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Semi-physiologically based pharmacokinetic modeling of paclitaxel metabolism and in silico-based study of the dynamic sensitivities in pathway kinetics

Martin N. Fransson^{a,*}, Jan Brugård^b, Peter Aronsson^b, Henrik Gréen^{c,d}

^a*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden*

^b*MathCore Engineering AB, Linköping, Sweden*

^c*Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden*

^d*Science for Life Laboratory, School of Biotechnology, Division of Gene Technology, KTH Royal Institute of Technology, Solna, Sweden*

Abstract

Purpose: To build a semi-physiologically based pharmacokinetic model describing the uptake, metabolism and efflux of paclitaxel and its metabolites and investigate the effect of hypothetical genetic polymorphisms causing reduced uptake, metabolism or efflux in the pathway by model simulation and sensitivity analysis.

Methods: A previously described intracellular pharmacokinetic model was used as a starting point for model development. Kinetics for metabolism, transport, binding and systemic and output compartments were added to mimic a physiological model with hepatic elimination. Model parameters were calibrated using constraints postulated as ratios of concentrations and amounts of metabolites and drug in the systemic plasma and output compartments. The sensitivity in kinetic parameters was tested using dynamic sensitivity analysis.

Results: Predicted plasma concentrations of drug and metabolites were in the range of what has been observed in clinical studies. Given the final model,

*Corresponding author. Present address: Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, PO Box 281, SE-171 77, Stockholm, Sweden. Tel.: +46 8 524 839 74; fax: +46 8 31 49 75.

Email address: Martin.Fransson@ki.se (Martin N. Fransson)

plasma concentrations of paclitaxel seems to be relatively little affected by changes in metabolism or transport, while its main metabolite may be largely affected even by small changes. If metabolites prove to be clinically relevant, genetic polymorphisms may play an important role for individualizing paclitaxel treatment.

Keywords: Paclitaxel metabolism, Mathematical modeling, Pharmacokinetics, Sensitivity analysis, CYP2C8, CYP3A4, OATP, ABCB1

1. Introduction

Typically, parametric pharmacokinetic modeling starts with fitting one- or several compartments to observed drug concentrations in plasma from repeated sampling from one or several individuals. The latter case is subject to so called population pharmacokinetics, where the use of nonlinear mixed effects models allow variability in the population to be tested against potential covariates, such as body-mass, gender or genetic polymorphisms. Finding significant covariates is especially important for chemotherapeutic drugs because of their potency and narrow therapeutic index (Undevia et al., 2005).

In the case of paclitaxel, a mitotic inhibitor used in treatment for a range of different tumors, the use of population pharmacokinetic models has indicated sex, age, body weight and bilirubin to be significant covariates (Henningsson et al., 2003; Joerger et al., 2006). However, these covariates explain only a smaller part of the population variability, and it is believed that pharmacogenetics could play an important role in further reducing variability (Sparreboom and Figg, 2006). For paclitaxel, potential genetic polymorphisms affecting pharmacokinetics may be associated with the organic anion-transporting polypeptide (OATP) at cellular uptake (Smith et al., 2005), cytochromes P450 2C8 and 3A4/5 (CYP2C8, CYP3A4/5) (Monsarrat et al., 1993; Cresteil et al., 1994; Harris et al., 1994b; Rahman et al., 1994) during hepatic metabolism or the transporter protein ABCB1 at cellular efflux (Jang et al., 2001). Two large studies have so far been investigating genetic polymorphisms in association to paclitaxel pharmacokinetics, using the population pharmacokinetic approach (Henningsson et al., 2005a; Bergmann et al., 2011). While Henningsson et al. (2005a) found no associations for polymorphisms in CYP2C8, CYP3A4/5 or ABCB1, Bergmann et al. (2011) reported the allele CYP2C8*3 to cause an 11% decrease in paclitaxel clearance. The same study also found evidence that the CYP2C8*4 and ABCC1

may have an effect on clearance, but found no significant influence of genetic variants in OATP, CYP3A4/5 or ABCB1. In contrast, a few minor studies have reported ABCB1 polymorphisms to affect either the kinetics of paclitaxel (Yamaguchi et al., 2006; Green et al., 2009) or of its hydroxy metabolites (Nakajima et al., 2005; Fransson et al., 2011).

In addition to the top-down pharmacokinetic modeling approach, where the study data may be a limiting factor for model complexity (Aarons, 2005), a complementary mechanistic approach may help to understand the relative importance of the targeted genes, and consequently what findings that may be expected from a clinical study. For this reason, the aim of the present work was to develop a semi-physiologically based pharmacokinetic model with detailed description of the uptake, metabolism and efflux of paclitaxel and its metabolites, by extending an existing in vitro derived intracellular pharmacokinetic model and integration of existing population pharmacokinetic models. The hypothetical effect of genetic polymorphisms causing reduced uptake, metabolism or efflux in the pathway was then investigated by the use of simulation-based dynamic sensitivity analysis.

2. Material and methods

2.1. Model development

A semi-physiologically based pharmacokinetic model was developed using the software MathModelica ver. 2.1 (MathCore Engineering AB, Linköping, Sweden), and combined low- and high-level pharmacokinetic models. An intracellular pharmacokinetic model used to assess the uptake and efflux of paclitaxel in tumor cells by Kuh et al. (2000) and Jang et al. (2001), was used as a starting point for model development. Pharmacodynamic effects on the number of cells were omitted. Kinetic parameters depending on cell number were up-scaled to represent a female liver of 1475 g (de la Grandmaison et al., 2001) with a hepatocellularity of 9.9×10^7 cells/g (Barter et al., 2007) resulting in a cell number of 1.5×10^{11} . The original model, comprising one compartment each for cells, considered here to be liver tissue, equation (3e), and cell medium, considered to be liver plasma, (3c), was then extended with a reservoir or systemic plasma compartment, (3a), and output compartment, (3g), to conceptually mimic a physiological model for hepatic elimination (Sirianni and Pang, 1997). A saturable transport mechanism, representing hepatic uptake facilitated by OATP (Smith et al., 2005), was introduced

between the liver plasma compartment, (3c), and liver tissue compartment, (3e).

In hepatocytes, paclitaxel is mainly metabolized by CYP2C8 to 6 α -hydroxypaclitaxel (Harris et al., 1994a; Kumar et al., 1994; Rahman et al., 1994), or by CYP3A4 to p-3'-hydroxypaclitaxel (Harris et al., 1994b). Both hydroxy metabolites can be further metabolized to 6 α -, p-3'-dihydroxypaclitaxel, by CYP3A4 or CYP2C8 (Sonnichsen et al., 1995). Hence, two metabolizing mechanisms, representing CYP2C8 and CYP3A4, were added in the liver tissue compartment, (3e), using Michaelis-Menten kinetics.

Because of its lipophilic properties, paclitaxel is usually administrated as an infusion, dissolved in the formulation vehicle Cremophor EL, which has been shown to affect the kinetics of the drug (Sparreboom et al., 1996), and most likely the kinetics of the two primary hydroxy metabolites (Fransson et al., 2011). Binding of drug to Cremophor EL and proteins in the systemic and liver plasma compartments, (3a) and (3c), was assumed to be instantaneous by including an equation derived from population pharmacokinetic modeling by Henningsson et al. (2001), describing the relation between total and unbound concentrations, (3b) and (3d). Cremophor EL concentrations were simulated using a previously reported three-compartment model (Henningsson et al., 2005b), also in the Appendix, equation A.5.

For each physiological compartment, four ordinary differential equations had to be used, each one representing the drug or one of the three metabolites. In total, the model consisted of a system of 19 ordinary differential equations, where the corresponding time-dependent variables are presented in Table 1. Initial estimates for physiological and kinetic parameters for paclitaxel were derived from various literature sources according to Table A.4. Because little kinetic data is available about paclitaxel metabolites, it was assumed that all binding parameters for metabolites are the same as for paclitaxel, with the exception of binding to Cremophor EL (Fransson et al., 2011). Initial values (pre-optimization) for enzyme kinetics of metabolites were also taken to be the same as the corresponding ones for the parent drug.

2.2. Constrained optimization

To mimic the in vivo situation as far as possible the semi-physiologically based model was subject to constrained optimization. Two time-points were chosen to constrain the model in such a way that the simulations would give reasonable ratios between metabolite and parent drug concentrations and amounts. To describe the relation between total concentrations of parent

Table 1: Time-dependent variables

Variable	Meaning
$x_1(t)$	Total conc. (μM) paclitaxel in systemic plasma
$x_2(t)$	Total conc. (μM) paclitaxel in liver plasma
$x_3(t)$	Total conc. (μM) paclitaxel in liver tissue
$x_4(t)$	Total conc. (μM) 6 α -hydroxypaclitaxel in systemic plasma
$x_5(t)$	Total conc. (μM) 6 α -hydroxypaclitaxel in liver plasma
$x_6(t)$	Total conc. (μM) 6 α -hydroxypaclitaxel in liver tissue
$x_7(t)$	Total conc. (μM) p-3'-hydroxypaclitaxel in systemic plasma
$x_8(t)$	Total conc. (μM) p-3'-hydroxypaclitaxel in liver plasma
$x_9(t)$	Total conc. (μM) p-3'-hydroxypaclitaxel in liver tissue
$x_{10}(t)$	Total conc. (μM) 6 α -, p-3'-dihydroxypaclitaxel in systemic plasma
$x_{11}(t)$	Total conc. (μM) 6 α -, p-3'-dihydroxypaclitaxel in liver plasma
$x_{12}(t)$	Total conc. (μM) 6 α -, p-3'-dihydroxypaclitaxel in liver tissue
$x_{13}(t)$	Amount (μmol) paclitaxel in output compartment
$x_{14}(t)$	Amount (μmol) 6 α -hydroxypaclitaxel in output compartment
$x_{15}(t)$	Amount (μmol) p-3'-hydroxypaclitaxel in output compartment
$x_{16}(t)$	Amount (μmol) 6 α -, p-3'-dihydroxypaclitaxel in output compartment
$x_{17}(t)$	Conc. (ml/l) Cremophor EL in central compartment
$x_{18}(t)$	Conc. (ml/l) Cremophor EL in first peripheral compartment
$x_{19}(t)$	Conc. (ml/l) Cremophor EL in second peripheral compartment

drug and metabolites in systemic plasma, an earlier developed model was simulated (Fransson et al., 2011), and the total concentrations of the population means at time (t) at three hours, end of infusion, was noted. Total paclitaxel concentration ($x_1(t)$) was taken as the reference level, and total concentrations of 6 α -hydroxypaclitaxel ($x_4(t)$), p-3'-hydroxypaclitaxel ($x_7(t)$) and 6 α -, p-3'-dihydroxypaclitaxel ($x_{10}(t)$) was used to determine an appropriate ratio for total concentrations. Ratios of the amounts of 6 α -hydroxypaclitaxel ($x_{14}(t)$), p-3'-hydroxypaclitaxel ($x_{15}(t)$) and 6 α -, p-3'-dihydroxypaclitaxel ($x_{16}(t)$) to parent drug ($x_{13}(t)$) in output were estimated using information about the mean extractable radioactivity from fecal collections as reported by Walle et al. (1995). Only the parent drug and three mentioned metabolites were considered, and the ratios were calculated from the sum of these four compounds. The constraint in the output compartment was set to $t = 18$ hours, a compromise between amounts being stable (flattened curves) and

computation time. Moreover, all the constraints were somewhat relaxed by allowing a 20% deviation from the reference level. The constraints are summarized in Table 2. Initial values for the model are presented in the Ap-

Table 2: Optimization constraints in systemic plasma and output compartment

Variable	Reference level	Constraint with 20% deviation
$x_4(t)$	$0.066x_1(t = 3)$	$0.053x_1(3) < x_4(3) < 0.079x_1(3)$
$x_7(t)$	$0.018x_1(t = 3)$	$0.014x_1(3) < x_7(3) < 0.022x_1(3)$
$x_{10}(t)$	$0.018x_1(t = 3)$	$0.014x_1(3) < x_{10}(3) < 0.022x_1(3)$
$x_{14}(t)$	$5.2x_{13}(t = 18)$	$4.1x_{13}(18) < x_{14}(18) < 6.2x_{13}(18)$
$x_{15}(t)$	$0.38x_{13}(t = 18)$	$0.30x_{13}(18) < x_{15}(18) < 0.46x_{13}(18)$
$x_{16}(t)$	$1.2x_{13}(t = 18)$	$0.94x_{13}(18) < x_{16}(18) < 1.4x_{13}(18)$

pendix in Table A.4 and Table A.5. A manual optimization step using the MathModelica Simulation Center was first carried out to put the constrained variables approximately within a 50% deviation from the reference level. In this step, only V_{max} parameters were adjusted. Constrained optimization was then carried out in Mathematica ver. 8 (Wolfram Research, Inc., Champaign, IL, USA) using the NDSolve and FindMinimum routines, with several subsequent steps until all constraints were fulfilled with 20% maximum deviation from the reference level. In this second step, all V_{max} and related K_M parameters, as well as the parameters describing binding to Cremophor EL, B_{CrEL} , were subject to the optimization procedure.

2.3. Dynamic sensitivity analysis

Dynamic sensitivity analysis was performed in MathModelica Simulation Center using the CVODES solver, which supports forward sensitivity analysis (Hindmarsh et al., 2005). The sensitivity s_i for a parameter p is calculated as

$$s_{i,p} = \frac{\partial x_i}{\partial p} \quad (1)$$

where x_i is the i th (state) variable. The forward sensitivity analysis will provide the local sensitivity for the parameters under consideration (Varma et al., 1999; Wu et al., 2008). This means that the results from the analysis are conditional on the specific parameter estimates, and that the sensitivity may not be valid for another set of estimates.

Sensitivity was tested on all V_{max} and related K_m parameters by first introducing a parameter, GE ("genetic effect"). This parameter effects either the V_{max} or the K_m independently of the substrate. For instance

$$V_{maxOATP}^{pac} \rightarrow GE_{OATP}^{V_{max}} \cdot V_{maxOATP}^{pac}, \quad GE_{OATP}^{V_{max}} = 1 \quad (2)$$

so that the sensitivity in the parameter $GE_{OATP}^{V_{max}}$ can be tested. This way, the sensitivity will be independent of the magnitude of the different V_{max} , and the effect of reduced capacity in transporters and enzymes can be compared.

3. Results

3.1. Model development and constrained optimization

The final model for paclitaxel is described by equations (3a)-(3g), where the differential equations (3a), (3c) and (3e) represent the kinetics for total concentrations of paclitaxel, x_1 - x_3 , and equations (3b), (3d) and (3f) represent the relation between total, x_1 - x_3 , and unbound concentrations, y_1 - y_3 . Equation (3g) represents the change in amount of drug, x_{13} , in the output compartment. The descriptions of each variable can be found in Table 1. The full model including kinetics for metabolites is represented by Figure 1, and all the underlying equations can be found in Appendix A.

$$V_{SysPl} \cdot \dot{x}_1 = -Q_{LivPl}(x_1 - x_2) + Dose^{pac} \quad (3a)$$

$$x_1 = y_1 + (B_{linPl} + B_{CrEL}^{pac} \cdot x_{17}) \cdot y_1 + \frac{B_{maxPl} \cdot y_1}{K_{mPl} + y_1} \quad (3b)$$

$$V_{LivPl} \cdot \dot{x}_2 = Q_{LivPl}(x_1 - x_2) - Q_{LivTi}(y_2 - y_3) - \frac{V_{maxOATP}^{pac} \cdot y_2}{K_{mOATP}^{pac} + y_2} \quad (3c)$$

$$x_2 = y_2 + (B_{linPl} + B_{CrEL}^{pac} \cdot x_{17}) \cdot y_2 + \frac{B_{maxPl} \cdot y_2}{K_{mPl} + y_2} \quad (3d)$$

$$\begin{aligned} V_{LivTi} \cdot \dot{x}_3 = & Q_{LivTi}(y_2 - y_3) + \frac{V_{maxOATP}^{pac} \cdot y_2}{K_{mOATP}^{pac} + y_2} \\ & - \frac{V_{max2C8}^{pac} \cdot y_3}{K_{m2C8}^{pac} + y_3} - \frac{V_{max3A4}^{pac} \cdot y_3}{K_{m3A4}^{pac} + y_3} - \frac{V_{maxABC}^{pac} \cdot y_3}{K_{mABC}^{pac} + y_3} \end{aligned} \quad (3e)$$

$$x_3 = y_3 + B_{linTi} \cdot y_3 + \frac{B_{maxTi} \cdot y_3}{K_{mTi} + y_3} \quad (3f)$$

$$\dot{x}_{13} = \frac{V_{maxABC}^{pac} \cdot y_3}{K_{mABC}^{pac} + y_3} \quad (3g)$$

Figure 1: The final model structure. x_1 - x_{19} , time-dependent variables according to Table 1 and y_1 - y_{12} , the corresponding unbound concentrations. Black dashed arrows represent binding, black solid arrows represent enzyme kinetics and double-edged gray arrows represent compartmental flows.

The parameter estimates from the constrained optimization is presented in Appendix A, Table A.5. The concentration of total paclitaxel and metabolites in the systemic plasma, liver plasma, liver tissue compartments, as well as the amount in the output compartment from a 20 hour simulation of the final model with a three-hour infusion is shown in Figure 2.

The final step during the optimization consisted of taking the estimates from a 30% to a 20% maximum deviation. Using the Mathematica Timing function, the last step was measured to approximately 6.1 hours of computational time on a Lenovo T61 with Intel Core Duo CPU 2.00 GHz and 1.96 GB RAM, using Windows XP.

3.2. Dynamic sensitivity analysis

Dynamic sensitivities for GE^{K_m} parameters behaved similar but in the opposite direction of those for the corresponding $GE^{V_{max}}$ parameters. The later ones are shown in Figure 3 for the systemic plasma compartment and in Figure 4 for the output compartment. A negative sensitivity means a decrease in $GE^{V_{max}}$ will result in an increased plasma concentration or amount. The effect on a concentration or amount of a change in a particular $GE^{V_{max}}$ can be estimated by approximating (1). For instance, a 10% decrease in $GE_{OATP}^{V_{max}}$ with $s_{1,GE_{OATP}^{V_{max}}} = -1.2$ at $t = 3$ hours (Figure 3, top left), will increase the total paclitaxel concentration in systemic plasma from $\hat{x}_1(3) = 5.25$ μM (Figure 2, top left) to approximately

$$\hat{x}_1(3) + s_{1,GE_{OATP}^{V_{max}}}(3) \cdot \partial GE_{OATP}^{V_{max}} \approx 5.25 + (-1.2) \cdot (-0.10) = 5.37 \mu\text{M} \quad (4)$$

Given the final model estimates, systemic plasma concentration of paclitaxel was clearly most sensitive to changes in $GE_{OATP}^{V_{max}}$, describing the uptake by OATP, where a change will have more than 10 times the effect compared to a change in the metabolism by CYP2C8 at $t = 3$ hours, which had the second most sensitive $GE^{V_{max}}$ (Figure 3, top left). For 6 α -hydroxypaclitaxel, the ABCB1 transporter is most sensitive, with increasing plasma concentrations for decreasing $GE_{ABC}^{V_{max}}$, and with CYP2C8 as being second most sensitive, with decreasing plasma concentrations for decreasing $GE_{2C8}^{V_{max}}$ (Figure 3, top right).

In the output compartment, the amount of paclitaxel was most sensitive to changes in ABCB1, where a decreasing $GE_{ABC}^{V_{max}}$ will give rise to a decreasing amount (Figure 4, top left). For the amount of 6 α -hydroxypaclitaxel, metabolism by CYP2C8 is most sensitive, although an equal change in

Figure 2: Simulations from the final model of total concentrations in systemic plasma (top left), liver plasma (top right), liver tissue (bottom left) and amounts in output compartment (bottom right) of paclitaxel (solid), 6 α -hydroxypaclitaxel (dashed), p-3'-hydroxypaclitaxel (dotted) and 6 α -, p-3'-dihydroxypaclitaxel (dash-dotted).

metabolism by CYP3A4 will have an almost as big but opposite effect (Figure 4, top right). For all compounds in the output compartment, effects from changed uptake by the OATP transporter are small in comparison to changes in metabolism or efflux.

A summary of the effect on paclitaxel and 6 α -hydroxypaclitaxel of a 10% decrease in $GE^{V_{max}}$ can be found in Table 3.

Table 3: Change in concentration and amount from a 10% decrease in different $GE^{V_{max}}$

Variable ^a	$GE_{OATP}^{V_{max}} = 0.9$	$GE_{2C8}^{V_{max}} = 0.9$	$GE_{3A4}^{V_{max}} = 0.9$	$GE_{ABC}^{V_{max}} = 0.9$
$x_1(3)$	2.3%	0.2%	0.1%	0.0%
$x_4(3)$	5.0%	-9.4%	6.7%	34.0%
$x_{13}(18)$	0.0%	4.1%	1.4%	-9.0%
$x_{14}(18)$	0.0%	-3.2%	2.3%	1.6%

^a $x_1(3)$: total paclitaxel concentration in systemic plasma at three hours; $x_4(3)$: total 6 α -hydroxypaclitaxel concentration in systemic plasma at 3 hours; $x_{13}(18)$: amount paclitaxel in output compartment at 18 hours; $x_{14}(18)$: amount 6 α -hydroxypaclitaxel in output compartment at 18 hours

4. Discussion

The simulated C_{max} of 5.25 μ M for total concentration of paclitaxel at $t = 3$ hours in Figure 1 is in the same range as the observed C_{max} from three-hour infusions reported previously by Walle et al. (1995), with 6-10 μ M; Karlsson et al. (1999), 2-10 μ M; Henningsson et al. (2001), 1-10 μ M; Joerger et al. (2006), 1-4 μ M. Because of the three-hour constraints in the systemic compartment, maximum total concentrations of metabolites are also in the right range (Czejka et al., 2003; Fransson et al., 2011). The flat phase following the initial rapid increase in concentration and preceding the end of infusion at three hours is not evident from clinical data (Walle et al., 1995; Karlsson et al., 1999; Czejka et al., 2003; Joerger et al., 2006), which could be a consequence of the limited compartmental space in systemic plasma with increased binding as result. Simulation using a ten times larger volume for systemic plasma removed the two distinct phases (data not shown). Previously described population pharmacokinetic models have used two (Henningsson et al., 2001, 2005a; Joerger et al., 2006) or at least one (Henningsson et al.,

Figure 3: Sensitivity in $GE^{V_{max}}$ for OATP (solid), CYP2C8 (dashed), CYP3A4 (dotted) and ABCB1 (dash-dotted) in systemic plasma concentrations of paclitaxel (top left), 6 α -hydroxypaclitaxel (top right), p-3'-hydroxypaclitaxel (bottom left) and 6 α -, p-3'-dihydroxypaclitaxel (bottom right).

Figure 4: Sensitivity in $GE^{V_{max}}$ for OATP (solid), CYP2C8 (dashed), CYP3A4 (dotted) and ABCB1 (dash-dotted) in output amounts of paclitaxel (top left), 6 α -hydroxypaclitaxel (top right), p-3'-hydroxypaclitaxel (bottom left) and 6 α -, p-3'-dihydroxypaclitaxel (bottom right).

2003; Bergmann et al., 2011) additional peripheral compartments, although in some cases, for total concentrations of paclitaxel, additional compartments may have been attributable to Cremophor EL binding (Karlsson et al., 1999; Joerger et al., 2006). The lack of peripheral compartments in the present model is also evident from the rapid decrease in total concentrations after the 3 hour infusion. Addition of peripheral compartments to the model were considered, but because previous models are either based on unbound concentrations (Henningsson et al., 2001, 2003, 2005a; Bergmann et al., 2011) or does not explicitly handle binding to Cremophor EL (Karlsson et al., 1999; Joerger et al., 2006), such an approach would mean even more assumptions would need to be accounted for. There are also few sources with parametric models describing the kinetics of paclitaxel metabolites (Fransson et al., 2011).

As a result of the rapid elimination of paclitaxel and metabolites from systemic plasma, amounts in the output compartment are likely to increase and stabilize more rapidly in the present model than in vivo. From Figure 2 (bottom right) it is evident that amounts in the output compartment are stable from approximately 8 hours, which would mean that selecting $t = 18$ hours for the constraint on metabolite-drug ratios is sufficient, although the constraint is derived from information on fecal collections between 24-48 hours (Walle et al., 1995). The efflux from liver tissue to the output compartment is fully dependent on the ABCB1 transporter, and no diffusion is assumed with the possibility of a back-flow. A mathematical model by Bartholome et al. (2007) describing vectorial transport by OATP1B3 and ABCC2 across polarized cells found an additional leakage component for efflux over the apical membrane. For the present model, this may indicate that the importance of the transporter ABCB1 will be overestimated for the output compartment. However, because an appropriate volume of the output compartment is difficult to estimate a concentration cannot be determined, something that would be required for a diffusion mechanism. Diffusion would also be affected by the accumulation of the amounts in the output compartment, and this accumulation would not be representative for the in vivo situation.

The total paclitaxel plasma concentration in the systemic compartment is most sensitive to changes in $GE_{OATP}^{V_{max}}$. However, the absolute effect on the plasma concentration is small. According to Table 3 a 10% decrease in $GE_{OATP}^{V_{max}}$ will only provide an 2.3% increase in concentration at the end of infusion at 3 hours. This can be compared to the effect of a 10% decrease in $GE_{ABC}^{V_{max}}$, which will cause total concentrations of 6 α -hydroxypaclitaxel to in-

crease with 34% at the same point in time. Given the final model estimates, total paclitaxel concentrations are relatively little affected by changes in GE parameters. If the model and final estimates are to be considered representative for the in vivo situation, it would mean that neither of the investigated transporters, nor the metabolizing enzymes, play a major role in the population variability of paclitaxel plasma concentrations. Hence, they would not show up as significant covariates in clinical studies using population pharmacokinetic data. This may explain the absence of findings by Henningsson et al. (2005a), and the relatively modest effect on decreased clearance by Bergmann et al. (2011). In the same way, the predicted relatively large effect on 6 α -hydroxypaclitaxel plasma concentrations from decreased capacity in the ABCB1 transporter is supported by our previous findings (Fransson et al., 2011), where individuals carrying the polymorphisms G/A or G/G (wild-type) showed a 30% increase, and individuals with polymorphism T/T showed a 27% decrease, relative individuals with polymorphism G/T. The result that genetic variation may influence the metabolite concentrations more than paclitaxel is in accordance with the hypothesis proposed by Leskela et al. (2011). They found that CYP2C8*3, CYP2C8 Haplotype C and CYP3A5*3 correlates to paclitaxel-induced neuropathy and suggested that the metabolites are affecting the risk of neuropathy.

Because local sensitivity analysis is used, the sensitivities in Figures 3 and 4 and the effects in Table 3 are only valid for small deviations from the final parameter estimates (Appendix, Table A.5). The validity of the sensitivity analysis can be tested by manually reducing the parameter estimates and then perform a new simulation. Such simulations showed that a 10% decrease in the estimate agree with the local sensitivity analysis, but that a 50% decrease in some cases are too big, and that the sensitivity plots in Figures 3 and 4 cannot be used to accurately predict such a large deviation (data not shown). This should be considered if effects from genetic polymorphisms are considered to be large, in which case the solution would be an additional simulation using a reduced estimate.

In conclusion, the developed model predicts plasma concentrations of drug and metabolites that are in the range of observations from clinical studies. Given the final model structure with parameter estimates derived from constrained optimization, while plasma concentrations of paclitaxel seems to be relatively little affected by changes in the capacity of transport or metabolism, its main metabolite 6 α -hydroxypaclitaxel may be largely affected even by small changes. If future studies can confirm that paclitaxel

metabolites are clinically relevant, the present work indicates that genetic polymorphisms may play an important role for individualizing paclitaxel treatment.

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Appendix A. Model equations and parameter estimates

$$V_{SysPl} \cdot \dot{x}_1 = -Q_{LivPl}(x_1 - x_2) + Dose^{pac} \quad (A.1a)$$

$$x_1 = y_1 + (B_{linPl} + B_{CrEL}^{pac} \cdot x_{17}) \cdot y_1 + \frac{B_{maxPl} \cdot y_1}{K_{mPl} + y_1} \quad (A.1b)$$

$$V_{LivPl} \cdot \dot{x}_2 = Q_{LivPl}(x_1 - x_2) - Q_{LivTi}(y_2 - y_3) - \frac{V_{maxOATP}^{pac} \cdot y_2}{K_{mOATP}^{pac} + y_2} \quad (A.1c)$$

$$x_2 = y_2 + (B_{linPl} + B_{CrEL}^{pac} \cdot x_{17}) \cdot y_2 + \frac{B_{maxPl} \cdot y_2}{K_{mPl} + y_2} \quad (A.1d)$$

$$\begin{aligned} V_{LivTi} \cdot \dot{x}_3 = & Q_{LivTi}(y_2 - y_3) + \frac{V_{maxOATP}^{pac} \cdot y_2}{K_{mOATP}^{pac} + y_2} \\ & - \frac{V_{max2C8}^{pac} \cdot y_3}{K_{m2C8}^{pac} + y_3} - \frac{V_{max3A4}^{pac} \cdot y_3}{K_{m3A4}^{pac} + y_3} - \frac{V_{maxABC}^{pac} \cdot y_3}{K_{mABC}^{pac} + y_3} \end{aligned} \quad (A.1e)$$

$$x_3 = y_3 + B_{linTi} \cdot y_3 + \frac{B_{maxTi} \cdot y_3}{K_{mTi} + y_3} \quad (A.1f)$$

$$\dot{x}_{13} = \frac{V_{maxABC}^{pac} \cdot y_3}{K_{mABC}^{pac} + y_3} \quad (A.1g)$$

$$V_{SysPl} \cdot \dot{x}_4 = -Q_{LivPl}(x_4 - x_5) \quad (A.2a)$$

$$x_4 = y_4 + (B_{linPl} + B_{CrEL}^{6\alpha} \cdot x_{17}) \cdot y_4 + \frac{B_{maxPl} \cdot y_4}{K_{mPl} + y_4} \quad (A.2b)$$

$$V_{LivPl} \cdot \dot{x}_5 = Q_{LivPl}(x_4 - x_5) - Q_{LivTi}(y_5 - y_6) - \frac{V_{maxOATP}^{6\alpha} \cdot y_5}{K_{mOATP}^{6\alpha} + y_5} \quad (A.2c)$$

$$x_5 = y_5 + (B_{linPl} + B_{CrEL}^{6\alpha} \cdot x_{17}) \cdot y_5 + \frac{B_{maxPl} \cdot y_5}{K_{mPl} + y_5} \quad (A.2d)$$

$$\begin{aligned} V_{LivTi} \cdot \dot{x}_6 = & Q_{LivTi}(y_5 - y_6) + \frac{V_{maxOATP}^{6\alpha} \cdot y_5}{K_{mOATP}^{6\alpha} + y_5} \\ & + \frac{V_{max2C8}^{pac} \cdot y_3}{K_{m2C8}^{pac} + y_3} - \frac{V_{max3A4}^{6\alpha} \cdot y_6}{K_{m3A4}^{6\alpha} + y_6} - \frac{V_{maxABC}^{6\alpha} \cdot y_6}{K_{mABC}^{6\alpha} + y_6} \end{aligned} \quad (A.2e)$$

$$x_6 = y_6 + B_{linTi} \cdot y_6 + \frac{B_{maxTi} \cdot y_6}{K_{mTi} + y_6} \quad (A.2f)$$

$$\dot{x}_{14} = \frac{V_{maxABC}^{6\alpha} \cdot y_6}{K_{mABC}^{6\alpha} + y_6} \quad (A.2g)$$

$$V_{SysPl} \cdot \dot{x}_7 = -Q_{LivPl}(x_7 - x_8) \quad (A.3a)$$

$$x_7 = y_7 + (B_{linPl} + B_{CrEL}^{p-3'} \cdot x_{17}) \cdot y_7 + \frac{B_{maxPl} \cdot y_7}{K_{mPl} + y_7} \quad (A.3b)$$

$$V_{LivPl} \cdot \dot{x}_8 = Q_{LivPl}(x_7 - x_8) - Q_{LivTi}(y_8 - y_9) - \frac{V_{maxOATP}^{p-3'} \cdot y_8}{K_{mOATP}^{p-3'} + y_8} \quad (A.3c)$$

$$x_8 = y_8 + (B_{linPl} + B_{CrEL}^{p-3'} \cdot x_{17}) \cdot y_8 + \frac{B_{maxPl} \cdot y_8}{K_{mPl} + y_8} \quad (A.3d)$$

$$\begin{aligned} V_{LivTi} \cdot \dot{x}_9 = & Q_{LivTi}(y_8 - y_9) + \frac{V_{maxOATP}^{p-3'} \cdot y_8}{K_{mOATP}^{p-3'} + y_8} \\ & + \frac{V_{max3A4}^{pac} \cdot y_3}{K_{m3A4}^{pac} + y_3} - \frac{V_{max2C8}^{p-3'} \cdot y_9}{K_{m2C8}^{p-3'} + y_9} - \frac{V_{maxABC}^{p-3'} \cdot y_9}{K_{mABC}^{p-3'} + y_9} \end{aligned} \quad (A.3e)$$

$$x_9 = y_9 + B_{linTi} \cdot y_9 + \frac{B_{maxTi} \cdot y_9}{K_{mTi} + y_9} \quad (A.3f)$$

$$\dot{x}_{15} = \frac{V_{maxABC}^{p-3'} \cdot y_9}{K_{mABC}^{p-3'} + y_9} \quad (A.3g)$$

$$V_{SysPl} \cdot \dot{x}_{10} = -Q_{LivPl}(x_{10} - x_{11}) \quad (A.4a)$$

$$x_{10} = y_{10} + (B_{linPl} + B_{CrEL}^{di} \cdot x_{17}) \cdot y_{10} + \frac{B_{maxPl} \cdot y_{10}}{K_{mPl} + y_{10}} \quad (A.4b)$$

$$V_{LivPl} \cdot \dot{x}_{11} = Q_{LivPl}(x_{10} - x_{11}) - Q_{LivTi}(y_{11} - y_{12}) - \frac{V_{maxOATP}^{di} \cdot y_{11}}{K_{mOATP}^{di} + y_{11}} \quad (A.4c)$$

$$x_{11} = y_{11} + (B_{linPl} + B_{CrEL}^{di} \cdot x_{17}) \cdot y_{11} + \frac{B_{maxPl} \cdot y_{11}}{K_{mPl} + y_{11}} \quad (A.4d)$$

$$\begin{aligned} V_{LivTi} \cdot \dot{x}_{12} = & Q_{LivTi}(y_{11} - y_{12}) + \frac{V_{maxOATP}^{di} \cdot y_{11}}{K_{mOATP}^{di} + y_{11}} \\ & + \frac{V_{max3A4}^{6\alpha} \cdot y_6}{K_{m3A4}^{6\alpha} + y_6} + \frac{V_{max2C8}^{p-3'} \cdot y_9}{K_{m2C8}^{p-3'} + y_9} - \frac{V_{maxABC}^{di} \cdot y_{12}}{K_{mABC}^{di} + y_{12}} \end{aligned} \quad (A.4e)$$

$$x_{12} = y_{12} + B_{linTi} \cdot y_{12} + \frac{B_{maxTi} \cdot y_{12}}{K_{mTi} + y_{12}} \quad (A.4f)$$

$$\dot{x}_{16} = \frac{V_{maxABC}^{di} \cdot y_{12}}{K_{mABC}^{di} + y_{12}} \quad (A.4g)$$

$$\begin{aligned} V_1^{CrEL} \cdot \dot{x}_{17} = & -Q_{12}^{CrEL}(x_{17} - x_{18}) - Q_{13}^{CrEL}(x_{17} - x_{19}) \\ & - \frac{V_{max}^{CrEL} \cdot x_{17}}{K_m^{CrEL} + x_{17}} + Dose^{CrEL} \end{aligned} \quad (A.5a)$$

$$V_2^{CrEL} \cdot \dot{x}_{18} = Q_{12}^{CrEL}(x_{17} - x_{18}) \quad (A.5b)$$

$$V_3^{CrEL} \cdot \dot{x}_{19} = Q_{13}^{CrEL}(x_{17} - x_{19}) \quad (A.5c)$$

Table A.4: Literature derived estimates used as initial estimates for optimization

Parameter	Estimate	Meaning	Reference
V_{SysPl} (l)	2.358	Volume systemic plasma	Hurley (1975): $S = 1.71$ and subtracting V_{LivPl}
V_{LivPl} (l)	0.2 ^a	Volume liver plasma	Taniguchi et al. (1996); Geraghty et al. (2004)
V_{LivTi} (l)	0.29 ^b	Volume liver tissue	Jang et al. (2001): BC19 volume of 1.96 pl
Q_{LivPl} (l/h)	60	Flow V_{SysPl} and V_{LivPl}	Molino et al. (1991)
Q_{LivTi} (l/h)	543 ^b	Flow V_{LivPl} and V_{LivTi}	Jang et al. (2001)
B_{maxTi} (μ M)	61.5	Maximal intracellular binding	Jang et al. (2001)
K_{mTi} (μ M)	0.00524	Concentration at half B_{maxTi}	Jang et al. (2001)
B_{linTi}	0.118	Linear intracellular binding	Jang et al. (2001)
B_{maxPl} (μ M)	0.0245	Maximal binding in plasma	Henningsson et al. (2001)
K_{mPl} (μ M)	0.000106	Concentration at half B_{maxPl}	Henningsson et al. (2001)
B_{linPl}	7.59	Linear binding in plasma	Henningsson et al. (2001)
$V_{maxOATP}^{pac}$ (μ mol/h)	9200 ^b	Maximum uptake rate OATP	Smith et al. (2005)
K_{mOATP}^{pac} (μ M)	6.79	Concentration at half $V_{maxOATP}^{pac}$	Smith et al. (2005)
V_{max2C8}^{pac} (μ mol/h)	170 ^c	Maximum reaction rate CYP2C8	Cresteil et al. (2002); Naraharisetti et al. (2010)
K_{m2C8}^{pac} (μ M)	15	Concentration at half V_{max2C8}^{pac}	Cresteil et al. (2002)
V_{max3A4}^{pac} (μ mol/h)	100 ^c	Maximum reaction rate CYP3A4	Cresteil et al. (2002); Kato et al. (2010)
K_{m3A4}^{pac} (μ M)	15	Concentration at half V_{max3A4}^{pac}	Cresteil et al. (2002)
V_{maxABC}^{pac} (μ mol/h)	41 ^b	Maximum efflux rate ABCB1	Jang et al. (2001)
K_{mABC}^{pac} (μ M)	0.0139	Concentration at half V_{maxABC}^{pac}	Jang et al. (2001)
B_{CrEL}^{pac}	3.78	Binding to CrEL in plasma	Henningsson et al. (2001)
V_1^{CrEL} (l)	4.54	Volume central comp. CrEL	Henningsson et al. (2005b)
V_2^{CrEL} (l)	1.32	Volume first periph. comp. CrEL	Henningsson et al. (2005b)
V_3^{CrEL} (l)	3.53	Volume second periph. comp. CrEL	Henningsson et al. (2005b)
Q_{12}^{CrEL} (l/h)	1.17	Flow between V_1^{CrEL} and V_2^{CrEL}	Henningsson et al. (2005b)
Q_{13}^{CrEL} (l/h)	0.479	Flow between V_1^{CrEL} and V_3^{CrEL}	Henningsson et al. (2005b)
V_{max}^{CrEL} (ml/h)	0.64	Maximum elimination rate of CrEL	Henningsson et al. (2005b)
K_{m}^{CrEL} (ml/l)	2.57	Concentration at half V_{max}^{CrEL}	Henningsson et al. (2005b)

^aCalculated from references and by using a hematocrit value of 0.4

^bUsing a cell number of 1.5×10^{11} (de la Grandmaison et al., 2001; Barter et al., 2007)

^cCalculated from references and by using 32 mg microsomal protein per gram of liver (Barter et al., 2007)

Table A.5: Estimates from constrained optimization for paclitaxel transport, metabolism and Cremophor EL binding

Parameter	Initial ^a	Manual ^b	Mathematica ^c
$V_{maxOATP}^{pac}$ ($\mu\text{mol/h}$)	9200	2300	2210
K_{mOATP}^{pac} (μM)	6.79	fixed	fixed
V_{max2C8}^{pac} ($\mu\text{mol/h}$)	170	68000	108000
K_{m2C8}^{pac} (μM)	15	fixed	fixed
V_{max3A4}^{pac} ($\mu\text{mol/h}$)	100	40000	35800
K_{m3A4}^{pac} (μM)	15	fixed	fixed
V_{maxABC}^{pac} ($\mu\text{mol/h}$)	41	fixed	fixed
K_{mABC}^{pac} (μM)	0.0139	fixed	fixed
$V_{maxOATP}^{6\alpha}$ ($\mu\text{mol/h}$)	9200	4600	4030
$K_{mOATP}^{6\alpha}$ (μM)	6.79	fixed	5.79
$V_{max3A4}^{6\alpha}$ ($\mu\text{mol/h}$)	100	100	114
$K_{m3A4}^{6\alpha}$ (μM)	15	fixed	16.3
$V_{maxABC}^{6\alpha}$ ($\mu\text{mol/h}$)	41	82	107
$K_{mABC}^{6\alpha}$ (μM)	0.0139	fixed	0.0130
$V_{maxOATP}^{p-3'}$ ($\mu\text{mol/h}$)	9200	18400	14000
$K_{mOATP}^{p-3'}$ (μM)	6.79	fixed	8.43
$V_{max2C8}^{p-3'}$ ($\mu\text{mol/h}$)	170	17000	16900
$K_{m2C8}^{p-3'}$ (μM)	15	fixed	11.0
$V_{maxABC}^{p-3'}$ ($\mu\text{mol/h}$)	41	10	10.4
$K_{mABC}^{p-3'}$ (μM)	0.0139	fixed	0.0143
$V_{maxOATP}^{di}$ ($\mu\text{mol/h}$)	9200	18400	15200
K_{mOATP}^{di} (μM)	6.79	fixed	7.37
V_{maxABC}^{di} ($\mu\text{mol/h}$)	41	41	34.8
K_{mABC}^{di} (μM)	0.0139	fixed	0.0119
B_{CrEL}^{pac}	3.78	fixed	fixed
$B_{CrEL}^{6\alpha}$	3.78	fixed	4.79
$B_{CrEL}^{p-3'}$	3.78	fixed	2.85
B_{CrEL}^{di}	3.78	fixed	3.34

^aFrom Table A.4. Estimates for parameters governing metabolite kinetics are assumed to be the same as for the parent drug.

^bUsing MathModelica Simulation Center by adjusting V_{max} parameters to meet an approximate 50% deviation from the constraints.

^cFinal estimates. Using Mathematica with NDSolve and FindMinimum to meet a maximum of 20% deviation from the constraints.