Cancer Chemother Pharmacol (2001) 47: 309–318
DOI 10.1007/s002800000215

ORIGINAL ARTICLE

**Lia van Zuylen · Mats O. Karlsson · Jaap Verweij
Eric Brouwer · Peter de Bruijn · Kees Nooter
Gerrit Stoter · Alex Sparreboom**

Pharmacokinetic modeling of paclitaxel encapsulation in Cremophor EL micelles

Received: 1 June 2000 / Accepted: 27 September 2000 / Published online: 16 February 2001

Abstract Nonlinear disposition of paclitaxel (Taxol) in cancer patients has been described in several studies, but the underlying mechanism is still a matter of speculation. Previously, we have shown in vitro that the paclitaxel formulation vehicle, Cremophor EL (CrEL), alters the blood distribution of paclitaxel as a result of entrapment of the compound in circulating CrEL micelles, thereby reducing the free drug fraction available for cellular partitioning. Based on these findings, we prospectively re-evaluated the linearity of paclitaxel disposition in patients using whole blood and plasma analysis, and sought to define a new pharmacokinetic model to describe the data. Seven patients with solid tumors were treated with paclitaxel infused over 3 h, each at consecutive 3-weekly dose levels of 225, 175 and 135 mg/m^2 (CrEL dose level, 18.8, 14.6, and 11.3 ml/m^2, respectively). Patient samples were collected up to 24 h after the start of infusion, and analyzed by high-performance liquid chromatography. Paclitaxel peak levels and areas under the curve in whole blood increased linearly with dose, whereas plasma levels showed substantial deviation from linearity. This was shown to be caused by a CrEL concentration-dependent decrease in paclitaxel uptake in blood cells, as reflected by the blood:plasma concentration ratios which altered significantly from 0.83 ± 0.11 (at 135 mg/m^2) to 0.68 ± 0.07 (at 225 mg/m^2). It is concluded that the nonlinear disposition of paclitaxel is related to paclitaxel dose-related levels of the formulation vehicle CrEL, leading to a disproportionate drug accumulation in the plasma fraction. The pharmacokinetic model developed accurately described the data, and will help guide future development and refinement of clinical protocols, especially in defining the exposure measure best linked to paclitaxel effects and toxicities.

Key words Paclitaxel · Cremophor EL · Nonlinear pharmacokinetics

L. van Zuylen (✉) · J. Verweij · E. Brouwer · P. de Bruijn
K. Nooter · G. Stoter · A. Sparreboom
Department of Medical Oncology,
Rotterdam Cancer Institute (Daniel den Hoed Kliniek)
and University Hospital Rotterdam, 3075 EA Rotterdam,
The Netherlands
E-mail: zuylen@onch.azr.nl
Tel.: +31-10-4391733
Fax: +31-10-4391003

M. O. Karlsson
Division of Biopharmaceutics and Pharmacokinetics,
Department of Pharmacy, Uppsala University,
SE-751 23 Uppsala, Sweden

Introduction

Nonlinear disposition of drugs is a pharmacokinetic characteristic that implies that a given increase in dose may lead to a disproportionate increase in systemic drug exposure. After i.v. drug administration, the phenomenon is most commonly caused by saturation of enzyme, binding or transporter capacity [36]. Although there are many drugs that display nonlinear elimination kinetics, resulting from either saturable metabolic pathways (e.g. phenytoin [1]) or saturable excretion pathways (e.g. cyclophosphamide [3]), capacity-limited distribution in humans is rare, although concentration-dependence has been described for the binding of some drugs to plasma proteins, blood cells and extravascular tissue or binding sites [20]. The pharmacokinetic behavior of the anticancer agent paclitaxel (Taxol), for example, has been shown to be distinctly nonlinear in several studies, apparently as a result of saturable distribution in combination with saturable elimination processes [9, 15, 24].

Interestingly, studies in mice have demonstrated that in the absence of Cremophor EL (CrEL), a polyoxyethylated castor oil derivative used as a formulation vehicle for i.v. drug administration, both distribution and elimination of paclitaxel appear to be linear processes [26]. Because plasma concentrations of CrEL in mice and humans are within the same range, it is likely that CrEL also plays a pivotal role in the nonlinear paclitaxel disposition in humans [27]. The cause of the

observed nonlinearity, however, has not yet been discovered, but possible explanations include CrEL-mediated inhibition of endogenous P-glycoprotein activity causing altered hepatobiliary secretion [7, 10] and altered protein binding resulting from CrEL-induced lipoprotein dissociation [32]. Recently, we have found in vitro that CrEL causes profound concentration-dependent alterations in paclitaxel distribution in human blood, possibly because the highly hydrophobic paclitaxel favors partitioning in CrEL micelles [31]. Based on these findings, we speculated that the nonlinear kinetics of paclitaxel are not related to saturable tissue binding or Michaelis-Menten elimination kinetics, but are caused by dose- and time-varying CrEL concentrations in the central compartment. In the present study, we carried out a comprehensive pharmacokinetic analysis of paclitaxel and CrEL in patients by measuring paired whole blood and plasma levels to discover the cause of the saturable kinetic processes, and present explicit modeling of the disposition of the compounds to permit a formal explanation of the observed phenomena.

Material and methods

Patients and treatment

Patients with a histologically confirmed diagnosis of a malignant solid tumor for whom paclitaxel as monotherapy was a viable therapeutic option or for whom other treatment options were not available, were candidates for this study. Additional eligibility criteria were: age ≥18 years; World Health Organization performance status < 3; life expectancy of at least 3 months; no previous treatment with taxanes; adequate bone marrow function (WBC count $> 3.0\times10^9$/l, platelet count $> 100\times10^9$/l), renal function (serum creatinine ≤ 140 μ*M* and creatinine clearance ≥60 ml/min), and hepatic function (serum bilirubin, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase concentrations within normal limits); and no preexisting peripheral neuropathy graded > 1 according to NCI Common Toxicity Criteria.

The study drug paclitaxel (Taxol; Bristol-Myers Squibb, Woerden, The Netherlands) was formulated in a mixture of CrEL and dehydrated ethanol USP (1:1, v/v) and diluted further into 500 ml isotonic sodium chloride prior to dosing. Paclitaxel was administered every 3 weeks as a 3-h i.v. infusion at consecutive dose levels of 225, 175 and 135 mg/m^2 (CrEL dose levels, 18.8, 14.6, and 11.3 ml/m^2, respectively). Patients continuing treatment thereafter were given paclitaxel at a dose of 175 mg/m^2 out of protocol. Premedication was uniform for all patients and consisted of dexamethasone (10 mg i.v.), clemastine (2 mg i.v.) and ranitidine (50 mg i.v.), all given 30 min before the start of paclitaxel administration. During chemotherapy, the patients did not use any other comedication that might have interfered with paclitaxel disposition. The clinical protocol was approved by the Rotterdam Cancer Institute Review Board, and all patients signed informed consent before entering the study.

Sample collection

Blood samples (about 5 ml) were obtained from all patients during all courses of treatment and collected into Vacutainer glass tubes containing 143 U USP lithium heparin as anticoagulant. Samples were obtained at the following time-points: before dosing and at 1, 2, 3, 3.08, 3.25, 3.5, 3.75, 4, 5, 7, 9, 11, 15, and 24 h after the start of infusion. After agitation, 2-ml aliquots of whole blood were snap-frozen at –20°C at the site of collection, and plasma was separated by centrifugation for 5 min at 4000 *g* (4°C) and then stored at –80°C. Complete stool collections were obtained from each patient up to 24 h after drug administration in polystyrene containers and stored immediately at –80°C. Weighed feces samples were homogenized individually in four volumes of deionized Milli-Q-UF water (Millipore, Milford, Mass.) using five 1-min bursts of an Ultra-Turrax T25 homogenizer (IKA-Labortechnik, Dottingen, Germany) operating at 20,500 rpm. Aliquots of feces homogenates (200 μl) were diluted fivefold with drug-free human plasma prior to further sample processing.

Drug analysis

Paclitaxel (batch 494034, purity 98.3% by reversed-phase high-performance liquid chromatography, HPLC) and docetaxel, used as internal standard, were provided by Bristol-Myers Squibb and Rhône-Poulenc Rorer (Vitry-sur-Seine, France), respectively. Reference stocks of pure 6α-hydroxypaclitaxel, 3′-*p*-hydroxypaclitaxel and 6α,3′-*p*-dihydroxypaclitaxel were obtained by isolation and purification of a patient's feces sample, as described previously [25]. Stock solutions of each of the taxanes were prepared at 1.0 mg/ml in dimethyl sulfoxide. Concentrations of paclitaxel in plasma were determined using isocratic reversed-phase HPLC with UV detection ($\lambda = 230$ nm) with a lower limit of quantitation of 10 ng/ml, as reported [28]. The same assay methodology was also used for determination of paclitaxel in whole blood, and was validated as described previously [28] using spiked quality control samples containing 40, 200, 400 and 15,000 ng paclitaxel/ml. The within-run precision and between-run precision ranged from 2.23% to 8.74% and 1.81% to 8.07% ($n = 14$ at each of the concentrations), respectively, with a mean percentage deviation from nominal values of less than ± 3.30%.

A potential CrEL concentration-dependency in extraction recovery of paclitaxel from whole blood was tested by repeated analysis of spiked whole blood (nominal concentration 1000 ng/ml) containing 0, 0.1, 0.5, 1.0, 5.0 or 10 μl CrEL/ml. The mean (± SD) recovered concentrations in these samples were 1037 ± 28.4, 1067 ± 33.7, 1064 ± 7.91, 1074 ± 1.90, 1041 ± 35.1 and 1057 ± 6.71, respectively, with no trend in significant deviations from the control value ($P = 0.194$; $n = 16$ at each CrEL concentration). The detector response of the internal standard docetaxel, as measured by the peak height, was also not significantly different during analysis of either plasma or whole blood samples with mean values of 44,019 ± 4739 μV and 43,728 ± 5410 μV, respectively ($P = 0.525$; $n = 164$ for each matrix). These data were considered acceptable for analysis of paclitaxel in whole blood samples obtained from patients for the conduct of the present study.

For determination of paclitaxel and its hydroxylated metabolites in feces homogenates, this HPLC methodology was further modified as described previously [8]. In brief, standard curves were prepared in drug-free human plasma and were expanded to encompass concentrations between 0.1 and 5 μg/ml. A 1-ml aliquot of standard or plasma-diluted sample was mixed for 5 min with 100 μl 10 μg/ml docetaxel in 50% (v/v) methanol in water and 5 ml 20% (v/v) acetonitrile in *n*-butyl chloride. After centrifugation for 5 min at 4000 *g* (4°C), the entire upper organic phase was separated and dried under nitrogen at 60°C. The dried residue was reconstituted in 125 μl of 50% (v/v) methanol in water by vortex-mixing, and following a brief centrifugation step, 100 μl of the clear supernatant was injected into the HPLC system from low-volume glass inserts via a temperature-controlled Waters 717Plus autosampling device (Milford, Mass.). Chromatographic separations were performed at 60°C on an Inertsil ODS-80A column (150×4.6 mm internal diameter, 5 μm particle size; GL Science, Tokyo, Japan) and a guard column (4.0×4.0 mm, 5 μm) packed with LiChrospher 100 RP-18 material (Merck, Darmstadt, Germany). The mobile phase consisted of methanol/tetrahydrofuran/0.2 *M* aqueous ammonium hydroxide (60:2.5:37.5, v/v/v) with the pH adjusted to 6.0 (formic acid), and was degassed by ultrasonication prior to use. The flow-rate was set at 1.0 ml/min, and the eluent was monitored at an

absorption wavelength of 230 nm with a SpectraPhysics UV-200 detector (San Jose, Calif.).

Integration of chromatographic data and calculation of calibration graphs was performed as described previously [28]. A formal method validation was performed by repeat analysis of quality control samples spiked to contain 0.50, 2.5, 10 and 20 μg paclitaxel/ml on four consecutive days along with a seven-point calibration curve processed in duplicate. There were no endogenous compounds in any of the studied patient specimens that could have interfered with the peaks of interest in the system. Standard curves were fitted by an equation with proportional weighting, and were strictly linear in the tested range, with an R^2 value greater than 0.995 in all cases. The within-run precision and between-run precision ranged from 2.49% to 7.82% and 1.68% to 10.6% ($n = 20$ at each of the concentrations), respectively, with a mean percentage deviation from nominal values of less than ± 8.23%.

The analytical method for determination of CrEL concentrations in plasma was based on binding of the surfactant to the Coomassie Brilliant Blue G-250 dye in protein-free plasma extracts by measuring the change in ratio of absorbances at 595 nm over 450 nm [2, 29]. For this purpose, 50-μl samples of plasma were deproteinized by the 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 then separated by centrifugation for 5 min at 4000 *g*, transferred to a clean 10-ml glass tube, and dried under nitrogen at 60°C for 30 min. The residue was reconstituted in 50 μl water by vortex-mixing, and a 25-μl volume was pipetted into a 96-well flat-bottom cluster (Costar Corporation, Cambridge, Mass.). Finally, 250 μl of water-diluted (1:4, v/v) Coomassie Brilliant Blue G-250 reagent was added, and the absorbance maximum of the dye at 595 nm after binding to CrEL, and the simultaneous decrease in absorbance at 450 nm were measured within 24 h against a reagent blank using a Bio-Rad Model 550 automated microplate reader (Bio-Rad Laboratories, Hercules, Calif.). The lower limit of this procedure was 0.50 μl/ml, and over the entire range studied (up to 10.0 μl/ml), the method has excellent specificity, accuracy (≤ 6.33% relative error), and precision (< 10%) [2].

Pharmacokinetic analysis

The pharmacokinetics of paclitaxel were initially evaluated by a noncompartmental method using the Siphar package (version 4.0; SIMED, Créteil, France). The actual times of drug intake and blood sampling were taken into account. Peak drug levels (C_{max}) were determined by visual inspection of the concentration-time data. The area under the concentration-time curve (AUC) from zero to the last sampling time-point with a detectable concentration (C_{last}) was calculated by the linear trapezoidal rule. The apparent half-life of the terminal disposition phase ($T_{1/2}$) was defined as $\ln 2/\lambda$, in which λ was the elimination rate constant of the terminal phase, estimated by a least-squares regression analysis of the final three data points of the concentration-time profiles. Total body clearance (CL) was calculated as the quotient of dose (expressed in mg/m^2) and AUC extrapolated to infinity by dividing C_{last} by λ. The relationships between paclitaxel dose and C_{max} or AUC in plasma and blood were evaluated in a scattered plot with linear and nonlinear fitting using Graphstatics Plus (Manugistics, Rockville, Mass.). The AUC of CrEL in plasma was also calculated using the Siphar package as described previously [30].

Statistical considerations

All pharmacokinetic parameters are reported as mean values ± SD. Since multiple measurements were performed at different times on the same patients, comparisons between the sets of observations were based on within subject differences. Therefore, variation between subjects, which is usually considerable, did not affect our ability to distinguish differences between the sets of observations, which here related to the three paclitaxel dose levels. The effect of drug dose on the apparent paclitaxel clearance in plasma and blood was evaluated using a Friedman's two-way analysis of variance test, with the level of significance set at $P < 0.05$. Statistical analysis was performed using the Number Cruncher Statistical System (NCSS) software package (version 5.X; J.L. Hintze, Kaysville, Utah).

Model development

Paclitaxel in blood was assumed to exist in the following pharmacokinetically distinguishable forms: unbound ($C_{unbound}$), bound to plasma protein (C_{bound}), in micellar form together with CrEL ($C_{micellar}$), and bound to or distributed into red blood cells (C_{rbc}). Further, $C_{unbound}$ was assumed to be in equilibrium with tissues. For some tissues and organs, the rate to attain this equilibrium may be fast and therefore part of the central volume of distribution. For other tissues and organs it may be slow, where the number of peripheral compartments will reflect the heterogeneity of the rate of equilibration between tissues of importance for the distribution of paclitaxel. As neither $C_{unbound}$ nor C_{bound} was measured separately and since the relationship between the two was assumed to be linear and instantaneous, there was no possibility of separately characterizing these based on pharmacokinetic data. However, no approximation of the model was necessary because of this, the only consequence being that the relevant parameters were based on the sum of the two concentrations rather than on $C_{unbound}$ alone. Thus, $C_{non\text{-}micellar}$ is the sum of $C_{unbound}$ and C_{bound}, C_{plasma} is the sum of $C_{micellar}$ and $C_{non\text{-}micellar}$, whereas C_{blood} is the hematocrit-weighted average of C_{rbc} and C_{plasma}.

The only feature that distinguishes the model used from standard linear compartmental pharmacokinetic models is that paclitaxel may be included in micelles and thereby undergo a change in its pharmacokinetic properties. In the present model, these CrEL-paclitaxel micelles were assumed to exist in plasma, and with plasma rapidly equilibrating organs and tissues. In the initial model for $C_{micellar}$ it was assumed to be proportional to both the unbound paclitaxel concentration and the total CrEL concentration. Several modifications to this assumption were explored, including nonlinear dependence on paclitaxel and/or CrEL concentrations as well as time-dependent changes in the proportionality constant governing the relationship. The information about the relationship between paclitaxel, CrEL and $C_{micellar}$ as entered into the model came from the nonlinear behavior of the plasma concentration-time curve and the varying ratios of C_{blood} to C_{plasma}. Therefore, the data were best utilized in a simultaneous fit of the model to both. The ratio between C_{rbc} and $C_{non\text{-}micellar}$, a necessary part of the model, was estimated as the parameter θ_{rbc}. Whereas nonlinear mixed effects (''population'') modeling is the accepted method of analyzing sparse pharmacokinetic data, rich pharmacokinetic data sets are more commonly analyzed by individual modeling. However, when complex models are used, there is a gain in analyzing data from several subjects together even if the data are rich [11, 23]. Indeed, if many parameters are to be estimated from a single individual's pharmacokinetic profile, there are usually some parameters for which the information is sparse. Thus, in a sense, with complex pharmacokinetic models, individual data are no longer ''rich'' in information.

This analysis was performed as population modeling using the first-order method as implemented in the NONMEM program (version V; S.L. Beal and L.B. Sheiner, San Francisco, Calif.). As the number of individuals was small, there was no model building in the traditional sense, where both structural, statistical and covariate models are refined in an integrated process. Covariates, apart from the CrEL concentrations, were absent. Interindividual variability parameters would be expected to be poorly estimated, but were included in the model as long as the termination was successful and could provide estimates of parameter precision. Interindividual variability was included as log normally distributed, and residual variability was initially modeled as combined additive and proportional, where either component may be omitted if superfluous. The assessment of statistical significance of the additional parameters was based on the difference between the objective

function values (part of the output from NONMEM) between the models being compared. This difference was minus twice the log likelihood for the models and was approximately χ-squared distributed. A difference of greater than 10.83 (one degree of freedom) was significant at the 0.1% level, which was used in discriminating between hierarchical models.

Introducing the CrEL concentrations into the model requires knowledge of CrEL at all time-points, including those between sampled CrEL observations. In the present modeling, we used the individual predictions from a two-compartment model generated as a part of a separate analysis of CrEL pharmacokinetics in a larger population that included 67 complete concentration-time courses (not shown). Although this model is a simplification of CrEL pharmacokinetics, it adequately describes the individual profiles, which is what was needed for the present purpose.

Results

Patient characteristics

Seven patients (three male, four female) with various malignant solid tumors were included in the study (Table 1). The median age was 54 years (range 32–64 years), and all patients had normal hematopoietic and liver functions at the time of study. One patient had slightly increased serum creatinine values and a lower creatinine clearance (138 μM and 57 ml/min, respectively), but was accepted based on the known limited role of renal function in paclitaxel disposition [8]. Two patients did not receive the last course (135 mg/m^2) because of progressive disease after two paclitaxel courses. Eventually, 19 courses were available for pharmacokinetic evaluation.

Table 1 Characteristics of patients undergoing concomitant blood and plasma sampling during and after paclitaxel infusion. Values are number of patients, unless otherwise stated. (*ALAT* alanine aminotransferase, *ASAT* aspartate aminotransferase)

Total	7
No. of courses	19
Age (years)	
Median	54
Range	32–64
Gender	
Male	3
Female	4
WHO performance status	
0	2
1	4
2	1
Primary tumor site	
Bladder	3
Breast	1
Lung	1
Unknown	1
Weak tissue (sarcoma)	1
Pretherapy clinical chemistry (mean, range)	
Serum creatinine (μM)	92 (69–138)
Total bilirubin (μM)	9 (3–15)
ASAT (U/l)	19 (10–28)
ALAT (U/l)	16 (6–31)
Total protein (mg/ml)	76 (74–78)
Serum albumin (mg/ml)	42 (34–49)

Paclitaxel blood distribution

The mean plasma and blood concentration-time profiles of paclitaxel at the three different dose levels tested are displayed in Fig. 1. The relationships between the administered dose and paclitaxel C_{max} and AUC in plasma indicated a substantial deviation from linearity (Fig. 2A), whereas this disproportionality was less pronounced with data based on whole blood measurements (Fig. 2B). In line with previous findings [9, 15, 24], the apparent plasma clearance of paclitaxel was clearly dose-dependent and significantly decreased from 16.7 ± 2.53 l/h per m^2 (at 135 mg/m^2) to 9.75 ± 2.78 l/h per m^2 (at 225 mg/m^2; $P = 0.030$; Table 2). The whole blood clearance of paclitaxel, however, appeared to be independent of the administered dose ($P = 0.063$), and averaged 17.5 ± 3.43 l/h per m^2. Examination of blood:plasma concen-

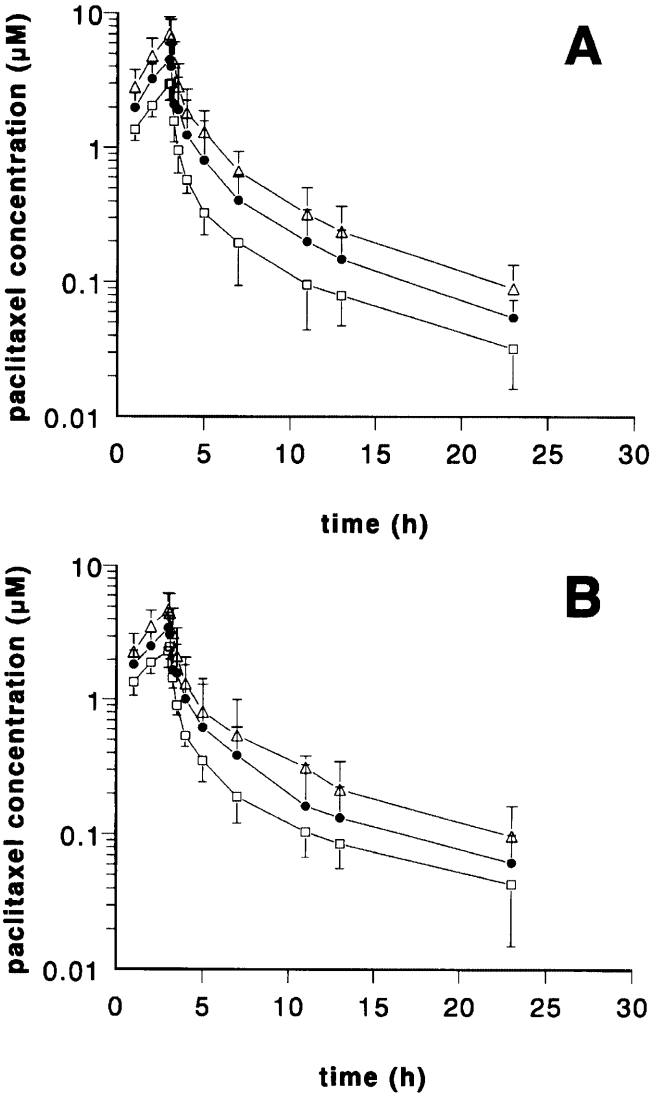

Fig. 1A,B Plasma (**A**) and blood (**B**) concentration-time profiles of paclitaxel in patients treated with paclitaxel at dose levels of 135 mg/m^2 (❑), 175 mg/m^2 (●) and 225 mg/m^2 (Δ). Data are presented as mean values and SD (error bars)

tration ratios (C_b/C_p) revealed that the discrepant kinetic profiles of paclitaxel in plasma and blood was most closely associated with a concentration-dependent change in movement of drug within the central compartment during the distribution phase. At C_{max}, C_b/C_p ratios decreased in a dose-dependent manner from 0.83 ± 0.11 (135 mg/m^2) to 0.80 ± 0.18 (175 mg/m^2) and 0.68 ± 0.07 (225 mg/m^2).

The estimated terminal half-life was relatively consistent in all subjects and not different between dose levels, exhibiting mean values of 7.21 ± 0.623 h and 6.91 ± 0.485 h for plasma and blood, respectively (plasma vs blood, $P = 0.69$). This finding is further substantiated by the fecal recovery patterns of paclitaxel and its three main hydroxylated metabolites during the first 24 h after initiation of the infusion, which were qualitatively and quantitatively independent of the dose given. Fecal excretion of unchanged drug accounted for only 2.8%, 3.7% and 3.7% of the total dose administered at the 135, 175 and 225 mg/m^2 dose levels, respectively. This is in line with previous findings [31], and with murine data indicating that fecal excretion pathways of paclitaxel are independent of the administered dose and the vehicle used for drug formulation [27].

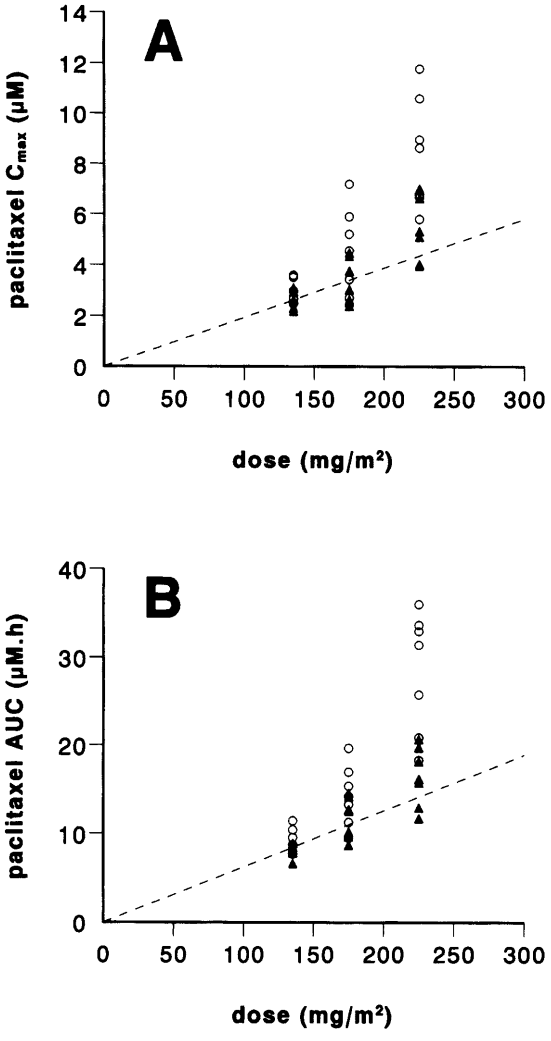

Fig. 2A,B Relationships between the administered dose and paclitaxel C_{max} (**A**) and paclitaxel AUC (**B**) in plasma (○) and whole blood (▲). Dotted lines indicate linearity of the relationships in the case of proportional increases in C_{max} and AUC with dose

CrEL pharmacokinetics

The plasma clearance of CrEL at the tested dose levels of 18.8, 14.6, and 11.3 ml/m^2 was independent of the administered dose ($P = 0.61$) and averaged 320 ± 53.2 ml/h per m^2, which is within the same range as described for this compound previously when based on calculations using $AUC_{0\text{-}t}$ [30]. Mean CrEL plasma concentration-time profiles at the various dose levels are shown in Fig. 3.

Pharmacokinetic model

We sought to define a pharmacokinetic model that would explain the nonlinearity observed in paclitaxel plasma disposition. The distribution of paclitaxel to tissues was best described by a three-compartment model (based on considerations presented in Fig. 4), and was characterized by the following parameters ($\pm$SD): intercompartmental clearance of paclitaxel for peripheral compartment one, 30 ± 8 l/h; volume of paclitaxel for peripheral compartment one, 51 ± 14 l; intercom-

Table 2 Summary of noncompartmental paclitaxel pharmacokinetics. Data were obtained from seven patients after treatment with a 3-h i.v. infusion of paclitaxel, each receiving consecutive 3-weekly doses of 225 ($n = 7$), 175 ($n = 7$) and 135 mg/m^2 ($n = 5$). Pharmacokinetic parameters are mean values $\pm$SD. (*AUC* area under the concentration-time curve up to the last sampling-time point with detectable paclitaxel concentration, C_{max} peak concentration, *CL* apparent clearance, $T_{1/2}$ apparent half-life of the terminal disposition phase)

Dose (mg/m^2)[a]	Matrix	C_{max} (μM)	AUC (μM·h)	CL (l/h/m^2)	$T_{1/2}$ (h)
135	Plasma	3.04 ± 0.493	9.62 ± 1.43	16.7 ± 2.54	7.70 ± 4.15
	Blood	2.63 ± 0.387	8.06 ± 2.92	18.8 ± 2.30	8.15 ± 2.92
175	Plasma	4.54 ± 1.73	14.3 ± 3.40	14.0 ± 3.76	7.17 ± 1.92
	Blood	3.31 ± 0.883	11.8 ± 2.30	17.9 ± 3.64	6.96 ± 1.63
225	Plasma	8.75 ± 2.76	28.7 ± 7.04	9.75 ± 2.78	6.77 ± 2.45
	Blood	5.58 ± 1.32	17.9 ± 4.81	15.7 ± 4.34	5.99 ± 1.67

[a]Corresponding dose levels of CrEL, 11.3, 14.6, and 18.8 ml/m^2, respectively

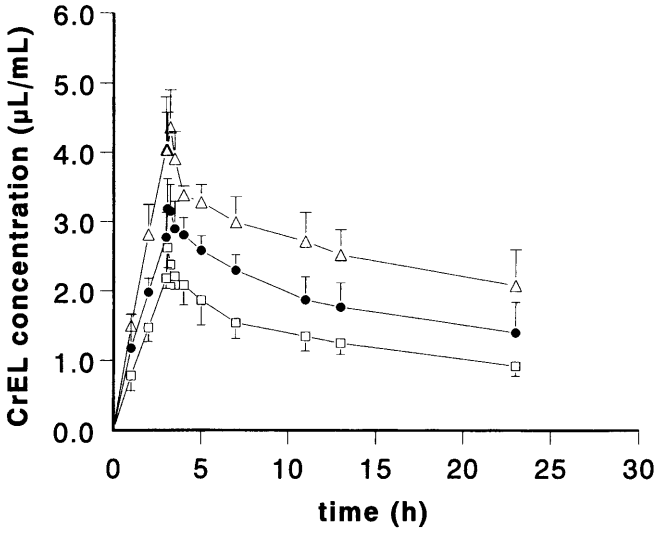

Fig. 3 Plasma concentration-time curves of CrEL in patients treated with paclitaxel at dose levels of 135 mg/m^2 (❑), 175 mg/m^2 (●) and 225 mg/m^2 (Δ). Data are presented as mean values and SD (error bars)

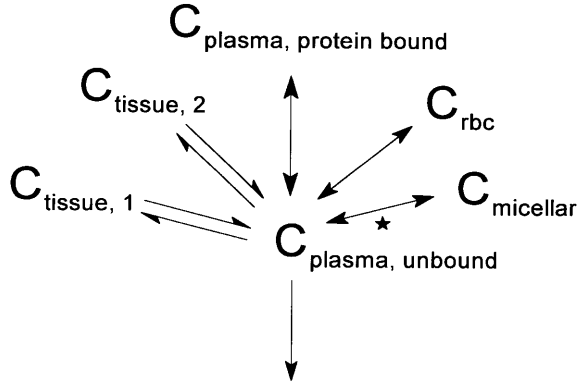

Fig. 4 Pharmacokinetically distinguishable forms of paclitaxel in the blood compartment assumed to exist in the proposed model for paclitaxel pharmacokinetics. *Double-headed arrows* denote processes with assumed instantaneous equilibrium. The *star* indicates a nonlinear process

partmental clearance of paclitaxel for peripheral compartment two, 34 ± 8 l/h; volume of paclitaxel for peripheral compartment two, 340 ± 81 l. The central volume of distribution of paclitaxel was 41 ± 14 l, whereas the proportionality constant relating C_{rbc} to $C_{non\text{-}micellar}$ was 1.7 ± 0.2. The overall clearance of paclitaxel based on $C_{non\text{-}micellar}$ was 71 ± 13 l/h. The relationship between $C_{micellar}$ and $C_{non\text{-}micellar}$ was well described by the following function:

$$C_{micellar} = C_{non-micellar} \times C_{CrEL} \times \theta_{micellar} \times \{1 - (T \times \theta_{time})\}$$

where C_{CrEL} is the CrEL plasma concentration in microliters per milliliter, T is the time after start of infusion, and $\theta_{micellar}$ and θ_{time} are parameters estimated to be 0.97 ± 0.22 and 0.039 ± 0.004, respectively.

Before arriving at this final model for the relationship, several others were tried, including those containing functions with nonlinear relationships for both $C_{non\text{-}micellar}$ and C_{CrEL}. The fit of the current model to the observed paclitaxel plasma and blood concentration gave a good description of the data without any trends in the residuals over time or paclitaxel concentration (Fig. 5). The presently developed model gave an unbiased prediction of the C_b/C_p ratio over time (Fig. 6A), and it could also predict the free fraction of paclitaxel at any CrEL concentration compared to the free fraction in the absence of CrEL. This estimated ratio showed good agreement with previously published data obtained from a single patient treated with paclitaxel at a dose level of 157.5 mg/m^2 [31] (Fig. 6B). An example of concentration-time data for the various pharmacokinetically distinguishable forms of paclitaxel in blood from a representative patient is given in Fig. 7.

Discussion

By prospectively measuring paclitaxel concentrations in whole blood and plasma in cancer patients given different dose levels of paclitaxel and serving as their own control, we demonstrated that the AUC of paclitaxel in whole blood was a linear function of the dose administered. The paclitaxel C_b/C_p ratios showed that the fraction of this drug that was bound in plasma changed appreciably with concentration in the concentration range of interest. Recently, we have shown in vitro that CrEL causes a profound alteration in paclitaxel accumulation in erythrocytes in a concentration-dependent manner, by reducing the free drug fraction available for cellular partitioning, thereby suggesting that paclitaxel trapping occurs in CrEL micelles [31]. These findings were later independently confirmed by Knemeyer et al. [17] and Van Tellingen et al. [33] in similar in vitro experiments.

Yet the findings of our present study demonstrate for the first time that CrEL affects the clinical pharmacokinetics of paclitaxel by a disproportionate accumulation process in plasma, and lends further support to our prior supposition that the ability of CrEL to modulate the murine disposition of paclitaxel may have important clinical ramifications. Moreover, our findings indicate that the nonlinear pharmacokinetics of paclitaxel are not related to saturable tissue binding but are caused by paclitaxel dose-related levels of CrEL in blood. Although our study population included only seven patients, the paclitaxel plasma pharmacokinetics were highly consistent with data previously reported by Gianni et al. [9] obtained from a larger group of patients. For example, the paclitaxel plasma AUC varied from 10.9 ± 1.1 to 18.5 ± 3.0 and 24.3 ± 6.8 μ*M*·h in their study versus 9.6 ± 1.4, 14.3 ± 3.4 and 28.7 ± 7.0 μ*M*·h in our study, at dose levels of 135, 175 and 225 mg/m^2, respectively, indicating that our small patient population was indeed representative.

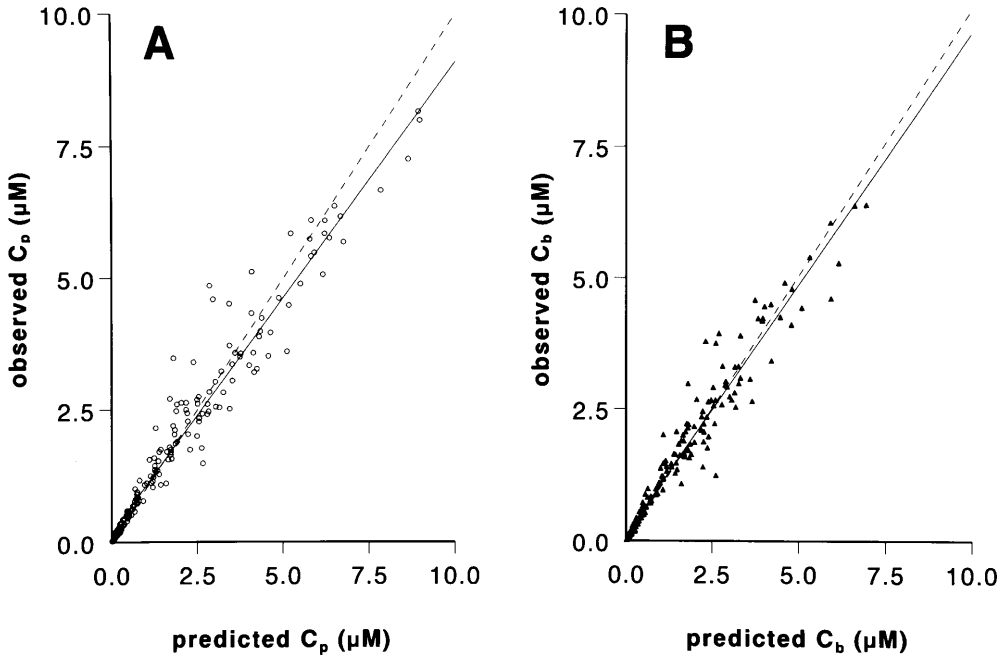

Fig. 5A,B Observed versus predicted plasma (**A**) and blood (**B**) concentrations using the proposed pharmacokinetic model. *Dotted lines* indicate the lines of identity, and *solid lines* indicate linear regression fits ($y = 1.0654x - 0.0802$ for plasma and $y = 1.0008x - 0.0263$ for blood)

Our findings indicate that the other suggested causes of the nonlinearity of paclitaxel mentioned in the Introduction are unlikely. The established decrease in C_b/C_p ratio of paclitaxel in our study excludes the role of P-glycoprotein [10], because modulation of endogenous P-glycoprotein expressed in certain blood cells (e.g. white blood cells) by CrEL should lead to an increase in this ratio. The hypothesis of altered P-glycoprotein-mediated hepatobiliary secretion is based on experiments in an isolated perfused rat-liver system in which paclitaxel is administered as a bolus dose both in the absence of CrEL and after the administration of either 80 or 800 μl CrEL [7]. Total and biliary clearance of paclitaxel decreased in a dose-dependent manner not through alterations in the metabolism of paclitaxel under the influence of CrEL, but logically by the same principle as defined in our study, i.e. by micelle formation preventing paclitaxel reaching sites of metabolism and excretion. Altered protein binding as the primary cause of the nonlinear paclitaxel pharmacokinetics [32] has been already excluded as an explanation by our previous in vitro study, in which the CrEL-induced alteration in paclitaxel accumulation in erythrocytes was also observed in the absence of any plasma proteins [31].

The model presented here accurately described the pharmacokinetics of paclitaxel in patients at all dose levels studied, and could be proven to be useful for prediction of paclitaxel disposition at as-yet-untested dose levels. In the past, the first attempts to model the nonlinear pharmacokinetics of paclitaxel used a nonlinear distribution mechanism and a nonlinear elimination based on plasma measurements [24]. Later a model in which the saturable process was replaced by saturable binding to sites outside plasma was shown to describe the data equally well, and to be more probable based on the known pharmacologic properties of paclitaxel [9]. In the present study we used an alternative approach for describing the nonlinear pharmacokinetics of paclitaxel. In contrast to the previous models, only one nonlinear component was required to describe both the apparent nonlinear distribution and the elimination kinetics.

The sequence of models developed for paclitaxel illustrates the effort to develop mechanistic models that is a dominant principle in contemporary modeling. It also illustrates the principle that to discriminate between complex models, there is often the necessity to obtain information on more than one component of the system. In this case, in addition to CrEL levels, we measured both blood and plasma concentrations. Although the possibility cannot be excluded that the saturable binding of paclitaxel to tubulin may play a role in its pharmacokinetic behavior [14], the present model was able to explain not only the nonlinear distribution and elimination of paclitaxel, but also the changing C_b/C_p ratio, something that no previous model has explained. We did not compare the fit of the present model to the data with the fit that previous models might have given. One reason for this is that the previous models from the nature of their development had no component for predicting the blood concentrations. As blood concentrations are an integral part of the present model, without which the model cannot be evaluated, no direct comparison could be made.

Several assumptions and approximations are made in our model that may not be necessary if additional information on these processes are being gathered. Three

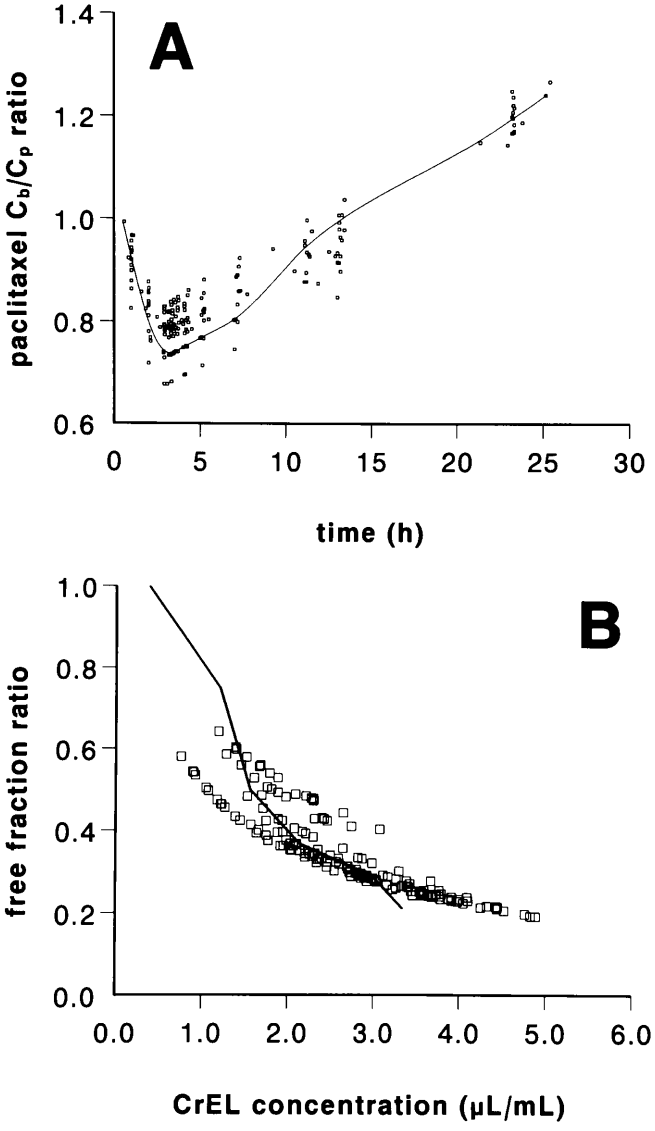

Fig. 6A,B Observed (*symbols*) and model-predicted (*line*) ratios (C_b/C_p) of the paclitaxel blood:plasma concentrations over time (**A**), and the ratio between the free fraction of paclitaxel at zero CrEL concentration to the free fraction of paclitaxel in the presence of CrEL at various concentrations [i.e. $C_{non\text{-}micellar}/(C_{nonmicellar} + C_{micellar})$]. The *continuous line* is based on data from reference 31 (see Table 3 therein), whereas the *squares* are model-based estimates for all observations up to 20 h after paclitaxel administration (**B**)

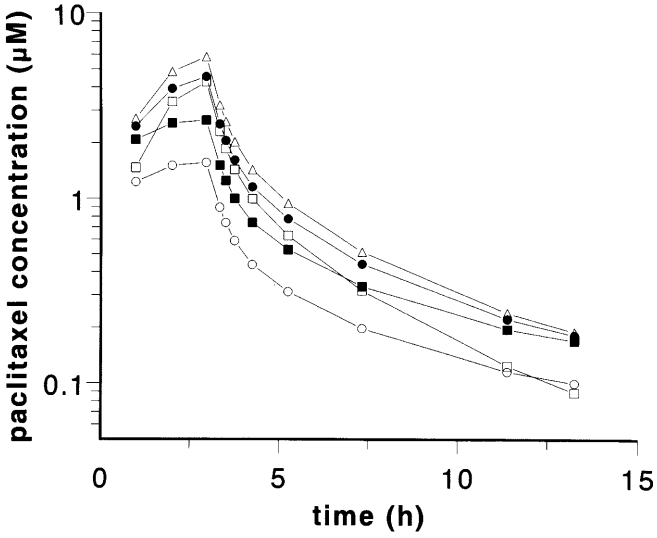

Fig. 7 Paclitaxel concentration-time profiles using model-fit curves in a representative patient treated with paclitaxel at a dose level of 175 mg/m^2 showing C_{plasma} (i.e. the sum of $C_{micellar}$ and $C_{non\text{-}micellar}$) (Δ), C_{blood} (i.e. the hematocrit-weighted average of C_{rbc} and C_{plasma}) (●), $C_{non\text{-}micellar}$ (i.e. the sum of $C_{unbound}$ and C_{bound}) (○), $C_{micellar}$ (❑) and C_{rbc} (■)

assumptions regarding the micellar binding may be particularly approximate. First, the amount of paclitaxel in micellar form is, at any time, assumed to be directly proportional to the total concentrations of the drug and CrEL. We know that at very low CrEL concentrations micelles cannot be formed, although it has been shown that in aqueous solution CrEL micelles can persist for several hours after dilution below the critical micellar concentration, estimated to be 0.009% (w/v) at equilibrium [16]. It appears, however, that this critical micellar concentration in plasma is low compared to the range of concentrations experienced during the observation period of these treatments. Second, even above the critical micellar concentration there may be deviation from the simple direct proportionality that was postulated. Although literature data on CrEL micellar kinetics are lacking, preliminary experiments indicated that the in vitro equilibrium between paclitaxel in micelles and free paclitaxel is reached within 1 min (A. Sparreboom and J. Verweij, unpublished data), and thus can be considered instantaneous on the time-scale of paclitaxel pharmacokinetics. It is possible, however, that with time CrEL is able to form micelles with other components so that less is available for forming paclitaxel micelles.

Third, the change in the micellar concentration with time is modeled as a linear relationship, although it may well be a more complex function of both degradation of CrEL and/or binding to other blood constituents. Indeed, we have found recently that compounds such as CrEL and polysorbate 80 are prone to a degradation pathway induced by serum carboxylesterases that causes a release of fatty acids (ricinoleic acid in the case of CrEL and oleic acid in the case of polysorbate 80) [34]. Although this metabolic route is likely to be very slow for CrEL, given the long terminal half-life of CrEL of around 80 h [30], it is likely to impact on the CrEL-paclitaxel interaction. Hence, the assumptions regarding linear plasma protein and red cell binding as well as linear tissue distribution and elimination may in the end prove false under further scrutiny. In the present data set, however, such nonlinearities were not indicated. It is also noteworthy in this context that plasma protein binding of paclitaxel in human samples, determined by both equilibrium dialysis and ultrafiltration techniques, has been shown previously to be independent of the paclitaxel concentration within the therapeutic range

associated with 3-h i.v. infusions [18]. We are currently exploring the relevance of this principle for the in vivo situation by defining free (unbound) area-dose relationships in cancer patients treated with paclitaxel.

Our current findings will have significant implications for the interpretation of the relationship between the pharmacokinetic parameters and pharmacodynamic outcome of paclitaxel treatment. Over the last few years, various hypotheses regarding relationships between plasma pharmacokinetics and hematological toxicity (e.g. neutropenia) have been postulated, including those using a step function (threshold model) [12, 15] and more general models that use a nonlinear continuous function for the time-dissociated component [13, 22]. A common feature of these models is the use of the total paclitaxel plasma concentration, which is, in view of our current findings, unlikely to be the exposure measure best linked to observed toxicity profiles. Since the nonlinear pharmacokinetics of paclitaxel in plasma can be explained by CrEL concentration-dependent changes in drug movement within the central compartment, the effects of entrapment of paclitaxel in the plasma compartment will be less with prolonged (e.g. 24-h) infusion schedules associated with lower concentrations of CrEL [35]. Thus, total plasma levels of paclitaxel measured in these schedules will represent a higher fraction of free paclitaxel, and this may, in part, explain the increased incidence of severe hematological toxicity seen in the 24-h infusion schedules as compared with infusion of the same dose over 3 h [6, 9].

The existence of CrEL in blood as large polar micelles with a highly hydrophobic interior may also have important consequences for other drugs that are formulated in this vehicle. CrEL is currently used in i.v. preparations of many hydrophobic drugs including other anticancer agents (e.g. teniposide, didemnin B, halomon), anesthetics, vitamins and immunosuppressive agents (e.g. cyclosporin A, tacrolimus). It is possible that the pharmacokinetic behavior of these drugs will also be influenced by the formulation vehicle, although the impact is likely to be smaller than for paclitaxel, since the amounts of this vehicle coadministered are much less. For example, cyclosporin A is known to exhibit disproportionate dose-AUC relationships, and it can be postulated this phenomenon is related, in part, to the presence of CrEL and micellar entrapment similar to that described here for paclitaxel, in addition to saturable binding to erythrocytes [19]. It can also be anticipated that CrEL may alter the distribution of some compounds coadministered with paclitaxel. For example, clinically significant pharmacokinetic interactions between paclitaxel and doxorubicin have been reported [10], and both preclinical and clinical evidence suggests that these are directly attributable to CrEL [4, 21, 37]. Similarly, differences in the plasma concentration-time profile of CrEL resulting from differences in the paclitaxel administration schedule could also account for the apparent sequence- and schedule-dependent effects of paclitaxel on doxorubicin pharmacokinetics [5].

In conclusion, we have shown that micellar encapsulation of paclitaxel in CrEL can, as a single principle, explain both the nonlinear distribution and nonlinear elimination processes of paclitaxel in plasma. This micellar formation is likely to affect other hydrophobic drugs, either those themselves formulated in CrEL, or those given in combination with paclitaxel, leading to changes in the pharmacokinetic and pharmacodynamic behavior of these compounds that could have significant clinical implications. Our pharmacokinetic model was able to accurately describe the generated paclitaxel blood and plasma data, and will help guide further development and refinement of clinical protocols. Future studies will focus on defining the exposure measure best linked to paclitaxel-induced antitumor effects and toxicities, not only to expand our understanding of paclitaxel pharmacology but to provide an insight into considerations of optimal dosage and schedule of drug administration. Ultimately, a more rational and selective chemotherapy with paclitaxel should be possible and thus the treatment of cancer improved.

Acknowledgements We are grateful to Dr. Luca Gianni (Istituto Nazionale Tumori, Milan, Italy) for many helpful scientific discussions, Walter J. Loos for his excellent support in arranging the assay of plasma CrEL concentrations, Linda Ouwens for her patience in providing all patient samples and related information, and the patients for their participation in the study.

References

1. Bauer LA, Blouin RA (1983) Phenytoin Michaelis-Menten pharmacokinetics in Caucasian paediatric patients. Clin Pharmacokinet 8:545
2. Brouwer E, Verweij J, Hauns B, Loos WJ, Nooter K, Mross K, Stoter G, Sparreboom A (1998) Linearized colorimetric assay for Cremophor EL: application to pharmacokinetics after one-hour paclitaxel infusions. Anal Biochem 261:198
3. Chen T-L, Passos-Coelho JL, Noe DA, Kennedy J, Black KC, Colvin OM, Grochow LB (1995) Nonlinear pharmacokinetics of cyclophosphamide in patients with metastatic breast cancer receiving high-dose chemotherapy followed by autologous bone marrow transplantation. Cancer Res 55:810
4. Colombo T, Parisi I, Zucchetti M, Sessa C, Goldhirsch A, D'Incalci M (1999) Pharmacokinetic interactions of paclitaxel, docetaxel and their vehicles with doxorubicin. Ann Oncol 10:391
5. Duska LR, Penson R, Supko JG, Finkelstein DM, Makastorsis T, Gallagher J, Borden K, Goodman A, Fuller AF, Nikrui N, Seiden MV (1999) A phase I study of continuous infusion doxorubicin and paclitaxel chemotherapy with granulocyte colony-stimulating factor for relapsed epithelial ovarian cancer. Clin Cancer Res 5:1299
6. Eisenhauer EA, Ten Bokkel Huinink WW, Swenerton KD, Gianni L, Myles J, Van der Burg MEL, Kerr I, Vermorken JB, Buser K, Colombo N, Bacon M, Santabárbara M, Onetto N, Winograd B, Canetta R (1994) European-Canadian randomized trial of paclitaxel in relapsed ovarian cancer: high-dose versus low-dose and long versus short infusion. J Clin Oncol 12:2654
7. Ellis AG, Webster LK (1999) Inhibition of paclitaxel elimination in the isolated perfused rat liver by Cremophor EL. Cancer Chemother Pharmacol 43:13
8. Gelderblom H, Verweij J, Brouwer E, Pillay M, De Bruijn P, Nooter K, Stoter G, Sparreboom A (1999) Disposition of [*G*-^{3}H]paclitaxel and Cremophor EL in a patient with severely impaired renal function. Drug Metab Dispos 27:1300

9. Gianni L, Kearns CM, Giani A, Capri G, Vigano L, Locatelli A, Bonadonna G, Egorin MJ (1995) Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. J Clin Oncol 13:180
10. Gianni L, Vigano L, Locatelli A, Capri G, Giani A, Tarenzi E, Bonadonna G (1997) Human pharmacokinetic characterization and in vitro study of the interaction between doxorubicin and paclitaxel in patients with breast cancer. J Clin Oncol 15:1906
11. Hempel G, Karlsson MO, De Alwis DP, Toublanc N, McNay J, Schaefer HG (1998) Population pharmacokinetic-pharmacodynamic modeling of moxonidine using 24-hour ambulatory blood pressure measurements. Clin Pharmacol Ther 64:622
12. Huizing MT, Keung AC, Rosing H, Van der Kuij V, Ten Bokkel Huinink WW, Mandjes IM, Dubbelman AC, Pinedo HM, Beijnen JH (1993) Pharmacokinetics of paclitaxel and metabolites in a randomized comparative study in platinum-pretreated ovarian cancer patients. J Clin Oncol 11:2127
13. Karlsson MO, Molnar V, Bergh J, Freijs A, Larsson R (1998) A general model for time-dissociated pharmacokinetic-pharmacodynamic relationship exemplified by paclitaxel myelosuppression. Clin Pharmacol Ther 63:11
14. Karlsson MO, Molnar V, Freijs A, Nygren P, Bergh J, Larsson R (1999) Pharmacokinetic models for saturable distribution of paclitaxel. Drug Metab Dispos 27:1220
15. Kearns CM, Gianni L, Egorin MJ (1995) Paclitaxel pharmacokinetics and pharmacodynamics. Semin Oncol 22:16
16. Kessel D (1992) Properties of cremophor EL micelles probed by fluorescence. Photochem Photobiol 56:447
17. Knemeyer I, Wientjes MG, Au JL-S (1999) Cremophor reduces paclitaxel penetration into bladder wall during intravesical treatment. Cancer Chemother Pharmacol 44:241
18. Kumar GN, Walle UK, Bhalla KN, Walle T (1993) Binding of Taxol to human plasma, albumin and alpha 1-acid glycoprotein. Res Commun Chem Pathol Pharmacol 80:337
19. Legg B, Rowland M (1988) Saturable binding of cyclosporin A to erythrocytes: estimation of binding parameters in renal transplant patients and implications for bioavailability assessment. Pharm Res 5:80
20. Ludden TM (1991) Nonlinear pharmacokinetics. Clin Pharmacokinet 20:429
21. Millward MJ, Webster LK, Rischin D, Stokes KH, Toner GC, Bishop JF, Olver IN, Linahan BM, Linsenmeyer ME, Woodcock DM (1998) Phase I trial of cremophor EL with bolus doxorubicin. Clin Cancer Res 4:2321
22. Minami H, Sasaki Y, Saijo N, Ohtsu T, Fujii H, Igarashi T, Itoh K (1998) Indirect-response model for the time course of leukopenia with anticancer drugs. Clin Pharmacol Ther 64:511
23. Schoemaker RC, Cohen AF (1996) Estimating impossible curves using NONMEM. Br J Clin Pharmacol 42:283
24. Sonnichsen DS, Hurwitz CA, Pratt CB, Shuster JJ, Relling MV (1994) Saturable pharmacokinetics and paclitaxel pharmacodynamics in children with solid tumors. J Clin Oncol 12:532
25. Sparreboom A, Huizing MT, Boesen JJB, Nooijen WJ, Van Tellingen O, Beijnen JH (1995) Isolation, purification and biological activity of mono- and dihydroxylated paclitaxel metabolites from human faeces. Cancer Chemother Pharmacol 236:299
26. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH (1996) Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 56:2112
27. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH (1998) Preclinical pharmacokinetics of paclitaxel and docetaxel. Anticancer Drugs 9:1
28. Sparreboom A, De Bruijn P, Nooter K, Loos WJ, Stoter G, Verweij J (1998) Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. J Chromatogr 705:159
29. Sparreboom A, Loos WJ, Verweij J, De Vos AI, Van der Burg MEL, Stoter G, Nooter K (1998) Quantitation of Cremophor EL in human plasma samples using a colorimetric dye-binding microassay. Anal Biochem 255:171
30. Sparreboom A, Verweij J, Van der Burg MEL, Loos WJ, Brouwer E, Vigano L, Locatelli A, De Vos AI, Nooter K, Stoter G, Gianni L (1998) Disposition of Cremophor EL in humans limits the potential for modulation of the multidrug resistance phenotype in vivo. Clin Cancer Res 4:1937
31. Sparreboom A, Van Zuylen L, Brouwer E, Loos WJ, De Bruijn P, Gelderblom H, Pillay M, Nooter K, Stoter G, Verweij J (1999) Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer Res 59:1454
32. Sykes E, Woodburn K, Decker D, Kessel D (1994) Effects of Cremophor EL on distribution of Taxol to serum lipoproteins. Br J Cancer 70:401
33. Van Tellingen O, Huizing MT, Nannan Panday VR, Schellens JHM, Nooijen WJ, Beijnen JH (1999) Cremophor EL causes (pseudo-) non-linear pharmacokinetics of paclitaxel in patients. Br J Cancer 81:330
34. Van Tellingen O, Beijnen JH, Verweij J, Scherrenburg EJ, Nooijen WJ, Sparreboom A (1999) Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. Clin Cancer Res 5:2918
35. Van Zuylen L, Gianni L, Verweij J, Mross K, Brouwer E, Loos WJ, Sparreboom A (2000) Interrelationships of paclitaxel disposition, infusion duration and Cremophor EL kinetics in cancer patients. Anticancer Drugs 11:331
36. Wagner JG (1979) Nonlinear pharmacokinetics. In: Fundamentals of clinical pharmacokinetics, 2nd edn. Drug Intelligence Publications, Hamilton, Ill., p 247
37. Webster LK, Cosson EJ, Stokes KH, Millward MJ (1996) Effect of the paclitaxel vehicle, Cremophor EL, on the pharmacokinetics of doxorubicin and doxorubicinol in mice. Br J Cancer 73:522