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Human OATP1B1, OATP1B3 and OATP1A2 can mediate the *in vivo* uptake and clearance of docetaxel

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Organic anion transporting polypeptides (human: OATPs and mouse: Oatps) are uptake transporters with important roles in drug pharmacokinetics and toxicity. We aimed to study the *in vivo* impact of mouse and human OATP1A/1B transporters on docetaxel plasma clearance and liver and intestinal uptake. Docetaxel was administered to Oatp1a/1b knockout and liver-specific humanized OATP1B1, OATP1B3 and OATP1A2 transgenic mice. Experiments were conducted with a low polysorbate 80 (2.8%) formulation, as 8% polysorbate somewhat inhibited docetaxel plasma clearance after intravenous administration. After intravenous administration (10 mg/kg), Oatp1a/1b knockout mice had an approximately threefold higher plasma area under the curve (AUC). Impaired liver uptake was evident from the significantly reduced (approximately threefold) liver-to-plasma AUC ratios. Absence of mouse Oatp1a/1b transporters did not affect the intestinal absorption of orally administered docetaxel (10 mg/kg), while the systemic exposure of docetaxel was again substantially increased owing to impaired liver uptake. Most importantly, liver-specific expression of each of the human OATP1B1, OATP1B3 and OATP1A2 transporters provided a nearly complete rescue of the increased plasma levels of docetaxel in Oatp1a/1b-null mice after intravenous administration. Our data show that one or more of the mouse Oatp1a/1b transporters and each of the human OATP1A/1B transporters can mediate docetaxel uptake *in vivo*. This might be clinically relevant for OATP1A/1B-mediated tumor uptake of docetaxel and for docetaxel clearance in patients in whom the transport activity of OATP1A/1B transporters is reduced owing to genetic variation or pharmacological inhibition, leading to potentially altered toxicity and therapeutic efficacy of this drug.

One of the most widely used chemotherapeutic drugs is docetaxel, a microtubule inhibitor approved for the treatment of breast, lung, ovarian, prostate, gastric and head and neck cancers.[1] An important problem in docetaxel therapy is the interpatient variability in docetaxel exposure, which in turn can lead to unpredictable dose-limiting toxicity (neutropenia and diarrhea) and/or variability in response to treatment.[2] Factors that control plasma exposure to docetaxel include drug-metabolizing enzymes and drug transporters.[2] One of the major clearance mechanisms of docetaxel is metabolism by cytochrome P450 3A (CYP3A), a drug-metabolizing enzyme complex expressed in both intestine and liver.[3] Although there are substantial interindividual differences in expression and activity of CYP3A enzymes, this alone cannot explain entirely the interpatient variability after intravenously administered docetaxel.[1] Recent studies pointed to low-activity polymorphic variants of drug transporters involved in the clearance of docetaxel as contributors to the

Key words: docetaxel, organic anion transporting polypeptides (OATPs), OATP1A/1B, OATP1B1, OATP1B3

Abbreviations: ABC: ATP-binding cassette; ABCB1: P-glycoprotein; ABCC2: multidrug resistance-associated protein 2; AUC: area under plasma concentration–time curve; CYP: cytochrome P450; i.v.: intravenous; LC-MS/MS: liquid chromatography coupled with tandem mass spectrometry; OATP: organic anion transporting polypeptide; SD: standard deviation; SEM: standard error of the mean; Slco1a/1b(−/−): Oatp1a/1b knockout mice; Slco1a/1b(−/−);1A2(Tg): Oatp1a/1b knockout mice with specific expression of human OATP1A2 in liver; Slco1a/1b(−/−);1B1(Tg): Oatp1a/1b knockout mice with specific expression of human OATP1B1 in liver; Slco1a/1b(−/−);1B3(Tg): Oatp1a/1b knockout mice with specific expression of human OATP1B3 in liver

Additional Supporting Information may be found in the online version of this article.

Conflict of interest: The research group of A.H.S. receives revenue from commercial distribution of some of the mouse strains used in this study. J.H.B. is an inventor on patents on the application of oral taxane formulations

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What's new?

Proteins of the organic anion-transporting polypeptide (OATP) superfamily serve major roles in drug transport, potentially influencing drug distribution, action, and toxicity. In the case of the anticancer agent docetaxel, transporters OATP1B1 and OATP1B3, which are expressed in tumors, may affect hepatic clearance and systemic exposure, according to the present study. Liver uptake of docetaxel was found to be impaired in Oatp1a/1b knockout mice, leading to significant increases in drug plasma levels. Plasma levels were reduced with liver-specific expression of human OATP1A/1B transporters. Reduced transporter activity, through genetic variation or pharmacological inhibition, could alter the therapeutic index of docetaxel.

unpredictable systemic exposure of docetaxel.[1,4,5] These transporters involve efflux transporters from the ATP-binding cassette (ABC) transporter family (ABCB1 or ABCC2), but also uptake transporters of the organic anion transporting polypeptide (OATP) family.[1,6]

The OATP superfamily of sodium-independent influx transporters consists of six families, of which the OATP1B subfamily is best studied with respect to clinical pharmacogenetics of drugs.[7] It is important to note that between mouse and human Oatps there are no straighforward orthologs; for example, humans have only one OATP1A transporter (OATP1A2), whereas in mouse there are at least four known transporters (Oatp1a1, Oatp1a4, Oatp1a5 and Oatp1a6). In contrast, humans have two OATP1B transporters (OATP1B1 and OATP1B3), whereas in mouse there is only one (Oatp1b2).[8]

Because of their localization in pharmacokinetically relevant tissues (liver, small intestine and kidney) and their capacity to transport many drugs, OATP1A/1B transporters are thought to play a major role in the distribution, pharmacodynamics and toxicity of many drugs.[9–11] In liver, OATP1B1 and OATP1B3 are highly expressed on the basolateral membrane of hepatocytes where they mediate the hepatic uptake, and therefore clearance of many xenobiotics.[8] OATP1A2 is mainly expressed in other tissues such as brain, kidney and small intestine, whereas in liver it is expressed in cholangiocytes but not in hepatocytes. Its role in drug distribution remains to be elucidated.[8] It is thought that in small intestine human OATP1A2 and/or mouse Oatp1a proteins are expressed on the apical membrane of enterocytes where they might mediate the intestinal uptake of drugs.[12] In addition to their role in the pharmacokinetics of drugs, many OATPs are expressed in breast, gastrointestinal and lung tumors, where they may contribute to the tumor uptake of anticancer drugs.[13]

Docetaxel has been described as a substrate of human OATP1B1 and OATP1B3, and rat and mouse Oatp1b2 *in vitro*,[14–16] but conflicting data have been reported on the *in vivo* contribution of Oatp1b2 to docetaxel pharmacokinetics[14,17] in Oatp1b2 knockout mice. The impact of the functional alterations in uptake capacity of OATP1B1 and/or OATP1B3 on docetaxel pharmacokinetics and toxicity (upon intravenous administration) has been studied in several pharmacogenetic studies, but the results are equivocal,[4,6,14] while the interaction between OATP1A2 and docetaxel has not been reported yet.

In recent years, substantial efforts have been made to obtain an oral formulation for docetaxel.[18] While oral dosing has substantial advantages over intravenous dosing (more patient-friendly, no hospitalization required and lower healthcare costs), it brings the challenge that the drug must pass an additional biological barrier, the intestinal epithelium. It might be that mouse Oatp1a or human OATP1A2 uptake transporters have a role in docetaxel intestinal uptake, while metabolizing enzymes and efflux drug transporters might limit its effective absorption.[8]

Here, we studied the impact of the combined deletion of the mouse *Oatp1a* and *Oatp1b* genes on the disposition of docetaxel after intravenous and oral administration, using Oatp1a/1b knockout mice.[19] We further assessed the *in vivo* impact of human OATP1B1, OATP1B3 and OATP1A2 on the uptake of docetaxel using humanized transgenic mice with liver-specific expression of OATP1B1, OATP1B3 or OATP1A2 in an Oatp1a/1b knockout background.[20,21]

Material and Methods

Animals

Animals were housed in groups as far as possible in a temperature-controlled environment with a 12-hr light/12-hr dark cycle. They received a standard diet (AM-II; Hope Farms) and acidified water *ad libitum*. All mouse experiments were approved by the Animal Experiments Review Board of the Netherlands Cancer Institute (Amsterdam), complying with Dutch legislation and in accordance with European Directive 86/609/EEC. Male wild-type, *Slco1a/1b(−/−)* (Oatp1a/1b knockout), *Slco1a/1b(−/−);1B1(Tg)*, *Slco1a/1b(−/−);1B3(Tg)* and *Slco1a/1b(−/−);1A2(Tg)* (*i.e.*, liver-specific OATP1B1-, OATP1B3- and OATP1A2-humanized transgenic) mice of comparable genetic background (>99% Friend virus B, FVB), between 8 and 14 weeks of age, were used.[20,22] The transgenic expression cassettes used for predominant liver-specific expression of the OATP proteins contained an ApoE promoter upstream, and an hepatic control region downstream of the OATP cDNA inserts.[20]

Chemicals and reagents

Docetaxel was obtained from Sequoia Research Products (Oxford, UK). Isoflurane (Forane) was purchased from Abbott Laboratories (Queenborough, Kent, UK) and heparin (5,000 IE/mL) was from Leo Pharma BV (Breda,

The Netherlands). Bovine serum albumin (BSA), Fraction V was from Roche (Mannheim, Germany) and drug-free lithium-heparinized human plasma was obtained from Bioreclamation LLC (New York, NY). All other reagents (polysorbate 80, ethanol) were from Sigma-Aldrich (Steinheim, Germany).

Pharmacokinetic studies

For intravenous studies a dosage of 10 mg/kg was injected in a volume of 5 μL per gram of bodyweight into the tail vein of mice using a solution containing 2 mg/mL docetaxel. Corresponding with 32.5 mg/m^2, this dose of 10 mg/kg in mice is considered clinically relevant because docetaxel is administered to patients at a weekly dose of 25–40 mg/m^2.[23] For solutions with low polysorbate concentrations (2.77% of polysorbate 80 in the final solution), docetaxel was dissolved in a mixture of ethanol:polysorbate 80 (50:50) to a concentration of 36 mg/mL, which was further diluted before injection with saline to a concentration of 2 mg/mL docetaxel. For solutions containing high polysorbate concentrations (8.3% in the final solution), docetaxel was dissolved in a mixture of ethanol:polysorbate 80 (50:50) to a concentration of 12 mg/mL, which was further diluted before injection with saline to 2 mg/mL docetaxel.

For oral studies, a dosage of 10 mg/kg was given by administration of a volume of 10 μL per gram of bodyweight by oral gavage to the mice using a solution containing 1 mg/mL docetaxel. We used the formulation containing low polysorbate 80 concentrations (2.77% in the final solution): docetaxel was dissolved in a mixture of ethanol:polysorbate 80 (50:50) to a concentration of 18 mg/mL, which was further diluted before dosing with saline to 1 mg/mL docetaxel.

Experiments were terminated (at $t = 3$, 15, 30, 60, 120 and 240 min after i.v. dosing and $t = 5$, 7.5 and 15 min after oral dosing) by isoflurane anesthesia and heparin-blood sampling by cardiac puncture, followed by cervical dislocation and tissue collection. For the oral studies, portal vein blood samples were taken before cardiac puncture. Blood samples were centrifuged at 5,200*g* for 5 min at 4°C and plasma was collected and stored at −30°C until analysis.

Drug analysis

Concentrations of docetaxel in plasma and livers [homogenized in 3 mL of ice-cold 4% (w/v) BSA] were determined by LC-MS/MS analysis as previously described.[24] D_9-labeled docetaxel was used as internal standard for docetaxel. In summary, mouse plasma or tissue homogenate samples of 20 μL were diluted with 180 μL of human plasma. Human plasma was used for dilution of the samples as the concentrations in the undiluted mouse plasma were outside the calibration range and also to mimic the calibration standards that were in human plasma. After dilution of the samples, 25 μL of internal standard working solution was added. Subsequently, the samples were mixed briefly, *tert*-butyl methyl ether was added and the samples were shaken for 10 min at 1,250 rpm. The samples were centrifuged at 23,000*g*, snap-frozen and the organic layer was collected. After evaporation of the organic layer, the samples were reconstituted with 100 μL of 10 mM ammonium hydroxide pH 5:acetonitrile (1:1, v/v) and an aliquot was injected into the LC-MS/MS system. Calibration standards in human plasma in a range of 0.25–500 ng/mL were used for quantification of docetaxel.

Pharmacokinetic and statistical analysis

Averaged plasma concentrations for each time point were used to calculate the area under the blood concentration *versus* time curve (AUC) from $t = 0$ to the last sampling time point by the linear trapezoidal rule; Standard error of the mean (SEM) was calculated by the law of propagation of errors.[25] Pharmacokinetic parameters were calculated using the software package PK Solutions 2.0.2 (SUMMIT, Research Services, Ashland, OH).

When variances were not homogeneous, the data were log-transformed to obtain equal variances. The two-sided unpaired Student's *t*-test was used throughout the study to assess the statistical significance of differences between two sets of data. Statistical significance of differences between wild-type and *Slco1a/1b(−/−)*, *Slco1a/1b(−/−);1B1(Tg)*, *Slco1a/1b(−/−);1B3(Tg)* or *Slco1a/1b(−/−);1A2(Tg)* or between *Slco1a/1b(−/−)* mice and *Slco1a/1b(−/−);1B1(Tg)*, *Slco1a/1b(−/−);1B3(Tg)* or *Slco1a/1b(−/−);1A2(Tg)* mice was assessed by one-way ANOVA followed by Tukey's multiple comparison test. Results are presented as the mean ± SD (standard deviation). Differences were considered to be statistically significant when $p < 0.05$.[20] Statistical analysis was performed using GraphPad (GraphPad Prism version 5.01; GraphPad Software, La Jolla, CA).

Results

Influence of polysorbate 80 concentration on pharmacokinetics of docetaxel

Docetaxel is very poorly soluble in water, and thus the formulation of docetaxel for intravenous administration contains ethanol and polysorbate 80 (or Tween 80), a detergent used to maintain docetaxel in solution, in concentrations between 0.75 and 2% in clinical formulations.[26,27] Recently, there has been an increasing number of reports showing that polysorbate 80 might have an inhibitory effect on the transport activity of OATP uptake transporters.[14,28] Therefore, we started by analyzing the effect of polysorbate 80 concentration in the final formulation on the plasma and liver levels of docetaxel after intravenous administration (10 mg/kg) to wild-type mice. We used two docetaxel formulations: one with high polysorbate 80 concentration (8.3%) and one with a low polysorbate 80 concentration (2.77%). At different time points after dosing, we compared the docetaxel plasma and liver levels and liver-to-plasma ratios in these mice (Fig. 1). After dosing with the high polysorbate 80 formulation, the plasma levels of docetaxel at later time points were modestly, but significantly higher than after dosing with the low polysorbate 80 formulation (Fig. 1*a*), suggesting that polysorbate

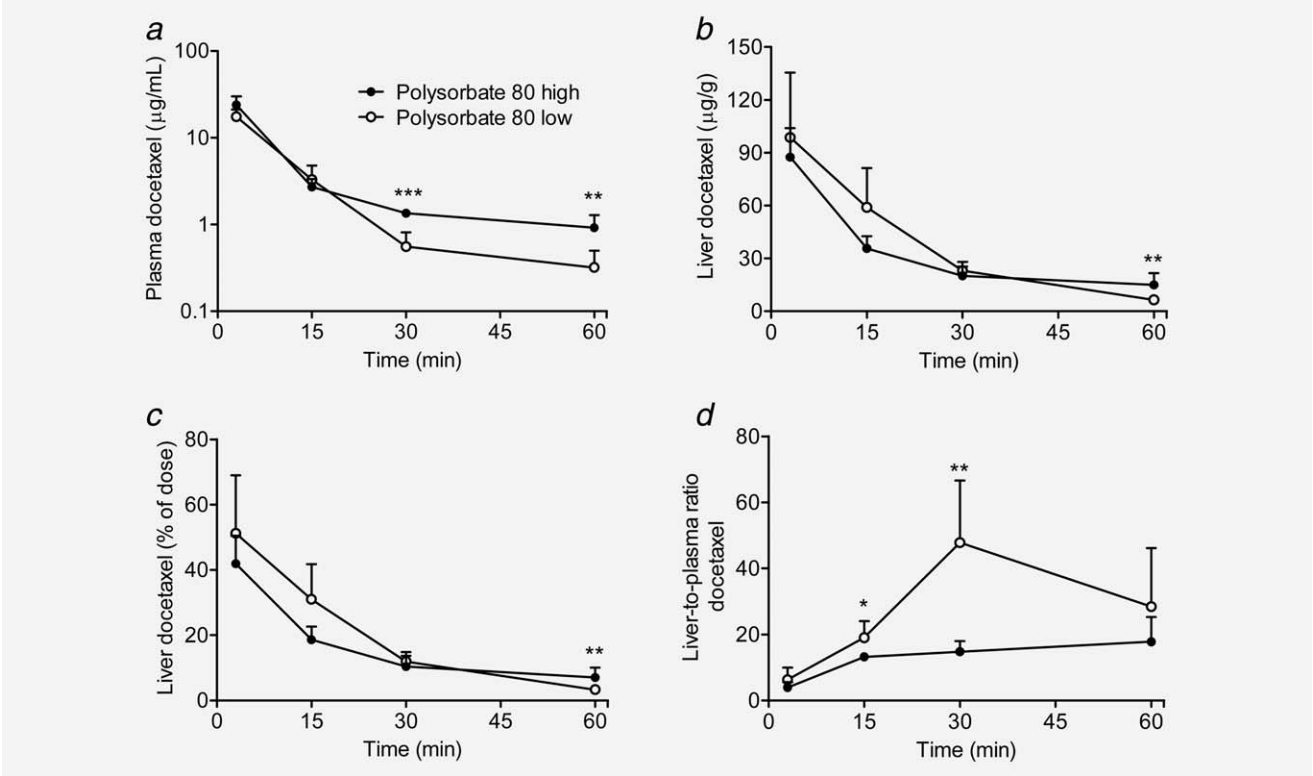

Figure 1. Impact of high (black bars) and low polysorbate (white bars) formulation on plasma and liver levels of docetaxel after administration of 10 mg/kg docetaxel i.v. to male wild-type mice. (*a*) Plasma concentrations of docetaxel, (*b*, *c*) docetaxel liver concentrations as µg/g and % of dose, respectively, and (*d*) liver-to-plasma ratios of docetaxel. Averaged liver-to-systemic plasma ratios were calculated from individual mouse data. A semilog graphical presentation was used for panel *a* to improve resolution at low plasma levels. Data are presented as mean ± SD ($n = 5–6$, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ when compared with wild-type high polysorbate formulation).

80 at high concentrations has a modest *in vivo* inhibitory effect on plasma clearance of docetaxel. The liver concentrations after high polysorbate concentrations were variably, but mostly not significantly affected by high *versus* low polysorbate 80 formulation (Figs. 1*b* and 1*c*). Assessed by the liver-to-plasma ratios, low polysorbate 80 formulation led to generally higher ratios than the high polysorbate 80 formulation (Fig. 1*d*), suggesting that high concentrations of polysorbate 80 might somewhat inhibit the uptake of docetaxel in the liver. On the basis of these results, we used the low (2.77%) polysorbate 80 formulation in our subsequent pharmacokinetic studies. Further reduction of polysorbate 80 in the formulation was not compatible with the comparatively high docetaxel dosages given.

Impact of Oatp1a/1b transporters on plasma and liver exposure of docetaxel after intravenous administration

Docetaxel was described as a mouse Oatp1b2 substrate *in vitro*, whereas conflicting data have been reported on the *in vivo* contribution of Oatp1b2 to docetaxel pharmacokinetics.[14,17] Here, we aimed to study the combined roles of the mouse Oatp1a/1b transporters in the plasma and liver exposure of docetaxel, using Oatp1a/1b knockout mice, which lack all Oatp1a and Oatp1b transporters. After intravenous dosing (10 mg/kg), plasma exposure of docetaxel was significantly increased in the Oatp1a/1b knockout mice in comparison with wild-type mice (Fig. 2*a*). The area under the curve (AUC) of the plasma concentrations in Oatp1a/1b-null mice was 2.9-fold higher than that in the wild-type mice (608.7 ± 25.2 *versus* 211.8 ± 19.8 µg min/mL, mean ± SEM; $p < 0.001$), indicating that disposition of docetaxel is impaired in the absence of Oatp1a/1b transporters.

OATP1A/1B transporters mediate mainly the liver uptake of drugs, thus controlling their clearance and disposition. Therefore, we also measured the liver concentrations. Similar to previous studies with rosuvastatin,[29] the liver exposure (AUC) of docetaxel was not markedly different between the two strains of mice (Figs. 2*b* and 2*c*), whereas the liver-to-plasma ratios were markedly reduced at virtually all time points after dosing (Fig. 2*d*). As discussed before for analogous rosuvastatin data,[29] and as explained in more detail in the Discussion section, this suggests that liver uptake of docetaxel is substantially impaired in the Oatp1a/1b-null mice. We note that at later time points, when plasma and liver concentrations were quite low, a somewhat higher liver concentration was observed in the Oatp1a/1b-null mice, likely mainly reflecting the markedly higher plasma concentrations in this strain (Figs. 2*b* and 2*c*).

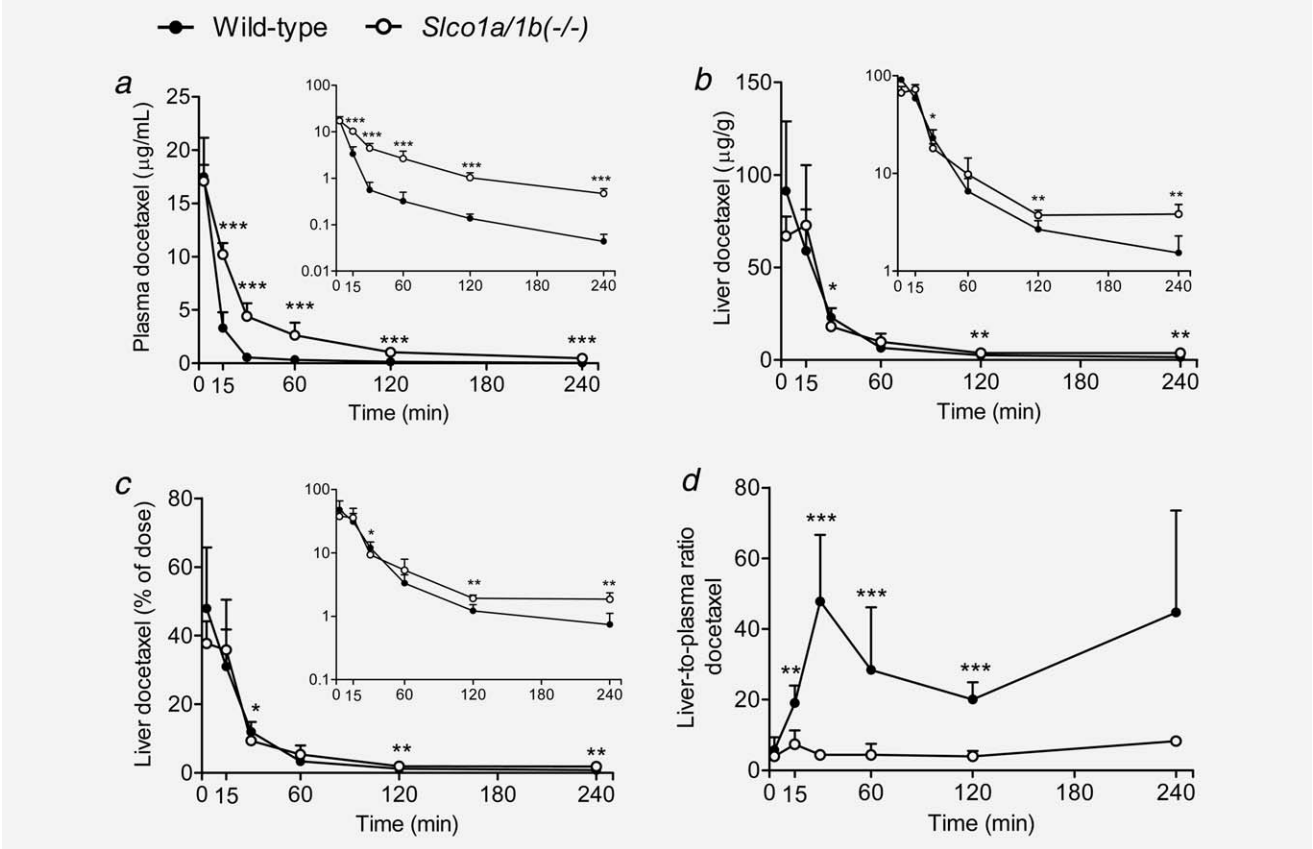

Figure 2. Role of Oatp1a/1b uptake transporters in the plasma and liver exposure of docetaxel after intravenous administration of docetaxel (10 mg/kg) to male wild-type and Oatp1a/1b knockout mice. (*a*) Plasma concentrations of docetaxel, (*b*, *c*) docetaxel liver concentrations as μg/g and % of dose, respectively, and (*d*) liver-to-plasma ratios of docetaxel. Averaged liver-to-systemic plasma ratios were calculated from individual mouse data. Data are presented as mean ± SD ($n = 5–6$, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ when compared with wild-type).

Oatp1a transporters are not essential for the intestinal absorption of docetaxel

In contrast to Oatp1b2 that is exclusively expressed in the liver, members of the Oatp1a family are also expressed in enterocytes,[12] where they are thought to contribute to the intestinal absorption of drugs. Therefore, we assessed the impact of Oatp1a on the intestinal absorption of docetaxel, again using the low polysorbate 80 formulation (2.77%). We compared portal vein concentrations in Oatp1a/1b knockout and wild-type mice, shortly after oral administration (10 mg/kg) of docetaxel. However, docetaxel portal vein concentrations were substantially and significantly higher at all time points in the Oatp1a/1b knockout mice, rather than lower (Fig. 3*a*). Note that the high interindividual variation necessitated log-transformation of the data before statistical testing (Supporting Information Fig. 1). These results suggest that Oatp1a transporters are not essential in the intestinal absorption of docetaxel. The increased portal vein concentrations at all time points after dosing likely reflect in part the higher systemic plasma concentrations of docetaxel in the Oatp1a/1b-null mice (Fig. 3*b*) resulting from impaired liver uptake, as seen previously in the intravenous experiment (Figs. 2, 3*d* and 3*e*). This impaired liver uptake was evident both in the liver-to-systemic plasma and liver-to-portal vein plasma ratios (Figs. 3*d* and 3*e*). The log-transformed data of these experiments required for statistical testing are presented in Supporting Information Figure 1.

Human OATP1B1, OATP1B3 and OATP1A2 can transport docetaxel in vivo

In the human liver, OATP1B1 and OATP1B3 are expressed on the basolateral membrane of hepatocytes. Although not straightforward homologs of the individual mouse Oatp1a/1b proteins, based on amino acid homology, substrate specificity and tissue localization, they are considered to fulfill the same roles as the basolateral mouse Oatp1a/1b transporters in the liver.[20,25,30] We recently generated and characterized humanized mice with hepatocyte-specific expression of OATP1B1, OATP1B3 and OATP1A2 [in an *Slco1a/1b(−/−)* background].[20,21] The transgenic proteins in these strains were primarily expressed in liver, although some OATP1B3 expression was also seen in kidney.[20,21] Note that *Slco1a/1b(−/−);1A2(Tg)* mice do not represent a physiological model for the role of OATP1A2 in hepatic uptake of drugs,

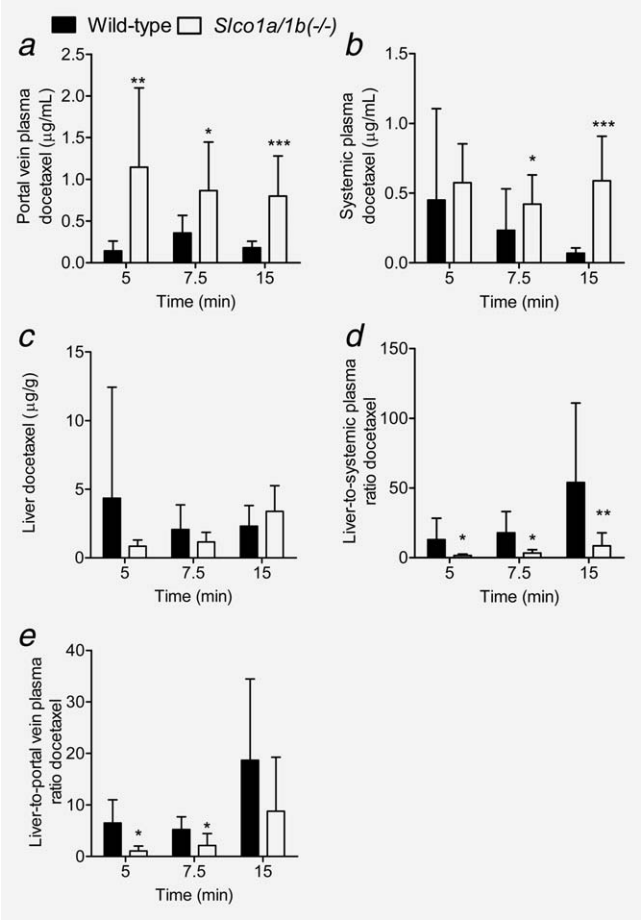

Figure 3. Mouse Oatp1a proteins are not essential for the intestinal absorption of docetaxel after oral administration (10 mg/kg) to male wild-type and Oatp1a/1b knockout mice. (*a*) Docetaxel portal vein plasma concentrations and (*b*) docetaxel systemic plasma concentrations, (*c*) docetaxel liver levels in μg/g, (*d*) liver-to-systemic plasma ratios and (*e*) liver-to-portal vein plasma ratios. Averaged liver-to-systemic plasma (or liver-to-portal vein plasma) ratios were calculated from individual mouse data. Data are presented as mean ± SD ($n = 5–6$, $*p < 0.05$; $**p < 0.01$ when compared with wild-type).

as hepatic OATP1A2 in humans is expressed only in cholangiocytes and not in hepatocytes. Nevertheless, this mouse model has proved to be useful in studying the *in vivo* transport capacity of OATP1A2, which might be relevant for its activity in other healthy or malignant tissues.[20]

We used these models to assess the capacity of the human OATP1A/1B proteins to transport docetaxel *in vivo*. After 10 mg/kg i.v. dosing, the increased docetaxel plasma concentrations in the Oatp1a/1b-null mice were partially or completely brought back to wild-type levels in all the humanized mouse strains (Fig. 4*a*). Only at 30 min after dosing, plasma levels in the OATP1B3-humanized mice were as high as in the Oatp1a/1b-null mice, perhaps owing to experimental variation (Fig. 4*a*). Also plotted as plasma AUC from 15 till 60 min in these strains (Fig. 5*a*), the plasma AUC in the Oatp1a/1b-knockout mice was fourfold increased in comparison with the wild-type mice. The rescue provided by the presence of OATP1B1 or OATP1A2 was obvious as the plasma AUC values were quite similar to the values in the wild-type mice and significantly lower than in the Oatp1a/1b knockout mice (Fig. 5*a*). The values in the OATP1B3-humanized mice were intermediate between the values in the wild-type and Oatp1a/1b-null mice, most probably owing to the high plasma concentrations in this strain at 30 min after administration (Fig. 4*a*).

As seen before (Fig. 2*b*), the liver levels were not significantly changed in the Oatp1a/1b-null mice in comparison with wild-type mice, and accordingly shifts in liver concentration due to the role of human OATP1A/1B transporters in rescuing the absence of mouse Oatp1a/1b transporters were modest, albeit significant for OATP1B1 and OATP1B3 at 30 min (Fig. 4*b*). However, the liver AUC levels from 15 to 60 min were not significantly altered in any of the tested strains (Fig. 5*b*).

Liver-to-plasma ratios in the humanized mice (in OATP1A2 mice especially) were also significantly higher than in the knockout mice (Fig. 4*c*). However, the liver-to-plasma ratios were not up to the levels seen in wild-type mice (Fig. 4*c*).

This partial rescue by human OATP1A/1B transporters in liver-to-plasma ratios of docetaxel is likely an underestimation of the actual OATP1B-mediated liver uptake in humans, as in human liver both OATP1B1 and OATP1B3 function at the same time, likely resulting in additive effects. Nevertheless, these results show that human OATP1B1, OATP1B3 and OATP1A2 can transport docetaxel *in vivo* and that all three transporters can compensate to an extent for the loss of the murine Oatp1a/1b transporters.

Discussion

In our study, we found that mouse Oatp1a/1b transporters contribute clearly to the plasma clearance of docetaxel, whereas impaired liver uptake in the absence of Oatp1a/1b transporters was obvious in the markedly reduced liver-to-plasma ratios. Importantly, liver-specific expression of human OATP1B1, OATP1B3 or OATP1A2 provided substantial rescue of the increased plasma levels of docetaxel in Oatp1a/1b knockout mice, albeit that, as assessed by liver-to-plasma ratios, this rescue was not entirely complete. Oatp1a/1b transporters did not appear to contribute to intestinal docetaxel uptake. Our findings indicate that all the human OATP1A/1B uptake transporters, when expressed in hepatocytes, can mediate the liver uptake of docetaxel *in vivo*, and can thus contribute to hepatic docetaxel clearance, and, by implication, possibly docetaxel tumor uptake *in vivo* as well.

Although it has been described that human OATP1B1 and OATP1B3 can transport docetaxel *in vitro*, there is wide variability dependent on the type of cellular uptake system used.[1,14,15] Our data demonstrate docetaxel transport by OATP1B1 and OATP1B3 in hepatocytes *in vivo*. Transport activity of OATP1B1 or OATP1B3 can be reduced as a

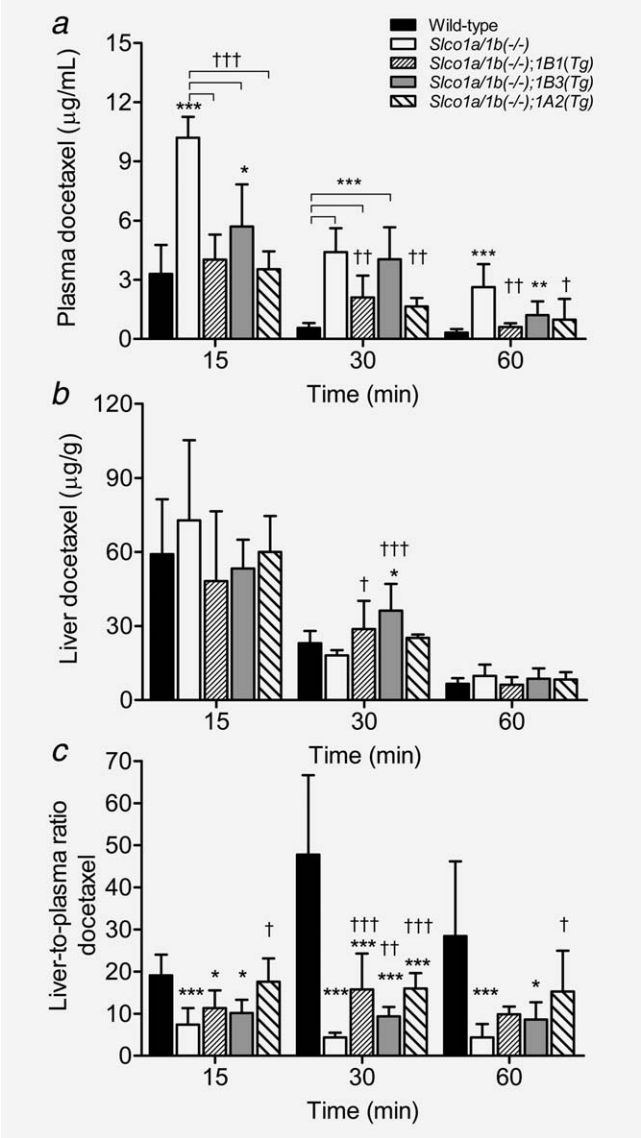

Figure 4. Human OATP1B1, OATP1B3 and OATP1A2 transport docetaxel *in vivo* after i.v. administration of docetaxel (10 mg/kg) to male wild-type, Oatp1a/1b knockout and OATP1B1, -1B3 and -1A2 transgenic mice. (*a*) Plasma concentrations of docetaxel, (*b*) docetaxel liver concentrations and (*c*) liver-to-plasma ratios of docetaxel. Averaged liver-to-systemic plasma ratios were calculated from individual mouse data. Data are presented as mean ± SD ($n = 5–6$, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ when compared with wild-type, $\dagger p < 0.05$; $\dagger\dagger p < 0.01$; $\dagger\dagger\dagger p < 0.001$ when compared with Oatp1a/1b knockout mice).

consequence of genetic variation (polymorphic variants) or pharmacological inhibition, leading to decreased liver uptake of docetaxel and thus impaired plasma clearance. Yet there are only two clinical studies that show an association between low-activity polymorphisms in the gene encoding OATP1B3 and altered docetaxel pharmacokinetics[4] or docetaxel-induced neutropenia,[31] whereas others investigating the impact of polymorphic variants of OATP1B1 or OATP1B3 on docetaxel pharmacokinetics and toxicity did not find any associations.[1,6,14,32] Our data suggest that in the case of single polymorphisms affecting only one of the human OATP1B transporters, the remaining OATP1B1 or OATP1B3 could at least partially compensate for the loss of function of the other transporter, as both can mediate docetaxel uptake into liver. Accordingly, single polymorphisms in the OATP1B1 or OATP1B3 genes were not clearly associated with altered docetaxel clearance.[14] This may be different for Rotor syndrome patients, which are deficient in both OATP1B1 and OATP1B3,[21] and might thus be at risk of developing life-threatening toxicity when treated with docetaxel. Also, clinically relevant drug–drug interactions might occur when docetaxel is co-administered with effective inhibitors of OATP1B transporters (*e.g.*, rifampicin, cyclosporine and statins) or other OATP1B substrates (*e.g.*, statins and methotrexate) that might compete for transport into the liver.[33]

To the best of our knowledge, we show here for the first time that OATP1A2 can transport docetaxel *in vivo* and that it can mediate docetaxel liver uptake. Because expression of OATP1A2 in human liver is restricted to cholangiocytes, the epithelial cells of the bile duct, the information provided by our humanized OATP1A2 mice (in which OATP1A2 is expressed in hepatocytes) has only qualitative meaning. Nevertheless, this information is relevant for the function of OATP1A2 in other tissues such as small intestine, kidney and the blood–brain barrier, where it might affect the oral uptake, urinary reabsorption or brain penetration of docetaxel.[34–36] The role of OATP1A2 in small intestine, and thus in the oral bioavailability of docetaxel, remains unclear as in our study we observed that mouse Oatp1a transporters are not essential for the intestinal absorption of docetaxel. Theoretically, underexpression or overexpression of other intestinal docetaxel transporters, such as Abcb1a (P-glycoprotein), Abcc2/Mrp2 or Oatp2b1 in Oatp1a/1b-null mice, might have masked an effect of removal of Oatp1a proteins on the net intestinal absorption of docetaxel (Fig. 3). However, we previously found that the intestinal RNA levels of these transporters are unchanged in Oatp1a/1b-null mice,[20,22] making this possibility less likely. Importantly, when expressed in tumor cells, OATP1A2 can likely affect the susceptibility of these cells to docetaxel chemotherapy *in vivo* by altering the effective intracellular docetaxel exposure.[13]

OATP1A/1B proteins have been found expressed in tumors of almost all the cancer types that are currently treated with docetaxel: colorectal, gastric, ovarian, breast and lung cancer (reviewed in Refs. 13 and 37−39) and OATP1A2 has also been found in the blood–brain barrier of gliomas.[40] Previous studies in our group showed that other anticancer drugs, namely methotrexate and paclitaxel, are also transported *in vivo* by human OATP1A/1B proteins.[20] These data suggest that *in vivo* expression and activity of OATP1A/1B transporters in various tumors may affect the tumor drug uptake, and hence influence their sensitivity to certain anticancer drugs.[37] Direct studies involving sensitivity to docetaxel in cell lines derived from these tumor types are lacking

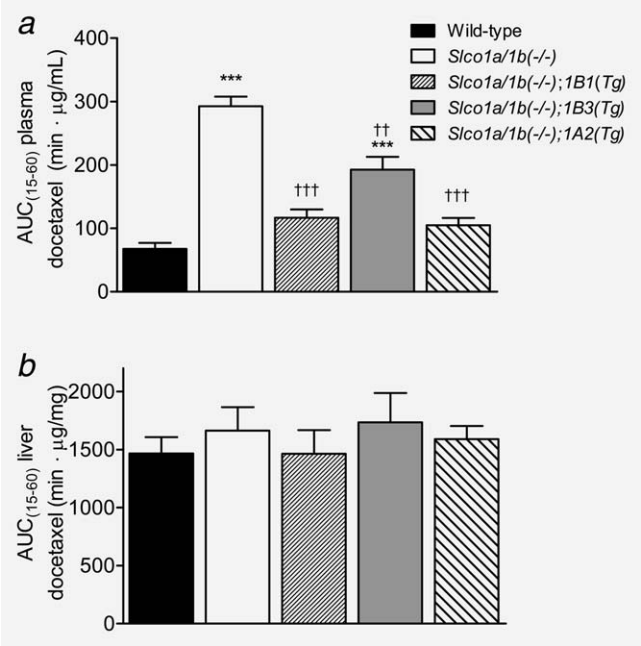

Figure 5. Area under the curve (15–60 min) of (*a*) plasma and (*b*) liver after i.v. administration of docetaxel (10 mg/kg) to male wild-type, Oatp1a/1b knockout and OATP1B1, -1B3 and -1A2 transgenic mice. Averaged liver-to-systemic plasma ratios were calculated from individual mouse data. Data are presented as mean ± SEM ($n = 5–6$, ***$p < 0.001$ when compared with wild-type, ††$p < 0.01$; †††$p < 0.001$ when compared with Oatp1a/1b knockout mice).

so far, although there are indications that for paclitaxel and methotrexate, expression and activity of OATP1B1 and/or OATP1B3 transporters can increase sensitivity of tumors cells to these drugs.[39,41] Moreover, there are several studies trying to correlate the tumor expression levels of OATPs with tumor development and prognosis.[42,43]

In our study, we aimed among others to investigate whether the combined mouse Oatp1a/1b transporters expressed on the basolateral membrane of hepatocytes have a substantial role in mediating the liver uptake, and hence plasma clearance of docetaxel. Conflicting results have been reported previously for i.v. docetaxel pharmacokinetics in single Oatp1b2 knockout mice. Although de Graan *et al.*[14] initially reported a 26-fold increase in plasma AUC in an Oatp1b2 knockout strain compared to its matched wild-type strain, a subsequent report by the same group found only a 1.7-fold increase in AUC in the same mice with the same i.v. dose of docetaxel.[17] No explanation was provided for this discrepancy, but a 13-fold higher reported wild-type plasma AUC in the second report compared to the first report appeared to be a major factor. The first report found a four-fold increase (rather than the expected equal level or perhaps decrease) in liver docetaxel AUC in the Oatp1b2 knockout mice compared to wild-type mice, whereas the second report provided no data on liver concentrations. The actual contribution of Oatp1b2 to docetaxel liver uptake and plasma clearance therefore remains open for further analysis. In view of the poor reproducibility of the reported data for Oatp1b2 knockout mice,[14,17] a detailed comparison of our findings with those data seems currently unwarranted.

We observed a threefold higher docetaxel plasma AUC between full Oatp1a/1b knockout mice and (FVB) wild-type mice, indicating a marked contribution of Oatp1a/1b transporters to docetaxel plasma clearance. At the same time, we observed very similar liver AUCs between FVB wild-type and Oatp1a/1b knockout mice and a markedly decreased liver-to-plasma ratio (Figs. 2*b*–2*d*). This latter pharmacokinetic behavior is very similar to what we previously reported for rosuvastatin,[29] where reduced liver uptake (owing to the absence of Oatp1a/1b transporters) resulted in marked changes in plasma exposure and liver-to-plasma ratios, without affecting the liver concentrations. This pharmacokinetic behavior is consistent with the physiologically base pharmacokinetic model of Watanabe *et al.* for drugs with a low renal clearance and for which the liver uptake is the main rate-limiting elimination step,[44–46] as appears to be the case for pravastatin, rosuvastatin and docetaxel. Put in simplified terms, when most of the drug is usually cleared through the liver, and there is no efficient alternative clearance route, strongly decreased liver uptake by knockout of sinusoidal drug uptake systems rapidly results in higher plasma drug levels. These subsequently drive relatively increased liver uptake, as long as there is still some residual sinusoidal uptake transporter activity for the drug left. In view of the abundance and often partial redundance between multispecific sinusoidal drug uptake transporters, these conditions are often met, even when three sinusoidal transporters (Oatp1a1, Oatp1a4 and Oatp1b2) are simultaneously knocked out. For instance, sinusoidal Oatp2b1 might be one of the uptake transporters taking over. The net effect often is increased plasma level of the drug, and clearly decreased liver-to-plasma ratios, but not much effect on the steady-state liver levels of the drug.[29,44–46]

In line with previous studies,[14,26,28] we here provide *in vivo* pharmacokinetic evidence that polysorbate 80 at high concentrations can have an inhibitory effect on the plasma clearance of docetaxel, probably by inhibiting Oatp1a/1b-mediated liver uptake. Perhaps new formulations, which have been studied recently, will provide a better alternative to current polysorbate 80 formulations (reviewed in Refs. 47 and 48).

Taken together, our results suggest that human OATP1A/1B uptake transporters can have multiple effects on the docetaxel therapeutic index, not only by controlling its general plasma and tissue pharmacokinetics and toxicity but also by possibly mediating its tumor uptake. OATP1A/1B transporters might thus represent a valuable target for modulators to improve chemotherapy. Further studies using humanized OATP1A/1B mice may help to better assess the *in vivo* impact of OATP1A/1B transporters on pharmacokinetics, toxicity and therapeutic outcome of anticancer drugs.

References

1. Baker SD, Verweij J, Cusatis GA, et al. Pharmacogenetic pathway analysis of docetaxel elimination. *Clin Pharmacol Ther* 2009;85:155–63.
2. Baker SD, Sparreboom A, Verweij J. Clinical pharmacokinetics of docetaxel: recent developments. *Clin Pharmacokinet* 2006;45:235–52.
3. van Herwaarden AE, Wagenaar E, van der Kruijssen CM, et al. Knockout of cytochrome P450 3A yields new mouse models for understanding xenobiotic metabolism. *J Clin Invest* 2007;117:3583–92.
4. Chew SC, Singh O, Chen X, et al. The effects of CYP3A4, CYP3A5, ABCB1, ABCC2, ABCG2 and SLCO1B3 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of docetaxel in nasopharyngeal carcinoma patients. *Cancer Chemother Pharmacol* 2011;67:1471–8.
5. Tran A, Jullien V, Alexandre J, et al. Pharmacokinetics and toxicity of docetaxel: role of CYP3A, MDR1, and GST polymorphisms. *Clin Pharmacol Ther* 2006;79:570–80.
6. Chew SC, Sandanaraj E, Singh O, et al. Influence of SLCO1B3 haplotype-tag SNPs on docetaxel disposition in Chinese nasopharyngeal cancer patients. *Br J Clin Pharmacol* 2012;73:606–18.
7. Kalliokoski A, Niemi M. Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* 2009;158:693–705.
8. Iusuf D, van de Steeg E, Schinkel AH. Functions of OATP1A and 1B transporters in vivo: insights from mouse models. *Trends Pharmacol Sci* 2012; 33:100–8.
9. Hagenbuch B, Meier PJ. Organic anion transporting polypeptides of the OATP/ SLC21 family: phylogenetic classification as OATP/ SLCO superfamily, new nomenclature and molecular/ functional properties. *Pflugers Arch* 2004;447:653–65.
10. Konig J, Seithel A, Gradhand U, et al. Pharmacogenomics of human OATP transporters. *Naunyn Schmiedebergs Arch Pharmacol* 2006;372:432–43.
11. Niemi M. Role of OATP transporters in the disposition of drugs. *Pharmacogenomics* 2007;8:787–802.
12. Klaassen CD, Aleksunes LM. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev* 2010;62:1–96.
13. Cutler MJ, Choo EF. Overview of SLC22A and SLCO families of drug uptake transporters in the context of cancer treatments. *Curr Drug Metab* 2011;12:793–807.
14. de Graan AJ, Lancaster CS, Obaidat A, et al. Influence of polymorphic OATP1B-type carriers on the disposition of docetaxel. *Clin Cancer Res* 2012;18:4433–40.
15. Smith NF, Acharya MR, Desai N, et al. Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. *Cancer Biol Ther* 2005;4: 815–18.
16. Yamaguchi H, Kobayashi M, Okada M, et al. Rapid screening of antineoplastic candidates for the human organic anion transporter OATP1B3 substrates using fluorescent probes. *Cancer Lett* 2008;260:163–9.
17. Hu S, Mathijssen RH, de Bruijn P, et al. Inhibition of OATP1B1 by tyrosine kinase inhibitors: in vitro-in vivo correlations. *Br J Cancer* 2014; 110:894–8.
18. Moes JJ, Koolen SL, Huitema AD, et al. Pharmaceutical development and preliminary clinical testing of an oral solid dispersion formulation of docetaxel (ModraDoc001). *Int J Pharm* 2011;420: 244–50.
19. van de Steeg E, van Esch A, Wagenaar E, et al. High impact of Oatp1a/1b transporters on in vivo disposition of the hydrophobic anticancer drug paclitaxel. *Clin Cancer Res* 2011;17:294–301.
20. van de Steeg E, van Esch A, Wagenaar E, et al. Influence of human OATP1B1, OATP1B3, and OATP1A2 on the pharmacokinetics of methotrexate and paclitaxel in humanized transgenic mice. *Clin Cancer Res* 2013;19:821–32.
21. van de Steeg E, Stranecky V, Hartmannova H, et al. Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. *J Clin Invest* 2012;122:519–28.
22. van de Steeg E, Wagenaar E, van der Kruijssen CM, et al. Organic anion transporting polypeptide 1a/1b-knockout mice provide insights into hepatic handling of bilirubin, bile acids, and drugs. *J Clin Invest* 2010;120:2942–52.
23. Engels FK, Verweij J. Docetaxel administration schedule: from fever to tears? A review of randomised studies. *Eur J Cancer* 2005;41:1117–26.
24. Kuppens IE, van Maanen MJ, Rosing H, et al. Quantitative analysis of docetaxel in human plasma using liquid chromatography coupled with tandem mass spectrometry. *Biomed Chromatogr* 2005;19:355–61.
25. Iusuf D, Sparidans RW, van Esch A, et al. Organic anion-transporting polypeptides 1a/1b control the hepatic uptake of pravastatin in mice. *Mol Pharm* 2012;9:2497–504.
26. Ten Tije AJ, Loos WJ, Verweij J, et al. Disposition of polyoxyethylated excipients in humans: implications for drug safety and formulation approaches. *Clin Pharmacol Ther* 2003;74:509–10.
27. van Tellingen O, Beijnen JH, Verweij J, et al. Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. *Clin Cancer Res* 1999;5:2918–24.
28. Engel A, Oswald S, Siegmund W, et al. Pharmaceutical excipients influence the function of human uptake transporting proteins. *Mol Pharm* 2012;9:2577–81.
29. Iusuf D, van Esch A, Hobbs MJ, et al. Murine Oatp1a/1b uptake transporters control rosuvastatin systemic exposure without affecting its apparent liver exposure. *Mol Pharmacol* 2013;83:919–29.
30. Iusuf D, van de Steeg E, Schinkel AH. Hepatocyte hopping of OATP1B substrates contributes to efficient hepatic detoxification. *Clin Pharmacol Ther* 2012;92:559–62.
31. Kiyotani K, Mushiroda T, Kubo M, et al. Association of genetic polymorphisms in SLCO1B3 and ABCC2 with docetaxel-induced leukopenia. *Cancer Sci* 2008;99:967–72.
32. Lewis LD, Miller AA, Owzar K, et al. The relationship of polymorphisms in ABCC2 and SLCO1B3 with docetaxel pharmacokinetics and neutropenia: CALGB 60805 (Alliance). *Pharmacogenet Genomics* 2013;23:29–33.
33. Karlgren M, Vildhede A, Norinder U, et al. Classification of inhibitors of hepatic organic anion transporting polypeptides (OATPs): influence of protein expression on drug-drug interactions. *J Med Chem* 2012;55:4740–63.
34. Gao B, Hagenbuch B, Kullak-Ublick GA, et al. Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J Pharmacol Exp Ther* 2000;294:73–9.
35. Glaeser H, Bailey DG, Dresser GK, et al. Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clin Pharmacol Ther* 2007;81:362–70.
36. Lee W, Glaeser H, Smith LH, et al. Polymorphisms in human organic anion-transporting polypeptide 1A2 (OATP1A2): implications for altered drug disposition and central nervous system drug entry. *J Biol Chem* 2005;280:9610–17.
37. Obaidat A, Roth M, Hagenbuch B. The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. *Annu Rev Pharmacol Toxicol* 2012;52:135–51.
38. Sissung TM, Reece KM, Spencer S, et al. Contribution of the OATP1B subfamily to cancer biology and treatment. *Clin Pharmacol Ther* 2012;92: 658–60.
39. Svoboda M, Wlcek K, Taferner B, et al. Expression of organic anion-transporting polypeptides 1B1 and 1B3 in ovarian cancer cells: relevance for paclitaxel transport. *Biomed Pharmacother* 2011;65:417–26.
40. Bronger H, Konig J, Kopplow K, et al. ABCC drug efflux pumps and organic anion uptake transporters in human gliomas and the blood-tumor barrier. *Cancer Res* 2005;65: 11419–28.
41. Abe T, Kakyo M, Tokui T, et al. Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *J Biol Chem* 1999;274:17159–63.
42. Maeda T, Irokawa M, Arakawa H, et al. Uptake transporter organic anion transporting polypeptide 1B3 contributes to the growth of estrogen-dependent breast cancer. *J Steroid Biochem Mol Biol* 2010;122:180–5.
43. Pressler H, Sissung TM, Venzon D, et al. Expression of OATP family members in hormone-related cancers: potential markers of progression. *PLoS One* 2011;6:e20372.
44. Watanabe T, Kusuhara H, Sugiyama Y. Application of physiologically based pharmacokinetic modeling and clearance concept to drugs showing transporter-mediated distribution and clearance in humans. *J Pharmacokinet Pharmacodyn* 2010; 37:575–90.
45. Yoon I, Han S, Choi YH, et al. Saturable sinusoidal uptake is rate-determining process in hepatic elimination of docetaxel in rats. *Xenobiotica* 2012; 42:1110–19.
46. Watanabe T, Kusuhara H, Maeda K, et al. Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. *J Pharmacol Exp Ther* 2009;328:652–62.
47. Engels FK, Mathot RA, Verweij J. Alternative drug formulations of docetaxel: a review. *Anticancer Drugs* 2007;18:95–103.
48. Hennenfent KL, Govindan R. Novel formulations of taxanes: a review. Old wine in a new bottle? *Ann Oncol* 2006;17:735–49.