Pergamon

European Journal of Cancer Vol. 32A, No. 6, pp. 985–990, 1996

Printed in Great Britain
0959-8049/96 $15.00+0.00

S0959-8049(96)00063-9

What Have We Learnt Thus Far From Mice With Disrupted P-glycoprotein Genes?

P. Borst and A.H. Schinkel

The Netherlands Cancer Institute, Division of Molecular Biology, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

INTRODUCTION

CLINICAL INTEREST in P-glycoproteins (Pgp) is still on the rise and not without reason. Extrusion of drugs from cancer cells by Pgp probably contributes to drug resistance of a substantial fraction of human tumours, up to 50% according to Gottesman [1]. Effective inhibitors of Pgp-mediated drug transport (also known as reversal agents or Pgp modulators) are now entering clinical trials, the cyclosporin A analogue PSC 833 being one of the most promising [2]. The safe use of these inhibitors requires knowledge of their potential side-effects. What will happen if Pgp in normal tissues is inhibited? Are there functions of Pgp indispensable to normal physiology? Will inhibition of Pgp alter the metabolism of the carcinostatic drugs given in combination with inhibitors of Pgp? Will the metabolism and thus toxicity of other drugs in use for the treatment of cancer patients be affected by these inhibitors?

To study the physiological functions of Pgp in depth, we have turned to mice. It is now possible by standard techniques to disrupt specific genes in the germline of mice. Mice homozygous for the disruption can be obtained if the defect is not lethal. As shown in Figure 1, mice have three linked Pgp genes. The *Mdr1a* (also called *Mdr3*) and *Mdr1b* (also called *Mdr1*) genes are functional homologues of the human *MDR1* gene, and are known to encode Pgp able to transport drugs. The murine *Mdr2* gene encodes a Pgp closely related to the Pgp encoded by the human *MDR3* (also called *MDR2*) gene. Until recently, no function for this gene was known.

We have obtained mice with disruptions of each of the three Pgp genes and in addition we have generated a double knockout in which both *Mdr1a* and *Mdr1b* are disrupted. Mice homozygous for these gene disruptions are viable and fertile. In this review, we shall summarise results obtained thus far with these mice, emphasising defects that could be relevant for treatment of cancer patients with inhibitors of drug-transporting Pgp. Some of these results have been discussed in more detail recently [3].

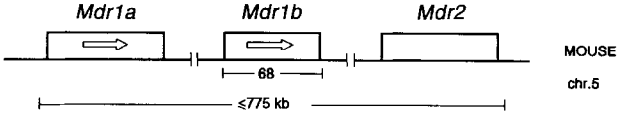

Figure 1. Schematic map of the mouse P-glycoprotein gene locus. Known distances and sizes are indicated in kilobases. The intergenic distance of the mouse genes, and the sizes of mouse *Mdr1a* (*Mdr3*) and *Mdr2* genes are not known exactly. Arrows indicate the direction of transcription. chr., chromosome. Adapted from Borst and associates [4].

PUTATIVE PHYSIOLOGICAL FUNCTIONS OF DRUG-TRANSPORTING P-GLYCOPROTEINS IN MAMMALS

Speculations, old and new

Some of the speculations on physiological functions of drug-transporting Pgp and the rationale for these speculations are summarised in Table 1. The indirect evidence for these

*Table 1. Possible physiological functions of drug-transporting P-glycoproteins in mammals**

1.	Protection against exogenous toxins ingested with food: expression in small intestine, colon, blood–tissue barrier sites
2.	Excretion of metabolites or toxins: expression in liver canalicular membrane, kidney (digoxin transport)
3.	Transport of steroid hormones: expression in adrenal gland, demonstrated transport of cortisol, corticosterone, aldosterone
4.	Extrusion of (poly-)peptides (cytokines) not exported from the cell via the classical signal/cleavage pathway: compare yeast *STE6*, *Escherichia coli HlyB*, mammalian endoplasmic reticulum peptide transporters
5.	Ion transport and cell volume regulation: activation of an endogenous Cl^- channel activity
6.	Lymphocyte cytotoxicity: possible involvement in NK-cell-mediated cytotoxicity
7.	Transport of prenylcysteine methyl esters
8.	Intracellular vesicular transport of cholesterol

*Modified from Borst and Schinkel [3].

Correspondence to P. Borst.

functions has been summarised by Borst and associates [4], by Lum and Gosland [5] and in other reviews in this issue. The most recent developments concern points 5–8 of Table 1. Recent evidence indicates that the MDR1 Pgp is not a Cl^- channel, as proposed by Higgins and Sepúlveda and coworkers, but that this Pgp activates an endogenous Cl^- channel [4, 6]. Point 6 of Table 1 concerns the role of Pgp in lymphocytes. The highest levels of MDR1 Pgp mRNA are found in natural killer (NK) cells, but substantial levels are also present in cytotoxic T-cells [7–9]. Chong and associates [10] found that NK-cell-mediated toxicity was inhibited by high concentrations of two MDR reversal agents and they have suggested that NK-cell action may require "the functional expression of an efflux molecule similar or identical to P-glycoprotein".

Casey and colleagues [11, 12] have recently found that prenylcysteine methyl esters interact with Pgp. These esters represent the C-terminal structures of prenylated proteins, such as cellular ras and most G-proteins, and they are formed when these proteins are degraded in the cell. Zhang and associates [11] have shown that these esters activate Pgp-mediated ATPase activity and compete for drug binding to Pgp. Although actual transport of the esters by Pgp has not been tested yet, Zhang and Casey [12] have suggested that Pgp might play a role in removing the toxic esters from cells.

Finally, Field and associates [13] have found an effect of inhibitors of Pgp on cholesterol transport. In cultured intestinal cells (CaCo-2 cells), they observed that esterification of plasma membrane cholesterol and triacylglycerol-rich lipoprotein secretion was inhibited by inhibitors of Pgp, such as verapamil, trifluoperazine, amiodarone and cyclosporin A. They tentatively conclude from these results that Pgp "might function to maintain the acidic environment of transport vesicles, and therefore, could play a role in the (vesicular) transport of lipids by the intestine" [13].

Properties of mice with disrupted drug-transporting Pgp genes

The general characteristics of these mice are presented in Table 2. In each case, we have shown that the disrupted allele is a null allele, that is, it does not give rise to synthesis of detectable Pgp (fragments). The phenotypes of these mice are therefore attributable to lack of Pgp only. The important result is that even the double knockout mice are healthy and fertile under laboratory conditions. It should be stressed that the *Mdr1b* (–/–) and double knockout mice have not been extensively tested yet. They may have subtle defects that are not apparent in gross anatomy and histology. We have not challenged any of these mice with bacterial or viral infections or other forms of stress. We have shown, however, that the *Mdr1a* (–/–) mice are hypersensitive to drugs transported by Pgp (see below). We expect a more severe hypersensitivity in the double knockout mice and a milder drug sensitivity in the *Mdr1b* (–/–) mice, as the Mdr1b Pgp has a more restricted tissue distribution than the Mdr1a Pgp (Table 2).

Mice with a disrupted Mdr1a *gene* [14–16]

Mice homozygous for a disruption of the *Mdr1a* gene have completely lost all detectable P-glycoprotein in gut epithelium and brain capillaries, showing that this Pgp is the predominant one present at these important barriers in mice and that the absence of the Mdr1a Pgp does not lead to an activation of the Mdr1b Pgp in these tissues. No substantial change (≥2-fold) in *Mdr1b* RNA was found either in the brain, heart, lung, muscle, spleen, thymus, testis, ovary and uterus of *Mdr1a* (–/–) mice, but in liver and kidney there was a substantial increase [14]. This upregulation of *Mdr1b* in liver and kidney probably compensates in part for the loss of excretory capacity caused by the *Mdr1a* disruption.

The complete loss of Mdr1a Pgp has no apparent deleterious effect on mice, as long as they are not challenged with drugs that are normally transported by Pgp. Figure 2 illustrates what happens if these mice are treated with a single dose of 6 mg vinblastine (VBL)/kg. The most striking effect is in the brain: 4 h after injection the level of VBL in *Mdr1a* (–/–) mice is 13-fold higher than in controls and this difference further increases with time because elimination of VBL is also slower in mutant than in wild-type mice (Figure 2b). The body clearance of VBL is also reduced in the mutant mice. This may in part be due to slow outflow of drug from large organs, normally protected by the Mdr1a Pgp, in part to a slower elimination of the drug via the faeces. Approximately 75% of a VBL dose is excreted as polar metabolites in the faeces in mice [17, 18] and this fraction is probably not affected in *Mdr1a* (–/–) mice. However, the excretion of unchanged VBL in faeces was reduced from 20–25% in wild-type mice to 9% in *Mdr1a* (–/–) mice (J. van Asperen and colleagues, unpublished results), and this decrease might be due to reabsorption of VBL in the gut that lacks the protection by Mdr1a Pgp in the deficient mice. The lethal dose of VBL in the *Mdr1a* (–/–) mice was only approximately 3-fold lower than in controls and the signs of general toxicity were similar in the knockout mice and in controls. Probably the organs most affected by the lack of Pgp, brain and heart, are not

Table 2. Mice with disrupted drug-transporting Pgp genes

Gene disruption	Life span	Fertility	Phenotype thus far	Normal tissue expression*	Refs†
Mdr1a (*Mdr3*)	Normal	+	Normal	Gut, liver, brain	a
Mdr1b (*Mdr1*)	Normal	+	Normal	Kidney, adrenals, pregnant uterus	b
Mdr1a + *Mdr1b*	Normal (at 12 months)	+	Normal	See *Mdr1a* and *Mdr1b*	b

*Only major tissues. See Borst [4] for more complete list.
†a, Schinkel and associates [14]; b, unpublished results.

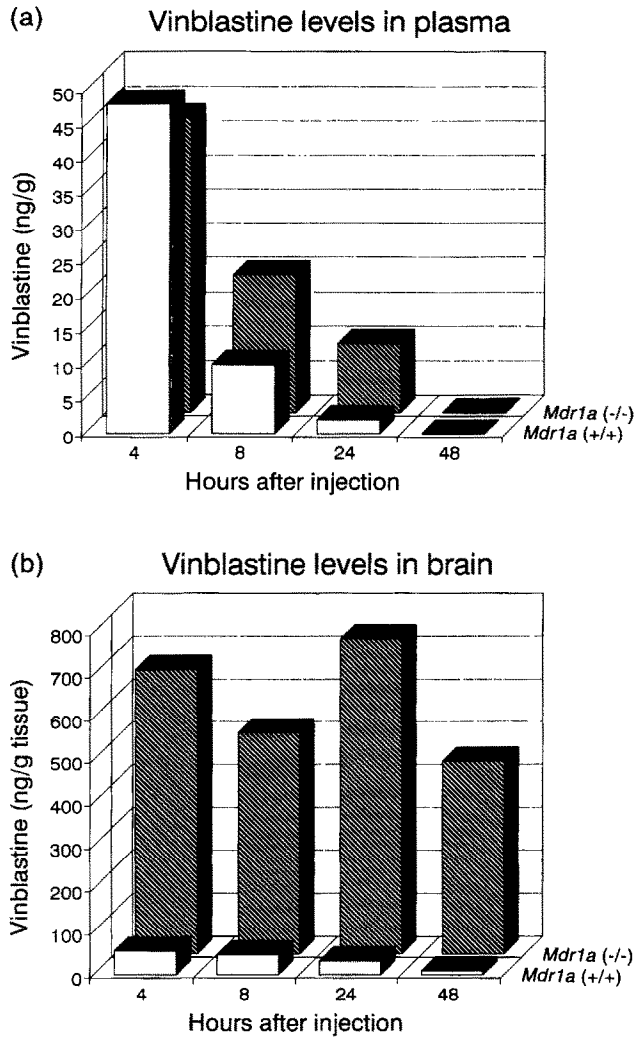

Figure 2. Plasma and tissue levels of vinblastine in mice at different time points after a single intravenous injection of 6 mg vinblastine/kg. *Mdr1a* (+/+), controls. Data from Schinkel and associates [14].

dose-limiting for VBL. Unpublished results of U. Mayer and associates with digoxin and of O. van Tellingen and associates with paclitaxel (personal communications), indicate that elimination of these drugs is also substantially slower in the *Mdr1a* (–/–) mice.

The situation is very different for a drug for which brain toxicity is limiting. An example is ivermectin, a widely used antihelminthic and acaricide drug, and an excellent Pgp substrate [15, 16]. Ivermectin interferes with neurotransmission in several helminths, in insects and in the mammalian brain, but normally it does not reach brain synapses, because Pgp in the mammalian blood–brain barrier excludes it. In the *Mdr1a* (–/–) brain, ivermectin concentrations are 100-fold higher than in the brain of control mice and the tolerance of *Mdr1a* (–/–) mice for ivermectin is also reduced by 100-fold [14]. The lethal ivermectin dose in *Mdr1a* (–/–) mice comes near the dose normally used in humans to treat river blindness.

We have found several other drugs that accumulate more in the brains of *Mdr1a* (–/–) mice than of (+/+) mice. A summary is presented in Table 3. For comparison, the ability of these compounds to be transported by Pgp is presented. This ability was assessed by measuring vectorial transport through a monolayer of pig kidney cells expressing a transfected *MDR1* or *Mdr1a* mini-gene [16]. In general, the drugs that are effectively transported accumulate to a much larger degree in brains of (–/–) mice than in those of (+/+) mice. For [^{3}H]-morphine, the difference was marginal, and for [^{3}H]dexamethasone, a drug transported well by kidney cells expressing mouse *Mdr1a*, the difference was modest. This may be due to rapid metabolism of these drugs, as only total radioactivity was measured. It should also be noted that some of these data are only based on a single drug dose and tissue measurements at a single time point (4 h after i.v. injection). Nevertheless, these results illustrate the power of Pgp as an active extruder of amphipathic molecules that pass the blood–brain barrier by diffusion.

Mice with a disrupted Mdr2 *gene*

The mouse Mdr2 Pgp and its human homologue, the MDR3 Pgp are only present in a few tissues. By far the highest concentrations are found in liver [19–23], where this Pgp is restricted to the canalicular membrane of the hepatocyte [23, 24], suggesting that it is involved in the secretion of compounds into bile.

This suggestion was confirmed by the phenotype of mice homozygous for a disruption of the *Mdr2* gene, resulting in a null allele [25]. The bile of these *Mdr2* (–/–) mice almost completely lacked phosphatidylcholine (PC), normally one of the major constituents of bile. Even more important was that the (+/–) mice, heterozygous for the defect, had approximately half the normal level of PC in their bile. As these heterozygotes had no other biochemical defects or pathological symptoms, this result established that the Mdr2 Pgp is required for PC transport through the canalicular membrane [25]. Several lines of evidence [26–29] now suggest (but do not prove) that this Pgp class is a PC translocator that flips PC from the inner to the outer leaflet of the canalicular membrane.

To test whether the Pgp encoded by the human *MDR3* gene is also a PC translocator, we generated mice carrying an *MDR3* mini-gene, driven by an albumin promoter (preferentially expressed in liver), and crossed the *MDR3* transgene into the *Mdr2* (–/–) line (A.J. Smith and colleagues, unpublished results). In one line with high and uniform *MDR3* expression in the liver, comparable to wild-type murine *Mdr2* expression, the PC levels in the bile were normal and no liver pathology was observed. This proves that the MDR3 Pgp can fully replace the function of its murine Mdr2 counterpart and that the liver defects found in the *Mdr2* (–/–) mice are indeed solely due to the absence of the Mdr2 Pgp.

Can the Mdr2/MDR3 Pgp transport drugs?

No increased transport of drugs was found in cells transfected with *Mdr2* or *MDR3* constructs [30, 31], even though the constructs used were recently shown to yield Pgp able to transport PC [26, 27]. Buschman and Gros [32] have shown that the Mdr2 Pgp does not even bind the drug analogue, iodoarylazidoprazosin, that binds to drug-transporting Pgp. The lack of drug transport seems adequately explained by the high specificity of this transporter for PC and its analogues.

Nevertheless, there are reports in the literature linking the Mdr2/MDR3 Pgp to drug transport. Nooter and associates [33] and Herweijer and associates [34] found good correlation between the level of *MDR3* (and *MDR3* + *MDR1*) RNA and reduced (cyclosporin-sensitive) daunorubicin accumulation in B-cell leukaemias. This is remarkable, but this correlation

Table 3. Increased levels of drugs in the brain of Mdr1a (–/–) *mice compared to brains of control mice**

Drug	Analysis	Increased accumulation in *Mdr1a* (–/–) brain	Transport by Pgp
Vinblastine	Vinblastine	+++	+++
[^{3}H]Ivermectin	Radioactivity	+++	+++
[^{3}H]Digoxin	Radioactivity	+++	+++
[^{3}H]Cyclosporin A	Radioactivity	+++	+++
[^{3}H]Dexamethasone	Radioactivity	+	+++
[^{3}H]Morphine	Radioactivity	+	+

*From Schinkel and associates [14, 16].

might (in theory at least) be explained by the simultaneous overexpression of *MDR3* and another unknown cyclosporin-sensitive transport system able to extrude daunorubicin. The second observation comes from Ruetz and Gros [26] who found that transport of a PC analogue by Mdr2 Pgp inserted into yeast vesicles is completely inhibited by the reversal agent, Verapamil. Inhibition requires binding, but binding does not imply transport. It is therefore possible that the Mdr2 Pgp is inhibited by (some) inhibitors of drug-transporting Pgp without being able to transport these compounds. More physiological systems are now available to test this interpretation.

LESSONS FROM KNOCKOUT MICE FOR THE USE OF INHIBITORS OF PGP (REVERSAL AGENTS) IN PATIENTS

Although we think that mice with disrupted Pgp genes can teach us something about the possible untoward effects that can be expected for the effective MDR reversal agents, the lessons are still incomplete, as long as the *Mdr1a* + *Mdr1b* double knockout has not been fully analysed. It is also possible that the consequences of a complete loss of drug-transporting Pgp are not the same in mice and humans, although we consider this unlikely. Nevertheless, some clear lessons are already emerging.

1. The fact that the double knockout mouse appears normal suggests that the drug-transporting Pgp have no essential function in normal metabolism, in contrast to earlier suggestions (see Table 1). Especially noteworthy is the absence of gross disturbances in corticosteroid metabolism, in pregnancy, and in bile formation. However, we have not looked in detail at NK-cell function, accumulation of prenylcysteine methyl esters, or intracellular cholesterol transport, and more subtle defects may be detected as the analysis of the double knockout mice progresses to completion. Nevertheless, we think that an important positive message from the knockout mice is that the complete absence of drug-transporting Pgp in at least one mammal is compatible with fairly normal physiology. We therefore expect that treatment with MDR reversal agents, which selectively block the function of the MDR1 P-glycoprotein, should not have major effects on human physiology, beyond the predictable effects on drug metabolism. A caveat should be added: drugs are never as specific as directed genetic lesions, and reversal agents may be expected to have side-effects beyond their interference with Pgp function. For instance, Böhme and colleagues [35] have shown that cyclosporin A (CsA) is a potent inhibitor of ATP-dependent bile salt transport through the canalicular membrane of the rat. The CsA analogue and reversal agent PSC 833 has the same side-effect, albeit less severe. In addition, there are transporters that have not yet been characterised for their sensitivity to reversal agents, such as Sister of Pgp [36], and that might also be inhibited by these agents. Reversal agents may therefore have more severe effects than those resulting from a total block of Pgp alone.

2. The results thus far obtained with knockout mice highlight the two types of problems that can be expected with effective reversal agents.

A. Decreased elimination of drugs. Two factors could contribute to this decrease: (a) decreased excretion of drug in liver, kidney or gut, depending on which route is important in the Pgp-mediated excretion of the drug; and (b) increased reabsorption of drug from bile, gut lumen, or urine before the drug can be eliminated from the body. For drugs that are excreted in unaltered form or as a conjugate that can be hydrolysed after secretion, Pgp in the epithelial surfaces of bile ducts, gut and kidney proximal tubules may be important to prevent reabsorption of the excreted drug. How important, remains to be determined.

Obviously, the importance of Pgp in drug elimination will depend on the fraction of drug that is normally excreted by Pgp. If this fraction is high, the rate of drug elimination will be decreased more by a block of Pgp than when most of the drug is metabolised and excreted as hydrophilic metabolites. From clinical results with the (relatively ineffective) reversal agents tested so far, it is already clear, for instance, that these agents interfere with the elimination of anthracyclines resulting in considerable increases in area under the curve (AUC) and concomitant toxicity [9, 37, 38]. The 3-fold decrease in tolerance for VBL in the *Mdr1a* (–/–) mice appears to be mainly due to this type of delayed drug elimination.

B. Increased toxicity of drugs in organs normally protected by Pgp. The drastically decreased tolerance of the *Mdr1a* (–/–) mice for ivermectin is a good example. It also illustrates that side-effects of reversal agents will not be limited to anticancer drugs, but will potentially extend to any other drug that is affected by Pgp. The accumulation of digoxin in the brain of *Mdr1a* (–/–) mice (Table 3) is a good example. Mice are intrinsically resistant to digoxin and the increased digoxin levels in the brain are therefore without severe toxic effects. We expect humans to be more affected by a drastic increase in brain digoxin.

The experiments with knockout mice suggest that several other organs apart from the brain are at risk when Pgp is eliminated. In the *Mdr1a* (–/–) mouse, drug removal was

consistently slowed down in skeletal muscle, heart, intestine, testis and lung. If the Mdr1b Pgp is also absent, one can expect problems in adrenals and ovary as well, whereas the toxicity for tissues that contain both Mdr1a and Mdr1b Pgp, e.g. kidney, muscle, lung and spleen should be exacerbated. Further analysis of the *Mdr1b* (−/−) and double knockout mice should clarify the situation.

OTHER LESSONS FROM KNOCKOUT MICE FOR PHARMACOLOGY

We think that our results show that knockout mice, in general, can become an important tool in pharmacology. Drug metabolism in mammals is a complex affair involving parallel pathways with overlapping drug specificities. Although these can often be distinguished by the use of inhibitors, few inhibitors act on a single target. In contrast, gene disruption is specific for a single gene and by combining multiple gene disruptions it should become possible to dissect complex pathways in detail. Obviously, the genetic approach is not perfect either. The absence of one gene product can result in the overproduction of another one and this overproduction may mask the genetic defect produced. A case in point is the upregulation of *Mdr1b* expression in the liver of the *Mdr1a* (−/−) mice, masking the decreased capacity for excretion of Pgp substrates into bile. However, the power of the gene disruption approach lies in the possibility to disrupt more and more genes and eventually—this is indeed a long-term goal—to combine all the null alleles in a single mouse. It is therefore an approach that gains in power as one proceeds. The human and mouse genome projects are also helping to provide new candidate genes for disruption.

CONCLUSION

Although it is early days, there are already a few general lessons to be drawn from our P-glycoprotein knockout mice.

1. We have found a novel and unexpected function, translocation of phosphatidylcholine, for the Mdr2 Pgp and its human MDR3 homologue.

2. The expectation that P-glycoprotein is an important component in the defence of mammals against amphipathic xenotoxins (Table 1) has been fully confirmed by our results with the *Mdr1a* (−/−) mutant, given the fact that most of the drugs tested thus far are biotoxins from nature.

3. The substantial amounts of drug-transporting Pgp present in the gut should have an important effect on the oral availability of drugs transported by Pgp. This is an important area for future research. It is conceivable that potent reversal agents with short half lives in the body might be used to temporarily block the gut Pgp and allow uptake of drugs from the gut that are otherwise excluded by Pgp.

4. We think that our demonstration of the importance of Pgp in the blood–brain barrier is a useful addition to brain pharmacology. It explains many (if not all) exceptions to the rule that the penetration of drugs into the brain tends to increase with increasing hydrophobicity. It has also been shown that the apparent cut-off for penetration into the brain at a molecular mass of 700 Da is another consequence of Pgp action. Large drugs are merely good Pgp substrates. The presence of functional Pgp in the blood–brain barrier obviously offers opportunities for new types of drug treatment. Drugs that are actively pumped out may gain entry in the presence of a potent reversal agent. In drug design, the presence of Pgp could be taken into account to make drugs with enhanced or decreased brain accessibility.

5. The fact that none of the mouse Pgp is required for normal fetal development, implies that inborn errors of Pgp synthesis could exist in humans. Defects in MDR3 Pgp synthesis should lead to liver disease early in life. As the human bile salts are more hydrophobic (and therefore aggressive towards membranes) than murine bile salts, we expect the human disease to be more severe than the murine disease and to lead rapidly to obliteration of bile ducts and cessation of bile production [25]. This may make the detection of this Pgp defect difficult.

We expect children born with a defect in the MDR1 Pgp to be healthy, until challenged with Pgp substrates. To our knowledge, no untoward reactions to drugs have been reported that would suggest a compromised Pgp function. A vast number of Africans has been treated with ivermectin for river blindness, without reports of severe side-effects [39]. It is possible that there are natural toxins in the environment that kill humans with a total defect of MDR1 Pgp at an early age. Such deaths might be recorded as "cot death" and remain unexplained. It is also possible (though unlikely in our opinion) that humans have additional protection mechanisms against amphipathic toxins, not present in mice, reducing the consequences of a defect in the MDR1 Pgp. It seems likely, however, that variations in MDR1 Pgp activity will exist in the human population and the consequences of these variations for pharmacotherapy remain an interesting area for future research.

1. Gottesman MM. How cancer cells evade chemotherapy: sixteenth Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res* 1993, **53**, 747–754.
2. Fisher GA, Sikic BI. Clinical studies with modulators of multidrug resistance. *Hematol Oncol Clin North Am* 1995, **9**, 363–382.
3. Borst P, Schinkel AH. Mice with disrupted P-glycoprotein genes. In Gupta S, Tsuruo T, eds. *Multidrug Resistance in Cancer Cells*. New York/London, John Wiley & Sons Ltd, 1996, in press.
4. Borst P, Schinkel AH, Smit JJM, *et al.* Classical and novel forms of multidrug resistance and the physiological functions of P-glycoproteins in mammals. *Pharmacol Ther* 1993, **60**, 289–299.
5. Lum BL, Gosland MP. MDR expression in normal tissues. Pharmacologic implications for the clinical use of P-glycoprotein inhibitors. *Hematology/Oncology Clinics of North America* 1995, **9**, 319–336.
6. Hardy SP, Goodfellow HR, Valverde MA, Gill DR, Sepúlveda FV, Higgins CF. Protein kinase C-mediated phosphorylation of the human multidrug resistance P-glycoprotein regulates cell volume-activated chloride channels. *EMBO J* 1995, **14**, 68–75.
7. Coon JS, Wang Y, Bines S, Markham PM, Chong AS-F, Gebel HM. Multidrug resistance activity in human lymphocytes. *Hum Immunol* 1991, **32**, 134–140.
8. Klimecki WT, Futscher BW, Grogan TM, Dalton WS. P-glycoprotein expression and function in circulating blood cells from normal volunteers. *Blood* 1994, **83**, 2451–2458.
9. Nooter K, Sonneveld P. Clinical relevance of P-glycoprotein expression in haematological malignancies. *Leuk Res* 1994, **18**, 233–243.
10. Chong AS-F, Markham PN, Gebel HM, Bines SD, Coon JS. Diverse multidrug-resistance-modification agents inhibit cytolytic activity of natural killer cells. *Cancer Immunol Immunother* 1993, **36**, 133–139.
11. Zhang L, Sachs CW, Fu H-W, Fine RL, Casey PJ. Characterization of prenylcysteines that interact with P-glycoprotein and inhibit drug transport in tumor cells. *J Biol Chem* 1995, **270**, 22859–22865.
12. Zhang FL, Casey PJ. Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem* 1996, in press.
13. Field FJ, Born E, Chen H, Murthy S, Mathur SN. Esterification

of plasma membrane cholesterol and triacylglycerol-rich lipoprotein secretion in CaCo-2 cells: possible role of P-glycoprotein. *J Lipid Res* 1995, **36**, 1533–1543.
14. Schinkel AH, Smit JJM, Van Tellingen O, *et al.* Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. *Cell* 1994, 77, 491–502.
15. Schinkel AH, Mol CAAM, Wagenaar E, Van Deemter L, Smit JJM, Borst P. Multidrug resistance and the role of P-glycoprotein knockout mice. *Eur J Cancer* 1995, **31A**, 1295–1298.
16. Schinkel AH, Wagenaar E, Van Deemter L, Mol CAAM, Borst P. Absence of the *mdr1a* P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest* 1995, **96**, 1698–1705.
17. Van Tellingen O, Beijnen JH, Nooijen WJ, Bult A. Tissue disposition, excretion and metabolism of vinblastine in mice as determined by high-performance liquid chromatography. *Cancer Chemother Pharmacol* 1993, **32**, 286–292.
18. Van Tellingen O, Beijnen JH, Nooijen WJ, Bult A. Plasma pharmacokinetics of vinblastine and the investigational vinca alkaloid n-(deacetyl-*O*-4-vinblastoyl-23)-L-ethyl isoleucinate in mice as determined by high-performance liquid chromatography. *Cancer Res* 1993, **53**, 2061–2065.
19. Croop JM, Raymond M, Haber D, *et al.* The three mouse multidrug resistance (*mdr*) genes are expressed in a tissue-specific manner in normal mouse tissues. *Mol Cell Biol* 1989, **9**, 1346–1350.
20. Teeter LD, Becker FF, Chisari FV, Li D, Kuo MT. Overexpression of the multidrug resistance gene *mdr3* in spontaneous and chemically induced mouse hepatocellular carcinomas. *Mol Cell Biol* 1990, **10**, 5728–5735.
21. van der Bliek AM, Baas F, Ten Houte de Lange T, Kooiman PM, Van der Velde-Koerts T, Borst P. The human *mdr3* gene encodes a novel P-glycoprotein homologue and gives rise to alternatively spliced mRNAs in liver. *EMBO J* 1987, **6**, 3325–3331.
22. Chin JE, Soffir R, Noonan KE, Choi K, Roninson IB. Structure and expression of the human MDR (P-glycoprotein) gene family. *Mol Cell Biol* 1989, **9**, 3808 3820.
23. Smit JJM, Schinkel AH, Mol CAAM, *et al.* Tissue distribution of the human MDR3 P-glycoprotein. *Lab Invest* 1994, **71**, 638–649.
24. Buschman E, Arceci RJ, Croop JM, *et al.* Mdr2 encodes P-glycoprotein expressed in the bile canalicular membrane as determined by isoform-specific antibodies. *J Biol Chem* 1992, **267**, 18093–18099.
25. Smit JJM, Schinkel AH, Oude Elferink RPJ, *et al.* Homozygous disruption of the murine *mdr2* P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* 1993, **75**, 451–462.
26. Ruetz S, Gros P. Phosphatidylcholine translocase: a physiological role for the *mdr2* gene. *Cell* 1994, 77, 1071–1083.
27. Smith AJ, Timmermans-Hereijgers JLPM, Roelofsen B, *et al.* The human MDR3 P-glycoprotein promotes translocation of phosphatidylcholine through the plasma membrane of fibroblasts from transgenic mice. *FEBS Lett* 1994, **354**, 263–266.
28. Oude Elferink RPJ, Ottenhoff R, Van Wijland M, Smit JJM, Schinkel AH, Groen AK. Regulation of biliary lipid secretion by mdr2 P-glycoprotein in the mouse. *J Clin Invest* 1995, **95**, 31–38.
29. Ruetz S, Gros P. Enhancement of Mdr2-mediated phosphatidylcholine translocation by the bile salt taurocholate. *J Biol Chem* 1995, **270**, 25388–25395.
30. Buschman E, Gros P. Functional analysis of chimeric genes obtained by exchanging homologous domains of the mouse *mdr1* and *mdr2* genes. *Mol Cell Biol* 1991, **11**, 595–603.
31. Schinkel AH, Roelofs MEM, Borst P. Characterization of the human *MDR3* P-glycoprotein and its recognition by P-glycoprotein specific monoclonal antibodies. *Cancer Res* 1991, **51**, 2628–2635.
32. Buschman E, Gros P. The inability of the mouse *mdr2* gene to confer multidrug resistance is linked to reduced drug binding to the protein. *Cancer Res* 1994, **54**, 4892–4898.
33. Nooter K, Sonneveld P, Janssen A, *et al.* Expression of the *mdr3* gene in prolymphocytic leukemia: association with cyclosporin-A-induced increase in drug accumulation. *Int J Cancer* 1990, **45**, 626–631.
34. Herweijer H, Sonneveld P, Baas F, Nooter K. Expression of *mdr1* and *mdr3* multidrug-resistance genes in human acute and chronic leukemias and association with stimulation of drug accumulation by cyclosporine. *J Natl Cancer Inst* 1990, **82**, 1133–1140.
35. Böhme M, Jedlitschky G, Leier I, Büchler M, Keppler D. ATP-dependent export pumps and their inhibition by cyclosporins. *Adv Enz Reg* 1994, **34**, 371–380.
36. Childs S, Yeh RL, Georges E, Ling V. Identification of a sister gene to P-glycoprotein. *Cancer Res* 1995, **55**, 2029–2034.
37. Sikic BI. Modulation of multidrug resistance: at the threshold. *J Clin Oncol* 1993, **11**, 1629–1635.
38. McLeod HL. Clinical reversal of the multidrug resistance phenotype: true tumour modulation or pharmacokinetic interaction? *Eur J Cancer* 1994, **30A**, 2039–2041.
39. Taylor HG, Pacqué M, Muñoz B, Greene BM. Impact of mass treatment of onchocerciasis on the transmission of infection. *Science* 1990, **250**, 116–118.

Acknowledgements—We thank our colleagues Judith van Asperen and Drs Ulrich Mayer, Olaf van Tellingen, Wim J. Nooijen and Jos H. Beijnen for permission to quote unpublished results on vinblastine and digoxin excretion in *Mdr1a* (–/–) mice. The experimental work in our laboratory is supported in part by grant NKI 92-41 of the Dutch Cancer Society.