Systems Pharmacological Analysis of Paclitaxel-Mediated Tumor Priming That Enhances Nanocarrier Deposition and Efficacy[§]

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ABSTRACT

Paclitaxel (PAC)-mediated apoptosis decompresses and primes tumors for enhanced deposition of nanoparticulate agents such as pegylated liposomal doxorubicin (DXR). A quantitative pharmacokinetic/pharmacodynamic (PK/PD) approach was developed to analyze efficacy and identify optima for PAC combined with sterically stabilized liposome (SSL)-DXR. Using data extracted from diverse literature sources, Cremophor-paclitaxel (Taxol[®]) PK was described by a carrier-mediated dispositional model and SSL-DXR PK was described by a two-compartment model with first-order drug release. A hybrid-physiologic, well-stirred model with partition coefficients (K_p) captured intratumor concentrations. Apoptotic responses driving tumor priming were modeled using nonlinear, time-dependent transduction functions. The tumor growth model used net first-order growth and death rate constants, and two transit compartments that captured the temporal displacement of tumor exposure versus effect, and

apoptotic signals from each agent were used to drive cytotoxic effects of the combination. The final model captured plasma and intratumor PK data, apoptosis induction profiles, and tumor growth for all treatments/sequences. A feedback loop representing PAC-induced apoptosis effects on K_{p_DXR} enabled the model to capture tumor-priming effects. Simulations to explore time- and sequence-dependent effects of priming indicated that PAC priming increased K_{p_DXR} 3-fold. The intratumor concentrations producing maximal and half-maximal effects were 18 and 7.2 $\mu\text{g/ml}$ for PAC, and 17.6 and 14.3 $\mu\text{g/ml}$ for SSL-DXR. The duration of drug-induced apoptosis was 27.4 h for PAC and 15.8 h for SSL-DXR. Simulations suggested that PAC administered 24 h before peak priming could increase efficacy 2.5-fold over experimentally reported results. The quantitative approach developed in this article is applicable for evaluating tumor-priming strategies using diverse agents.

Introduction

Inadequate delivery of anticancer drugs to solid tumors remains a major challenge in oncology (Jang et al., 2001). The processes controlling tumor drug delivery and, therefore, treatment efficacy include tumor perfusion, extravasation of drug into the tumor tissue, and transport within the interstitium (Jain, 1987, 1989). These processes are influenced by the physicochemical properties of drugs, such as molecular weight, diffusion coefficient, and receptor affinity. Tumor physiologic properties also affect deposition. Tumor stroma and cellular density impede drug diffusion and constrict microvasculature. High tumor interstitial fluid pressure (IFP) opposes diffusion into the tumor, and permeability of

the tumor microvasculature governs both influx and efflux of drug (Jang et al., 2001).

Numerous strategies have been explored to enhance drug deposition and distribution within solid tumors (Jang et al., 2003). These include approaches that use tumor-priming agents that alter tumor vascular perfusion/permeability, interstitial pressure, or stromal properties to enhance deposition and efficacy of subsequently administered agents. Effectors of tumor-priming include cytotoxic drugs (Griffon-Etienne et al., 1999; Lu et al., 2007), physical modulation of the tumor stromal matrix with enzymes such as collagenase or hyaluronidase (Eikenes et al., 2004, 2005; Provenzano et al., 2012), inhibitors of select signaling pathways (Tong et al., 2004; Olive et al., 2009), hyperthermia (Kong et al., 2000; Xu et al., 2007; Choe et al., 2011; Sen et al., 2011), and drug delivery vehicles (Zhou et al., 2002; Arnold et al., 2005; Baker et al., 2008; Cho and Kwon, 2011).

Paclitaxel (PAC) treatment decompresses tumors, increasing vascular perfusion (Griffon-Etienne et al., 1999) and enhancing delivery and efficacy of nanoparticulates such as SSL-DXR

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ABBREVIATIONS: CMDD, carrier-mediated drug disposition; CL_{Cre} , CreEL linear first-order elimination rate; CL_D , intercompartment clearance; CreEL, Cremophor EL; DXR, doxorubicin; E_{max} , maximum effect signal; IFP, interstitial fluid pressure; K_g , first-order net tumor growth rate constant; k_{off} , first-order dissociation rate constant of paclitaxel from Cre-pac; k_{on} , second-order association rate constant for the partitioning of free PAC into CreEL; K_p , partition coefficient; k_{rel} , first-order release rate constant of free DXR from liposomes; MTT, mean transit time; PAC, paclitaxel; PBPK, physiologically based pharmacokinetics; PD, pharmacodynamics; PK, pharmacokinetics; R_{ss} , maximum percentage change from initial tumor volume; SSL, sterically stabilized liposome; τ_d , mean transit time to cell death for cancer cells; TMDD, target-mediated drug disposition; V_{Cre} , CreEL volume of distribution.

(Doxil; Azla, Palo Alto, CA), a sterically stabilized liposome (SSL) formulation containing doxorubicin (DXR; Lu et al., 2007). The mechanism proposed for PAC-mediated tumor-priming suggests that a wave of drug-induced apoptosis reduces tumor cell density, thereby increasing tumor perfusion and reducing IFP. The elevation of tumor permeability and perfusion, and reduction in outward convective fluid flow, result in enhanced tumor deposition and retention of subsequently administered chemo/adjuvant agents such as SSL-DXR or nanoparticulate vehicles carrying small interfering RNA (Millenbaugh et al., 1998; Griffon-Etienne et al., 1999; Lu et al., 2007).

To date, the dose, time, and sequence dependence of priming combination therapies have been established empirically. However, a quantitative, system-based pharmacological model that captures both the pharmacokinetics (PK) and antitumor PD of the combined agents could identify determinants of tumor drug delivery in priming strategies, and simulations based on accurate models could identify strategies for optimizing deposition and efficacy of tumor-priming drug combinations. The primary goal of this work was to develop a PK and PD analysis approach based on available experimental data that would capture data for both PK and therapeutic outcomes of paclitaxel in Cremophor-EL (Cre-pac; Taxol®; Bristol-Myers Squibb, Princeton, NJ) sequenced with a nanoparticulate drug formulation (SSL-DXR). The integrated, mechanism-based mathematical model developed identifies key factors that influence the drug deposition and tumor response observed with this combination (Lu et al., 2007), and enables simulations to explore the optimal temporal sequence for administration of this tumor-priming agent and the subsequent cytotoxic nanoparticulate.

Data were extracted from diverse literature sources to develop PK models for Cre-pac and SSL-DXR, alone and in combination. The PK analysis allowed characterization of plasma and tumor concentration-time relationships for both drugs, including total and free (i.e., released from carrier) concentrations (Gabizon et al., 1989; Xiong et al., 2005; Desai et al., 2006; Lu et al., 2007). The model integrated the PK behavior of the Cremophor EL vehicle (CreEL), which circulates as a microemulsion and, therefore, behaves as a pharmacokinetic “compartment,” to capture Cre-pac data accurately (Sparreboom et al., 1996b). Available PD data included the time course and magnitude of apoptotic responses mediated by Cre-pac and SSL-DXR, both in vivo and in vitro (Milas et al., 1995; Sparreboom et al., 1996a;

Xiong et al., 2005; Desai et al., 2006; Lu et al., 2007; Aroui et al., 2009), as well as the effects of Cre-pac and SSL-DXR on tumor progression as single agents, and in priming and nonpriming sequences (Lu et al., 2007). Simulations with the model yielded nonintuitive relationships between antitumor efficacy and the temporal sequencing of the priming and nanoparticulate agent, and support the hypothesis that efficacy of this tumor-priming strategy may be highly dependent on the interdose interval.

Materials and Methods

Drugs

Data for the tumor-priming sequence of PAC combined with SSL-DXR were extracted from (Lu et al., 2007). The PAC formulation was Cre-pac, which consists of PAC solubilized in a 1:1 (v/v) mixture of polyethoxylated castor oil (CreEL) and ethanol. The SSL-DXR formulation was Doxil, which consists of DXR encapsulated at high concentration and efficiency by remote loading (Mayer et al., 1986; Lasic et al., 1992) into the core of 65- to 80-nm liposomes composed of hydrogenated soy phosphatidylcholine, cholesterol, and polyethylene glycol-bearing phosphatidylethanolamine.

Sources of Preclinical Data

Relevant preclinical data (Table 1) included six studies in which Cre-pac and/or SSL-DXR were administered i.v. to mice at varying doses (Milas et al., 1995; Sparreboom et al., 1996a; Xiong et al., 2005; Desai et al., 2006; Lu et al., 2007) or cells were treated in vitro with free DXR (Aroui et al., 2009). Where necessary, data were extracted by digitization. For all studies, Cre-pac and SSL-DXR concentrations were available only as mean data. The PK of the CreEL carrier was obtained from Sparreboom et al. (1996b), and the dose of CreEL administered in the tumor-priming study of Lu et al. (2007) was calculated from the PAC dose administered and the PAC/CreEL ratio for Cre-pac stated by the manufacturer. A study that reported plasma concentrations of total and SSL-encapsulated DXR after administration of both SSL-DXR and an equivalent dose of unencapsulated (free) DXR (Gabizon et al., 1989) was excluded from analysis during model building and then used as an independent validation data set for the final parameters that were obtained by modeling.

Structural Pharmacokinetic Models

The pharmacokinetic model for Cre-pac is shown within Fig. 1. All corresponding differential equations are reported in Supplemental Data and describe the plasma concentrations for Cre-pac (Supplemental Equations 1 and 2) and free PAC (Supplemental Equations

TABLE 1

Studies from which data were extracted for in silico PK/PD analysis of combination chemotherapy with Cre-pac (Taxol) and SSL-DXR (Doxil)

Drug Formulation	Dose	Available Experimental Data	References ^a
Cre-pac	20 mg/kg	PAC intratumor concentrations (μg/g)	Desai et al. (2006)
Cre-pac	2, 10, 20 mg/kg	Plasma CreEL concentrations (μg/ml)	Sparreboom et al. (1996b)
Cre-pac	40 mg/kg	Total plasma and intratumor SSL-DXR and free plasma PAC concentrations (μg/ml)	Lu et al. (2007) ^b
SSL-DXR	20 mg/kg	Tumor growth from baseline (%) Fraction of apoptosis cells (%)	Lu et al. (2007) ^b
Cre-pac	60 mg/kg	Fraction of apoptotic cells (%)	Milas et al. (1995) ^b
DXR	5 mg/kg	Free DXR concentration (μg/ml)	Xiong et al. (2005)
DXR	0.1, 0.5, 2.5 nM	Fraction of apoptotic cells (%)	Aroui et al. (2009) ^b

CreEL, Cremophor EL; PAC, paclitaxel.

^a A publication from Gabizon et al. (1989) that contains time-course data for total and sterically stabilized liposome (SSL)-encapsulated plasma doxorubicin (DXR), as well as for an equivalent dose of free (unencapsulated) DXR was not used in the analysis but was used as an external data set for validation of the final model.

^b Cell types used for assessment of apoptosis induction by Cre-pac and SSL-DXR were FaDu hypopharyngeal carcinoma cells (Lu et al., 2007), MCA-4 murine breast carcinoma cells (Milas et al., 1995), and MDA-MB231 human breast cancer cells (Aroui et al., 2009).

Fig. 1. Schematic of the PK/PD model for combination chemotherapy with Cre-pac and SSL-DXR in various combinations. Symbols, parameters, and corresponding equations are reported in *Materials and Methods*, Tables 1 and 2, and Supplemental Data. PK was captured using a CMDD model for Cre-pac (green) and a three-compartment model for SSL-DXR (yellow), incorporating linear release of drug from liposomes (Ait-Oudhia et al., 2012). Free plasma concentrations and blood flow (Q) were used to estimate K_p of drugs from plasma to tumor (blue; K_{p_PAC} and K_{p_DXR}). Apoptotic signals (orange for Cre-pac, pink for SSL-DXR) elicited by each agent were driven by their observed intratumor concentrations in a delayed, nonlinear, time-dependent manner. Tumor growth inhibition is modeled with the two transit compartments (gray; R_1 , dividing tumor cells; R_2 , cells committed to death), with a first-order growth rate constant (K_g). The cytotoxic effects of each agent (α and β) determine the magnitude of tumor cell death (τ_d). Red dashed arrow denotes feedback loop capturing the priming effect of PAC-induced apoptosis that alters SSL-DXR deposition (K_{p_DXR}).

3–5). The base model was developed for the analysis of PAC release rates from diverse formulations, and additional details are published (Ait-Oudhia et al., 2012). The model accounts for key characteristics of the CreEL-based PAC formulation, including circulation time of the carrier, rate of drug release or equilibration, and the biodistribution of both the carrier (CreEL) and the released, unbound drug. Because CreEL exists as a microemulsion that can exchange drug with plasma, CreEL in blood behaves as a circulating compartment and affects PAC PK. Therefore, conceptual elements of a target-mediated drug disposition (TMDD) model (Mager and Jusko, 2001) were used to accommodate “carrier-mediated drug disposition” (CMDD) effects on the biodistribution of Cre-pac. The PK of CreEL (Fig. 1) was captured by a one-compartment model (A_{cre} , V_{cre}) having a volume of distribution V_{cre} and a linear first-order elimination rate (CL_{cre}). Partitioning of free PAC into CreEL was described using a second-order association rate constant (k_{on}) and a first-order dissociation rate constant (k_{off}) of PAC from Cre-pac.

A three-compartment model described the PK of released PAC. It includes a central compartment (A_p), having a volume of distribution (V_c), from which free, unbound PAC was cleared linearly, with a clearance CL_{pac} . Two peripheral compartments (A_1 and A_2), having volumes V_1 and V_2 and intercompartmental clearances CL_{D1} and CL_{D2} , serve as distribution sites for free PAC released from the carrier (Bulitta et al., 2009).

A simplified pharmacokinetic model for SSL-DXR is also shown as a component of Fig. 1. The injected dose of SSL-DXR produces the initial concentration of encapsulated drug (A_L) in the central compartment. Drug is released from liposomes according to a first-order release rate constant of free DXR from liposomes, k_{rel} . The PK

model for released drug consists of central (A_{DXR} , V_{DXR}) and peripheral (A_{p_DXR} , V_{p_DXR}) compartments having an intercompartment distribution rate (CL_D) and linear clearance from central compartment (CL_{DXR}). Supplemental Data provides the set of differential equations describing SSL-DXR PK (Supplemental Equations 8–10).

Hybrid Physiologically Based PK Model of Drug Concentrations in Tumor. A hybrid physiologically based PK (PBPK) model (Fig. 1) was developed based on data for tumor-bound and unbound plasma concentrations of PAC and DXR after i.v. administration of Cre-pac and SSL-DXR. Intratumor concentrations of both drugs (C_{T_pac} and C_{T_DXR}) were described using a semiphysiologic, well-stirred model with estimated partition coefficients (K_{p_PAC} and K_{p_DXR}) and tumor blood flow (Q_T):

$$\frac{dC_{T-d}}{dt} = \frac{Q_T}{V_T} \cdot \left(CU_d - \frac{C_{T-d}}{K_{p-d}} \right) \quad (1)$$

where the subscript d represents the individual drug (PAC or DXR), CU is the concentration of unbound drug, V_T is the tumor volume, calculated from a growth function $g(R) = \frac{V_0 - V_T}{V_0} \cdot 100$, where V_0 is the baseline tumor volume of 600 mm³ (Lu et al., 2007), and $g(R)$ is the percentage change in tumor volume with time (eq. 5).

PD Model for Apoptosis and Tumor Growth

PK profiles of the drugs in tumor were used to drive tumor cell apoptotic responses, which were modeled using nonlinear, time-dependent signal transduction functions consisting of parameters

$E_{\max}$ (the maximum effect signal), and EC_{50} (concentration of free drug mediating half-maximal effect) for each drug (d):

$$\frac{dAPO_{d-1}}{dt} = \frac{1}{\tau_{APO-d}} \cdot \left(\frac{E_{\max-d} \cdot C_{T-d}^{\gamma}}{EC_{50-d}^{\gamma} + C_{T-d}^{\gamma}} - APO_{d-1} \right) \quad (2)$$

$$\frac{dAPO_{d-2}}{dt} = \frac{1}{\tau_{APO-d}} \cdot (APO_{d-1} - APO_{d-2}) \quad (3)$$

$$\frac{dAPO_{d-3}}{dt} = \frac{1}{\tau_{APO-d}} \cdot (APO_{d-2} - APO_{d-3}) \quad (4)$$

The tumor growth model (Fig. 1) used two transit compartments to accommodate the delay between drug exposure and apoptotic effects, and first-order rate constants described net tumor cell growth and death rates. The apoptotic signal arising from the cytotoxicity of each individual agent was integrated into the model via linear interaction functions having concentration-dependent slopes for PAC and DXR:

$$\frac{dR_1}{dt} = g(R) - \left(\frac{1}{\tau_d} \right) \cdot (1 + \alpha \cdot APO_{pac-3}) \cdot (1 + \beta \cdot APO_{DXR-3}) \cdot R_1 \quad (5)$$

$$\frac{dR_2}{dt} = \left(\frac{1}{\tau_d} \right) \cdot (R_1 - R_2) \quad (6)$$

where $g(R) = K_g \cdot R_1 \cdot (1 - \frac{R_1}{R_{ss}})$ is the logistic growth function of cycling cells (R_1), K_g is the first-order net tumor growth rate constant mediated by cycling cells, R_{ss} is the maximum percentage change from initial tumor volume in untreated animals, R_2 represents the compartment of cells committed to death, τ_d is the mean transit time (MTT) to cell death for cancer cells, α and β represent the slopes of the concentration-dependent PAC and DXR effects, and APO_{pac1-3} and APO_{DXR1-3} are the transit compartments that account for the nonlinear time dependence of PAC and DXR apoptotic effects. The total tumor volume is the sum of the two transit compartments $V_T = R_1 + R_2$ (Fig. 1).

PAC-induced priming was modeled as a time-dependent feedback loop, such that the apoptosis signal of PAC enhanced the tumor deposition of SSL-DXR (i.e., increased K_{p_DXR}). The feedback loop was described with the following equation:

$$K_{p_DXR}(t) = K_{p_DXR} \cdot (1 + \theta \cdot APO_{3-pac}) \quad (7)$$

where θ is a proportionality coefficient. An analogous feedback loop, in which SSL-DXR affected Cre-pac deposition, was not required to fit the experimental data.

Data Analysis

Models describing the PK data of both drugs in plasma and tumor, and the PD data for tumor volume progression and apoptosis, were implemented in MATLAB R2011a (Mathworks Inc., Natick, MA). Parameter estimates were obtained after minimization of a likelihood-derived function using the Nelder-Mead Simplex algorithm as implemented in the *fminsearch* function. Ordinary differential equations were solved using the *ode45* solver. Residual variability in the PK- and PD-dependent variables was modeled with both proportional (eq. 8), and additive and proportional (eq. 9) models:

$$V_{i,j} = \sigma_1^2 \cdot Y_{i,j}^2 \quad (8)$$

$$V_{i,j} = \sigma_1^2 \cdot Y_{i,j}^2 + \sigma_2^2 \quad (9)$$

where V_i and $V_{i,j}$ are the variance of the residual for the i^{th} observation data point and the variance of the residual for the i^{th} data point of j^{th} PD response, Y_i is the prediction for the i^{th} observation data point, and σ_1 and σ_2 are the variance parameters.

Model Simulations

Simulations were conducted with the PK and PD models to explore model and system sensitivity to various parameters and conditions, including alternative priming sequences and their effect on antitumor efficacy. Dosing schemes simulated included SSL-DXR administered 0, 6, 12, 18, 24, 28, 36, 48, 72, and 96 h after PAC administration. End points simulated included the effect of interdose interval on tumor drug exposure, progression, and tumor volume nadir after treatment.

Results

PBPK models for Cre-pac and SSL-DXR

Hybrid PBPK models (Fig. 1) were developed and evaluated to describe Cre-pac and SSL-DXR temporal profiles in mouse plasma and tumors. The best-fitting model for SSL-DXR consisted of a two-compartment systemic model. For Cre-pac, the superior model incorporated a CMDD component, conceptually analogous to TMDD models (Mager and Jusko, 2001), in which the affinity of PAC for a circulating CreEL microemulsion compartment of continuously changing amount affects PAC biodisposition (Ait-Oudhia et al., 2012). Unbound plasma drug concentrations were linked to a single blood flow-limited tumor compartment. The predicted plasma and tumor profiles for Cre-pac (Fig. 2, A and C) and SSL-DXR (Fig. 2, B and D) concentrations agreed well with observed values (Danesi et al., 2002; Desai et al., 2006; Lu et al., 2007), and the fitted parameters (Table 2) were estimated reliably with low coefficients of variation.

To reduce the number of fitted parameters, we obtained values for fb_{DXR} , B_p , B_{sat} , and k_d from published reports (Danesi et al., 2002; Bulitta et al., 2009) and fixed them in the final model. The remaining parameters were estimated by simultaneous fitting of available data for drug concentrations in plasma and tumor in parallel with PD analysis of tumor volume and the time course of apoptosis for five treatment groups that were compared in Lu et al. (2007): no drug, both drugs alone, Cre-pac before SSL-DXR, and SSL-DXR before Cre-pac. The final parameters are summarized in Table 2. The estimated values of CL_{pac} , V_{pac} , CL_{cre} , and V_{cre} are comparable with those from noncompartmental analysis of Cre-pac PK (Sparreboom et al., 1996b). Furthermore, the parameters CL_{DXR} and V_{DXR} obtained in this study are similar to reported values (Xiong et al., 2005).

Drug release from the Cre-pac microemulsion or from SSL-DXR nanoparticles produces biologically active free drug. The half-life of release was calculated using the formula $t_{1/2} = \frac{0.693}{k_i}$, where $k_i = k_{off}$ for Cre-pac was estimated for 10 min after administration, because of its comparatively rapid release rate, and $k_i = k_{rel}$ for SSL-DXR was estimated for 15 h after dosing. Few experimentally determined estimates of PAC release rates have been published for Cre-pac. By simultaneous analysis of published clinical data for Cre-pac and CreEL, a $t_{1/2}$ of 8 min was estimated for PAC release from Cre-pac (Ait-Oudhia et al., 2012). Data for CreEL-bound and free (unbound) PAC concentrations in plasma enabled the estimation of a PAC binding constant for the circulating CreEL microemulsion ($5.15 \text{ h} \times \mu\text{g} \times \text{ml}^{-1}$). The final model captured CreEL data well (Fig. 2E). The estimated $t_{1/2}$ for DXR release from SSL-DXR was 15 h, which is consistent with several studies of drug release rates in vitro and in vivo for

Fig. 2. Pharmacokinetics of Cre-pac, CreEL, and SSL-DXR in plasma and tumor. In all panels, symbols represent observed data (Lu et al., 2007), except where noted. Lines represent model predictions. (A) PAC plasma concentrations. (B) total (i.e., free + bound) plasma concentrations of DXR after SSL-DXR administration from data of Danesi et al. (2002) and Desai et al. (2006). (C) PAC tumor concentrations (Lu et al., 2007) after Cre-pac administration. (D) Total tumor DXR concentrations after administration of SSL-DXR alone ($\circ$) or SSL-DXR administered 48 h after dosing with Cre-pac ($\bullet$). (E) CreEL plasma concentrations from data of Sparreboom et al. (1996b), in which varying doses of Cre-pac were administered: green squares (2 mg/kg), blue circles (10 mg/kg), and red triangles (20 mg/kg). (F) total plasma concentrations of DXR after administration of 5 mg/kg unencapsulated (free) drug. Data from Xiong et al. (2005).

similar liposome formulations (Charrois and Allen, 2004; Xiong et al., 2005). Good agreement between predicted and observed DXR concentrations after administration of free DXR (Fig. 2F) adds confidence to the estimated release rate constant. Model-simulated SSL-DXR concentration-time profiles for total and encapsulated drug were also in excellent agreement with an external data set (Supplemental Fig. 1) that reported plasma concentration-time data for total and SSL-encapsulated drug after i.v. administration of a structurally similar SSL-DXR formulation (Gabizon et al., 1989), thus contributing validity to the final SSL-DXR PK model.

Tumor blood flow was estimated as 3.38 ml/h, consistent with estimates of drug flow in tumor-bearing mice that range from 4.5 (Wang et al., 2009) to 6 ml/h (Baxter et al., 1994). The tumor:plasma K_p estimated from experimental data for Cre-pac and SSL-DXR were low (<1), signifying low tumor deposition when administered as single agents. Overall, the PK model predicts greater deposition of DXR than PAC for the

two formulations, as shown by comparison of the K_p values ($K_{p_DXR} = 0.085$ vs. $K_{p_PAC} = 0.044$).

Simultaneous modeling of the entire PK and PD data sets also enables model prediction of the experimentally observed increase in drug tumor deposition of the tumor-priming combination (Lu et al., 2007). By incorporating a PAC-mediated feedback loop that modulates SSL-DXR deposition (Fig. 1), the model correctly predicts the observed increase in tumor deposition of SSL-DXR with the priming regimen (Cre-pac followed by SSL-DXR; Fig. 2D). This enhanced drug deposition is consistent with the greater tumor cell-killing effect observed experimentally with PAC tumor priming sequences and estimated by PD analysis of tumor responses (Fig. 3C). Although experimental data for PAC deposition are not available, an analogous feedback loop, by which SSL-DXR pretreatment would modulate PAC tumor concentrations, was not required to predict the efficacy observed for the reverse, nonpriming sequence (SSL-DXR followed by Cre-pac).

TABLE 2

Pharmacokinetic and pharmacodynamic parameters and coefficients of variations

The mean residence time (MRT) for sterically stabilized liposome doxorubicin (SSL-DXR), calculated as $MRT = V_{ss_DXR}/CL_{DXR}$ with $V_{ss_DXR} = V_{DRX} + V_{p_DXR}$, was ~42 h.

Parameter	Definition	Estimate
		% CV
Pharmacokinetics		
CL_{pac} (10^{-1} l/h)	PAC linear clearance	0.85 (24.9)
CL_1 (l/h)	PAC clearance for peripheral compartment 1	0.65 (21.8)
CL_2 (l/h)	PAC clearance for peripheral compartment 2	0.24 (31.4)
V_{pac} (l)	PAC central volume of distribution	0.82 (5.15)
V_{p1-pac} (l)	PAC volume for peripheral compartment 1	1.21 (27.6)
V_{p2-pac} (l)	PAC volume for peripheral compartment 2	1.5 (17.4)
CL_{cre} (10^{-2} l/h)	Cremophor EL clearance	0.92 (6)
V_{cre} (10^{-2} l)	Cremophor EL volume of distribution	0.53 (2.41)
k_{rel} (h^{-1})	Release rate constant for SSL-DXR	0.046 (2.8)
CL_{DXR} (10^{-1} l/h)	SSL-DXR linear clearance	0.42 (11.2)
k_{on} ($h \mu g/ml$) $^{-1}$	Second-order binding affinity constant for PAC	5.15 (25.3)
k_{off} (h^{-1})	First-order dissociation constant for PAC	4.09 (141)
CL_D (l/h)	SSL-DXR clearance for peripheral compartment	0.85 (39.8)
V_{DXR} (l)	SSL-DXR central volume of distribution	0.7 (39.8)
V_{p_DXR} (l)	SSL-DXR volume for peripheral compartment	1.06 (39.8)
Q_T (ml/h)	Tumor blood flow	3.38 (7.36)
K_{p_PAC}	Partition coefficient for PAC	0.044 (24.8)
K_{p_DXR}	Partition coefficient for SSL-DXR	0.085 (7.6)
B_p	Linear binding of PAC to plasma proteins	21 ^a
B_{sat}	Saturable binding of PAC to plasma proteins	35 ^a
k_d ($\mu g/ml$)	Dissociation constant from plasma proteins	4.38 ^a
fb_{DXR} (%)	Fraction of DXR bound to plasma proteins	85 ^b
Pharmacodynamics		
E_{max_PAC} (% apoptosis)	Maximum effect for PAC apoptotic signal	18.04 (10.01)
EC_{50_PAC} ($\mu g/ml$)	Cre-pac concentration inducing 50% of E_{max_pac}	7.217 (12.7)
γ	Hill coefficient for Cre-pac	2.22 (45.7)
E_{max_DXR} (% apoptosis)	Maximum effect for SSL-DXR apoptotic signal	70.59 (3.08)
EC_{50_DXR} (nM)	SSL-DXR concentration inducing 50% of E_{max_DXR}	14.34 (5.24)
$\tau_{Apo-PAC}$ (h)	Single-compartment mean transit time for PAC	9.14 (42.6)
$\tau_{Apo-DXR}$ (h)	Single-compartment mean transit time for SSL-DXR	5.273 (4.45)
K_g (10^{-2} , h^{-1})	Net growth rate constant	0.565 (2.55)
R_{ss} (% change)	Maximum unperturbed % change in tumor size	1857 (7.3)
τ_d (h)	Time to death	22.59 (13.7)
α (1/% apoptosis)	Slope of PAC concentration dependence of apoptosis induction	4.31 (15.2)
β (1/% apoptosis)	Slope of SSL-DXR concentration dependence of apoptosis induction	0.93 (21.5)
θ (1/% apoptosis)	Coefficient to K_{p_DXR} after tumor priming	0.27 (38.4)

CV, coefficient of variance; PAC, paclitaxel.

^a B_p , B_{sat} , and k_d parameters were fixed from Bulitta et al. (2009).^b fb_{DXR} parameter was fixed from Danesi et al. (2002).

PD Models for Apoptosis Induced by Cre-pac and SSL-DXR

The time course of DXR- and PAC-induced apoptosis was estimated from available ex vivo and in vitro data (Milas et al., 1995; Lu et al., 2007; Aroui et al., 2009). In the final model, drug effects were expressed as the fraction of apoptotic cells as a function of time after administration. A delay between drug exposure and onset of apoptosis was observed for both drugs (Fig. 3, A and B), consistent with the temporal cascade of events that ensue in cells (Au et al., 1999). Therefore, a nonlinear, time-dependent signal transduction model for apoptosis was used (Mager and Jusko, 2001) that included three transit compartments. The overall MTT (MTT_{Apo}) for propagation of the apoptotic signal, which describes the delay between drug exposure and apoptosis, was calculated as $MTT_{Apo} = N \cdot \tau_{Apo_d}$, where N is the number of transit compartments and τ_{Apo_d} represents the mean time of signal transduction between compartments. The estimated MTT_{Apo} for Cre-pac was nearly 2-fold greater than for SSL-DXR (27.4 versus 15.8 h). After propagation through the transduction compartments, the drugs produce in compartment APO_{DXR-3} or APO_{PAC-3} (Fig. 1) the apoptotic signal that determines the time course and magnitude of apoptosis

produced in the tumor. In the case of PAC-mediated priming sequences, the signal from APO_{PAC-3} feeds back to influence deposition of SSL-DXR (Fig. 1).

Notably, it was necessary to develop the model using data from different tumor cell types and tumor systems. Despite differences that may be intrinsic to each, the final model captured well the data for the time course and magnitude of apoptosis mediated by Cre-pac (Fig. 3A) (Milas et al., 1995; Lu et al., 2007) and SSL-DXR (Fig. 3B) (Aroui et al., 2009).

Models for Xenograft Tumor Growth and Therapeutic Effects of Cre-pac and SSL-DXR

A tumor growth model was developed to characterize the dynamic tumor therapeutic effects. The available data reported changes in tumor volume relative to the initial volume at the start of treatment (Lu et al., 2007). In the tumor growth model (Fig. 1), the drug signal mediating cytotoxicity was assumed to reflect the kinetic characteristics and magnitude of the apoptotic response observed for each individual drug. For simplicity, it was assumed that K_g and maximum percentage change in tumor size R_{ss} were constant across all treatment groups, and that the mechanism of killing for both drugs was transmitted through the apoptotic

Fig. 3. Temporal pharmacological effects of Cre-pac and SSL-DXR. Symbols represent observed data; lines represent model predictions. (A) percentage apoptotic cells in tumor after in vivo Cre-pac treatment. Filled circles/solid line represent data from Lu et al. (2007) using FaDu hypopharyngeal carcinoma cells; open circles/dashed line represent data from Milas et al. (1995) using MCA-4 murine breast carcinoma. (B) percentage apoptotic MDA-MB231 human carcinoma cells as a function of DXR concentration after treatment in vitro (Aroui et al., 2009). DXR concentrations: circles/solid line, 0.1 μM ; diamonds/dashed line, 0.5 μM ; triangles/dotted line, 2.5 μM . (C) treatment-mediated effects of Cre-pac and SSL-DXR on FaDu tumor volume. Symbols represent data from Lu et al. (2007), expressed as percentage change from the pretreatment tumor volume baseline for the drugs alone and in combination. Lines represent fitting of the model of Fig. 1 to the data. Blue hexagons, saline-treated controls; red triangles, Cre-pac alone administered at 40 mg/kg; green diamonds, SSL-DXR administered at 20 mg/kg; purple circles, SSL-DXR followed 48 h later with Cre-pac (non-priming sequence); olive squares Cre-pac followed 48 h later with SSL-DXR (priming sequence). (D) model-based simulation of apoptotic signals in tumor elicited by Cre-pac or SSL-DXR. Dashed lines represent relative magnitude of apoptotic signal for Cre-pac (red) and SSL-DXR (blue) in the final signal transduction compartment, $\text{APO}_{\text{PAC-3}}$ or $\text{APO}_{\text{DXR-3}}$. Solid lines represent kinetics and magnitude of apoptotic signal emanating from transduction compartments $\text{APO}_{\text{PAC-3}}$ (red) and $\text{APO}_{\text{DXR-3}}$ (blue) for the doses used in (C), calculated as $(\alpha \times \text{APO}_{\text{PAC-3}})$ for Cre-PAC and $(\beta \times \text{APO}_{\text{DXR-3}})$ for SSL-DXR. Parameters α and β listed in Table 2.

signaling cascade. All data (control and treatment groups) were modeled simultaneously, and excellent fittings were obtained (Fig. 3C). The final analysis recapitulated with remarkable fidelity the greater efficacy of the priming sequence (Cre-pac followed by SSL-DXR) compared with the reverse, nonpriming sequence. The enhanced cell-killing effect in the PAC-primed treatment group, captured by introducing a feedback loop into the model by which Cre-pac-mediated apoptosis increased SSL-DXR tumor deposition, is quantitatively consistent with the tumor priming mechanisms hypothesized by Lu et al. (2007).

In the final model, the variables estimated include the slopes of the concentration dependence of Cre-pac and SSL-DXR cytotoxic effects as single agents (α and β), the τ_d , and the proportionality coefficient by which Cre-pac priming increased K_{p_DXR} (θ). Table 2 provides final estimates of the model variables, and all parameters were determined with good precision. K_g for the tumor was 0.0057 h^{-1} , θ was 0.27 (units = 1/% of baseline-apoptosis), and τ_d was 24 h. The model predicted tumor growth accurately for each treatment arm (Fig. 3C), and as reported in the experimental data, the model-predicted tumor volume decrease was greater for the priming sequence (Cre-pac followed by SSL-DXR) than for the reverse, nonpriming sequence or the drugs as the single agents.

Simulations and investigation of model sensitivity to specific parameters yielded insight into the interaction of PAC and DXR in priming and nonpriming sequences. According to the model, K_{p_DXR} , the relative fraction of DXR undergoing tumor deposition, was 2-fold greater than K_{p_PAC} (Table 2). Furthermore, $E_{\text{max-DXR}}$ (maximum apoptotic signal for SSL-DXR in compartment $\text{APO}_{\text{DXR-3}}$) was 4-fold greater than $E_{\text{max-PAC}}$ (Fig. 3D). Conversely, α (slope of Cre-pac concentration dependence of signaling) was 4-fold greater

than β (slope of SSL-DXR concentration dependence of signaling), consistent with the greater magnitude of PAC-induced apoptosis reported experimentally (Lu et al., 2007). The net result of these interacting influences is the model output for the magnitude and time course of the apoptotic signal, which is the product $(\beta \times \text{APO}_{\text{DXR-3}})$ for SSL-DXR or $(\alpha \times \text{APO}_{\text{PAC-3}})$ for Cre-PAC. Figure 3D shows that the apoptotic signal from Cre-pac priming exceeds that of the reverse sequence.

Optimal Interdose Interval for Tumor Priming by PAC

With simultaneous fitting of all data for all treatment groups, the final hybrid-PBPK/PD model captured observed data well for tumor responses (Fig. 3C). Therefore, simulations were performed to investigate modifications of the priming regimen that might enhance tumor response. The model parameter identified as the most important determinant of efficacy was the interdose interval between Cre-pac priming and SSL-DXR administration. Figure 4A shows simulated time courses of tumor volume response to identical doses of Cre-pac with different interdose intervals for administration of SSL-DXR. The observed data (Lu et al., 2007), which used a 48-h interdose interval, are overlaid on the results. Simulations predict that an interdose interval of 24 h would increase tumor exposure (area under the curve; AUC) of SSL-DXR (Fig. 4B) and time to tumor progression (Fig. 4C) by 2.5-fold, and decrease the residual tumor volume at nadir to half the volume observed experimentally with a 48-h interdose interval (Fig. 4D). Simulations also suggest that the kinetics by which SSL-DXR exposure in tumor (Fig. 4B) and antitumor efficacy (Fig. 4C) increase in the first 24 h after Cre-pac dosing may be abrupt, and the enhancement resulting from priming may subside rapidly over the subsequent 24 h.

Fig. 4. Model-based simulations of the effect of interdose interval on the efficacy of Cre-pac/SSL-DXR tumor priming sequence based on data for FaDu tumor model of Lu et al. (2007). (A) predicted treatment-mediated change in tumor volume, expressed as percentage change from initial tumor volume mediated by 40 mg/kg Cre-pac and 20 mg/kg SSL-DXR administered simultaneously (dark blue line), or with Cre-pac followed by SSL-DXR after 6 (dark green line), 12 (blue line), 18 (gray line), 24 (burgundy line), 28 (olive line), 36 (light blue line), 48 (dashed mauve line), 72 (green line), and 96 h (red line). Symbols represent experimental data of Lu et al. (2007) in which SSL-DXR was administered 48 h after Cre-pac; dashed line represents model fit to experimental data; horizontal line from ordinate represents one doubling of tumor volume from the baseline at initiation of treatment. (B) drug exposure ($AUC_{0-\infty}$) as a function of interdose interval for Cre-pac and SSL-DXR; symbols represent model predictions at discrete time points; lines connect symbols. Vertical dashed red line represents interdose interval (48 h) used experimentally by Lu et al. (2007). (C) time to tumor progression to twice the baseline volume as a function of interdose interval for Cre-pac and SSL-DXR; symbols and lines same as in (B). (D) maximum tumor cell-killing response (i.e., nadir of tumor volume) as a function of interdose interval for Cre-pac and SSL-DXR; symbols and lines are the same as in (B).

Discussion

Combination chemotherapy is standard practice in clinical oncology, but identification of optimal therapeutic combinations poses numerous challenges, including the selection of agents that may exert complementary mechanisms of action, and identification of the sequence and dose ranges of the agents. A variety of pharmacological approaches for modulating barriers to tumor drug delivery have emerged (Griffon-Etienne et al., 1999; Eikenes et al., 2005; Lu et al., 2007; Choe et al., 2011; Sen et al., 2011; Provenzano et al., 2012), and these suggest mechanistically rational candidates for combination therapy with cytotoxic agents. PAC has been reported to compromise barriers to drug delivery via tumor decompression and relief of interstitial tumor pressure (Griffon-Etienne et al., 1999). A preclinical study investigating PAC-mediated tumor priming (Lu et al., 2007) demonstrated enhanced efficacy with

an appropriately timed sequence of Cre-pac followed by Doxil. The mechanism of tumor priming was ascribed to changes in tumor permeability and intratumor diffusion mediated by a wave of PAC-mediated apoptosis occurring within a 24- to 72-h time window after Cre-pac administration (Au et al., 1999; Jang et al., 2001; Lu et al., 2007). Compromise of tumor barrier properties enhanced the deposition and pharmacological activity of Doxil within a defined temporal window, but the reverse sequence was less efficacious.

Combination therapies often are determined empirically. We sought to develop a quantitative framework that includes pharmacokinetic analysis of drug deposition and PD analysis of antitumor efficacy to evaluate tumor-priming approaches combined with nanoparticle delivery strategies. A PBPK/PD model was developed to capture underlying mechanisms of drug interaction in a quantitative and semimechanistic

fashion. A second objective was to use this model-based framework to explore by simulation the key factors that might control efficacy of a tumor-priming strategy.

Development of linked hybrid PBPK/PD models for analyzing tumor priming required data beyond those reported in the original study (Lu et al., 2007). Essential data included the PK of both drugs, and either direct or ancillary data that would permit estimation of unbound drug concentrations in plasma and tumor. Required PD data included the magnitude and time course of drug-mediated cell death via apoptosis, and the dynamics of cell death and tumor growth after diverse treatment sequences of the drugs alone and in combination (Milas et al., 1995; Sparreboom et al., 1996b; Xiong et al., 2005; Desai et al., 2006; Lu et al., 2007; Aroui et al., 2009).

The final model (Fig. 1) links drug exposure in plasma and tumor with pharmacological effects such as the time course and magnitude of apoptotic responses, as well as treatment effects on tumor volume progression. It captures experimental data for the two drugs individually and in combination. Several unique features were incorporated into the final model. One is the inclusion of a CMDD component to describe the PK of Cre-pac, which is a conceptual adaptation of target-mediated dispositional effects (Mager and Jusko, 2001). CMDD and TMDD phenomena share several characteristics; the equilibrium association/dissociation (k_{off} and k_{on}) of PAC with CreEL was modeled in a manner analogous to ligand-receptor binding, and captured the effect of the dynamically changing “receptor” abundance (CreEL concentration) on PAC PK. Other novel components include the nonlinear signal transduction model describing the apoptosis-promoting activity of each agent, driven by intratumor drug exposures, as well as the model for tumor growth, in which a signal transduction cascade of two transit compartments links drug concentrations with the temporally delayed apoptotic responses. Finally, a feedback loop was developed to describe Cre-pac priming as a temporal apoptosis-driven phenomenon modulating tumor deposition of nanoparticulate SSL-DXR.

Several key assumptions were made to reduce the complexity of the system. One was that apoptosis is responsible for tumor cell death (Danesi et al., 2002; Bulitta et al., 2009). Although apoptosis is not the sole mechanism of cell killing by PAC and DXR, it plays a predominating role, as has been demonstrated experimentally (Lu et al., 2007; Aroui et al., 2009).

The minimal PD model developed to describe treatment effects on tumor progression fitted all observed tumor response profiles reliably for all treatment groups, including responses to the drugs as single agents, as well as to the forward (priming) and reverse (nonpriming) sequence combinations. Several tumor progression/drug effect models were compared during model development, including a cell distribution model (Simeoni et al., 2004; Magni et al., 2006) and a signal distribution model (Lobo and Balthasar, 2002); both failed to capture the sequential drug combination data in their published form. For the tumor progression model implemented in this study, two transit compartments were sufficient to characterize adequately the delay between dosing and effect. The final model is mechanistically realistic, in that the dynamics of apoptotic responses resulting from treatment with Cre-pac and SSL-DXR alone were sufficient as drivers for the observed therapeutic effects of the combinations.

The final hybrid-PBPK/PD model captured observed data for tumor responses to the single and combined agents well, which supported the feasibility of exploratory simulations to explore model sensitivity, as well as strategies to optimize therapeutic outcome. The interdose interval between the priming agent and therapeutic agent was identified as the most influential factor in determining efficacy as predicted by the model. The interdose interval selected experimentally (Lu et al., 2007) timed administration of SSL-DXR to coincide with the peak time of tumor vascular permeability (48 h). Simulations in this study suggest that the optimal interdose interval may be ~24 h after Cre-pac priming. A possible explanation as to why administration of the nanoparticle agent before the development of peak tumor priming may mediate greater efficacy is that a greater fraction of the tumor AUC of SSL-DXR, which has a long mean residence time (42 h; Table 2), may be centered temporally over the tumor-priming window, which develops and peaks over a 24- to 72 h interval after PAC administration.

It is interesting to note that SSL-DXR also exerts tumor-priming effects and enhancement of its own tumor deposition (Arnold et al., 2005). However, the temporal relationships between SSL-DXR dosing and tumor priming differ significantly from those of Cre-pac (Chaudhuri et al., in press): in an intracranial brain tumor model, SSL-DXR treatment produced a transient decrease in tumor perfusion, permeability, and nanoparticle deposition in a 3- to 4-day window after dosing, followed by a gradual increase in tumor vascular permeability and nanoparticle deposition that peaked 7–10 days after dosing (Arnold et al., 2005). Thus, the interdose interval used in priming combinations must account for the temporal PD of the priming agent.

In conclusion, an integrated, hybrid-physiologic PBPK/PD model was developed that captured pharmacokinetic and PD data for a tumor-priming agent in sequence with a nanoparticulate drug delivery vehicle. The final model is based on the mechanisms of action and interaction of the two agents used. Using the available data, the model integrates quantitatively the plasma PK for single agents, the effects of intratumor drug exposure on cellular responses such as apoptosis, and the ultimate effect of apoptosis on tumor growth when drugs were administered individually, combined in a priming-inducing sequence, or combined in the reverse, nonpriming sequence. Simulations yielded unanticipated predictions regarding the optimal interdose interval. Given the additional 2.5-fold increase in SSL-DXR deposition and therapeutic efficacy that simulations predict for alternative interdose intervals, the simulations yielded intriguing testable hypotheses for experimental investigation. An important characteristic of the model is its ability to capture and integrate the tumor priming effect of Cre-pac into combination therapy, which was achieved by incorporation of a drug interaction feedback loop, such that the tumor apoptotic response elicited by Cre-pac drove enhanced deposition of SSL-DXR in the tumor. Although further experiments are needed to test these predictions of the model, this PBPK/PD analysis approach has considerable potential for assessing the role of a broader range of tumor-priming treatments.

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Authorship Contributions

Participated in research design: Ait-Oudhia, Straubinger, Mager.

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