RESEARCH ARTICLE

Two-Mechanism Peak Concentration Model for Cellular Pharmacodynamics of Doxorubicin[1]

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Abstract

A mathematical model is presented for the cellular uptake and cytotoxicity of the anticancer drug doxorubicin. The model assumes sigmoidal, Hill-type dependence of cell survival on drug-induced damage. Experimental evidence indicates distinct intracellular and extracellular mechanisms of doxorubicin cytotoxicity. Drug-induced damage is therefore expressed as the sum of two terms, representing the peak values over time of concentrations of intracellular and extracellular drugs. Dependence of cell kill on peak values of concentration rather than on an integral over time is consistent with observations that dose−response curves for doxorubicin converge to a single curve as exposure time is increased. Drug uptake by cells is assumed to include both saturable and unsaturable components, consistent with experimental data. Overall, the model provides better fits to *in vitro* cytotoxicity data than previous models. It shows how saturation of cellular uptake or binding with concentration can result in plateaus in the dose−response curve at high concentrations and short exposure, as observed experimentally in some cases. The model provides a unified framework for analyzing doxorubicin cellular pharmacokinetic and pharmacodynamic data, and can be applied in mathematical models for tumor response and treatment optimization.
Neoplasia (2005) **7,** 705−713

Introduction

Despite its toxicity, doxorubicin has been in clinical use since the 1970s and remains a widely used anticancer drug. No fully satisfactory mathematical model for doxorubicin cellular pharmacokinetics and pharmacodynamics has been proposed and validated. Cellular pharmacokinetic models play an important role in predicting the penetration of drugs into tumor tissues. The penetration of doxorubicin from microvessels into the tumor tissue is limited by binding to extracellular sites as well as by cellular uptake. This is especially important in regions of low vascular density. Cellular pharmacodynamic models are needed in the development of whole-body pharmacodynamic models to predict drug response as a function of administration dosage and scheduling. Such predictions can then be used to optimize therapy.

The use of prolonged infusion or fractionated doses of doxorubicin has been justified on the grounds that the major dose-limiting side effect, cardiotoxicity, is believed to depend on peak drug levels, whereas the antitumor effect is assumed to be a function of area under the concentration−time curve (AUC) [1]. In actuality, the predictive value of AUC alone for antitumor effect has not been thoroughly established. Doxorubicin is classified by some researchers as cell cycle phase−nonspecific, whereas others consider it a cell cycle phase−specific drug. Ozawa et al. [2] proposed that cell kill for cell cycle phase−nonspecific drugs is a function of the (extracellular) AUC. AUC dependence had also been proposed by Eichholtz-Wirth [3], who fit the following model for cytotoxic effect of doxorubicin to data for Chinese hamster and HeLa cells:

$$S = \exp(-k t_{\exp} c_{e}) \tag{1}$$

In Eq. (1), S is survival relative to controls, k is a model parameter that depends on the cell line, $t_{\exp}$ is exposure time, and c_{e} is extracellular concentration. El-Kareh and Secomb [4] noted that several data sets in the literature do not support the idea that cell kill is a function purely of AUC, but imply an additional dependence on exposure time. Lankelma et al. [5] also noted that, in general, cell uptake and cytotoxicity of anticancer drugs depend on the shape of the concentration−time curve.

Alternative models in the literature for drugs that do not conform to AUC-dependent cytotoxicity include the $C^n T$ model [6], the Hill equation−based model proposed by Levasseur et al. [7], and the exponential cell kill model of Gardner [8]. All these models involve extracellular drug concentration only, and do not account for transport of drug into the cell. Although

there is evidence that doxorubicin can kill cells without entering them, intracellular drug is believed to make a significant contribution to cell kill [9]. Cellular uptake data [10] show that intracellular drug levels take hours to equilibrate with extracellular levels, suggesting that the kinetics of uptake affects cytotoxicity.

Some cellular pharmacodynamic models proposed in the literature have included cellular pharmacokinetics. Lobo and Balthasar [11] used a transit compartment model for methotrexate that accounts for delays such as may result from uptake, binding, or other processes that must occur before a drug can act lethally. Their model is general in that the delays are introduced as first-order reactions without assigning any particular physical interpretation to any of these processes. The mathematical assumption of first-order kinetics rules out saturability in extracellular concentration, a feature shown by the cellular uptake data [10]. Lankelma et al. [5] recently proposed a pharmacodynamic model for doxorubicin that involves two intracellular compartments, with cell kill a function of the concentration history in the second compartment. In their model, concentration is raised to a constant power n and appears in a "fading-memory" integral giving cumulative cell damage. El-Kareh and Secomb [12] proposed a cellular pharmacodynamic model for cisplatin that related cell kill to the peak value of time of an intracellular species, with the concentration of this species determined by kinetic equations for drug uptake and binding. Such a model could be applied to other drugs by modifying the kinetic equations to reflect differences in cellular uptake and target binding between drugs.

Of the abovementioned cellular pharmacodynamic models, only that of Levasseur et al. [7] has been tested for doxorubicin with cytotoxicity data that cover both multiple exposure times and multiple concentrations. In that study, the data included exposure times from 1 to 24 hours. Exposure time dependence over this range was partly fitted by an exponent (γ) in their model, which is quadratic in exposure time. This has the effect of causing the model to break down if times beyond the range fitted are considered. At some exposure time beyond 24 hours, the exponent changes sign, causing the sigmoidal dose–response curves to invert and become unrealistic [12]. This model also has the limitation that it cannot readily be generalized to cases in which extracellular exposure is a function $c_e(t)$ of time, as occurs in vivo. In the study of Lankelma et al. [5], the exposure times considered were 1, 3, and 7 days, and for each exposure time only one extracellular concentration was used. As discussed by El-Kareh and Secomb [4], cellular uptake and cytotoxicity models can only be validated with data sets that include multiple exposure times and multiple concentration values.

The need remains for a cellular pharmacokinetic and pharmacodynamic model for doxorubicin that can be validated with data for several different cell lines, that can be used when the extracellular concentration varies with time, and that is sufficiently simple to be of practical use. The purpose of this paper is to develop such a model. Experimental evidence implies two mechanisms of doxorubicin

cytotoxicity. The primary mechanism under clinical conditions is believed to involve topoisomerase II inhibition by intracellular drug, although a number of other mechanisms have been proposed [1,9]. Drug that is rendered incapable of entering cells also shows cytotoxicity, indicating a second mechanism involving extracellular drug. Based on these observations, a two-mechanism cellular pharmacodynamic model is proposed.

Materials and Methods

Literature searches were performed to find cytotoxicity data sets for doxorubicin that included at least three values of exposure time, and for which the concentration covered a range adequate to show the sigmoidal shape of the dose–response curves. The data sets of Eliaz et al. [13] for murine melanoma cells, Levasseur et al. [7] for wild-type A2780 human ovarian carcinoma cells, Link et al. [14] for the HT29 and NMG64/84 human colorectal carcinoma cell lines, Nguyen-Ngoc et al. [15] for mouse sarcoma cells, Rupniak et al. [16] for human ovarian carcinoma cells isolated from ascitic fluid, Vrignaud et al. [17] for rat glioblastoma cells, and Walker et al. [18] for human bladder cancer cells were found to meet the criteria. The analysis was restricted to in vitro studies because in vivo data on drug uptake and cytotoxicity are confounded by many factors, including transport of drug from plasma to the tumor cells, altered drug response in the tumor interior due to the low-pH, low-oxygen environment, and multicellular and confluent-dependent resistance. In all cases, data points were read manually from the graphs provided in the papers.

The pharmacodynamic model was developed based on the following considerations. The damage done to a cell by drug action is represented by a single continuous measure D that depends on drug exposure. For an individual cell, the possible outcomes are repair and survival if the damage is below some threshold value, or death if the damage exceeds the threshold. Mathematically, this can be represented by a step function dependence of survival on D. For a population of cells, the threshold value is heterogeneous, resulting in a sigmoidal variation in survival as a function of dose or damage [7]. This distribution of damage threshold values over the population is not known. However, it has been found empirically that survival curves are well fitted by a Hill equation, when plotted against drug concentration or other variables that are likely to measure or correlate with damage. For this reason, a Hill equation is used here to relate survival relative to controls to damage:

$$S = \frac{1}{1 + A[D]^n} \tag{2}$$

where A and n are constants.

The damage D is a function of drug concentration and exposure time. A study including long exposure times [13] showed that dose–response curves (response versus concentration for fixed exposure times) for doxorubicin converge to a single curve as exposure time is increased. This

behavior is inconsistent with models in which D is a function of AUC (area under the curve) of concentration only, because such dependence would cause the dose–response curves to continue to shift to the left as exposure time increases. By a similar argument, functions of either extracellular or intracellular concentration integrated over the exposure time can be ruled out as choices for D because such functions would increase with exposure time, contradicting the observed behavior. In the present model, D is assumed to depend on peak values over time of concentrations of the species leading to cell kill. For a given extracellular concentration, increases in exposure time beyond that required to reach equilibrium intracellular drug levels do not result in any further cell kill. As a result, the survival curves converge to a single asymptotic curve in the limit of long exposure time, as observed.

Much evidence points to topoisomerase II inhibition as the main mechanism of doxorubicin-induced cell kill [9], but some experimental studies have provided evidence for an extracellular mechanism. Maestre et al. [19] compared free drug with immobilized drug, which could not enter the cell, and found that both were cytotoxic, with the former inducing apoptosis, and the latter inducing necrosis. Based on these observations, D is assumed to be the sum of two terms—one dependent on the peak concentration c_i^{peak} of an intracellular species, and one on the peak concentration c_{eb}^{peak} of an extracellular membrane-bound species:

$$D = (c_i^{peak})^m + c_{eb}^{peak} \qquad (3)$$

The exponent m allows for the fact that the heterogeneity in sensitivity need not be the same for both cell kill mechanisms.

As shown by uptake data of Kerr et al. [10], intracellular levels of doxorubicin take hours to equilibrate with extracellular levels. The kinetics of cellular uptake should therefore be considered. Doxorubicin is believed to enter cells by passive transport mechanisms. The uptake data indicate saturation in extracellular concentration, which has been interpreted as an indication either of carrier-mediated transport [20] or of self-association in the extracellular environment [1]. Based on these observations, the following cellular pharmacokinetic model is proposed, in which the uptake rate is a combination of a linear diffusive component and a saturable, carrier-mediated component:

$$\frac{dc_i}{dt} = k_3 \left(k_1 c_e + \frac{k_2 c_e}{K_i + c_e} - c_i \right) \qquad (4)$$

In Eq. (4), c_e is the concentration of free extracellular drug. The parameter k_1 gives the ratio of intracellular to extracellular concentration at which the net rate of passive exchange is zero. This parameter is not necessarily equal to one because intracellular and extracellular concentrations are generally measured in different units, and conversion often involves assumptions about cell volume, or the relation between fluorescence and concentration, which are not precisely known. Furthermore, intracellular binding or sequestration allows intracellular concentrations of doxorubicin to be much higher than extracellular concentrations at equilib-

rium. The model of Eq. (4) was fitted to cellular uptake data [10] to obtain values for the parameters k_1, k_2, K_i, and k_3. In fitting these model parameters to the data, the root mean square (RMS) deviation of the predicted values and the data points was minimized by using a numerical function minimization routine of the Mathematica software package (Wolfram Research, Champaign, IL).

For cases in which extracellular concentration $c_e(t)$ varies with time, c_i^{peak} is determined by solving Eq. (4) for $c_i(t)$ and by determining the peak value that this function takes over time. For the *in vitro* experimental cytotoxicity data sets used to test the model, c_e was held constant for an exposure time t_{exp}, and then reduced to zero. For this type of exposure:

$$c_i^{peak} = \left(k_1 c_e + \frac{k_2 c_e}{K_e + c_e} \right)(1 - \exp[-k_3 t_{exp}]) \qquad (5)$$

Because formation of an extracellular bound species involves binding and no transport across the membrane, the concentration of extracellular membrane-bound species is assumed to equilibrate rapidly and therefore to be described by a Michaelis-Menten equation:

$$c_{eb}^{peak} = \frac{k_4 c_e}{K_e + c_e} \qquad (6)$$

The saturability inherent in Eqs. (5) and (6) accounts for the fact that the numbers of membrane transporters and extracellular binding sites available for doxorubicin are finite. It is compatible with the plateaus seen in some dose–response curves for doxorubicin at high concentrations. Substituting the expressions for peak concentrations obtained from Eqs. (5) and (6) in Eqs. (2) and (3) gives an expression for survival relative to controls:

$$S = \frac{1}{1 + A\left[\left(\left(k_1 + \frac{k_2 c_e}{K_i + c_e} \right)(1 - \exp(-k_3 t_{exp})) \right)^m + \frac{k_4 c_e}{K_e + c_e} \right]^n} \qquad (7)$$

This two-mechanism peak concentration model was fit to each of the eight cytotoxicity data sets from the literature to estimate the unknown parameters by minimizing the mean square deviation between predicted and experimental data points as described above. When uptake parameters are not available from separate data, the parameter A is not independent of the other parameters in Eq. (7). The value 1 was therefore assigned to A, and the eight remaining parameters were obtained by fitting. For purposes of comparison, seven other previous models were also fitted to the same data sets. These include the AUC-dependent model [3] (Eq. (1)) and the $C^n T$ Hill model [6]:

$$S = \frac{1}{1 + A(C^n T)^m} \qquad (8)$$

Because dose–response curves are often fitted to Hill equations rather than exponentials, another AUC-dependent model, the "extracellular AUC Hill model":

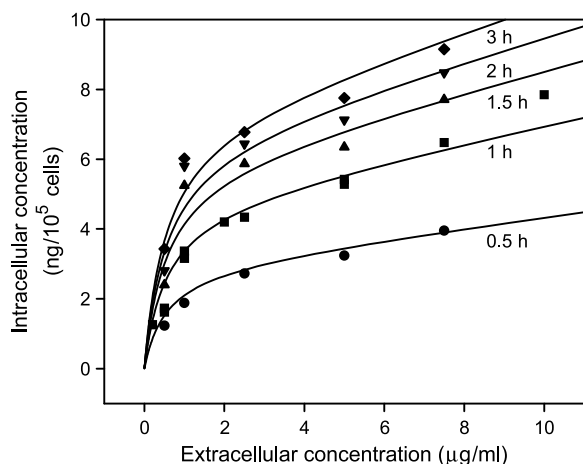$$S = \frac{1}{1 + A(AUC_e)^n} \qquad (9)$$

Figure 1. *Cellular pharmacokinetic model fit to cellular uptake data of Kerr et al. [10] for doxorubicin in non-small cell lung tumor cells.*

was tested as an alternative to Eichholtz-Wirth's model. The models of Levasseur et al. [7] and Gardner [8] were also tested. Gardner's study proposes two models—one for cell cycle phase–specific drugs and one for phase–nonspecific drugs. Because doxorubicin has, at times, been classified either way in the literature, both models were tested.

Results

Figure 1 shows the fit of the uptake model (Eq. (4)) to data for the cellular uptake of doxorubicin by non-small cell lung tumor cells [10]. The data clearly show the initial saturation, followed by continued linear uptake, in agreement with Eq. (4). The best model fit, with parameter values $k_1 = 0.00631$ (ng/10^5 cells)/(μg/ml), $k_2 = 0.126$ ng/10^5 cells, $K_i = 0.528$ μg/ml, and $k_3 = 1.01$ hr^{-1}, shows good agreement. When either the saturable or the nonsaturable uptake term was omitted in Eq. (4), the resulting fits were significantly inferior, confirming that both terms are needed.

RMS residual errors for the fit of the present two-mechanism peak concentration model and seven other models to eight data sets for doxorubicin cytotoxicity are

shown in Table 1. In all but two cases, the present model gave lower residual errors than the other models. For the data set of Levasseur et al. [7], the C^nT model and the single and double Hill models [7] gave slightly smaller values for the RMS residual. For the data set of Vrignaud et al. [17], the double Hill model [7] gave slightly smaller residuals. In no case did any other model give a significantly (more than .01) lower residual than the present model. The Wilcoxon signed rank one-tailed test was performed using the RMS deviation values in Table 1, from which it was determined that the two-mechanism peak concentration model was superior to the C^nT model (significance level $P = .004$), the Levasseur et al. single Hill model ($P = .008$), and the Levasseur et al. double Hill model ($P = .012$).

The fitted parameters for the two-mechanism peak concentration model are given in Table 2. In each case, $A = 1$. The parameter K_i, which indicates the concentration at which the intracellular uptake saturates, is small in all cases, indicating that this binding or transport step has a high affinity for the drug. In the data sets of Rupniak et al. [16] and Walker et al. [18], high values of K_e were obtained, indicating that the effect of extracellularly bound drug is nonsaturating over the range of concentrations considered.

Model fits to the data for the present two-mechanism peak concentration model, the C^nT model, and the single or double Hill equation model [7], are shown for several data sets in Figures 2–6. The present model is able to represent the "plateau" in survival that is seen in some data sets at high concentrations and short exposure times (Figures 2 and 5). This arises in the model because the saturable component of cellular uptake reaches its maximal level, and increased uptake can only be achieved through the nonsaturable pathway, which is relatively inefficient.

The improved ability of the two-mechanism peak concentration model to fit the data, relative to previously proposed models, is most evident for data sets covering broad ranges of exposure times [13,15,17] (Figures 2, 4, and 5). Conversely, the data of Walker et al. [18] (Figure 6) cover a limited range of exposure times (30 minutes to 2 hours), and all the models considered yield RMS deviations of about .05 or less. This is likely due to the fact that, over

Table 1. RMS Deviations of Model Fits to Cytotoxicity Data for Doxorubicin Acting on Various Cancer Cell Lines.

Data Set	Two-Mechanism Peak Concentration Model	Extracellular AUC (Hill Equation)	Extracellular AUC (Exponential; Eichholtz-Wirth [3])	C^nT	Levasseur et al. [7] (Single Hill Equation)*	Levasseur et al. [7] (Double Hill Equation)*	Gardner [8] Cell Cycle–Nonspecific	Gardner [8] Cell Cycle–Specific
Number of parameters	8	2	1	3	6*	12*	2	3
Eliaz et al. [13]	0.0317	0.102	0.113	0.0996	0.0950	0.0937	0.101	0.0961
Levasseur et al. [7]	0.119	0.131	0.130	0.113	0.112	0.110	0.128	0.128
Link et al. [14] HT29 cells	0.0397	0.0943	0.115	0.0921	0.0803	0.0595	0.115	0.0668
Link et al. [14] NMG64/84 cells	0.0814	0.0952	0.136	0.0937	0.0998	0.0998	0.131	0.131
Nguyen-Ngoc et al. [15]	0.0426	0.123	0.148	0.0689	0.0644	0.0598	0.148	0.0711
Rupniak et al. [16]	0.0425	0.201	0.246	0.0541	0.0535	0.0535	0.246	0.101
Vrignaud et al. [17]	0.0494	0.169	0.186	0.0972	0.0560	0.0471	0.186	0.186
Walker et al. [18]	0.0141	0.0269	0.0537	0.0261	0.0255	0.0255	0.0537	0.0537

*The Levasseur et al. [7] single and double Hill models have 7 and 13 parameters, respectively, but the parameter E_{con} was constrained to force survival relative to controls to equal 1 at zero concentration.

Table 2. Model Parameter Values Obtained from Fit to Data.

Data Set	k_1	k_2	K_i	k_3	k_4	K_e	m	n
Eliaz et al. [13]	0.0004926	0.9626	0.0003385	0.2932	1.191	0.2198	8.444	5.511
Levasseur et al. [7]	5.527	3.350	0.01593	0.06189	2.881	3.817	0.3950	5.878
Link et al. [14] HT29 cells	22.76	5.840	0.0009478	0.01450	1.495	0.1185	0.4578	3.979
Link et al. [14] NMG64/84 cells	5.952	0.8406	0.00003644	0.7725	0.2842	0.005309	0.2147	10.68
Nguyen-Ngoc et al. [15]	0.2257	1.299	5.711×10^{-6}	0.1549	1.240	0.6210	0.3594	4.809
Rupniak et al. [16]	34.78	0.6003	1.252×10^{-7}	0.03845	2784	429.7	4.845	0.7418
Vrignaud et al. [17]	9.227×10^{-6}	2.417	0.07207	1.205	1.581	1.148	0.6818	3.886
Walker et al. [18]	0.1048	0.9885	0.002017	3.026	2301	1352	25.89	2.136

Parameter values are based on fits to Eq. (7) with concentrations measured in micrograms per milliliter, and time in hours, except for the Levasseur et al. [7] data set, for which concentration is in micromolars.
In each case, $A = 1$.

Discussion

The present cellular pharmacokinetic model (Eq. (4)) provides a good fit to data for uptake by non-small cell lung cancer cells [10]. The model is further supported by data on doxorubicin uptake by Chinese hamster ovary cells [21], which also show uptake as a function of extracellular concentration at a fixed exposure time first increasing rapidly and nonlinearly, and then linearly at a slower rate at higher concentrations. Bates et al. [21] hypothesized that doxorubicin enters the cell only by passive diffusion, but at lower concentrations much of it is taken up rapidly by high-affinity binding sites. At higher concentrations, these binding sites are saturated and additional drug taken up by the cell remains unbound in the intracellular space, giving a linear uptake behavior. The model proposed here predicts the same behavior observed by Bates et al. [21], although here the saturability is interpreted in terms of a carrier for transmembrane transport rather than intracellular binding sites.

The model of Eq. (7) relates drug effect to the peak value of total intracellular concentration (as well as that of an extracellular species). In reality, nuclear doxorubicin is likely responsible for the primary intracellular mechanism of cell kill, namely topoisomerase II inhibition. The fact that we found peak total intracellular concentration to be a good predictor of drug activity may reflect a rapid equilibration of nuclear, DNA-bound doxorubicin with the free intracellular drug, so that the peak value of DNA-bound drug is proportional to that of total intracellular drug.

A unique feature of the model proposed here is that the dose–response curves converge to a single asymptotic curve as the exposure time is increased. This behavior has been observed for doxorubicin and other chemotherapeutic drugs including paclitaxel [22]. In the model, it results from the assumption that cell kill depends on peak concentrations. In models in which cell kill depends on a time integral of concentration, the survival fraction approaches zero as exposure time is increased. Several researchers [8,11,23–25]

have described the rate of chemotherapeutic drug-induced cell kill relative to the population size as a function of the instantaneous extracellular drug concentration only. In such models, the survival fraction similarly approaches zero as exposure time increases.

Relative to the present model, the Hill equation models of Levasseur et al. [7] give comparable fits to some experimental cytotoxicity data sets [7,17] based on the RMS residual values (Table 1). In some cases, however, the resulting survival curves intersect (Figures 2 and 5). This implies behavior in which increasing dose leads to increasing survival, which can only occur if the drug is self-inhibiting. The double Hill equation model of Levasseur et al. [7] can also show abrupt changes in survival with concentration (Figure 5), which are unlikely to be realistic. The two-mechanism peak concentration model does not show these types of behavior. Of the other models considered, the $C^n T$ model generally gives the next best fits to the experimental data. When $n > 1$, this model gives a cell kill that depends more strongly on concentration than on exposure time. In this sense, it approaches the behavior of the present model, in which cell kill depends only on concentration when the exposure time is long enough.

The present model contains a relatively large number of unknown parameters (eight). This number of parameters is needed to represent the observed behavior in cases where a plateau in survival occurs at high concentrations and short exposure times [13,17]. By comparison, the double Hill model [7], which can also represent such behavior, requires 12 unknown parameters. For some of the data sets [7,18], it was found that a simplified version of Eq. (7) with three independent unknown parameters:

$$S = \frac{1}{1 + A\left[k_1\left(1 - \exp(-k_3 t_{\exp})\right)\right]^n} \tag{10}$$

could fit the data almost equally well. In these cases, the plateau in survival was not seen, either because the range of exposure times was too limited or because different cell lines were used. The saturation in extracellular concentration shown by the Kerr et al. [10] uptake data is absent in Eq. (10), probably reflecting the fact that the data sets [7,18] covered limited ranges of concentration, over which uptake could be well approximated as nearly linear in c_e. In Table 2,

all the parameters for the model of Eq. (7) were given for consistency. For those data sets for which the simplified model of Eq. (10) provides an equally good fit, not all the parameters in Table 2 are individually meaningful, in the sense that other combinations of parameters could provide an equally good fit to the data.

Plateaus in the survival curve at high concentrations, as seen in Figures 2 and 5, have been discussed previously [26]. Disappointing results from high-dose chemotherapy with agents such as doxorubicin have been attributed to
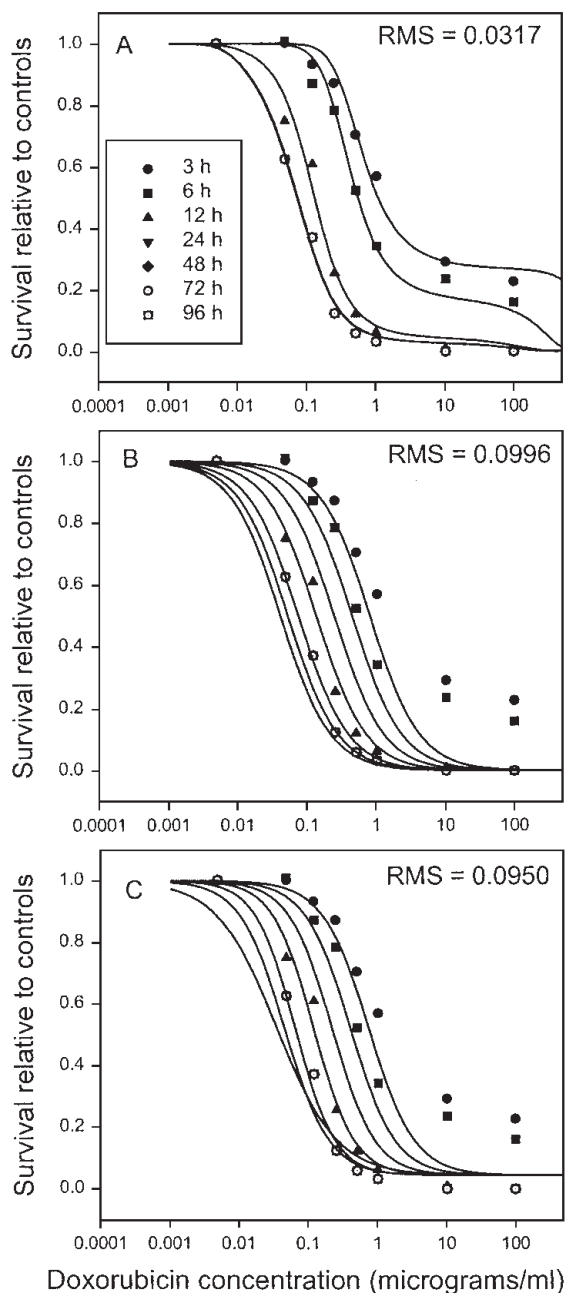

Figure 2. *Cellular pharmacodynamic model fits to data of Eliaz et al. [13] for B16F10 murine melanoma cells. (A) Two-mechanism peak concentration model. (B) C^nT model. (C) Single Hill model of Levasseur et al. [7]. For the exposure times 24, 48, 72, and 96 hours, the data overlapped and only one symbol is visible. The model prediction curves also coincide.*
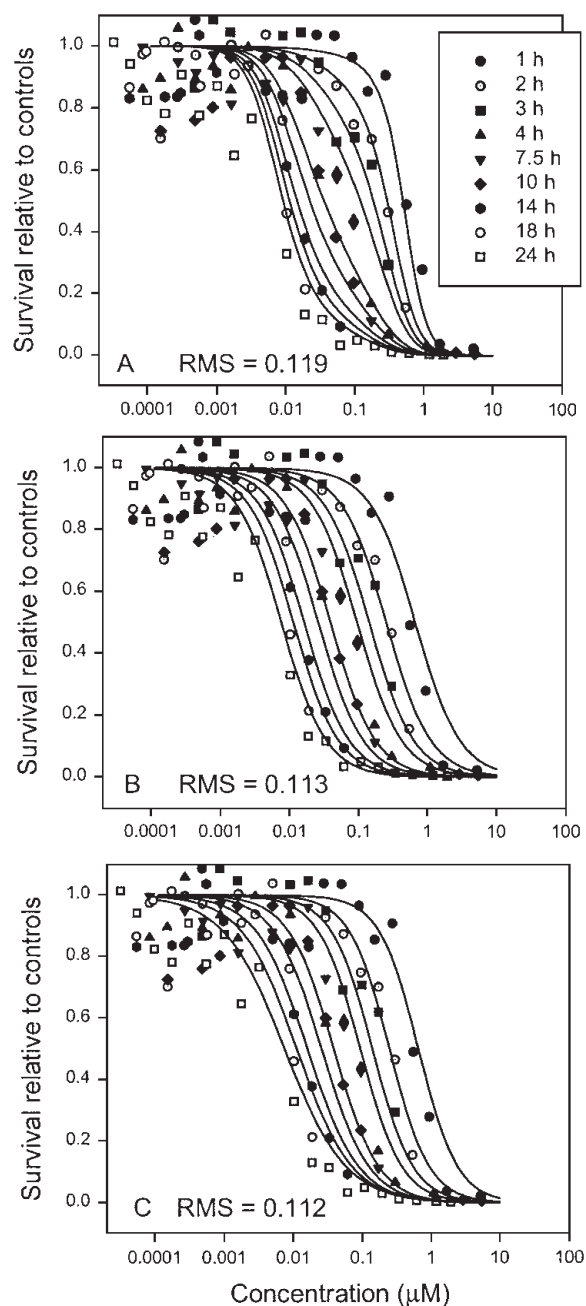

Figure 3. *Cellular pharmacodynamic model fits to data of Levasseur et al. [7] for doxorubicin effect on wild-type A2780 human ovarian carcinoma cells. (A) Two-mechanism peak concentration model. (B) C^nT model. (C) Single Hill model of Levasseur et al. [7].*

this effect [27]. Gardner [8] interpreted such plateaus as evidence of cell cycle phase specificity, reasoning that, at short exposure times, a fraction of the cells is not in the sensitive phase during exposure, so that cell kill is incomplete even at high concentrations. However, the cell cycle phase–specific model based on this concept [8] does not give a good fit to the data sets considered here showing such behavior [13,17].

In some cases, a further decline in survival at higher concentrations is seen, giving a "double sigmoidal" curve with two inflection points. Such behavior was observed by

Levasseur et al. [7] for paclitaxel and for the response of resistant cell lines to methotrexate, raltitrexed, and AG2034, although not for doxorubicin. They remarked that this phenomenon could be attributed to the existence of two or more intracellular drug targets, but considered it more likely to result from cellular heterogeneity. Their model is based on the superposition of two Hill equations representing two populations of different drug sensitivities. Such behavior can occur in the present model, as seen in Figure 2*A* for data at 6 hours [13]. It results from including both satu-
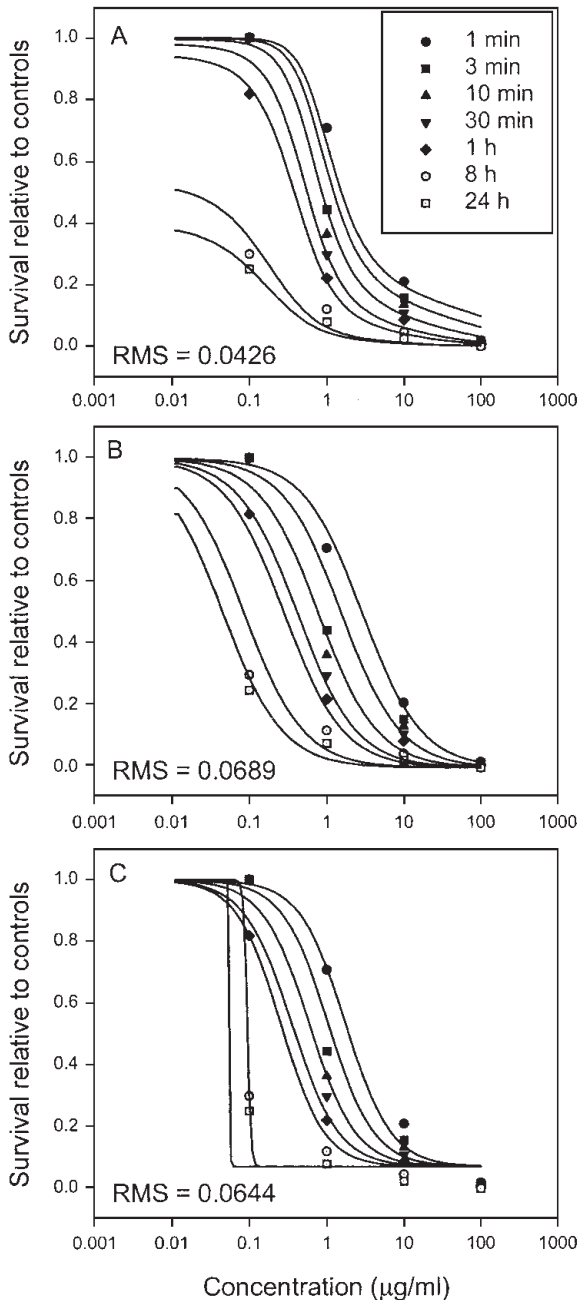

Figure 4. *Cellular pharmacodynamic model fits to doxorubicin cytotoxicity data of Nguyen-Ngoc et al. [15] for mouse sarcoma cells. (A) Two-mechanism peak concentration model. (B) C^nT model. (C) Single Hill model of Levasseur et al. [7].*
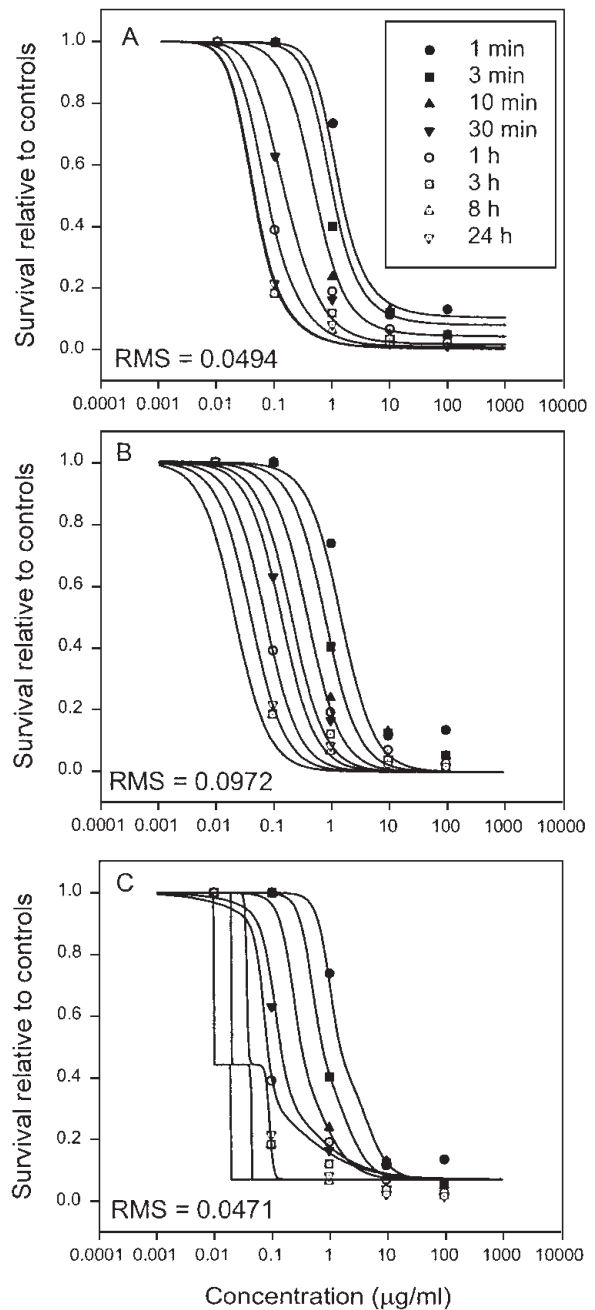

Figure 5. *Cellular pharmacodynamic model fits to data of Vrignaud et al. [17] data for doxorubicin cytotoxic effect on rat glioblastoma cells. (A) Two-mechanism peak concentration model. (B) C^nT model. (C) Double Hill model of Levasseur et al. [7].*

rable and nonsaturable terms in the cellular uptake kinetics (Eq. (4)). At low concentrations, the saturable uptake term in Eq. (4) is dominant. The dose–response curve initially levels off when this saturable uptake term reaches saturation with increasing concentration. Then, as the nonsaturable uptake term becomes dominant, the survival curve declines again. Given that wild cell populations are likely to have a continuous spectrum of sensitivities rather than two distinct subpopulations, the present model appears to provide a more satisfactory explanation for such behavior.
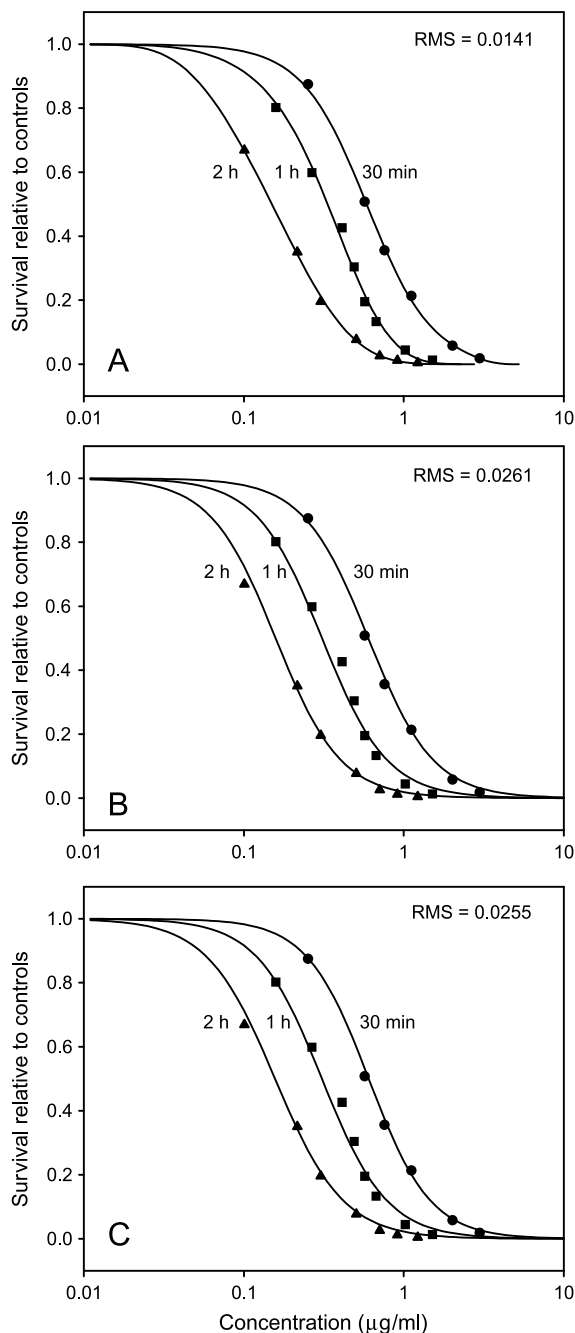
Figure 6. *Cellular pharmacodynamic model fits to data of Walker et al. [18] for doxorubicin effect on human bladder cancer cells. (A) Two-mechanism peak concentration model. (B) C^nT model. (C) Single Hill model of Levasseur et al. [7].*

According to the present model, survival is predicted to approach zero for sufficiently high concentrations if a non-saturable uptake mechanism is available (i.e., if k_1 is non-zero in Eq. (7)). This is the case for the fit to the data of Eliaz et al. [13], as shown in Figure 2A. However, the data of Vrignaud et al. [17] give a value of k_1 very close to zero and the survival curves show a plateau with no second inflection point for the range of concentrations used (Figure 5A). Neither data set has sufficient data points at very high concentrations to definitively show the eventual behavior. Also, none of the data sets considered here

resolves survival fractions relative to controls below 1%, and plateaus may be present at very low survival. Further data are needed to determine the possible role of this plateau effect in clinical trials of high-dose doxorubicin.

At high drug concentrations, significant cytotoxicity is observed even for exposure times of 1 hour or less (Figures 3–6). Such exposure times are much shorter than cell cycle times, which are typically about 20 hours or more for the cell lines considered [7,13,14,17,18]. Topoisomerase II inhibition is considered to be the main mechanism of doxorubicin-induced cell kill [9], and this mechanism may only be effective if the drug is present when cells pass through a specific phase of the cell cycle. Therefore, it might seem surprising that a large cell kill could be achieved with short exposure times. However, it should be noted that the measurements of cell survival are made well after the time of exposure, typically 1 to 4 days [13,15,16,18]. The present peak concentration model is consistent with the concept that a drug remains bound to nuclear structures after cellular exposure to the drug has ended, and has its effect during subsequent cell cycles.

The present model is a step toward understanding and quantifying the relation between drug administration and tumor response for the widely used anticancer drug, doxorubicin. The model is for cells in culture, where oxygenation, pH, cell density, cell attachment, and proliferation status are generally different than within tumors. Such factors may influence cytotoxicity [28,29]. In addition, plasma exposure and cellular exposure may differ significantly *in vivo* because of limitations in drug transport. Changes in cellular uptake or efflux of drug are important factors in cellular resistance to doxorubicin [30]. By explicitly representing the cellular uptake process and the sensitivity to the drug at a given intracellular level, the model proposed here provides a basis for analyzing experimental data obtained under conditions more representative of the physiological tumor environment. It is expected that this model will have use in future mathematical modeling of the response and optimization of doxorubicin chemotherapy.

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