human MRP1 and MRP2 are composed of 1531 and 1545 amino acids, respectively. The MRP1 gene is localized to chromosome 16p13.1 and the MRP2 gene is found at chromosome 10q24.

Table 1. Extent of Modulation of the Function of P-Glycoprotein by Different Drugs

<table>
<thead>
<tr>
<th>Drug class/Drug name</th>
<th>Drug concentration, ng/ml</th>
<th>% Inhibition of Pgp</th>
<th>Clinical drug blood level, ng/ml</th>
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<td><strong>Analgetics</strong></td>
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<td>Propoxyphene</td>
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<td>Loperamide</td>
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<td><strong>H1-receptor blockers</strong></td>
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<td>Terfenadine</td>
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<td>Clemastine</td>
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<td>Metoclopramide</td>
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<td><strong>Ca</strong>(^{2+}) channel blockers</td>
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<td>Verapamil</td>
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<td><strong>Antipsychotics</strong></td>
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<td>Fluphenazine</td>
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<td>Triflupromazine</td>
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Dose dependent inhibition of Pgp in NIH3T3/MDR1 or Caco-2/MDR1 cells by drugs of different treatment classes. Inhibition of Pgp function was measured by flow cytometric assessment by retention of rhodamin123 and daunorubicin. Dashes indicate that no measurements were made. For calculation of % inhibition both NIH3T3/MDR1 and the parental NIH3T3 cells were used. % Inhibition = FI of MDR1 with drug – FI of MDR1 without drug / FI of parental without drug – FI of MDR1 without drug, where FI = Fluorescence Intensity.
Both of them are amplified in various anti-tumor drug-resistant cell lines [50, 51]. Analysis of their amino acid sequences identified the MRP1 and MRP2 proteins as members of the ATP-binding cassette (ABC) superfamily of transporter proteins. However, the well-known Pgp is only distantly related to MRP1 and MRP2. The amino acid sequence identity between MRP1 and Pgp is only 15%, while comparison of the MRP2 sequence with that of Pgp indicates that there is 25% identity between them. MRP2 has an overall amino acid sequence identity of 49% to MRP1, and the region with highest identity of amino acid sequence is located in the carboxy-terminal domain [52]. Human MRP1 and MRP2 have a 172kDa [53] and 174 kDa molecular mass of unglycosylated form, respectively [54]. Characteristics of both MRP1 and MRP2 are listed in Table 2. Both MRP1 and MRP2 are N-glycosylated in their mature form and have an apparent molecular mass of about 190kDa. MRP1 and MRP2 have a similar core structure to that of MDR, i.e. an internally duplicated structure of two cytosolic nucleotide ATP-binding sites and two putative six transmembrane segments. However, MRP1 and MRP2 have an extra N-terminal transmembrane domain containing 5 membrane-spanning helices compared to the traditional topology of MDR Pgp.

MRP1 structure to 22Å resolution has been obtained by electron microscopy studies of negatively stained single particles as well as 2-dimensional crystals formed after reconstitution of native MRP1 with lipids [55]. A functional analysis of the nucleotide binding domains of MRP1 has identified several mutations that are critical for MRP-mediated multidrug resistance [56]. Serine is the single amino acid phosphorylated in MRP1, whose phosphate groups are metabolically active and undergo cycles of phosphorylation and dephosphorylation in the cells [57]. However, the detailed structure of MRP1 still needs be solved by higher resolution of MRP1 X-ray studies.

The structure of MRP2 has been predicted by comparing the pattern of the hydrophatic plot of the deduced amino acid sequence with that of MRP1. MRP1 and MRP2 contain a 13 amino acid long “deletion” between the Walker A and Walker B motifs of the N-terminal nucleotide binding domain, in comparison with the nucleotide binding domains of Pgp. The predicted topology of MRP2 is similar to that of MRP1 with two nucleotide binding domains and 17 transmembrane helices divided into three transmembrane-spanning domains (TMDs). The N-terminal TMD$_0$ is composed of five transmembrane helices, and TMD$_1$ and TMD$_2$ are followed by amino-and carboxy-terminal ABC regions, respectively. Recent studies have shown that TMD$_0$ is important for the proper routing of MRP2 to the apical membrane in that transfection of polarized Madine-Darby canine kidney (MDCKII) cells with an MRP2 expression vector construct lacking TMD$_0$ resulted in localization of a functional MRP2 protein in intracellular compartments [58]. Chimeric polypeptides composed of the various combinations of NH$_2$ and COOH-proximal portions of MRP1 and MRP2 expressed in polarized epithelial cells also suggest the critical importance of the COOH-terminal region of MRP2 for apical sorting.

### Distribution and Localization of MRP1 and MRP2 in Normal Tissue and Tumors

MRP1 and MRP2 are predominantly localized to the plasma membrane, particularly in cancer cells, with detectable levels present in intracellular membrane compartments of some cell types. In addition to an MDR1-like core, MRP1 and MRP2 contain an additional N-terminal segment of about 280 amino acids embedded with
five transmembrane helices, while a small cytoplasmic loop (≈ 80 amino acids) connects this area to the core (detailed above). Studies revealed a special role of the small cytoplasmic loop region for the transport activity and the proper intracellular routing of the MRP1 and MRP2 proteins [58, 59]. MRP1 is expressed at a low level in the liver, and the expression is restricted to the basolateral membrane and sinusoidal cells. The expression of MRP2 is predominant in the canalicular membrane of hepatocytes, and highly expressed MRP2 is found in the apical membranes of kidney proximal tubules [60]. MRP2 is predominantly expressed in the liver and to a lesser extent in the kidney, whereas none is detectable in the brain, heart, lung, testis or skeletal muscle [61, 62].

In humans, MRP1 appears to be more ubiquitously distributed than MRP2; it has been detected in several types of epithelia, lung bronchioles, smooth and skeletal muscle cells, and in the heart, adrenal cortex, epidermis, salivary gland ducts and alveolar macrophages. This generalized tissue distribution makes it difficult to evaluate normal functions of MRP1. Because of the presence of MRP1 in many epithelia, MRP1 may have an excretory function in protecting the organism against xenobiotics. The presence of MRP1 in bronchiolar epithelium, heart muscle, and macrophages would agree with the glutathione S-conjugate carrier activity detected previously [63]. Furthermore, MRP staining was seen in 46 of 119 untreated tumors from various histogenetic origins. This suggests that MRP1 may contribute to the intrinsic resistance against treatment with chemotherapeutic drugs.

MRP1 is also expressed in blood cells [64]. Mice homozygous for a disrupted MRP1 gene have an impaired response to an inflammatory stimulus, probably as a consequence of decreased leukotriene C4 secretion from leukocytes [65]. Murine MRP1 is localized to the basolateral membrane of various epithelial cells, and human MRP1 is routed to the basolateral membrane when expressed in the epithelial cell lines LLC-PK1 and Madin-Darby canine kidney (MDCK) II [66]. In the murine kidney, MRP1 is expressed at the basolateral membrane of cells of Henle’s loop and the cortical collecting duct [67]. Under certain conditions and in certain cell types, MRP1 can also be found in intracellular membranes of vesicles, possibly derived from the Golgi apparatus [68]. Recent evidence indicates that subcellular MRP1 is active but its physiological function in this subcellular location, if any, is not well understood [69]. In addition, MRP1 expression is detected in almost every cell type, including lung, gastrointestinal, urothelial carcinoma, neuroblastomas, glomas, retinoblastomas, melanomas, tumors of the breast, endometrium, ovary, prostate and thyroid, as well as in hematological malignancies (see Table 2). MRP1 is particularly highly expressed in the major histologic forms of non-small cell lung cancer. MRP2, described as the canalicular multispecific organic anion transporter, is also expressed at the renal proximal tubules and small intestinal villi [70]. A killifish ortholog of MRP2 has been located at the brush-border membrane by immunohistochemistry with a polyclonal antibody raised against rabbit [71]. MRP2, like MRP1, is also expressed in some tumor tissues such as ovarian and colorectal carcinomas, mesotheliomas, hepatocarcinomas, and in leukemic cells.

**Physiological Functions of MRP1 and MRP2**

MRP1 and MRP2 proteins play an important physiological role in the protection of the body against xenobiotics occurring in the environment, which is accomplished by active efflux of these toxic agents. In order to characterize the normal physiological functions of MRP1, knockout mice have been generated to lack a functional MRP1 gene. These mice are healthy and fertile, have a normal lifespan and show no physiological, clinical or histological abnormalities [72, 73]. Therefore, inhibition of MRP1 would not probably lead to severe abnormalities. However, it cannot be excluded that humans lacking MRP1 might show a phenotype due to a broad exposure to toxins in food and the use of various drugs. Analysis of MRP1 knockout mice has demonstrated that a high level of MRP1 in the oropharyngeal mucosa protects against drug-induced oral mucositis caused by direct damage of the epithelium of the tongue and cheek [74, 75]. MRP1 deficient mice also exhibit markedly increased damage of bone marrow, oropharyngeal mucosal surfaces, and the testes by cytotoxic drugs and impaired inflammatory response [65, 67, 74]. A high level of MRP1 in the epithelium of the urinary collecting ducts protects against diabetes insipidus, and in the basal plasma membrane of the epithelium of the testicular tubules and sertoli cells, it protects against the abrogation of spermatogenesis.

The brain is one of the most critical and sensitive organs in vertebrates. Pgp makes an important contribution to the blood-brain barrier, a selective barrier to nutrient uptake and compound transport from the bloodstream to brain tissue (detailed above). MRP1 contributes to another barrier, the blood-CSF (cerebrospinal fluid) barrier together with Pgp, which separates the brain from the spinal cord and prevents many compounds from moving from the bloodstream into the CSF. In fact, MRP1 is an important contributor to the blood-tissue barrier since high levels of MRP1 have been detected in the choroid plexus. The absence of MRP1 in knockout mice results in a 10-fold higher transport of compounds into the CSF, whereas the plasma and brain concentration in both mice was not significantly different [75, 68].

Transport across the hepatocyte canalicular membrane into bile is a decisive step in the elimination of endogenous and xenobiotic substances from the mammalian organism. Transferrases in the hepatocyte catalyze the conversion of many of these substances into amphiphilic anionic conjugates with glutathione, glucuronate, or sulfate. Excretion of these conjugates into bile is mediated by MRP2. Functional studies in normal and transport-deficient mutant rats indicated a broad spectrum of endogenous and xenobiotic amphiphilic anions as substrates for MRP2, including many glutathione S-conjugates, glucurononides, and sulfate conjugates. MRP2 is a canalicular multispecific organic anion transporter, and mutations of it could lead to errors in biliary secretion resulting in the mild liver disease termed Dubin-Johnson syndrome, a hereditary disorder characterized by modest elevation in serum conjugated bilirubin and a rare autosomal recessive liver disorder with a similar phenotype [76, 77]. In the liver, MRP2 plays an important role in the biliary excretion of multiple conjugated and unconjugated organic anions across the canalicular and urethral epithelial membranes. Another physiological role of MRP2 is to maintain the homeostasis of reduced folates. Biliary excretion of folates is less in Esai hyperbilirubinemic mice, suggesting that these substrates are transported via MRP2. MRP2 provides a major route for the secretion of organic anions from the liver, and rats and humans lacking this transporter develop a mild liver disease, mainly due to the inability of the liver to excrete bilirubin-glucurononides.

Furthermore, studies with membrane vesicles from MRP2-expressing Sf9 cells have identified p-aminohippurate as an MRP2 substrate, suggesting that MRP2 might be involved in renal clearance of this compound [70]. Impairment of cholestasis can be associated with a down-regulation of MRP2 expression, and MRP2 contributes to bile flow as an important driving force.

**Substrates Transported by MRP1 and MRP2**

Drug absorption across the gut tract can be highly dependent upon its affinity with transport proteins. MRP1 functions as an ATP-dependent transporter of anionic conjugates such as leukotriene C₄, S-(dinitrophenyl)-glutathione, estradiol-17β-D-glucuronide, and etoposide-glucuronide [78-81]. The most thoroughly studied MRP1 substrate is leukotriene C₄, which is transported by MRP1 in an ATP-dependent fashion with high efficiency (Km approx. 100nM) [80, 82]. Aflatoxin B1 is characterized as a hepatotoxin and hepatocarcinogen in many vertebrate species [83]. MRP1 may play a potential protective role in mammalian chemical car-
cinogenesis by transporting aflatoxin B1 and its glutathione (GSH) conjugates. Like Pgp, MRP1 has been shown to confer cellular resistance to various natural products by transporting the agents out of the cells. MRP1-associated oncolytic drugs include the anthracyclines (doxorubicin and daun-orubicin), epipodophyllotoxins (etoposide), and vinca alkaloids (vincristine). Usually, MRP1 needs a cellular co-factor, the ubiquitous, negatively-charged tripeptide GSH in order to pump out hydrophobic anticancer drugs such as etoposide, vincristine and doxorubicin. Further analysis demonstrated that MRP1 primarily transports amphipathic organic anions, including GSH-, glucuronide-, and sulfate-conjugates of many drugs, toxins and endogenous compounds, including the inflammatory mediator leukotriene C4 [84].

MRP2-mediated transport has been investigated in isolated rat hepatocytes and killifish renal proximal tubules. Studies with killifish renal proximal tubules have indicated the presence of an MRP-like transporter involved in efflux of fluorescein-methotrexate and lucifer yellow [85]. MRP2 is involved in renal excretion of organic anions. Efflux of lucifer yellow from killifish proximal tubules is only partly inhibited by the MRP2 substrates leukotriene C4 and S-(dinitrophenyl)-glutathione [71]. Also, recent clearance of the MRP2 substrates α-naphthyl-β-D-glucuronide, E3040-glucuronide, cephramide, the quinolone HSR-903, and lucifer yellow is not impaired in MRP2-deficient rats. Biochemical studies using the cloned cDNA indicate that MRP2, like MRP1, directly mediates the transport of glucuronate and GSH conjugates albeit at affinities significantly lower than those of MRP1 [86, 87]. Recent studies have shown that MRP2, like MRP1, transports GSH itself. Efflux of GSH from MDCKII cells overexpressing MRP2 is sensitive to depletion of ATP [88]. In addition, both cAMP and PKC stimulated MRP2-mediated efflux of an anionic conjugate across the hepatocyte apical membrane [89]. In the killifish proximal tubule, efflux of fluorescein-methotrexate is negatively correlated with PKC activity.

**MRP1- AND MRP2-MEDIATED RESISTANCE TO ANTI-TUMOR DRUGS**

MRP1 was discovered in doxorubicin-resistant lung H69AR cancer cells that lack Pgp expression. H69AR was derived from the parental NCI-H69 (H69) small cell lung cancer cell line by repeated transient exposure to doxorubicin. H69AR cells display moderately high levels of resistance (=100-fold) to the Vinca alkaloids, epipodophyllotoxins, doxorubicin, and mitoxantrone. In addition, these cells are highly resistant to daunorubicin, epirubicin, and colchicine [90]. HeLa cells overexpressing MRP1 display a typical multidrug resistance phenotype accompanied by lower drug intracellular accumulation. The most intriguing characteristic of MRP1 is its wide substrate specificity. Transfection of the MRP1 cDNA conferred resistance in tumor cells to anthracyclines, Vinca alkaloids, etoposide, arsenical and antimicrobial agents but not to cisplatin and mitoxantrone. Similar findings were reported by several independent laboratories using different cell types [91, 92]. It has also been shown that MRP1 antisense oligonucleotides can decrease MRP1 levels and reverse drug resistance in transfected and drug-selected cells [93].

Mouse embryonic stem cells and bone marrow-derived mast cells in which the MRP1 gene was inactivated showed an increased sensitivity to anthracyclines. MRPI does not confer resistance to taxol, a clinically important agent that is a substrate of Pgp. MRP1 can transport methotrexate, but not its polyglutylated metabolites [94]. MRP1 lies at the intersection between the GSH-dependent cellular machinery of phase II detoxification and drug efflux. BSO can modulate drug resistance by MRP1 expression with pacemaker enzymes (gamma-glutamylcysteine synthetase (gamma-GCS) and glutathione S-transferases (GSTs)) for GSH synthesis and S-conjugation. The forced overexpression of MRP1 with gamma-GCS and/or GSTs confers significantly enhanced levels of drug resistance compared to expression of single components alone, which indicates that coordinate expression regulation in tumors may be of clinical significance [95].

Like MRP1, human MRP2 not only transports anionic conjugates but also confers resistance to various cationic chemotherapeutic drugs [85, 96]. The biochemical mechanism whereby MRP2 confers resistance to natural products appears to be similar to that of MRP1. Studies with membrane vesicles from cells expressing human MRP2 have indicated that MRP2 confers resistance to a variety of natural products such as anthracyclines, vinca alkaloids, etoposide as well as camptothecins and methotrexate [85, 86]. Furthermore, transportation of cationic drugs, such as vincristine, daunorubicin, and aflatoxin B1, only occurs in the presence of physiological concentrations of GSH with MRP2 [97]. A similar GSH dependency of ATP-dependent vinblastine transport has also been shown for rabbit MRP2 [98]. For human MRP1, the mechanism involved in GSH-stimulated ATP-dependent transport of vincristine has been identified as a GSH-vincristine cotransport mechanism. Daunorubicin and etoposide, unlike vincristine, did not stimulate GSH transport, indicating the involvement of a mechanism different from cotransport [97]. However, unlike MRP1, MRP2 may be competent in the transport of GSH alone without the requirement for simultaneous cotransport or binding of other compounds. To date, there is little knowledge about the mechanism by which GSH participates in MRP2-mediated drug resistance.

MRP1 and MRP2 both seem to be key players in drug transport in various tissues and share the ability of recognizing a large number of various, mainly hydrophobic compounds. Although they mostly transport hydrophobic anionic conjugates, MRP1 and MRP2 also extrude hydrophobic uncharged drugs [99, 100].

**MRP1 AND MRP2 AS POTENTIAL DRUG TARGETS**

In the clinical setting, patients who overexpress MRP1 or MRP2 in tumors are usually not responsive to anticancer agents. Thus, many of these patients progress to advanced diseases and have poorer prognoses. The search for MRP1 or MRP2 modulators or inhibitors has only recently begun, and only a few potent and selective modulators or inhibitors are known to be effective in the clinic.

There has been intense search for compounds which can act to reverse the MDR phenotype exhibited by MRP1 or MRP2 transporters. Reversing agents can be designed to target MRP1 and MRP2 at different levels such as at the protein, mRNA and DNA levels. The function of MRP1 and MRP2 can be inhibited at the protein level by chemical modulators or other reversing agents, and by suppression of protein expressed via translational repression. Using monoclonal antibodies is a novel strategy to target therapy against malignant cells. Previous studies indicated the existence of different MRP1 or MRP2 conformations associated with different stages of transport-associated ATP hydrolysis and substrate translocation. This suggests the possibility of generating specific antibodies capable of trapping MRP1 or MRP2 in a transient conformation as a mechanism for antibody-mediated inhibition of drug efflux activity, thereby potentiating the efficacy of chemotherapeutic agents. Several MRP1-specific monoclonal antibodies have been developed to detect various intracellular epitopes that require prior permeabilization of cells. Recently, it was found that a monoclonal antibody directed towards an extracapillary epitope of the human MRP1 could specifically recognize a cell surface domain located at the N-terminus of this anticancer drug efflux transporter [101]. In addition, intracellular expression of an antibody fragment could be used for intracellular sequestration of the MRP1 or MRP2, thereby preventing its expression on the cell surface, resulting in a phenotypic knockout. It was demonstrated previously that antibody fragments directed against the EGF and IL-2 receptors were able to reverse the transformed phenotype of the transfected cells and suppressed tumor growth in vivo almost completely, preventing the
presentation of these receptors on the cell surface [102, 103]. It is thought that intracellular expression of antibodies may be a more effective method for gene inactivation compared to anti-sense RNA and dominant negative mutants because of its high specificity [104].

The function of MRP1 and MRP2 can be affected by suppression at the mRNA level, by gene expression via transcriptional repression or induced mRNA degradation. This type of inactivation of MRP1 and MRP2 is a potential powerful therapeutic strategy. However, unlike the rigorous search for agents that directly modulate the transporter activity, there has been relatively little progress in discovering agents capable of reversing MDR at the mRNA level. The specific and relatively stable antisense phosphorothioate oligonucleotide could promote degradation of targeted mRNA to downregulate gene expression. It was reported that the antisense phosphorothioate oligonucleotide, ISIS 7597, could reduce MRP1 mRNA and protein expression in MRP1-transfected HeLa cells. Unfortunately, the decrease in MRP1 expression was somehow short-lived and only partially sensitized the drug-resistant cells to doxorubicin [93].

The recently developed posttranscriptional expression blocking method, known as RNA interference (RNAi) is considered a revolutionary method of silencing targeted genes. RNAi, mediated by double-stranded RNA, is cleaved into 21 to 23-nt duplexes. These duplexes, small interfering RNAs (siRNAs), can be incorporated into a protein complex called the RNA-induced silencing complex (RISC), which can recognize and cleave the target mRNA. Downregulated MRP2 by antisense cDNA expression or an antisense oligonucleotide-mediated strategy may be involved in the sensitivity of cells to certain anticancer agents [96]. Chemically synthesized siRNA targeted against MRP2 showed gene-silencing activities and reversed the drug-resistant phenotype in the ovari an carcinoma cell line A2780RCIS [105].

Most of the effort aimed at reversing MDR has focused on small molecules inhibiting MRP1 or MRP2 function. Most modulators reverse MDR by restoring the intracellular accumulation of drugs, either by direct competition for their drug binding sites, or by non-comprehensive inhibition by causing conformational changes to the protein upon binding. Agosterol A, isolated from a marine sponge, was found to reverse vincristine resistance, and reduce MRP mediated [3H]-leukothriene C4 transport using membrane vesicles prepared from the MRP overexpressing CV60 cells [106]. PAK-104P, a pyridine analog, acts on a non-competitive manner in inhibiting MRP-mediated efflux of anthracyclins [107]. Bathonium sulfoximine, an agent used to reduce glutathione levels, appears to be a good inhibitor to sensitize MRP-overexpressing cells to natural products [108]. GS-EA derivatives, synthesized glutathione conjugates of ethacrynic acid, partially reversed the resistance of MRP1-overproducing SI9 cells to methotrexate [109]. Tricyclic isoxazoles were identified as a promising series of selective MRP1 inhibitors [110]. Furosemide, an anionic diuretic, has a strong stimulatory effect on the MRP2–ATPase activity in high concentration [111], while furosemide has little effect on MRP1, pointing in the direction of selective inhibition of MRP2. Benzylxarboxone has been reported to be an inhibitor of both MRP1 and MRP2 [59]. MK 571, an efficient antagonist of a leukotriene receptor, inhibited both MRP1-dependent transport and ATPase activity. GSF-0017, a potent MRP1 inhibitor, effectively inhibited the MRP1 transporter of the GS-PG (prostaglandin) conjugate in plasma membrane vesicles prepared from HL-60/R-CP cells [112]. The uricosuric agent, sulfinpyrazone, can inhibit MRP1 transport activity but stimulates MRP2 transport activity, again a possibility for development of a selective agent. Highly specific small molecular inhibitors of MRP2 would be valuable additions to studies attempting to delineate the contribution of different transporters in in vivo models of drug disposition and elimination.

Some agents inhibit both MRP1 and MRP2. The farnesyl protein transferase inhibitor SCH-66336 (Ionoafarnib) can inhibit the function of MRP1 and MRP2 [113]. A clinical study indicated that Ionoafarnib causes a synergy with cisplatin and 5-fluorouracil by inhibiting MRP1 and MRP2. Coadministration of these drugs could thus reduce chemotherapeutic and produce less toxicity. The dye of the turmeric curcumin contained as a food ingredient in curry inhibits both MRP1- and MRP2-mediated transport in cells [114]. It should be noted that interactions between cross-stimulatory or inhibitory compounds appear very complex.

Most high-affinity substrates for MRP1 and MRP2 are organic anions with a substantial hydrophobic moiety. Potent competitive inhibitors should share this characteristic as high-affinity substrates, such as leukotriene Cs, S-decylglutathiones, and the leukotriene D4 antagonist MK571. Other inhibitors of MRP1 are organic acids that were originally developed to inhibit transport of uric acid, like sulfinpyrazone, benzylxarboxone, and propencene. Although MRP1 and MRP2 have similar substrate specificity, inhibitors of MRP1 are not necessarily good inhibitors of MRP2. Sulfinpyrazone, for instance, does not inhibit transport of the model substrate nitrophenyl S-glutathione by MRP2. Negatively charged compounds do not readily enter cells. Therefore, they do not provide an obvious advantage for drug development. It is thought that good inhibitors have to be administered as pre-drugs in which the charged moiety is shielded [60].

**PROSPECTIVE DEVELOPMENTS IN MRP1 AND MRP2 STUDIES**

Classic physiological and biochemical studies have shown that MRP1 and MRP2 play an important role in the adsorption, distribution, and elimination of drugs and xenobiotics. In normal tissue, MRP1 and MRP2 play a role in the maintenance of cellular homeostasis by modulating intracellular concentration of various molecules of physiological significance. The overlapping resistance profiles of MRP1 and MRP2 have important ramifications for the clinical use of modulators designed to inhibit the action of MRP1 and MRP2. More detailed studies of MRP1 and MRP2 expression in specific tumors may be useful to determine which inhibitors to use and when to use them during the course of clinical treatments. Interestingly, patients without treatment with chemotherapeutic agents frequently overexpress MRP1 and MRP2 mRNA. Very low levels of MRP1 and MRP2 in cells and tissues may significantly affect their basal resistance to cytotoxic drugs. Protein sorting to the plasma membrane represents an important component of the regulation of MRP1 or MRP2 expression, and has become an intensively studied issue. The cellular localization of MRP1 and MRP2 greatly influences their function. This is exemplified by the intracellular retention of MRP2 observed in certain cases of Dubin–Johnson syndrome and by the endocytic retrieval of MRP2 in endotoxin-induced cholestasis [77, 115]. It has also been demonstrated that defective and recycled MRP1 is mislocalized in the cytoplasm in adenocarcinoma and hepatocarcinoma cells resistant to cisplatin [69]. In conclusion, it is important to understand the regulation of MRP1 and MRP2 in different cancer systems so that effective strategies could be developed to circumvent intrinsic resistance to cancer chemotherapy.

Accumulating evidence demonstrates that MRP1 and MRP2 play a critical role in the absorption of oral medicines across the gastrointestinal tract, in the distribution of drugs used for therapy such as at the blood-CSF barrier and in tumor cells, and in the excretion of metabolites into the bile or urine. In addition, characterization of gene polymorphisms will allow the prediction of individual response variability to specific drugs. The clinical relevance of genetic polymorphisms of MRP1 and MRP2 and their impact on the pharmacological response of individual patients remain to be elucidated. So far, it has been difficult to identify efficient MRP1 inhibitors that can be applied at sufficiently high dosages to inhibit
MRP1 activity in vivo. Recently, it was shown that the use of photoactive probes allows for rapid screening of MRP1- or MRP2-specific high affinity inhibitors or activators. Correlations among structurally and functionally dissimilar photocative probes allow for the rapid identification of interacting drugs, either as substrates or reversing agents [16]. Ultimately, higher resolution analysis of the structure of MRP1 or MRP2 is needed to determine the architecture of their binding sites and their exact mechanisms of transportation.

SUMMARY

The study of drug transporters is a rapidly expanding area in drug metabolism, pharmacokinetics, and cancer chemotherapy, both in terms of molecular characterization of transport proteins and in understanding their contribution to drug absorption, drug excretion and cellular and tumoral distribution of drugs, metabolites and xenobiotics. In the first section of this review, we described certain aspects of drug-drug interactions related to the transporter Pgp, or ABCB1. These interactions occur in most cases of polypharmacy of the elderly, and in HIV and cancer patients. There is no definite evaluation of potential toxic side effect in case of polypharmacy despite of some efforts by the USFDA and several research laboratories. Results of some of these studies are highlighted above. More such studies would be needed and the results of these studies should be implemented into clinical practice. In cancer chemotherapy, one of the major problems hindering the resolution of Pgp related drug-drug interactions is that we have not yet been able to differentiate between Pgp overexpression in cells versus constitutively expressed Pgp. Therefore, a risk-benefit ratio estimation is necessary in cancer chemotherapy when blockers of Pgp are employed.

The expression of MRP1 and MRP2, both in normal and malignant tissues, also influences the pharmacokinetics of drugs and metabolites. We have summarized in the second part of this review some of the most important related studies. More detailed knowledge of the structure and function of these transporters could help to develop specific substrates that do not affect Pgp. In general, the presence of MRP molecules separately but especially together with Pgp complicates pharmacology and the possible avoidance of drug-drug interactions.

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ABBREVIATIONS

ATP = Adenosine triphosphate
MRP = Multidrug-resistance associated protein
MDR = Multidrug resistance
TR/GY rat = Transport deficient/groningen yellow rat
GSH = Glutathione
LTC4 = Leukotriene C4
GSSG = Glutathione disulfide
TM = Transmembrane domain
MSD = Membrane-spanning domain
cMOAT = Canalicular multispecific organic anion transporter
ABC = ATP-binding cassette

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