Pharmacodynamic Modeling of Chemotherapeutic Effects: Application of a Transit Compartment Model to Characterize Methotrexate Effects in Vitro

Submitted: May 15, 2002; Accepted: September 22, 2002; Published October 29, 2002

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ABSTRACT The time course of chemotherapeutic effect is often delayed relative to the time course of chemotherapeutic exposure. In many cases, this delay is difficult to characterize mathematically through the use of standard pharmacodynamic models. In the present work, we investigated the relationship between methotrexate (MTX) exposure and the time course of MTX effects on tumor cell growth in culture. Two cancer cell lines, Ehrlich ascites cells and sarcoma 180 cells, were exposed for 24 hours to MTX concentrations that varied more than 700-fold (0.19-140 μg/mL). Viable cells were counted on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 20, 22, and 24 for Ehrlich ascites cells and on days 1, 2, 3, 5, 7, 9, 11, 13, 14, 15, 17, 19, and 21 for sarcoma 180 cells, through the use of a tetrazolium assay. Although MTX was removed 24 hours after application, cell numbers reached nadir values more than 100 hours after MTX exposure. Data from each cell line were fitted to 3 pharmacodynamic models of chemotherapeutic cell killing: a cell cycle phase-specific model, a phase-nonspecific model, and a transit compartment model (based on the general model recently reported by Mager and Jusko, *Clin Pharmacol Ther.* 70:210-216, 2001). The transit compartment model captured the data much more accurately than the standard pharmacodynamic models, with correlation coefficients ranging from 0.86 to 0.999. This report shows the successful application of a transit compartment model for characterization of the complex time course of chemotherapeutic effects; such models may be very useful in the development of optimization strategies for cancer chemotherapy.

KEYWORDS: methotrexate, cell growth inhibition, modeling, chemotherapeutic effect, transit compartment model.

INTRODUCTION Although several chemotherapeutics demonstrate short elimination half-lives (eg, less than 12 hours),[1, 2] maximal clinical effects (and toxicities) often occur 14 to 21 days following drug administration.[3-5] As such, peak drug concentrations often precede peak drug effects by days or weeks, greatly complicating attempts to relate the time course of drug exposure to the time course of drug effect. Such pharmacokinetic-pharmacodynamic (PKPD) relationships may be of great value, as they may facilitate the individualization and optimization of chemotherapy.

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Perhaps because of the inherent difficulties, relatively few PKPD modeling approaches have been developed to characterize the time course of chemotherapeutic effects.[6-8] In lieu of time-course models, PKPD individualization efforts have largely attempted to relate a time-averaged value of drug exposure (eg, the area under the plasma concentration–time curve) to peak effect or peak toxicity (eg, nadir white blood cell count).[9-13] Unfortunately, such "static" approaches are of limited value, as these models do not predict the time course of effect and, thus, may not be used to predict optimal schedules of drug administration. Additionally, it is desirable to relate effects to the entire time course of drug exposure, because chemotherapeutic effects are often found to be "protocol dependent" (ie, dependent on the duration of drug administration and on the duration of drug exposure),[14-16] where identical areas under the curve produce markedly different effects.

Recently, a simple, robust approach has been introduced to model PKPD time delays through the use of transit compartments.[17] In the present study, we wished to evaluate the transit compartment model for use in relating the time course of chemotherapeutic exposure to the time course of chemotherapeutic effect. Representative data were collected following investigations of the cytotoxic effects of MTX, an established anticancer drug, against 2 cancer cell lines in vitro. Consistent with expectations, the peak effect (ie, the time to reach the lowest cell number following MTX treatment) was delayed tremendously relative to the time course of MTX exposure in this experimental system. These data were modeled with the transit compartment model and with 2 established PKPD models of chemotherapeutic effects (ie, a phase-specific model and a phase-nonspecific model).[17-20] Relative to the established models, the transit compartment model was found to provide superior fitting of the data; consequently, this model may find broad application in the characterization of chemotherapeutic effects.

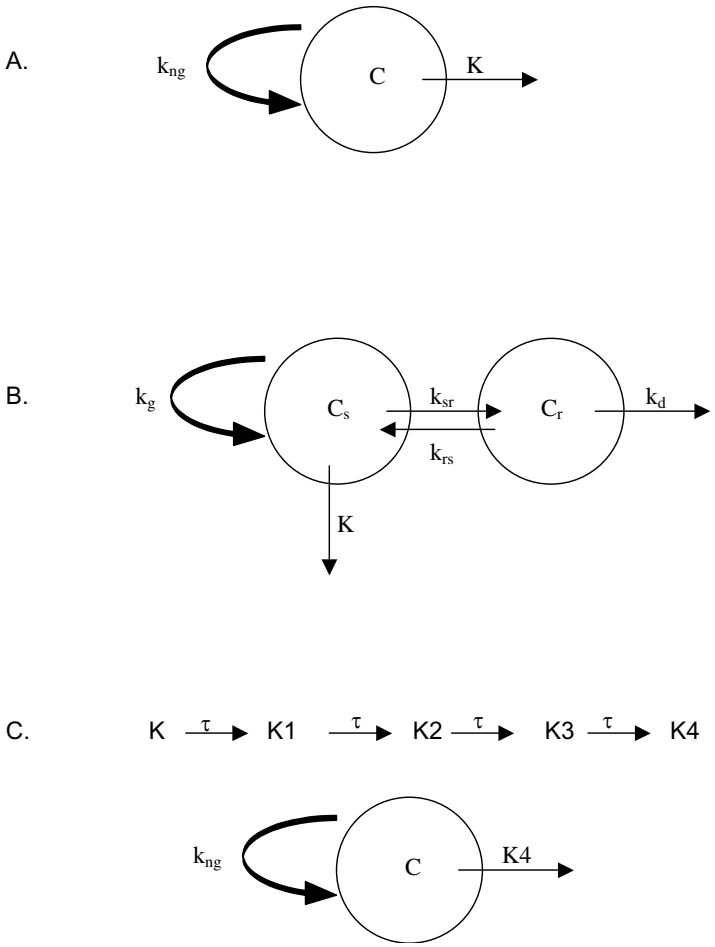

Figure 1. Schematic representation of the models evaluated for characterization of MTX cytotoxicity. (A) Phase-nonspecific model: C, viable cells; k_{ng}, net growth rate constant; K, cell kill constant. (B) Phase-specific model: C_s, cells sensitive to MTX; C_r, cells resistant to MTX; k_g, cell proliferation rate constant; k_d, cell loss rate constant; k_{sr} and k_{rs}, cell cycling rate constants. (C) Transit compartment model: K1, K2, K3, and K4 refer to the cell kill rate constants in the transit compartments; τ, transit time; k_{ng}, net growth rate constant; C, viable cells. For each model, the cell kill constant is a nonlinear function of MTX concentration: $K=K_{max}•M/(EC_{50}+M)$, where K_{max} is the maximal value of the cell kill constant, EC_{50} is a Michaelis constant, and M refers to the MTX concentration, which is a time-dependent variable.

MATERIALS AND METHODS

Materials

MTX and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma Chemical (St Louis, MO). Sodium dodecyl sulfate (SDS) was obtained from Bio-Rad Laboratories (Hercules, CA). Cell culture media (RPMI 1640), certified fetal bovine serum, and gentamicin were obtained from Invitrogen Corporation (Grand Island, NY). Ehrlich ascites cells and sarcoma 180 cells were obtained from American Type Cell Culture (Manassas, VA).

In vitro cell growth inhibition

The cancer cell lines were grown within a humidified, 5% CO_2 incubator at 37°C. RPMI 1640 media was prepared to contain approximately 10 ng/mL folic acid and was supplemented with 10% fetal bovine serum and gentamicin (100 μg/mL). Cell suspensions containing 10 000 cells/mL and 5000 cells/mL were prepared for Ehrlich ascites cells and sarcoma 180 cells, respectively, and 0.1 mL of suspension (ie, containing 1000 or 500 cells) was dispensed into wells of 12 96-well plates. After allowing the cells to attach for 48 hours, media was aspirated through a 25-gauge needle. Preliminary studies demonstrated that this method of aspirating media did not result in significant loss of cells (recovery was found to be 100.7 ± 6.3%, n = 12). Following aspiration, 100 μL of media containing MTX (0.19 μg/mL, 2.0 μg/mL, 14.0 μg/mL, or 140.0 μg/mL) was added. Each plate was prepared in an identical fashion, with 4 wells used for each concentration of MTX. Cells were incubated with MTX for 24 hours, and media was then aspirated. Each well was then washed 4 times with 100 μL of MTX-free media. Cell number was determined on days

1, 3, 5, 7, 9, 11, 13, 15, 17, 20, 22, and 24 for Ehrlich ascites cells and on days 1, 2, 3, 5, 7, 9, 11, 13, 14, 15, 17, 19, and 21 for sarcoma 180 cells, using 1 plate per assay, via the tetrazolium assay.[21] Briefly, on the day of analysis, media was aspirated from all wells of the assay plate. Exactly 100 μL of fresh media and 25 μL of MTT solution (5 mg/mL in phosphate saline buffer, pH 7.4) were added to each well, and the plate was then incubated for 4.5 hours at 37°C in the incubator. After incubation, 100 μL of 10% SDS-0.01 M hydrochloric acid (10%SDS-HCl) was added to each well, and the plate was incubated overnight at 37°C. Absorbance in each well was determined at 590 nm using a plate reader (Spectromax, Molecular Devices Sunnyvale, CA). Cell number was determined through the use of a standard curve (run on each plate) that related absorbance to cell count (linear ranges: 156 to 10 000 cells for sarcoma 180 cells, and 156 to 12 500 for Ehrlich ascites cells). Cell number was determined for each treatment group until assay response exceeded the upper limit of the standard curve (ie, where assay response indicated cell number greater than 10 000 cells/well or 12 500 cells/well for sarcoma 180 cells or Ehrlich ascites cells, respectively).

Pharmacodynamic modeling

Three pharmacodynamic models were used to fit the time course of MTX effects: (1) a cell cycle phase-nonspecific model of cytotoxicity, (2) a phase-specific model of cytotoxicity, and (3) a cell kill model that was based on the general transit compartment model of Mager and Jusko.[17] Schematic representations of the models are shown in **Figure 1**. Differential equations were as follows:

Phase-nonspecific model

$$\frac{dC}{dt} = k_{ng} \cdot C - K \cdot C$$

$$K = \frac{K_{\max} M}{EC_{50} + M}$$

Where C represents the cell number, k_{ng} is a first-order rate constant of net growth (mathematically equivalent to a first-order growth rate constant [k_g] minus a first-order death rate constant [k_d]; ie, $k_{ng} = k_g - k_d$), K is a nonlinear function of MTX cell kill, which is dependent on the MTX concentration in media, M; the maximal MTX cell kill rate, K_{max}; and a Michaelis constant, EC_{50}.

Phase-specific model

$$\frac{dC_s}{dt} = k_g \cdot C_s - k_{sr} \cdot C_s + k_{rs} \cdot C_r - K \cdot C_s$$

$$\frac{dC_r}{dt} = k_{sr} \cdot C_s - k_{rs} \cdot C_r - k_d \cdot C_s$$

$$K = \frac{K_{\max} M}{EC_{50} + M}$$

Two cell populations are assumed: a sensitive population (where C_s refers to the number of sensitive cells) and a resistant population (where C_r refers to the number of resistant cells). The first-order rate constants k_{sr} and k_{rs} refer to rates of cell cycling between the populations; k_g and k_d refer to rates of cell growth and cell death, respectively. K is as defined above.

Transit compartment model

$$\frac{dC}{dt} = k_{ng} \cdot C - K4 \cdot C$$

$$\frac{dK1}{dt} = \frac{1}{\tau}(K - K1)$$

$$\frac{dK2}{dt} = \frac{1}{\tau}(K1 - K2)$$

$$\frac{dK3}{dt} = \frac{1}{\tau}(K2 - K3)$$

$$\frac{dK4}{dt} = \frac{1}{\tau}(K3 - K4)$$

$$K = \frac{K_{\max} M}{EC_{50} + M}$$

C, K_{max}, EC_{50}, M, and K are as defined above; however, the operative rate function of MTX-induced cell killing, K4, is related to K via a series of transit compartments (ie, K1-K4). τ refers to the mean transit time in each transit compartment. As shown, the transit compartments delay the time course of cell kill, relative to the time course of drug exposure. Four transit compartments are employed in this model; however, only 1 parameter is used to describe transit kinetics (τ). As such, the model is both flexible (because of the number of transit compartments) and highly stable (because of the use of a small number of parameters [k_{ng}, K_{max}, EC_{50}, τ]).

Because K is a function of M, a time-dependent variable, each model is a dynamic model of drug effect. That is, the cell kill constant changes with time, as influenced by factors that control the time course of drug exposure (eg, the dosing regimen, the relevant pharmacokinetics of the system). Additionally, cell number is also a time-dependent variable. As such, each model allows dynamic characterization of cell growth and drug-induced cell killing. All model parameters were fitted to mean cell number versus time data, where mean number was determined from 4 wells assayed at each time point. Data for all MTX exposures were fitted simultaneously, for each cell line, with ADAPT II software.[22]

RESULTS

In vitro MTX cell inhibition

The time courses of cell growth for the 2 tumor cell lines are shown in **Figures 2, 3, and 4.** In the absence of MTX, each cell line demonstrated exponential growth. MTX induced concentration-dependent inhibition in apparent cell growth; however, MTX effects were significantly delayed relative to the time course of drug exposure. Little change in cell number was observed during the 24-hour incubation

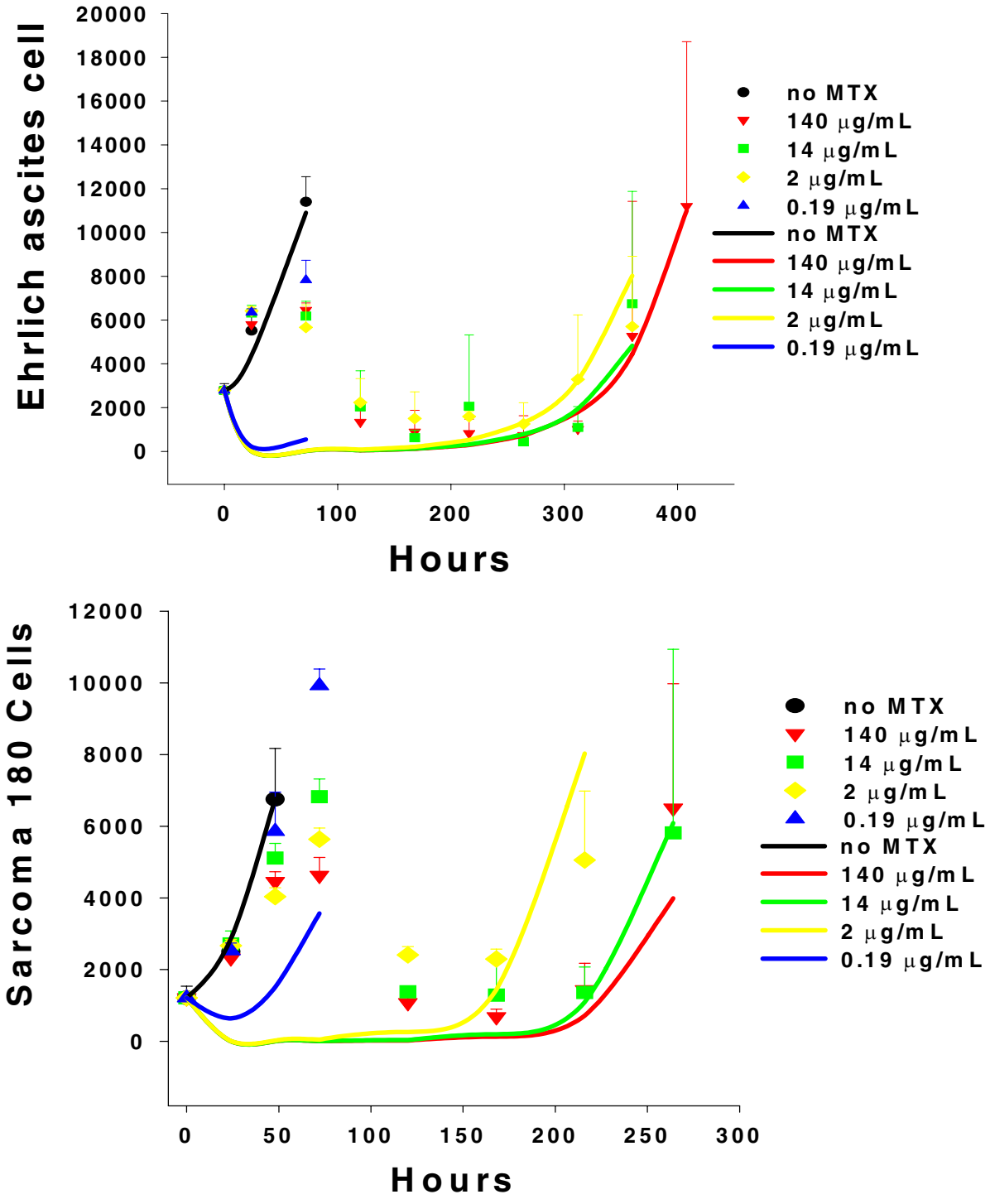

Figure 2. Phase-nonspecific model predictions of MTX effects on the time course of cell growth. Ehrlich ascites cells (A) and sarcoma 180 cells (B) were treated with MTX for 24 hours (concentrations ranging from 0 to 140 µg/mL). After removal of MTX, cells were fed with fresh media every 48 hours. Solid symbols and bars refer to the mean and SD (n = 4). Model-predicted profiles are shown as solid lines.

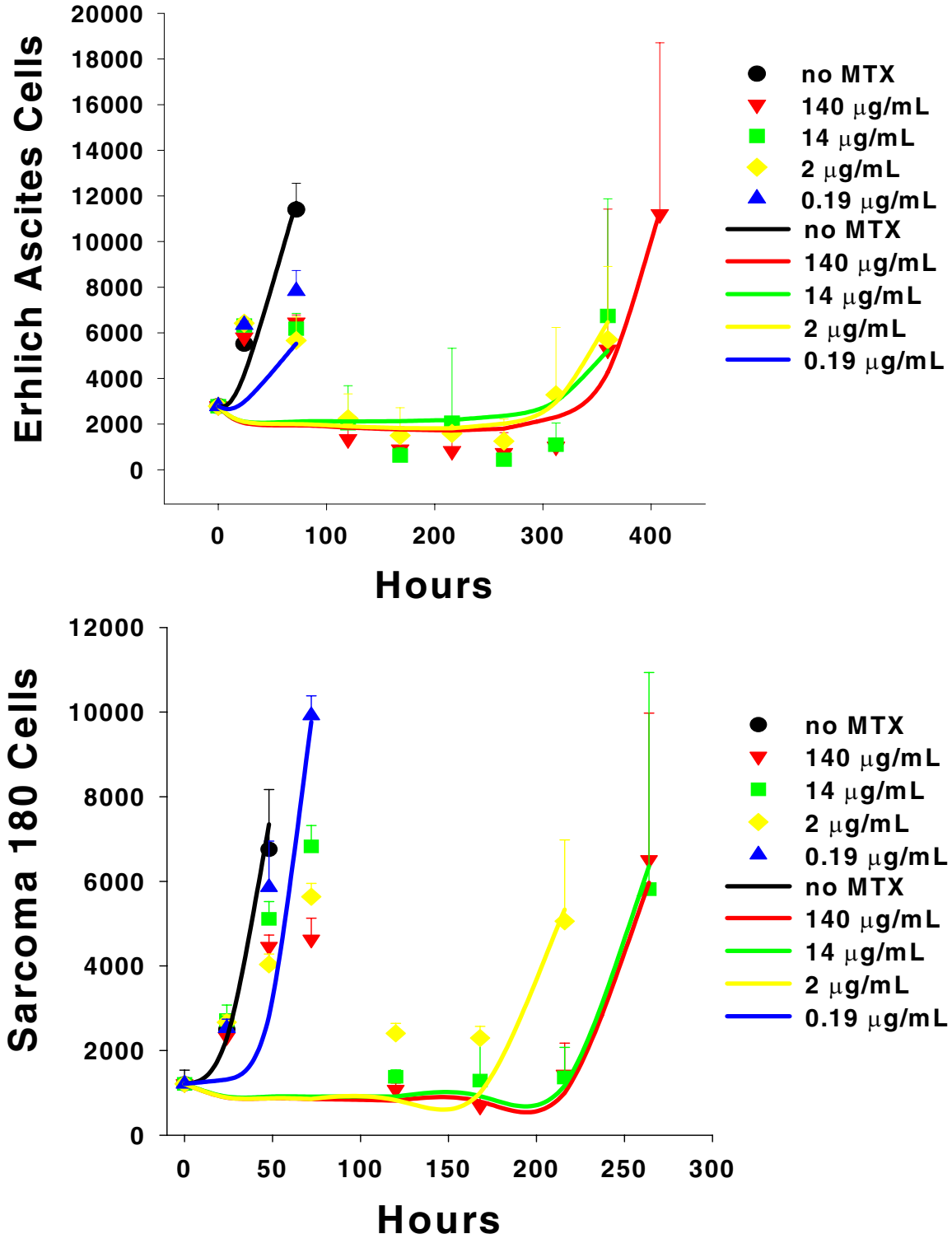

Figure 3. Phase-specific model predictions of MTX effects on the time course of cell growth. Ehrlich ascites cells (A) and sarcoma 180 cells (B) were treated with MTX for 24 hours (concentrations ranging from 0 to 140 μg/mL). After removal of MTX, cells were fed with fresh media every 48 hours. Solid symbols and bars refer to the mean and SD (n = 4). Model-predicted profiles are shown as solid lines.

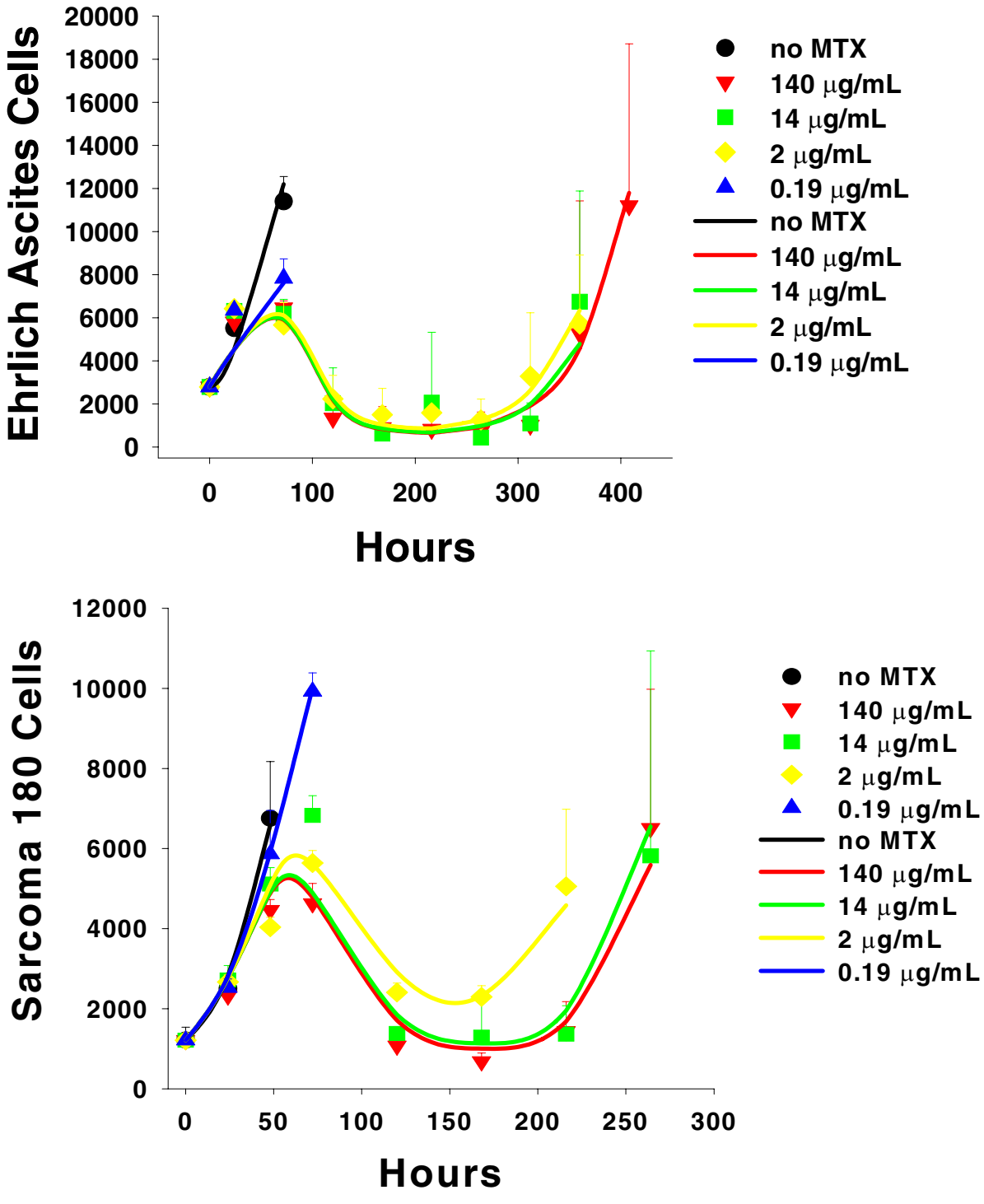

Figure 4. Transit compartment model predictions of MTX effects on the time course of cell growth. Ehrlich ascites cells (A) and sarcoma 180 cells (B) were treated with MTX for 24 hours (concentrations ranging from 0 to 140 μg/mL). After removal of MTX, cells were fed with fresh media every 48 hours. Solid symbols and bars refer to the mean and SD (n = 4). Model-predicted profiles are shown as solid lines.

of MTX (ie, immediately following MTX removal, cell number was not significantly different from the untreated cells, $P > .05$ for each cell line). Cell number reached nadir values at 168 hours for sarcoma 180 cells and at 264 hours for Ehrlich ascites cells. The rate of cell growth after recovery from MTX treatment appeared to be similar to that of the untreated cells.

Pharmacodynamic modeling

Best-fit predictions of the phase-nonspecific model, following simultaneous fitting to the data, are shown in **Figure 2**. The model provided satisfactory characterization of cell growth for untreated cells (r^2 = 0.999, for each cell line). Model predictions are close to observed values of cell number in the "recovery phase" (ie, after 150-200 hours); however, the model was unable to capture the observed increase in cell number that occurred during MTX incubation, severely underpredicting cell number between 24 hours and 120 hours for each cell line. Model predictions of cell number data following MTX treatment were generally poor (**Figure 2**), with correlation coefficients (r^2) between predicted and observed values of cell number as low as 0.099 (eg, for 2 μg/mL MTX applied to Ehrlich ascites cells). Additionally, parameter estimates were associated with high variability (eg, EC_{50} = 0.21 [percentage coefficient of variation, %CV, = 89%] for Ehrlich ascites cells, EC_{50} = 0.84 [%CV = 35%] for sarcoma 180 cells).

Predictions of the phase-specific model are shown in **Figure 3**. As with the nonspecific model, the phase-specific model provided poor predictions of the cell counts in MTX-treated wells between 24 hours and 120 hours. Correlation coefficients for the fitted curves were generally poor, ranging from 0.16 to 0.99, and parameter estimates were associated with high variability (ie, %CV values and 95% confidence intervals were too large for estimation by ADAPT II). The phase-specific model, which had twice the number of fitted parameters of the phase-nonspecific model (ie, 6 vs 3 parameters), was found to have a superior Akaike criterion value (ie, 480 vs 489 for fitting to sarcoma 180 data, and 556 vs 574 for fitting to Ehrlich ascites data), which suggests that this model may be superior to the phase-nonspecific model for fitting to these data.

Predictions of the transit compartment model are presented in **Figure 4**. As shown, the transit compartment model provided satisfactory characterization of the entire time course of cell count data, for each cell line. Correlation coefficients ranged from 0.86 to 0.999. We also evaluated models with 2, 3, and 5 transit compartments (not shown), and we found that the present model, with 4 transit compartments, provided superior fitting to the data. Estimated parameter values for fitting to the Ehrlich ascites cell line were τ = 34.1 hours (%CV = 3.4), K_{max} = 0.29 h^{-1} (%CV = 5.8), EC_{50} = 0.1 μg/mL (%CV = 48.7), and k_{ng} = 0.02 h^{-1} (%CV = 4.9). Doubling time for Ehrlich ascites cells was estimated from k_{ng} to be 34.6 hours. Estimated parameter values following fitting to the sarcoma 180 data were τ = 30.0 hours (%CV = 2.5), K_{max} = 0.34 h^{-1} (%CV = 2.1), EC_{50} = 0.32 μg/mL (%CV = 14.2), and k_{ng} = 0.035 h^{-1} (%CV = 1.9). The doubling time for sarcoma 180 was estimated to be 19.8 hours.

DISCUSSION The pharmacodynamics of chemotherapeutics have been extensively investigated in vitro and in vivo. In most experimental settings, chemotherapeutic effects are significantly delayed relative to chemotherapeutic exposure. The time course of drug effect is of great interest in the field of cancer chemotherapy; however, because of the lack of simple mathematical models capable of characterizing the time course of effect, most analyses have reported relationships of drug exposure to drug effect at a fixed time point (eg, often at the time of peak effect for in vivo studies, and often at an arbitrary time point for in vitro studies).[10, 12, 13, 23, 24] When drug effect is examined at a fixed time point, exposure-effect relationships may be easily characterized through the use of simple, static relationships (eg, the Hill function: $Effect = \frac{E_{max} \bullet C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}$, where E_{max}, EC_{50}, and γ are constants, and where C may refer to the chemotherapeutic dose, steady-state concentration, or, most commonly, area under the concentration vs time curve). Static exposure-effect relationships have been successfully applied for the optimization of chemotherapy in several cases;[9, 25, 26] however, characterization of the entire time course of chemotherapeutic effect may provide additional information to allow further optimization. For example, modeling of the time course of drug effect may facilitate the scheduling of subsequent courses of chemotherapy and may assist in the selection of optimal drug combinations.

In the early 1970s, Jusko proposed 2 models to describe the kinetics of chemotherapeutic effects (as shown in **Figure 1**).[18, 19] For each of these models, the kinetics of cell killing is defined to be directly related to drug concentrations in plasma. As such, these models are not well suited to characterize chemotherapeutic effects in cases where effect is substantially delayed relative to the time course of drug exposure. Perhaps because chemotherapeutic effects often appear days or weeks following drug exposure, these models have not found wide use in the field of cancer chemotherapy.

Time delays between the time course of drug exposure and the time course of drug effects have been typically described through the use of "effect site" models or "indirect response" models. The effect site model attributes the delay in effect to the delay associated with drug distribution to the site of effect.[27] Indirect response models predict a delayed time course of apparent drug response as a consequence of indirect mechanisms of drug action (eg, where the drug may act via stimulation or inhibition of the processes involved in the production or loss of the measured response).[28] Recently, Sun and Jusko proposed a transit compartment model to characterize delayed drug effects, where the time delay is captured through the use of

Table 1. Akaike Information Criterion Values for the Models Evaluated

Model	Ehrlich Ascites	Sarcoma 180
Phase nonspecific	574	489
Phase specific	556	480
Transit compartment	501	407

a series of transit compartments.[20] The model, which was originally developed to describe the kinetics of signal transduction, was later shown to have general utility in characterizing effects that occur via a cascade.[17] We were interested in evaluating this model for utility in characterizing chemotherapeutic effects, which often occur via a complicated cascade of events.

In the present work, the time course of MTX effects on cancer cell growth was assessed in vitro, following 24 hours of MTX exposure to sarcoma 180 cells and Ehrlich ascites cells, grown in culture. Consistent with the results of studies investigating MTX effects in vivo,[29] we observed a significant delay between the time course of MTX exposure and the time course of MTX effects in this model system. For example, at the conclusion of MTX exposure (ie, 24 hours after the initiation of MTX treatment), no difference was found when comparing cell number in MTX-treated wells and untreated wells (**Figure 4**, $P > .05$), and the apparent "peak effect" (ie, the nadir cell count) occurred at 168 hours and at 264 hours for sarcoma 180 cells and Ehrlich ascites cells, respectively.

Data were fitted to 3 pharmacodynamic models, including a transit compartment model and 2 established models of chemotherapeutic effect, the cell cycle phase-specific model and the phase-nonspecific model. The 3 models are similar in many respects. For example, each model assumes that cell growth follows first-order kinetics. This assumption appears appropriate for our data, as cell number increased exponentially in the absence of MTX treatment (and also following recovery from MTX treatment). However, each model may be modified to incorporate more complex growth functions, as may be needed to describe cell growth in certain experimental settings. Additionally, each model relates MTX effect (ie, cell killing) to MTX concentration in media. It is quite likely that there is a complex relationship between MTX concentration in media and MTX concentrations at the biophase (eg, the intracellular site of MTX effect), and it is plausible that the kinetics that define this relationship may contribute to the observed dissociation between the time course of MTX treatment and the time course of MTX effect. We have made no attempt to characterize the kinetics of MTX uptake or intracellular processing, and the transit compartmental model does not attempt to infer MTX concentrations at the biophase (ie, this is not a variant of an "effect site" model). As such, the models may be described as "traditional" pharmacodynamic models because they relate drug effect to drug concentrations in an accessible fluid (eg, media, plasma). Nonetheless, the cell kill rate function of each model may be easily modified to be a function of MTX concentrations at the biophase (if these concentrations are known).

The most important difference between the transit compartment model and the standard models of chemotherapeutic effect is that the transit compartment model incorporates a series of first-order transfer steps to allow characterization of delays between drug exposure and cell killing. The phase-nonspecific model and the phase-specific model assume a direct relationship between MTX concentration and cell killing; consequently, these models predicted a rapid decrease in cell number during the course of MTX exposure (**Figures 2** and **3**). However, as noted above, and as shown in **Figures 2** through **4**, no decrease in cell number was observed during the 24 hours of MTX treatment. As such, the standard chemotherapeutic models dramatically under predict cell numbers at early points in the study. The cell cycle phase-specific model appeared to characterize the data better than the phase-nonspecific model both visually and based on model-fitting criteria (**Table 1**); however, neither model provided predictions consistent with the observation of continued cell growth during the 24-hour treatment with MTX. On the other hand, we found that the transit compartment model could adequately describe the entire cell count versus time profile, for each cell line, for each treatment (ie, without MTX exposure, or for 24-hour MTX exposure at concentrations ranging from 0.19 to 140 μg/mL). Consistent with the observed data, this model predicted increases in cell number during the MTX treatment, with nadir cell counts occurring 100 to 200 hours after MTX removal.

Perhaps because of the simplicity of the transit compartment model, parameters were estimated with good precision (**Tables 2** and **3**). The estimated %CV of EC_{50} for Ehrlich ascites cells was 49%; however, estimated %CV values were lower than 15% for all other fitted parameters. The high %CV associated with the Ehrlich ascites EC_{50} is likely due, in part, to unavailability of data near or below the estimated EC_{50} (ie, the estimated EC_{50} was 0.1 μg/mL, which was lower than the lowest MTX concentration used in this study, 0.19 μg/mL). The transit time was found to be similar for the 2 cell lines and was estimated to be 30 hours and 34 hours for sarcoma 180 cells and Ehrlich ascites cells, respectively. The values of the parameters K_{max} and EC_{50} were dependent on the tumor cell lines. The EC_{50} for Ehrlich ascites cells (0.1 μg/mL) was 3 times lower than the EC_{50} of sarcoma 180 (0.32 μg/mL), suggesting that the Ehrlich ascites cell line was more sensitive to the cell-killing effect of MTX.

Table 2. Parameter Estimates Obtained Following Analysis of Ehrlich Ascites Data*

Parameter	Estimated Value	SD	%CV
Phase nonspecific			
k_{ng} (h^{-1})	0.02	0.0001	6.3
K_{max} (h^{-1})	0.26	0.01	3.9
EC_{50} (μg/mL)	0.21	0.19	88.8
Phase specific			
k_g (h^{-1})	0.06	NE	—
k_d (h^{-1})	0.001	NE	—
k_{sr} (h^{-1})	0.036	NE	—
k_{rs} (h^{-1})	0.25×10^{-5}	NE	—
K_{max} (h^{-1})	1.9	NE	—
Transit compartment			
k_{ng} (h^{-1})	0.02	0.001	4.9
τ (h)	34.1	1.2	3.4
K_{max} (h^{-1})	0.29	0.017	5.8
EC_{50} (μg/mL)	0.1	0.05	48.7

*CV indicates coefficient of variation; NE, not estimated

Table 3. Parameter Estimates Obtained Following Analysis of Sarcoma 180 Data*

Parameter	Estimated Value	SD	%CV
Phase nonspecific			
k_{ng} (h^{-1})	0.036	0.001	3.0
K_{max} (h^{-1})	0.35	0.02	6.5
EC_{50} (μg/mL)	0.84	0.29	35
Phase specific			
k_g (h^{-1})	0.06	NE	—
k_d (h^{-1})	0.0009	NE	—
k_{sr} (h^{-1})	0.0009	NE	—
k_{rs} (h^{-1})	0.87×10^{-7}	NE	—
K_{max} (h^{-1})	2.9	NE	—
EC_{50} (μg/mL)	10.5	NE	—
Transit compartment			
k_{ng} (h^{-1})	0.035	0.0007	1.9
τ (h)	30.0	0.78	2.6
K_{max} (h^{-1})	0.34	0.007	2.1
EC_{50} (μg/mL)	0.32	0.05	14.2

*CV indicates coefficient of variation; NE, not estimated

Of note, this model utilizes a series of transit compartments to describe delays between the time course of drug exposure and the time course of drug effect. The model has a mechanistic foundation, where transit compartments are related to a cascade of kinetic events that precipitate drug effects. However, the model structure also serves as an exceptionally robust, empiric framework for characterization of delayed drug effects. For example, it is likely that a cascade of events precipitate MTX-induced cellular toxicity (eg, MTX uptake into cells, MTX diffusion to the site of action, MTX binding to and competitive inhibition of dihydrofolate reductase, depletion of reduced folates, interruption of cellular synthetic processes). The transit compartment model structure is amenable to the characterization of the time course of each event, where different τ values might be used in conjunction with a transit compartment associated with each event. However, in lieu of data sufficient for characterizing each event in the cascade, the transit compartment model may be used empirically, where the number of compartments is determined by the fitting of the data (ie, similar to the approach used for fitting data to a 1-, 2-, or 3-compartment pharmacokinetic model). In the present application, the model has been used empirically, and only pure

speculation would allow discussion of the biologic meaning of each compartment in the present model. Nonetheless, the present study demonstrates the robustness of the model, as we found that a simple transit compartment model, consisting of only 4 parameters, provided very good characterization of the relatively complex time course of cell growth following MTX exposure in vitro. This simple model may be easily modified to characterize in vivo data by defining the cell kill function (ie, $K = \frac{K_{max} M}{EC_{50} + M}$) as a function of plasma MTX concentrations (eg, M = MTX plasma concentration), where MTX concentrations in plasma may be defined by an appropriate pharmacokinetic function.

In this work, we have investigated the usefulness of the transit compartment model to characterize MTX cytotoxic effects in vitro. We successfully characterized the complex time course of cell growth in the presence of MTX with a simple pharmacodynamic model. The transit compartment model may find utility as a general model to characterize chemotherapeutic effects that are delayed relative to chemotherapeutic exposure. Modeling the time course of chemotherapeutic effects is desirable because it may facilitate the development of individualization and optimization strategies for chemotherapy.

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