Influence of Cremophor EL and Genetic Polymorphisms on the Pharmacokinetics of Paclitaxel and Its Metabolites Using a Mechanism-Based Model

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ABSTRACT:
The formulation vehicle Cremophor EL has previously been shown to affect paclitaxel kinetics, but it is not known whether it also affects the kinetics of paclitaxel metabolites. This information may be important for understanding paclitaxel metabolism in vivo and in the investigation of the role of genetic polymorphisms in the metabolizing enzymes CYP2C8 and CYP3A4/CYP3A5 and the ABCB1 transporter. In this study we used the population pharmacokinetic approach to explore the influence of predicted Cremophor EL concentrations on paclitaxel (Taxol) metabolites. In addition, correlations between genetic polymorphisms and enzyme activity with clearance of paclitaxel, its two primary metabolites, 6α-hydroxypaclitaxel and p-3'-hydroxy-paclitaxel, and its secondary metabolite, 6α-p-3'-di-hydroxy-paclitaxel were investigated. Model building was based on 1156 samples from a study with 33 women undergoing paclitaxel treatment for gynecological cancer.

Introduction
Paclitaxel is a natural substance from the Pacific yew tree that was recognized for its property of causing apoptosis by promoting microtubule stabilization (Schiff et al., 1979) and is today used for the treatment of a number of different types of cancer. Because of the large interindividual differences observed in the pharmacokinetics of the drug, identification of patient factors that can be used to individualize the dose in clinical practice is desirable. The dose of paclitaxel is determined by body surface area, but the measure of body size explains only a small part of the variability (Mathijsen et al., 2007). Other demographic characteristics previously proposed to be attributable to population variability include gender, age, body weight, and bilirubin (Heningsson et al., 2003; Joerger et al., 2006). Several of the genes involved in the pharmacokinetics of paclitaxel contain single nucleotide polymorphisms (SNPs) that may result in interindividual variability of paclitaxel clearance. Previously considered polymorphisms for paclitaxel clearance or exposure include SNPs coding for the metabolizing enzymes CYP2C8, CYP3A4, and CYP3A5 and also for the ABCB1 transporter (Heningsson et al., 2005; Nakajima et al., 2005; Sissung et al., 2006; Yamaguchi et al., 2006; Gréen et al., 2009; Bergmann et al., 2010). So far, results are ambiguous, with a few positive findings for SNPs affecting clearance of paclitaxel (Yamaguchi et al., 2006; Gréen et al., 2009; Bergmann et al., 2010). Although paclitaxel metabolites are considered not to be active against tumors and have been shown to be up to 30-fold less cytotoxic than the parent compound (Harris et al., 1994a; Sparreboom et al., 1995), the particular metabolic pattern for paclitaxel may be of interest for identifying correlations between individual clearance and SNP
variants for participating enzymes. The metabolites may also be involved in the neuropathy seen after paclitaxel-containing chemotherapy (Leskelä et al., 2010).

Elimination of paclitaxel occurs mainly as hepatic metabolism (Walle et al., 1995), performed by cytochrome P-450 monoxygenases as a one- or two-step hydroxylation process in either of two metabolic paths (Monsarrat et al., 1993). In the first step, paclitaxel is oxidized by CYP2C8 to 6α-hydroxypaclitaxel (6αOH-pac) or by CYP3A to p-3'-hydroxypaclitaxel (p3OH-pac) (Cresteil et al., 1994; Harris et al., 1994b; Rahman et al., 1994). 6αOH-pac is the predominant metabolite in most cases (Harris et al., 1994a; Kumar et al., 1999; Sonnichsen et al., 1995), with an estimated overall formation in vitro of 63% (Cresteil et al., 2002). In the second step, 6αOH-pac and p3OH-pac may be further oxidized to the secondary metabolite 6α-p-3'-dihydroxypaclitaxel (diOH-pac) by CYP3A and CYP2C8, respectively (Sonnichsen et al., 1995; Monsarrat et al., 1998). Together, paclitaxel and the three metabolites constitute a diamond-shaped pathway with each cytochrome P450 monoxygenase in parallel on the two arms (Fig. 1).

Distribution and/or elimination of total concentrations of paclitaxel (Taxol) have earlier been suggested to be saturable (Sonnichsen et al., 1994; Gianni et al., 1994; Karlsson et al., 1999). The observed nonlinear kinetics of total concentrations, when administered as Taxol, have been attributed primarily to micelle encapsulation by the formulation vehicle Cremophor EL (CrEL) (Sparreboom et al., 1996). When binding of paclitaxel to CrEL and plasma proteins is taken into account, unbound concentrations of paclitaxel will fit a model with linear kinetics (Henningsson et al., 2001). The use of this more mechanism-based model ideally requires measurements of unbound concentrations of paclitaxel and concentrations of CrEL in addition to total plasma concentrations. However, the model has been shown to be at least as good as using a more empirical explanation of the nonlinear behavior (Gianni et al., 1995) when only total paclitaxel concentrations are available for analysis (Fransson and Grèen, 2008). CrEL concentrations were predicted from a model developed previously (Henningsson et al., 2005b), and a similar approach can be taken to investigate whether and how CrEL also is influencing the metabolite kinetics of paclitaxel.

The purpose of the present study was to investigate the influence of CrEL on paclitaxel metabolite kinetics and to investigate correlations of clearance of paclitaxel and its metabolites with enzyme activity and SNP variants of the metabolizing enzymes CYP2C8, CYP3A4, and CYP3A5 and the transporter protein ABCB1. In particular, the metabolic pattern suggests that a correlation may exist for the proportion of clearance of paclitaxel to 6αOH-pac and p3OH-pac and clearance of these metabolites to diOH-pac. Use of information on both parent and metabolite concentrations, in addition to predictions of CrEL concentrations, in a population pharmacokinetic model, can provide a better understanding for the elimination of paclitaxel, and potential correlations may be easier to elucidate.

Materials and Methods

Patients and Study Design. Thirty-three white women, 36 to 75 years of age (median age 62 years), with different types of gynecological cancers (epithelial ovarian cancer, n = 26; peritoneal cancer, n = 4; ovarian or peritoneal cancer, n = 1; carcinoma in corpus uteri, n = 1; and carcinoma in cervix uteri, n = 1), were included in the study as described previously (Grèen et al., 2009). The patients received combination therapy of paclitaxel (Taxol; Bristol-Myers Squibb, Wallingford, CT), administered as an intravenous infusion with a target time of 3 h and dosing at 175 mg/m² (n = 30) or 135 mg/m² (n = 3, due to poor general condition), and carboplatin, with target area under the plasma concentration-time curve of 5 or 6 mg · min/ml according to the formula of Calvert et al. (1989). For 31 patients, treatment consisted of at least six cycles of chemotherapy. One patient received only a single cycle due to septicemia, and another patient was withdrawn after four cycles because of severe neurotoxicity. Blood samples for pharmacokinetic analysis were obtained during one cycle per patient from the first to eighth cycle (median cycle number 3) and collected in EDTA tubes. Target times for sampling were immediately before infusion, at 30 min and 1 h after the start of infusion, immediately before the infusion was stopped, and at 5, 15, and 30 min and 1, 2, 4, 8, and 24 h after the infusion was stopped. Centrifuged plasma samples were stored at −80 °C until analysis. Regional ethics committees approved the study and written informed consent was obtained from each patient.

Assays and Samples. Total concentrations of paclitaxel, 6αOH-pac, and p3OH-pac from all patients and of diOH-pac from 15 patients were analyzed in plasma samples using solid-phase extraction, reverse-phase high-performance liquid chromatography, and an ion trap mass spectrometer with a sonic spray ionization interface as described elsewhere (Grèen et al., 2006b). For paclitaxel, 6αOH-pac, and p3OH-pac, docetaxel (Aventis Pharma, Vitry Alfortville, France) was used as an internal standard, and for diOH-pac, paclitaxel was used as reference. The lower limit of quantification was 0.5 ng/ml for paclitaxel and 2 ng/ml for hydroxymetabolites (Grèen et al., 2006b), but observations below the lower limit of quantification were included in the analysis if detected. Twenty-seven observations were considered nondetectable. For one patient, 15 observations of paclitaxel (n = 5), 6αOH-pac (n = 4), p3OH-pac (n = 3), and diOH-pac (n = 3) were removed because of large (>30%) within-sample variability for paclitaxel. In total, 1156 observations of paclitaxel (n = 345), 6αOH-pac (n = 332), p3OH-pac (n = 336), and diOH-pac (n = 143) remained for pharmacokinetic analysis.

Enzyme Activity and Genotyping. The selection of the CYP3A4 phenotyping method was based on finding a nonradioactive assay without any risk of sedation and with an reaction similar to that of paclitaxel, i.e., a hydroxylation. We finally settled on the use of oral quinine tablets followed by a simple high-performance liquid chromatography method for the quantification of the ratio in plasma. Enzyme activity for CYP3A4 in vivo was determined by administration of a 250-mg quinine tablet to the patients 24 to 48 h before the start of chemotherapy followed by blood sampling 16 h later (Mirmihan et al., 1999, 2001). The CYP3A4 activity was expressed as the metabolic ratio of

![FIG. 1. Metabolic pathways for paclitaxel by CYP2C8 and CYP3A.](image-url)
Concentrations of paclitaxel. The mechanism-based model consists of a linear
compiled using the G77 compiler.

Foundation for Statistical Computing, Vienna, Austria). FORTRAN files were
sourceforge.net) for R (version 2.10.0, 2009; http://www.r-project.org, R

graphical analysis were performed in Xpose (version 4.1.0; http://xpose.
Solutions, Ellicott City, MD) was used in combination with PsN (version 3.1.0;

analysis, NONMEM software (version VI, level 2.0, 2006; Icon Development

that the mechanism-based model structure is not identifiable (with high prob-

of various models was applied to find the best structures in terms of fit (see

sponding unbound concentration, 

\[ \frac{[\text{pac}]}{[\text{pac}]_\text{u}} \text{, where } [\text{pac}]_\text{u} \text{ is the corre-

B

max is the maximum nonlinear binding to plasma components, and } K_m \text{ is the concentration at half of the maximal binding to plasma components.}

ability) without information on unbound concentrations of paclitaxel (Fransson

and Green, 2008). Therefore, the approach with frequentist priors (Gislekog

et al., 2002) was used in the current study to stabilize the analysis. The priors

(mean and S.E.) used in the final model are presented in Table 2. Because

CrEL concentrations were not available from the study, a model describing

the population pharmacokinetics of CrEL, with three compartments and saturated

elimination, was implemented and used to predict CrEL concentrations at any

time point (Henningsson et al., 2005b). Model parameters for CrEL were tried

as priors or as fixed estimates. Earlier identified covariate relations were not used

in the CrEL model. The paclitaxel model and the CrEL model were

specified using the ADVAN 6 subroutine in NONMEM VI, and all estimations

were performed with the first-order conditional estimation method with

interaction.

Interindividual variability (IIV) for an individual i was, in general, modeled

exponentially:

\[ \theta_i = \theta_{\text{pop}} \cdot \exp(\eta_i) \]  

(2)

where \( \theta_{\text{pop}} \) is the parameter for the typical value for the population and \( \eta \sim N(0, \sigma^2) \). Residual errors for observed concentrations (\( \epsilon_{\text{obs}} \)) versus predicted concentrations (\( \epsilon_{\text{pred}} \)) were modeled using a proportional error in combination, if needed, with an additive error according to

\[ \epsilon_{\text{obs}} = \epsilon_{\text{pred}} \cdot \left( 1 + \frac{\text{RSE}}{100} \right) \]  

(3)

where \( \text{RSE} \) is the residual standard error and \( \epsilon_{\text{obs}}, \epsilon_{\text{pred}} \sim N(0, \sigma^2) \).

The combined paclitaxel-CrEL model was first fitted to total paclitaxel concentrations only. Priors were used on all parameters previously reported at the population level, and for IIV the prior for clearance of unbound paclitaxel was included (Henningsson et al., 2001). Significance of IIV in the remaining parameters was tested one at a time without additional priors. The mechanism-based model structure of paclitaxel was extended to also include the metabolites. Initially the paclitaxel parameters were fixed to the estimates obtained in the first step. Total metabolite concentrations were fitted sequentially in the following order: only 6\alpha\text{-OH-pac}, only 3\alpha\text{-OH-pac}, 6\alpha\text{-OH-pac in combination with } 3\alpha\text{-OH-pac, and 6\alpha\text{-OH-pac and } 3\alpha\text{-OH-pac in combination with diOH-pac. For metabolites, the issue of identifiability caused by not knowing the unbound fraction implies that the size of any linear terms cannot be estimated in an equation connecting unbound and total concentrations. Hence, a parameter corresponding to } B_{\text{lin}} \text{ for metabolites cannot be estimated. All metabolite parameters will therefore be scaled by the (unknown) fraction that is linearly bound.}

The NONMEM objective function value (OFV), which is proportional to

\(-2 \times \log \text{likelihood of the data, was used to guide model development. For}

nested models, the OFV is } ^2 \text{ distributed, and a new model was selected if the}

addition of one parameter caused a drop in OFV by at least 10.83, which

corresponds to a significance level of } p < 0.001. In addition, the new

parameter should not cause problems with identifiability. Individual plots and

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele</th>
<th>Polymorphism</th>
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<td>C/C 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
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<td></td>
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</tr>
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<td></td>
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<td></td>
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<td>*1C</td>
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<td>*3</td>
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<td>*4</td>
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<td>CYP3A4</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prior Estimate</th>
<th>Prior RSE</th>
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<tbody>
<tr>
<td>V1 (liters)</td>
<td>229</td>
<td>17</td>
</tr>
<tr>
<td>V2 (liters)</td>
<td>856</td>
<td>15</td>
</tr>
<tr>
<td>V3 (liters)</td>
<td>203</td>
<td>21</td>
</tr>
<tr>
<td>Q1 (l/h)</td>
<td>134</td>
<td>22</td>
</tr>
<tr>
<td>Q2 (l/h)</td>
<td>213</td>
<td>23</td>
</tr>
<tr>
<td>B_{\text{lin}}</td>
<td>7.59</td>
<td>12</td>
</tr>
<tr>
<td>B_{\text{CrEL}}</td>
<td>3.78</td>
<td>10</td>
</tr>
<tr>
<td>B_{\text{max}} (\mu mol/l)</td>
<td>0.0245</td>
<td>27</td>
</tr>
<tr>
<td>K_m (\mu mol/l)</td>
<td>0.000106</td>
<td>43</td>
</tr>
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</table>

V1, V2, and V3, volumes of the central, first, and second peripheral compartment for unbound concentrations of paclitaxel; Q1 and Q2, intercompartmental clearances between the central and peripheral compartments for unbound concentrations of paclitaxel; B_{\text{lin}} linear binding to plasma components; B_{\text{CrEL}}, binding directly proportional to CrEL concentration; B_{\text{max}} maximal binding to plasma components; K_m concentration at half of the maximal binding to plasma components.
plots of conditional weighted residuals versus time (Hooker et al., 2007) were also used to determine the importance of the new parameter.

For parameter estimates not supported by priors, the S.E.s provided by NONMEM were complemented by log-likelihood profiling (LLP). By fixing each parameter at a time in the original model, LLP will calculate the parameter value that results in an increase in OFV for a prespecified significance level. Because a model with a fixed parameter is nested relative to the original model, the lower and higher parameter value that each cause an increase in OFV by 3.84 will constitute a 95% confidence interval (CI) for the specific parameter. Because the search is performed on both sides of the final estimate, no symmetry assumptions are made. For parameter estimates supported by priors, the informativeness of the study-specific data was investigated by calculating the quota of model relative standard errors (RSSEs) to prior RSSEs. The predictive performance of the final model was evaluated using visual predictive checks based on 1000 simulations.

Sensitivity analysis was also performed to determine the influence of frequentist priors on parameter estimates and significance of covariates by 1) increasing the S.E. for each prior by 50%, 2) decreasing and 3) increasing the estimate for one of the priors with $-i/2 \times$ the S.E., and 4) using a different set of priors (Henningson et al., 2005a).

Covariate Testing. Shrinkage in the random effects was calculated to evaluate the appropriateness of using the empirical Bayes estimates of investigation of potential covariate relationships (Savic and Karlsson, 2009). Testing for significant covariates was performed directly in NONMEM, using stepwise covariate modeling in PsN with forward selection and backward elimination. Because of the relatively small population ($n = 33$), covariate testing was considered to be the secondary aim of the work and was hence performed only as a univariate analysis. Covariates were to be included in the final model only if they were significant in both the forward-selection ($p < 0.01$) and backward-elimination step ($p < 0.001$). Inclusion of priors was not compatible with stepwise covariate modeling as implemented in PsN (version 3.1.0), and, therefore, parameters supported by priors (i.e., all structural paclitaxel parameters except clearance) were fixed to the estimates of the final model. In addition, significance testing was performed in R software, using one-way ANOVA on empirical Bayes estimates derived from the POSTHOC step in NONMEM, because it was reported earlier that ANOVA tests in some cases may be more reliable for dealing with type 1 errors than the likelihood ratio test obtained by NONMEM OFV (Bertrand et al., 2009). All IIV parameters, except those that were fixed, were tested against age. In addition, clearance of paclitaxel and metabolites and the relative fraction parameter, $f_{pOHL}/fmpac$, were tested for relationships to SNP variants and CYP3A4 enzyme activity.

Results

Model for Paclitaxel Metabolites. Observed total concentrations of paclitaxel and its three metabolites are shown in Fig. 2. The previously developed paclitaxel-CrEL model adequately described total paclitaxel concentrations, using IIV in clearance for the three-compartment model structure of unbound paclitaxel. Inclusion of IIV in $B_{\text{lin}}$ (eq. 1) further improved the fit ($\Delta$OFV = −85), and after inclusion IIV in other population parameters was not significant ($p < 0.001$). Use of frequentist priors instead of fixed parameter values in the CrEL model had little effect on estimates in the combined paclitaxel-CrEL model. Therefore, the CrEL parameters were fixed to stabilize the analysis.

The final parameter estimates are presented in Table 3. The ratio of relative standard errors for estimates and priors was less than 1 for all population parameters, confirming that the current data set on paclitaxel contained new information and was in line with the data the priors were derived from. The priors for clearance of unbound paclitaxel and the related IIV parameter were omitted in the final model so as not to interfere with covariate testing.

The mechanism-based model was extended with compartments to fit metabolite concentrations. The fraction eliminated by different elimination routes cannot be estimated unless the metabolites are administered. To keep the model structure identifiable, it was therefore assumed that all parent drug and primary metabolites were converted into the metabolites observed here.

![Fig. 2. Observed total plasma concentrations of paclitaxel ($n = 345$), 6α-hydroxypaclitaxel ($n = 332$), $p$-3′-hydroxypaclitaxel ($n = 336$), and 6α-$p$-3′-dihydroxypaclitaxel ($n = 143$). Paclitaxel, 6α-hydroxypaclitaxel, and $p$-3′-hydroxypaclitaxel were observed in 33 individuals and 6α-$p$-3′-dihydroxypaclitaxel was observed in 15 individuals.](image-url)
A one-compartment model with linear elimination and binding of predicted unbound concentrations of 6αOH-pac to predicted CrEL concentrations resulted in a drop in OFV of −230 units and improved conditional weighted residual plots. A nonlinear binding component, similar to $B_{\text{max}}$ and $K_m$ in eq. 1, was found to improve the OFV further (−61) but could be simplified to a single nonspecific binding parameter ($B_{\text{up}}$, 6αOH) without an increase in OFV. A small decrease (−4) in OFV was found by multiplying the constant with CrEL concentrations. To explain the steep increase in total 6αOH-pac concentration, the Hill coefficient decreased OFV further with 65 units. The final model structure for unbound 6αOH-pac was also determined.

## Table 3: Parameter Estimates for the Final Model Without Covariate Relations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (l/h)</th>
<th>RSE %</th>
<th>Lower</th>
<th>Upper</th>
<th>LLP 95% CI Lower</th>
<th>LLP 95% CI Upper</th>
<th>RSE/RSE Prior</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_1$</td>
<td>188</td>
<td>11</td>
<td>144</td>
<td>262</td>
<td>0.245</td>
<td>0.769</td>
<td>0.00149</td>
</tr>
<tr>
<td>$Q_1$</td>
<td>123</td>
<td>10</td>
<td>92</td>
<td>143</td>
<td>0.261</td>
<td>0.762</td>
<td>0.00149</td>
</tr>
<tr>
<td>$Q_2$</td>
<td>181</td>
<td>13</td>
<td>147</td>
<td>215</td>
<td>0.265</td>
<td>0.769</td>
<td>0.00149</td>
</tr>
<tr>
<td>$B_{\text{up}}$</td>
<td>7.89</td>
<td>10</td>
<td>6.89</td>
<td>8.89</td>
<td>0.285</td>
<td>0.769</td>
<td>0.00149</td>
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<tr>
<td>$B_{\text{CeEL}}$</td>
<td>3.62</td>
<td>9.1</td>
<td>1.96</td>
<td>5.26</td>
<td>0.271</td>
<td>0.772</td>
<td>0.00149</td>
</tr>
<tr>
<td>$B_{\text{nsp},6\alpha OH}$</td>
<td>0.0207</td>
<td>22</td>
<td>0.0196</td>
<td>0.0219</td>
<td>0.0081</td>
<td>0.00149</td>
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<tr>
<td>$K_m$</td>
<td>0.000123</td>
<td>36</td>
<td>0.000119</td>
<td>0.000126</td>
<td>0.00149</td>
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<tr>
<td>$f_1$</td>
<td>24.4</td>
<td>4.7</td>
<td>21.9</td>
<td>27.0</td>
<td>0.233</td>
<td>0.769</td>
<td>0.00149</td>
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<tr>
<td>$f_2$</td>
<td>15.3</td>
<td>39</td>
<td>10.0</td>
<td>22.3</td>
<td>0.233</td>
<td>0.769</td>
<td>0.00149</td>
</tr>
</tbody>
</table>

where $B_{\text{CeEL},6\alpha OH}$ is the maximal binding rate to CrEL, $B_{\text{nsp},6\alpha OH}$ is the nonspecific binding parameter to CrEL, and $B_{\text{CeEL},6\alpha OH}$ is the CrEL concentration at half-maximal binding rate and Hill $H_{\text{CeEL}}$ is the Hill coefficient for CrEL concentration. Allowing an additive error at this time of the first sample improved the stability of the model and lowered the OFV with 15 units.

The final model structure for unbound p3OH-pac was also described with a one-compartment model with linear elimination, and the relationship between the total and unbound metabolite and CrEL concentration was identical to eq. 4. The parameters $H_{\text{CeEL}}$ and $CrEL_{50}$ for eq. 4 were set to be the same for both primary metabolites. There was no need for an additive error on the first samples for p3OH-pac.

The distribution of clearance of unbound paclitaxel to the formation of the primary metabolites was modeled using a relative fraction parameter. Preliminary modeling attempts indicated that this parameter was not identifiable at a population level and was therefore fixed to a corresponding literature value describing in vitro formation of p3OH-pac, which has been estimated to be 37% of the paclitaxel
metabolized to 6αOH-pac and p3OH-pac (Cresteil et al., 2002). To allow IIV for the relative fraction parameter, \( f_{p3OH/fmpac} \), but to keep individual estimates in the range of 0 to 1, logit transformation was used, so that

\[
\frac{f_{p3OH/fmpac}}{\text{exp}(\beta_{\text{pop}} + \eta)} = \frac{\text{exp}(\beta)}{1 + \text{exp}(\beta)}
\]

where IIV was described additively:

\[
\beta = \beta_{\text{pop}} + \eta
\]

and \( \beta_{\text{pop}} \) is the population value corresponding to \( f_{p3OH/fmpac} = 0.37 \) and \( \eta \sim N(0, \sigma^2) \). Using a block structure with three additional covariance parameters for IIV in \( CL_{p3OH/fmpac} \), \( CL_{p3OH/fmpac} \), and \( \beta \) in eq. 6 did not convey a significant change \((p < 0.05)\) in OFV. When the two primary metabolites were fitted without using a block structure, IIV in \( CL_{p3OH/fmpac} \) was not significant \((p < 0.05)\) and was consequently removed.

Total concentrations of the secondary metabolite diOH-pac were included using a one-compartment model with linear elimination. No association between total diOH-pac and CrEL was found. An additive error, on top of a proportional, reduced the OFV with \( 71 \) units.

For the full model, the log-likelihood profiling for each parameter not supported by a prior is presented in Table 3. The boundaries of the 95% CIs are in agreement with the symmetric 95% confidence intervals calculated from the standard errors reported by NONMEM, but they showed a trend of being right-skewed. Observed concentrations versus population predictions, stratified on parent drug and the different metabolites, are shown in Fig. 3, and visual predictive checks are shown in Fig. 4.

**Covariate Analysis.** Rare SNP variants (Table 1) were pooled with larger groups before covariate analysis. CYP2C8*1B A/A was pooled with C/A, and CYP2C8 haplotype C/G was pooled with C/G.

Shrinkage for nonfixed random effects ranged from 6.2 to 34%. For compatibility reasons, all parameters using priors had to be fixed to their respective estimate from the final model (Table 3) when the automatic stepwise covariate model building procedure in PsN was applied. All covariates significant at \( p < 0.1 \) were tested using one-way ANOVA. The results from the covariate analysis are presented in Table 4. No parameters were significantly correlated to the investigated covariates for \( p < 0.01 \), which was considered to be the required level for inclusion in the final model during the forward-selection step. However, \( CL_{\text{diOH/fmmet}} \) which was significantly \((p < 0.05)\) affected by \( GM_{2677T/A} \), was also tested by inclusion in the final model using frequentist priors instead of fixed estimates. Individuals with SNP variant G/A or G/G (pooled) showed a 30% \((2.8–62%, 80\% \) confidence interval\) increase compared with the reference group constituted by the G/T heterozygous patients, whereas individuals with variant T/T showed a 27% \((9.4–42%, 80\% \) confidence interval\) decrease in \( CL_{\text{diOH/fmmet}} \) relative to that of the reference group G/T. Patients with the wild-type G/G or G/A variant had the highest \( CL_{\text{diOH/fmmet}} \), whereas G/T individuals were intermediate and T/T homozygous patients in position 2677 had the lowest \( CL_{\text{diOH/fmmet}} \).

Inclusion of the covariate decreased the IIV \((\text{coefficient of variation (CV) percentage})\) in \( CL_{\text{diOH/fmmet}} \) from 36.6 to 30.5%. \( CL_{\text{pac}} \) also showed a significant \((p < 0.05)\) correlation with CYP3A5*3 and was tested separately in the final model. Individuals with SNP variant G/A showed a 20% \((4.7–32%, 95\% \) confidence interval\) decrease relative to that of the reference group G/G. Inclusion of the covariate decreased the IIV \((\text{CV percentage})\) in \( CL_{\text{pac}} \) from 15.3 to 12.7%. In addition, there was a tendency that \( CL_{\text{pac}} \) and \( CL_{\text{diOH/fmmet}} \) were correlated \((p < 0.1)\) to CYP2C8*1C and CYP3A4*1B, respectively.
Because inclusion of the covariates only resulted in a minor reduction in OFV, two alternative models that included IIV in $V_1$ and $V_2$ were evaluated to investigate the consistency in covariate effects. In alternative model 2, the IIV in the linear binding of paclitaxel to plasma proteins ($B_{lin}$) also was excluded to exactly mimic the parameterization in the original model (Henningsson et al., 2001). Only GM2677T/A showed a significant effect for all three models ($p < 0.05$ for ANOVA and $p < 0.1$, based on a drop in OFV).

The results from the sensitivity analysis are presented in Table 5. $\text{CL}_{6\alpha\text{-OH}}/\text{fm}_{\text{pac}}$ was significantly ($p < 0.05$) correlated to GM2677T/A regardless of the tested sets of prior estimates. The parameter $\text{CL}_{6\alpha\text{-OH}}/\text{fm}_{\text{pac}}$, without inclusion of covariate, is presented to show the effect on a parameter estimate when different sets of frequentist priors are used.

**Discussion**

To our knowledge, this is the first time the pharmacokinetics of both paclitaxel and its most acknowledged metabolites have been characterized using a population approach with nonlinear mixed-effects modeling. Previously, paclitaxel and 6\alpha-hydroxy-paclitaxel were modeled using nonlinear kinetics for both parent drug and metabolite (Gianni et al., 1995), without considering the formulation vehicle CrEL. It was later shown that the nonlinear kinetics for paclitaxel most likely is a result of binding to CrEL and plasma components and that unbound concentrations follow linear kinetics (Henningsson et al., 2001). In this work, we presented strong indications that the same may be true for the two primary metabolites, because total concentrations of

![Graphs](image.png)

**Fig. 4.** Visual predictive checks based on 1000 simulations. Dashed lines, 95% confidence interval of the 5th, 50th, and 95th percentiles of simulated data; solid lines, 5th, 50th, and 95th percentiles of observed data.

**Table 5**

Sensitivity analysis for significant ABCB1 allele G2677T/A using empirical Bayes estimates

<table>
<thead>
<tr>
<th>Model</th>
<th>$p$ (ANOVA) G2677T/A on $\text{CL}<em>{\text{6\alpha\text{-OH}}}/\text{fm}</em>{\text{pac}}$</th>
<th>$\text{CL}<em>{\text{6\alpha\text{-OH}}}/\text{fm}</em>{\text{pac}}$ (l/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final model</td>
<td>0.012</td>
<td>2830</td>
</tr>
<tr>
<td>Test 1</td>
<td>0.012</td>
<td>2790</td>
</tr>
<tr>
<td>Test 2</td>
<td>0.010</td>
<td>2800</td>
</tr>
<tr>
<td>Test 3</td>
<td>0.014</td>
<td>2870</td>
</tr>
<tr>
<td>Test 4</td>
<td>0.011</td>
<td>2290</td>
</tr>
</tbody>
</table>

Test 1, increased S.E. for each prior by 50%; test 2, prior for $B_{\text{CrEL}}$ set to prior estimate $-2 \times \text{S.E.}$; test 3, prior for $B_{\text{CrEL}}$ set to prior estimate $+2 \times \text{S.E.}$; test 4, prior estimates from Henningsson et al. (2005a) for unbound paclitaxel parameters only ($V_1$, $V_2$, $V_3$, $Q_2$, and $Q_3$).
6αOH-pac and p3OH-pac both seemed to be dependent on the predicted time course of CrEL and by extensive and nonlinear binding, whereas no such association was apparent for the secondary metabolite diOH-pac.

A Hill equation was earlier used to describe micelle kinetics (Piszkieczewicz, 1977), although for catalytic micelles rather than for substrate binding only. Still, the estimate of 2.71 in Table 3 for the Hill coefficient is well within the range of the previously derived coefficients for a number of combinations of detergents and substrates, which has been reported to range from approximately 1 to 6 with the majority less than 3 (Piszkieczewicz, 1977).

The visual predictive checks in Fig. 4 show good adequacy of the models, although for total diOH-pac concentration, the lower percentile was overpredicted. It is plausible that the low number of patients (n = 15) contributing with observations to diOH-pac, in combination with relatively noisy data, contributed to the relatively poor visual predictive check for diOH-pac.

The maximum binding rate to CrEL in the Hill term was five times higher for 6αOH-pac than for p3OH-pac (Table 3), explaining the steeper increase in total 6αOH-pac concentrations than for total p3OH-pac concentrations during the infusion (Fig. 2).

No significant correlation between the pathways of paclitaxel to p3OH-pac and 6αOH-pac to diOH-pac or paclitaxel to 6αOH-pac and p3OH-pac to diOH-pac could be detected. In addition, the first two pathways were not significantly correlated to the CYP3A4 phenotype. The absence of these associations is noteworthy but may depend on the relatively small study group. For parameters in which shrinkage in random effects was relatively high (CLp,m, 21%; CLdOH/Fmm, 34%), the approach with ANOVA may not help in detecting false-negative results (Bertrand et al., 2009). It was also shown earlier that study groups with less than 50 to 100 individuals may cause problems in characterization of covariate effects (Ribbing and Jonsson, 2004).

This issue should also be considered for the finding in this work of reduced clearance (fraction metabolized) of 6αOH-pac/Fmm for carriers of the ABCB1 G2677T/A G/T and T/T polymorphisms. The study group under consideration here has previously been used to show (nonparametrically) a significantly higher clearance for total paclitaxel concentrations in individuals carrying the G/A variant of G2677T/A (Gréen et al., 2009), but this is the first time G2677T/A has been significantly correlated to the primary metabolite 6αOH-pac. Because 6αOH-pac is the major metabolite for most patients, it may also reflect paclitaxel clearance, which makes it plausible that the finding in this model-based analysis is related to the same source as the earlier finding for the parent drug. In a previous study, Yamaguchi et al. (2006) found a positive correlation of the ABCB1 total mutant allele number to the clearance of paclitaxel in 13 Japanese patients with ovarian cancer. Nakajima et al. (2005) did not present a similar finding in 23 patients with ovarian cancer, although they found that carriers of the ABCB1 genotype C3435T had a significantly higher area under curve of p3OH-pac. In addition, Sissung et al. (2006) did not find a correlation between the ABCB1 genotypes C3435T and G2677T/A and paclitaxel pharmacokinetics, although that study was also small (n = 26). Correlations between kinematics for unbound paclitaxel and the genotypes CYP2C8*2, CYP2C8*3, CYP2C8*4, and CYP3A4G*3 and the C3435T genotype were not found in a large study with 97 individuals (Henningsson et al., 2005a). However, that study was based on a less homogeneous group of patients than those in the current study, because it included a large number of dosing schedules, infusion times, and both male and female patients being treated for different kinds of tumors (Henningsson et al., 2005a). In a more recent study that was relatively large (n = 93) and more homogeneous, a significant correlation between CYP2C8*3 and clearance of unbound paclitaxel was reported (Bergmann et al., 2010), but no correlation between ABCB1 genotypes and clearance was found. In the present study, the CYP2C8*3 correlation could not be detected, which may be the result of too few individuals (n = 6) carrying the specific genotype.

All correlations significant at p < 0.1 from the stepwise covariate modeling are summarized in Table 4, together with their corresponding p value from ANOVA using empirical Bayes estimates. Although effects can be considered small, they are consistent between NONMEM OFV and ANOVA, which would reduce the probability of a type 1 error (Bertrand et al., 2009). The significant correlation of CLpac to CYP3A5*3 may correspond with a recent finding (Leskelä et al., 2010), in which the allele was shown to have a protective effect against neurotoxicity. In the current study, carriers of CYP3A5*3 G/A showed a 20% decrease in clearance of unbound paclitaxel relative to that of the reference group G/G. This observation is somewhat surprising, because the G/G group is carrying an inactive allele and would thus be expected to have lower clearance. However, it should be pointed out that only five individuals were carriers of the G/A genotype and that a larger population would be desirable to further clarify this outcome, and, for the two alternative models, the genotype was not statistically significant (Table 4).

In contrast to the finding for CYP3A5*3, the correlation of CLtotOH- pac/Fmm of G2677T/A was significant over all models and also consistent over different sets of frequentist priors as presented in Table 5. The latter finding implies that the significant correlation is not an artifact of a particular set of priors. The estimated covariate effects for G2677T/A on clearance of 6αOH-pac/Fmm were relatively large, with an increase and decrease of 30 and 27% for G/G pooled with G/A and T/T, respectively, compared with the effect of CYP2C8*3 on clearance of unbound paclitaxel reported by Bergmann et al. (2010), which was only a decrease of 11%. However, the effect estimates of G2677T/A in this study were associated with a high uncertainty, and the 95% and 90% CIs (but not the 80% CI) for the estimates included zero.

The correlation of clearance of diOH-pac/Fmm could be an artifact, because only three individuals carried the specific genotype. To our knowledge, there is no information in the literature concerning the role of CYP3A4 in clearance of diOH-pac. The correlation of CYP2C8*1C to clearance of unbound paclitaxel was not found in Bergmann et al. (2010) and was not consistent for the alternative models.

In conclusion, our study supports the previous findings of the ABCB1 transporter playing a part in paclitaxel metabolism (Nakajima et al., 2005; Yamaguchi et al., 2006) or paclitaxel treatment outcome (Greén et al., 2006a). For the first time, we have also presented strong indications that the formulation vehicle Cremophor EL, similar to the parent drug, affects the pharmacokinetics of the two primary metabolites. Finally, the finding by Leskelä et al. (2010) that alleles associated with decreased paclitaxel metabolism seem to contribute to a lower risk for neurotoxicity could imply that paclitaxel metabolites play a role in adverse effects in paclitaxel treatment. If so, paclitaxel metabolites could be clinically relevant, in which case the description of metabolite kinetics developed in this work may be important.

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Authorship Contributions
Participated in research design: Fransson, Gréen, and Friberg.
Conducted experiments: Gréen.
Performed data analysis: Fransson, Grén, and Friberg.
Wrote or contributed to the writing of the manuscript: Fransson, Grén, Litton, and Friberg.

Other: Fransson, Grén, Litton, and Friberg.

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