Rapid Esterase-sensitive Breakdown of Polysorbate 80 and Its Impact on the Plasma Pharmacokinetics of Docetaxel and Metabolites in Mice

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ABSTRACT

We have developed and validated an analytical methodology for the quantification of docetaxel and its four major human oxidation metabolites in mouse plasma. We have used this procedure to study the pharmacokinetics and metabolism of docetaxel in female FVB mice, receiving 2.5, 10, or 33 mg/kg of docetaxel by i.v. injection. We have also studied the pharmacokinetics of polysorbate 80, because it was shown previously that the vehicle substance Cremophor EL, which is used in the formulation of paclitaxel, exerts a profound effect on the pharmacokinetics of this compound. Linear pharmacokinetics of docetaxel was observed at dose levels between 2.5 and 10 mg/kg, where plasma levels corresponded to those in patients receiving the maximum tolerated dose. At the highest dose level of 33 mg/kg, a deviation from the linear kinetics was observed. Compared with humans, mice could tolerate much higher plasma levels, suggesting that the toxic side effects are related to a certain plasma threshold concentration instead of area under the curve or Cmax. At the highest dose level, three docetaxel metabolites could be detected in the plasma samples of mice for up to 4 h after drug administration. The hydroxy metabolite of the tert-butoxy group (metabolite II) was the major metabolite, followed by the two epimeric hydroxyoxazolone-type compounds (metabolites I and III). A fourth putative metabolite (e.g., the cyclic oxazolidinedione derivative) was not detected. Because of rapid degradation of polysorbate 80 by esterases in plasma, the concentration of this vehicle substance declined very rapidly. Consequently, this substance was not able to interfere in the disposition of docetaxel.

INTRODUCTION

Docetaxel is a semisynthetic taxane analogue, prepared by chemical manipulation of 10-deacetyl baccatin-III, an inactive precursor isolated from the needles of the European yew tree, Taxus baccata L. (1). The compound is a potent inhibitor of cell replication, blocking cells in the late G2-M phase of the cell cycle, by stabilization of the microtubule cytoskeleton (2). In clinical trials, good response rates have been documented in patients with breast, lung, head and neck, and advanced platinum-refractory ovarian carcinomas (3, 4). The pharmacokinetics of docetaxel in patients has been established in early clinical trials (5). Unlike for paclitaxel, docetaxel has shown a linear pharmacokinetic behavior over a wide range of dose levels (20–115 mg/m²). By using mass spectrometry and nuclear magnetic resonance spectroscopy, Monier et al. (6) identified four major metabolites in feces of patients, which were formed by oxidation reactions of the tert-butoxyl moiety in the C13-side chain of the parent compound (Fig. 1).

Mice are the primary species used in in vivo anticancer drug screening. They provide essential information about the safety and efficacy of new cytotoxic entities. To make an optimal and a more rational use of the preclinical data, information about the pharmacokinetic behavior of the experimental agent is required. Thus far, detailed studies on the pharmacokinetics of docetaxel and metabolites in mice are scarce (7). We have reported previously about the pharmacokinetics of paclitaxel in mice and demonstrated that its nonlinear pharmacokinetic behavior was due to the vehicle substance Cremophor EL (8). Because the more than proportional increase in plasma AUC did not result in an equivalent increase in tissue levels (9), we postulated that Cremophor EL reduces the “free” fraction of paclitaxel in mice and demonstrated that its nonlinear pharmacokinetic behavior was due to the vehicle substance Cremophor EL (8). Because the more than proportional increase in plasma AUC did not result in an equivalent increase in tissue levels (9), we postulated that Cremophor EL reduces the “free” fraction of paclitaxel in mice and demonstrated that its nonlinear pharmacokinetic behavior was due to the vehicle substance Cremophor EL (8). Because the more than proportional increase in plasma AUC did not result in an equivalent increase in tissue levels (9), we postulated that Cremophor EL reduces the “free” fraction of paclitaxel in mice and demonstrated that its nonlinear pharmacokinetic behavior was due to the vehicle substance Cremophor EL (8). Because the more than proportional increase in plasma AUC did not result in an equivalent increase in tissue levels (9), we postulated that Cremophor EL reduces the “free” fraction of paclitaxel in mice and demonstrated that its nonlinear pharmacokinetic behavior was due to the vehicle substance Cremophor EL (8). Because the more than proportional increase in plasma AUC did not result in an equivalent increase in tissue levels (9), we postulated that Cremophor EL reduces the “free” fraction of paclitaxel in mice and demonstrated that its nonlinear pharmacokinetic behavior was due to the vehicle substance Cremophor EL (8). Because the more than proportional increase in plasma AUC did not result in an equivalent increase in tissue levels (9), we postulated that Cremophor EL reduces the “free” fraction of paclitaxel in mice and demonstrated that its nonlinear pharmacokinetic behavior was due to the vehicle substance Cremophor EL (8). Because the more than proportional increase in plasma AUC did not result in an equivalent increase in tissue levels (9), we postulated that Cremophor EL reduces the “free” fraction of paclitaxel in mice and demonstrated that its nonlinear pharmacokinetic behavior was due to the vehicle substance Cremophor EL (8).
Cremophor EL, we have also established the pharmacokinetics of this excipient by using a colorimetric dye-binding assay.

**MATERIALS AND METHODS**

**Animals.** Female FVB mice, 10–14 weeks of age, were used in all experiments. The animals were maintained at constant temperature (20–24°C) and humidity and were given food (Hope Farms BV, Woerden, the Netherlands) and acidified water ad libitum.

**Chemicals and Reagents.** Docetaxel reference compound and the clinical docetaxel formulation in PS-80 (Taxotere, 40 mg/ml) originated from Rhône-Poulenc Rorer (Anthony Cedex, France). Docetaxel metabolites, isolated and purified from patient feces as described in detail previously (11), were available in small quantities as solutions of 1 mg/ml in methanol. The internal standard, cephalomannine, was kindly provided by Dr. L. K. Webster (Peter McCallum Cancer Institute, Melbourne, Australia). PS-80, oleic acid, PMSF, essentially fatty free human serum albumins and carboxyl esterase (EC 3.1.1.1) were purchased from Sigma Chemical Co. (St. Louis, MO). Coomassie Brilliant Blue G-250 was purchased from Bio-Rad Laboratories (Munich, Germany) as a concentrated solution in 85% (w/v) phosphoric acid-95% (v/v) ethanol (2:1, v/v). All other chemicals and reagents were of analytical or Lichrosolv gradient grade and were purchased from E. Merck (Darmstadt, Germany). Filtered and deionized water (Milli-Q Plus system; Millipore, Milford, MA) was used in all aqueous solutions. Drug-free human plasma originated from the Central Laboratory of the Blood Transfusion Service (Amsterdam, the Netherlands). Blank mouse plasma was obtained from untreated animals.

**HPLC Assay for Docetaxel.** The HPLC system was composed of a Model 300C solvent delivery system (Gynkotek, Germering, Germany), a Marathon HPLC autosampler equipped with a 100-μl sample loop (Spark Holland, Emmen, the Netherlands), a Spectroflow SF759 variable wavelength detector (Kratos, Ramsey, NJ), and a SP4600 Datalet integrator connected to a WINner/286 chromatography work station (Thermo Separations Products, San Jose, CA). Chromatographic separation was performed with an analytical column (15 × 0.46 cm, inside diameter) packed with 5 μm APEX-octyl material (Jones Chromatography, Littleton, CO). The mobile phase was a mixture of acetonitrile-methanol-0.02 M ammonium acetate buffer, pH 5.0 (35:10:55, v/v/v). The flow rate of the mobile phase was 1.0 ml/min. The column was maintained at ambient temperature, and the column effluent was monitored at a wavelength of 231 nm with a filter rise time of 5 s.

**Preparation of Calibration Standards and Quality Control Samples.** Calibration samples of docetaxel were prepared in blank human plasma. The final concentrations were 5000, 1000, 500, 250, 100, 50, and 25 ng/ml of docetaxel. Quality control samples were prepared in blank mouse plasma at three levels, i.e., 50, 250, and 1000 ng/ml of docetaxel. A fourth quality control sample in blank mouse plasma containing docetaxel and all metabolites was prepared by dilution of the mixture of docetaxel and metabolites in blank mouse plasma. The final concentrations of metabolites M1, M2, M3, and M4 were 144, 230, 188, 172, and 277 ng/ml, respectively.

**Sample Preparation Procedure.** Quality control samples or unknown mouse plasma samples (50–250 μl) and 50 μl of the internal standard (20 μg/ml cephalomannine in methanol) were twice extracted with 4 ml of diethyl ether. The combined ethereal fractions were dried by vacuum concentration in a Speed-Vac Plus SC210A system (Savant, Farmingdale, NY) at 43°C, and the residues were reconstituted in 500 μl of 0.01 M ammonium acetate buffer, pH 5.0 and further processed by automated solid phase extraction using the ASPEC system (Gilson Medical, Middleton, WI). The solid phase extraction columns (Bond Elut Cyanopropyl columns; 100 mg; Varian, Harbour City, CA) were washed with 2 ml of methanol and 2 ml of 0.01 M ammonium acetate buffer, pH 5.0. Volumes of 500 μl of sample were loaded onto the solid phase extraction column. After consecutive washings with 2 ml of 0.01 M ammonium acetate buffer (pH 5.0) and 1 ml of methanol-0.01 M ammonium acetate buffer, pH 5.0 (2:8, v/v), docetaxel
and the metabolites were eluted with 500 µl of acetonitrile-
triethylamine (1000:1, v/v). The samples were dried by vacuum
concentration, and the residues were redissolved in 200 µl of
acetonitrile-methanol-water (35:10:55, v/v/v). Aliquots of 100
µl were subjected to HPLC. Peak area ratios of the analytes and
the internal standard were used for quantitative computations.
Calibration graphs were calculated by weighted (1/Y) least-
squares linear regression analyses. The validation was per-
duced as described previously for paclitaxel (10).

Pharmacokinetic Study of Docetaxel. Docetaxel formulated
in PS-80 and diluted with ethanol and sterile isotonic
sodium chloride solution was given to mice at dose levels of 2.5,
10, and 33 mg/kg by i.v. bolus injection (3.3 µl/g of body
weight) in the tail vein. Blood samples from three to four mice
per time point were collected in heparinized syringes at 5, 15,
30, and 45 min and 1, 2, 4, 6, 8, and 12 h after drug adminis-
tration into 1.5-ml polypropylene microtubes (Eppendorf, Ham-
burg, Germany). Plasma was obtained by centrifugation at 4°C
(10 min; 2100 × g) and stored at −20°C until analysis within 4
weeks.

The plasma concentration-time curves of docetaxel were
fitted using a two-compartmental model with the MW/PHARM
software program (12). The AUC of docetaxel was calculated by
noncompartmental analysis using the linear trapezoidal rule
with the formula:

\[
AUC = \sum_{i=2}^{n} \text{Concentration}_i \cdot (\Delta \text{time}_{i-1} + \Delta \text{time}_i)/2
\]

with \( \Delta \text{time}_0 = 0 \). The SE of the AUC was calculated with the
law of propagation of errors using the formula:

\[
\text{SE}_{\text{AUC}} = \left[ \sum_{i=2}^{n} \text{SE}_{i}^2 \cdot (\Delta \text{time}_{i-1} + \Delta \text{time}_i)^2 \right]^{1/4}
\]

The slope of the final log-linear part of the concentration-time
profile \((K_{el}, \text{elimination rate constant})\) was obtained by un-
weighted linear regression analysis after logarithmic transfor-
mation of the individual mouse concentrations versus time.

The terminal half-life \([t_{1/2} = \ln (2) / K_{el}]\), the clearance
(\(CL = \text{Dose/AUC}\)), and the apparent volume of distribution
(\(Vd = \text{Dose}/\text{AUC} \cdot K_{el}\)) were calculated by classical pharma-
kokineti
c equations.

The concentrations of docetaxel metabolites were quanti-
fied using the calibration curve of docetaxel in plasma and were
corrected for their differences in recovery relative to docetaxel.
The AUCs of the metabolites were calculated by the linear
trapezoidal rule as outlined above.

Blood Sampling for the in Vivo Kinetics of PS-80. The
in vivo kinetics of PS-80 in mice was performed in a different
set of animals than used for the pharmacokinetics study of
docetaxel and metabolites. The animals received an i.v. bolus
dose of 3.3 µl of PS-80:ethanol:saline (1:1:2, v/v/v) per g of
body weight, corresponding to the amount of vehicle adminis-
tered to animals receiving 33 mg/kg of docetaxel. All blood
samples were collected in heparinized microtubes containing 10
µl of 10% PMSF in isopropanol to prevent continued
degradation of PS-80 after sampling. The plasma fraction was
separated by centrifugation (3000 × g for 5 min) and was stored
at −80°C until analysis.

For the analysis of PS-80 in humans, blood samples were
obtained following receipt of informed consent from five pa-
tients with advanced colorectal cancer receiving 100 mg/m² of
docetaxel (single agent) as a 1-h i.v. infusion (13). Blood sam-
ple were obtained prior to and up to 48 h after the start of the
infusion from an indwelling i.v. cannula and collected in He-
mogard vacutainer tubes (Becton Dickinson, Meylan, France)
containing PMSF (see above) and EDTA as anticoagulant. All
samples were centrifuged immediately after sampling, and the
plasma fraction was snap-frozen and stored at −20°C until
analysis.

PS-80 Assay. The analytical procedure for PS-80 in plasma
was a modification of our colorimetric dye-binding micro assay for
Cremophor EL (14). Briefly, 50-µl samples of plasma were deproteinized by addition of 500 µl of acetonitrile
in Teflon-capped, 12-ml tubes, followed by vortex mixing for 1
min. Next, 2 ml of n-butyl chloride was added, followed by
vigorous mixing for 5 min. The organic layer was separated by
centrifugation (4000 × g for 5 min), transferred to a clean 10-ml
glass tube, and dried under nitrogen at 60°C for 30 min. The
dried residue was reconstituted in 50 µl of water by vortex
mixing, and a 25-µl aliquot was pipetted into a 96-well flat
bottomed cluster (Costar Corp., Cambridge, MA). Finally, 250
µl of water-diluted (1:4, v/v) Coomassie Brilliant Blue G-250
reagent were added, and the absorbance maximum of the dye at
595 nm after binding to PS-80 and the simultaneous decrease in
absorbance at 450 nm were measured within 5 h against a
reagent blank using a Model 550 automated microplate reader
(Bio-Rad Laboratories, Hercules, CA).

Calibration standards were prepared in drug-free human or
murine plasma on the day of analysis by pipetting 10 µl of
diluted aqueous standard of PS-80 to 490 µl of plasma to yield
final concentrations of 0.01, 0.05, 0.10, 0.25, 0.50, and 1.0%
(v/v/v). Calibration curves were calculated by weighted (concentra-
tion ²) least squares linear regression analysis using the
equation: \(Y = a \cdot \text{concentration} + b\). The response \(Y\) is
calculated from the absorption \(A\) measured at 595 and 450 nm
using the equation: \(Y = (A_{595}/A_{450})_{\text{standard}} - (A_{595}/A_{450})_{\text{water}}\).

The validation was performed as described previously for
Cremophor EL (14), and analytical runs included a calibration
curve in duplicate and quality control samples in quintuplicate,
performed on four separate occasions. The lower limit of detec-
tion was established at 0.01% (v/v). Typical calibration curves
rendered correlation coefficients better than 0.995, with the
relative deviation from nominal concentrations and values for
within-run and run-to-run variability <15%.

In Vitro Degradation of PS-80. The hydrolysis kinetics
of PS-80 was established in PBS, human plasma, and PBS
containing human serum albumin, all with or without carboxyl
esterase, using the dye-binding assay. PS-80, human serum
albumin, and esterase concentrations of 1.0% (v/v), 40 mg/ml,
and 0.1 units/ml, respectively, were used. All experiments
were conducted at pH 7.4 and 37°C and were performed in duplicate
on three separate occasions. Levels of oleic acid were measured
using precolumn esterification with 9-anthryldiazomethane and
reversed-phase HPLC, as described previously (15).
RESULTS

Analytical Procedure for Docetaxel and Metabolites.
We have developed and validated an HPLC method for the determination of docetaxel and four metabolites in mouse plasma. Representative chromatograms of a blank and a spiked plasma sample are shown in Fig. 2. The elution order of the compounds was metabolites I, II, III, IV, cephalomannine (internal standard, V), and docetaxel (VI), with retention times of approximately 6.6, 7.0, 7.5, 12.7, 14.2, and 17.1 min, respectively. No interfering endogenous peaks were observed in blank mouse plasma samples. Fig. 2C depicts a mouse plasma sample obtained 30 min after i.v. administration of 33.3 mg/kg of docetaxel. Docetaxel and metabolites I, II, and III but not metabolite IV were detected.

The lower limit of quantitation for docetaxel was 25 ng/ml using 250 μl of plasma. The precision and accuracy of the method were acceptable (Table 1). Calibration curves were linear over the range of 25–5000 ng/ml. The absolute recovery of the extraction procedure for docetaxel was 75.0%. The recoveries of metabolites I, II, III, and IV were 70.3, 75.4, 73.3, and 91.4%, respectively, relative to docetaxel.

Pharmacokinetics of Docetaxel and Metabolites. We have established the plasma concentration-time profiles of docetaxel, following a single i.v. bolus injection of docetaxel at dose levels of 2.5, 10, and 33 mg/kg (Fig. 3). We used noncompartmental methods to calculate the pharmacokinetic parameters (Table 2).

The AUC of docetaxel increased linear between dose levels of 2.5–10 mg/kg. The CL at the 33 mg/kg dose level was significantly lower than at the two lower dose levels, indicative for nonlinear pharmacokinetic behavior. The course of the concentration-time curves suggests that this nonlinearity resulted from the first part of the curve, when the concentrations are relatively high.

At the highest dose level (33 mg/kg), three of the four metabolites of docetaxel could be detected in the plasma samples (Table 2). Metabolite II was the major metabolite observed in plasma, followed by metabolites I and III, each at about similar levels. Metabolite IV was not detected in any of the mouse plasma samples. Moreover, no additional putative metabolite peaks were observed. The Cmax of the metabolites occurred between 15 and 45 min after drug administration, and the compounds could no longer be detected in samples taken at 6 h or later. Only metabolites II and III were detected at 10 mg/kg; however, their concentrations remained much lower relative to those found at 33 mg/kg.

Pharmacokinetics of PS-80. The plasma concentration-time curve of PS-80 in animals receiving this vehicle compound at a dose level that is equivalent to 33 mg/kg of docetaxel is depicted in Fig. 4. Within 15 min after bolus injection, the concentration rapidly declines to levels <0.05% (v/v) of the plasma volume. The results obtained by studying the in vitro kinetics of PS-80 breakdown strongly suggest that esterases in plasma, catalyzing the cleavage of the oleic acid side chain from the PS-80 molecule, are responsible for this rapid decay (Table 3). On the basis of the colorimetric assay, about 66 and 55% of the initial concentration of PS-80 were recovered 15 min and 4 h, respectively, after the start of the incubation. The apparent slowing down of PS-80 breakdown after 15 min was due to the fact that the breakdown product was also detected by the colorimetric assay, albeit with a lower molar absorption coefficient. The rapid esterase-sensitive breakdown of PS-80 was accompanied by a quantitative release of oleic acid from the surfactant.

The PS-80 peak plasma levels observed in five patients with advanced colorectal cancer receiving 100 mg/m² of do-

Table 1 The accuracy (%DEV), within-day precision (WDP%), and between-day precision (BDP%) of docetaxel in mouse plasma determined in three analytical runs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Nominal concentration (ng/ml)</th>
<th>%DEV</th>
<th>WDP%</th>
<th>BDP%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docetaxel</td>
<td>50</td>
<td>−9.3</td>
<td>13.7</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>−6.5</td>
<td>6.6</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>−6.4</td>
<td>3.5</td>
<td>2.1</td>
</tr>
</tbody>
</table>
As a single-agent 1-h i.v. infusion, the concentration of docetaxel in plasma was 0.016% (v/v; mean ± SD; n = 5) and 0.005% (v/v) at later time points, the levels had already fallen below the detection limit of the assay.

### DISCUSSION

We have established the pharmacokinetics of docetaxel in mice receiving this drug at three dosages covering a wide range of dose levels. This study shows that the plasma concentrations in mice receiving 2.5 and 10 mg/kg resemble those in patients receiving 100 mg/m² (see example in Fig. 3), and the AUC increased proportionally within this range of dose levels. This result is in line with our previous preclinical pharmacokinetics study of paclitaxel, where we showed that the nonlinear pharmacokinetics of paclitaxel in mice (and probably also in humans) results from the vehicle substance Cremophor EL (8).

When paclitaxel was administered in the same formulation as docetaxel [i.e., PS-80:ethanol (1:1; v/v) diluted with saline], the pharmacokinetic behavior was linear, and the Cl of 2.6 l/h/kg was in the same range as established for docetaxel in this study. Cremophor EL levels in plasma were relatively high (0.5–2%, v/v), as this compound seems to be distributed in the central compartment exclusively and is eliminated at a very slow rate (8). The pharmacokinetics of PS-80 is very different from that of Cremophor EL. The plasma concentration of PS-80 was 0.05% (v/v) within 15 min after drug administration. Because of the rapid breakdown by esterases, PS-80 is unable to exert similar effects as Cremophor EL on the pharmacokinetics of docetaxel.

#### Table 2

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>2.5</th>
<th>10</th>
<th>33</th>
</tr>
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<tbody>
<tr>
<td><strong>Docetaxel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>4.85 ± 0.07</td>
<td>9.76 ± 0.56</td>
<td>65.9 ± 8.6</td>
</tr>
<tr>
<td>$AUC$</td>
<td>1.49 ± 0.05</td>
<td>4.80 ± 0.15</td>
<td>30.6 ± 1.5</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>1.68 ± 0.06</td>
<td>2.08 ± 0.07</td>
<td>1.08 ± 0.05</td>
</tr>
<tr>
<td>$K_{el}$</td>
<td>0.575 ± 0.092</td>
<td>0.418 ± 0.024</td>
<td>0.432 ± 0.048</td>
</tr>
<tr>
<td>$V_d$</td>
<td>2.9</td>
<td>5.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Metabolite I**
- $C_{\text{max}}$ ---
- $AUC$ ---
- $t_{1/2}$ 0.64 ± 0.14
- $V_d$ 0.38 ± 0.04

**Metabolite II**
- $C_{\text{max}}$ ---
- $AUC$ 0.11 ± 0.04
- $t_{1/2}$ 2.18 ± 0.32
- $V_d$ 1.73 ± 0.15

**Metabolite III**
- $C_{\text{max}}$ ---
- $AUC$ 0.08 ± 0.03
- $t_{1/2}$ 0.80 ± 0.15
- $V_d$ 0.80 ± 0.07

---

*a* $C_{\text{max}}$, maximum plasma level; $AUC$, area under the plasma concentration versus time curve; $K_{el}$, elimination rate constant; $Cl$, clearance; $V_d$, apparent volume of distribution; $t_{1/2}$, elimination half-life; --, plasma levels below lower limit of quantitation.
In patients, interactions by PS-80 are even less likely. Because of the fact that patients receive docetaxel by a 1-h i.v. infusion instead of a bolus injection, the plasma levels remain much lower (16). In fact, this much more rapid breakdown of PS-80 makes this compound a much more favorable component for the formulation/solubilization of poorly water-soluble agents than Cremophor EL.

In contrast to a previous report (7), indications for nonlinear pharmacokinetic behavior were observed at dose levels between 10 and 33 mg/kg. From the course of the plasma concentration-time curve, it can be perceived that this more than proportional increase in the AUC was caused mainly during the first hours after drug administration. During this drug distribution phase, the plasma concentration declines slower than at the 10-mg/kg dose level, whereas the terminal half-life is similar. However, it is obvious that the plasma level at which this effect was observed is much higher than the plasma concentrations, which are achieved in patients. Testing of additional dose levels might have aided in further clarifying this nonlinear pharmacokinetic behavior. A dose level of 33 mg/kg of docetaxel could be administered to mice without drug-related toxic side effects. Lethal effects, which were encountered when giving higher dose levels, occurred within seconds after drug administration and resulted from the vehicle. With a more concentrated formulation, a maximum tolerated dose of docetaxel as high as 95 mg/kg has been reported in mice (7). The much higher tolerance of mice toward very high plasma levels fits with the idea that the toxic side effects of taxanes are not directly related with AUC or C_max but are more related with the time that the plasma concentration is maintained above a certain threshold level. This relationship has been well established for paclitaxel in patients (17–19) but has not yet been thoroughly investigated for docetaxel. The relatively short elimination half-life in mice makes that the initially high plasma levels decline rapidly to below the toxic threshold.

In vivo antitumor efficacy studies with new drugs such as docetaxel are established in tumor-bearing mice usually treated at or near the 10% lethal dose. When trying to extrapolate the results from such studies to the clinic, it is important to realize that in case of docetaxel, these experimental tumors are being exposed to drug levels, which can never be achieved in humans. Moreover, previous data suggest that the amount of docetaxel penetrating into the experimental tumors depends upon the plasma levels reached shortly after (bolus) administration (7). The relatively low peak plasma levels in humans versus mice may partly explain the discrepancy between the dramatic (i.e., curative) efficacy in mouse models and the useful albeit modest (palliative) results in patients.

The structures of the docetaxel metabolites have been elucidated by Monegier et al. (6) and Gaillard et al. (20). Despite their apparent structural similarities, metabolic breakdown of docetaxel and paclitaxel occurs through very different pathways. The major metabolite of paclitaxel in mice and humans is 6α-hydroxy-paclitaxel, and the formation of this product is catalyzed by the P-450 isoenzyme CYP2C8 (21, 22). The substrate specificity of this enzyme depends on the R2 group in the molecule (Fig. 1), and replacement of the acetyl moiety by a hydroxy group as in doxetaxel explains why no such analogous metabolic product has been detected for this drug. Metabolic conversion at the C13 side chain is catalyzed by CYP3A4 (21, 23). Hydroxylation at the p-position of the phenyl moiety attached to the C3’ of the C13-side chain, which is a second major metabolite of paclitaxel in mice and humans (9, 24–26), is only a very minor product of docetaxel in rats and is not found in humans (6, 20). In contrast, the pathway of docetaxel metabolism involves changes in the tert-butyl ester group on the C13-side chain (Fig. 1). In a first step, oxidation results in the formation of a primary alcohol (metabolite II). A second oxidation step gives the corresponding aldehyde (structure not shown), an unstable intermediate that immediately reacts further to yield the isomers I and III by spontaneous cyclization, or may be oxidized into a carboxylic acid derivative (not shown), which would be readily transformed to metabolite IV (6). The latter metabolite, however, was not detected in mouse plasma. The levels of metab-

**Table 3** In vitro stability of PS-80 under various conditions

<table>
<thead>
<tr>
<th>Solvent/Condition</th>
<th>Amount of PS-80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>PBS</td>
<td>ND</td>
</tr>
<tr>
<td>Human plasma/0°C</td>
<td>ND</td>
</tr>
<tr>
<td>Human plasma/37°C</td>
<td>66.5 ± 4.2</td>
</tr>
<tr>
<td>Human plasma/37°C; heat pretreated^b</td>
<td>ND</td>
</tr>
<tr>
<td>Human plasma/+PMSF^a</td>
<td>ND</td>
</tr>
<tr>
<td>Human serum albumin</td>
<td>ND</td>
</tr>
<tr>
<td>Esterase</td>
<td>58.4 ± 7.5</td>
</tr>
<tr>
<td>Esterase/+PMSF^a</td>
<td>ND</td>
</tr>
</tbody>
</table>

^a All experiments were conducted in PBS (pH 7.4) at 37°C unless specified otherwise. Amounts are presented as the percentage of the initial concentration (mean ± SD), ND, not done.

^b Plasma was pretreated by heating at 35°C for 30 min.

Because of inactivation of PMSF (28), the addition of PMSF to achieve a final concentration of 100 μM was repeated every hour.
olite II in plasma increased more than proportional with dose, suggesting that the further conversion or excretion of this product may be saturated. Because a previous study showed that the excretion of radiolabeled docetaxel in mice occurred mainly in the feces (>85%), with <10% of the dose being recovered as parent drug (27), it is likely that the major part of the metabolites formed in the liver are secreted directly into the bile. Together with the fact that these metabolites are much less cytotoxic than the parent drug in clonogenic assays (11), these data indicate that metabolic breakdown represents an important detoxification route.

In conclusion, the pharmacokinetic behavior of docetaxel in mice is linear within the dose range of 2.5–10 mg/kg. Because of rapid esterase-dependent degradation of PS-80 in both mice and humans, PS-80 is unlikely to play a role in the disposition of docetaxel. A dose level of 10 mg/kg seems to be most appropriate when designing antitumor efficacy studies or drug interaction studies in mice because the plasma concentration falls within the range that is clinically relevant. The concentrations of metabolites remain very low. The indications that toxicity appears to be related to a threshold drug level in plasma may be important for further pharmacokinetic-pharmacodynamic studies in patients.

**ACKNOWLEDGMENTS**

The excellent biotechnical assistance of Ton Schrauwers, Walter Loos, and Eric Brouwer is gratefully acknowledged.

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In conclusion, the pharmacokinetic behavior of docetaxel in mice is linear within the dose range of 2.5–10 mg/kg. Because of rapid esterase-dependent degradation of PS-80 in both mice and humans, PS-80 is unlikely to play a role in the disposition of docetaxel. A dose level of 10 mg/kg seems to be most appropriate when designing antitumor efficacy studies or drug interaction studies in mice because the plasma concentration falls within the range that is clinically relevant. The concentrations of metabolites remain very low. The indications that toxicity appears to be related to a threshold drug level in plasma may be important for further pharmacokinetic-pharmacodynamic studies in patients.

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Olaf van Tellingen, Jos H. Beijnen, Jaap Verweij, et al.


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