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Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine

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ABSTRACT In mice, the *mdr1a* and *mdr1b* genes encode drug-transporting proteins that can cause multidrug resistance in tumor cells by lowering intracellular drug levels. These P-glycoproteins are also found in various normal tissues such as the intestine. Because *mdr1b* P-glycoprotein is not detectable in the intestine, mice with a homozygously disrupted *mdr1a* gene [*mdr1a*($-/-$) mice] do not contain functional P-glycoprotein in this organ. We have used these *mdr1a*($-/-$) mice to study the effect of gut P-glycoprotein on the pharmacokinetics of paclitaxel. The area under the plasma concentration-time curves was 2- and 6-fold higher in *mdr1a*($-/-$) mice than in wild-type (wt) mice after i.v. and oral drug administration, respectively. Consequently, the oral bioavailability in mice receiving 10 mg paclitaxel per kg body weight increased from only 11% in wt mice to 35% in *mdr1a*($-/-$) mice. The cumulative fecal excretion (0–96 hr) was markedly reduced from 40% (after i.v. administration) and 87% (after oral administration) of the administered dose in wt mice to below 3% in *mdr1a*($-/-$) mice. Biliary excretion was not significantly different in wt and *mdr1a*($-/-$) mice. Interestingly, after i.v. drug administration of paclitaxel (10 mg/kg) to mice with a cannulated gall bladder, 11% of the dose was recovered within 90 min in the intestinal contents of wt mice vs. <3% in *mdr1a*($-/-$) mice. We conclude that P-glycoprotein limits the oral uptake of paclitaxel and mediates direct excretion of the drug from the systemic circulation into the intestinal lumen.

P-glycoproteins, encoded by the human *MDR1* or the murine *mdr1a* (also called *mdr3*) and *mdr1b* (also called *mdr1*) genes are drug efflux pumps localized in the plasma membrane. They can reduce the intracellular accumulation of a wide range of compounds, including cytotoxic drugs such as vinca alkaloids and anthracyclines (1–3). Although initially discovered by their ability to cause multidrug resistance in mammalian tumor cells, these P-glycoproteins are also present in many normal tissues (4–13).

The normal physiological function of these P-glycoproteins is still a matter of conjecture, but the idea that they serve to protect the organism against toxins is supported by several studies (4–6). Drug-transporting P-glycoproteins are found in particular in organs that have an excretory function, with P-glycoprotein being localized at the apical side of the epithelial layer (e.g., the bile canalicular membrane and the renal proximal tubules) (4, 7–9). Furthermore, P-glycoproteins are found in tissues that act as a barrier, such as the brain capillary endothelial cells that form the blood-brain barrier and the

brush border of the intestine (5, 10–13). The latter is of particular interest because food ingestion exposes the body to a wide variety of potentially harmful compounds (14). P-glycoprotein in the gut epithelium could therefore protect against these xenotoxins, and indirect evidence for this hypothesis has been reported (15–18).

Recently, Schinkel *et al.* (10) generated mice with a disruption of the *mdr1a* gene [*mdr1a*($-/-$) mice] to study the physiological and pharmacological role of P-glycoproteins. The extreme sensitivity of these *mdr1a*($-/-$) mice for the central neurotoxic pesticide ivermectine has clearly demonstrated the important protective function of P-glycoprotein in the blood-brain barrier (10). Further studies with vinblastine in these mice demonstrated a lower body clearance and a reduced fecal excretion (19), which suggested that P-glycoprotein may contribute to drug elimination by mediating biliary excretion and/or limiting (re-)uptake from the intestinal lumen after hepatobiliary excretion. We have studied this possibility in more detail with the cytotoxic agent paclitaxel (Taxol). (The United States Adopted Name Council has given the name paclitaxel to refer to the registered trade name Taxol; Bristol-Myers Squibb.) Paclitaxel has become a very important drug in the treatment of cancer (20, 21), and it is also a very good substrate of P-glycoprotein, as was shown by *in vitro* transport studies in polarized monolayers of an *MDR1*-transfected pig kidney epithelial cell line (LLC-PK1; unpublished results). Our pilot experiments confirmed an earlier study, reporting that the concentration of paclitaxel in plasma of mice remained very low after oral drug administration (22). These observations raised the possibility that this low bioavailability may result from P-glycoprotein activity in the gut. We have, therefore, tested the fate of orally and i.v.-administered paclitaxel in wild-type (wt) and *mdr1a*($-/-$) mice. Our results show that P-glycoprotein in the intestine limits the uptake of orally administered paclitaxel and suggest that P-glycoprotein may serve to protect the body against a broad range of orally ingested xenotoxins that are substrates of this transport protein.

MATERIALS AND METHODS

Chemicals. Paclitaxel (batch 80617492D), 2'-methylpaclitaxel, and paclitaxel for injection (Taxol) supplied as a concentrated sterile solution with 6 mg/ml in a 5 ml vial in Cremophor EL and dehydrated ethanol (United States Pharmacopeia; 1:1, vol/vol) originated from Bristol-Myers Squibb. [³H]Paclitaxel (batches 114-195-014 and 125-075-0116; specific activities 16.4 and 14.5 mCi/mg; radiochemical purity >99%) was supplied by Moravek Biochemicals (Brea, CA). The major

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Abbreviations: wt, wild type; plasma AUC, area under the plasma concentration-time curves.

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part of the tritium label is at the *m*- and *p*-positions in the aromatic rings, whereas a minor fraction is either at the 2- or 10-position in the taxane ring. Metabolite I (3'-*p*-hydroxypaclitaxel) and metabolite II (6 α -hydroxypaclitaxel) were isolated from patient feces samples, as described in detail elsewhere (23). Hypnorm was from Janssen (Tilburg, The Netherlands) and Dormicum from Roche Nederland (Mijdrecht, The Netherlands). Lyophilized BSA was obtained from Organon Teknika-Cappel. Acetonitrile and methanol were of Lichrosolv gradient grade (Merck). All other chemicals were of the highest grade available commercially and were used as received. Water was deionized by the Milli-Q Plus system (Millipore). Blank human plasma was obtained from healthy volunteers.

Drug Analyses. Paclitaxel and its hydroxylated metabolites were determined by liquid-liquid and solid-phase extraction, followed by reversed-phase HPLC with UV detection as described (24). Aliquots of 10–200 μ l of plasma, bile, and homogenates of intestinal contents were also added to 3 ml of Ultima Gold scintillation fluid (Packard), and radioactivity was determined by liquid scintillation counting in a Tri-Carb Series 4000 Minaxi model B4430 counter (Packard) using quench correction by external standardization. Cremophor EL levels were determined by a novel HPLC method described in detail elsewhere (25).

Pharmacokinetic Studies. Animals were housed and handled according to institutional guidelines complying with Dutch legislation. Female FVB wt and *mdr1a*($-/-$) mice weighing 25 ± 5 g (10–14 weeks of age) were used in all experiments. They were given food (Hope Farms, Woerden, The Netherlands) and acidified water *ad libitum*. Plasma pharmacokinetic studies were carried out with animals receiving 10 or 5 mg/kg (body weight) of unlabeled paclitaxel, while other experiments were performed with a drug solution supplemented with [3 H]paclitaxel (≈ 1 μ Ci per animal; 1 Ci = 37 GBq).

For oral administration, the commercially available paclitaxel formulation was used. This solution was diluted 10- or 20-fold with saline for the 10 and 5 mg/kg experiments, respectively. The solution used for the 5 mg/kg experiment was supplemented with Cremophor EL and ethanol to achieve the same vehicle exposure in both dose groups. Volumes of 16.7 ml/kg (body weight) of these solutions were administered by direct injection into the stomach using a blunt-ended needle inserted via the esophagus, resulting in dose levels of 10 and 5 mg/kg (body weight).

For i.v. administration, paclitaxel was formulated in polysorbate-80 and ethanol (1:1, vol/vol). This solution was diluted 2- or 4-fold with saline for the 10 and 5 mg/kg experiments, respectively. The required injection volume for both dose levels was 3.3 ml/kg and was administered into a tail vein. Blood samples for plasma pharmacokinetic studies were collected under diethyl ether anaesthesia. Animals receiving 10 mg/kg (body weight) of paclitaxel were sacrificed at 5, 15, 30, and 45 min, and at 1, 1.5, 2, 4, 6, and 8 hr after treatment, with 2–10 animals per time point. After oral administration of 5 mg/kg (body weight) blood samples were taken at 1, 2, 4, 6, and 8 hr, with 4 animals per time point. Samples were collected in heparinized tubes and centrifuged at $1500 \times g$ for 10 min. Plasma was separated and stored at -20°C until analysis. Urine and feces were collected from wt and *mdr1a*($-/-$) mice receiving 10 mg/kg (body weight) of paclitaxel by i.v. or oral administration ($n = 4$ –6 per group). They were kept in Ruco type M/1 stainless-steel metabolic cages (Valkenswaard, The Netherlands), and specimens were collected during time intervals of 0–8, 8–24, 24–48, 48–72, and 72–96 hr.

To monitor the biliary excretion of the drug, mice were anaesthetized with a mixture of Hypnorm (fentanyl 0.2 mg/ml, fluanisone 10 mg/ml), Dormicum (midazolam 5 mg/ml), and water (1:1:2, vol/vol/vol). The volume of the mixture injected i.p. was 5–7 ml/kg body weight. After median laparotomy and

ligation of the common bile duct, the gall bladder was cannulated using a polyethylene tube (Portex, Hythe, U.K.) with a 0.28 mm internal diameter. The tube was fixed in the gall bladder with additional ligation to prevent leakage. The temperature of the animals was monitored with a rectal probe and maintained at $36 \pm 1^\circ\text{C}$ using a standard heating pad and a lamp. The tissues at the open surface of the abdominal cavity were moistened with saline. Additional anaesthesia (≈ 30 μ l) was also given directly in the opening of the abdominal cavity, if needed. Bile was collected for 90 min after drug administration ($n = 4$ per group). Next, blood was obtained from the axillary venous plexus and intestinal contents were removed. Intestinal contents and feces were homogenized in 4% (wt/vol) BSA and frozen at -20°C until analysis.

Pharmacokinetic Analysis. The area under the plasma concentration-time curves (plasma AUC) after oral (AUC_{oral}) and i.v. ($\text{AUC}_{\text{i.v.}}$) administration was calculated by the linear trapezoidal rule, without extrapolation to infinity. The standard error of the AUC was calculated with the law of propagation of errors. The $t_{1/2}$ after i.v. injection was calculated by linear regression analysis of the log-linear part of the plasma concentration-time curves. The clearance (Cl) was calculated by the formula $\text{Cl} = \text{dose}/\text{AUC}$ and the oral bioavailability (F) by the formula $F = \text{AUC}_{\text{oral}}/\text{AUC}_{\text{i.v.}} \times 100\%$. The two-sided unpaired Student's *t*-test was used for statistical analysis.

RESULTS

If P-glycoprotein in the intestinal mucosa of mice would limit the bioavailability of paclitaxel, this should result in higher plasma levels in *mdr1a*($-/-$) mice than in wt mice after oral drug administration. This is borne out by the results shown in Fig. 1A. After administration of 10 mg paclitaxel/kg body weight, the plasma AUC is about 6-fold higher in *mdr1a*($-/-$) mice than in wt mice. This difference in plasma AUC is reflected by the maximum plasma concentration, which is ≈ 10 -fold higher in *mdr1a*($-/-$) mice (Table 1). During the elimination phase the plasma levels remain ≈ 3 -fold higher in *mdr1a*($-/-$) mice.

Because the plasma AUC is determined by both uptake and elimination, we also treated mice with paclitaxel given by i.v. injection. After i.v. administration, maximum plasma levels of paclitaxel were equal in wt and *mdr1a*($-/-$) mice (Fig. 1B). The plasma concentration-time curves coincide but diverge ≈ 30 min after drug administration, with a more rapid decay in wt animals. During the elimination phase the plasma levels are about 5-fold higher in *mdr1a*($-/-$) mice. The difference in plasma concentration-time profiles after i.v. administration results in an approximately 2-fold higher AUC in *mdr1a*($-/-$) mice (Table 1). Relative to their respective plasma AUC values after i.v. drug administration, the estimated bioavailability after oral administration ($\text{AUC}_{\text{oral}}/\text{AUC}_{\text{i.v.}} \times 100\%$) is 11.2% for wt and 35.2% for *mdr1a*($-/-$) mice. Macroscopic signs of toxicity were absent in both *mdr1a*($-/-$) and wt mice and thus cannot explain the increased bioavailability. Drug uptake after oral administration appears to be a nonlinear process in both wt and *mdr1a*($-/-$) mice. When the dose was reduced by 50% to 5 mg/kg (body weight), the plasma AUC was about 4-fold lower [namely, 0.88 ± 0.04 mg/(l·hr)] in the *mdr1a*($-/-$) mice. In wt mice the plasma AUC could not be calculated after oral administration of 5 mg/kg because the plasma levels hardly exceeded the lower limit of quantitation (25 ng/ml) of the HPLC assay.

In mice, excretion of paclitaxel via the feces is an important route of drug elimination (26). To test whether the pattern of drug excretion had been altered in *mdr1a*($-/-$) mice, we studied the cumulative excretion in feces and urine over a period of 96 hr after i.v. administration of [3 H]paclitaxel (Table 2), analyzing both total radioactivity and unchanged drug. In wt mice, $\approx 90\%$ of the radioactivity was recovered in the feces, much of it as unchanged drug (40%) or as hydroxy-

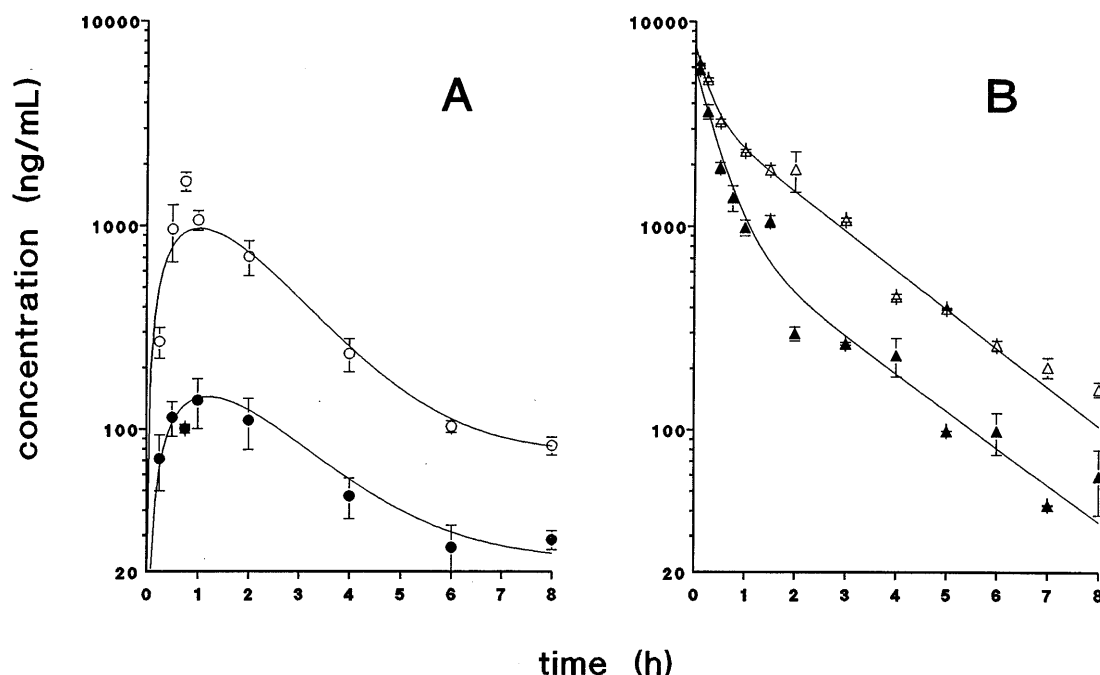


FIG. 1. Plasma concentration-time curves of paclitaxel in female FVB wt (●/▲) and *mdr1a*(-/-) (○/△) mice after oral (A) and i.v. (B) administration of paclitaxel (10 mg/kg). Data are shown as the mean concentration, and error bars represent the SEM ($n = 2-10$ per time point).

lated metabolites (15%). The remaining fraction of the administered radioactivity left the body via the feces or the urine as unknown breakdown products. In *mdr1a*(-/-) mice receiving paclitaxel by i.v. injection, the fecal excretion of unaltered drug was markedly reduced to only 1.5% of the dose. In contrast, however, the fecal excretion of the hydroxylated metabolites was increased. In this group of animals a major part of the radioactivity ($\approx 45\%$) was excreted in the urine, but as in wt animals, only a very minor fraction of this was unchanged drug or one of the known hydroxylated metabolites.

In line with the higher bioavailability in *mdr1a*(-/-) mice, a large difference in fecal excretion was also observed after oral administration of paclitaxel. In *mdr1a*(-/-) mice, only 2% of the orally delivered dose was recovered in the feces as unchanged drug, whereas in wt mice almost 90% was excreted unchanged. In *mdr1a*(-/-) mice, the amounts of hydroxylated metabolites recovered in the feces were not dependent on the route of administration (oral or i.v.). In wt mice, however, fecal excretion of hydroxylated metabolites after oral administration of paclitaxel was about 3-fold lower than after i.v. administration (Table 2).

To test whether diminished biliary excretion or increased (re-)uptake from the intestinal lumen was responsible for the reduced fecal excretion of paclitaxel in the *mdr1a*(-/-) mice, we analyzed the excretion of drug in mice in which the common bile duct was ligated and the bile was collected via a cannula

located in the gall bladder (Table 3). After i.v. administration of [^3H]paclitaxel (10 mg/kg body weight), about one-quarter of the recovered radioactivity was unchanged drug. Furthermore, the biliary excretion of unchanged drug and of hydroxylated metabolites was not significantly different in *mdr1a*(-/-) and wt mice ($P = 0.13$). These findings suggest that the higher bioavailability in the *mdr1a*(-/-) mice results from increased (re-)uptake in the intestine. However, the anaesthesia and surgical procedures in combination with the administered dose of 10 mg/kg influenced the condition of the animals. They developed respiratory and cardiac problems (transient bradycardia) immediately after injection of the drug solution. The paclitaxel plasma levels observed in these mice were >2 -fold higher than those observed in "intact" wt animals (Fig. 1). We therefore repeated the same experiment with animals receiving half the dose of paclitaxel (namely, 5 mg/kg). Major toxicities were no longer observed in this set of animals. The plasma levels found in these animals were similar to those found in intact animals given the same dose. At this lower dose level the biliary excretion of unchanged paclitaxel was also similar in wt and *mdr1a*(-/-) mice.

A remarkable effect was noticed in wt mice with a cannulated gall bladder. Although the excretion of bile into the intestinal lumen is blocked in these mice, $\approx 11\%$ of the dose of 10 mg/kg was recovered as unchanged drug in the intestinal lumen within 90 min after i.v. drug administration. In *mdr1a*(-/-) mice, however, this direct excretion into the

Table 1. Plasma pharmacokinetic parameters after i.v. or oral administration of paclitaxel at 10 mg/kg to mice

	i.v. administration		Oral administration	
	Wild type	<i>mdr1a</i> (-/-)	Wild type	<i>mdr1a</i> (-/-)
AUC ₍₀₋₈₎ , mg/(l·hr)	4.45 ± 0.15	9.10 ± 0.33*	0.50 ± 0.05	3.20 ± 0.25*
Cl, l/hr/kg	2.24 ± 0.08	1.10 ± 0.04*	—	—
$t_{1/2}$, hr	1.66 ± 0.31	1.71 ± 0.08	—	—
C _{max} , mg/l	5.84 ± 0.24	6.23 ± 0.02	0.14 ± 0.04	1.6 ± 0.17*
F, %	—	—	11.2	35.2

AUC₍₀₋₈₎, area under the plasma concentration-time curve up to 8 hr; C_{max}, maximum plasma levels; Cl, clearance; F, oral bioavailability.

* $P < 0.05$.

Table 2. Amounts of paclitaxel and metabolites excreted by intact mice within 96 hr after i.v. or oral administration of 10 mg/kg (body weight) of [³H]paclitaxel

Biological matrix	Compound	i.v. administration		Oral administration	
		Wild type	<i>mdr1a</i> (-/-)	Wild type	<i>mdr1a</i> (-/-)
Feces	Paclitaxel	39.8 ± 2.6	1.5 ± 0.2*	87.1 ± 8.8	2.2 ± 0.30*
	3'- <i>p</i> -hydroxy paclitaxel	8.6 ± 0.5	16.1 ± 0.8	2.4 ± 0.2	17.6 ± 1.9*
	6 α -hydroxy paclitaxel	6.3 ± 0.4	13.8 ± 0.4	1.9 ± 0.5	11.3 ± 1.2*
	³ H label	90.4 ± 5.5	34.3 ± 1.2*	NA	NA
Urine	Paclitaxel	0.97 ± 0.10	0.78 ± 0.11	0.09 ± 0.03	0.07 ± 0.03
	3'- <i>p</i> -hydroxy paclitaxel	<0.01	<0.01	ND	ND
	6 α -hydroxy paclitaxel	<0.01	<0.01	ND	ND
	³ H label	15.9 ± 1.7	44.6 ± 4.7*	NA	NA

Excretion is given as percentage of the dose (mean ± SEM; *n* = 4–6) NA, Not available; ND, not detectable.
**P* < 0.05.

intestinal lumen was <3% of the dose (Table 3). At the 5 mg/kg dose level the direct intestinal excretion in wt mice was reduced to 3.5% of the dose, but was still significantly higher than in *mdr1a*(-/-) mice (*P* = 0.02).

DISCUSSION

Our results show that the disruption of the *mdr1a* P-glycoprotein gene in mice results in a drastic alteration in the pharmacokinetics of paclitaxel, a cytotoxic drug increasingly used in the treatment of cancer patients. P-glycoprotein at the luminal side of the intestinal epithelium appears to be an important component of the defense against substrate drugs such as paclitaxel. The disruption of this defense leads to a substantial increase in bioavailability after oral administration (35.2 vs. 11.2%) in the *mdr1a*(-/-) mice. The ability to transfer paclitaxel from the circulation into the gut is greatly reduced, establishing a major role for P-glycoprotein in this process. The overall result is a 6-fold increase in the area under the paclitaxel plasma AUC in the *mdr1a*(-/-) mice after oral administration, and a 2-fold increase after i.v. administration. Clinical trials are underway in which amphipathic drugs, such as paclitaxel, are combined with inhibitors of P-glycoprotein. These combinations have led to diminished drug clearances (27–29) similar to our observations in *mdr1a*(-/-) mice.

The murine pharmacokinetics of paclitaxel have been studied extensively by us using sensitive and selective HPLC methods (24, 26, 30). Because the normal pharmaceutical formulation contains Cremophor EL, which causes nonlinear pharmacokinetic behavior of paclitaxel (30), we have used an i.v. formulation that does not contain this additive. For experiments with animals receiving paclitaxel orally, we have used the commercially available formulation of paclitaxel in Cremophor EL: ethanol (1:1, vol/vol; Taxol). Nonlinear pharmacokinetic behavior of paclitaxel after oral administration due

to Cremophor EL is not likely to occur because the levels of Cremophor EL in plasma remain very low [<0.1% (vol/vol)]. Furthermore, our recent experiments with different amounts of Cremophor EL demonstrated that the oral bioavailability was unaffected (results not shown).

The *mdr1a*(-/-) mice are particularly useful for studying the role of P-glycoprotein in the intestine, because the *mdr1a* gene is the only murine P-glycoprotein expressed in this tissue (10). The biliary output of paclitaxel after i.v. administration was not reduced in *mdr1a*(-/-) mice, which may seem unexpected because the *mdr1a* gene is expressed in the liver. Although the *mdr1b* gene is expressed in the liver as well, and Schinkel *et al.* (10) have shown that the *mdr1b* expression is elevated in the *mdr1a*(-/-) mice, recent experiments in mice with both disrupted *mdr1a* and *mdr1b* genes showed an unaltered biliary excretion too (unpublished results). This result implies that alternative transport systems for paclitaxel are present in the liver.

Because biliary excretion is not different in *mdr1a*(-/-) and (+/+) mice, the low bioavailability in wt mice should result from the presence of P-glycoprotein in the intestine. Our finding that the amount of unchanged drug recovered in the feces of *mdr1a*(-/-) mice after oral administration is very low and similar to that after i.v. administration, whereas in wt mice almost the complete dose of orally administered paclitaxel is recovered in the feces, indicates that (re-)absorption of drug from the intestinal lumen is very efficient if not prevented by P-glycoprotein. The fact that the bioavailability of paclitaxel does not equal 100%, in spite of the almost complete (re-)absorption from the intestinal lumen, is probably due to first-pass hepatic extraction and/or metabolism in the liver and intestinal mucosa. The nonlinear increase of the plasma AUC at higher dose levels may be due to saturation of the enzymes involved.

Whereas in wt mice a substantial fraction of the dose (40%) is excreted as unchanged drug in the feces, the elimination of

Table 3. Amounts of paclitaxel and metabolites excreted by mice with cannulated gall bladder within 90 min after i.v. administration of [³H]paclitaxel

Sample	Compound	Dose, 10 mg/kg		Dose, 5 mg/kg	
		Wild type	<i>mdr1a</i> (-/-)	Wild type	<i>mdr1a</i> (-/-)
Plasma (level, ng/ml)	Paclitaxel	2020 ± 420	2370 ± 280	289 ± 38	327 ± 44
Bile	Paclitaxel	4.81 ± 0.27	2.56 ± 0.58	5.94 ± 1.70	4.10 ± 0.54
	3'- <i>p</i> -hydroxy paclitaxel	2.86 ± 0.28	2.48 ± 0.26	4.74 ± 0.69	4.10 ± 0.18
	6 α -hydroxy paclitaxel	2.00 ± 0.25	1.29 ± 0.24	3.25 ± 0.51	1.98 ± 0.22
	³ H label	17.9 ± 0.69	15.0 ± 1.5	25.7 ± 4.5	26.6 ± 2.90
Intestinal contents	Paclitaxel	10.77 ± 0.25	2.49 ± 0.86*	3.53 ± 0.38	0.98 ± 0.28*
	3- <i>p</i> -hydroxy paclitaxel†				
	6 α -hydroxy paclitaxel	0.37 ± 0.10	0.37 ± 0.16	0.16 ± 0.05	<0.1
	³ H label	12.22 ± 0.28	3.79 ± 1.11*	4.63 ± 0.49	1.52 ± 0.05

Excretion is given as percentage of the dose (mean ± SEM; *n* = 4). ND, Not detectable.
**P* < 0.05.

†This compound could not be accurately quantified in the intestinal contents due to the presence of an interfering substance.

paclitaxel in *mdr1a*($-/-$) mice occurs almost exclusively through metabolic breakdown. In wt mice, 3'-*p*-hydroxypaclitaxel and 6 α -hydroxypaclitaxel, which are probably formed in the liver by the cytochrome P450 enzymes (31, 32), are the two principal metabolites identified thus far. Because these compounds could not be detected in plasma or tissues except for small quantities in the liver and the intestines, it is very likely that they are efficiently excreted via the bile into the gut, and that reabsorption is low (26). In *mdr1a*($-/-$) mice the excretion of these known metabolites increased 2-fold. Furthermore, a large amount (45%) of the administered radioactivity was recovered in the urine of these mice. The identity of this metabolic breakdown product (or products) is unknown. Because the ^3H label is mainly located (>95%) in the aromatic side chains of the molecule, it is not certain whether this urinary excretion product contains the basic taxane skeleton, or whether this part of the molecule is still excreted into the feces.

A remarkable finding is the substantial fraction of the dose recovered in the intestinal contents of wt animals with cannulated gall bladder receiving paclitaxel by i.v. administration. This fraction must be excreted directly through the mucosal cells into the intestinal lumen. Excretion is drastically reduced in *mdr1a*($-/-$) mice, implicating P-glycoprotein as an essential component in this excretion process. The reduced excretion at the lower dose level suggests that this P-glycoprotein-mediated transport occurs only when the plasma level is sufficiently high. The basis of this threshold effect is unknown. Our findings clearly show, however, that direct excretion of compounds through the gut wall can be an important pathway of drug elimination, as also indicated by earlier observations (33–35).

We expect that the results presented here for paclitaxel are representative for other amphipathic drugs transported by P-glycoprotein. This is supported by our recent demonstration that the predominantly fecal excretion of the cardiac glycoside digoxin in wt mice shifted to predominantly urinary excretion in *mdr1a*($-/-$) mice. Moreover, after interruption of biliary excretion, we found substantial excretion of digoxin into the gut of wt mice, but not in *mdr1a*($-/-$) mice (36).

In conclusion, P-glycoprotein in the epithelium of the gut limits the bioavailability of orally administered paclitaxel, and it is likely that a similar protection is afforded to many other orally ingested toxic substances transported by P-glycoprotein. Intestinal P-glycoprotein also contributes to the elimination of parenterally administered substrate drugs by a direct secretion of drug into the intestinal lumen (37). These findings provide a rationale for attempts to improve the low and variable oral bioavailability of paclitaxel and other substrate drugs (e.g., etoposide) by concomitant administration of P-glycoprotein blockers.

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- Danø, K. (1972) *Cancer Chemother. Rep.* **56**, 701–708.
- Riordan, J. R. & Ling, V. (1979) *J. Biol. Chem.* **254**, 12701–12705.
- Ueda, K., Cardarelli, C., Gottesman, M. M. & Pastan, I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3004–3008.
- Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M. M., Pastan, I. & Willingham, M. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7735–7738.
- Cordon-Cardo, C., O'Brien, J. P., Casals, D., Rittman-Grauer, L., Biedler, J. L., Melamed, M. R. & Bertino, J. R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 695–698.
- Fojo, A. T., Ueda, K., Slamon, D. J., Poplack, D. G., Gottesman, M. M. & Pastan, I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 265–269.
- Croop, J., Raymond, M., Haber, D., Devault, A., Arceci, R. J., Gros, P. & Housman, D. (1989) *Mol. Cell. Biol.* **9**, 1346–1350.
- Sugawara, I., Kataoka, I., Morishita, Y., Hamada, H., Tsuruo, T., Itoyama, S. & Mori, S. (1988) *Cancer Res.* **48**, 1926–1929.
- Cordon-Cardo, C., O'Brien, J. P., Boccia, J., Casals, D., Bertino, J. R. & Melamed, M. R. (1990) *J. Histochem. Cytochem.* **38**, 1277–1287.
- Schinkel, A. H., Smit, J. J., Van Tellingen, O., Beijnen, J. H., Wagenaar, E., Van Deemter, L., Mol, C. A., Van der Valk, M. A., Robanus-Maandag, E. C., Te Riele, H. P., Berns, A. J. M. & Borst, P. (1994) *Cell* **77**, 491–502.
- Arceci, R. J., Croop, J. M., Horwitz, S. B. & Housman, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4350–4354.
- Teeter, L. D., Becker, F. F., Chisari, F. V., Li, D. J. & Kuo, M. T. (1990) *Mol. Cell. Biol.* **10**, 5728–5735.
- Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M. M., Pastan, I. & Willingham, M. C. (1989) *J. Histochem. Cytochem.* **37**, 159–164.
- Ames, B. N., Profet, M. & Gold, L. S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7782–7786.
- Hunter, J., Hirst, B. H. & Simmons, N. L. (1993) *Pharm. Res.* **10**, 743–749.
- Leu, B. L. & Huang, J. D. (1995) *Cancer Chemother. Pharmacol.* **35**, 432–436.
- Hsing, S., Gatmaitan, Z. & Arias, I. M. (1992) *Gastroenterology* **102**, 879–885.
- Hunter, J., Hirst, B. H. & Simmons, N. L. (1991) *Br. J. Cancer* **64**, 437–444.
- van Asperen, J., Schinkel, A. H., Beijnen, J. H., Nooijen, W. J., Borst, P. & van Tellingen, O. (1996) *J. Natl. Cancer Inst.* **88**, 994–999.
- McGuire, W. P., Hoskins, W. J., Brady, M. F., Kucera, P. R., Partridge, E. E., Look, K. Y., Clarke-Pearson, D. L. & Davidson, M. (1996) *N. Engl. J. Med.* **334**, 1–6.
- Huizing, M. T., Sewberath Misser, V. H., Pieters, R. C., Ten Bokkel Huinink, W. W., Veenhof, C. H. N., Vermorcken, J. B., Pinedo, H. M. & Beijnen, J. H. (1995) *Cancer Invest.* **13**, 381–404.
- Eiseman, J. L., Eddington, N. D., Leslie, J., MacAuley, C., Sentz, D. L., Zuhowski, M., Kujawa, J. M., Young, D. & Egorin, M. J. (1994) *Cancer Chemother. Pharmacol.* **34**, 465–471.
- Sparreboom, A., Huizing, M. T., Boesen, J. J. B., Nooijen, W. J., Van Tellingen, O. & Beijnen, J. H. (1995) *Cancer Chemother. Pharmacol.* **36**, 299–304.
- Sparreboom, A., van Tellingen, O., Nooijen, W. J. & Beijnen, J. H. (1995) *J. Chromatogr.* **664**, 383–391.
- Sparreboom, A., van Tellingen, O., Huizing, M. T., Nooijen, W. J. & Beijnen, J. H. (1996) *J. Chromatogr.* **681**, 355–362.
- Sparreboom, A., van Tellingen, O., Nooijen, W. J. & Beijnen, J. H. (1996) *Anti-Cancer Drugs* **7**, 78–86.
- Lum, B. L., Kaubisch, S., Yahanda, A. M., Adler, K. M., Jew, L., Ehsan, M. N., Brophy, N. A., Halsey, J., Gosland, M. P. & Sikic, B. I. (1992) *J. Clin. Oncol.* **10**, 1635–1642.
- Bartlett, N. L., Lum, B. L., Fischer, G. A., Brophy, N. A., Ehsan, M. N., Halsey, J. & Sikic, B. I. (1994) *J. Clin. Oncol.* **12**, 835–842.
- Berg, S. L., Tolcher, A., O'Shaughnessy, J. A., Denicoff, A. M., Noone, M., Ognibene, F. P., Cowan, K. H. & Balis, F. M. (1995) *J. Clin. Oncol.* **13**, 2039–2042.
- Sparreboom, A., van Tellingen, O., Nooijen, W. J. & Beijnen, J. H. (1996) *Cancer Res.* **56**, 2112–2115.
- Harris, J. W., Rahman, A., Kim, B.-R., Guengerich, F. P. & Collins, J. M. (1994) *Cancer Res.* **54**, 4026–4035.
- Rahman, A., Korzekwa, K. R., Grogan, J., Gonzalez, F. J. & Harris, J. W. (1994) *Cancer Res.* **54**, 5543–5546.
- Bezek, S., Scasnar, V., Kukan, M. & Trnovec, T. (1990) *Bio-pharm. Drug Dispos.* **11**, 403–409.
- Georg, C. F. & Gruchy, B. S. (1979) *J. Pharm. Pharmacol.* **31**, 643–651.
- Fabre, J., Rudhardt, M., Dayer, P., Trimble, E. R. & Male, P. J. (1984) *Arzneim.-forsch.* **34**, 789–791.
- Mayer, U., Wagenaar, E., Beijnen, J. H., Smit, J. W., Meijer, D. K. F., van Asperen, J., Borst, P. & Schinkel, A. H. (1996) *Br. J. Pharmacol.* **119**, 1038–1044.
- Wacher, V. J., Salphati, L. & Benet, L. Z. (1996) *Adv. Drug Deliv. Rev.* **20**, 99–112.