Taking Account of Drug Transporter Function in Chemotherapy

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P-glycoprotein (P-gp), the archetypical multidrug resistance protein, was discovered over 30 years ago. Major pharmaceutical companies invested heavily in developing P-gp inhibitors that are potent and effective in their intended effects on the target. However, after generally unfavourable clinical trials of these compounds, the drug companies put them and their drug resistance reversal programs on the shelf, and the FDA has never approved any chemosensitiser for clinical use. This reaction, while understandable, is unfortunately premature, and will very likely prove temporary. There is growing understanding of just how much drug transporters influence the problems that limit the efficacy of chemotherapy: drug resistance, pharmacokinetic variability, toxicity and drug-drug interactions. There are correspondingly rich opportunities for intervention in drug transport to improve cancer treatment outcomes. A lot has been written about P-gp in this context; here, ABCG2 will be used as an example to illustrate these points, as this transporter is proving to be of similar significance for chemotherapy as P-gp.

Background

One of the normal functions of ATP-binding cassette (ABC) transporters is protection of organisms against xenobiotic toxins, particularly those encountered in the diet. It is P-gp and ABCG2 in the intestinal epithelium that protects you from carcinogens such as 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) generated on the barbecue (1). A functional toxin has to be soluble enough in lipids that it can diffuse into cells, and at least sparingly soluble in water. This sets them apart from most substances in the body and it means that a plasma membrane pump that expels amphiphilic compounds, like P-gp or ABCG2, will serve well in a protective role. Many cytotoxic drugs are derived from natural toxins, so it is not surprising that transporters are prominent sources of drug resistance.

Many of the chemotherapeutic drugs in use today are substrates of one or more multidrug transporter proteins. The latter are all members of the ABC family and include P-gp, the breast cancer resistance protein (BCRP/ABCG2) and the multidrug resistance-associated proteins 1 through 5 (MRP1-5/ABCC1-5). The status of MRPs 6 and 7 (ABCC6 and 10) as multidrug transporters is yet to be confirmed by more than one group. ABCG2 was identified in 1996 by Doyle and Ross (2) as the source of a previously unexplained spectrum of multidrug resistance in drug-selected cell lines.

ABCG2 transports a wide range of drugs (3) (Fig. 1). The best therapeutic drug substrates are the topoisomerase I inhibitors topotecan and SN-38 (the active metabolite of irinotecan/CPT-11) and the antifolate methotrexate. Although ABCG2 was originally identified in a doxorubicin-resistant line, where induction of P-gp-mediated resistance was inhibited by co-exposure to verapamil, it was later realised that the gene had mutated, enabling superior transport of anthracyclines (4,5). The mutation appeared in lines derived independently by several groups, including mouse lines (6), always altering R482. Three points are consequently worth noting. The first is the danger of relying on drug-selected models for understanding transporter behaviour. The second is that the mutation has not been observed as a consequence of chemotherapy and does not correspond to any natural single nucleotide polymorphism in ABCG2. Finally, although the anthracyclines are also substrates of wild-type ABCG2, they are relatively poor ones – an under-appreciated fact. Other chemotherapeutic drugs that are relatively poor substrates include flavopiridol and the epipodophyllotoxins such as etoposide. ABCG2 may nevertheless mediate resistance to these drugs, especially when other mechanisms are inoperative or prevented (7).

It has recently become clear that several of the new targeted kinase inhibitors, such as gefitinib (Iressa™) and imatinib (Gleevec®), are also substrates of ABCG2.

The informed reader will have noticed the overlap between the substrate specificity of ABCG2 and those of P-gp, MRP1 and MRP2. This of course complicates attempts to manipulate drug transport and accounts in part for the prior problems in application of P-gp inhibitors to drug resistance reversal.

Drug Resistance

There is little doubt about the influence of multidrug transporters on innate or primary drug resistance, as is shown by the effects of genetically inactivating transporters in mice – fibroblasts nullizygous for the Mdr1 and Mrp1 genes were substantially sensitised to numerous drugs, 16-fold in the case of paclitaxel (8). Strikingly, the cells were more than 20-fold more sensitive than those for vincristine, a good substrate of both transporters. The LD50 for the mice themselves was approximately 100-fold lower (9).

Expression of multidrug transporters is increased in drug-resistant tumours, either as a result of selection by the treatment, or induction by the drugs themselves, in some cases by the same pregnane X receptor-mediated system as for the cytochrome P450 oxidases that metabolise the drugs. Upregulation of transporters is a mechanism that tumour cells seem able to accomplish easily and with little or no detriment to their physiology.
The evidence that overexpression of transporters is a clinically significant drug resistance mechanism in vivo is accumulating slowly. This endeavour has been hampered by the technical difficulties entailed in obtaining adequately pure tumour cell populations before and after treatment for comparison. The evidence is best for haematologic cancers, where such problems are minimised; P-gp expression is a confirmed drug resistance mechanism and a marker of poor treatment outcomes in several haematologic cancers (10). ABCG2 expression has been surveyed in an increasing number of tumours and related to treatment outcomes, as reviewed recently by Robey (3). Most such studies must be considered pilots and many were not well designed to give clear answers. However, there are intriguing indications that should prompt more comprehensive studies of ABCG2 as a marker of poor drug response, for example, in acute myelogenous and lymphocytic leukaemias.

There is much recent interest in ABCG2 expression as a source of resistance to imatinib in chronic myeloid leukaemia (CML), especially the work of Hughes and colleagues in Adelaide (11). Imatinib and many other kinase inhibitor drugs in use or under development have relatively low toxicity (by virtue of specificity for the targets) and are taken orally, on a daily basis, over long periods of time. This is of course an ideal recipe for emergence of acquired drug resistance and it means that, regardless of the benefits of the new agents, drug resistance is not going to become any less of a problem in the near future.

Prospects of Reversal of Drug Resistance

The rationale for drug resistance reversal, or chemosensitisation, was simple: some tumours overexpress a multidrug transporter, so inhibiting it should sensitize them to drugs more than normal cells. That sounds reasonable but the experience with P-gp inhibitors has usually been that toxicity to normal tissues is increased so much that dose reduction is necessary, with no net improvement in the therapeutic index. Given the variety of cells in the body, perhaps that should have been anticipated. Nevertheless, a few well-designed trials have shown clear benefits (3) and it certainly does not follow that transporter inhibitors are useless for chemotherapy. It does mean that considerable work is needed to find the combinations of cancers and drugs and the individual patients that will show a net benefit from inhibiting cellular drug efflux systemically. Big Pharma has been visibly reluctant to embrace this task, let alone any kind of personalised therapy, but that will inevitably change now that expression profiling of tumour biopsies is technically feasible. It should not be long before screening for expression of genes affecting drug resistance becomes a part of routine molecular pathology for cancer patients to inform their treatment.

Since soon after the transporter's discovery, efficient inhibitors of ABCG2 have been available. It is hard to overstate the importance of these agents for understanding the function of the protein and its significance for chemotherapy, not to mention its normal physiological roles. Our colleagues who work on transport of cholesterol by other members of the ABCG subfamily are not so fortunate. Fumitremorgin C, a toxin from Aspergillus fumigatus, was the first ABCG2 inhibitor identified (12). The neurotoxic properties of the fumitremorgins (responsible for tremors in cattle caused by eating Aspergillus-infected hay) may be separable from the effects on ABCG2, as we were able to show that an analogue, Ko143, is potent for inhibiting ABCG2 in mice and altering the oral pharmacokinetics of a substrate drug without overt toxicity (13). The analogue, with an EC₅₀ below 10 nanomolar, remains the most potent and specific inhibitor available.

**Fig. 1.** ABCG2 is a homodimeric plasma membrane efflux pump of broad substrate specificity that includes numerous anticancer and other drugs. The lists shown are far from exhaustive. All substrates inhibit transporter activity to some degree; the tyrosine kinase inhibitor (TKI) drugs are significant ABCG2 substrates and also relatively potent inhibitors. This can complicate inferences about how their interaction with the transporter affects drug resistance, pharmacokinetics or toxicity, particularly in combination chemotherapy with other ABCG2 substrate drugs.

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Natural</td>
<td>Cimetidine, Glucocorticoids, Chrysin, PhIP, Cytotoxic drugs</td>
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<tr>
<td>Cytotoxic drugs</td>
<td>Mitoxantrone, Bisantrene, Camptothecin analogues, Methotrexate, Flavopiridol, Anthracyclines, Epipodophyllotoxins, TKIs: Imatinib, Gefitinib, Erlotinib, CI-1033, TKIs: Imatinib, Nilotinib,</td>
</tr>
<tr>
<td>Other drugs</td>
<td>Nitrofurantoin, Fluroquinolones, Other drugs: Fluoroquinolones, Nitrofurantoin, Glucocorticoids, PDT photosensitisers, Cimetidine</td>
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Of more immediate utility was the P-gp inhibitor elacridar (GF120918) from GlaxoSmithKline, which also proved active against ABCG2 at nanomolar concentrations (14), as did structurally-related compounds from other companies (e.g. XR9576/tariquidar from the Xenova group). They have already been used in human trials for inhibiting P-gp and so were available for studies of mouse and human ABCG2 function and its impact on substrate drug pharmacology, as discussed further below. However, the eventual application of ABCG2 inhibitors to reversal of drug resistance will depend on prior identification of cancers where ABCG2 activity is a clear prognostic factor. In the meantime, another approach is being pursued: there would be obvious advantages if the transporter inhibitors have chemotherapeutic action in themselves.

Drug companies have been slow to adopt systematic screening for interactions of new agents with ABC transporters as part of the drug development process. One barrier may be that too much knowledge about a drug complicates the approval process, as became apparent when we informed one company that their lead compound was also a significant inhibitor of ABCG2. This news was received with interest but permission to investigate the compound’s potential for improving the oral pharmacokinetics of substrate drugs was politely declined.

Some years ago, a number of research groups, including us, began to screen new chemotherapeutic drugs for interactions with the multidrug transporters in a more or less systematic fashion, testing them not only as substrates, but as modifiers of transporter activity and expression. A major fruit of the work has been the finding that several new chemotherapeutic agents inhibit ABCG2 substantially at physiologically attainable concentrations. This immediately suggested the possibility of synergism between them and other drugs that are substrates of ABCG2 in tumours that express the transporter.

Targeted receptor tyrosine kinase inhibitors are prominent in that list, including the EGFR inhibitors gefitinib (15), erlotinib (Tarceva®) and CI-1033 (Pfizer), and the Abelson (Abl) kinase inhibitor imatinib (16), the mainstay of treatment for CML. There were clear synergies between such drugs and ABCG2 substrates such as irinotecan and topotecan, both in vitro and in preclinical models (17). Such combinations should be applicable to colon, lung and ovarian cancers, but only a few Phase I-II trials have been conducted, so it is too early to judge whether there will be benefits. A clear therapeutic synergy has been observed between imatinib and nilotinib, another Abl kinase inhibitor used to treat CML. This is in part explained by the finding that the former drug increases the intracellular concentration of the latter, indicating inhibition of transport (11).

Another group of compounds that inhibit ABCG2 are the flavonoids, such as chrysin and quercetin, which are prominent in the diet. An Australian company, Novogen, is conducting trials with the isoflavone phenoxydol, which has activity against a variety of tumours. We found that this compound is also a serviceable inhibitor of ABCG2. It is currently under trial as a chemosensitiser in combination with topotecan for ovarian carcinoma.

### Manipulating Drug Pharmacokinetics

Most anticancer drugs have narrow therapeutic windows, with too little being ineffective and too much overly toxic. Achieving optimal plasma pharmacokinetics is thus a challenge. Like P-gp, ABCG2 is widely expressed but is most abundant in epithelial and endothelial cells, like those of the hepatic canaliculi, the intestinal epithelium, the proximal tubules of the kidney, the blood-brain barrier (BBB) and the syncytial trophoblasts of the placenta (18)—in other words, at sites that strongly influence the uptake, excretion and disposition of substrate drugs.

The consequences of ABCG2 activity for these processes were demonstrated by inhibiting the transporter in mice with elacridar. Confounding effects of the compound on P-gp activity were neatly obviated by performing the experiments in Mdr1 knockout mice. Elacridar increased the plasma availability of orally co-administered topotecan 6-fold, decreased its hepatobiliary excretion 2-fold and decreased the concentration in the intestinal lumen 3-fold (19). Administered to wild-type mice, where both P-gp and ABCG2 were inhibited, elacridar had correspondingly greater effects on the pharmacokinetics of topotecan. This type of manipulation has several striking potential benefits for chemotherapy: it makes oral administration of certain drugs more feasible, it flattens their plasma concentration curves and, by lifting the (oral) bioavailability of a drug substantially closer to 100%, it reduces the room for inter- and intra-patient variability in drug response. Moreover, by reducing drug excretion into the intestinal lumen, it can reduce a common dose-limiting toxicity: diarrhoea. Although the pharmacokinetic effects are somewhat less spectacular than in mice, these principles have been demonstrated in humans treated with topotecan for ovarian cancers (20).

There is still another potential application of manipulating transporter activity: enhanced drug penetration of tissue compartments protected by transporters. Take, as an example, the use of elacridar or pantoprazole (another ABCG2 inhibitor) to improve the passage of imatinib through the BBB. This could usefully increase the exposure of malignant gliomas to the drug (21). ABCG2 is present on the apical (blood) side of the endothelial cells of the BBB and actively opposes diffusion of substrate drugs into the brain (Fig. 2). As noted earlier, it is an inhibitor of ABCG2 and, like many transporter inhibitors, it turned out to be a substrate as well.

### Concerning Toxicity

The long term use of new, low toxicity, anticancer drugs that incidentally inhibit transporter function raises the question of whether new toxicities will arise from this source. An example is imatinib therapy for CML, which might cause chronic systemic inhibition of ABCG2. The Apcg2 knockout mice provides some clues. Although overtly healthy, these mice developed necrotic lesions on exposed skin (ears, tail and snout) when fed particular batches of chow and placed on the top shelf nearer the room lights. This was eventually traced to protoporphyria and associated phototoxicity arising from accumulation of metabolites of haemoglobin and dietary chlorophyll (22). It emerged that ABCG2 efficiently transports porphyrins, removing them from red blood cells and then from the body. It turns out
that total porphyrins in the urine of CML patients on daily imatinib are lower than in the normal reference population and red blood cell porphyrins are elevated (unpublished results, Ling, S., Centenary Institute and Poulos, V., Royal Prince Alfred Hospital). The changes are related to imatinib dose. Although too small to produce symptoms, the changes confirm systemic inhibition of ABCG2. Intriguingly, one patient on a high dose of imatinib had previously suffered severe phototoxicity while taking cyclosporin A concurrently for an unrelated condition. The latter is a P-gp inhibitor and P-gp also contributes to porphyrin excretion, suggesting that the combined block on porphyrin elimination resulted in overt symptoms. This work requires more data, particularly from patients on high doses of imatinib, but it does illustrate the point that the effects of chronic inhibition of transporter activity might produce toxicities in patients predisposed to them, or via interactions with other drugs.

On the other hand, as suggested earlier, it might be possible to alleviate the toxicity of some chemotherapeutic drugs by manipulating their transport. An example arises from work done at the Sydney Cancer Centre by S. Clarke and L. Rivory a few years ago. They successfully used the flavonoid chrysin as an alternative to high dose loperamide for palliating the diarrhoea that bedevils treatment with irinotecan. The active metabolite, SN-38, is secreted into the bile and through the intestinal epithelium by the action of several transporters - MRP2, P-gp and ABCG2. Chrysin is a potent inducer of UGT1A1, a UDP glucuronosyltransferase that inactivates SN-38 by glucuronidation. However, as noted above, chrysin is also a serviceable inhibitor of ABCG2 and may thus be expected to inhibit excretion of SN-38 into the intestinal lumen and to increase its resorption. Previous tests with loperamide in an isolated perfused rat liver system showed that loperamide has a similar effect by inhibiting P-gp (Tobin, P. et al., unpublished data).

These examples illustrate that drug transport affects all of the major problems of chemotherapy: drug resistance, pharmacokinetics, toxicities and drug-drug interactions. While clinicians need to be cognisant of the possible negative consequences arising from transporter activity, it should also be clear that many opportunities arise to take advantage of drug-transporter interactions, for synergistic combination chemotherapy, for reducing the variability in bioavailability of drugs and for alleviating drug toxicities.

References