

GENES AND PROTEINS INVOLVED IN ANTICANCER DRUG RESISTANCE AND PHARMACOKINETICS

Our research focuses on genes and proteins that cause drug resistance or drug susceptibility in tumors, or influence the pharmacological and toxicological behavior of anticancer and many other drugs and toxins, including carcinogens. Insight into these systems may: i) improve chemotherapy and more generally pharmacotherapy approaches for cancer and other diseases; ii) increase insights into factors determining susceptibility to carcinogens, and; iii) allow elucidation of physiological functions. To study the physiological, pharmacological and toxicological roles of the proteins involved, and their interactions, we generate and analyze knockout or transgenic mice lacking or overexpressing the relevant genes.

Impact of drug transporters We have a long-standing interest in plasma membrane proteins of the ATP binding cassette (ABC) multidrug transporter family, including P-glycoprotein (P-gp, ABCB1/MDR1), MRP2 (ABCC2) and BCRP (ABCG2) (figure 6). These proteins actively export a wide range of anticancer, anti-HIV/AIDS, and many other drugs from cells. This ATP-dependent drug extrusion can cause multidrug resistance (MDR) in tumor cells. P-gp, MRP2 and BCRP all localize to the apical membrane of polarized epithelial cells, resulting in apically directed export of drug substrates, and there is considerable overlap in substrate specificity between these transporters. Previous experiments in P-gp and Bcrp1 knockout mice indicated that these transporters can protect an organism against exogenous toxins and drugs by limiting penetration of substrates into brain, testis, and fetus, by restricting uptake of orally administered substrates, and by mediating excretion of substrates via liver and intestine. To extend these analyses we have generated MRP2 knockout mice, and crossed these with existing P-gp, Bcrp1 and MRP3 knockout mice in order to elucidate the separate and combined contributions of these transporters to pharmacological, toxicological and physiological functions. MRP3 is expressed in the basolateral membrane of hepatocytes and enterocytes, so transporting in the opposite direction of P-gp, Bcrp1 and MRP2.

P-gp, Bcrp1, MRP2 and MRP3 (compound) knockout mice We generated and characterized Bcrp1/MRP2/MRP3 combination knockout mice, which were viable and fertile, and had a normal life span. We then used these mice, in conjunction with existing P-gp/MRP2, MRP2/MRP3, and single knockout mice to investigate the roles of P-gp, Bcrp1, MRP2 and MRP3 in the elimination and toxicity of a number of different drugs and drug conjugates. We found that Bcrp1 and MRP2 deficiency increases the plasma levels of acyl glucuronide conjugates of the anti-inflammatory drug diclofenac, and that MRP3 deficiency decreases these levels, presumably because of their opposite roles in excreting the conjugates from the liver towards bile and blood, respectively. Bcrp1/MRP2/MRP3 deficiency resulted in mildly increased liver toxicity of diclofenac, presumably resulting from the increased liver accumulation of diclofenac conjugates. Rather similar results were found for the anticancer drug etoposide, which displayed decreased biliary excretion of etoposide in MRP2 knockout mice, resulting in increased formation of etoposide glucuronide in the liver, which was secreted into blood by MRP3. In MRP2/MRP3 combination knockout mice this resulted in highly increased liver levels of etoposide glucuronide. P-gp restricted the oral availability of etoposide, and mediated its direct intestinal, but not its hepatobiliary excretion. The anticancer drug methotrexate showed dramatically decreased elimination in Bcrp1/MRP2/MRP3 knockout mice, and highly increased liver levels of this drug and its primary nephrotoxic metabolite 7OH-methotrexate. These effects were far more pronounced than observed in the single or double knockout mice. Collectively, these studies indicate that there can be substantial overlap and redundancy in the detoxifying (protective) functions of these drug transporters for a number of anticancer and other drugs. This probably explains why it is often still comparatively safe to administer these potentially highly toxic drugs to patients, as chances are low that all of these systems would be simultaneously compromised by genetic polymorphisms or drug-drug interactions, thus resulting in toxic overdosing.



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Publications

Van Waterschoot RA, Lagas JS, Wagenaar E, van der Kruijssen CM, van Herwaarden AE, Song JY, Rooswinkel RW, van Tellingen O, Rosing H, Beijnen JH, Schinkel A.H. *Absence of both cytochrome P450 3A and P-glycoprotein dramatically increases docetaxel oral bioavailability and risk of intestinal toxicity. Cancer Res.* 2009;69:8996-9002

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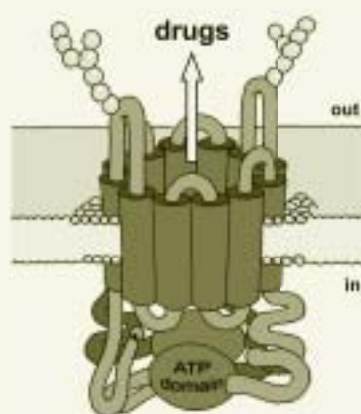
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Van de Steeg E. *Physiological and pharmacological functions of OATP1A/1B transporters. PhD thesis, University of Utrecht, April 2010.*

Insights from Cyp3a, Cyp3a/P-gp and Cyp3a/P-gp/Mrp2 combination knockout mice Cytochrome P450 3A (CYP3A) enzymes metabolize >50% of prescribed drugs, and represent one of the most important detoxifying systems. As CYP3A activity shows high inter- and intra-patient variability, it can have a profound influence on variable drug behavior (pharmacodynamics) and drug toxicity. Moreover, its substrates overlap extensively with those of the drug transporters P-gp, BCRP and MRP2. To investigate the physiological and pharmacological roles of CYP3A in combination with P-gp, we previously generated Cyp3a knockout mice, and showed a pronounced effect of Cyp3a deficiency on the oral bioavailability, i.v. clearance and toxicity of the anticancer drug and CYP3A substrate docetaxel. In combination Cyp3a/P-gp knockout mice the oral availability of docetaxel was disproportionately increased, suggesting a partly overlapping and compensatory activity of both detoxifying systems in docetaxel handling. We now extended these studies to lopinavir, an anti-HIV/AIDS drug known to be a substrate of both CYP3A and P-gp, and possibly MRP2. We found that lopinavir was transported by MDR1 but not by MRP2 in vitro. Accordingly, the lopinavir AUC_{oral} was significantly increased in P-gp knockout mice (~9-fold vs. wild-type) but not in Mrp2 knockout mice. A more than 2000-fold increase in lopinavir AUC_{oral} was observed in Cyp3a knockout mice compared to wild-type mice. Interestingly, no significant difference in AUC_{oral} between Cyp3a knockout and Cyp3a/P-gp/Mrp2 knockout mice was observed. CYP3A4 activity in the intestine or liver could each already reduce the lopinavir AUC_{oral} by more than 100-fold compared to the Cyp3a knockout situation. The results demonstrate that CYP3A is the major determinant of lopinavir pharmacokinetics, far more than P-gp. Both intestinal and hepatic CYP3A activity contribute profoundly to the low oral bioavailability of lopinavir. The impact of P-gp on oral lopinavir kinetics was only detectable in the presence of CYP3A, suggesting saturation of P-gp in the absence of CYP3A activity.

Insights from Oatp1a/1b (Slco1a/1b) knockout mice Organic anion transporting polypeptides (OATP, gene name: SLCO) belong to the superfamily of the solute carrier class of organic anion transporters. OATPs facilitate sodium-independent uptake transport of a wide variety of organic endo- and exogenous compounds, such as bile salts, steroid and thyroid hormones and their conjugates, and numerous drugs and toxins. Members of the OATP1A/1B family have a broad substrate specificity and are highly expressed in the sinusoidal membrane of hepatocytes, or in the apical membrane of enterocytes where they might affect liver or intestinal uptake, respectively, of drugs, xenobiotics, and endogenous substances. They might thus play an important role in drug disposition. To investigate the physiological and pharmacological roles of Oatp1a/1b transporters in vivo, we generated mice lacking all (5-7) Slco1a/1b genes. Slco1a/1b knockout mice were viable and fertile but, surprisingly, suffered from marked conjugated hyperbilirubinemia, due to impaired hepatic (re-)uptake of bilirubin glucuronide, and increased plasma unconjugated bile salt levels. The resulting hypothesis that sinusoidal secretion, presumably by MRP3 and related transporters, and subsequent re-uptake of glucuronidated compounds into hepatocytes by OATPs occurs under physiological conditions might alter our perspective on normal liver functioning. It may prevent saturation of biliary excretion and other hepatocyte detoxification processes by spreading these processes from the most heavily exposed periportal hepatocytes over the entire liver lobule. Slco1a/1b knockout mice further showed drastically decreased hepatic uptake and



consequently increased systemic exposure of the drugs methotrexate and fexofenadine upon i.v. and oral administration. Slco1a/1b knockout mice did not display reduced intestinal absorption of oral methotrexate or fexofenadine. Rifampicin was an effective and specific Oatp1a/1b inhibitor in controlling methotrexate pharmacokinetics. Our results indicate that Oatp1a/1b transporters play an essential role in hepatic (re-)uptake of conjugated bilirubin, unconjugated bile salts and drugs.

Figure 5: Putative structure of a prototypic ABC drug transporter.