

Influence of Polymorphic OATP1B-Type Carriers on the Disposition of Docetaxel

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Abstract

Purpose: Docetaxel is extensively metabolized by CYP3A4 in the liver but mechanisms by which the drug is taken up into hepatocytes remain poorly understood. We hypothesized that (i) liver uptake of docetaxel is mediated by the polymorphic solute carriers OATP1B1 and OATP1B3 and (ii) inherited genetic defects in this process may impair systemic drug elimination.

Experimental Design: Transport of docetaxel was studied *in vitro* using various cell lines stably transfected with OATP1B1*1A (wild-type), OATP1B1*5 [c.521T>C (V174A); rs4149056], OATP1B3, or the mouse transporter Oatp1b2. Docetaxel clearance was evaluated in wild-type and Oatp1b2-knockout mice as well as in two cohorts of patients with multiple variant transporter genotypes ($n = 213$).

Results: Docetaxel was found to be a substrate for OATP1B1, OATP1B3, and Oatp1b2 but was not transported by OATP1B1*5. Deficiency of Oatp1b2 in mice was associated with an 18-fold decrease in docetaxel clearance ($P = 0.0099$), which was unrelated to changes in intrinsic metabolic capacity in mouse liver microsomes. In patients, however, none of the studied common reduced function variants in OATP1B1 or OATP1B3 were associated with docetaxel clearance ($P > 0.05$).

Conclusions: The existence of at least two potentially redundant uptake transporters in the human liver with similar affinity for docetaxel supports the possibility that functional defects in both of these proteins may be required to confer substantially altered disposition phenotypes. In view of the established exposure–toxicity relationships for docetaxel, we suggest that caution is warranted if docetaxel has to be administered together with agents that potently inhibit both OATP1B1 and OATP1B3. *Clin Cancer Res*; 18(16); 4433–40. ©2012 AACR.

Introduction

The antimicrotubular agent docetaxel is a widely used chemotherapeutic agent that has been approved for the treatment of multiple malignant diseases, including cancers of the breast, lung, head and neck, stomach, and prostate. The disposition properties of docetaxel are characterized by up to 10-fold differences in drug clearance between patients

receiving the same therapeutic regimen (1). The high degree of interindividual pharmacokinetic variability observed with docetaxel has important toxicologic ramifications. In particular, it was previously shown that a mere 50% decrease in docetaxel clearance is associated with a more than 4-fold increase in the odds of developing severe neutropenia, the dose-limiting toxicity (2, 3).

Despite the established exposure–pharmacodynamic relationships for docetaxel, the mechanisms underlying the agent's unpredictable pharmacokinetics remain largely unexplained. It has been speculated that a critical determinant of docetaxel's pharmacokinetic variability is associated with differential expression of polymorphic drug-metabolizing enzymes and/or transporters at sites of elimination. However, several recent analyses indicated that the contribution of genetic variants in obvious candidate genes encoding enzymes or ATP-binding cassette transporters to explaining pharmacokinetic variability of docetaxel is rather limited (4–11).

The mechanisms by which docetaxel is taken up into human liver cells are still largely unknown. Previous *in vitro* screens have provided evidence that cellular uptake of the related compound paclitaxel may be regulated, in part, by the polymorphic organic anion transporting polypeptides

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Translational Relevance

Docetaxel is widely used for the treatment of multiple solid tumors, including cancers of the breast, lung, head and neck, stomach, and prostate. The interindividual pharmacokinetic variability seen with docetaxel treatment remains high, and this phenomenon may have important ramifications for the agent's clinical activity and toxicity. We speculated that differential expression of polymorphic transporters involved in the hepatic elimination of docetaxel plays a crucial role in explaining this pharmacologic variability. Here, we investigated the contribution of organic anion transporting polypeptides to the disposition of docetaxel using an array of *in vitro* and *in vivo* model systems. Our results indicate the existence of at least two potentially redundant uptake transporters in the human liver with similar affinity for docetaxel (OATP1B1 and OATP1B3) that regulate the initial, rate-limiting step in the elimination of docetaxel. In view of the established exposure–toxicity relationships for docetaxel, our results suggest that caution is warranted if docetaxel has to be administered together with agents that potentially inhibit both these transporters.

OATP1B1 (gene name, *SLCO1B1*) and/or OATP1B3 (gene name, *SLCO1B3*; refs. 12, 13). These transporters are expressed at high levels in the liver, where their localization is restricted to the basolateral membrane of hepatocytes, and they have been implicated in the liver uptake of multiple structurally diverse endogenous molecules and xenobiotics (14). In the current study, we tested the hypothesis that inherited variation in OATP1B1 and OATP1B3 is associated with the disposition of docetaxel and that these transporters collectively contribute to interindividual differences in the clearance of docetaxel in patients with cancer.

Materials and Methods

In vitro transport studies

Xenopus laevis oocytes injected with OATP1B1, OATP1B3, or rat Oatp1b2 cRNA along with water-injected controls were obtained from BD Biosciences. Chinese hamster ovary (CHO) cell lines stably expressing OATP1B1 or OATP1B3 (15) and Flp-In T-Rex293 cells transfected with OATP1B1*1A (wild-type), OATP1B1*1B [c.388A>G (N130D); rs2306283], OATP1B1*5 [c.521T>C (V174A); rs4149056], or OATP1B1*15 (N130D, V174A) have been described previously (16). OATP1B1 or OATP1B3 overexpressing human embryonic kidney (HEK293) cells were created by stably transfecting the respective cDNA fragments spliced from TrueClone plasmids (OriGene Technologies), cloned into a pIRES2-EGFP vector (BD Biosciences). Mouse Oatp1b2 overexpressing HEK293 cells were created similarly from a commercial cDNA cloned into a pDream2.1/MCS vector (GenScript). Overexpression of transporters in

HEK293 cells was confirmed using TaqMan probes (Applied Biosystems).

Uptake experiments were carried out as described previously (3), with results normalized to uptake values in cells transfected with an empty vector. Preliminary experiments indicated that Phenol Red, a pH indicator in trypsin used to resuspend cultured cells, influenced OATP1B-mediated uptake of docetaxel in Flp-In T-Rex293 cells (Supplementary Fig. S1), and therefore these studies were conducted in Phenol Red-free conditions.

Animal experiments

Adult male Oatp1b2-knockout mice (17) and age-matched wild-type mice (Taconic), both on a DBA1/lacJ background, were housed in a temperature-controlled environment with a 12-hour light cycle and given a standard diet and water *ad libitum*. Experiments were approved by the Institutional Animal Care and Use Committee of St. Jude Children's Research Hospital. Docetaxel, formulated in polysorbate 80 (Taxotere) and diluted in normal saline, was administered by tail vein injection at a dose of 10 mg/kg, and plasma, liver, and kidney from each animal were collected at 5, 15, 30, 60, 120, 240, and 480 minutes. Urine was collected from animals housed in metabolic cages for 48 hours after docetaxel administration. Samples were analyzed by liquid chromatography/tandem mass spectrometry (LC-MS-MS; see Supplementary Methods for details; ref. 18), and noncompartmental parameters calculated using PK Solutions 2.0 (Summit Research Services). Tissue concentrations of docetaxel were corrected for contaminating plasma (19). Gene expression patterns in livers were assessed using the Mouse 430v2 GeneChip array (Affymetrix). Microsomal metabolism of docetaxel in liver samples from wild-type and Oatp1b2-knockout mice was conducted as described (20) in the presence or absence of the Cyp3a inhibitor, ketoconazole.

Patient studies

Patients were enrolled onto a prospective pharmacokinetic study (Dutch trial registry: NTR2311). Inclusion criteria included a confirmed diagnosis of a solid tumor for which docetaxel (formulated in polysorbate 80; Taxotere) was a reasonable therapeutic option, age 18 years or older, World Health Organization (WHO) performance score of 0 or 1, and adequate hematopoietic, hepatic, and renal functions, as described previously (21). Concurrent use of agents known to induce or inhibit CYP3A4 was not allowed. The study was approved by the Erasmus University Medical Center review board, and all patients provided written informed consent.

Blood collection for pharmacokinetic analyses was conducted using a limited-sampling strategy where 4 or 5 samples were obtained over a 24-hour period after the end of infusion. Docetaxel concentrations in plasma were determined as described (Supplementary Methods). Pharmacokinetic parameters were estimated using a previously developed population model (22), in NONMEM version 7

(Icon Development Solutions). There was no statistically significant influence of sex, administered dose, or tumor type on the clearance of docetaxel ($P > 0.05$), and thus pharmacokinetic data of all patients were pooled in subsequent correlation studies without further correction or consideration of subgroup analyses.

Genomic DNA was isolated from whole blood using MagnaPure LC (Roche Diagnostics GmbH). Allelic discrimination analysis was conducted for the determination of several variants in OATP1B1 and OATP1B3 that were selected from the literature on the basis of their relatively high predicted allelic frequency and/or the known or suspected influence on functional properties of the encoded proteins (Supplementary Table S1; ref. 16). The analyses were conducted using TaqMan assays on an ABI PRISM 7500 system (Applied Biosystems) according to the manufacturer's instructions. Confirmatory genotyping analyses on select candidate variants, including the c.388G>A and c.521T>C variants in OATP1B1, and the c.334T>G and c.699G>A variants in OATP1B3, were done on previously collected samples from a similar, predominantly white cohort of 72 patients that had received docetaxel-based chemotherapy (4).

Statistical considerations

Data are presented as mean with SD, unless stated otherwise. Statistical calculations were done using analysis of variance or Student *t* test in SPSS version 17 (SPSS Inc), depending on the number of groups, and $P < 0.05$ was considered significant.

Results

Docetaxel transport *in vitro*

Experiments assessing the interaction of docetaxel with human OATP1B1 and OATP1B3 indicated that drug uptake is dependent on cell context, with both proteins being able to take up docetaxel when expressed in HEK293 cells or CHO cells, but no noticeable transport occurring by OATP1B1 when expressed in *Xenopus laevis* oocytes (Fig. 1A). Docetaxel was also found to be transported into cells expressing the mouse mOatp1b2 or rat rOatp1b2 transporters (Fig. 1B).

The transport of docetaxel into CHO cells transfected with OATP1B1 or OATP1B3 was found to be time-dependent and saturable with a Michaelis–Menten constant (K_m) of 7.6 ± 3.0 and 2.2 ± 0.6 $\mu\text{mol/L}$, respectively, and a maximum velocity (V_{max}) of 30.7 ± 5.7 and 27.2 ± 2.4 pmol/mg/min, respectively (Figs. 1C–F), and similar results were obtained for paclitaxel (Supplementary Fig. S2). Compared with cells overexpressing the wild-type OATP1B1 (OATP1B1*1A), *in vitro* transport activity of cells transfected with constructs carrying the c.521C substitution (OATP1B1*5 and OATP1B1*15) was completely lost (Fig. 2). Interestingly, the presence of the docetaxel excipient polysorbate 80 (Tween 80), at levels that can be achieved in patients (23), abrogated the OATP1B1-genotype-dependent transport of docetaxel (Fig. 2).

Docetaxel pharmacokinetics in Oatp1b2-knockout mice

We next evaluated the possible importance of these transporters for docetaxel in mice with a genetic deletion of Oatp1b2 [Oatp1b2(–/–) mice]. The area under the curve (AUC) for docetaxel in these animals was dramatically increased compared with that observed in wild-type mice ($8,826 \pm 845$ vs. 336 ± 96.9 ng \times h/mL; $P = 0.0066$) as a result of a more than 18-fold decrease in systemic clearance (1.08 ± 0.097 vs. 19.9 ± 7.08 L/h/kg; $P = 0.0099$). The respective concentration–time profiles of docetaxel in mice (Fig. 3A) suggests that the slow clearance in the Oatp1b2(–/–) mice is due to a distribution defect rather than an event occurring in the terminal elimination phase. Indeed, the terminal half-lives of docetaxel were not significantly different in Oatp1b2(–/–) mice and wild-type mice (2.41 ± 0.151 vs. 2.44 ± 0.533 h; $P = 0.87$).

As anticipated, the liver/plasma AUC ratio was significantly reduced in Oatp1b2(–/–) mice (1.32 ± 0.088 vs. 8.14 ± 2.39 ; $P = 0.0079$). The kidney/plasma AUC ratio was also reduced in Oatp1b2(–/–) mice (5.14 ± 0.363 vs. 43.0 ± 12.5 ; $P = 0.0063$), although there was limited shunting of docetaxel in the knockout mice to urine (urinary excretion, 1.68 ± 0.758 %dose vs. 1.03 ± 0.676 %dose; $P = 0.15$). To rule out potentially altered, compensatory expression of enzymes and transporters in the liver of Oatp1b2(–/–) mice at baseline, microarrays were used to evaluate differential expression profiles of 839 probe sets for 463 genes, including 49 ATP-binding cassette transporters, 78 cytochrome P450 enzymes, and 336 solute carriers. Compared with levels in liver of wild-type mice, besides probe sets for the Oatp1b2 gene *Slco1b2*, only transcripts of the enzyme *Cyp2b10* were decreased in the Oatp1b2(–/–) mice (Fig. 3B). Because taxanes are not known to be metabolized by *Cyp2b10*, this genetic alteration is unlikely to directly or indirectly influence docetaxel handling by the liver. Furthermore, there were no potentially compensatory changes in hepatic *Cyp3a* activity, the main metabolic route for docetaxel, as Oatp1b2-knockout had no influence on the hepatic microsomal metabolism of docetaxel *ex vivo* in the presence or absence of the *Cyp3a* inhibitor ketoconazole (Fig. 3C).

Docetaxel pharmacokinetics in patients with different transporter genotypes

To provide preliminary evidence for a possible role of OATP1B1 and OATP1B3 in the clinical pharmacology of docetaxel, an exploratory pharmacogenetic association analysis was conducted in human subjects with cancer undergoing docetaxel-based chemotherapy. To this end, pharmacokinetic and pharmacogenetic data were obtained from 141 predominantly white patients (87 females and 54 males) with a median age of 55 years (Supplementary Table S2). The average clearance of docetaxel in the study population was 41.8 ± 12.3 L/h, with a 6.3-fold difference between the lowest and highest values.

The relative frequencies of the variant alleles in our patient population were comparable with previously reported estimates (16), the distributions of all polymorphisms were in

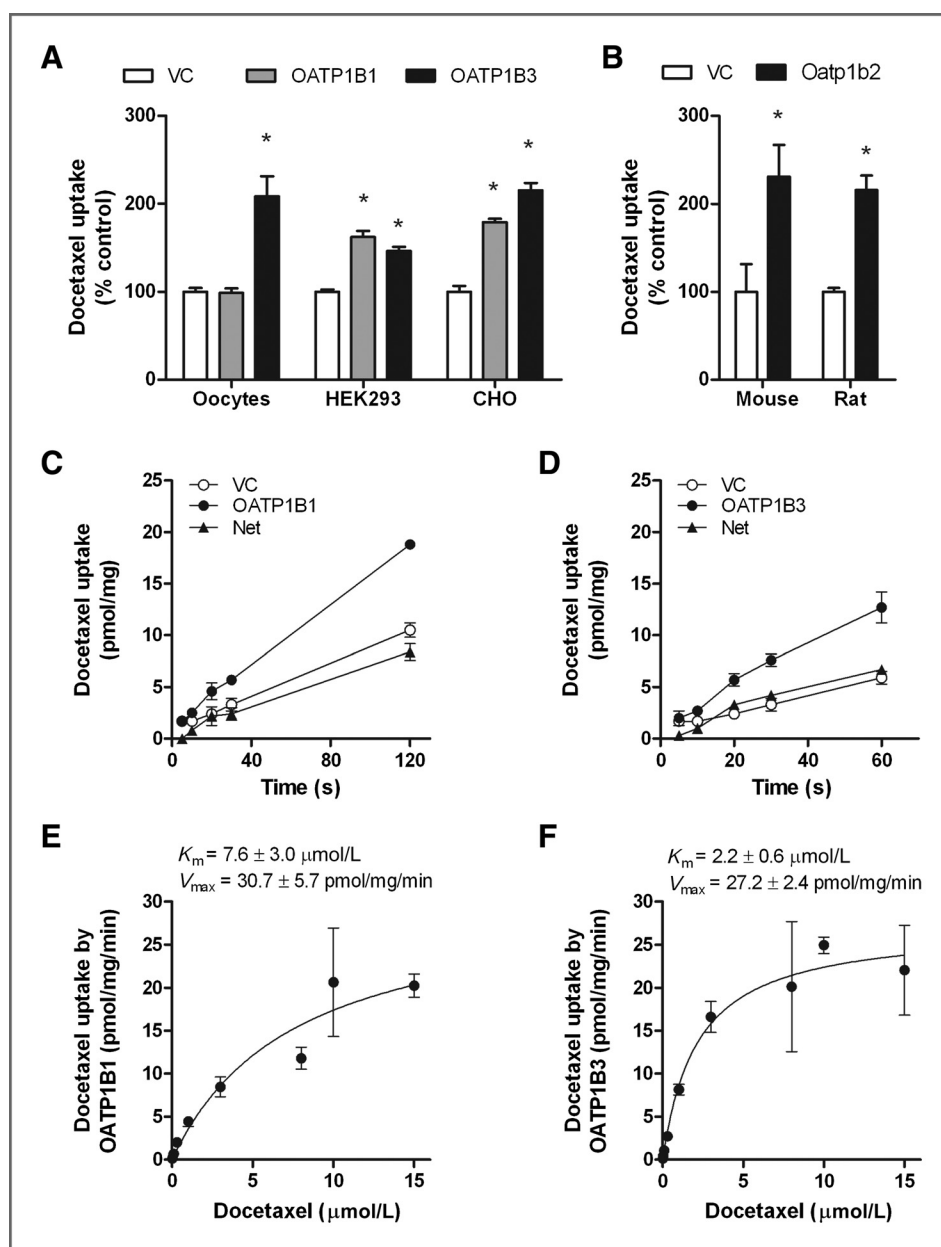


Figure 1. *In vitro* transport studies of docetaxel. A, transport of docetaxel by human OATP1B1 and OATP1B3 was evaluated with constructs transfected in *Xenopus laevis* oocytes (docetaxel concentration, 2 $\mu\text{mol/L}$; 30-minute incubations), HEK293 cells (2 $\mu\text{mol/L}$; 30 minutes), or CHO cells (1 $\mu\text{mol/L}$; 2 minutes). B, transport of docetaxel by mouse Oatp1b2 transfected in HEK293 cells (0.1 $\mu\text{mol/L}$; 60 minutes) or rat Oatp1b2 transfected in *Xenopus laevis* oocytes (2 $\mu\text{mol/L}$; 30 minutes). Data represent the mean of 2 to 32 observations and are expressed as the average percent of uptake values in cells transfected with an empty vector (VC). The star (*) denotes a significant difference from VC ($P < 0.05$). Time dependence of transport by OATP1B1 (C) and OATP1B3 (D) and concentration dependence of transport by OATP1B1 (E) and OATP1B3 (F) was evaluated in CHO cells, where data represent the mean of 2 to 4 independent experiments in cells stably expressing OATP1B1, OATP1B3, or in control cells (VC), and the net difference. K_m denotes the Michaelis-Menten constant and V_{max} the maximum velocity. Error bars represent the SE.

Hardy-Weinberg equilibrium, and demographic characteristics at baseline were similar for individuals carrying 0, 1, or 2 variant alleles at the loci of interest. Despite the observed functional impact of the OATP1B1 c.521C substitution *in vitro*, none of the individual polymorphisms in OATP1B1 were found to be associated with the clearance of docetaxel *in vivo* (Fig. 4). Significant associations were also not observed between docetaxel clearance and the studied variants in OATP1B3 (Fig. 5), and associations did not improve when individuals were clustered on the basis of observed diplotypes (Supplementary Fig. S3). In consideration of the relatively few individuals carrying polymorphism predicted to be associated with altered docetaxel transport, we conducted additional genotyping

on the c.388G>A and c.521T>C variants in OATP1B1 and the c.334T>G and c.699G>A variants in OATP1B3 in a separate cohort of 72 patients (4). However, enrichment of these candidate polymorphisms also did not result in statistically significantly improved genetic associations with docetaxel clearance (Supplementary Fig. S4).

Discussion

The current study provides support for a growing body of knowledge that solute carriers belonging to the family of organic anion transporting polypeptides can have a dramatic impact on the hepatic accumulation and systemic clearance of CYP3A4 substrates. Using an array of *in vitro*

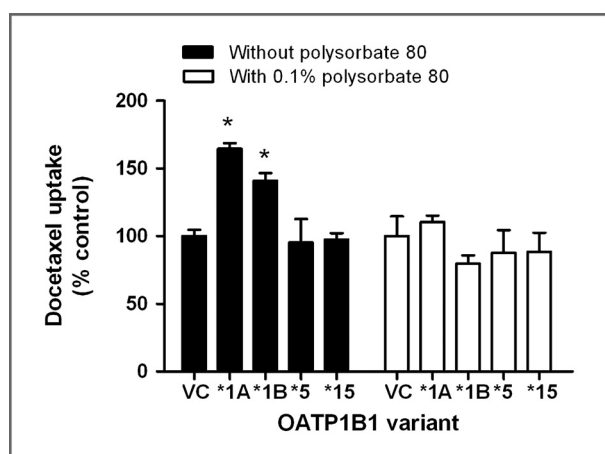


Figure 2. Influence of OATP1B1 variants on docetaxel transport *in vitro*. Transport of docetaxel (concentration, 0.1 $\mu\text{mol/L}$; 60-minute incubations) was evaluated in Flp-In T-Rex293 cells transfected with OATP1B1*1A (wild-type), OATP1B1*1B [c.388A>G (N130D); rs2306283], OATP1B1*5 [c.521T>C (V174A); rs4149056], or OATP1B1*15 (N130D, V174A). Data represent the mean of 6 observations and are expressed as the average percent of uptake values in cells transfected with an empty vector (VC) in the absence or presence of 0.1% of polysorbate 80. Error bars represent the SE. The star (*) denotes a significant difference from VC ($P < 0.05$).

transport assays, including intracellular accumulation studies in multiple transfected model systems, docetaxel was identified as a high-affinity substrate for human OATP1B1 and OATP1B3. We found that the interaction of docetaxel with OATP1B1 and OATP1B3 was strongly dependent on cell context and culture medium composition, and this has obvious implications for future screening strategies aimed at identifying novel substrates for these transporters.

Our *in vitro* studies also suggest that docetaxel is a transported substrate of mouse Oatp1b2 and rat Oatp1b2. The rodent Oatp1b2 transporters share more than 60% amino acid sequence homology to human OATP1B1 and OATP1B3, and on the basis of their shared basolateral localization in hepatocytes and overlapping substrate specificity (24), it is possible that in rodents Oatp1b2 fulfills the same function in the liver as OATP1B1 and OATP1B3 in humans. On the basis of this premise, we evaluated the pharmacokinetic properties of docetaxel in a mouse model with a genetic deletion of Oatp1b2. One possible limitation of this model is that fact that, unlike in humans, mouse hepatocytes express multiple members of Oatp1a, a related subfamily of transporters that can potentially provide compensatory restoration of function when Oatp1b2 is lost (25). Despite this limitation, compared with wild-type mice, the systemic exposure to docetaxel in the Oatp1b2 ($-/-$) mice was remarkably increased by more than 26-fold. Gene expression profiling and Cyp3a activity measurements in liver samples excluded alterations in alternate transport mechanisms or metabolic pathways as a possible cause of the delayed clearance phenotype in Oatp1b2 ($-/-$) mice. These findings suggest that Oatp1b2-mediated transport of docetaxel is likely a critically important rate-limiting process in the elimination of this drug in mice. This supposition is consistent with the notion that the change in clearance of docetaxel observed here in Oatp1b2 ($-/-$) animals is at least as dramatic as compared with phenotypic changes associated with complete deficiency of metabolic Cyp3a activity in mice (20). Nonetheless, considering the relatively low amino acid homology between OATP1B1 or OATP1B3 and Oatp1b2 (about 64%) and between CYP3A4 and Cyp3a11 (about 73%), additional investigation is

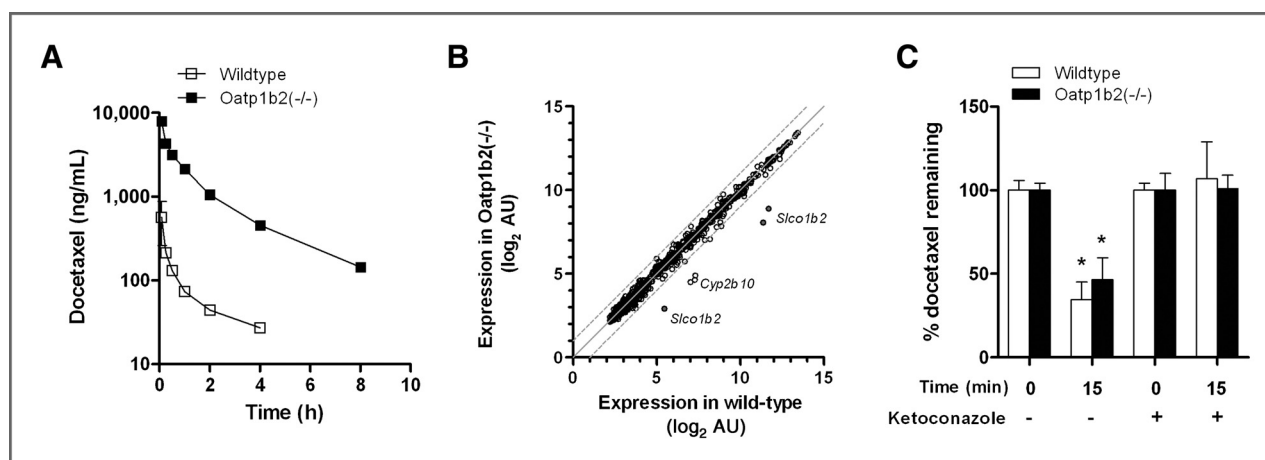


Figure 3. Influence of Oatp1b2-knockout on docetaxel pharmacokinetics. A, plasma concentration–time profile of docetaxel in wild-type and Oatp1b2 ($-/-$) mice (i.v. dose, 10 mg/kg). Data represent the mean of at least 6 observations per time point, and error bars represent the SE. B, comparative expression of 839 probe sets for 463 genes, including 49 ATP-binding cassette transporters, 78 cytochrome P450 enzymes, and 336 solute carriers, at baseline in livers of wild-type mice and Oatp1b2 ($-/-$) mice ($n = 5$ per group). Each symbol represents a single probe set, the solid line is the line of identity, and the dotted lines are the 95% confidence intervals. C, influence of Oatp1b2 knockout on the *ex vivo* liver microsomal metabolism of docetaxel (concentration, 2 $\mu\text{mol/L}$; 15-minute incubations) in the absence or presence of ketoconazole (20 $\mu\text{mol/L}$). Under these conditions, formation of the main murine metabolite of docetaxel (M2) was not different between liver microsomes from wild-type or Oatp1b2 ($-/-$) mice ($P = 0.29$). Data represent the mean of 8 independent observations per group and are expressed as the percent of drug added to the microsomes at time zero. Error bars represent the SE. The star (*) denotes a significant difference from time zero ($P < 0.05$).

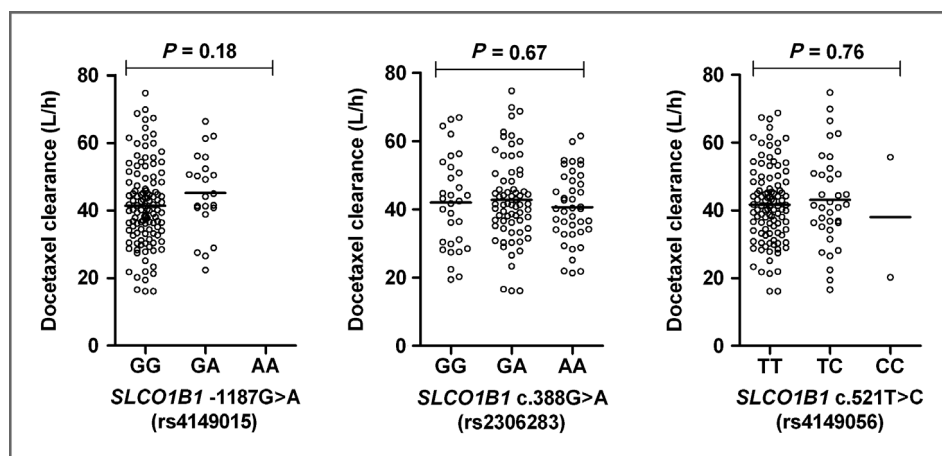


Figure 4. Docetaxel clearance as a function of observed OATP1B1 (*SLCO1B1*) genotypes. Data were obtained in 141 predominantly white patients with cancer receiving docetaxel-based chemotherapy. Each symbol represents an individual patient, and horizontal lines indicate median values. The *P* value denotes a statistical comparison of the clearance of docetaxel in the different genotype group.

required using humanized models for these proteins to provide direct evidence for involvement of OATP1B-type carriers in the hepatic uptake of docetaxel.

It is interesting to note that a previous study showed that mice deficient for all *Oatp1a* and *Oatp1b* genes display only a rather modest 2-fold increase in concentrations of paclitaxel in plasma, presumably due to decreased uptake of the drug into the liver compared with wild-type mice (26). The reasons underlying the apparent differences in outcome of the study with paclitaxel and our current results for docetaxel are not entirely clear. It is possible that the background strains onto which these respective knockout mice were developed (FVB vs. DBA1/lacJ, respectively) differentially impact any resulting phenotypes for structurally similar xenobiotics. Regardless of the exact mechanism, the observations made in the mice provide further evidence that hepatic OATP transporters can affect the pharmacokinetic properties of a remarkably broad range of substrates that include charged organic anions (e.g., methotrexate), charged organic cations (e.g., imatinib), polar zwitterions (e.g., fexofenadine), and uncharged hydrophobic agents (e.g., taxanes).

On the basis of *in vitro* uptake studies, multiple functionally different haplotypes, including OATP1B1*5 and

OATP1B1*15, were found to have a detrimental impact on docetaxel transport. This finding is consistent with previously studies showing substantially diminished transport activity of several OATP1B1 substrates by these particular variants when transfected into mammalian cells (27). *In vivo*, these variants have been associated with altered systemic exposure and toxicity in response to multiple substrate drugs (28).

Interestingly, the relevance of these genetic variants in OATP1B1 could not be confirmed in our prospectively conducted pharmacogenetic-association study done in a group of predominantly white patients with cancer receiving treatment with docetaxel. It is possible that additional rare genetic variants or haplotypes in OATP1B1 of importance to the transport docetaxel in this population are yet to be discovered and that much larger numbers of patients are then needed to more precisely quantify genotype-phenotype associations.

We also considered the possibility that the interaction of docetaxel with OATP1B1 may be masked by the pharmaceutical vehicle polysorbate 80 (Tween 80), which is used to solubilize docetaxel in clinical preparations. Indeed, the presence of polysorbate 80, even in relatively low amounts, completely nullified the genotype-dependent transport of

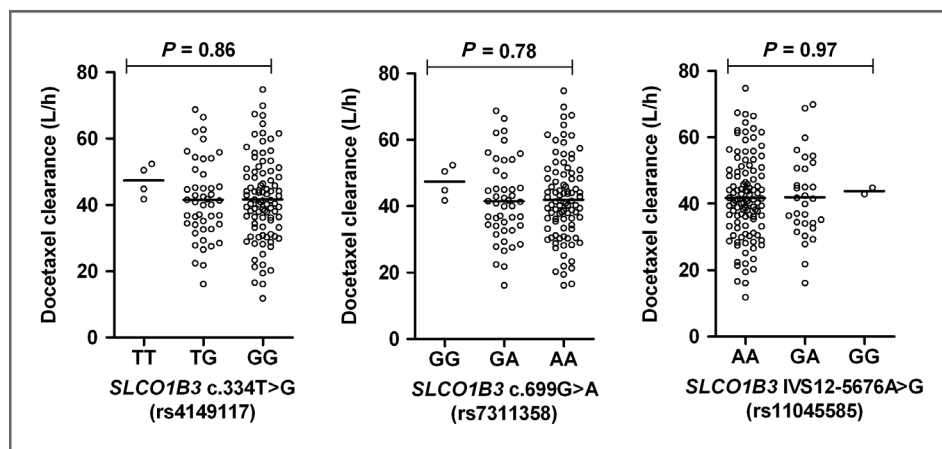


Figure 5. Docetaxel clearance as a function of observed OATP1B3 (*SLCO1B3*) genotypes. Data were obtained in 141 predominantly white patients with cancer receiving docetaxel-based chemotherapy. Each symbol represents an individual patient, and horizontal lines indicate median values. The *P* value denotes a statistical comparison of the clearance of docetaxel in the different genotype group.

docetaxel by OATP1B1 observed in the absence of polysorbate 80. Although further investigation is required to confirm direct involvement of polysorbate 80-mediated inhibition of OATP1B1 as the primary mechanistic basis for the observed *in vivo* effects, it is of note that similarly altered hepatic uptake has been described for colchicine in the presence of Solutol HS15 (29) and for paclitaxel in the presence of Cremophor (12). If confirmed, these observations suggest that the impact of reduced function variants of OATP1B1 on the clearance of docetaxel may be much more pronounced for polysorbate 80-free formulations of the drug, such as nab-docetaxel (ABI-008).

In our study, several genetic variants in OATP1B3 were also not significantly associated with the pharmacokinetics of docetaxel. This is in line with previously published data that we collected in another predominantly white, independent cohort of patients (4). It should be pointed out that this finding is at odds with several other investigations conducted in patients of Asian descent. For example, homozygosity (GG) for rs11045585 was associated with reduced clearance of docetaxel, compared with patients carrying the AA or AG genotypes (5). In another study, a particular OATP1B3 genotype combination comprising the reference allele at IVS4+76G>A (rs4149118) and variant alleles at 699G>A (rs7311358), IVS12+5676A>G (rs11045585), and *347_*348insA (rs3834935)indel was also linked with reduced clearance of docetaxel (30). It is possible that differences in outcome with our study are associated with the fact that such variants may occur at different frequencies between Asians and Caucasians, and/or on different, ethnicity-dependent haplotype structures.

Regardless of any potential ethnic considerations, the existence of at least 2 potentially redundant uptake transporters in the human liver with similar affinity for docetaxel supports the possibility that functional defects in both of these proteins may be required to confer substantially altered disposition phenotypes such as those seen in the *Oatp1b2*(-/-) mice. While complete functional deficiency of either OATP1B1 or OATP1B3 has been recorded to occur (31), deficiency of both transporters is very rare, with an estimated frequency in the human population of about 1 in a million (32). It can thus be postulated that intrinsic physiologic and environmental variables influencing OATP1B1- or OATP1B3-mediated uptake of docetaxel into hepatocytes may have a more profound influence on the clearance of docetaxel in the general population than do defective genetic variants. This recognition is particularly relevant in the context of the recent guidelines offered by The International Transporter Consortium regarding pre-clinical criteria needed to trigger the conduct of clinical

studies to evaluate drug-transporter interactions (33). Indeed, it is conceivable that instances of idiosyncratic hypersensitivity to docetaxel are the result of currently unrecognized drug-drug interactions at the level of hepatic uptake mechanisms (see Supplementary Table S3 for examples).

Collectively, our findings show the importance of OATP1B-type solute carriers as the initial, rate-limiting step in the elimination of docetaxel. Our results suggest that genetic defects leading to impaired function of both OATP1B1 and OATP1B3 may be required to confer substantially reduced clearance of this drug in humans. In view of the established exposure-toxicity relationships for docetaxel, we suggest that caution is warranted if docetaxel has to be administered together with agents that potentially inhibit both of these transporters.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

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