Low Systemic Exposure of Oral Docetaxel in Mice Resulting from Extensive First-Pass Metabolism Is Boosted by Ritonavir[1]

Heleen A. Bardelmeijer, Mariët Ouwehand, Tessa Buckle, Maarten T. Huisman, Jan H. M. Schellens, Jos H. Beijnen, and Olaf van Tellingen[2]

The Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, 1066 CX Amsterdam [H. A. B., M. O., T. B., M. T. H., J. H. M. S., J. H. B., O. V. T.]; Department of Pharmacy and Pharmacology, The Netherlands Cancer Institute/Slotervaart Hospital, 1066 EC Amsterdam [J. H. B.]; and Division of Drug Toxicology, Faculty of Pharmaceutical Sciences, Utrecht University, 3584 CA Utrecht [J. H. M. S., J. H. B.], the Netherlands

ABSTRACT

P-glycoprotein seems to be the most important factor limiting the oral absorption of paclitaxel. We have now explored the mechanisms responsible for the low oral bioavailability of docetaxel, a structurally related taxane drug. The recovery of 33% of oxidative metabolites and only 39% of unchanged drug in the feces of FVB wild-type mice receiving 10 mg/kg of oral docetaxel indicates that the major part of the oral dose has been absorbed. The feces and bile of mice receiving 10 mg/kg of i.v. docetaxel contained large amounts of metabolites and only minor quantities of unchanged drug, highlighting the importance of metabolism as an elimination route for this drug. In wild-type and P-glycoprotein knockout mice, dose escalation of p.o. administered docetaxel from 10 to 30 mg/kg resulted in a more than proportional increase in plasma levels, which suggested saturation of first-pass metabolism. Moreover, coadministration of 12.5 mg/kg of the HIV protease inhibitor ritonavir, also a strong inhibitor of cytochrome P4503A4 with only minor P-glycoprotein inhibiting properties, increased the plasma levels after oral docetaxel by 50-fold. *In vitro* transport studies across monolayers of LLC-PK1 cells (parental and transduced with *MDR1* or *Mdr1a*) suggested that docetaxel is a weaker substrate for P-glycoprotein than paclitaxel is. In conclusion, docetaxel is well absorbed from the gut lumen in mice despite the presence of P-glycoprotein in the gut wall. Subsequent first-pass extraction is the most important factor determining its low bioavailability. The inhibition of docetaxel metabolism by ritonavir provides an interesting strategy to improve the systemic exposure of oral docetaxel.

INTRODUCTION

Cytotoxic drugs are administered to patients by mostly i.v. infusion. However, there are several arguments in favor of exploring the oral route for the administration of these drugs, including patients' preference for oral treatment, facilitated repeated dosing for chronic treatment regimens, and pharmacoeconomic advantages. Frequently, however, the oral route cannot be used because of a low and variable oral bioavailability. Next to pharmaceutical properties of a drug (*e.g.*, dissolution rate, stability within the intestinal tract), both limited passage through the intestinal wall and limited first-pass metabolism are important factors in reducing the oral bioavailability of drugs (1). We have previously shown that P-gp,[3] a cell membrane localized transport protein that was initially discovered to confer multidrug resistance in mammalian cancer cells (2), limits the absorption of p.o. administered paclitaxel (3). This study using mice carrying a homozygous disruption of the *Mdr1a* gene [*Mdr1a* (−/−) mice], also demonstrated that the plasma levels of paclitaxel were increased by 6-fold

relative to wild-type mice. Moreover, almost 90% of the oral dose was recovered unchanged in the feces of wild-type mice, whereas this was less than 3% in *Mdr1a* (−/−) mice, which showed that P-gp in the gastrointestinal tract forms an efficient barrier against paclitaxel absorption (3). On the basis of these findings, we have tested the usefulness of compounds that inhibit P-gp function to increase the systemic exposure of p.o. administered paclitaxel in mice and patients. Concomitant administration of the P-gp inhibitors cyclosporin A, SDZ PSC 833, and GF120918 to mice resulted in 7- to 10-fold higher paclitaxel plasma levels (4–6). In patients, the combination of 60 mg/m² of oral paclitaxel and 15 mg/kg of oral cyclosporin A resulted in nearly 7-fold higher plasma levels compared with patients who received paclitaxel alone, thereby reaching potentially therapeutic levels (7, 8). These results with paclitaxel encouraged us to perform (pre)clinical studies investigating the usefulness of cyclosporin A to increase the systemic exposure of p.o. administered docetaxel. Docetaxel is a semisynthetic taxane analogue that is prepared from 10-deacetyl baccatin III, a noncytotoxic precursor isolated from the needles of the European yew tree (*Taxus baccata* L.). It is currently available as a formulation for i.v. infusion (Taxotere) and has shown activity against lung, head and neck, breast, and ovarian cancer (9). Like paclitaxel, docetaxel is a potent inhibitor of cell replication by blocking the cells in the late G_2-M phase of the cell cycle because of inhibition of the microtubule dynamics (10). Thus far, four major metabolites (M-1, M-2, M-3, and M-4; Fig. 1) formed by CYP3A4 have been identified in human feces, which is the major route of elimination of docetaxel and its metabolites (11). We have detected three of these metabolites (M-1, M-2, and M-3) in mouse plasma after i.v. administration of a high dose (33 mg/kg) of docetaxel (12), which indicates that the metabolism of docetaxel in mice and humans occurs through comparable pathways. Docetaxel is also a substrate of P-gp, as was first shown in 1994 by Wils *et al.* (13) using Caco-2 cell monolayers and was recently confirmed using parental and *MDR1*-transduced LLC-PK1 cells (14).

Preliminary results showed that the pharmacokinetic handling of paclitaxel and docetaxel appeared to be less similar than anticipated on the basis of their structural similarities. Consequently, we performed a comprehensive pharmacokinetic analysis of docetaxel in mice, with the aim of unraveling the critical biological factors involved in the oral bioavailability of this compound.

MATERIALS AND METHODS

Laboratory Animals

The experiments were performed using female FVB wild-type and *Mdr1a/1b* (−/−) mice (15). All of the mice were between 10 and 17 weeks of age and were housed and handled according to institutional guidelines that comply with Dutch legislation. They were given food (Hope Farms B.V., Woerden, the Netherlands) and acidified water *ad libitum*.

Drugs and Chemicals

Docetaxel (10 mg/ml) formulated in polysorbate 80/ethanol/saline [20:13:67 (v/v/v); Taxotere] and docetaxel, pure compound, originated from Aventis

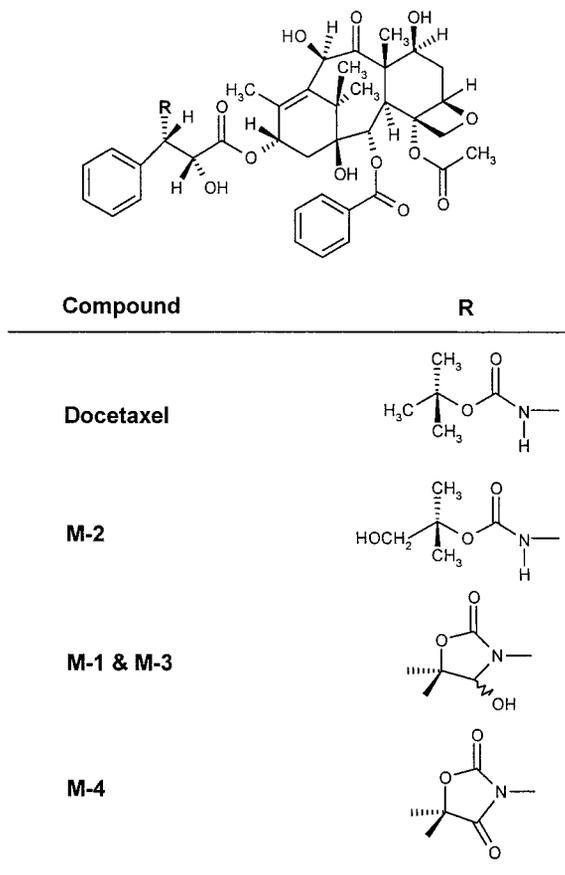| Compound	R
Docetaxel	
M-2	
M-1 & M-3	
M-4	

Fig. 1. Structures of docetaxel and its four known metabolites.

(Anthony Cedex, France). Paclitaxel (Yewtaxan), pure compound, originated from Yew Tree Pharmaceuticals (Haarlem, the Netherlands). [³H]Docetaxel (1.0 mCi/ml in ethanol; specific activity, 7.3 Ci/mmol) and [³H]paclitaxel (1.0 mCi/ml in ethanol; specific activity. 6.5 Ci/mmol) were from Moravek Biochemicals Inc. (Brea, CA). Inuline [¹⁴C]carboxylic acid, pure compound, (specific activity, 5.8 mCi/mmol) was from Amersham (Little Chalfont, United Kingdom). Cyclosporin A (50 mg/ml) in cremophor EL/ethanol [67:33 (v/v); Sandimmune] was obtained from Novartis Pharma AG (Bazel, Switzerland) and ritonavir (80 mg/ml, Norvir oral solution) was from Abbott (Abbott Park, IL). The P-gp inhibitor LY335979, pure compound, was a gift of Eli Lilly and Company (Indianapolis, IN). Polysorbate 80 (Tween 80) and polyoxyethyleneglycerol triricinoleate 35 (cremophor EL) were from Sigma Chemical Co (St. Louis, MO) and propyleneglycol was from Bufa B.V. (Uitgeest, the Netherlands). Lyophilized BSA (fraction V) was obtained from Roche Diagnostics GmbH (Mannheim, Germany). Saline was purchased from Braun (Emmenbrücke, Germany). Hypnorm was from Janssen Pharmaceuticals (Beerse, Belgium), Dormicum from Roche Nederland (Mijdrecht, the Netherlands), and methoxyflurane (Methofane) originated from Medical Developments Australia Pty Ltd (Springvale, Victoria, Australia). All other chemicals originated from E. Merck (Darmstad, Germany) and were of analytical or Lichrosolv gradient grade. Water obtained by the Milli-Q Plus system (Millipore, Milford, MA) was used throughout the experiments. Blank human plasma originated from the Blood bank (Amsterdam, the Netherlands).

Plasma Pharmacokinetic Studies

Combination with Cyclosporin A. Wild-type mice and *Mdr1a/1b* (−/−) mice were given docetaxel (10 mg/kg) p.o. by gavage or i.v. by injection in the lateral tail vein with either p.o. administered cyclosporin A (50 mg/kg) or cyclosporin A vehicle solution [cremophor EL/ethanol/saline, 6.7:3.3:90 (v/v/v)]. Cyclosporin A was administered 30 min in advance when docetaxel was administered i.v., or at the same time when docetaxel was given p.o. Blood samples were obtained by cardiac puncture under methoxyflurane anesthesia and were collected in tubes containing EDTA as anticoagulant. At least five

and three animals per time point were used for cohorts receiving oral and i.v. docetaxel, respectively.

Combination with Ritonavir. Wild-type mice and *Mdr1a/1b* (−/−) mice (*n* = 6 per group) with a cannulated jugular vein (see cannulation of the Jugular Vein) received docetaxel (10 mg/kg) p.o. 30 min after oral ritonavir (12.5 mg/kg) or ritonavir vehicle solution [ethanol/cremophor EL/propylene glycol/water, 43:9.8:25:22.2 (v/v/v/v)].

Dose-escalation. Wild-type mice and *Mdr1a/1b* (−/−) mice (*n* = 6 per group) with a cannulated jugular vein received oral docetaxel at dose levels of 10, 20, and 30 mg/kg.

Blood samples of 120 μl were taken from cannulated animals at serial time-points, and this volume was replaced with drug-free heparinized mouse blood obtained from donor mice.

Plasma was separated by centrifugation at 4°C for 5 min at 3000 × *g* and stored at −20°C until analysis.

Mass Balance Studies

Combination with Cyclosporin A. Wild-type and *Mdr1a/1b* (−/−) mice (*n* = 4 per group) were housed in Ruco type M/1 metabolic cages (Valkenswaard, the Netherlands) with free access to food and water. After an acclimatization period of 2 days, docetaxel (10 mg/kg) either as single agent or with cyclosporin A (50 mg/kg) was administered p.o. Cyclosporin A was administered at the same time as docetaxel. Feces and urine were collected for up to 96 h.

Combination with Ritonavir. The influence of ritonavir (12.5 mg/kg) was studied in a similar experiment; however, ritonavir was administered 30 min in advance of docetaxel.

i.v. Docetaxel. Docetaxel (10 mg/kg) was administered i.v. as single agent to four wild-type and four *Mdr1a/1b* (−/−) mice.

Feces samples were homogenized at 4°C in 10 volumes of 40 g/liter BSA in water using a Polytron PT1200 homogenizer (Kinematika AG, Littau, Switzerland). Urine samples were diluted 5-fold with drug-free human plasma. All of the samples were stored at −20°C until analysis.

Intraluminal Stability

The contents of the stomach, small intestine, and cecum of two wild-type mice were collected in separate vials. To each vial was added: 200 μl of saline, 1 μg of docetaxel per 100 mg of intestinal contents, and either 20 μl of saline or 20 μl of a 1-mg/ml solution of NADPH in saline. After vortex mixing, the vials were incubated at 37°C. Samples were taken at 0, 15, and 70 min and stored at −20°C until analysis.

Intraluminal Kinetics

Wild-type mice received docetaxel (10 mg/kg) p.o. Gastrointestinal tissues (stomach, small intestine cut into three pieces of equal length, cecum and colon) including their contents were collected from mice killed at 0.25, 0.5, 1, and 3 h after administration. Liver and gall bladder including the bile were also collected. All of the samples were homogenized at 4°C in 2 ml of 40 g/liter BSA in water as described above and stored at −20°C until analysis.

Biliary and Intestinal Secretion

The gall bladders of groups of four wild-type and *Mdr1a/1b* (−/−) mice were cannulated as described earlier (3). Docetaxel (10 mg/kg) was administered by i.v. injection. Bile was collected up to 90 min after drug administration. Next, the contents of the small intestine, cecum, and colon were collected. Bile samples were diluted with 1 ml of drug-free human plasma. The intestinal contents were homogenized in 2 ml of 4% BSA as described above. All of the samples were stored at −20°C until analysis.

In Vitro Transport Assays

Pig-kidney LLC-PK1 cells and subclones transduced with human *MDR1* (LLC-*MDR1* cells) or mouse *Mdr1a* (LLC-*Mdr1a* cells; Ref. 16) were seeded on Transwell microporous (3.0 μm) polycarbonate membrane filters (Corning, NY) at a density of 1 × 10⁶ cells per well and grown for 3 days in 2 ml of complete M199 medium (Life Technologies, Inc., Breda, the Netherlands). Transport experiments were performed in OptiMEM medium containing 11.7

6159

μM [³H]docetaxel or [³H]paclitaxel (both, ~5 kBq per well) at the donor compartment. In some experiments, the P-gp inhibitor LY335979 (17) was added at 0.5 or 5 μM to the apical compartment immediately before docetaxel or paclitaxel, whereas ritonavir was used at concentrations of 0 and 100 μM. Cells were incubated at 37°C in 5% CO_2, and samples of 50 μl were taken every hour, up to 4 h after the start of the experiment. The radioactivity in the samples was counted after mixing with 4 ml of UltimaGold scintillation cocktail (Packard, Meriden, CT) using model Tricarb β-scintillation counter (Packard). Inuline [¹⁴C]carboxylic acid (~1 kBq per well) was added to the apical compartment to check the integrity of the cell layer. Wells demonstrating a leakage of more than 1.5%/h were excluded from the analyses.

Cannulation of the Jugular Vein

The technique of cannulation of the jugular vein has been developed to allow serial blood sampling from an animal. Details will be described elsewhere.[4] In short, after anesthesia with 7 ml/kg Hypnorm/Dormicum/water [1:1:2 (v/v/v)], an incision was made in the skin just above the right clavicle. After preparation of the jugular vein, it was ligated at the distal side. A 2 French silicone cannula (Access Technologies, Skokie, IL) filled with heparin solution was inserted until the tip reached into the right atrium. The cannula was fixed to the vessel, tunneled to the head of the animal, fixed to the skin between the ears and further protected by a metal spring surrounded by polyvinyl chloride tubing. After recovery, the animal was placed in a specially designed cage that allowed free movement of the animal. The experiment was started after a recovery period of 2 days after the operation.

Analytical Methods

Docetaxel and metabolites in biological samples (plasma, feces, urine, tissue, and intestinal contents homogenates) were determined by HPLC, according to a previously developed assay (18).

To allow the quantification of docetaxel in the small plasma samples obtained from cannulated animals, we have developed a HPLC method with a lower limit of detection of 50 ng/ml that requires only 50 μl of sample volumes. Details of this method will be presented elsewhere.[5] In short, the assay utilizes liquid-liquid extraction of the plasma samples with *tert*-butyl-methyl ether. Next, docetaxel and the internal standard cephalomannine were separated by reversed-phase HPLC using a narrow-bore stainless steel analytical column (150 × 2.1 mm) packed with Symmetry C18 material. The mobile phase was composed of acetonitril/methanol/0.01 M ammonium acetate buffer [pH 5.0; 30:25:43 (v/v/v)] and was delivered at a flow rate of 0.2 ml/min. Peak detection was performed using a UV detector operating at a wavelength of 231 nm.

Pharmacokinetic Analysis

The AUC, the oral bioavailability and their respective SE values were calculated by noncompartmental methods as described previously (5, 6). The oral bioavailability is quoted "apparent" under the circumstance that oral docetaxel was given together with a modifier of drug uptake or inhibitor of drug metabolism, which may result in a change in the clearance of docetaxel from the body. To determine the "true" bioavailability, the same combination should be investigated with i.v. docetaxel.

Statistical Analysis

The unpaired Student *t* test (two-tailed) was used to compare the pharmacokinetic parameters. A $P < 0.05$ was considered statistically significant.

RESULTS

To support the initiatives for clinical studies with oral docetaxel in the Netherlands Cancer Institute, we performed a preclinical study to

---

[4] H. A. Bardelmeijer, T. Buckle, M. Ouwehand, J. H. Beijnen, J. H. M. Schellens, and O. van Tellingen. Chronic cannulation of the jugular vein in mice as a method for serial withdrawal of blood samples, submitted for publication.

[5] H. A. Bardelmeijer, M. Ouwehand, J. H. Beijnen, J. H. M. Schellens, and O. van Tellingen. A reversed-phase high-performance liquid chromatography method for determination of docetaxel in small volumes of murine plasma, submitted for publication.
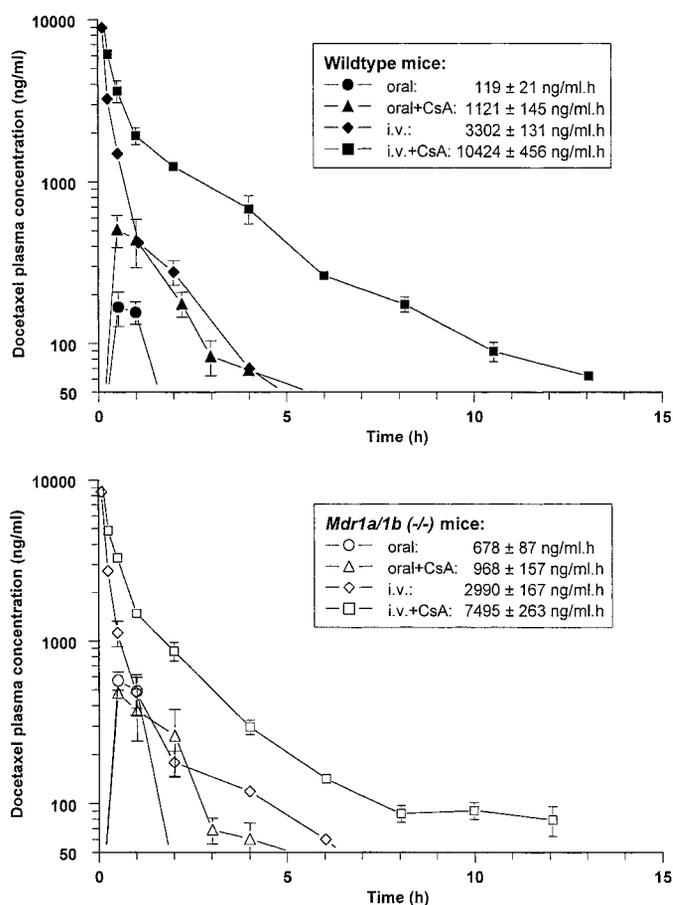

Fig. 2. Impact of oral cyclosporin A on the plasma concentration-time curves of docetaxel. A dose of 10 mg/kg docetaxel was administered i.v. as single agent (♦ and ◇) or in combination with 50 mg/kg p.o. administered cyclosporin A (■ and □); or docetaxel was given p.o. as a single agent (● and ○) or together with 50 mg/kg p.o. administered cyclosporin A (▲ and △). Data are expressed as means ± SE (*n* = 3–10 animals per time point). *Insets*, the calculated AUC values of each curve (means ± SE). *CsA*, cyclosporin A.

evaluate the effects of concomitant oral cyclosporin A on the bioavailability of docetaxel in wild-type and *Mdr1a/1b* (−/−) mice (Fig. 2). The bioavailability of docetaxel was increased by 6-fold from 3.6% in wild-type mice to 22.7% in *Mdr1a/1b* (−/−) mice ($P < 0.001$). There was no difference in the AUC of docetaxel in wild-type and *Mdr1a/1b* (−/−) mice after i.v. administration of the drug as a single agent (Fig. 2). In wild-type mice, the AUC of oral docetaxel was increased 9-fold from 119 ± 21 ng/ml·h to 1121 ± 145 ng/ml·h by coadministration of cyclosporin A ($P < 0.001$). Calculated relative to the AUC of docetaxel achieved after i.v. administration of this drug given without cyclosporin A, the apparent oral bioavailability of docetaxel plus concomitant cyclosporin A was 33.9%. However, coadministration of cyclosporin A also increased the AUC of i.v. administered docetaxel by 3-fold, both in wild-type and in *Mdr1a/1b* (−/−) mice (Fig. 2).

We next performed a mass-balance study in wild-type and *Mdr1a/1b* (−/−) mice to get additional insight into the disposition of p.o. administered docetaxel with and without cyclosporin A. Hardly any docetaxel was recovered in the feces of *Mdr1a/1b* (−/−) mice or wild-type mice receiving concomitant cyclosporin A (Table 1). However, in wild-type mice receiving docetaxel as single agent, only 40% of the dose was recovered in the feces as unchanged drug and mainly within the first 8 h after dosing. Moreover, about 30% of the dose was recovered as oxidative metabolites. This result suggests that a substantial uptake of docetaxel from the gastrointestinal tract occurs in

Table 1 *Fecal excretion of unchanged drug and metabolites after oral administration of docetaxel (10 mg/kg) with or without concomitant oral cyclosporin A (50 mg/kg) to wild-type and Mdr1a/1b (−/−) mice*

Data are expressed as percentage of administered dose ± SE ($n = 4$).

Mouse strain	Cyclosporin A	Docetaxel	Metabolites
Wild-type	−	38.6 ± 2.0	32.7 ± 2.8
Wild-type	+	5.4 ± 1.3	37.3 ± 2.0
Mdr1a/1b (−/−)	−	1.4 ± 0.6	45.2 ± 2.9
Mdr1a/1b (−/−)	+	0.1 ± 0.1	25.8 ± 4.6

wild-type mice, although the very low plasma levels observed after oral administration of docetaxel to wild-type mice do not support this assumption. Because an alternative explanation could be oxidative degradation of the drug within the gastrointestinal lumen, we studied the intraluminal kinetics of docetaxel in more detail. Docetaxel was administered p.o. to groups of wild-type mice, which were sacrificed after 0.25, 0.5, 1, and 3 h. Intestinal tissues (including their contents), the liver and gallbladder (with bile) were collected and analyzed for docetaxel and metabolites (Fig. 3). Within 1 h, ~90% of the dose of docetaxel had already disappeared from the stomach into the small intestine. At this time, only a very small quantity (~2%) had reached the colon, which suggested that excretion in the feces within 1 h after administration is negligible. Within 3 h after administration, hardly any drug was present in the small intestines, thus showing a very rapid disappearance from the major site in which drug uptake usually takes place. Given the rapid traversal of docetaxel through the gut, excretion of part of the dose will have occurred at this time point. Oxidative metabolites were present throughout the intestines. However, the finding that the ratio of the metabolites, relative to unchanged docetaxel, remains more or less constant over time throughout the intestines does not support the model of intraintestinal degradation. Moreover, the results from the *in vitro* experiment showed no degradation of docetaxel in the intestinal contents, either in the absence or in the presence of NADPH. Consequently, a more likely explanation for our observations is a rapid absorption from the intestines followed by extensive first-pass metabolism and effective hepatobiliary clearance. This is also supported by the finding that the liver contained 5.0% of the dose as docetaxel and 4.3% of the dose as metabolites at 1 h after drug administration.

Further evidence that metabolism plays an important role in the elimination of this drug from the body comes from *in vivo* experiments with i.v. administered docetaxel. In animals with a cannulated bile duct, the fraction of the dose that was recovered as metabolites in bile was 27.0% in wild-type mice and almost 50% in *Mdr1a/1b* (−/−) mice, whereas only 3–4% of unchanged drug was recovered in both of the mouse strains. The results of a mass balance study showed that, in both wild-type and *Mdr1a/1b* (−/−) mice, only a very small fraction of the dose was recovered in the feces as unchanged docetaxel (4.6 and 0%, respectively), whereas relatively large amounts of known metabolites were recovered in both of the mouse strains (42.6 and 25.6%, respectively).

The *in vitro* transport characteristics of docetaxel and paclitaxel were compared using monolayers of LLC-*MDR1* and LLC-*Mdr1a* cells. The difference between transport in the basolateral-to-apical and apical-to-basolateral direction (sometimes used to indicated active transport) was smaller for docetaxel than for paclitaxel (Fig. 4). An accurate measurement of the K_m was not feasible because of the relatively low aqueous solubility of paclitaxel and docetaxel. The inhibition of docetaxel transport over the LLC-*MDR1* cell line was achieved using 0.5 μM LY335979, whereas a concentration of 5 μM was needed to inhibit transport of paclitaxel. The inhibiting properties of LY335979 were slightly lower in the LLC-*Mdr1a* cell line; however, when 5 μM was enough to inhibit docetaxel transport, it was not sufficient to inhibit paclitaxel transport completely. When the active component of transport of docetaxel and paclitaxel is fully inhibited, the residual apical-to-basolateral diffusion of both compounds appears to be about equal, which indicates no major differences in their membrane permeability. The potential participation of another transporter besides P-gp was excluded by the finding that vectorial transport of both drugs over the parental LLC-PK1 monolayers (by endogenous porcine P-gp (16), was fully inhibited at 0.5 μM LY335979.

To further investigate the linearity of uptake after oral administration of docetaxel, we performed a dose-escalation study in wild-type and *Mdr1a/1b* (−/−) mice. An increase of the oral dose from 10 to 20 and 30 mg/kg in wild-type mice resulted in AUCs of 161 ± 49, 916 ± 127, and 2539 ± 279 ng/ml·h and, in *Mdr1a/1b* (−/−) mice, resulted in AUCs of 574 ± 112, 1625 ± 214, and 3705 ± 441 ng/ml·h, respectively, thus showing a more than proportional increase. This can be caused by the saturation of uptake and/or metabolism, with the latter probably being the more important factor, given the already substantial uptake at the lowest dose level.

Metabolism of docetaxel in humans occurs mainly via CYP3A4 and, in mice, probably through similar reactions using the CYP3a pathway (19, 20). We have, therefore, tested the effects of ritonavir, a very strong inhibitor of CYP3A4, on the oral bioavailability of docetaxel. Administration of ritonavir results in a major increase in
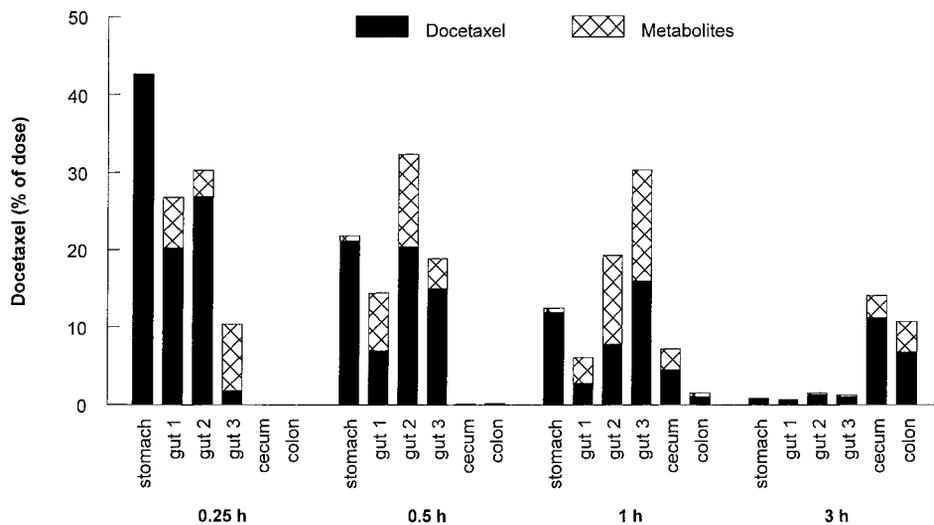

Fig. 3. Intraluminal kinetics of docetaxel. Fractions of docetaxel (*black bars*) and metabolites (*hatched bars*), expressed as percentage of administered dose (mean ± SE; $n = 4$ animals per time point) found in segments of the intestines at 0.25, 0.5, 1, and 3 h after oral administration of 10 mg/kg of docetaxel to wild-type mice.
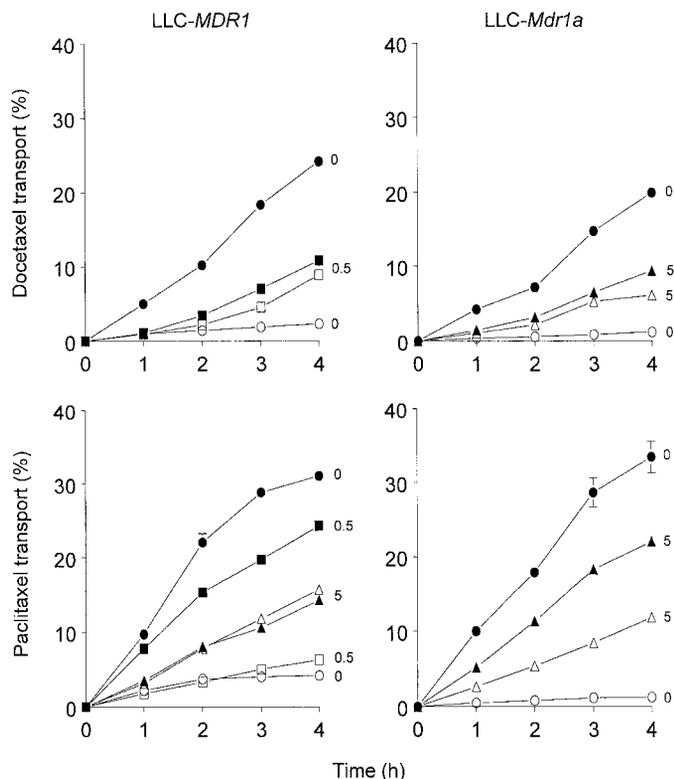
6161

Fig. 4. *In vitro* transport studies. Translocation of 11.8 μM [³H]paclitaxel or [³H]docetaxel, expressed as percentage of the dose added to the donor compartment (mean ± SE, assayed in triplicate) over monolayers of LLC-*MDR1* and LLC-*mdr1a* cells as single agent (● and ○) or in the presence of 0.5 μM (■ and □) or 5 μM of LY335979 (▲ and △). *Closed symbols*, basolateral-to-apical transport; *open symbols*, apical-to-basolateral transport. The concentrations of LY335979 are annotated in the graphs.

administered dose is recovered as unchanged drug in the feces of wild-type mice (3). In line with paclitaxel, the oral bioavailability of docetaxel was very low and coadministration of cyclosporin A increased the AUC of docetaxel by 9-fold. However, after i.v. administration of docetaxel to *Mdr1a/1b* (−/−) mice, there was no difference in its plasma clearance as compared with wild-type mice, whereas the clearance of paclitaxel was reduced by 50% in *Mdr1a/1b* (−/−) mice (3). Moreover, although the bioavailability of docetaxel was 6-fold higher in *Mdr1a/1b* (−/−) mice, it was still only 23%. Interestingly, we recovered only about 40% of the p.o. administered dose in the feces of wild-type mice as unchanged drug, and we also detected about 30% of oxidative metabolites. On the basis of the *in vitro* degradation experiment in intestinal juices and the *in vivo* intraluminal kinetics, these observations could not be explained by degradation of docetaxel within the gastrointestinal lumen. Hence, these results seem to indicate a substantial uptake of docetaxel despite the presence of P-gp in the intestinal wall, which would imply that docetaxel is a weaker substrate of P-gp than paclitaxel is. The results of *in vitro* transport studies strongly support this idea.

The rapid disappearance of docetaxel from the gut lumen and the short t_{max} in plasma both imply that the uptake of docetaxel occurs readily. Effective first-pass extraction and metabolic breakdown, subsequently followed by hepatobiliary excretion of unchanged drug and metabolites into the gut lumen, could thus explain the low docetaxel plasma levels. The important role of hepatic metabolism in the disposition of docetaxel is highlighted by the large amounts of metabolites recovered from the bile after i.v. administration of docetaxel to mice with cannulated gallbladders. Similarly, the fecal recovery of unchanged drug after i.v. administration of docetaxel was negligible. Given these results, we have tested the effects of the protease inhibitor
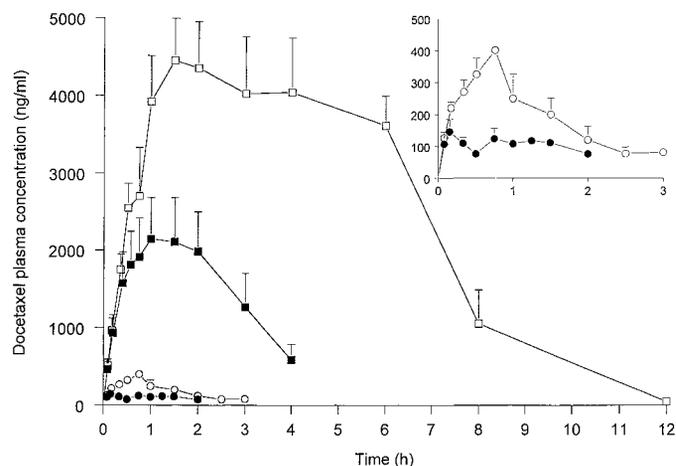
the plasma levels of docetaxel in wild-type and *Mdr1a/1b* (−/−) mice (Fig. 5). Calculated relative to the AUC of i.v. administered docetaxel without ritonavir, the systemic exposure in wild-type mice increased about 50-fold from 123 ± 39 ng/ml·h to 6,043 ± 1,572 ng/ml·h (*P* = 0.013) and in *Mdr1a/1b* (−/−) mice from 574 ± 112 ng/ml·h to 33,322 ± 1,753 ng/ml·h (*P* < 0.003). A mass balance study performed with this combination showed that 65.3% of unchanged docetaxel and 3.6% of metabolites were recovered in the feces of wild-type mice, whereas in *Mdr1a/1b* (−/−) mice, this was 5.5 and 46.0%, respectively (Table 2). The influence of ritonavir on the transport characteristics of docetaxel was studied *in vitro* over LLC-*MDR1* and LLC-*Mdr1a* cells. It was found that a concentration of 100 μM had no effect on the vectorial transport of docetaxel (Fig. 6), which suggested that ritonavir is not a very potent P-gp inhibitor.

DISCUSSION

This study shows that the presence of P-gp in the epithelial cells lining the gastrointestinal tract is not able to prevent the uptake of docetaxel from the intestinal lumen of mice. However, subsequent and very efficient first-pass metabolism, probably by CYP3a enzymes, results in very low plasma levels and, thus, in a low bioavailability. The HIV protease inhibitor ritonavir, a very effective inhibitor of human CYP3A4, dramatically increases the systemic exposure to docetaxel by 50-fold.

The mechanism that determines the low oral bioavailability of docetaxel is different from what we had expected, based on our experience with oral paclitaxel. The oral uptake of the latter, a structurally closely related taxane drug, is almost completely inhibited by P-gp in the intestinal wall, because virtually the complete p.o.

Fig. 5. Impact of oral ritonavir on the plasma concentration-time curves of docetaxel. Plasma concentration-time curves of 10 mg/kg p.o. administered docetaxel in wild-type mice (*closed symbols*) and in *Mdr1a/1b* (−/−) mice (*open symbols*) as single agent (● and ○) or in the presence of 12.5 mg/kg p.o. administered ritonavir (■ and □). *Inset*, the results without ritonavir in more detail. Data are expressed as means ± SE; *n* = 3–7 animals per curve.

Table 2 *Fecal excretion of unchanged drug and metabolites after oral administration of docetaxel (10 mg/kg) with or without oral ritonavir (12.5 mg/kg) to wild-type and Mdr1a/1b (−/−) mice*

Data are expressed as percentage of administered dose ± SE (*n* = 4).

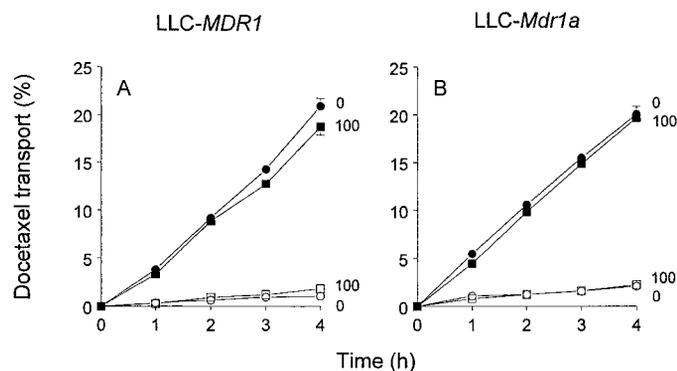| Mouse strain	Ritonavir	Docetaxel	Metabolites
Wild-type	−	37.8 ± 3.6	22.5 ± 1.6
Wild-type	+	65.3 ± 2.7	3.6 ± 0.6
Mdr1a/1b (−/−)	−	1.1 ± 0.4	28.6 ± 2.6
Mdr1a/1b (−/−)	+	5.5 ± 1.5	46.0 ± 4.0

Fig. 6. Inhibition of *in vitro* transport by ritonavir. Translocation of 11.8 μM [^{3}H]docetaxel expressed as mean percentage of the dose added to the donor compartment (mean $\pm$ SE; assayed in triplicate) over monolayers of LLC-*MDR1* and LLC-*Mdr1a* cells as single agent (● and ○) or in the presence of 100 μM ritonavir (■ and □). *Closed symbols*, basolateral-to-apical transport; *open symbols*, apical-to-basolateral transport. The concentration of ritonavir is annotated in the graphs.

ritonavir, a very effective inhibitor of CYP3A4, the key enzyme responsible for the metabolic breakdown of docetaxel in humans (21). Ritonavir increased the systemic exposure of oral docetaxel dramatically by 50-fold (Fig. 5). The inhibition of P-gp by ritonavir does not seem to contribute to this effect. Although several studies have suggested that ritonavir may act as a P-gp inhibitor (22–26), ritonavir was not potent enough to inhibit P-gp mediated *in vitro* transport of docetaxel. Moreover, there was no evidence for an increased uptake of docetaxel from the gastrointestinal tract when given with ritonavir. The fecal excretion of unchanged docetaxel in wild-type and *Mdr1a/1b* (−/−) mice receiving ritonavir was even higher than in animals receiving docetaxel as single agent, which may be caused by the strong inhibition of docetaxel metabolism resulting in a larger hepatobiliary excretion of unchanged drug.

Our results thus show that both P-gp and metabolism cause the low oral bioavailability of docetaxel with the latter, however, being the major determinant. It is well established that there is a striking overlap between substrates of P-gp and CYP3A4 (27–31). Bennet *et al.* (29) and Zhang and Bennet (31) have proposed a model in which P-gp and CYP3A4 cooperate as a barrier against the drug uptake from the gut lumen, with P-gp limiting the access of the drug to CYP3A4, thus preventing the saturation of the latter. Docetaxel appears to be an excellent model compound for this so-called "gate-keeper" theory. It was found that P-gp is moderately efficient in preventing the intestinal uptake of docetaxel at a dose of 10 mg/kg, with about 40% of the unchanged drug being recovered in the feces. However, of the 60% of the dose that is absorbed from the intestines, only 4% managed to bypass metabolism and reach the systemic circulation. When P-gp was absent, as in *Mdr1a/1b* (−/−) mice, a higher uptake of only about 2-fold from the gut resulted in about a 6-fold higher systemic plasma level. Moreover, a similar nonproportional increase was observed with increasing doses, whereas docetaxel displayed linear pharmacokinetics in mice after i.v. administration in the same dose range (18). Consequently, these results reflect the saturation of enzymes involved in first-pass metabolism with an increasing supply of drug.

In patients, the combination of oral cyclosporin A and docetaxel has proven to be very effective because it increased the apparent bioavailability of docetaxel from 8 to 90% (32). The reasons for the rather modest effect of cyclosporin A on the bioavailability of docetaxel in mice are not clear. Cyclosporin A acts as a double-edged sword, inhibiting both P-gp and CYP3A4 (33), and it may be that cyclosporin A is more effective in inhibiting human CYP3A4 than it is in murine CYP3a. Our experiments show that ritonavir is a very effective inhibitor of metabolism of docetaxel in mice. In HIV patients, ritona-

vir is now commonly used as a booster for saquinavir and indinavir oral bioavailability, and studies have shown that a low oral so-called "baby" dose of 100 mg is already sufficient to increase the bioavailability to therapeutic plasma levels (34, 35). Moreover, the use of ritonavir seems to be safe, even when taken for a long period of time, as is common in anti-HIV therapy. Consequently, the clinical use of ritonavir as a booster for the oral bioavailability of docetaxel appears to be an attractive alternative over concomitant cyclosporin A. Moreover, boosting with ritonavir may even reduce the costs of therapy, because it is likely that dose reductions of docetaxel are required when using ritonavir.

In conclusion, our study shows that both the P-gp and the CYP3a metabolizing enzymes determine the low oral bioavailability of docetaxel in mice with the CYP3a, however, being quantitatively most important. Ritonavir boosts the systemic exposure of docetaxel extensively and provides a promising combination for further testing in patients.

REFERENCES

1. Wacher, V. J., Salphati, L., and Benet, L. Z. Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv. Drug Delivery Rev., *46:* 89–102, 2001.
2. Juliano, R. L., and Ling, V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim. Biophys. Acta, *455:* 152–162, 1976.
3. Sparreboom, A., van Asperen, J., Mayer, U., Schinkel, A. H., Smit, J. W., Meijer, D. K. F., Borst, P., Nooijen, W. J., Beijnen, J. H., and van Tellingen, O. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc. Natl. Acad. Sci. USA, *94:* 2031–2035, 1997.
4. van Asperen, J., van Tellingen, O., van der Valk, M. A., Rozenhart, M., and Beijnen, J. H. Enhanced oral absorption and decreased elimination of paclitaxel in mice with cyclosporin A. Clin. Cancer Res., *4:* 2293–2297, 1998.
5. van Asperen, J., van Tellingen, O., Sparreboom, A., Schinkel, A. H., Borst, P., Nooijen, W. J., and Beijnen, J. H. Enhanced oral bioavailability of paclitaxel in mice treated with the P-glycoprotein blocker SDZ PSC 833. Br. J. Cancer, *76:* 1181–1183, 1997.
6. Bardelmeijer, H. A., Beijnen, J. H., Brouwer, K. R., Rosing, H., Nooijen, W. J., Schellens, J. H., and van Tellingen, O. Increased oral bioavailability of paclitaxel by GF120918 in mice through selective modulation of P-glycoprotein. Clin. Cancer Res., *6:* 4416–4421, 2000.
7. Meerum Terwogt, J. M., Beijnen, J. H., ten Bokkel Huinink, W. W., Rosing, H., and Schellens, J. H. Co-administration of cyclosporin enables oral therapy with paclitaxel. Lancet, *352:* 285, 1998.
8. Meerum Terwogt, J. M., Malingre, M. M., Beijnen, J. H., ten Bokkel Huinink, W. W., Rosing, H., Koopman, F. J., van Tellingen, O., Swart, M., and Schellens, J. H. Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. Clin. Cancer Res., *5:* 3379–3384, 1999.
9. Huizing, M. T., Misser, V. H., Pieters, R. C., ten Bokkel Huinink, W. W., Veenhof, C. H., Vermorken, J. B., Pinedo, H. M., and Beijnen, J. H. Taxanes: a new class of antitumor agents. Cancer Investig., *13:* 381–404, 1995.
10. Ringel, I., and Horwitz, S. B. Studies with RP 56976 (Taxotere): a semisynthetic analogue of Taxol. J. Natl. Cancer Inst. (Bethesda), *83:* 288–291, 1991.
11. Monegier, B., Gaillard, C., Sable, S., and Vuilhorgne, M. Structures of the major metabolites of docetaxel (RP 56976- Taxotere). Tetrahedron Lett., *35:* 3715–3718, 1994.
12. Sparreboom, A., van Tellingen, O., Nooijen, W. J., and Beijnen, J. H. Determination of paclitaxel and metabolites in mouse plasma, tissues, urine and faeces by semi-automated reversed-phase high- performance liquid chromatography. J. Chromatogr. B. Biomed. Appl., *664:* 383–391, 1995.
13. Wils, P., Phung-Ba, V., Warnery, A., Lechardeur, D., Raeissi, S. D., Hidalgo, I. J., and Scherman, D. Polarized transport of docetaxel and vinblastine mediated by P-glycoprotein in human intestinal epithelial cell monolayers. Biochem. Pharmacol., *48:* 1528–1530, 1994.
14. Shirakawa, K., Takara, K., Tanigawara, Y., Aoyama, N., Kasuga, M., Komada, F., Sakaeda, T., and Okumura, K. Interaction of docetaxel ("Taxotere") with human P-glycoprotein. Jpn. J. Cancer Res., *90:* 1380–1386, 1999.
15. Schinkel, A. H., Mayer, U., Wagenaar, E., Mol, C. A. A. M., van Deemter, L., Smit, J. J. M., van der Valk, M. A., Voordouw, A. C., Spits, H., van Tellingen, O., Zijlmans, J. M., Fibbe, W. E., and Borst, P. Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc. Natl. Acad. Sci. USA, *94:* 4028–4033, 1997.
16. Schinkel, A. H., Wagenaar, E., van Deemter, L., Mol, C. A., and Borst, P. Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J. Clin. Investig., *96:* 1698–1705, 1995.

6163

17. Dantzig, A. H., Shepard, R. L., Law, K. L., Tabas, L., Pratt, S., Gillespie, J. S., Binkley, S. N., Kuhfeld, M. T., Starling, J. J., and Wrighton, S. A. Selectivity of the multidrug resistance modulator. LY335979, for P-glycoprotein and effect on cytochrome P-450 activities. J. Pharmacol. Exp. Ther., *290:* 854–862, 1999.

18. van Tellingen, O., Beijnen, J. H., Verweij, J., Scherrenburg, E. J., Nooijen, W. J., and Sparreboom, A. Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. Clin. Cancer Res., *5:* 2918–2924, 1999.

19. Yanagimoto, T., Itoh, S., Sawada, M., and Kamataki, T. Mouse cytochrome P450 (Cyp3a11): predominant expression in liver and capacity to activate aflatoxin B1. Arch. Biochem. Biophys., *340:* 215–218, 1997.

20. Yanagimoto, T., Itoh, S., Sawada, M., Hashimoto, H., and Kamataki, T. Molecular cloning and functional expression of a mouse cytochrome P-450 (Cyp3a-13): examination of Cyp3a-13 enzyme to activate aflatoxin B1 (AFB1). Biochim. Biophys. Acta, *1201:* 405–410, 1994.

21. Marre, F., Sanderink, G. J., Desousa, G., Gaillard, C., Martinet, M., and Rahmani, R. Hepatic biotransformation of docetaxel (Taxotere) *in vitro*: involvement of the CYP3A subfamily in humans. Cancer Res, *56:* 1296–1302, 1996.

22. Alsenz, J., Steffen, H., and Alex, R. Active apical secretory efflux of the HIV protease inhibitors saquinavir and ritonavir in Caco-2 cell monolayers. Pharm. Res. (NY), *15:* 423–428, 1998.

23. Drewe, J., Gutmann, H., Fricker, G., Torok, M., Beglinger, C., and Huwyler, J. HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporine analog SDZ PSC 833. Biochem. Pharmacol., *57:* 1147–1152, 1999.

24. Profit, L., Eagling, V. A., and Back, D. J. Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors. AIDS (Hagerstown), *13:* 1623–1627, 1999.

25. Gutmann, H., Fricker, G., Drewe, J., Toeroek, M., and Miller, D. S. Interactions of HIV protease inhibitors with ATP-dependent drug export proteins. Mol. Pharmacol., *56:* 383–389, 1999.

26. Lee, C. G., Gottesman, M. M., Cardarelli, C. O., Ramachandra, M., Jeang, K. T., Ambudkar, S. V., Pastan, I., and Dey, S. HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. Biochemistry, *37:* 3594–3601, 1998.

27. Wacher, V. J., Wu, C. Y., and Benet, L. Z. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Mol. Carcinog., *13:* 129–134, 1995.

28. Watkins, P. B. The barrier function of CYP3A4 and P-glycoprotein in the small bowel. Adv. Drug Delivery Rev., *27:* 161–170, 1997.

29. Benet, L. Z., Izumi, T., Zhang, Y., Silverman, J. A., and Wacher, V. J. Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. J. Controlled Release, *62:* 25–31, 1999.

30. Kim, R. B., Wandel, C., Leake, B., Cvetkovic, M., Fromm, M. F., Dempsey, P. J., Roden, M. M., Belas, F., Chaudhary, A. K., Roden, D. M., Wood, A. J. J., and Wilkinson, G. R. Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. Pharm. Res. (NY), *16:* 408–414, 1999.

31. Zhang, Y., and Benet, L. Z. The gut as a barrier to drug absorption: combined role of cytochrome P450 3A and P-glycoprotein. Clin. Pharmacokinetics, *40:* 159–168, 2001.

32. Malingré, M. M., Richel, D. J., Beijnen, J. H., Rosing, H., Koopman, F. J., Bokkel Huinink, W. W., Schot, M. E., and Schellens, J. H. Coadministration of cyclosporine strongly enhances the oral bioavailability of docetaxel. J. Clin. Oncol., *19:* 1160–1166, 2001.

33. Wacher, V. J., Silverman, J. A., Zhang, Y., and Benet, L. Z. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. J. Pharm. Sci., *87:* 1322–1330, 1998.

34. Veldkamp, A. I., van Heeswijk, R. P., Mulder, J. W., Meenhorst, P. L., Schreij, G., van der Geest, S., Lange, J. M., Beijnen, J. H., and Hoetelmans, R. M. Steady-state pharmacokinetics of twice-daily dosing of saquinavir plus ritonavir in HIV-1-infected individuals. Journal of Acquired Immune Deficiency Syndromes, *27:* 344–349, 2001.

35. van Heeswijk, R. P., Veldkamp, A. I., Hoetelmans, R. M., Mulder, J. W., Schreij, G., Hsu, A., Lange, J. M., Beijnen, J. H., and Meenhorst, P. L. The steady-state plasma pharmacokinetics of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1-infected individuals. AIDS (Hagerstown), *13:* F95–F99, 1999.

Low Systemic Exposure of Oral Docetaxel in Mice Resulting from Extensive First-Pass Metabolism Is Boosted by Ritonavir

Heleen A. Bardelmeijer, Mariët Ouwehand, Tessa Buckle, et al.

Updated version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/62/21/6158

Cited articles	This article cites 33 articles, 10 of which you can access for free at: http://cancerres.aacrjournals.org/content/62/21/6158.full#ref-list-1
Citing articles	This article has been cited by 17 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/62/21/6158.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/62/21/6158. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.