Minireview

Multidrug Resistance and Pharmacological Protection Mediated by the Breast Cancer Resistance Protein (BCRP/ABCG2)[1]

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Introduction

Over the past 15 years it has become clear that drug exporters of the ABC[3] family (1) can both confer MDR to cancer cells and mediate extensive protection of intact organisms against the toxic action of (anticancer) drugs and toxins. The P-gp, MRP1, and MRP2 transporters have all been demonstrated to actively export many anticancer drugs from cells, thus conferring MDR. They also protect certain cells, tissues, and even the central blood compartment in the body from penetration of drugs and toxins (2–12). Useful overviews of properties and nomenclature of mammalian ABC transporters can be found at several websites.[4,5] Given the profound impact of a number of these transporters on MDR in tumor cells and on *in vivo* pharmacology and toxicology, there was great interest when recently a new drug transporter of the ABC class, the BCRP, was discovered. This review deals with current insights in the structure, function, and pharmacological impact of BCRP.

Structure and Function of BCRP

BCRP was initially cloned by Doyle *et al.* (13) from a highly doxorubicin-resistant MCF7 breast cancer cell line (MCF-7/AdrVp) developed in the groups of Fojo and Bates (14, 15). Importantly, transfection of the cloned *BCRP* cDNA directly demonstrated that BCRP itself could confer resistance to mitoxantrone, doxorubicin, and daunorubicin, and that it acted by energy-dependent (most likely through ATP hydrolysis) extrusion of its drug substrates (13, 16). Because the gene was isolated from a breast cancer cell line, it was called the BCRP gene, but there is now little reason to think it is specific for, or even usually expressed in, breast cancer cells (see below). Partial and full-length *BCRP* cDNA sequences were also cloned by Miyake *et al.* (17) and Allikmets *et al.* (18), who had recognized that this gene was overexpressed in MCF-7/AdrVp cells. These groups dubbed the gene *MXR* and *ABCP*, respectively. cDNA sequence analysis revealed that BCRP is a 655 amino acid ABC protein, containing a single NH_2-terminal ABC, followed by six putative TMSs (see Fig. 1). Structurally, *BCRP* belongs to the *ABCG* gene family, containing among others the *Drosophila white*, *brown*, and *scarlet* protein genes, and the human *white* homologue (*ABCG1*). Therefore, the Human Genome Nomenclature Committee recently renamed *BCRP* to *ABCG2*. Shortly after the identification of *BCRP*, Allen *et al.* (19) established that a murine homologue, *Bcrp1*, was highly overexpressed in mouse fibroblasts selected for resistance to doxorubicin, mitoxantrone, or topotecan. *Bcrp1* cDNA encodes a 657 amino acid protein with 81% identity (87% similarity) to BCRP and a virtually superimposable hydrophobicity profile (Fig. 1). Like human BCRP, murine Bcrp1 mediates drug resistance through energy-dependent efflux of drug substrates (19).

The human *BCRP* gene, containing 16 exons spanning 66 kb (20), is located on chromosome 4q22, and the mouse *Bcrp1* gene lies on chromosome 6, 28–29 cM from the centromere (18, 21). In most but not all drug-selected cell lines overexpressing *BCRP* or *Bcrp1*, the gene locus was found to be heavily amplified, indicating that overexpression can result both from *in situ* gene activation and gene amplification (17, 19, 21).

Because P-gp, MRP1, and MRP2 all contain an internally duplicated core structure with two ABC elements and two times six putative TMSs (next to an NH_2-terminal 5-TMS extension present in MRP1 and MRP2), and as the *Drosophila* brown and scarlet proteins each have to heterodimerize with white to transport substrates (22), it is very likely that BCRP functions as a dimer. The observation that high heterologous overexpression of *BCRP* cDNA itself in mammalian or insect cells yields functional drug transport and drug-dependent ATP hydrolysis (16, 23) strongly suggested that BCRP can act as a homodimer (and confirmed that the energy source for drug transport is ATP hydrolysis). Subsequently, BCRP homodimer formation was demonstrated directly using coimmunoprecipitation, and the partial dominant-negative effect of a BCRP mutant on drug resistance mediated by unmutated BCRP also indicates that the protein functions as a homodimer (24). Whether BCRP can also heterodimerize with other proteins, either of the ABCG class or others, is an open question.

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[3] The abbreviations used are: ABC, ATP binding cassette; MDR, multidrug resistance; P-gp, P-glycoprotein; MRP, multidrug resistance protein; BCRP/Bcrp1, breast cancer resistance protein; MXR, mitoxantrone resistance gene; ABCP, placental ABC protein; TMS, transmembrane segment; GSH, glutathione; FTC, fumitremorgin C; SP, side population.
[4] Internet address: http://www.humanabc.org.
[5] Internet address: http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html.

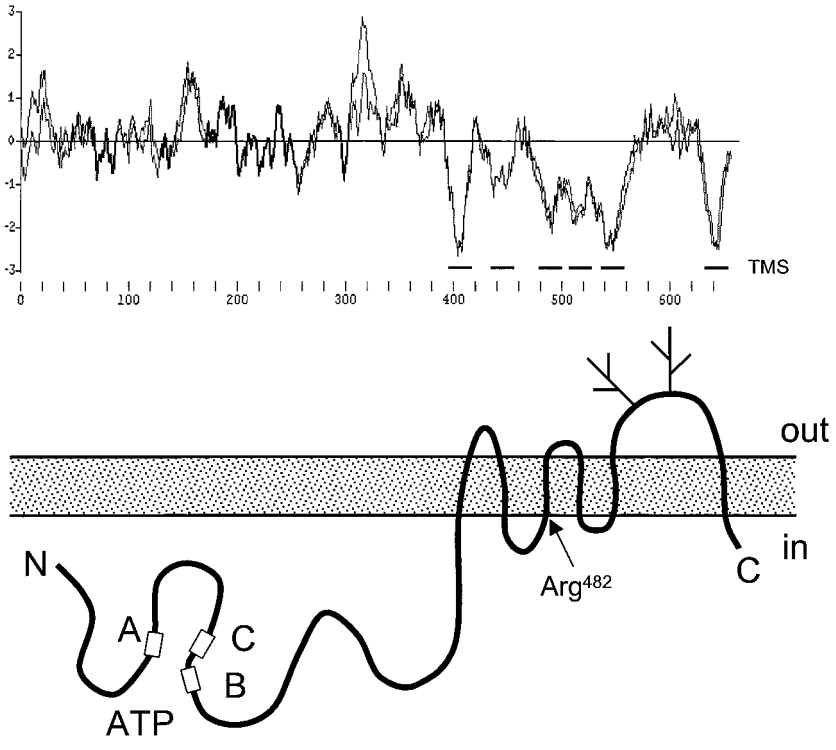


Fig. 1. Hydrophobicity plot and putative two-dimensional structure of BCRP/Bcrp1. The *top panel* shows superimposed hydrophobicity plots of human BCRP (655 amino acids) and mouse Bcrp1 (657 amino acids). The *horizontal bars below the plots* indicate the approximate position of putative TMSs. Amino acid positions are listed below the diagram. The *bottom panel* shows the putative two-dimensional structure of BCRP/Bcrp1 in the plasma membrane, roughly lined up with the hydrophobicity plots in the top panel. *Boxes marked A, B,* and *C* indicate the conserved sequence elements of the ABC; *N* and *C* denote the NH_2- and COOH-terminus of the polypeptide. Branched structures at the third putative extracellular loop indicate the probable position of N-linked glycosylation trees (amino acid positions 596 and possibly 557 in human BCRP, and 596 and 600 in murine Bcrp1). The position of Arg^{482}, mutated in BCRP variants conferring high anthracycline resistance, is indicated. BCRP most likely functions as a homodimer (24).

In rodents, the gene most related to *Abcg2* is *Abcg3*, the murine variant encoding a 650 amino acid protein with 54% identity to Abcg2 (25). *Abcg3* (also called *Bcrp2*) was not found to be up-regulated (and indeed was expressed only at a very low level) in murine drug-selected cells overexpressing *Abcg2* (19), indicating that Abcg3 is not necessary for Abcg2-mediated drug resistance. The usually conserved A and C sequence motifs (1) in the ABC of Abcg3 contain several substitutions at amino acid positions that are otherwise absolutely conserved within the family of ABC transporters, casting doubt even on the ability of Abcg3 to hydrolyze ATP. A human gene or cDNA homologue of *Abcg3* has not been identified thus far, despite the extensive human genomic and EST sequence data available. Finally, the tissue distribution of murine Abcg3 (mainly in spleen and thymus) is quite different from that of Abcg2 (see below). Altogether, it appears very unlikely that Abcg3 is necessary for the normal function of Abcg2. Whether Abcg3 itself has any other function, either alone or in partnership with another Abcg protein, remains uncertain.

Subcellular Localization of BCRP/Bcrp1

In view of the efficient drug extrusion capacity of BCRP and Bcrp1 in a variety of drug-resistant cell lines it was reasonable to expect that the protein would be located primarily in the plasma membrane of these cells, just like P-gp and MRP1 (6, 10). Development of several polyclonal and monoclonal antibodies recognizing BCRP allowed confirmation of this idea in both drug-selected and *BCRP*-transfected cells, all displaying primarily plasma-membrane located BCRP staining (26–28). As frequently seen with multispanning plasma membrane proteins, BCRP was found to be heavily *N*-glycosylated (Ref. 28; Fig. 1). When murine *Bcrp1* was ectopically expressed in polarized canine or porcine epithelial cell lines, it mediated apically directed transport of its drug substrates, indicating that Bcrp1 localizes to the apical membrane in polarized cells (29). This was later additionally corroborated in immunohistochemical studies of human BCRP and pharmacological studies in mice (see below).

Specificity and Mechanism of Drug Resistance Mediated by BCRP/Bcrp1

There is considerable, albeit not complete, overlap in anticancer drug substrate specificity among BCRP, P-gp, MRP1, and MRP2 (12, 30). Because this overlap may have clinical consequences for both MDR in tumors and anticancer drug pharmacology, it was important to establish the exact substrate preference of BCRP. Soon after *BCRP* and *Bcrp1* were cloned it became apparent that many cell lines, derived from a variety of tissues, had developed MDR on drug selection by overexpressing *BCRP* or *Bcrp1*. For instance, cell lines directly selected for resistance to mitoxantrone, topotecan, doxorubicin, SN-38 (the active metabolite of the camptothecin analogue irinotecan/CPT-11), flavopiridol, and the experimental indolocarbazole topoisomerase I inhibitors NB-506 and J-107088 all overexpressed *BCRP* or *Bcrp1*, indicating that all of these drugs are likely to be transported substrates (19, 31–35). In addition, several cytological dyes such as rhodamine 123, Lysotracker Green, and BBR3390, as well as the fluorescent conjugate BODIPY-prazosin, demonstrated decreased accumulation in *BCRP*-overexpressing cells (13, 30, 36–38). Consistently,

little or no resistance was found to the anticancer drugs vincristine, paclitaxel, or cisplatin, indicating that these are not transported substrates for BCRP/Bcrp1.

Additional analysis of various drug-selected *BCRP*- or *Bcrp1*-overexpressing cell lines revealed high cross-resistance to daunorubicin, epirubicin, 9-aminocamptothecin, bisantrene, and in some cases etoposide and teniposide (13, 19, 30, 32, 36, 39, 40). However, there are well-known pitfalls in the interpretation of cross-resistance data obtained from heavily drug-selected cells. Other drug transporters with partially overlapping substrate specificity may have become overexpressed (36, 41), alterations in the cytotoxic targets of the selecting drug can contribute to resistance, and mutations in the selected drug transporter may alter the substrate specificity (42). Moreover, there can be marked differences in substrate preference of homologous drug transporters across species (43). Therefore, caution is required in interpreting such cross-resistance data, and reliable determination of a drug resistance profile can only come from controlled ectopic expression of wild-type cDNA in drug-sensitive cell lines.

One discrepancy that became obvious quite early on was that in some selected cell lines BCRP conferred relatively high resistance to anthracyclines (and bisantrene), whereas in other cell lines there was hardly any resistance to anthracyclines compared with mitoxantrone resistance, which was consistently high. In addition, topotecan resistance was generally quite high but low in some *BCRP*-overexpressing lines (13, 32, 37, 39, 44). This paradox was resolved recently by Robey *et al.* (38) and Honjo *et al.* (23), who recognized that a few *BCRP*-expressing lines (MCF-7/AdVP3000 and S1-M1–80, each with clear anthracycline resistance) could efflux rhodamine 123 efficiently, whereas all of the other lines tested could not. cDNA sequence analysis revealed that the rhodamine-extruding lines contained a BCRP sequence deviating from the "wild-type" BCRP sequence at arginine 482, which was replaced with either threonine (in the MCF-7/AdVP3000 line) or glycine (in the S1-M1–80 line). The MCF-7/AdVp3000 line was the source of the first cloned *BCRP* cDNA (13), which thus turned out to be mutant. Subsequent short-term expression of wild-type *BCRP* (Arg^{482}), and engineered Arg^{482}-Thr and Arg^{482}-Gly mutants in HeLa cells confirmed that the wild-type BCRP could efficiently extrude mitoxantrone but not rhodamine 123 or doxorubicin, whereas both mutants efficiently extruded all three of the compounds (23). In all of the parental cell lines, only the wild-type BCRP sequence was found, indicating that in some lines the extended drug selection must have resulted in selection of mutant BCRPs that were more efficient in conferring resistance to the selecting drug. As Arg (482) is positioned just NH_2-terminal of the putative third TMS of BCRP, and as both mutations result in a 2–5 amino acid shift of the predicted TMS in the NH_2-terminal direction, it may be that the altered TMS configuration determines the change in substrate specificity of BCRP, possibly by lowering steric constraints on TMS3 (and therefore the whole protein, allowing it to accommodate a wider range of substrates). Alternatively, Arg^{482} may contribute directly to the drug-binding site of BCRP.

A related situation could exist for murine *Bcrp1*, which was also reported to confer either a narrow resistance phenotype, characterized by high resistance to mitoxantrone and topotecan, but (very) low resistance to anthracyclines, bisantrene, and etoposide, or a broad resistance phenotype conferring high resistance to all of these drugs (19).

In view of the above, it is somewhat surprising that Komatani *et al.* (35) found only substantial resistance to two experimental topoisomerase I inhibitors (NB-506 and J-107088) but not to mitoxantrone or topotecan when they transfected sequence-confirmed, wild-type (Arg^{482}) human *BCRP* cDNA to a PC13 human lung cancer cell line. One can think of several explanations for this observation, including cell line-dependent differences in ancillary proteins, or drug conjugation or complex formation affecting substrate preference. However, the most obvious one is that NB-506 and J-107088 are simply exceptionally good substrates for wild-type BCRP as compared even with mitoxantrone and topotecan. Consequently, low overexpression of BCRP would already confer substantial resistance to NB-506 and J-107088 before resistance to the other drugs becomes detectable. This could be tested easily.

However this may be, it is clear that a careful analysis of the cross-resistance profile of wild-type *BCRP* and *Bcrp1* cDNAs overexpressed in drug-sensitive cells is still needed to obtain a clean picture of the drug resistance conferred by these genes.

In contrast to P-gp, MRP1 and MRP2 are known to need intracellular GSH to transport weakly basic anticancer drugs like *Vinca* alkaloids and anthracyclines, and it appears that the drugs are exported together with GSH, possibly as a complex, or in a cotransport mode (12). Therefore, depletion of cellular GSH by treatment with buthionine sulfoximine reverses such drug resistance mediated by MRP1 and MRP2. However, buthionine sulfoximine treatment of *BCRP*-overexpressing cell lines did not affect the resistance to and/or efflux of anthracyclines or topotecan, indicating that GSH is not a necessary cofactor for BCRP/Bcrp1-mediated transport of these drugs (14, 32). Thus, it appears that BCRP functions more like P-gp than like the MRPs in this respect, although definitive proof that BCRP can transport its drug substrates without drug-associated or drug-conjugated cofactors must await reliable *in vitro* drug transport experiments, for instance using inside-out membrane vesicles.

Inhibitors of BCRP/Bcrp1

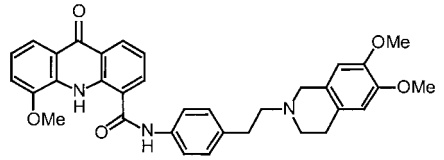
Small molecule inhibitors of P-gp have been very useful in basic cell pharmacological studies, and they are currently tested in clinical trials to reverse P-gp-mediated MDR in several malignancies, in the hope of improving chemotherapy response (see *e.g.,* Ref 45). Moreover, they may be useful in modulating the pharmacokinetic behavior of substrate drugs by enhancing their oral bioavailability, plasma half-lives, and possibly brain and fetal penetration (8, 46). Inhibitors of BCRP can be of value for similar reasons, and several have been described thus far, including some promising compounds that have emerged recently (Table 1).

A very useful finding was the demonstration by de Bruin *et al.* (47) that GF120918 (Fig. 2), a highly efficient P-gp inhibitor

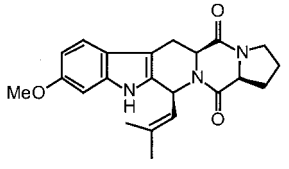
Table 1 Reported inhibitors of BCRP/Bcrp1

Compound	Effective concentration[a]	Comments	Refs
Reserpine	used at 5 μM	also inhibits P-gp	53
CI1033	low micromolar	HER tyrosine kinase inhibitor	52
GF120918	$EC_{90} \sim 0.05$ μM	potent P-gp inhibitor, well tolerated *in vivo*	19,47,48
FTC	1–5 μM	low activity against P-gp and MRP1, neurotoxic	37,40,50
Ko134	$EC_{90} \sim 0.1$ μM	tolerated *in vivo*, low activity against P-gp and MRP1	51

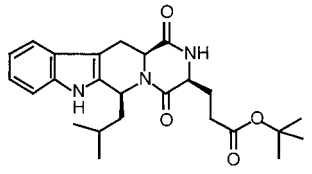
[a] EC_{90}, effective concentration of inhibitor that reduces drug resistance by 90%.



GF120918



Fumitremorgin C



Ko134

Fig. 2. Structures of the effective BCRP/Bcrp1 inhibitors GF120918, FTC, and Ko134.

that can readily be used in animals and patients (48, 49), is also quite effective in inhibiting BCRP. A subsequent study showed that GF120918 also inhibits mouse Bcrp1 (19). Although GF120918 lacks specificity as a BCRP inhibitor, for many potential clinical applications this may be an advantage, because two multidrug transporters, BCRP and P-gp, each with potentially adverse activity, can be blocked at the same time with one drug. Taking proper precautionary measures, GF120918 could even be applied to study the physiological functions of BCRP (see below). It is interesting to note that some other proprietary P-gp inhibitors structurally related to GF120918 also inhibit Bcrp1 substantially in our hands. Nevertheless, whereas it is not entirely surprising that a P-gp antagonist can act on another ABC transporter with overlapping substrate specificities, this is not the rule; verapamil, cyclosporin A, PSC833 and most other P-gp inhibitors we have tried have little effect on BCRP.

Rabindran *et al.* (37, 40) discovered early on that FTC (see Fig. 2), a tremorgenic mycotoxin produced by the fungus *Aspergillus fumigatus*, could effectively reverse drug resistance and increase cellular drug accumulation in BCRP-expressing cells. FTC effectively inhibited BCRP *in vitro* at concentrations (1–5 μM) well below those toxic to cultured cells ($IC_{50} > 80$ μM) and had little effect on P-gp- or MRP1-mediated drug resistance, making it very useful for cell pharmacological studies of BCRP. However, its neurotoxic effects precluded any meaningful use in animals, let alone patients. A series of FTC structural analogues (mostly pentacyclic diketopiperazines) was generated and analyzed by the same group (50). Unfortunately, none of the compounds tested had substantially better BCRP-inhibiting activity than native FTC, and they were frequently more cytotoxic. Whether any of these analogues might be applied in animals or patients is unclear.

In a related study, Van Loevezijn *et al.* (51) tested 42 mainly tetracyclic indolyl diketopiperazine analogues of FTC as inhibitors of murine Bcrp1 and human BCRP. As in the study by He *et al.* (50), it was found that the steric configuration of the molecules could have a major effect on inhibitory efficacy, which appears to contrast with the situation for P-gp inhibitors. The most potent analogues (Ko132 and Ko134) appeared to have comparable or greater activity than FTC, although they lacked a methoxy substituent group present on the aromatic ring system of native FTC (see Fig. 2). Demethoxy-FTC is far less effective than FTC in inhibiting BCRP (50, 51), suggesting that this methoxy group might be important for optimal interaction with BCRP. Analysis of a methoxylated derivative of Ko134 is currently ongoing in our laboratory and suggests considerable potential of this compound. Thus, FTC analogues of this type may be promising leads for development of clinically useful BCRP inhibitors.

Another experimental agent, the HER tyrosine kinase inhibitor CI1033, presently under evaluation for treating a variety of cancers, inhibits BCRP and, thus, at least *in vitro*, can synergize with SN-38 and related topoisomerase I inhibitors (52). Representing, as it does, an interesting and unexpected drug interaction that may prove favorable for chemotherapy, it is to be hoped that this is not the last example of such serendipity.

The Tissue Distribution of BCRP and Bcrp1

BCRP-overexpressing drug-resistant cell lines have been obtained from parent lines of diverse origins, including fibroblasts, breast, colon, gastric, lung, or ovarian carcinomas, fibrosarcomas, and myelomas, indicating that BCRP may be relevant in tumors arising from many different tissue types (31–33, 35). However, overexpression in drug-selected tumors does not necessarily reflect normal expression in the tissues of origin. Early studies on the (RNA) expression of

Table 2 Tissue distribution of human BCRP, as detected with the monoclonal antibodies BXP-21 and BXP-34, and some presumed functions of BCRP at these locations

Data are from Ref. 28.

Tissue	(Sub-)cellular localization	Function
Small intestine	Epithelium, apical membrane	Reduced uptake of xenobiotics
Colon	Epithelium, apical membrane	Reduced uptake of xenobiotics
Liver	Bile canalicular membrane	Excretion of xenobiotics
Mammary gland (breast)	Lobules, apical membrane Lactiferous ducts, apical membrane	?
Placenta	Syncytiotrophoblast, membrane facing maternal blood	Reduced fetal uptake of xenobiotics
Veins	Endothelium	?
Blood capillaries	Endothelium	?
Blood progenitor cells	Plasma membrane	Dye extrusion
Other major organs	Only in veins, capillaries	

BCRP and *Bcrp1* in tissues yielded somewhat variable outcomes (13, 18, 19), and are perhaps best considered in conjunction with later studies to arrive at a consistent picture (28, 29). Very high *BCRP* RNA expression was found in human placenta, but in murine placenta the expression was quite moderate. On the other hand, mice displayed highest expression of *Bcrp1* RNA in kidney, where humans appear to have low *BCRP* expression (13, 18, 29). Most revealing for possible physiological functions of BCRP thus far are the immunohistochemical studies of Maliepaard *et al.* (28), which made use of two independently derived monoclonal antibodies recognizing human BCRP, BXP-21, and BXP-34 (Table 2). BCRP was found in the placental syncytiotrophoblast plasma membrane, which faces the maternal bloodstream, and thus effectively forms part of the barrier between maternal and fetal circulation. It was also localized to the bile canalicular membrane of the liver hepatocytes and to the luminal membrane of villous epithelial cells in the small and large intestine, suggesting an excretory and/or uptake-limiting role for its substrates. BCRP was additionally found in the apical side of part of the ducts and lobules in the breast. Besides this tissue-specific distribution, BCRP staining was detected in the venous and capillary endothelial cells of practically all of the tissues analyzed but at best sporadically in arterial endothelium. In fact, high *BCRP* RNA levels detected in some tissues (cervix, ovary, and kidney) appeared to relate mainly to the high BCRP levels in the blood vessels of these tissues.

By immunohistochemistry BCRP was not generally detected in human erythrocytes, leukocytes, or platelets (28), but Zhou *et al.* (53) found that *Bcrp1* mRNA as detected by reverse transcription-PCR was present at high levels in primitive murine hematopoietic stem cells and sharply downregulated on differentiation. In more differentiated hematopoietic lineages, little or no expression of *Bcrp1* was seen in thymocytes, B-cells, granulocytes, or macrophages, but erythroid precursor cells and natural killer lymphocytes did express some *Bcrp1* (53). This expression pattern has led to speculation on a possible role of BCRP/Bcrp1 in maintaining the undifferentiated state of hematopoietic stem cells (see below).

Some of the possible functional implications of the observed tissue distribution of BCRP and Bcrp1 are discussed below.

Pharmacological and Physiological Functions of BCRP and Bcrp1

The tissue distribution of BCRP and Bcrp1 demonstrates very extensive overlap with that of P-gp, which led us to the supposition that BCRP/Bcrp1, like P-gp, might have an important role in the pharmacological handling of substrate drugs in animals and humans. In particular, BCRP/Bcrp1 found in the apical membranes of small and large intestinal epithelium, the biliary canalicular membranes, and the placental syncytiotrophoblasts, might function in limiting intestinal uptake of p.o. administered drugs, in hepatobiliary excretion of drugs, and in restricting the fetal penetration of drugs, respectively. To address these questions, we made use of the BCRP/Bcrp1 and P-gp inhibitor GF120918, which can be given at high oral dosage to animals and humans without obvious toxicity. To prevent confounding effects of P-gp inhibition, we performed pharmacological experiments in Mdr1a/1b knockout mice, which lack drug-transporting P-gp. Therefore, any pharmacological effect of GF120918 observed in these mice would most likely result from Bcrp1 inhibition.

Indeed, when GF120918 was p.o. coadministered with oral topotecan, the plasma availability of topotecan increased ~6-fold (29). The hepatobiliary excretion of i.v. administered topotecan was ~2-fold decreased by oral GF120918, consistent with an excretory role for bile canalicular Bcrp1. The initial plasma clearance of i.v. administered topotecan was also decreased ~2-fold, whereas the amount of topotecan in the intestinal contents was reduced 3-fold 1 h after topotecan administration. Taken together, the data indicate that the increased topotecan plasma availability after oral GF120918 treatment resulted from decreased hepatobiliary excretion and more efficient (re-)uptake of topotecan from the intestinal lumen (29). Thus, it appears that intestinal Bcrp1 plays an important role in limiting reuptake of substrate drugs from the intestine, hence limiting their oral bioavailability much the same as intestinal P-gp does (8). Given this functional role, it is noteworthy that in human jejunum, the mRNA level of *BCRP* is substantially higher than that of *MDR1* (54).

When [^{14}C]topotecan was administered i.v. to pregnant *Mdr1a/1b* knockout mice pretreated with oral GF120918, the fetal levels of radioactivity were increased 3.2-fold, whereas the maternal plasma level increased only 1.6-fold, indicating

a net 2-fold higher fetal accumulation of radioactivity (29). These data strongly suggest that Bcrp1 in the placental trophoblast counteracts the entry of topotecan into the fetus, making Bcrp1 a protective element of the maternal-fetal barrier, just as P-gp is one (46).

P-gp is abundant in the luminal membrane of endothelial cells of blood capillaries in brain and testis where it contributes to the blood-brain and blood-testis barriers (8). Human BCRP is found in many venous and capillary endothelial cells (28), and the same might be true in mice. However, we could not improve brain or testis penetration of topotecan or mitoxantrone with GF120918 administration. This could imply that Bcrp1 does not play a role in these barriers or that other transporters can take over such a function for the drug substrates tested.

Overall, the data are strongly suggestive of a pharmacological and toxicological protective role for Bcrp1. Hence, it may well be that protection from naturally occurring xenobiotic toxins is (one of) the main normal biological function(s) of BCRP/Bcrp1, just as appears to be the case for P-gp.

Another physiological role for BCRP/Bcrp1 was suggested by the work of Zhou *et al.* (53). This showed that Bcrp1 expression is high in a so-called SP of murine bone marrow cells, characterized by low accumulation of the dye Hoechst 33342. SP cells are highly enriched for undifferentiated stem cells, which makes the SP phenotype an important tool in stem cell characterization and research. Bcrp1 expression was found to be low or absent in more differentiated hematopoietic cells. It was additionally demonstrated that BCRP can effectively extrude Hoechst 33342 from cells, indicating that BCRP is at least in part responsible for the low dye accumulation in SP cells. Similar data were reported by Scharenberg *et al.* (55) in the human hematopoietic compartment. Transplantation of BCRP-transduced bone marrow cells into lethally irradiated recipient mice resulted in lower repopulation capacity than seen with mock-transduced bone marrow cells. In addition, in a competitive repopulation assay for peripheral RBCs, thymus, and bone marrow, BCRP-transduced bone marrow cells were less effective than mock-transduced cells (53). The authors speculate that these effects might be because of a general differentiation-inhibiting role of BCRP/Bcrp1 (for instance by extrusion of a differentiation-inducing substrate), but a general moderately adverse effect of high BCRP overexpression on cell growth and proliferation could also explain the results. Whatever the biological role of BCRP/Bcrp1 expression in bone marrow stem cells, it seems very likely that it is responsible for the appearance of the SP-phenotype. Interestingly, a similar SP phenotype was also observed in skeletal muscle stem cells and embryonic stem cells, and again associated with relatively high Bcrp1 expression (53). Therefore, it could be that Bcrp1 expression is a more general characteristic of various stem cell populations.

The Clinical Significance of BCRP

On the basis of the insights obtained thus far, BCRP function may be relevant in two areas of cancer chemotherapy: (*a*) it may be expressed in tumors and other malignancies treated with anticancer drugs that are BCRP substrates, and therefore render these cancer cells relatively resistant to chemotherapy; and (*b*) it may be an important factor in the pharmacology of substrate anticancer drugs, affecting their oral bioavailability and their plasma clearance through hepatobiliary and intestinal elimination. Most anticancer drugs have narrow therapeutic windows, with excessive exposure rapidly leading to unacceptable toxicity, and underexposure causing therapeutic inefficacy. Thus, clear insight into the mechanisms governing their plasma pharmacokinetics, and opportunities to modulate these mechanisms with inhibitors are highly relevant. For instance, the use of BCRP inhibitors to improve the oral bioavailability of BCRP substrate drugs such as topotecan is now the subject of ongoing clinical studies in the group of J. H. M. Schellens in this institute. During pregnancy, the protective role of BCRP in the placenta may also be relevant for cancer chemotherapy and, indeed, pharmacotherapy in general.

The expression of drug transporters such as P-gp and MRP1, even at low levels, contributes at least to some extent to intrinsic resistance to chemotherapeutic drugs (56), so we expect that the same will be true for BCRP in tumors or in normal tissues. Studies addressing BCRP expression in clinical tumors are few at this moment. Ross *et al.* (57) found considerable *BCRP* mRNA levels in a fraction (7 of 20) of acute myeloid leukemias, and Sargent *et al.* (58) reported that 6 of 22 acute myeloid leukemias blast cells had >10% positive staining for BCRP using BXP-34 immunodetection. In a study of 43 untreated breast carcinomas, Kanzaki *et al.* (59) found uniformly low levels of *BCRP* mRNA. Clearly, far more extensive clinical studies, preferentially also using reliable immunohistochemical detection, will be needed to establish the presence of BCRP in various tumor types and its potential contribution to clinical drug resistance. If so, the availability of efficacious, *in vivo* applicable inhibitors of BCRP such as GF120918 and perhaps Ko134(-analogues) may be of value in improving the response of tumors to chemotherapy with BCRP substrate drugs. Given the similarities between BCRP and P-gp, it is noteworthy that there is now considerable evidence that P-gp levels both predict and influence the outcome of chemotherapy in several types of tumors and that inhibiting P-gp activity can be beneficial in circumventing clinical drug resistance (45).

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