Cancer Chemother Pharmacol (2009) 63:1035–1048
DOI 10.1007/s00280-008-0828-1

ORIGINAL ARTICLE

Multiple-pool cell lifespan models for neutropenia to assess the population pharmacodynamics of unbound paclitaxel from two formulations in cancer patients

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Received: 1 April 2008 / Accepted: 18 August 2008 / Published online: 13 September 2008

Abstract
Purpose Our objective was to build a mechanism-based pharmacodynamic model for the time course of neutropenia in cancer patients following paclitaxel treatment with a tocopherol-based Cremophor-free formulation (Tocosol Paclitaxel®) and Cremophor® EL-formulated paclitaxel (Taxol®).
Methods A randomized two-way crossover trial was performed with 35 adult patients who received 175 mg/m^2 paclitaxel as either 15 min (Tocosol Paclitaxel) or 3 h (Taxol) intravenous infusions. Paclitaxel concentrations were measured by LC–MS/MS. NONMEM VI was used for population pharmacodynamics.
Results The cytotoxic effect on neutrophils was described by four mechanism-based models predicated on known properties of paclitaxel that used unbound concentrations in the central, deep peripheral or an intracellular compartment as forcing functions. Tocosol Paclitaxel was estimated to release 9.8% of the dose directly into the deep peripheral compartment (DPC). All models provided reasonable fitting of neutropenic effects. The model with the best predictive performance assumed that this dose fraction was released into 22.5% of the DPC which included the site of toxicity. The second-order cytotoxic rate constant was 0.00211 mL/ng per hour (variability: 52% CV). The relative exposure at the site of toxicity was 2.21 ± 0.41 times (average ± SD) larger for Tocosol Paclitaxel compared to Taxol. Lifespan was 11.0 days for progenitor cells, 1.95 days for maturating cells, and 4.38 days for neutrophils. Total drug exposure in blood explained half of the variance in nadir to baseline neutrophil count ratio.
Conclusions The relative exposure of unbound paclitaxel at the site of toxicity was twice as large for Tocosol Paclitaxel compared to Taxol. The proposed mechanism-based models explained the extent and time course of neutropenia jointly for both formulations.

Prior presentation: The pharmacokinetic modeling analysis was in part presented as a poster at the American Conference on Pharmacometrics (ACoP) 2008, Tucson, AZ. The data and non-compartmental pharmacokinetic analysis of this study were in part presented at the 2005 Annual Meeting of the American Society of Clinical Oncology (Abstract No. 2045), Orlando, FL. Financial support: This clinical study was performed by Sonus Pharmaceuticals, Inc., and the modeling was supported by Sonus Pharmaceuticals. Jürgen Bulitta was supported by a post-doctoral fellowship from Johnson & Johnson.

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Keywords Paclitaxel · Population pharmacokinetics and pharmacodynamics · Non-hematological malignancies · Multiple-pool cell lifespan model · Monte Carlo simulation · Neutrophils/toxicity · LC–MS/MS

Introduction

Paclitaxel is a key oncology drug and has broad antitumor activity against refractory ovarian, breast, bladder, lung, and head and neck cancers [43]. The standard paclitaxel formulation (Taxol®) uses Cremophor EL and ethanol (1:1, w/w) to solubilize paclitaxel. As neutropenia is the principal toxicity of paclitaxel [42], it is important to understand the relationship between unbound paclitaxel concentrations and neutrophil counts. Most patients show severely decreased nadirs for neutrophil counts due to paclitaxel treatment. Neutrophil toxicity is usually observed approximately 8–10 ten days after paclitaxel dosing and resolves between 2–3 weeks post-treatment [38, 42]. The severity of toxicity has been described by the duration of paclitaxel concentration above a critical plasma concentration [6, 11, 14, 15], the total exposure to total or unbound paclitaxel [14, 15, 38], or a more general model [25]. Minami et al. [37] used an indirect response model to describe the toxicity of paclitaxel. Friberg et al. [7], Kloft et al. [29], Joerger et al. [24], and Ozawa et al. [40] described the time course of neutrophil and leukocyte counts for several chemotherapeutic drugs by a semi-mechanistic model with three transit compartments.

To predict the time course of neutrophil counts for various paclitaxel formulations and doses, it is important to incorporate the mechanism of action into a pharmacokinetic/pharmacodynamic (PKPD) model. We applied the semi-physiologic multiple-pool cell lifespan model proposed by Krzyzanski and Jusko [32] to relate the time course of unbound paclitaxel concentrations to the time course of neutrophil counts. This model is based on the cell lifespan concept and includes myeloid cells in two stages in the bone marrow (progenitor and maturating cells), and neutrophils in the circulating pool. The drug effect is specified by an irreversible cytotoxic effect of paclitaxel on progenitor cells in bone marrow.

Cremophor EL is an important "binding" site for paclitaxel [47, 48]. Therefore, it is important to use unbound concentrations as the forcing function for PD effects. Earlier studies found nonlinear PK of total paclitaxel [11, 23, 26, 28, 46]. More recent studies [10, 14–16, 47, 48] explained the nonlinearity of total paclitaxel by entrapment of paclitaxel within Cremophor micelles and these studies [10, 14–16] successfully described the PK of unbound paclitaxel by models with linear disposition.

In this study a new tocopherol-based, Cremophor-free paclitaxel formulation (Tocosol Paclitaxel) was compared to Taxol. Tocosol Paclitaxel nanodroplets showed sustained release of paclitaxel [13] that supports a 15 min Tocosol Paclitaxel infusion compared to 3 h (or longer) infusions for Taxol (see companion article). This is a key advantage of Tocosol Paclitaxel in addition to the absence of Cremophor-related toxicity. Tocosol Paclitaxel nanodroplets can potentially release a fraction of the dose directly at peripheral sites. If the equilibrium between drug at a peripheral site accessible to nanodroplets and plasma is slow, the unbound paclitaxel exposure at this peripheral site can be substantially higher than the plasma exposure due to direct drug release from nanodroplets.

Our first objective was to build a mechanism-based population PKPD model that describes the extent and time course of neutrophil toxicity. This model should explain the differences in neutrophil counts between Tocosol Paclitaxel and Taxol and was predicated on our population PK model for paclitaxel. Secondly, we sought to predict the time course of neutropenia for various paclitaxel dosage regimens by this model.

Materials and methods

Details on the clinical and bioanalytical procedures that are not described herein can be found in the companion article.

Study design and patients

The study was a randomized, two-period, open label, multicenter (six clinical sites), crossover trial in 36 adult patients. The inclusion criteria included an absolute neutrophil count (ANC) at screening $>2 \times 10^9$ L^{-1} and a platelet count $> 100 \times 10^9$ L^{-1}. The study was approved by the Ethics Committees and Institutional Review Boards at all participating sites and conducted according to the revised version of the Declaration of Helsinki. All patients signed written informed consent before entering the study.

Drug administration, blood sampling, and drug analysis

Each patient received 175 mg m^{-2} paclitaxel (about 300 mg for a patient with 1.73 m^2 body surface area, BSA) as Tocosol Paclitaxel in study period I followed by the same dose of paclitaxel as Taxol in study period II or vice versa. The washout period was 3 weeks. Paclitaxel was given as a constant rate infusion over approximately 15 min for Tocosol Paclitaxel and 3 h for Taxol. In each study period, 4 mL of blood were taken at 18 specified time points up to

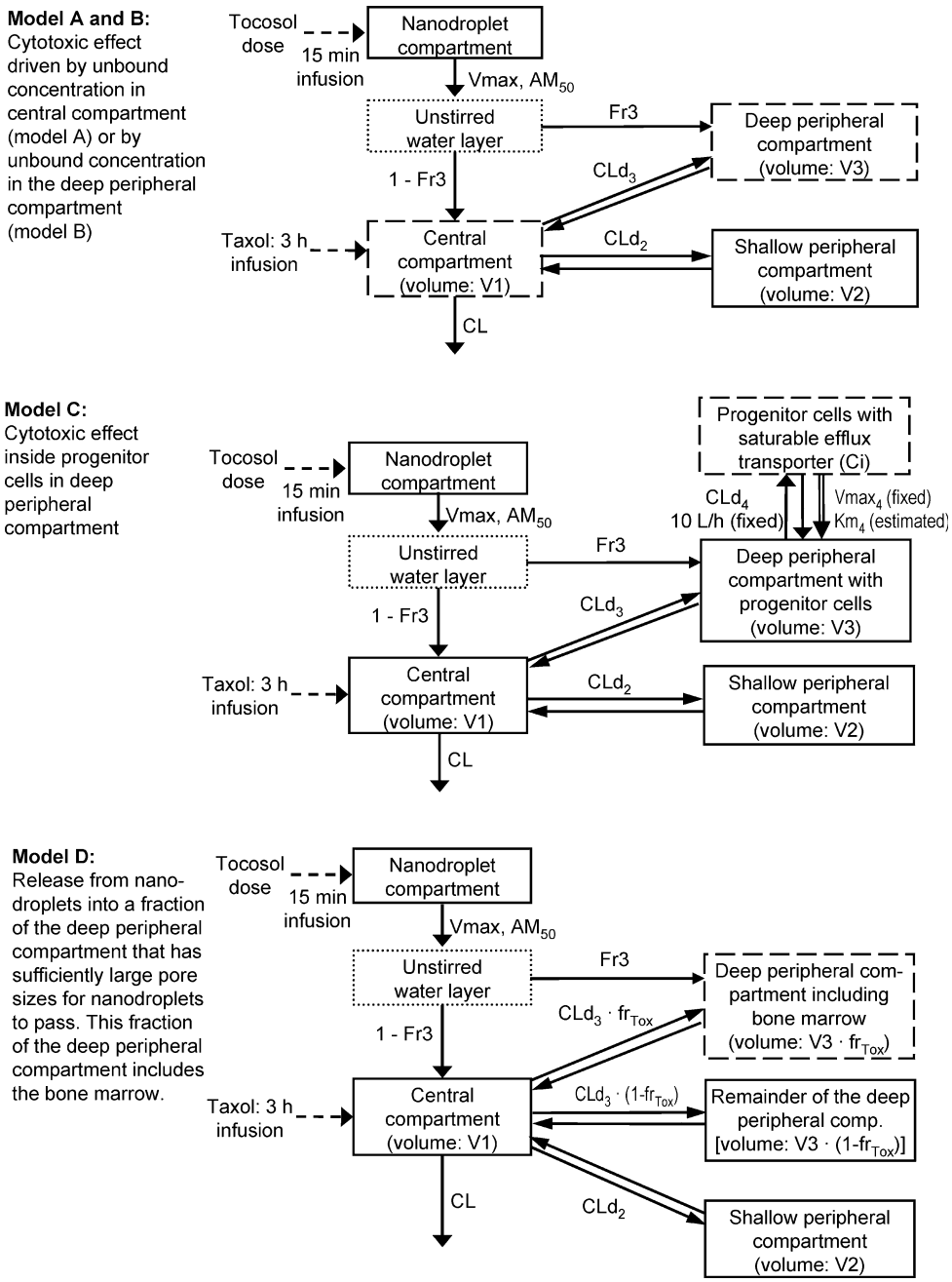

Fig. 1 Structural PKPD models for unbound paclitaxel (compartments associated with the cytotoxic effect shown with a *dashed border*). *Vmax* maximum rate of paclitaxel release from nanodroplet compartment, AM_{50} amount of paclitaxel in nanodroplet compartment that results in 50% of Vmax, *Fr3* fraction of dose directly entering the deep peripheral compartment from nanodroplets, *V1* volume of distribution of central compartment, *V2*: volume of distribution of shallow peripheral compartment, *V3*: volume of distribution of deep peripheral compartment, *CL* total plasma clearance, CLd_2 distributional clearance from central compartment to V2, CLd_3 distributional clearance from central compartment to V3, Cld_4 distributional clearance from V3 to progenitor cells, $Vmax_4$ maximum rate of efflux from progenitor cells, Km_4 intracellular concentration associated with a half-maximal rate of efflux, *Ci* concentration in progenitor cells, fr_{Tox} fraction of deep peripheral compartment that has sufficiently large pore sizes to permit nanodroplets to pass and release drug inside the tissue; this fraction of the deep peripheral compartment is expected to include bone marrow. All volumes and clearances refer to unbound paclitaxel

120 h post end of infusion. Sample handling and analysis are described in the companion article.

Laboratory testing

A complete blood count, chemistry panel, prothrombin time, partial thromboplastin time, and pregnancy test (if applicable) were performed within 1 week before studying drug administration in study period I and within 1 day before administration in study period II. Additionally, a complete blood count including ANC and platelet count was performed on days 1, 4, 7, 10, 13, and 16 in each study period.

Population PKPD analysis

Population PK model

Several structural PK models were assessed with details provided elsewhere. In brief, drug input was described by zero-order input into the central compartment for Taxol and by zero-order input into the nanodroplet compartment for Tocosol Paclitaxel (Fig. 1). For Tocosol Paclitaxel, 9.8% [4.4–15.3%; 90% confidence interval] of the amount in the nanodroplet compartment was estimated to be directly released into the deep peripheral compartment

(DPC). The PK parameters for the disposition of unbound paclitaxel were assumed to be the same for Tocosol Paclitaxel and Taxol within each patient.

Specification of the cytotoxic effect

An initial descriptive analysis of the neutrophil counts showed more pronounced toxicity for Tocosol Paclitaxel than for Taxol. Various structural models were compared to describe the differences in toxicity without invoking a different mean cytotoxic rate constant for each formulation. It was assumed that the interaction of unbound paclitaxel and progenitor cells at the site of toxicity was not affected by the formulation.

Structural model A and B

The PK model was not modified for models A and B (Fig. 1) and the cytotoxicity was determined by an irreversible function which used the unbound concentration in the central compartment (model A) or unbound concentration in the DPC (model B) raised to a power of γ (Fig. 2). If two formulations achieve the same unbound drug exposure, this model predicts a greater (lesser) extent of toxicity for the formulation with higher peak concentrations, if γ is above (below) 1.

Structural model C

This model included a compartment for progenitor cells that were assumed to reside in the DPC. These cells were assumed to comprise a small fraction of the DPC and to contain an efflux transporter for paclitaxel. It was assumed that the drug concentration at the progenitor cells was in rapid equilibrium with the concentration of the DPC (Fig. 1, model C). As Tocosol Paclitaxel was predicted to achieve higher peak concentrations in the DPC and in the progenitor cell compartment, the extent of saturation of the efflux transporter and therefore the extent of neutrophil toxicity was more pronounced for Tocosol Paclitaxel than for Taxol. The neutrophil toxicity was assumed to be driven by the intracellular paclitaxel concentration (C_i, Fig. 2).

Structural model D

This model assumed that Tocosol Paclitaxel nanodroplets could only enter a fraction of the DPC. The diameter of the nanodroplets is about 40–80 nm [4, 5] which is smaller than the large pores of bone marrow that allow passage of particles up to a size of 1,000 to 10,000 nm [31, 49]. The DPC was split into two parts (Fig. 1). It was assumed that the half-life of re-distribution of unbound drug from the DPC to the central compartment was the same for both parts of the DPC within a patient. It was assumed that a fraction (Fr3) of the paclitaxel dose from nanodroplets was exclusively released into the fraction (fr_{Tox}) of the DPC that comprises the bone marrow as the site of toxicity (Fig. 2). As 9.8% of the Tocosol Paclitaxel dose (see above) entered into this fraction of the DPC, the unbound drug exposure in this fraction of the DPC was larger than in the remainder of the DPC leading to a larger extent of neutrophil toxicity for Tocosol Paclitaxel. The ratio of unbound area under the curve in this fraction of the DPC ($fAUC_{DPC,Tox}$) and unbound AUC in the central compartment ($fAUC_{Central}$) is:

$$\frac{fAUC_{DPC,Tox}}{fAUC_{Central}} = \frac{fAUC_{Central} + fAUC_{EPR}}{fAUC_{Central}} = 1 + \frac{Fr3 \cdot CL}{fr_{Tox} \cdot CLd_3}$$

The CL is the unbound total clearance and CLd_3 the unbound distributional clearance between the central and deep peripheral compartment (Fig. 1). This ratio is larger than 1 due to the additional unbound area under the curve caused by the enhanced permeability and retention (EPR) effect ($fAUC_{EPR}$).

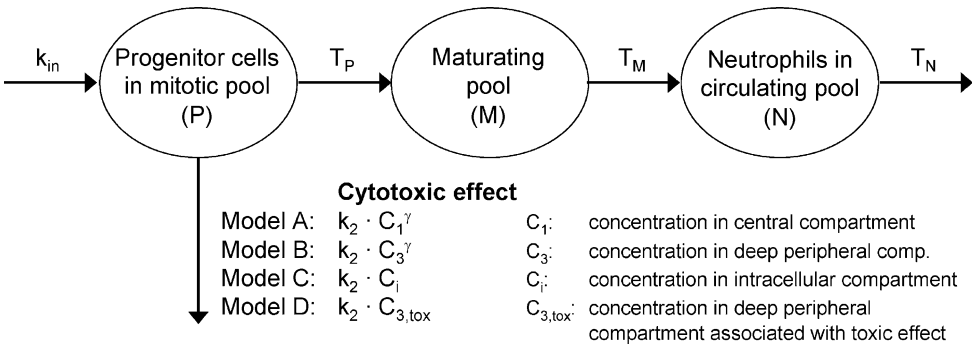

Fig. 2 Lifespan model for cytotoxic effect of paclitaxel on neutrophils. The progenitor cells (P) are produced at a zero-order input rate k_{in}. The progenitor cells reside in the mitotic pool for the lifespan (T_P). Unbound paclitaxel is assumed to cause a cytotoxic effect on progenitor cells (rate constant: k_2). The surviving progenitor cells reach the maturation pool (M) in which they reside for their lifespan T_M before they reach the circulating neutrophil pool (N). The lifespan of neutrophils is denoted as T_N

Population PKPD model

The multiple-pool cell lifespan model proposed by Krzyzanski and Jusko [32] was used to describe the cytotoxic effect of unbound paclitaxel on neutrophils (Fig. 2). The progenitor cells are produced by a zero-order process (rate constant: k_{in}). Either k_{in} or the baseline ANC can be estimated as model parameters, since the baseline can be calculated from the ratio of k_{in} and the lifespan of neutrophils (T_N). Baseline ANC was estimated instead of k_{in}, since baseline ANC can be directly read from the observed ANC. Progenitor cells reside in the mitotic pool for their lifespan T_P. Cells can leave the progenitor cell compartment either by conversion to maturating cells or by loss due to the cytotoxic effect of paclitaxel. For model D, the equation for fraction of cells (SF) surviving the cytotoxic effect in the progenitor cell compartment is:

$$SF(t) = \exp\left[-\int_{t-T_P}^{t} k_2 \cdot C_{3,tox}(z)dz\right] \quad (1)$$

The k_2 is the second-order rate constant for the cytotoxic effect, $C_{3,tox}$ is the unbound paclitaxel concentration at the site of toxicity, T_P is the lifespan of progenitor cells and t is time. The integral in equation (1) was calculated by use of the "dummy variable" Int(t):

$$\frac{dInt}{dt} = k_2 \cdot C_{3,tox}(t) \quad \text{IC: Int}(0) = 0 \quad (2)$$

The survival fraction [SF(t)] can be described as a function of Int(t) with the limits of integration depending on cell lifespan parameters:

$$SF(t) = \exp\{-[Int(t) - Int(t - T_P)]\} \quad (3)$$

The equations for SF(t) and Int(t) of models A, B, and C describe the cytotoxic effect by the equations shown in Fig. 2 instead of $k_2 \cdot C_{3,tox}$. In addition to these models with a linear (non-saturable) cytotoxic effect, models with a saturable cytotoxic effect (concentration associated with 50% of maximal effect: KC_{50}) were considered:

$$\text{cytotoxic effect} = \frac{k_2 \cdot KC_{50} \cdot C_{3,tox}}{KC_{50} + C_{3,tox}} \quad (4)$$

The surviving progenitor cells reach the maturation pool in which they reside for their lifespan T_M before they are released to the circulating neutrophil pool. Neutrophils stay in the circulating pool for their lifespan T_N. The equation for neutrophils (N) is:

$$\frac{dN}{dt} = k_{in} \cdot SF(t - T_M) - k_{in} \cdot SF(t - T_M - T_R) \quad \text{IC: } N_0 = k_{in} \cdot T_N \quad (5)$$

Equations (2) and (5) comprise a system of delay differential equations that can be solved by the method of steps (see Perez-Ruixo et al. [41] for details). Solving the equations for cells in the progenitor or maturating cell compartment is not necessary to calculate the time course of neutrophils.

Implementation

The 42 day time course of ANC data including treatment by both formulations was fitted simultaneously. To apply the method of steps, four replicates of the PK model were specified for study period I and additional four replicates of the PK model were specified for study period II. The PK models within each study period shared all PK parameters except lag-time.

Parameter variability model for PD

The between-subject variability (BSV) was estimated by assuming a log-normal distribution of PD parameters. It was assumed that the system parameters (baseline ANC, T_P, T_M, and T_N) did not change within a patient over both study periods. The mean k_2 was assumed to be the same for both treatments, however, k_2 could vary between occasions.

Observation model and computation

The 'transform both sides method' was applied to model the ANC data. The residual unidentified variability was described by an additive error model on natural log-scale. WinNonlin™ Professional (version 5.0.1, Pharsight Corp., Mountain View, CA) was used for descriptive statistics and ANOVA. All PKPD models were built in NONMEM version VI level 1.1 (Feb 2007, NONMEM Project Group, University of California, San Francisco, CA) [1]. The first-order conditional estimation (FOCE) method was applied for estimation of PD model parameters and the ADVAN6 or ADVAN9 differential equation solvers were used. A sequential estimation strategy was used. Previously estimated individual PK parameters were fixed during estimation of the population PD model parameters. The Individual PK Parameters (IPP) method was described in detail by Zhang et al. [51].

Model qualification

The models were compared by their predictive performance, the objective function in NONMEM, individual model fits, observed versus predicted plots, and other standard diagnostic plots. The predictive performance was assessed by visual predictive checks. At least 5,000 virtual patients were simulated from each population PKPD model for Tocosol Paclitaxel and Taxol in the presence of residual

error. These virtual patients had the same demographic characteristics as the patients in our study. From the simulated ANC profiles, the median, 10, 25, 75, and 90% percentiles were calculated at each time point by qualified Perl scripts. These percentiles were then plotted on top of the observed ANC data.

Simulations

The ANC profiles were predicted for doses of 80, 100, or 120 mg m^{-2} Tocosol Paclitaxel or 175 mg m^{-2} Taxol. Simulations included a single dose, two doses given on days 0 and 14, or three doses given on days 0, 7, and 14. The ANC profiles were simulated in absence of residual error.

Correlation of drug exposure and neutrophil toxicity

The ratio of nadir to baseline ANC was plotted against various measures of drug exposure to explore potentially clinically useful surrogates for the drug exposure at the site of toxicity. The individual fitted baselines and fitted nadirs were used for this correlation, as those were expected to be more reliable than observed baseline ANC and observed nadirs.

Results

Patients

Demographic and clinical details for the 35 patients evaluable for PKPD analysis are provided in the companion article. On study day 0, hematocrit was 0.350 ± 0.047 (average $\pm$ SD) for patients receiving Tocosol Paclitaxel in period I and 0.337 ± 0.038 for patients receiving Taxol in period I. On day 0, platelet count was $280 \pm 85 \times 10^9$ L^{-1} for patients receiving Tocosol Paclitaxel in period I and $325 \pm 145 \times 10^9$ L^{-1} for patients receiving Taxol in period I. For patients in the Tocosol Paclitaxel-Taxol sequence, ANC was $4.70 \pm 2.65 \times 10^9$ L^{-1} on day 0 and $6.56 \pm 5.12 \times 10^9$ L^{-1} on day 20. For patients in the Taxol-Tocosol Paclitaxel sequence, ANC was $5.84 \pm 3.42 \times 10^9$ L^{-1} on day 0 and $5.64 \pm 3.62 \times 10^9$ L^{-1} on day 20. For the Tocosol Paclitaxel group, 56% of the patients experienced a NCI-CTC (Version 2.0) grade 3 or 4 neutropenia. This percentage was 21% for Taxol. None of the patients experienced a thrombocytopenia of grade 3 or 4. There were no episodes of febrile neutropenia or sepsis following either drug and there were no grade 4 non-hematologic toxicities. The severity and frequency of grade 1–3 non-hematologic adverse events was comparable for both treatments.

Population PKPD

Figure 3 shows the observed and individual fitted ANC for both study periods. The predicted lag-time in ANC profiles of about 2 days represents the lifespan of maturating cells. The duration of decrease of ANC from baseline to nadir and the duration of increase back to baseline are primarily determined by the lifespan of neutrophils and cytotoxic effect. The duration of ANC at nadir is primarily determined by the difference in lifespan of progenitor cells and neutrophils. Most ANC profiles returned to baseline before the ANC decreased due to the second treatment after 21 days. A (slight) rebound in ANC was seen in about half of the patients (see ANC at about 26 and 42 days in Fig. 3).

Models A to D had virtually indistinguishable curve fits for about two-thirds of the patients. A linear regression of the observed ANC versus individual fitted ANC on natural log-scale yielded a range of slopes of [1.014–1.016], range of intercepts of [−0.0154 to −0.0141], and range of correlation coefficients of [0.954–0.955] for models A to D. This plot showed no bias throughout the whole range of ANC for models A to D (data not shown).

Table 1 compares the parameter estimates of models A–D. The objective function was 27–38 points better for models B, C, and D than for model A. All four models yielded consistent estimates for baseline ANC and the three lifespan parameters. These system parameters had standard errors (SE) relative to the mean below 30%. The between-subject variability was the largest for baseline ANC (57% CV) and smaller for lifespans of maturating cells (33%) and progenitor cells (20%). The data did not support estimation of the variability in lifespan of neutrophils. The SE of the between-subject variability (variances) was below 40%, except for the variability in lifespan of maturating cells. Models A–D use unbound drug concentrations in different compartments to describe the cytotoxic effect on progenitor cells. Therefore, parameter estimates for the cytotoxic effect cannot directly be compared among models A–D.

Figure 4 shows the visual predictive checks. The observed ANC are plotted on top of the percentiles of the predicted ANC for model D in panels a.1 and a.2. Tocosol Paclitaxel showed a more pronounced decline in the ANC compared to Taxol. Model D explained this greater toxicity by a 2.21 ± 0.41 times (average $\pm$ SD) as large extent of availability of unbound paclitaxel at the site of toxicity compared to Taxol. For model D, the site of toxicity was the fraction of the DPC that included the bone marrow. Visual predictive checks showed that model D captured the central tendency and the variability of the observed ANC for Tocosol Paclitaxel and Taxol very well (Fig. 4, panels a.1 and a.2). Fig. 4 compares the visual predictive checks for model D (panels b.1 and b.2), model C (panels c.1 and

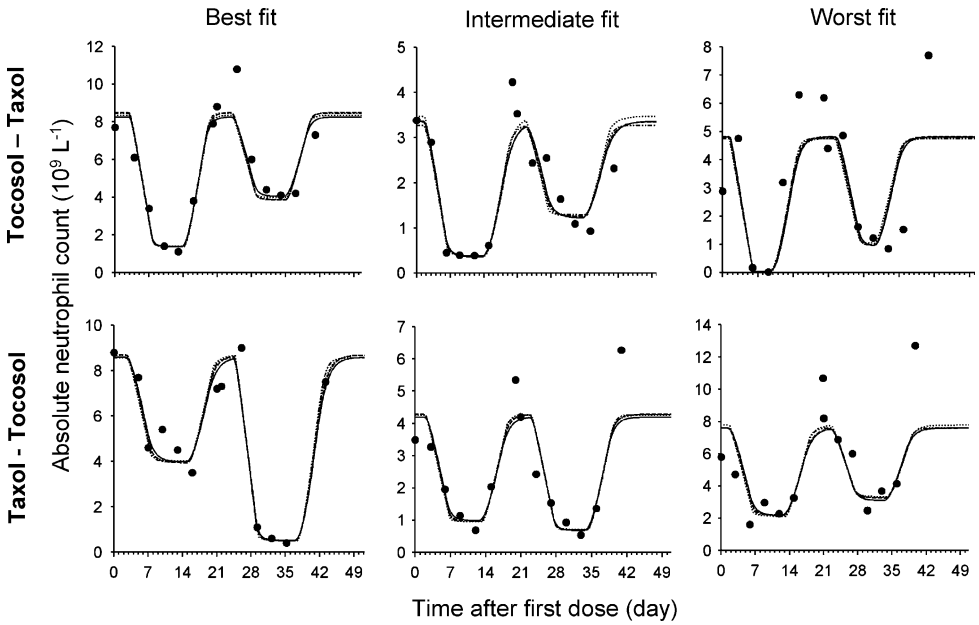

Fig. 3 Time course of neutrophil counts following single doses of 175 mg m^{-2} Tocosol Paclitaxel as 15 min infusion and Taxol as 3 h infusion (*top row* sequence Tocosol Paclitaxel—Taxol, *bottom row* sequence Taxol—Tocosol Paclitaxel). Individual curve fits for model A (*dotted line*), model B (*dashed-dotted line*), model C (*dashed line*), and model D (*continuous line*) for the patients with the best, intermediate, and worst curve fit. The curve fits were ranked by the average residual on log-scale. This criterion deems misfits at high ANC less important compared to misfits at low ANC

c.2), model B (panels d.1 and d.2), and model A (panels e.1 and e.2). Models C and B slightly under-predicted the toxicity for Tocosol Paclitaxel and slightly over-predicted the toxicity for Taxol. Mispredictions were more pronounced for model A.

As model D had an excellent predictive performance for both treatments, this model was used to simulate ANC profiles. The predicted ANC profiles for various dosage regimens are shown in Fig. 5. As Tocosol Paclitaxel had a 2.21 times larger availability at the site of toxicity, simulated ANC profiles for 80 mg m^{-2} Tocosol Paclitaxel (Fig. 5, panels a–c) were very similar to the profiles for 175 mg m^{-2} Taxol (Fig. 5, panels j–l). As expected, ANC profiles decreased more for the larger Tocosol Paclitaxel doses of 100 and 120 mg m^{-2}. The model predicted almost no accumulation in toxicity, if Tocosol Paclitaxel was dosed every 14 days, whereas there was some accumulation in toxicity for dosing every 7 days. The predicted median ANC for 120 mg m^{-2} Tocosol Paclitaxel dosed every 7 days had its nadir at about 1×10^9 L^{-1} (Fig. 5, panel i). As this value is the upper limit for NCI–CTC grade 3 neutropenia, about 50% of the patients are expected to have a grade 3 or 4 neutropenia for this dosage regimen. Lower frequencies between 25 and 50% for grade 3 or 4 neutropenia were predicted for 80 and 100 mg m^{-2} Tocosol Paclitaxel dosed every 7 days (Fig. 5, panels c and f).

For the multiple-pool cell lifespan model with linear second-order cytotoxicity, the ratio of nadir to baseline ANC can be approximated by the AUC of unbound paclitaxel at the site of toxicity with an exponential relationship (Fig. 6). For model D, this AUC is the AUC in the fraction of the DPC that includes the bone marrow. This correlation is shown in Fig. 6 (bottom). As the AUC at the site of toxicity is not directly observable, the correlation of the ratio of nadir to baseline ANC with the total AUC in whole blood and with the AUC in plasma ultrafiltrate was also assessed. The total drug AUC explained about half (Fig. 6, top) of the variability in the nadir to baseline ANC ratio and the AUC in plasma ultrafiltrate explained about one-third (Fig. 6, middle) of this variability. As expected, the slopes of the latter two empiric relationships were different from the slope for the unbound AUC at the site of toxicity.

Discussion

This work presents the first application of a multiple-pool cell lifespan model with population PKPD methodology in anticancer chemotherapy. This mechanism-based PD model was combined with four PK models to describe the cytotoxic effect of paclitaxel on neutrophils (Figs. 1 and 2). The curve fits of those models were precise and virtually indistinguishable from each other for most patients (Fig. 3). We applied a sequential analysis strategy based on individual PK parameter estimates [51]. This approach seems reasonable for our dataset, since the onset of neutrophil toxicity is seen after about 1 week, ANC was measured every 3 days, and the terminal half-life of paclitaxel was about 25 h.

A more pronounced neutrophil toxicity was observed for Tocosol Paclitaxel compared to Taxol at the same nominal dose (Fig. 4), as the extent of availability to the site of toxicity was 2.21 times as large for Tocosol Paclitaxel than for Taxol (estimated by model D). Models A–D

Table 1 Population PD parameter estimates for the effect of unbound paclitaxel on neutrophils after a dose of 175 mg m^{-2} paclitaxel

Parameter	Symbol	Unit	Estimate (standard errors relative to mean)			
			Model A	Model B[a]	Model C	Model D
Objective function value			−192.5	−219.7	−223.4	−230.5
Fixed effects						
Rate constant for cytotoxic effect[b]	k_2	mL ng^{-1} h^{-1}	2.27×10^{-3} (108%)	0.633×10^{-3}	15.3×10^{-3} (38%)	2.11×10^{-3} (15%)
Exponent for cytotoxic effect	γ		1.09 (34%)	1.82	1 (fixed)	1 (fixed)
Affinity constant for intracellular efflux transporter	Km_4	ng mL^{-1}			0.936 (62%)[c]	
Fraction of volume subject to EPR effect	fr_{Tox}					0.225 (35%)
Baseline ANC	N_0	10^9 cells L^{-1}	5.21 (12%)	5.23	5.26 (11%)	5.22 (12%)
Lifespan of progenitor cells	T_P	Days	11.7 (7.4%)	11.7	11.5 (7.2%)	11.0 (7.6%)
Lifespan of maturating cells	T_M	Days	2.23 (28%)	1.85	1.87 (23%)	1.95 (27%)
Lifespan of neutrophils	T_N	Days	4.71 (13%)	4.67	4.63 (11%)	4.38 (15%)
Between-subject variability for						
Rate constant for cytotoxic effect[d]			67%[e] (35%[f])	53%	52% (29%)	52% (34%)
Baseline ANC			57% (37%)	57%	57% (34%)	57% (39%)
Lifespan of progenitor cells			19% (57%)	20%	21% (39%)	20% (38%)
Lifespan of maturating cells			31% (107%)	34%	35% (104%)	33% (104%)
Additive error on log scale (SD)			0.329 (6.2%)	0.329	0.326 (5.6%)	0.327 (5.8%)

[a] NONMEM converged successfully for this model, but was unable to compute standard errors

[b] The unit of the cytotoxic rate constant is $(mL/ng)^{\gamma}$ h^{-1}

[c] CLd_4 was fixed to 10 L/h, V4 fixed to 1 L, and $Vmax_4$ fixed to 100 mg/h

[d] The k_2 could differ between subjects and between study periods and the population parameter variability of k_2 was estimated. A model with different variances for each formulation was not statistically superior to the model with a common variance

[e] This value is the apparent coefficients of variation of a normal distribution on log-scale representing the population parameter variability for k_2 and representing the between-subject variability for baseline ANC, lifespan of progenitor and lifespan of maturating cells

[f] All standard errors for random effects were calculated as standard error relative to the estimated variance

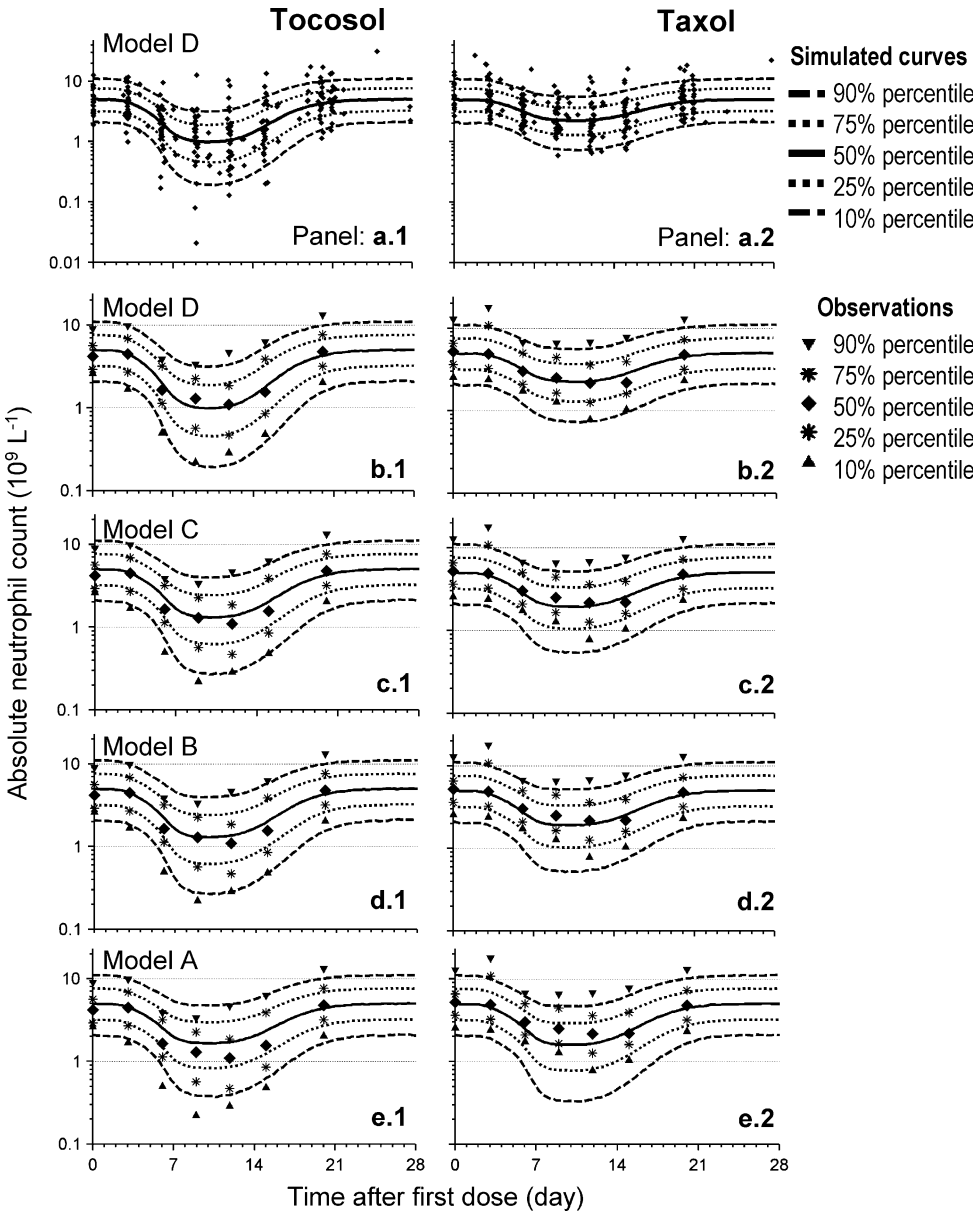

Fig. 4 Visual predictive checks for Tocosol Paclitaxel and Taxol. The plot shows the median, 10, 25, 75, and 90% percentiles of the simulated ANC profiles plotted on top of the individual observations (panels a.1 and a.2) or on top of the respective percentiles of the observed ANC counts (panels b.1 to e.2). Ideally, 20% of the observations should fall outside the 80% prediction interval at each time point in panels a.1 and a.2; in panels b.1–e.2, the simulated percentile lines should ideally fall on top of the markers representing the observed ANC for each percentile

incorporated different mechanisms by which the greater toxicity of Tocosol Paclitaxel was explained. The four models used the unbound paclitaxel concentration in different compartments to describe the cytotoxic effect on progenitor cells (Fig. 1). The increased toxicity of Tocosol Paclitaxel was explained by exponents (γ) above 1 for models A and B (Table 1 and Fig. 2), since Tocosol Paclitaxel achieved higher peak concentrations compared to Taxol, although Tocosol Paclitaxel achieved a similar unbound AUC in the central compartment as well as in the DPC. This exponent is equivalent to the common use of a power coefficient in the Hill function for the limiting case that unbound concentrations are far below the affinity constant (KC_{50}). In this case, the cytotoxic rate constant is the maximal cytotoxic rate constant (Kmax) divided by KC_{50}^{Hill}.

Model C included an efflux transporter that is more saturated at the higher intracellular paclitaxel concentrations achieved by Tocosol Paclitaxel than by Taxol. Paclitaxel is a substrate of P-glycoprotein and this transporter might play an important role in the efflux of cytotoxic anticancer drugs from progenitor cells [35, 44]. As P-glycoprotein-mediated efflux comprises the major efflux mechanism at least for some cells [22], we assumed that the efflux clearance from progenitor cells is ten times larger at low concentrations than the distributional clearance by passive diffusion. This choice only affected the estimated KC_{50} and the rate constant for cytotoxicity, as

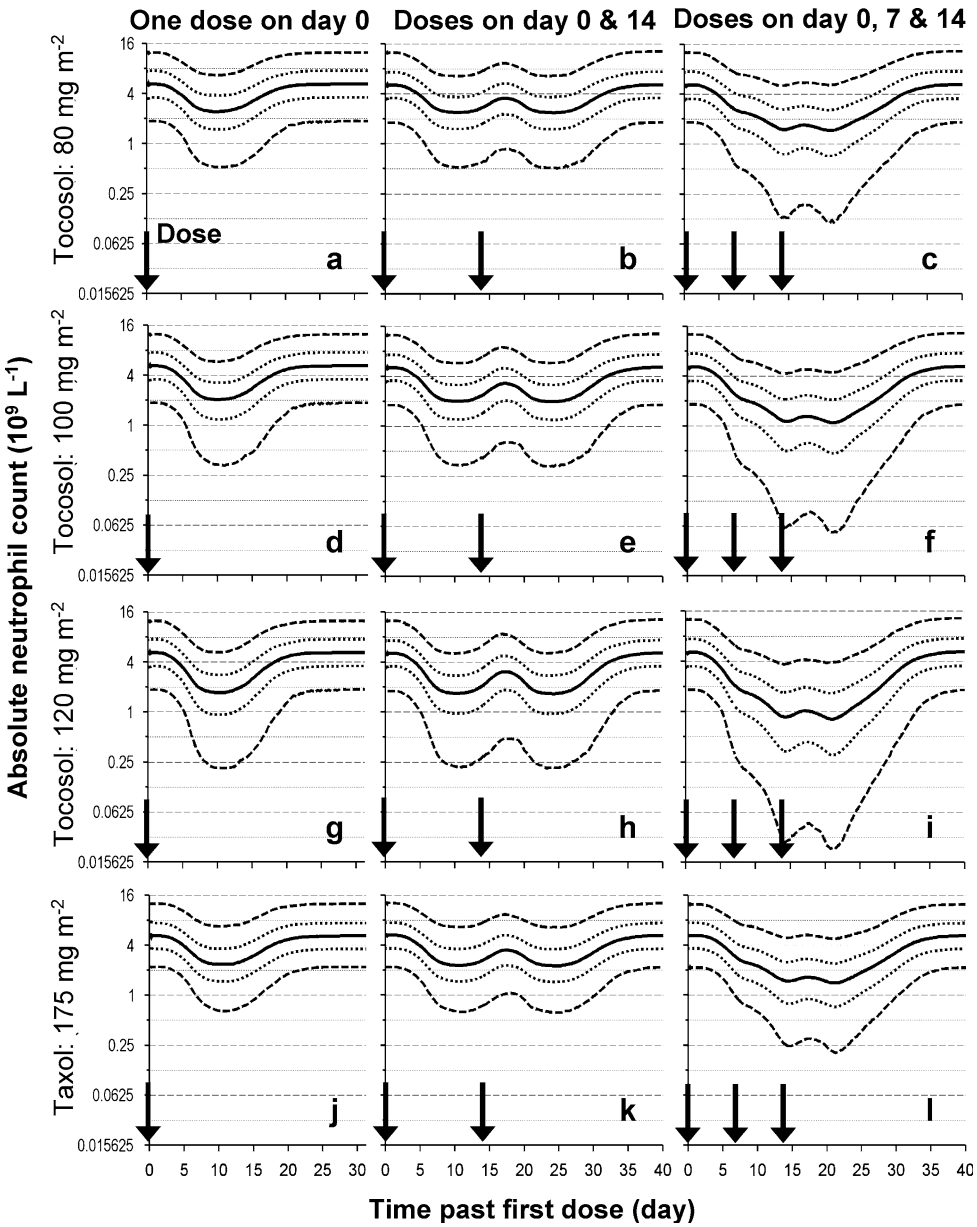

Fig. 5 Simulated ANC for one dose (*left*), two doses given every 14 days (*middle*), or three doses given every 7 days (*right*) of 80, 100, or 120 mg m^{-2} Tocosol Paclitaxel or of 175 mg m^{-2} Taxol (lines show the 5, 25, 50, 75, and 95% percentiles; simulations in absence of residual error, *arrows* indicate doses)

long as the P-glycoprotein-mediated efflux clearance was large. The intracellular distribution was assumed to be in a rapid equilibrium with the extracellular concentration and the steady-state solution for the intracellular concentration was implemented. Models that fitted the full time course of intracellular distribution yielded comparable results.

Model D was based on the EPR effect [21, 39]. In the population PK analysis, 9.8% of the dose was estimated to be released into the DPC. This leads to slightly higher concentrations in the DPC. Direct release from the formulation into the DPC is a mechanism by which a higher unbound AUC in the DPC relative to the central compartment can be achieved. A slow release from the DPC to the central compartment will cause this difference in unbound AUC to be more pronounced. However, if the 9.8% dose were released into the whole DPC, the unbound AUC was only slightly higher in the DPC (Fig. 5, panel e, in the companion paper) and this could explain only part of the differences in toxicity between Tocosol Paclitaxel and Taxol. It was therefore assumed for model D that the 9.8% dose was released into a fraction of the DPC and this fraction (fr_{Tox}) was an estimated model parameter. As the Tocosol Paclitaxel nanodroplets have a diameter of about 40–80 nm [4, 5] and as the fenestrae in bone marrow allow particles up to a size of 1,000–10,000 nm to pass [31, 49], it seems possible that the EPR effect might only affect those tissues lumped in the DPC that have a sufficiently large pore size that can be penetrated by Tocosol Paclitaxel

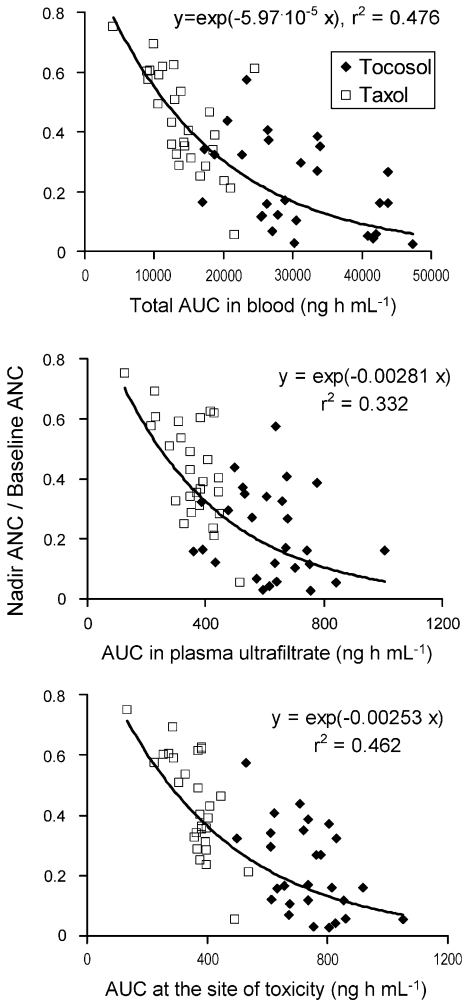

Fig. 6 Ratio of nadir ANC to baseline ANC plotted against the area under the curve (AUC) of the total paclitaxel in whole blood (*top*), the AUC in plasma ultrafiltrate (*middle*), and the AUC at the site of toxicity of model D (*bottom*). According to the multiple-pool cell lifespan model with a linear second-order cytotoxic rate constant, the ratio of nadir ANC to baseline ANC can be approximated by $\exp(-k_2 \cdot AUC_{effect\ site})$. Data from six subjects were excluded from this plot, since the estimated baseline was outside 0.66–1.5 times the observed baseline potentially due to a rebound in ANC. For these six patients the baseline ANC could not be reliably estimated. The AUC in whole blood and AUC in plasma ultrafiltrate were taken from non-compartmental analysis

nanodroplets. The differences in the average cytototoxic effect between both formulations were explained by an fr_{Tox} of 0.225 (Table 1).

Our data did not allow concluding that any of the four proposed models was mechanistically most appropriate. However, model D had the best predictive performance for Tocosol Paclitaxel (Fig. 4, panels a.1 and b.1) and good predictive performance for Taxol (Fig. 4, panels a.2 and b.2). The lower range of the prediction interval for Taxol was slightly too low during the first 10 days (Fig. 4, panel b.2). This might be attributed to the slightly higher observed baseline ANC at day 0 for Taxol (see results section) or to the slight rebound in ANC. However, the nadir ANC was well predicted by model D for both treatments.

The simulated ANC profiles for 80 mg m^{-2} Tocosol Paclitaxel (Fig. 5, panels a–c) or 175 mg m^{-2} Taxol (Fig. 5, panels j–l) were very similar, since the paclitaxel exposure at the site of toxicity was predicted to be 2.21 ± 0.41 times (average ± SD) as large for Tocosol Paclitaxel relative to Taxol. When paclitaxel was given every 14 days, almost no accumulation of neutrophil toxicity was predicted (Fig. 5, middle column), whereas some accumulation was predicted for dosing every 7 days. The median predicted ANC for 120 mg m^{-2} Tocosol Paclitaxel showed a nadir at about 1×10^9 L^{-1} which is the upper limit for NCI-CTC grade 3 neutropenia. This simulation result suggests that about 50% of patients will experience grade 3 or 4 neutropenia for this dosage regimen. This prediction was in excellent agreement with the observed toxicity in four phase II trials [2, 3, 12, 13, 36]. Across the whole phase 2A program including 145 patients and Tocosol Paclitaxel doses from 80 to 120 mg/m^2 per week, grade 3–4 toxicities observed in more than 5% of the patients included neutropenia (17% grade 3, 16% grade 4, 2% febrile), anemia (11% grade 3, 1% grade 4), peripheral neuropathy (9% grade 3, 0% grade 4), and dyspnea (4% grade 3, 1% grade 4). Infusion related symptoms were generally mild and resolved without treatment after interruption of the infusion for several minutes. If the EPR effect is restricted to tissues with large pore sizes, potentially less toxicity of Tocosol Paclitaxel might be achieved in tissues with smaller pore sizes.

Additionally, the EPR effect might lead to better deposition of Tocosol Paclitaxel nanodroplets to the tumor, since tumor cells have pore sizes that allow nanodroplets to distribute into and release drug directly in tumor tissue [21, 39]. However, the initial analysis of a phase III pivotal trial of Tocosol Paclitaxel in women with metastatic breast cancer did not meet its primary endpoint of non-inferiority on objective response rate when compared to the TAXOL control arm. The objective response rate was 37% for Tocosol Paclitaxel versus 45% for TAXOL (p value = 0.085). While the final analysis of this phase III trial is ongoing, this result points to the difficulty of clinical drug development in oncology which has a high failure rate compared to other therapeutic areas [30].

Models for neutrophil toxicity predicting the nadir ANC and models fitting the whole time course of ANC were previously proposed. The former models predict the nadir ANC based on various measures of drug exposure [6, 11, 14, 15, 25, 38]. Minami et al. [37] developed a model to fit the time course of toxicity for paclitaxel based on an indirect response model. Friberg et al. [7] developed a

semi-mechanistic model that can describe and predict the time course of neutrophil and leukocyte counts for several chemotherapeutic agents. This model includes three transit compartments for the delay between the cytotoxic effect and the decline in neutrophil counts. The drug effect is specified as an inhibition of replication of progenitor cells by a linear or saturable function and their model can incorporate a rebound by addition of one model parameter. The main difference between the model from Friberg et al. [7] and the multiple-pool cell lifespan model is the assumed distribution of cell lifespans within a patient. If one assumes a median lifespan of 120 h, the 5–95% percentiles for this lifespan distribution ranges from 41 to 235 h for $n = 3$ transit compartments, from 67 to 186 h for $n = 10$ transit compartments, and from 101 to 140 h for $n = 100$ transit compartments.

In the multiple-pool cell lifespan model (Fig. 2) the drug effect was specified by a cytotoxic effect on progenitor cells. This model allows one to implement the lifespan of progenitor cells, maturating cells, and neutrophils as estimated model parameters. The lifespan model used here assumes that all cells of the same type within one patient have the same lifespan. Extensions to this model including a distribution of lifespans have been developed [33, 34]. The lifespan of neutrophil progenitor cells in bone marrow (sum of T_P and T_M) of 13–14 days for our models (Table 1) is similar to the literature value of 14 days in humans [45]. The literature value of 6 days for T_P is shorter than our estimate of 11–12 days, whereas the literature estimate for T_M of 8 days is longer than our estimate of approximately 2 days. This suggests that neutrophil progenitor cells remain sensitive to paclitaxel in the early maturating phase, which has also been concluded by Krzyzanski and Jusko [32]. Our estimated lifespan of neutrophils of 4–5 days is longer than the mean transit time of 10 h for neutrophils in the circulating pool in humans [45]. Further studies are required to resolve this difference.

A rebound in ANC was not built into our model (Fig. 2), since this model was intended to primarily describe the neutrophil toxicity. Incorporating a rebound into the PD model shown in Fig. 2 is possible with a feedback function [7–9, 27, 50]. For about 25% of our patients, there was an initial rise in ANC by 20% or more after the first dose. As this rise was not as pronounced as for the docetaxel study from Ozawa et al. [40], we did not include this feature into our model. We did not assess an effect compartment to drive the neutrophil toxicity as described by Hing et al. [17], since this would not have allowed us to specify the mechanism of the EPR effect.

Models with a saturable cytotoxic function [32, 41] did not reduce the objective function in our analysis significantly (results not shown). However, our study only included one dose level and therefore probably had a low power to detect a saturable cytotoxic effect. Models with a linear cytotoxic effect (Fig. 2) are expected to yield conservative predictions, as these models would predict more severe toxicity for high doses or for high peak concentrations. Previous studies found the duration of total paclitaxel concentrations above a threshold concentration to best predict neutrophil toxicity [11, 14, 15, 20]. However, Mould et al. [38] identified the AUC of total paclitaxel as an independent predictor of patient survival and granulocytopenia. As pointed out by Henningsson et al. [14], the AUC and the threshold concentration model performed similarly if unbound instead of total concentrations were used to describe toxicity. It is generally accepted that only unbound drug can bind to a receptor, although the relative affinity of the drug to its receptor and to other binding sites (e.g. proteins) may need to be considered.

The correlation of various measures of paclitaxel exposure with the ratio of nadir to baseline ANC is shown in Fig. 6. Data from six subjects were excluded from this plot, since the estimated baseline was outside 0.66–1.5 times the observed baseline potentially due to a rebound in ANC. This ratio can be approximated by a direct function of the cytotoxicity rate constant and the AUC at the target site for models C and D (Figs. 1, 2) [32]. Thus, this approach allows one to select doses based on the baseline ANC. Interestingly, both the individual fitted unbound AUC at the site of toxicity and the total AUC in whole blood explained about half of the variability for this ratio. The latter empiric correlation suggests that the total AUC in whole blood can be used as a surrogate measure for drug exposure at the site of toxicity for Tocosol Paclitaxel. This empiric relationship should not be applied for paclitaxel formulations other than Tocosol Paclitaxel.

The total variability of the nadir to baseline ANC ratio had a coefficient of variation of 57% which was in part reflected by the population parameter variability of 52% for the cytotoxicity rate constant (Table 1). The average k_2 in study period I and II were similar. An ANOVA of k_2 from model D yielded a coefficient of variation of 27% for between-subject variability and of 34% for between-occasion variability. These results suggest that the time course of ANC should be monitored during therapy, since the between-subject variability of k_2 comprises a major portion of its total variability. However, the between-occasion variability of 34% in k_2 will cause random (non-controllable) variability of the nadir ANC for a future dose within a patient [18, 19]. These results are in good agreement with the nadir ANC predictions in other studies [15, 25].

In conclusion, four mechanism-based population PKPD models that yielded precise and unbiased fits were proposed. The model with the best predictive performance assumed that a fraction of the Tocosol Paclitaxel dose was directly released into part of the deep peripheral compartment.

A dose of about 80 mg m^{-2} Tocosol Paclitaxel was predicted to result in a similar toxicity as 175 mg m^{-2} Taxol, since the relative exposure of unbound paclitaxel at the site of toxicity for Tocosol Paclitaxel was 2.21 ± 0.41 times (average ± SD) as large as for Taxol in our study. Tissues with tight junctions that do not allow nanodroplets to pass are expected to show less toxicity with Tocosol Paclitaxel compared to Taxol. This hypothesis needs to be confirmed in clinical trials. Total drug AUC in whole blood was found to be a clinically useful predictor of neutrophil toxicity, as it explained about half of the variability in the ratio of nadir ANC to baseline ANC.

Acknowledgments We thank Drs Louis Goedhals, Young Lee, and Jan Vermorken for leading the clinical study at their study sites. We thank Drs Wojciech Krzyzanski, Cornelia Landersdorfer, and Sébastien Bihorel for fruitful discussions about this project. This study was supported by Sonus Pharmaceuticals, Inc.

Conflicts of interest statement The work presented in this manuscript was supported by Sonus Pharmaceuticals, Inc.

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