Computational Modeling to Predict Effect of Treatment Schedule on Drug Delivery to Prostate in Humans

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Abstract  Purpose: To evaluate a computational approach that incorporates experimental data in preclinical models to depict doxorubicin human tissue pharmacokinetics.

Experimental Design: Beagle dogs were given 2 mg/kg doxorubicin as i.v. bolus, 4-h infusion, or 96-h infusion. Concentrations in plasma, prostate (target tissue), heart (toxicity), and major tissues for disposition were determined and modeled. Model parameters were obtained after the bolus injection with model validation based on the 4-h and 96-h infusion data. Clinical pharmacokinetic data and scale-up gave doxorubicin profiles in human prostate and heart.

Results: In agreement with in vitro results, tissues were best modeled with two compartments, one rapidly and one slowly equilibrating. The developed tissue distribution model predicted concentrations for all three administration regimens well, with an average deviation of 34% (median, 29%). Interspecies scale-up to humans showed that the change from a bolus injection to a slow, 96-h infusion (a) had different effects on the drug partition and accumulation in heart and prostate, and (b) lowered the peak concentration in the plasma by ~100-fold but had relatively little effect on maximal heart concentration (~33% lower). The simulated drug exposure in a human prostate was above the exposure required to inhibit tumor proliferation but was 30 to 50 times below that needed for cell death.

Conclusion: The present study shows a computation-based paradigm for translating in vitro and in vivo preclinical data and to estimate and compare the drug delivery and pharmacokinetics in target tissues after different treatment schedules.

Adequate delivery of chemotherapeutic agents to intended targets is a prerequisite for antitumor activity. The identification of effective treatment schedules to attain the desired pharmacodynamics further requires knowledge of the drug concentration-time profile at the target site. Target site pharmacokinetic data in humans are not readily available due, in large part, to the inability to obtain appropriate tissues. Hence, there is a need to develop an alternative approach to obtain these data.

The goal of the present study was to evaluate a computational approach that incorporates experimental preclinical tissue pharmacokinetic data and clinical plasma pharmacokinetic data to depict target site pharmacokinetics in humans. We selected prostate as the target organ for the following reasons. First, prostate cancer is the most common cancer and the second most common cause of cancer-related death in American men (1). A better understanding of the kinetics of drug delivery to the prostate may help future therapy development. Second, the prostate presents as an isolated organ and can be readily accessed for pharmacokinetic analysis. Third, the important physiologic parameters necessary for interspecies scaling from preclinical models to humans are well established in the literature.

The present study describes the establishment of a physiologically based tissue pharmacokinetic (PBPK) model using doxorubicin as the model drug. We selected dogs as the animal model because of the following physiologic and pathologic similarities to humans. Spontaneous development of benign prostatic hypertrophy and prostatic neoplasm is found only in humans and dogs. Similar to human prostates, dog prostates undergo age-related structural changes (2). The shape, size, and architecture of human and dog prostates are similar; both show significant differences between dog and human prostates are size and blood flow rate. Depending on the breed, the prostate of a dog can be 0.5 to 1.5 times the size of human prostate and has a higher blood perfusion (31-79 versus 15.7 ml/min/100 g in humans; refs. 4, 5).

PBPK modeling has been used since the 1970s to depict or predict the concentration-time profiles of drugs and metabolites in tissues (6–10). A PBPK model uses the anatomically correct tissue arrangement, physiologically relevant tissue volumes and tissue perfusion rates, tissue binding, and metabolism to describe the relationship between drug concentrations...
in plasma and tissues. In contrast, the commonly used compartmental pharmacokinetic models, in which the plasma and rapidly perfused tissues are lumped into a central compartment and the slowly equilibrating tissues are lumped into one or more peripheral compartments, lack anatomic or physiologic relevance and are unable to distinguish the fate or predict the drug disposition in different tissues. After a PBPK model is established for a drug in one mammalian species, it can be used to predict the data in another species by substituting the model (physiologic and pharmacokinetic) parameters for one species with those for another (e.g., humans). It is noted that whereas PBPK models for doxorubicin had been established in rodents and rabbits, these earlier studies did not include the prostate and therefore cannot be used to predict the tissue concentration in human prostate. Furthermore, the previous PBPK models depicted each tissue as a single homogeneous compartment. In contrast, the present model accounted for the biphasic drug uptake/transfer processes in tissues. This modification significantly improved the model performance such that it could be used to predict the tissue profiles after diverse treatment schedules (ranging from a rapid bolus injection to a slow, 96-h infusion).

We describe here a hybrid PBPK model to predict the doxorubicin concentration in prostate and other tissues after rapid (i.e., bolus) and slow (i.e., infusion over 4 or 96 h) i.v. administrations. After validation using experimental data, the model was then used for interspecies scale-up to depict the drug concentration-time profiles in human prostate tissues after rapid and slow i.v. injections. These simulated data were compared with our previously reported in vitro pharmacodynamic data in human prostate tumors to infer the effects of altering treatment schedules on antitumor activity.

### Materials and Methods

**PBPK model development and validation: overview.** The four steps for establishing the PBPK model were to (a) include in the model tissues or organs that are of pharmacologic interest; (b) define the process of drug distribution from blood to organs (i.e., flow rate limited or membrane limited); (c) establish the mass balance equations for each organ and obtain the required physiologic and pharmacokinetic parameters either experimentally or by calculation using previously published data; and (d) substitute the model parameters into the differential mass balance equations for plasma and tissues to generate computer simulations of plasma and tissue concentration-time profiles. Model validation was accomplished by evaluating model performance after administration of dosing regimens different from those used in the model development studies.

**Model selection.** Two PBPK models (i.e., global and hybrid models) were considered. The global model requires accounting for the mass balance and therefore knowledge of drug disposition in all organs. The hybrid model requires only knowledge of drug disposition in specific organs, which is less demanding experimentally. Furthermore, establishing a global model for doxorubicin is not feasible due to the fact that the disposition of doxorubicin is not fully elucidated, with metabolism accounting for only 50% to 60% of the dose and renal excretion for 0.7% to 23% (average of ~5%; refs. 11, 12). Hence, we selected the hybrid model. The model included the prostate because it is the intended target site; the heart because it is the site of the dose-limiting cardiac toxicity of doxorubicin (13); the liver because it is the major metabolizing organ (8); and the spleen because it represents a rapidly perfused organ (8). The remaining carcass is represented by muscle. Plasma concentrations served as the drug input function for the simulation of tissue concentration profiles.

Based on the rapid cellular uptake of doxorubicin via both carrier-mediated processes and passive diffusion (12), we selected a perfusion rate-limited model for blood-to-tissue transfer. We observed biphasic doxorubicin accumulation in prostate tumor histocultures, with a rapid initial increase where drug concentrations approached 25% to 35% of the maximal concentration within 12 h, followed by a slow increase over the next 84 h (see Results). This data suggested the presence of two compartments in a tissue: a compartment where equilibrium between unbound and bound drugs is reached instantaneously and a second slow binding compartment. Further assumptions for the PBPK model follow widely accepted pharmacologic principles, which are as follows: (a) intercompartmental transport occurs via blood flow; (b) the instantaneous equilibrium between tissue of the rapidly equilibrating compartment and blood within the tissue; (c) blood concentrations in the tissue blood compartment are equal to the effluent blood concentration; (d) the arterial drug concentrations that perfuse the tissues are identical for all tissues; and (e) only unbound drug molecules are eliminated. The observed slow drug accumulation in tissues was described by a second tissue compartment that only slowly equilibrates with the first tissue compartment. As shown in Results, this step significantly improved the quality of the data fitting and the usefulness of the PBPK model.

**Differential mass balance equations.** Figure 1 is the schematic representation of a hybrid PBPK model for an organ of interest. Using the assumptions outlined above, differential mass balance equations were written to describe the changes in concentrations with time in plasma and different organs according to standard methods (7). Plasma concentrations instead of blood concentrations were used because we found constant blood-to-plasma ratios (i.e., 2.12) at four concentrations (0.006-3.5 μg/mL) that spanned the concentration range found in the present study. The equations describing the arterial plasma concentration (Cp) for the bolus and infusion administration are shown in Appendix 1.

**Obtaining model parameters for dogs.** The eight parameters in the PBPK model, depicted in Fig. 1 (see figure legends for definitions of parameters), were obtained as follows. The values for V and Q were obtained from the literature (5, 14–16). The unbound fraction in plasma (fplasma) was measured using equilibrium dialysis. Briefly,

![Diagram of a hybrid PBPK model for doxorubicin.](image)
commercial dialysis cells (Spectrum, Los Angeles, CA) were used and separated by a Spectra/por 1 membrane (10,000 MW cutoff; Spectrum). Plasma (1.2 mL) was added to doxorubicin and added to one side and an equal volume of phosphate buffer, previously described (17), was added to the other side. Dialysis was then done at 37°C in the dark for 18 h. Plasma and buffer samples were analyzed for doxorubicin concentrations. The blood-to-plasma concentration ratio ($R_p$) was determined experimentally as previously described (18). Briefly, 10 μL of a doxorubicin solution were incubated with heparinized dog blood at 37°C for 5 min. Plasma samples were obtained and analyzed by high-performance liquid chromatography and the blood-to-plasma concentration ratio was calculated. The values of $k_{pu}$, $k_{appu}$, and $k_{su}$ were estimated by WinNonlin analysis as parameters of the model describing the plasma/tissue concentration-time profiles obtained after a bolus injection. The value of $Cl_{j,t}$ was taken from the literature (see Appendix 1).

**Evaluation of PBPK model performance in dogs.** The model was evaluated in two ways. The first method used the same experimental data for establishing and evaluating the model (i.e., the data were used to obtain the model parameters and the plasma and tissue concentration-time profiles simulated using these parameters were compared with the same experimental data to determine the goodness of fit). The second validation used additional, independent experimental data that were not used to generate the model parameters. For this purpose, we used the model to simulate the plasma and tissue concentration-time data for two different dosing rates (i.e., infusions over 4 or 96 h) and compared the simulated data with additional, subsequently obtained experimental data. Note that these three treatment schedules are used clinically.

**Pharmacokinetic data analysis.** WinNonlin was used to perform computer fitting and simulations. Plasma concentration-time profiles were analyzed using a two-compartment open body model and a weight function of $1/(concentration)^2$. No weighting was used for the analysis of tissue concentration-time profiles. The initial values were estimated with SAS (SAS Institute, Cary, NC; see Appendix 1).

**Scale-up of dog data to predict doxorubicin tissue concentration-time profiles in humans.** Interspecies scale-up of the dog data to humans requires obtaining and substituting the dog-specific physiologic and pharmacokinetic model parameters with the human-specific parameters (i.e., tissue volume and blood flow, tissue clearance, pharmacokinetic macroconstants). The remaining parameters (i.e., free drug fraction in plasma or tissue, blood-to-plasma partition, transfer between rapidly and slowly equilibrating tissue subcompartments) were considered drug/tissue properties and not species dependent. Hence, the values obtained for dogs were used in the human model. For body surface area and blood flow rates, we used the values for older men because prostate cancer occurs more often in men at the age of 60 years or older (4, 19). As the volume of prostate is highly variable in patients in the same age group, ranging from 13 to 244 mL (20), we used the mean value of 35.7 g (21, 22). The calculated total blood flow to prostate tissues was 29.4 mL/min/100 g (4). The human plasma pharmacokinetic macroconstants for bolus administration and infusions were obtained from the literature (23). $Cl_{j,t}$ was estimated from literature data (ref. 12; see Appendix 1).

**Animal protocols.** Doxorubicin and epirubicin were gifts from Pharmacia, Inc. (Milan, Italy). All other chemicals and solvents were reagent grade or better. Male beagle dogs ($n = 20$; Spencerville, OH), 1 to 1.5 years of age, were maintained in accordance with institutional guidelines and housed in a facility with controlled 12-h light cycle for a week before experiments and allowed free access to food and water. Animal protocols were approved by The Ohio State University Institutional Animal Care and Use Committee and followed the guidelines set by the Institute of Laboratory Animal Resources.

Animals were divided into three study groups according to the route of drug administration. One group of animals ($10.0 ± 1.2 kg; n = 13$) received doxorubicin by bolus i.v. injection over 0.5 min through an angiocatheter (16 gauge, 2 in. in length) inserted into a cephalic vein. The remaining animals received doxorubicin by continuous i.v. infusion over 4 h (12.3 ± 1.11 kg; $n = 2$) or 96 h (10.6 ± 0.8 kg; $n = 5$). The doxorubicin dose was 2 mg/kg, equivalent to ~40 mg/m². A flexible polyurethane catheter (15 cm long) was placed in the right jugular vein of the animals previously sedated with 1 mg/kg of s.c. administered acepromazine. Drug solution was then infused using a portable infusion CADD-PULS pump (SIMS Deltec, St. Paul, MN) placed in an animal jacket. Blood samples within the first 24 h were obtained through an indwelling cephalic vein catheter and subsequent samples via needle puncture into alternate peripheral veins. For tissue samples, animals were anesthetized with i.v. pentobarbital (26 mg/kg) and subsequently euthanized by pentobarbital overdose. Plasma and tissue samples were stored frozen at −20°C until analysis.

**Analysis of doxorubicin concentrations.** Plasma samples were analyzed using a previously described high-performance liquid chromatographic assay (24). Briefly, a mixture of plasma and the internal standard epirubicin was extracted using solid-phase extraction. Tissue samples were analyzed according to the method of Shinkai et al. (25) with the only exception that tissue homogenates were diluted with 2-mL saturated sodium bicarbonate instead of potassium phosphate buffer. Doxorubicin and epirubicin were detected using excitation and emission wavelengths of 480 and 550 nm, respectively. The detection limit for doxorubicin was 0.3 nmol/L in plasma. Standard curves, constructed separately for prostate tissue and plasma, were linear within the range of 0.05 to 6 μg/mL (i.e., 0.09-10 μmol/L) for prostate tissue and 1 to 80 ng/mL (i.e., 0.0017-0.0136 μmol/L) for plasma. The recovery of doxorubicin from tissue was ≥90%.

**Results**

**Plasma and tissue pharmacokinetics after i.v. bolus injection: experimental data.** Figure 2 shows the concentration-time profiles in plasma and tissues in dogs given an i.v. bolus dose of 2 mg/kg, equivalent to ~40 mg/m². The plasma concentration profile is adequately described by a two-compartment model. A three-compartment model yielded a better fit as indicated by a ~10% decrease of the Akaike Information Criterion, a measurement of goodness of fit (26). However, the pharmacokinetic parameters such as area under the curve (AUC) and clearance differed by <1% between the two models. Hence, we elected to use a two-compartment model in PBPK analysis for its relative ease. Tissue concentrations in prostate, heart, spleen, and muscle increased gradually, reaching peak levels between 60 and 120 min; only the liver showed the highest concentration at 5 min (first time point).

**Biphasic doxorubicin uptake in vitro: experimental data.** Figure 3 shows the biphasic increase of doxorubicin concentrations under in vitro conditions in xenograft prostate tumor histocultures (27). The concentration increased to 25% to 35% of the maximal plateau value within 12 h, followed by a slower incline over the next 84 h. The kinetics of uptake also depended on the extracellular drug concentration; the time to reach 50% of the maximal drug concentration in histocultures increased with decreasing initial drug concentration in culture medium, ranging from ~10 h at >2 μmol/L initial concentration to ~40 h at 0.2 to 0.12 μmol/L. The tumor-to-medium concentration ratios, obtained at the end of 96-h incubation, remained relatively constant at ~100 (range, 84-119; mean ± SD, 105 ± 13) over the 500-fold concentration range (0.02-10 μmol/L). These findings indicated significant drug binding to tissues/tumors. The biphasic drug uptake further suggested two transfer processes in tissues: a rapid exchange from plasma to a rapidly equilibrating compartment and a slow
exchange to a slowly equilibrating compartment. These data indicated that the relationship between tissue and plasma concentrations, especially for the early time points, could not be adequately described by a single tissue-to-plasma concentration ratio as was assumed in previous doxorubicin PBPK models. Accordingly, in the present PBPK model, each organ was divided into fast and slow tissue-drug interaction compartments and two rate constants were used to describe the drug transfer.

Development and validation of hybrid PBPK model in dogs. The i.v. bolus data were used to obtain the required parameters for the hybrid PBPK model; the results are shown in Table 1. The model depicted linear kinetics for all transfer processes. 

\( R_B \) and \( f_{\text{plasma}} \) remained constant at blood concentrations between 0.006 and 0.04 \( \mu g/mL \), at 2.12 and 0.4, respectively. The \( f_{\text{plasma}} \) value is similar to the 50% binding of doxorubicin to human plasma proteins (8). Model performance was evaluated in two ways. The first method evaluated the goodness-of-fit by comparing the model-simulated data and the experimental data obtained after an i.v. bolus injection; the average deviation was 19.6 ± 15.3% (mean ± SD; range, 0.65-69.1%; median, 16.5%; see Fig. 2, Plasma). The second method evaluated the predictive power. For this purpose, we obtained additional experimental data (not used to generate the model parameters) after administering the drug at two different dose rates (i.e., 4-h and 96-h infusions of the same total dose). In these two cases, the model-predicted data deviated from the experimental data by 36.7 ± 25.9% (range, 2.76-107%; median, 29.5%; see Fig. 2, Plasma). The generally good agreement between model predictions and experimental data indicates the applicability of the PBPK model for vastly different dosing rates.

Note that changes in the dosing rates did not alter the AUC\(_{0-\infty}\) in the plasma to a great extent (<40% deviation) but significantly decreased the peak concentration (peak concentrations after 4-h and 96-h infusions were ~18% and ~0.4% of the peak concentration after a bolus injection). The concentrations in tissues were also reduced, but to a much lower extent (Fig. 2). For example, the peak concentration in the heart after a 96-h infusion was ~70% of that after a bolus injection.

Comparison of present and previous PBPK models. It is noted that a previous PBPK model failed to predict the doxorubicin concentrations in the spleen of rabbits (8). The present study showed that the experimentally determined average spleen

![Fig. 2. Doxorubicin concentration-time profiles in plasma and tissues. Doxorubicin (2 mg/kg, equivalent to 40 mg/m\(^2\)) was administered by i.v. bolus injection (\( \bullet \); n = 1-3 dogs per time point), 96-h infusion (\( \Delta \); n = 4), or 4-h infusion (\( \bigcirc \); n = 2, insets). Lines, model-simulated total concentrations. Points, mean of the experimental observations; bars, SD.](image)

![Fig. 3. Biphasic doxorubicin uptake in vitro. In vitro uptake into histocultures of CWR22 tumor. Points, mean (n ≥ 3); bars, SD. A part of this data has been previously reported (40).](image)
weight was significantly higher compared with the literature value (90 versus 25 g), and that correcting the splenic blood flow rate using the higher spleen weight yielded good agreement between the model-predicted and observed data.

We further examined the importance of incorporating both the rapid and slow transfer processes in tissues on the overall model performance. For comparison, a second PBPK model using only one transfer function between plasma and tissue (i.e., the traditional $R_{tissue}$-based method) was established (not shown). Figure 4 shows a plot of the model prediction versus experimental data for the two models. The data predicted using this second model deviated from the experimental data by $126 \pm 166\%$ (mean $\pm$ SD; range, 0.20-679%; median, 34%), which was six times higher compared with the first model using two transfer processes.

**Application of PBPK model: scale-up from dogs to humans.** Tables 1 and 2 summarize the physiologic and pharmacokinetic parameters used to establish the PBPK model in humans. As a preliminary evaluation of the performance of the PBPK model in humans, we calculated the model-predicted volume of distribution at steady state, which was within the range reported in the literature (1,657 versus 1,400-3,000 liters) in human patients (12).

Figure 5 shows the simulated doxorubicin concentration-time profiles in human heart and cancerous prostate tissues after rapid and slow i.v. injections of 60 mg/m². The simulated profiles showed different profiles in the two tissues. For the rapid injection, the heart tissue showed an initial rapid increase to a peak level at $t = 12$ h, followed by a continuous decline, whereas the prostate tissue showed an initial peak level at 1 h, followed by a decline and a slower increase to a second peak level reached between 24 and 48 h. The latter peak in the prostate was due to the drug uptake into the slow equilibrating compartment. For the 96-h infusion, both tissues showed

Table 1. Plasma pharmacokinetic macroconstants and parameters

<table>
<thead>
<tr>
<th></th>
<th>$A$ (µg/mL)</th>
<th>$B$ (µg/mL)</th>
<th>$\alpha$ (h⁻¹)</th>
<th>$\beta$ (h⁻¹)</th>
<th>AUC (µg/mL·h)</th>
<th>$\text{Cl}$ (mL/h/kg)</th>
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<tr>
<td><strong>Dogs</strong> (2 mg/kg, equivalent to ~ 40 mg/m² dose)</td>
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<tr>
<td>Bolus</td>
<td>1.09114</td>
<td>0.01036</td>
<td>2.38985</td>
<td>0.01395</td>
<td>1.20</td>
<td>1,670</td>
</tr>
<tr>
<td>96-h infusion</td>
<td>0.00635</td>
<td>0.01025</td>
<td>0.30339</td>
<td>0.00612</td>
<td>1.59</td>
<td>1,260</td>
</tr>
<tr>
<td>4-h infusion</td>
<td>0.16578</td>
<td>0.04107</td>
<td>1.57080</td>
<td>0.00252</td>
<td>1.17</td>
<td>1,710</td>
</tr>
<tr>
<td>Humans (60 mg/m² dose)</td>
<td></td>
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<tr>
<td>Bolus*</td>
<td>2.53</td>
<td>0.0698</td>
<td>3.25</td>
<td>0.0242</td>
<td>3.67</td>
<td>404</td>
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<td>96-h infusion*</td>
<td>0.00809</td>
<td>0.0301</td>
<td>3.25</td>
<td>0.0242</td>
<td>3.67</td>
<td>404</td>
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</table>

*Data from ref. 40, calculated from microconstants using equations in Appendix 1.
†Dose conversion used a body surface area of 1.73 m² for a 70-kg patient.

Fig. 4. Predicted concentration versus observed concentration. ●, data predicted using the two plasma-to-tissue transfer processes. △, data predicted using a single transfer process. Solid line, perfect correlation with a slope of 1.
slowly increasing concentrations to reach the peak levels at 96 h, followed by a decline. In the heart, the peak doxorubicin concentration was \(~12\) µg/g after the bolus injection and \(~7.5\) µg/g after the 96-h infusion. In comparison, the two peak concentrations were \(~12\) and \(~8\) µg/g in the prostate after the bolus injection and \(~6\) µg/g after the slow infusion.

Within each tissue, the two treatments yielded identical cumulative AUCs (\(~740\) µg/g h for prostate and \(~820\) µg/g h for the heart), reflecting the linear transfer processes. Compared with the prostate, the initial slope of the plot of cumulative AUC of heart tissue versus time was more rapid due to the faster drug partitioning from the plasma into heart tissue.

Discussion

Our laboratory has a long-standing interest in using computational tools to simulate dynamic biological processes for the purpose of facilitating *in vitro* to *in vivo* and preclinical to clinical translations. We are particularly interested in developing computational paradigms for designing maximally effective clinical treatments. An example is our work in intravesical bladder cancer therapy, wherein computer simulations based on mathematical models of drug transport and tissue pharmacokinetics were done to identify the treatment conditions that would significantly improve treatment efficacy (28). The optimized treatment was tested in a randomized phase III trial; the results (19%) improvement in recurrence-free rate) are identical to the model predictions (18-20% improvement; refs. 29, 30).

For intravesical therapy in bladder cancer, wherein the drug solution is instilled directly into the urinary bladder, the mathematical models depict the drug partitioning from urine into bladder tissues and the drug transport within bladder tissues. The present study addresses a different situation where the drug travels from the i.v. entry point through the blood to the target organs. Accordingly, the mathematical models used in the present study are different from the earlier models for regional therapy. As discussed below, the results support using this computational approach to depict the kinetics of drug delivery to prostate tissues after an i.v. treatment.

The present study showed that the newly established PBPK model successfully predicted the doxorubicin concentration-time profiles in multiple organs, including the prostate, after vastly different dosing rates, and thereby offers several advantages over the previous PBPK models established for rodents and rabbits. Our results indicate the importance of separating a tissue into rapidly and slowly equilibrating compartments. Further, the present PBPK model was developed in dogs, a large animal species with a comparable anatomy, physiology, and pathology to humans and is therefore more clinically relevant and enables the interspecies scaling up to humans. These key findings are further discussed below.

**PBPK models of doxorubicin.** A previous PBPK doxorubicin model (8), established using rabbits as the animal model and the assumption that drug partition into tissue is rate limited by tissue perfusion rate, overestimated the drug concentrations in all tissues before 1 h but was able to predict the concentrations at later time points in all tissues except red marrow and spleen. An alternative PBPK model, established using a global model and rats as the animal model and the assumption of a membrane transport-limited drug partition into tissues (31), was able to predict the concentrations in blood and small intestine but underestimated the heart and lung concentrations at early time points (<4 h) and the liver concentrations after 2 h. These two earlier models used a single tissue-to-plasma ratio \(R_{\text{tissue}}\) to describe the plasma-to-tissue transfer. In an attempt to improve the model, and based on the observation that \(R_{\text{tissue}}\) is dependent on the amount of tissue DNA (18) and specific lipids (32, 33), a third PBPK model (mice as the animal model), which did not use a constant \(R_{\text{tissue}}\) value but instead described doxorubicin binding to DNA and phospholipids as saturable nonlinear processes (34), was developed. However, this model did not significantly improve the quality of data fitting over the previous two models.

In the present study, the observation of biphasic drug uptake and accumulation in prostate tumors and organs suggested the presence of two kinetic subcompartments: rapidly and slowly equilibrating compartments. This modification significantly improved the model predictions and eliminated the severalfold overprediction of tissue concentrations at early time points.

### Table 2. Doxorubicin PBPK model parameters

<table>
<thead>
<tr>
<th>Species-dependent model parameters</th>
<th>Prostate</th>
<th>Muscle</th>
<th>Heart</th>
<th>Spleen</th>
<th>Liver</th>
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<tr>
<td>Tissue blood flow, mL/min per organ</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Dog</td>
<td>4.04</td>
<td>227</td>
<td>69.6</td>
<td>87.3</td>
<td>280</td>
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<tr>
<td>Human</td>
<td>10.5</td>
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<td>Hepatic intrinsic clearance, mL/min</td>
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<tr>
<td>Dog</td>
<td></td>
<td></td>
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<tr>
<td>Tissue volume, g (wet weight)</td>
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<td></td>
</tr>
<tr>
<td>Dog</td>
<td>7.37</td>
<td>5751</td>
<td>88.1</td>
<td>87.3</td>
<td>289</td>
</tr>
<tr>
<td>Human</td>
<td>35.7</td>
<td>310</td>
<td></td>
<td></td>
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</table>

| Species-independent model parameters | | | | | |
| \(k_{\text{fast}}\) (%) | 1.45 | 12.7 | 0.521 | 0.526 | 0.747 |
| \(K_{\text{ass}, h}^{-1}\) | 24.0 | 13.05 | 227 | 70.5 | 50.3 |
| \(K_{\text{dis}, \times 10^{-2} h^{-1}}\) | 5.49 | 9.36 | 62.4 | 9.51 | 8.13 |

**Note:** Tissue volumes for prostate, heart, spleen, and liver of dogs were determined experimentally in the study animals using a density of 1.0. Mean body weight of the 18 dogs used in the study was 10.4 kg. Other values were from the literature (15). Human values were based on 70-kg body weight.
There are other examples of drugs that are extensively bound to tissues (e.g., cyclosporines) where PBPK models incorporating fast and slow drug-tissue interacting subcompartments yielded better data fitting (35, 36) as compared with a model that used a single plasma-to-tissue transfer process (37). With respect to the biological relevance of the two tissue subcompartments, we propose that the rapidly equilibrating compartment reflects the rapid exchange of doxorubicin between blood/plasma and interstitial fluid, whereas the slowly equilibrating compartment reflects the slow binding to macromolecules in tissues.

The previously published PBPK models were based on data obtained after an i.v. bolus dose and not after slow infusion, presumably due to the technical difficulty associated with i.v. infusion studies in small animals. To our knowledge, the present model is the first that predicted the kinetics of doxorubicin delivery to the prostate and the only model that predicted the tissue concentration-time profiles after different treatment schedules (rapid and slow injections).

Interspecies scale-up to humans using the PBPK model yielded simulated drug concentration-time profiles in prostate and heart tissues in humans after bolus and slow i.v. administrations. These data are useful for making inferences on the doxorubicin pharmacodynamics in these tissues. With respect to the cardiac toxicity of doxorubicin, a general belief is that the acute cardiac toxicity is due to the peak concentration and the chronic cardiac toxicity results from the cumulative AUC (13). A slow, 96-h doxorubicin infusion regimen was advocated as a means to delay the onset of toxicity (12). The simulated results showed that for the two treatment routes, the peak concentrations showed ~100-fold difference in plasma, but the difference in heart tissue was much smaller, at only 33% (12 versus 8 μg/g). Furthermore, the two treatments yielded identical cumulative AUCs in the heart. These results do not support the rationale of using the 96-h infusion regimen to lower the peak concentration in the heart.

To infer on the comparative antitumor activity of the bolus and slow infusions of doxorubicin, we compared the simulated doxorubicin concentration-time profiles in human prostates to our previously established concentration-effect relationships in histocultures of human prostate tumors (38). The results are shown in Table 3. The comparison indicated that the bolus injection, by yielding a greater AUC_{0-96 h}, would have resulted in a greater inhibition of DNA precursor incorporation compared with the 96-h infusion. However, neither treatment yielded sufficient AUC_{0-96 h} to induce appreciable cell death. Note that the comparison did not take into account the drug C × T after 96 h, and therefore may underestimate the later drug effects, and did not account for the differences under the in vitro and in vivo conditions. Examples for the latter are (a) in vitro pharmacodynamic study reported the extracellular drug concentrations as the effective concentration, whereas the in vivo tissue distribution study measured the sum of extracellular and intracellular concentrations; and (b) the static nature of the in vitro experiment (i.e., constant drug concentration in the culture medium) versus the dynamic nature of the in vivo experiment (i.e., changing concentrations due to distribution and elimination). Conversion of an effective C × T under in vitro condition to an effective C × T under in vivo condition

Fig. 5. PBPK model – simulated doxorubicin delivery to human heart and cancerous prostate tissues, after rapid or slow i.v. infusion, over time. Simulated profiles after a 60 mg/m² dose administered by a bolus injection over 0.5 min (solid lines) or a slow infusion over 96 h (dashed lines). A, concentration-time profiles. B, cumulative AUC. Note the different scales used in the insets under Plasma.
requires solving the equation of $C^a \times T = \text{effect}$, which, in turn, requires additional experimentalizations to establish the relationship between concentration, treatment time, and effect, as described in our earlier study designed to enable the translation of the in vitro mitomycin C pharmacodynamics in human bladder tumors into defining the effective treatment parameters in patients (39–41).

In summary, the present study showed the use of PBPK, together with human plasma pharmacokinetic data, to translate in vitro and in vivo preclinical data to predicting the in vivo kinetics of drug delivery to target tissues after different treatment schedules. Further research on separating tissues into the targeted compartment (e.g., molecular targets) and non-targeted compartment (e.g., extracellular fluid) and integrating PBPK in the target subcompartments with in vitro pharmacodynamic data may provide a means to simulate the in vivo pharmacodynamics.

### Appendix 1

**Differential mass balance equations describing the hybrid PBPK model**

Plasma concentration after i.v. bolus input is described by

$$C_{\text{plasma}} = A \cdot e^{-\beta \cdot t} + B \cdot e^{-\alpha \cdot t}$$  \hspace{1cm} (A)

where

$$A = \frac{\text{Dose}(x-k_{21})}{(x-\beta)f_1} \quad \text{and} \quad B = \frac{\text{Dose}(k_{21}-\beta)}{(x-\beta)f_1}$$

Plasma concentrations during i.v. infusion are

$$C_p = A \cdot (1 - e^{-\alpha \cdot t}) + B \cdot (1 - e^{-\beta \cdot t})$$  \hspace{1cm} (B)

Plasma concentrations after termination of an i.v. infusion are

$$C_p = A \cdot (e^{-\alpha \cdot t} - e^{-\alpha \cdot (t - \tau)}) + B \cdot (e^{-\beta \cdot t} - e^{-\beta \cdot (t - \tau)})$$  \hspace{1cm} (C)

where $A = \frac{Dose(x-k_{21})}{(x-\beta) f_1}$, $B = \frac{Dose(k_{21}-\beta)}{(x-\beta)f_1}$, $f_{\text{in}}$ is infusion rate, $\tau$ is infusion duration (4 or 96 h), $\alpha = k_{10} - k_{21}$, and $\beta = k_{10} + k_{12} + k_{21}$.

Tissue concentration-time profiles for nonelimination organs (prostate, heart, muscle, and spleen) are defined by Eqs. D.

<table>
<thead>
<tr>
<th>Time</th>
<th>$\text{In vivo pharmacokinetics}$</th>
<th>$\text{In vitro pharmacodynamics in human tumors}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$AUC$ ($\mu\text{g/mL h}$)</td>
<td>$\text{Effective AUC}$ ($\mu\text{g/mL h}$)</td>
</tr>
<tr>
<td></td>
<td>Bolus injection</td>
<td>96-h infusion</td>
</tr>
<tr>
<td>0–96 h</td>
<td>3.28</td>
<td>2.54</td>
</tr>
<tr>
<td>0–$\infty$</td>
<td>3.67</td>
<td>3.67</td>
</tr>
</tbody>
</table>

NOTE: The in vivo pharmacokinetic data were obtained from the simulated concentration-time profiles in humans (see Fig. 5). The in vitro pharmacodynamic data in human tumors were obtained from our previous publication, which measured the median AUC of doxorubicin that produced 50% inhibition of DNA precursor incorporation or cell kill in 17 tumors obtained from prostate cancer patients (41).

For the elimination organ, liver, Eq. D was modified to add an elimination term.

$$V \cdot \frac{dC_{fast}}{dt} = Q \cdot R_B \cdot (C_{\text{plasma}} - C_{fast} \cdot f_{fast}/f_{\text{plasma}}) - C_{fast} \cdot f_{fast} \cdot k_{assoc} \cdot V + C_{slow} \cdot k_{dis} \cdot V -$$

$$Cl_{1,i} \cdot C_{fast} \cdot f_{fast}$$  \hspace{1cm} (G)

where $C_T$ is the total drug concentration in the organ; $C_{fast}$ and $C_{slow}$ describe the drug concentrations in the rapidly and slowly equilibrating compartments, respectively; $f_{\text{plasma}}$ is the free fraction of doxorubicin in plasma; $f_{fast}$ is the free doxorubicin fraction in the rapidly equilibrating compartment; and $R_B$ is the blood-to-plasma ratio. In the absence of data to suggest that the two subcompartments have different volumes, we assumed that the two values were both equal to the organ volume $V$. The association constant ($k_{assoc}$) describes the drug transfer from the rapidly equilibrating compartment to the slowly equilibrating compartment, and the dissociation constant ($k_{dis}$) describes the reverse process. $Q$ is the blood flow perfusing the organ. $Cl_{1,i}$ is intrinsic clearance of liver.

**Calculation of model parameters**

$Cl_{1,i}$ was approximated as:

$$Cl_{1,i} = \frac{Q \cdot R_B}{(Q \cdot R_B - 1) \cdot f_{\text{plasma}}}$$  \hspace{1cm} (H)

where $Cl_{1,p}$ is the hepatic plasma clearance, which equaled 50% of the systemic clearance (12).

$f_{fast}$, $k_{assoc}$, and $k_{dis}$ were obtained by computer fitting the model to the experimentally obtained plasma or tissue concentration-time profiles, done in three steps. The first step
was to obtain initial estimates of \( f_{fast} \) and the ratio of \( k_{assoc}/k_{dis} \) from analysis of the plasma and tissue concentration-time profiles was obtained after a bolus injection and 96-h infusion. For this purpose, we assumed equal free drug concentrations in plasma and the rapidly equilibrating compartment (Eq. I).

\[
C_{fast} \cdot f_{fast} = C_{plasma} \cdot f_{plasma} \tag{I}
\]

\( f_{fast} \) was estimated using the data at 5 min with the further assumption of negligible concentration in the slow compartment at this early time point. Thus, the two tissue compartments would appear as a single compartment and Eq. I becomes:

\[
C_{T,5\, \text{min}} \cdot f_{fast} = C_{plasma,5\, \text{min}} \cdot f_{plasma}
\]

Reorganization yielded Eq. I,

\[
f_{fast} = \frac{C_{plasma,5\, \text{min}} \cdot f_{plasma}}{C_{T,5\, \text{min}}} \tag{I}
\]

As the terminal half-life of doxorubicin in dogs is 30 h (12), we assumed that, at the end of the 96-h infusion, a steady-state condition was reached in all compartments, including the slow tissue compartments. Hence, \( k_{assoc} \cdot \frac{C_{plasma}}{C_{T,96\, \text{h}}} = 0 \), and rearrangement of Eq. E yielded

\[
\frac{k_{assoc}}{k_{dis}} = \frac{C_{slow,96\, \text{h}}}{C_{plasma,96\, \text{h}} \cdot f_{plasma}} \tag{K}
\]

\( C_{slow,96\, \text{h}} \) was obtained by combining Eqs. F and I.

\[
C_{slow} = C_T - C_{fast} = C_T - C_{plasma} \cdot f_{plasma} \tag{L}
\]

Substitution into Eq. K yielded an approximate value for \( k_{assoc}/k_{dis} \) (renamed \( k_{ratio} \)). The second step was to find the initial parameter estimates for \( f_{fast} \) and \( k_{assoc} \) by fitting of approximated integral equations using SAS. Substitution, rearrangement, and Laplace transformation of Eqs. D, E, F, and I yielded Eqs. M and N, where \( k_{dis} \) was substituted with \( k_{ratio}/k_{assoc} \).

\[
f_{fast} = \frac{f_{plasma}}{C_{plasma}} \cdot (A \cdot e^{-\frac{\beta}{k_{ratio}/k_{assoc}} \cdot t} + B \cdot e^{-\frac{r}{k_{ratio}/k_{assoc}} \cdot t}) \tag{M}
\]

\[
C_{slow} = \frac{f_{plasma}}{C_{plasma}} \cdot \left( -\frac{B}{\beta - k_{ratio}/k_{assoc}} \cdot e^{-\frac{\beta}{k_{ratio}/k_{assoc}} \cdot t} + \frac{A}{\alpha - k_{ratio}/k_{assoc}} + \frac{B}{\beta - k_{ratio}/k_{assoc}} \cdot e^{-\frac{\beta}{k_{ratio}/k_{assoc}} \cdot t} \right) \tag{N}
\]

Experimentally obtained tissue concentration-time profiles were fitted with Eqs. F, M, and N to obtain parameter estimates for \( k_{assoc} \) and \( f_{fast} \). Nonlinear regression fitting was done using SAS. The initial value for \( k_{dis} \) was calculated as \( k_{assoc}/k_{ratio} \). The initial parameter estimates were then used with WinNonlin (Pharsight Corp., Mountain View, CA) to fit Eqs. D–G to the experimental data and solve for the best-fit values for \( k_{assoc}, k_{dis} \), and \( f_{fast} \).

References


Computational Modeling to Predict Effect of Treatment Schedule on Drug Delivery to Prostate in Humans

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