Advances in Brief

Role of Intestinal P-glycoprotein in the Plasma and Fecal Disposition of Docetaxel in Humans[1]

Lia van Zuylen,[2] Jaap Verweij, Kees Nooter, Eric Brouwer, Gerrit Stoter, and Alex Sparreboom

Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital, 3008 AE Rotterdam, the Netherlands

Abstract

Multidrug resistance (MDR)-1-P-glycoprotein (P-gp) is a drug-transporting protein that is abundantly present in biliary ductal cells and epithelial cells lining the gastrointestinal tract. Here, we have determined the role of P-gp in the metabolic disposition of the antineoplastic agent docetaxel (Taxotere) in humans. Pharmacokinetic profiles were evaluated in five cancer patients receiving treatment cycles with docetaxel alone (100 mg/m^2 i.v. over a 1-h period) and in combination with a new potent inhibitor of P-gp activity, R101933 (200–300 mg b.i.d.). The terminal disposition half-life and total plasma clearance of docetaxel were not altered by treatment with oral R101933 ($P \geq 0.27$). The cumulative fecal excretion of docetaxel, however, was markedly reduced from 8.47 ± 2.14% (mean ± SD) of the dose with the single agent to less than 0.5% in the presence of R101933 ($P = 0.0016$). Levels of the major cytochrome P450 3A4-mediated metabolites of docetaxel in feces were significantly increased after combination treatment with R101933 ($P = 0.010$), indicating very prominent and efficient detoxification of reabsorbed docetaxel into hydroxylated compounds before reaching the systemic circulation. It is concluded that intestinal P-gp plays a principal role in the fecal elimination of docetaxel by modulating reabsorption of the drug after hepatobiliary secretion. In addition, the results indicate that inhibition of P-gp activity in normal tissues by effective modulators, and the physiological and pharmacological consequences of this treatment, cannot be predicted based on plasma drug monitoring alone.

Introduction

Acquired or intrinsic resistance of malignant cells to taxanes and other naturally occurring anticancer drugs has been linked to the so called "classical" mechanism of MDR,[3] which can cause decreased intracellular concentrations of these drugs (1). This MDR phenotype is characterized by increased levels of P-gp, a member of the ATP binding cassette superfamily of transmembrane transport proteins with M_r 170,000 encoded by the *MDR1* gene, which acts as an energy-dependent drug-efflux pump with broad substrate specificity (1). P-gp expression has been found in nearly all of the tumor types, ranging from leukemia to carcinoma, and it has also been reported in a number of normal tissues, mainly in specialized epithelial cells with secretory functions including the apical biliary surface of hepatocytes and epithelial cells of the intestinal tract (2). The physiological functions of P-gp are still speculative at this time, but possibilities include involvement in the protection of epithelial mucosal cells in the gastrointestinal tract from xenobiotics, in transport of steroids in the adrenals and bile salts in the liver, in protein secretion in the kidneys (3), and, most recently discovered, in migration of dendritic cells and T-lymphocytes out of the skin (4). In addition, the expression of P-gp in the capillary endothelial cells forming the blood-brain and the blood-testis barrier apparently protects these organs from exogenous toxins, and thus contributes to the role of these anatomical sites as drug sanctuaries (reviewed in Ref. 5).

Several studies have shown that P-gp-mediated MDR is of clinical significance, and this observation has stimulated a search for noncytotoxic agents that can reverse this resistance phenomenon (6, 7). Clinical trials performed thus far with identified P-gp inhibitors given in combination with anticancer drugs, however, have raised important issues regarding the safety of the chemotherapeutic treatment because of drug interactions that increased or changed the spectrum of associated toxic side effects (8, 9). The results of these clinical trials in combination with the poorly defined pharmacological and physiological function of P-gp in humans emphasize the need to perform studies to further evaluate the role of P-gp in the disposition of substrate drugs. The importance of these studies is further underscored by the previous use of anticancer drugs that are rather poor substrates of P-gp given with nonspecific inhibitors of P-gp (9). Thus, in the present study, we evaluated the role of P-gp in the plasma disposition and fecal elimination

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[2] To whom requests for reprints should be addressed, at Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, P.O. Box 5201, 3008 AE Rotterdam, the Netherlands. Phone: 31-10-4391338; Fax: 31-10-4391003; E-mail: zuylen@onch.azr.nl.

[3] The abbreviations used are: MDR, multidrug resistance; P-gp, MDR1 P-glycoprotein; M1 and M3, diastereoisomers of 3′-de-*tert*-butoxycarbonylamino-3′-[3-(5,5-dimethyl-4-hydroxy-2-oxo-1, 3-oxazolidinyl)]-docetaxel; M2 (3′-de-*tert*-butoxycarbonyl-3′-2-(1-hydroxy-2-meth-yl)-propyloxycarbonyl-docetaxel; M4 (3′-de-*tert*-butoxycarbonylamino-3′-[3-(5,5-di-methyl-2,4-dioxo-1,3-oxazolidinyl)]-docetaxel; AUC, area under the concentration *versus* time curve; HPLC, high-performance liquid chromatography.

pathways of docetaxel, one of the best known substrates of P-gp (10), in humans using a combined treatment with the new p.o. administered P-gp inhibitor R101933 as a model compound.

Materials and Methods

Drug Administration. Docetaxel (Rhône-Poulenc Rorer, Antony Cedex, France) was supplied as a concentrated solution in polysorbate 80 (40 mg/ml; Taxotere) and was administered to five cancer patients at a dose of 100 mg/m^2 as a 1-h i.v. infusion. Three weeks later, the same dose was administered to each patient 1 h after intake of R101933 on day 3. All of the patients received premedication with dexamethasone (8 mg b.i.d.), starting 24 h prior to infusion of docetaxel and continuing for 4 days thereafter. R101933 (methyl 6,11-dihydro-11-(1-[2-[4-(2-quinolinyl-methoxy)phenyl]ethyl]-4-piperidinylidene]-5*H*-imidazo[2,1-b][3]benzazepine-3-carboxylate), an agent acting as a specific antagonist of P-gp, was supplied by Janssen Research Foundation (Beerse, Belgium) as an oral solution containing 10 mg/ml of the active compound in 15% hydroxypropyl-β-cyclodextrin. The drug was administered p.o. twice daily for 5 consecutive days (*i.e.*, days 1–5) with 200 ml of water, at least 1 h after a meal. The clinical protocol was approved by the institutional ethics committee, and signed informed consent was obtained from each participant before entering the study.

Sample Collection and Processing. Blood specimens were acquired in all of the patients during both treatment courses. Sample volumes of ~6 ml were drawn directly from a peripheral venous access device into tubes containing lyophilized sodium heparin as an anticoagulant. Blood samples were collected immediately before infusion, and at 0.5, 1, 1.25, 1.5, 2, 3, 7, 11, 23, and 31 h after the start of infusion. All of the samples were centrifuged immediately for 10 min at 1000 × *g* to yield plasma, which was stored frozen at −20°C in polypropylene vials until the time of analysis.

Complete stool collections for docetaxel analysis were obtained for the duration of the study (*i.e.*, up to 31 h after the start of drug administration). This space of time was chosen based on previous fecal excretion studies with the structural related agent paclitaxel (11). Fecal specimens were collected in polystyrene containers and stored immediately at −20°C. Weighted feces samples were homogenized individually in 3 volumes of a phosphate buffer [containing 0.01 M potassium phosphate, 0.137 M sodium chloride and 2.7 mM potassium chloride in the presence of 0.05% (w/v) glucose (pH 7.4)] using five 1-min bursts of an Ultra-Turrax T25 homogenizer (IKA-Labortechnik, Dottingen, Germany) operating at 20,500 rpm. Aliquots of 500 μl of feces homogenates were diluted with human plasma (1:1, v/v) and stored frozen at −80°C prior to further processing.

Analytical Methods. A pure reference standard of docetaxel (batch: 14PROC9230; purity: 98.0% by HPLC) was kindly supplied by Rhône-Poulenc Rorer, and was used as received. Plasma concentrations of docetaxel were determined by a validated HPLC assay with mass-spectrometric detection, with a lowest limit of quantitation of 1 ng/ml. Samples (200 μl) were pretreated by solid-phase extraction using end-capped Bond Elut nitrile microcolumns (Varian, Harbor City, CA). A

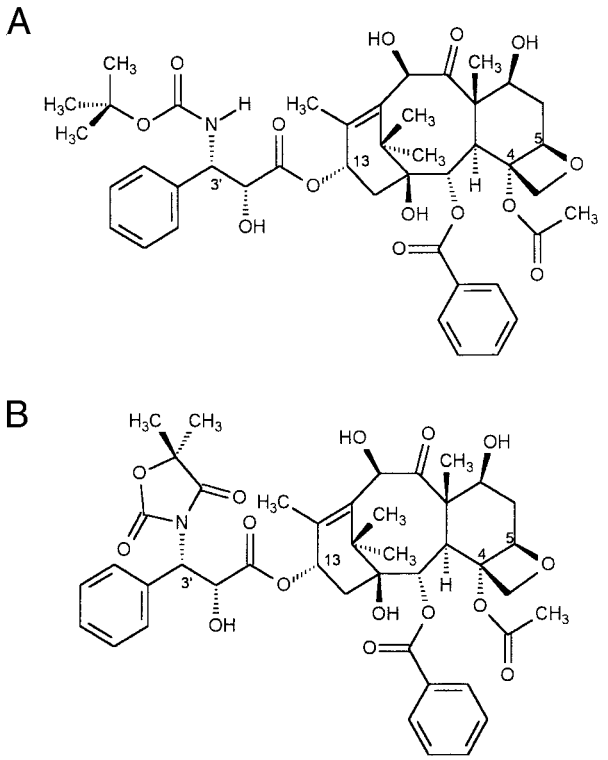

Fig. 1 Chemical structures of docetaxel (*A*) and its major cytochrome P450 3A4-mediated metabolite M4 (*B*).

stainless steel analytical column (100 × 4.6 mm, internal diameter) packed with 3 μm of Hypersil BDS C18 material (Alltech, Breda, the Netherlands) was used for chromatographic separation, and gradient elution was performed with a mixture of acetonitrile and 0.02 M ammonium acetate (pH 4.0) at a flow rate of 0.8 ml/min. Paclitaxel (Bristol-Myers Squibb, Wallingford, CT) was used as internal standard.

Authentic reference standards of the docetaxel metabolites M1, M2, M3, and M4 (Fig. 1) were obtained after isolation and purification of a patient fecal sample, as described previously (12), and their concentrations in feces homogenates were determined by reversed-phase HPLC with UV detection using a modification of a procedure described elsewhere (13). In brief, quantitative extraction was achieved by a single solvent extraction of 0.5-ml samples with a mixture of acetonitrile-*n*-butyl chloride (1:4, v/v). Chromatography was performed at 60°C using an Inertsil ODS-80A column (150 × 4.6 mm; 5-μm particle size; GL Science, Tokyo, Japan) protected by a Lichrospher 100 RP-18 end-capped-guard column (4.0 × 4.0 mm; 5-μm particle size; Merck, Darmstadt, Germany), and a 1-h exponential gradient elution (45–75%; 1 ml/min) of methanol in water-tetrahydrofuran-aqueous ammonium hydroxide (97.4:2.5:0.1, v/v/v) at pH 6.0. The column effluent was monitored at a wavelength of 230 nm.

Concentrations of R101933 and its esterase-mediated carboxylic acid metabolite, R102207, were determined by a validated HPLC method involving solid-phase extraction on Bond-Elut Certify microcolumns (Varian). Standard curves were

prepared in drug-free human plasma and were expanded to encompass concentrations between 2 and 10,000 ng/ml A 1-ml aliquot of standard or plasma sample was mixed with 3 ml of 1 M aqueous acetic acid and 100 μl of internal standard solution (R125026 in acetonitrile at 10 μg/ml). Before loading, columns were preconditioned with 3 ml of ethanol, 3 ml of water, and 1 ml of 1 M of aqueous acetic acid. Consecutive washing steps involved 3 ml of water, 1 ml of aqueous acetic acid, and 3 ml of ethanol, and elution was performed with 3 ml of a mixture of ethanol-ammonia (98:2, v/v). Samples were dried under nitrogen at 65°C and redissolved in 100 μl of 0.02 M ammonium formate (pH 4.0)-acetonitrile-ethanol (50:25:25, v/v/v). Chromatography was performed on a column (100 × 4.6 mm) packed with 3-μm Hypersil BDS C8 material (Alltech), and gradient elution (0.8 ml/min) with a mixture of 0.02 M ammonium formate (pH 4.0), acetonitrile, and ethanol. UV absorption measurements were performed at a wavelength of 270 nm. Determination of analyte concentrations was based on log-transformed peak areas of R101933 and R102207 and the internal standard *versus* nominal concentrations by interpolation using linear regression analysis.

Pharmacokinetic Analysis. Individual plasma concentration-time curves of docetaxel were analyzed by a two-compartmental model using the software package WinNonlin (Pharsight, Mountain View, CA). All of the curves were fitted using the actual infusion duration and blood sampling times. Pharmacokinetic parameters were calculated by standard methods. To test parameter differences for statistical significance among treatment courses, a two-tailed paired Student's *t* test was performed. *P* values of less than 0.05 were considered statistically significant.

***In Vitro* Studies.** A functional *in vitro* study was performed to evaluate whether oral R101933 in humans yields plasma levels that are capable of inhibiting P-gp function in tumor cells. The parental human ovarian carcinoma cell line A2780 (P-gp-negative) and the derived anthracycline-resistant lines $A2780_{T100}$ and 2780AD (both P-gp-positive), developed by transfection of P-gp and step-wise exposure to doxorubicin, respectively, were grown and maintained in colorless RPMI 1640 (Brunschwig, Amsterdam, the Netherlands). Cells were kept in continuous logarithmic growth at 37°C in a humidified atmosphere in 5% CO_2/95% air in medium supplemented with 10% (w/v) heat-inactivated bovine calf serum (Hyclone, Logan, UT), penicillin, streptomycin, and L-glutamine. Exponentially grown cells were trypsinized, packed by centrifugation for 5 min at 1500 × *g*, and washed twice with medium. The cells were counted microscopically and transferred to protein-free RPMI to dilutions containing 4.0×10^5 cells per ml. Inhibition of P-gp-mediated drug efflux in each of the cell lines was evaluated using a daunorubicin (final concentration, 1 μg/ml) retention assay, in the presence of various concentrations of R101933, ranging from 1 to 1000 ng/ml. Preliminary time course experiments revealed that at a given R101933 concentration, daunorubicin uptake in the cells did not change after 90 min, at which time equilibrium was reached. Thus experiments were carried out with a 2-h incubation period at 37°C, using 0.5-ml aliquots of the cell suspension in 4.5-ml polypropylene screw-cap tubes (Greiner, Alphen aan den Rijn, the Netherlands). The addition of R101933 was done after extraction of the compound from a human plasma matrix or patient sample using a mixture of acetonitrile and *n*-butyl chloride (1:4, v/v), and reconstitution of dried extracts in neat medium by agitation. During the incubation period, tubes were gently vortex-mixed at 30-min intervals to prevent clogging of the cells. Intracellular levels of daunorubicin were measured using flow cytometry with the aid of a fluorescence-activated cell sorter. The fluorescence intensity in the resistant cell lines relative to that observed in the A2780 parental cell line in the absence of R101933, expressed as percentage, was plotted relative to the R101933 concentration.

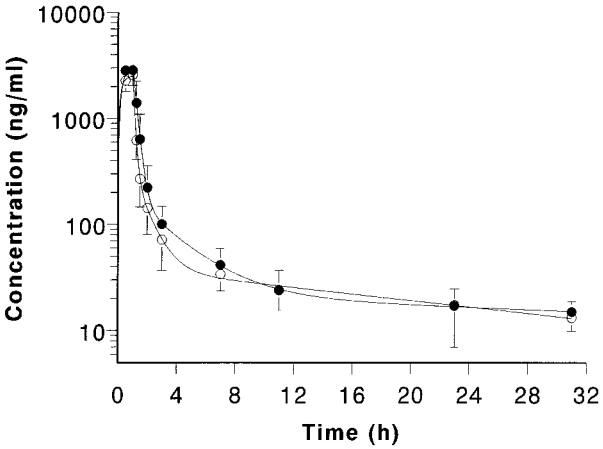

Fig. 2 Plasma concentration *versus* time profiles of docetaxel in five patients treated with docetaxel alone (○;100 mg/m^2 as a 1-h i.v. infusion) or in combination with oral R101933 (●; 200 or 300 mg b.i.d.). Data are presented as mean values (*symbols*) ± SD (*error bars*).

Results

Plasma Pharmacokinetics. The results of paired plasma concentration-time profiles of unchanged docetaxel given with and without cotreatment of oral R101933 were remarkable similar for all of the patients studied (Fig. 2). The mean pharmacokinetic parameters of docetaxel for both treatment courses are summarized in Table 1. There were no statistically significant differences in any of the studied docetaxel kinetic parameters, including the AUC and terminal disposition half-life, which suggested that R101933 did not influence the disposition of the taxane in the systemic circulation. The observed peak plasma concentrations of R101933 were in the range of 25.3–168 ng/ml and 83.1–142 ng/ml in the absence and presence of docetaxel, respectively. Concentrations of R101933 in this range were observed to inhibit P-gp-mediated daunorubicin efflux in two cell lines with known expression of P-gp and resistance to anthracyclines (Fig. 3). In all of the patients, there was extensive formation of the pharmacologically inactive compound, R102207, resulting from an ester hydrolysis, with peak levels approximately 75-fold higher (range, 33–124) than that of the parent drug.

Fecal Elimination. In humans, excretion of docetaxel via the feces is a major route of drug elimination (12, 14). To evaluate the effects of P-gp inhibition on elimination pathways of docetaxel, feces samples were collected over a

Table 1 Disposition and elimination kinetics of docetaxel in the absence or presence of oral R101933[a]

Parameter	Without R101933	With R101933	P^b
Plasma			
Docetaxel			
AUC^c (μg·h/ml)	3.62 ± 0.66 (3.04–4.50)	4.22 ± 0.97 (3.09–5.63)	0.33
$t_{½(\beta)}$ (h)	11.2 ± 2.10 (8.60–13.3)	8.70 ± 4.80 (3.15–14.4)	0.27
Feces			
Docetaxel			
fe_f (%)	8.47 ± 2.14 (5.92–11.3)	0.45 ± 0.24 (0.13–0.72)	0.0016
M4			
fe_f (%)	13.3 ± 1.81 (11.3–15.5)	20.1 ± 4.39 (16.8–27.4)	0.010

[a] Data were obtained from five cancer patients receiving a 1-h i.v. infusion of docetaxel at 100 mg/m^2 either alone or in combination with 200 or 300 mg of R101933 given p.o. The kinetic terms are mean values ± SD, with ranges in parentheses.

[b] Probability value *versus* control without R101933 (paired two-sided Student's *t* test).

[c] *AUC*, area under the plasma concentration of docetaxel versus time curve; $t_{½(\beta)}$, terminal disposition half-life of docetaxel; fe_f, percentage of the absolute docetaxel dose excreted in the feces within 31 h after administration of indicated drug.

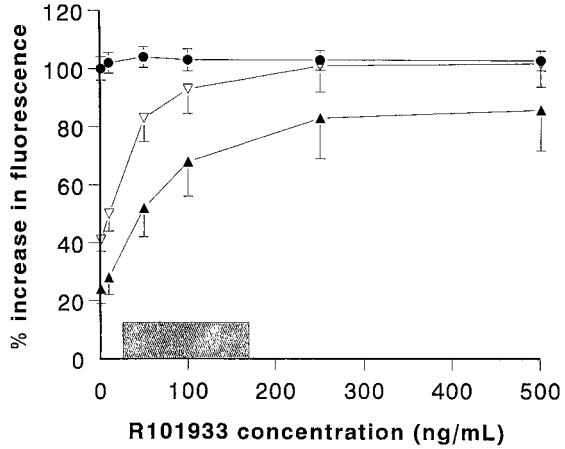

Fig. 3 Modulation of cellular daunorubicin uptake by R101933 as measured by a change in the fluorescence intensity in the P-glycoprotein-expressing and anthracycline-resistant cell lines $A2780_{T100}$ (▽) and 2780AD (▲) relative to that observed in the A2780 parental cell line (●) in the absence of R101933. Experiments were carried out with a 2-h incubation period at 37°C with R101933 present at various concentrations. Data points, expressed as percentage, are mean values of at least three independent experiments performed in duplicate; *bars*, SEs. *Gray box*, the range of R101933 peak levels observed in the five patients after treatment with a p.o. dose of 200 or 300 mg. Over the entire drug concentration range, substantial increases in intracellular fluorescence, induced by R101933-mediated inhibition of P-glycoprotein activity, could be detected.

period of 31 h from all five of the patients during both treatment courses with and without R101933. Preliminary insight obtained from work of our laboratory into the composition of docetaxel metabolites present in feces samples indicated that apart from docetaxel, seven chromatographic peaks absorbed at 230 nm, the peak wavelength of taxane derivatives. Structural identification using HPLC and off-line mass spectrometry showed that the major peak was M4, a known metabolite of docetaxel resulting from hydroxylation reactions on the *tert*-butyl propionate side chain (15). Using reference standards, three of the minor peaks were identified as metabolites resulting from oxidation of one of the methyl groups on the *tert*-butyl propionate side chain (M2), and spontaneous cyclization of this alcohol derivative to the two diastereoisomers M1 and M3.

In patients treated with docetaxel alone, 8.47 ± 2.14% (mean ± SD; $n = 5$) of the administered dose was excreted in the first 31 h as the parent drug, whereas 13.3 ± 1.81% of the dose could be accounted for by metabolite M4 (Table 1). The total fecal recovery of the metabolites M1, M2, and M3 was very low in all of the patients (less than 0.5% of the dose; data not shown) and apparently the compounds play only a minor role in the overall drug disposition. In the same group of patients, coadministration with R101933 resulted in markedly reduced fecal excretion of unchanged drug to less than 0.5% of the administered dose ($P = 0.0016$). In contrast, however, the excretion of metabolite M4 was significantly increased in all of the patients to 20.1 ± 4.39% of the dose ($P = 0.010$). These data clearly show that R101933 administered p.o. causes a profound alteration of P-gp-mediated intestinal reabsorption of docetaxel, without modifying the drug's kinetic behavior in plasma.

Discussion

In the present study we have shown that intestinal P-gp plays a principal role in the fecal elimination pathways of docetaxel without modifying the pharmacokinetic behavior of the drug in the systemic circulation. Previous investigations have shown a major role of the cytochrome P450 3A4 isozymes in docetaxel metabolism. In humans, the principal biotransformation routes involve hydroxylation of the *tert*-butoxy function in the C13 side chain, followed by a spontaneous cyclization reaction (15). The four principal metabolites resulting from this pathway have substantially less cytotoxic activity on tumor and nonmalignant hematopoietic cells as compared with the parent drug (12). Fecal excretion of docetaxel and its metabolites has been previously reported in two other cases (14, 15), and in both, M4 was the main metabolite, very similar to our present findings, and accounted for a larger fraction of the recovered dose than docetaxel itself. Interestingly, this metabolite can only be detected in the systemic circulation in about 30% of the patients treated with docetaxel at a dose of 100 mg/m^2 and reaches peak plasma levels ranging form only 22 to 230 ng/ml (16). This makes it very likely that metabolite M4 is very efficiently excreted through a biliary secretion pathway into the intestinal lumen immediately after its formation, and that reabsorption is low. In our patients, we observed that coadministration with R101933 had a marked effect on the fecal elimination of docetaxel, changing from excretion partly as unchanged drug in case of single-agent administration to almost exclusively through metabolic breakdown (mainly to M4) in the combination treatment. The similarity of the terminal disposition phases

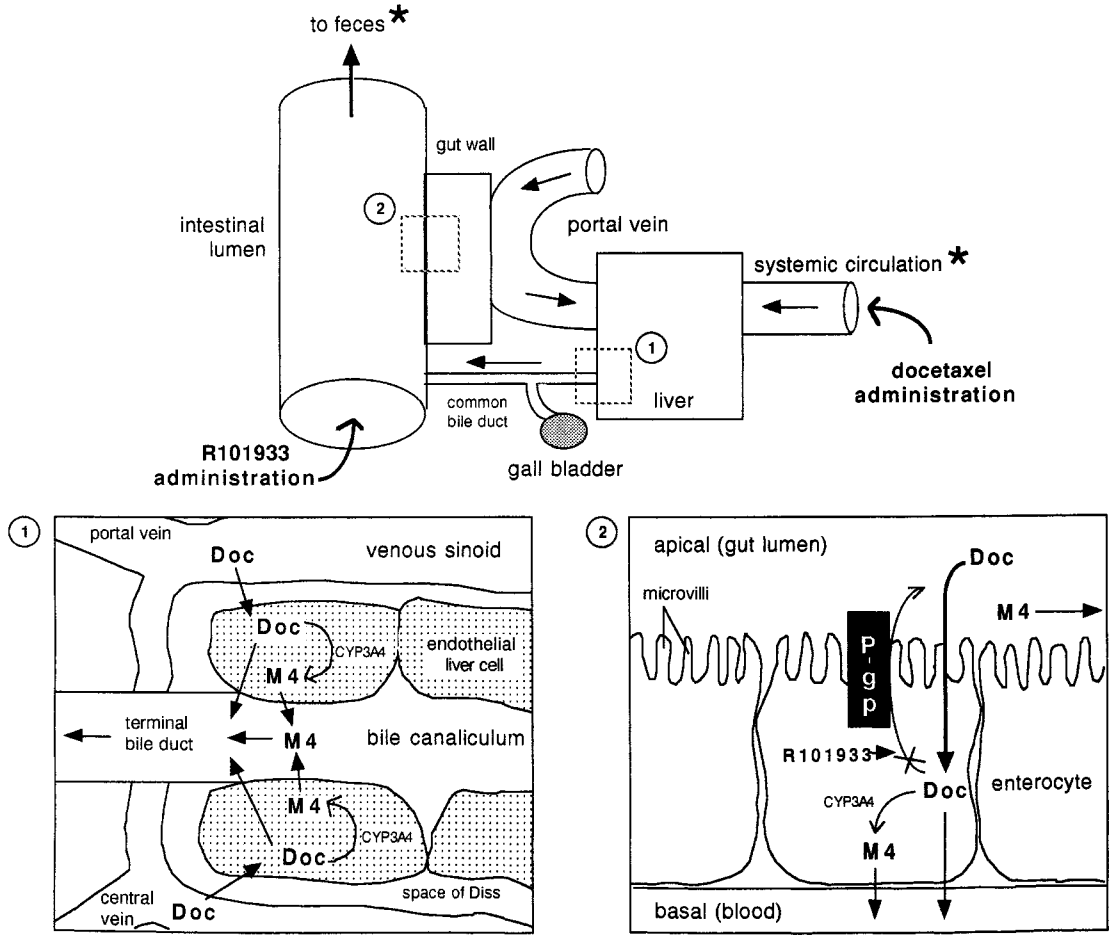

Fig. 4 Schematic representation of the role of intestinal P-glycoprotein in docetaxel metabolism and elimination in humans. ①, model of the hepatic-cellular plate showing the bile collecting system with hepatic metabolism of docetaxel from the systemic circulation or the portal vein after reabsorption following the biliary secretion by cytochrome P450 3A4 isozymes into metabolite M4 and its subsequent secretion in the terminal bile duct. ②, model of the intestinal epithelium showing reuptake of docetaxel into the intestinal lumen, P-glycoprotein-mediated efflux of docetaxel and its inhibition by R101933 and intestinal metabolism by the cytochrome P450 3A4 enzyme system. ∗, sites of drug measurement; *Doc*, docetaxel; *CYP3A4*, enzymes of the cytochrome P450 family isoform 3A4.

in plasma of docetaxel between treatment courses indicate that the reduced fecal excretion of the parent drug in the patients receiving R101933 is unlikely to be related to diminished (P-gp-mediated) biliary secretion. This is also in line with recent preclinical studies performed in mice lacking mdr1 (drug-transporting) P-gp, which indicates that these proteins are not essential *per se* for normal hepatobiliary secretion of the related compound, paclitaxel (17). After biliary secretion of docetaxel given in combination with R101933, reabsorption of drug from the intestinal lumen seems to be a very efficient process. This does not, however, result in increased plasma levels because of a virtually complete first-pass extraction and/or docetaxel metabolism in the liver and intestinal mucosa, which also has significant expression of cytochrome P450 3A isozymes (Ref. 9; see Fig. 4 for a schematic representation of the role of intestinal P-gp in docetaxel metabolism and excretion). These findings lend further support to previous observations that the absorption of substrate drugs from the intestines can be increased by concomitant administration with P-gp inhibitors (reviewed in Ref. 18).

The present data also raise important questions regarding the role of endogenously expressed P-gp (for instance in the bile canaliculi, kidneys, and intestinal epithelial cells) in the pharmacokinetics of substrate drugs in plasma. It has been shown previously that R101933 does not interfere with docetaxel metabolism in preclinical systems, it does not induce cytochrome P450 isozymes, and the major metabolic routes of the modulator itself is ester hydrolysis, which is independent of cytochrome P450 activity.[4] Our results of unaltered plasma concentrations of docetaxel in the combination with R101933 is consistent with the postulated concept that pharmacokinetic interference between P-gp modulators and anticancer drugs is the result of

[4] G. Mannens, unpublished data (on file, Janssen Research Foundation).

competition for (cytochrome P450) enzymes involved in drug metabolism (8). This is also in keeping with previous knowledge from data generated in P-gp knock-out mice (5, 17–19) and sheds light on some important mechanistic aspects of drug-drug interactions. Most importantly, our data clearly indicate that inhibiting P-gp function in normal tissues by administration of an effective modulator, the physiological and pharmacological consequences of this treatment cannot be predicted based on plasma drug measurement alone.

Given the dominant pharmacological role of intestinal P-gp activity in the efficiency and pattern of fecal excretion of docetaxel, we expect that the possibility to inhibit its activity completely with a p.o. administered P-gp modulator as demonstrated in this study can have important pharmacological applications. One of these applications is to increase the oral bioavailability of taxane drugs, which are known to display poor absorption characteristics after oral drug administration (19). Indeed, recent experimental data have shown that coadministration of oral PSC833 or cyclosporin A (both substrates of cytochrome P450 3A4 isozymes) increased the AUC for paclitaxel in mice more than 10-fold (20), and preliminary findings from the same group indicate that the same applies to paclitaxel administered to cancer patients (21). On the basis of our present data, which show a very prominent and efficient detoxification of reabsorbed docetaxel into hydroxylated metabolites before it can reach the systemic circulation, we expect that this approach of increasing drug bioavailability is unlikely to succeed unless the modulator significantly interferes with docetaxel metabolism.

Acknowledgments

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