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## Research Paper

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# Quasi-Equilibrium Pharmacokinetic Model for Drugs Exhibiting Target-Mediated Drug Disposition

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**Purpose.** The aim of this study is to derive and evaluate an equilibrium model of a previously developed general pharmacokinetic model for drugs exhibiting target-mediated drug disposition (TMDD).

**Methods.** A quasi-equilibrium solution to the system of ordinary differential equations that describe the kinetics of TMDD was obtained. Computer simulations of the equilibrium model were carried out to generate plasma concentration-time profiles resulting from a large range of intravenous bolus doses. Additionally, the final model was fitted to previously published pharmacokinetic profiles of leukemia inhibitory factor (LIF), a cytokine that seems to exhibit TMDD, following intravenous administration of 12.5, 25, 100, 250, 500, or 750  $\mu\text{g}/\text{kg}$  in sheep.

**Results.** Simulations show that pharmacokinetic profiles display steeper distribution phases for lower doses and similar terminal disposition phases, but with slight underestimation at early time points as theoretically expected. The final model well-described LIF pharmacokinetics, and the final parameters, which were estimated with relatively good precision, were in good agreement with literature values.

**Conclusions.** An equilibrium model of TMDD is developed that recapitulates the essential features of the full general model and eliminates the need for estimating drug-binding microconstants that are often difficult or impossible to identify from typical *in vivo* pharmacokinetic data.

**KEY WORDS:** leukemia inhibitory factor; mathematical model; nonlinear pharmacokinetics; target-mediated drug disposition.

## INTRODUCTION

The traditional paradigm of pharmacokinetic/pharmacodynamic (PK/PD) systems analysis typically involves a separation principle, whereby the time courses of plasma drug concentrations are modeled first and then fixed as driving functions in appropriate mechanism-based PD models (1,2). It is generally assumed that the amount of drug distribution to a biophase or drug interacting with a pharmacological target is negligible and does not affect PK profiles. Target-mediated drug disposition (TMDD) represents a distinct case wherein this assumption is invalid, and a significant proportion of the drug (relative to dose) is bound with high affinity to a receptor, enzyme, or transporter (3). Such interactions may result in nonlinear dose-dependent effects on drug disposition, with a decreasing apparent volume of distribution with increasing dose most commonly observed. When drug-target binding is also implicated in the elimination of the drug, such as receptor-mediated endocytosis, then concentration-dependent drug elimination may manifest as well (4).

A general PK model of TMDD has been described (5) and has since been utilized to characterize the PK of several

compounds in humans and animals, including: bosentan and imirestat (5), interferon- $\beta$ 1a (6,7), vascular endothelial growth factor (8), warfarin (9), thrombopoietin (10), and leukemia inhibitory factor (LIF) (11). This modeling approach essentially integrates the thermodynamics of drug-target binding within the system of ordinary differential equations that describe drug disposition (see Theoretical), and drug-binding microconstants ( $k_{\text{on}}$  and  $k_{\text{off}}$ ) are included in the model. However, these parameters are sometimes difficult to estimate from routine *in vivo* PK data. It has been shown, for example, that  $k_{\text{on}}-k_{\text{off}}$  parameters were not uniquely identifiable when the TMDD model was applied to abciximab PK/PD profiles, and a final stable model was achieved by assuming a quasi-equilibrium between free and bound drug concentrations (12,13). For the abciximab model, assuming a quasi-equilibrium state, along with some additional modifications, reduces one case of the TMDD model into one of the general nonlinear binding models originally described by Wagner (14). Although such a model was successfully applied to abciximab, these models do not allow for temporal changes in the homeostasis of the pharmacological target (e.g., production, degradation, and density up- and down-regulation). Drug exposure after relatively large doses or following multiple dosing may result in functional adaptation processes, and changes in receptor or enzyme kinetics may further impact the disposition of compounds exhibiting TMDD. Here we derive a quasi-equilibrium solution to the general TMDD model and evaluate its

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properties through computer simulations and nonlinear regression fitting to previously published LIF PK data resulting from the administration of a large range of intravenous (IV) doses in sheep (11).

## THEORETICAL

The details of the general pharmacokinetic model of TMDD have been introduced previously (5). As shown in Fig. 1, the key feature of this model responsible for observable nonlinear pharmacokinetic behavior is the saturable, high-affinity binding of the drug to its pharmacological target. Drug in the central compartment ( $C$ ) binds at the second-order rate ( $k_{on}$ ) to free receptors ( $R$ ) to form a drug receptor complex ( $RC$ ). The  $RC$  complex may dissociate at the first-order rate ( $k_{off}$ ) or be internalized and degraded by the first-order rate process of endocytosis ( $k_{int}$ ). Free drug can also be directly eliminated at a first-order rate ( $k_{el}$ ) or be distributed to a nonspecific tissue-binding site ( $A_T$ ) by first-order processes ( $k_{tp}$  and  $k_{pt}$ ). Free receptor can be synthesized at a zero-order rate ( $k_{syn}$ ) and degraded at a first-order rate ( $k_{deg}$ ). The input rate [ $In(t)$ ] to the free drug compartment accounts for any process (IV bolus, zero-order infusion, first-order absorption, etc.) that may require additional model components. Free drug  $C$ , free receptor  $R$ , and drug-receptor complex  $RC$  are expressed in molar concentrations, whereas  $A_T$  denotes amount (moles) of nonspecifically tissue bound or distributed drug. The model equations are as follows (5):

$$\frac{dC}{dt} = In(t) - k_{on}R \cdot C + k_{off}RC - (k_{el} + k_{pt})C + k_{tp} \cdot A_T/V_c \quad (1)$$

$$\frac{dA_T}{dt} = k_{pt}CV_c - k_{tp} \cdot A_T \quad (2)$$

$$\frac{dR}{dt} = k_{syn} - k_{on}R \cdot C + k_{off}RC - k_{deg}R \quad (3)$$

$$\frac{dRC}{dt} = k_{on}R \cdot C - (k_{off} + k_{int})RC \quad (4)$$

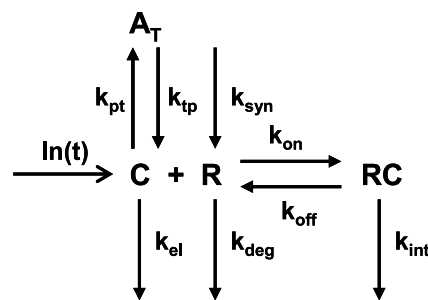
where  $V_c$  denotes the apparent volume of the free drug compartment. If the free drug is endogenously present (e.g., hormone or enzyme), then the initial conditions for the above system will be defined by the steady-state (baseline) values:

$$\begin{aligned} C(0) &= Dose/V_c + C_{ss}; \quad A_T(0) = A_{Tss}; \\ R(0) &= R_{ss}; \quad RC(0) = RC_{ss} \end{aligned} \quad (5a, b, c, d)$$

assuming that the free drug is administered as an IV bolus dose.

Often, the binding of drug to free receptor and dissociation of the drug-receptor complex are of several orders of magnitude faster than the remaining system processes. Thus, equilibrium between the binding and dissociation is achieved almost instantly and observable data contain limited information about these processes. Consequently, the estimation of the parameters  $k_{on}$  and  $k_{off}$  becomes difficult, if not impossible. To resolve this problem, we additionally assume that free drug, free receptor, and drug-receptor complex are at quasi-equilibrium or rapid equilibrium (15):

$$\frac{R \cdot C}{RC} = \frac{k_{off}}{k_{on}} \equiv K_D \quad (6)$$



**Fig. 1.** General pharmacokinetic model of target-mediated drug disposition. Adapted from Mager and Jusko (5). Symbols are defined in Theoretical.

where  $K_D$  denotes the equilibrium dissociation constant. The quasi-equilibrium assumption [Eq. (6)] adds to the system [Eqs. (1)–(4)] and results in five equations describing four unknowns. To reduce the number of equations, one can add side by side Eq. (1) to Eq. (4) and Eq. (3) to Eq. (4) and introduce new system variables:

$$C_{tot} = C + RC \quad (7a)$$

and

$$R_{tot} = R + RC \quad (7b)$$

where  $C_{tot}$  can be interpreted as the total drug concentration and  $R_{tot}$  as the total receptor concentration. Then the system will be described by four variables  $C$ ,  $C_{tot}$ ,  $R_{tot}$ , and  $A_T$  and the following four equations:

$$\frac{dC_{tot}}{dt} = In(t) - k_{int}C_{tot} - (k_{el} + k_{pt} - k_{int})C + k_{tp} \cdot A_T/V_c \quad (8)$$

$$\frac{dA_T}{dt} = k_{pt}CV_c - k_{tp}A_T \quad (9)$$

$$\frac{dR_{tot}}{dt} = k_{syn} - (k_{int} - k_{deg})(C_{tot} - C) - k_{deg}R_{tot} \quad (10)$$

$$\begin{aligned} C &= 1/2 \left[ (C_{tot} - R_{tot} - K_D) \right. \\ &\quad \left. + \sqrt{(C_{tot} - R_{tot} - K_D)^2 + 4K_D C_{tot}} \right] \end{aligned} \quad (11)$$

Derivations are provided in Appendix A. The initial conditions for Eqs. (8)–(10) are determined by the steady-state (baseline) values:

$$\begin{aligned} C_{tot}(0) &= Dose/V_c + C_{totss}; \\ A_T(0) &= A_{Tss}; \\ R_{tot}(0) &= R_{totss} \end{aligned} \quad (12a, b, c)$$

which are calculated in Appendix B and will be nonzero only if the endogenous production of free drug is present.

The assumption of fast drug-receptor binding allows the TMDD model to be simplified to its quasi-equilibrium form. However, the equilibrium model may contain parameters, which might not be identifiable from real data sets, and further simplifications should follow. If the receptor turnover is slow with respect to the experimental time scale, then one can

postulate to let the  $k_{\text{syn}}$  and  $k_{\text{deg}}$  parameters to be small such that the baseline value for the free receptors  $R_0$  is constant:

$$k_{\text{syn}} \rightarrow 0, \quad k_{\text{deg}} \rightarrow 0, \quad \text{and} \quad k_{\text{syn}}/k_{\text{deg}} = R_0 \quad (13a, b, c)$$

This simplifies Eq. (10) to yield:

$$\frac{dR_{\text{tot}}}{dt} = -k_{\text{int}}(C_{\text{tot}} - C) \quad (14)$$

Also, the steady state for  $R_{\text{tot}}$  [Eq. (12c)] will be undetermined. Providing that there is no prior exposure of receptors to drug, the initial condition for  $R_{\text{tot}}$  should be:

$$R_{\text{tot}}(0) = R_0 \quad (15)$$

If the receptors were partially occupied prior to the experiment, then  $R_{\text{tot}}(0)$  should become a model parameter because determination of the free receptor baseline values would not be possible.

Further simplifications can be made by assuming that the internalization rate is slow ( $k_{\text{int}} \approx 0$ ). Then Eq. (14) implies that the total receptor concentration will remain constant ( $R_{\text{tot}} \equiv R_0$ ), and the model equations will consist of Eqs. (8), (9), and (11). Such a model has been introduced previously by Wagner to describe drug concentration in plasma that is eliminated by fast binding to tissue and a parallel first-order process as well as distributed to a peripheral compartment (14). In this terminology, our free receptor compartment represents the tissue, and  $R_{\text{tot}}$  is the maximum receptor (tissue) capacity. Additionally, the differential equation for the variable  $C$  was derived. We can obtain it by differentiating with respect to time the equilibrium Eq. (6) (see Appendix C):

$$\frac{dC}{dt} = \frac{\ln(t) - (k_{\text{el}} + k_{\text{pt}})C + k_{\text{tp}} \cdot A_{\text{T}}/V_{\text{c}}}{1 + \frac{R_{\text{tot}}C}{(K_{\text{D}} + C)^2}} \quad (16)$$

with the initial condition  $C(0) = \text{Dose}/V_{\text{c}}$ . Thus, the quasi-equilibrium model with slow receptor turnover and internalization reduces to the Wagner nonlinear tissue-binding model that can be described by a single equation [Eq. (16)].

## METHODS

### Quasi-Equilibrium Approximation

The quasi-equilibrium assumption [Eq. (6)] is based on the observation that target-binding processes are much faster than all other processes described by the model. Comparison of the rates of change of the model variables requires a reference (time scale). From the point of view of observable data, the characteristic time ( $t_{\text{char}}$ ) would be the length of the time interval between measurements. If the sampling times are of order of minutes, one should not expect to collect data containing information about processes that last seconds (e.g., receptor binding). A characteristic time for the binding process can be inferred from the receptor-binding rate constant,  $k_{\text{on}}$ . If  $C_{\text{char}}$  is the characteristic ligand concentration (for IV bolus drug administration,  $C_{\text{char}} = \text{Dose}/V_{\text{c}}$ ), then  $C_{\text{char}}k_{\text{on}}$  will be the first-order rate constant for the depletion

of the receptor pool, and its reciprocal could be interpreted as the mean time a receptor resides in the receptor compartment prior to binding with its ligand:

$$\tau_{\text{B}} = 1/(C_{\text{char}}k_{\text{on}}) \quad (17)$$

The binding is fast if  $\tau_{\text{B}} \ll t_{\text{char}}$ , and hence the ratio  $\tau_{\text{B}}/t_{\text{char}} \equiv \varepsilon \ll 1$ . The time and time-dependent variables of the TMDD model can be scaled using  $t_{\text{char}}$ ,  $\tau_{\text{B}}$ , and other characteristic scales (e.g., a characteristic concentration of free receptors,  $R_{\text{char}}$ ), and the assumption of fast binding ( $\varepsilon \rightarrow 0$ ) can be used to find 0th-order approximations of the model by the means of singular perturbation theory (16,17). Such a technique has been used to derive the equilibrium equation in enzyme kinetics (15). In our case, it will yield Eq. (6) along with the quasi-equilibrium model [Eqs. (8)–(10)]. The change of variables from  $C$ ,  $R$ , and  $RC$  to  $C$ ,  $C_{\text{tot}}$ , and  $R_{\text{tot}}$  in the singular perturbation analysis will be dictated by the presence of higher-order approximate solutions in equations for the 0th-order approximate equations which can be removed in the same way as the binding terms were disposed of for Eqs. (1)–(4) (see Appendix A). Presenting details of this procedure exceeds the scope of this work, but the general conclusion of accuracy of the approximation provided by the singular perturbation theory can be drawn. If the ratio  $\tau_{\text{B}}/t_{\text{char}}$  is small, the approximation is usually accurate (assuming that the remaining scaling assumptions not discussed here hold). If  $\tau_{\text{B}}/t_{\text{char}} \approx 1$  or  $\tau_{\text{B}}$  is greater than  $t_{\text{char}}$ , then one might not observe good agreement between TMDD and equilibrium models. Because of the presence of the unknown parameters  $V_{\text{c}}$  and  $k_{\text{on}}$  in the definition of  $\tau_{\text{B}}$ , this criterion has limited practical value. However, if prior knowledge of such parameters is available, then an assessment can be made and  $\varepsilon$  may be a criterion for model selection. It should be noted that the error of the quasi-equilibrium approximation of the full model solution is  $O(\varepsilon)$ , which can be interpreted as the error being proportional to  $\varepsilon$  without specifying how big the proportionality constant is. In practice, this precludes any definite values of  $\varepsilon$  that might serve as criteria except that they should be less than 1.

Another assumption for ensuring accuracy of the equilibrium model as required by the singular perturbation approach is:

$$R_{\text{char}}/C_{\text{char}} = O(1) \quad (18)$$

which can be interpreted as the level of receptors available for binding must be comparable to or lower than the drug concentration [the symbol  $O(1)$  means that the left-hand side of Eq. (18) is bound by a constant]. If the free receptor concentration exceeds the drug concentration by an order of magnitude, then the accuracy of the quasi steady-state approximation might not be maintained and the full model should be used. Simulations will be performed where the differences between solutions of the equilibrium and full model with decreasing dose can be demonstrated.

### Simulations

Computer simulations of the quasi-equilibrium model [Eqs. (8)–(12)] were conducted using the ADAPT II software

program (18). To make a direct comparison with previous simulations of the general TMDD model (5), identical system parameters and conditions were selected. Two distinct cases of drug elimination were evaluated, where drug is eliminated from the central compartment alone (Case A,  $k_{el} = 1 \text{ h}^{-1}$ ,  $k_{int} = 0$ ) or in parallel with drug-target internalization (Case B,  $k_{el} = 1 \text{ h}^{-1}$ ,  $k_{int} = 0.1 \text{ h}^{-1}$ ). Nonspecific tissue binding ( $A_T$ ) was excluded ( $k_{pt} = k_{tp} = 0$ ), and total receptor density was assumed to be constant ( $dR_{tot}/dt = 0$  assuming  $k_{int} = k_{deg}$ ). The remaining parameters were as follows:  $R_{tot} = 100$  units,  $K_D = 1$  unit (for the general model,  $k_{on}$  was  $0.1 \text{ unit}^{-1} \text{ h}^{-1}$  and  $k_{off}$  was  $0.1 \text{ h}^{-1}$ ), and  $V_c = 10$  units. Escalating IV doses ranged from 100 to 4,000 units, and the initial condition for Eq. (8) was  $C_{tot}(0) = \text{Dose}/V_c$  [i.e., no endogenous drug is present in the system or  $\ln(t) = 0$ ].

### Data Analysis

Leukemia inhibitory factor plasma concentration-time profiles were obtained from a recent preclinical study conducted in sheep (11). Briefly, six groups of sheep ( $n = 2$  per group) received single IV doses of recombinant human LIF of 12.5, 25, 100, 250, 500, or 750  $\mu\text{g}/\text{kg}$  into the jugular vein. Blood samples (3 mL) were withdrawn from the indwelling jugular vein catheter at predose and at various times over 24 h. Plasma was separated and stored at  $-20^\circ\text{C}$  until analyzed for immunoreactive LIF using a commercially available ELISA that was validated for use with sheep plasma (Quantikine; R&D Systems, Minneapolis, MN, USA). The intra- and interassay variability were less than 15%, and the limit of quantification was defined to be 50  $\text{pg}/\text{mL}$  (from the lowest spiked plasma sample that demonstrated acceptable accuracy and precision).

Traditional methods of noncompartmental analysis (19) were applied to the PK profiles, revealing dose-dependent elimination (total clearance ranged from 5.18 to 1.09  $\text{mL}/\text{min}/\text{kg}$ ) and distribution (volume of the central compartment ranged from 68.9 to 39.1  $\text{mL}/\text{kg}$ , although no clear trend was observed for the steady-state volume of distribution) as dose increased (11). After evaluating several nonlinear PK models, it was determined that the full TMDD model best characterized the data [Eqs. (1)–(4)]. Minor modifications to the general model included setting the first-order rate terms of nonspecific binding equal to one another ( $k_{pt} = k_{tp} = k_{nsb}$ ) and allowing a separate total receptor density parameter for the largest IV dose ( $R_{totss2}$ ). No drug was detected in predose blood samples, and so initial conditions for Eqs. (1)–(4) are  $C(0) = \text{Dose}/V_c$ ,  $A_T(0) = 0$ ,  $R(0) = R_{totss}$ , and  $RC(0) = 0$ . The parameters  $k_{syn}$  and  $k_{off}$  were fixed as secondary parameters according to the relationships:  $k_{syn} = k_{deg}R_{totss}$  and  $k_{off} = k_{on}K_D$ , where the  $K_D$  term was fixed to an *in vitro* literature estimate (0.1 nM). Final model parameters thus included  $k_{nsb}$ ,  $k_{el}$ ,  $k_{deg}$ ,  $k_{on}$ ,  $k_{int}$ ,  $R_{totss1}$ ,  $R_{totss2}$ , and  $V_c$ , which were estimated using the maximum likelihood estimator in ADAPT II (*nota bene*: additional compartments and parameters were included to comodel PK profiles resulting from subcutaneous administration of LIF that are not considered in this present analysis). The variance model was defined as:

$$\sigma^2(t_j) = (\sigma y(t_j))^2 \quad (19)$$

where  $\sigma$  is a variance model parameter to be estimated and  $y(t_j)$  represents model-predicted values of drug concentration.

In this study, the LIF concentration-time profiles were refitted with the quasi-equilibrium model [Eqs. (8)–(11)]. The previous minor modifications were included, namely,  $k_{pt} = k_{tp} = k_{nsb}$  and a separate  $R_{totss}$  for the highest IV dose. The zero-order production of receptors ( $k_{syn}$ ) was specified as a secondary parameter as before; however, the  $K_D$  term was allowed to be estimated during the fitting process. Again, the maximum likelihood estimator in ADAPT II was used to estimate model parameters, along with the identical variance model [Eq. (19)].

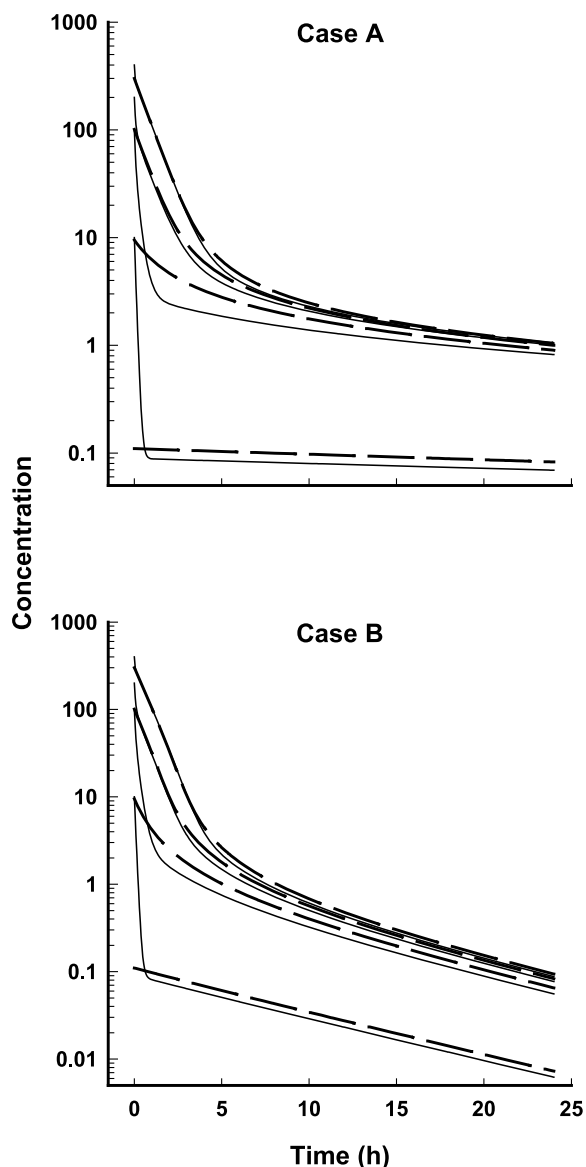
## RESULTS AND DISCUSSION

Drugs that demonstrate TMDD produce complex nonlinear pharmacokinetic profiles that necessitate the incorporation of drug-target binding into models used to characterize the PK and sometimes PD properties of such compounds. Here we present a new modeling approach based on the quasi-equilibrium conditions of a general TMDD model (Fig. 1). The major advantage of this equilibrium model is that drug-binding microconstants ( $k_{on}$  and  $k_{off}$ ), which are often difficult to estimate from typical *in vivo* PK experiments, are replaced by the equilibrium dissociation constant ( $K_D$ ).

A comparison of simulated pharmacokinetic profiles from the general and quasi-equilibrium TMDD models is shown in Fig. 2. The cases differ depending on whether drug elimination occurs only from the central compartment (Case A) or in parallel with drug-target complex internalization or metabolism (Case B). For both cases, there is good agreement between the models for relatively large doses, with only slight deviations at very early time points near the initial concentration and at inflection points around 5 h. In contrast, simulated data for low doses using the equilibrium model show systematic deviations from the general model over the entire time course. The immediate impact of this difference is difficult to interpret because error in measuring drug concentrations is not considered here. Regardless, the implementation of the equilibrium model clearly may be affected by the administered dose levels and the frequency of blood sampling, where the basic assumptions of quasi-equilibrium may not be satisfied.

If the free receptor concentration exceeds the drug concentration by an order of magnitude, then the accuracy of the quasi steady-state approximation might not be maintained and the full model should be used. This situation is depicted in Fig. 2 where the differences between solutions of the equilibrium and full model increase with decreasing dose. Assuming  $R_{char} = R_{tot}$ , the ratios of  $R_{char}/C_{char} = 10$  and 1 result in apparent differences, whereas the accuracy is acceptable for  $R_{char}/C_{char} < 1$ . For doses 100 and 1,000, the  $\tau_B$  values were 1 and 0.1 h, which also contributed to observed discrepancies.

The quasi-equilibrium model was applied to LIF concentration-time profiles following escalating IV doses in sheep. The LIF data set was chosen because the general model was shown to well characterize the profiles previously, and the data seem to satisfy the above equilibrium criteria. Using the previously estimated parameter values for LIF and the molecular weight of 19,710 (11), for the lowest dose of



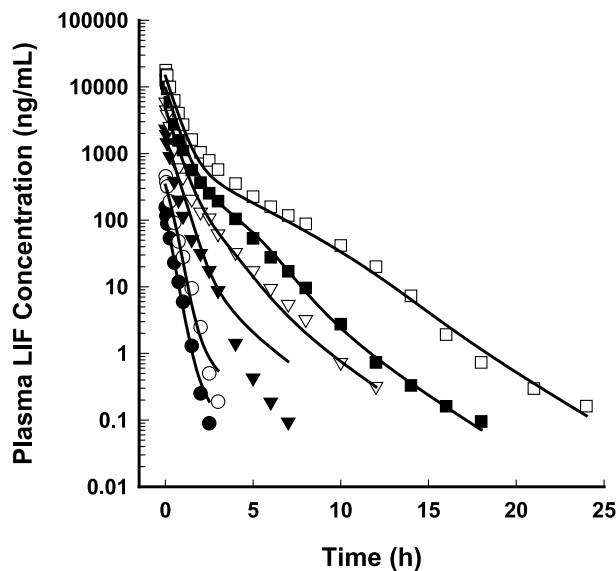
**Fig. 2.** Simulated concentration-time profiles for escalating intravenous doses (100, 1,000, 2,000, and 4,000 units) using target-mediated drug disposition models including binding microconstants [Eqs. (1)–(4); solid lines] or quasi-equilibrium conditions [Eqs. (8)–(11); dashed lines]. Cases correspond to elimination from the central compartment only (Case A) or in parallel with drug-target complex internalization/metabolism (Case B). Model parameterizations are defined in Methods.

12.5  $\mu\text{g}/\text{kg}$ ,  $\tau_B = 2.7$  s, whereas the characteristic time for data sampling was  $t_{\text{char}} = 1$  min. In addition, the initial concentration produced by the lowest dose was about 181 ng/mL, or approximately 9.18 nM, which exceeds the prior estimated  $R_{\text{totss}}$  of 5.30 nM. The mean data and model-predicted profiles from simultaneously fitting the model to all of the IV data are shown in Fig. 3. The equilibrium model well captured the LIF pharmacokinetic data, and the predicted profiles are essentially identical to prior fittings using the general TMDD model [see Fig. 2A in Ref. (11)].

Final estimated pharmacokinetic parameters for LIF using either the general or quasi-equilibrium model are listed

in Table I. Overall, there is good agreement between parameter estimates, with the exception of the equilibrium dissociation constant. The  $K_D$  estimate from the equilibrium model (1.22 nM) is about 10-fold higher than the fixed value used for fitting the general TMDD model. Although the  $K_D$  term was fixed in the previous analysis, the authors note that allowing both  $k_{\text{on}}$  and  $k_{\text{off}}$  to be estimated resulted in a similar  $K_D$  value of around 0.1 nM, which is similar to *in vitro* measurements (11). There are several potential sources for the discrepancy in this parameter estimate. First, the prior analysis also included three additional PK profiles resulting from the subcutaneous administration of 10, 20, and 50  $\mu\text{g}/\text{kg}$  of LIF. It is unclear whether these data influence  $K_D$  estimates, but were excluded from this analysis to avoid any complexities associated with the subcutaneous absorption of therapeutic proteins. The second and more probable cause for disagreement in  $K_D$  estimates is that the model may be insensitive to  $K_D$  values. Simulations of TMDD models for two drugs, abciximab and tissue plasminogen activator, reveal that a 100-fold variation in the  $K_D$  value had little effect on the resulting time course of plasma drug concentrations (13,20). A more complete sensitivity analysis of the equilibrium model is thus warranted and the subject of current study.

In summary, a new modeling approach for characterizing the pharmacokinetics of drugs showing target-mediated drug disposition has been developed based on quasi-equilibrium conditions of the general TMDD model. The equilibrium model eliminates the need to estimate the often problematic microconstants of drug-target binding, alternatively incorporating the equilibrium dissociation constant. Care must be taken to ensure that assumptions of equilibrium conditions have been satisfied, for which the newly described metrics of characteristic time ( $t_{\text{char}}$ ) and ligand



**Fig. 3.** Plasma leukemia inhibitory factor (LIF) concentration-time profiles following intravenous administration in sheep. Lines represent predicted profiles from fitting the quasi-equilibrium target-mediated drug disposition model [Eqs. (8)–(11)] to all of the data simultaneously. Symbols represent previously reported mean data (11) and correspond to LIF doses of 12.5 (●), 25 (○), 100 (▼), 250 (▽), 500 (■), and 750 (□)  $\mu\text{g}/\text{kg}$  ( $n = 2$  per observation).

**Table I.** Parameter Estimates for Leukemia Inhibitory Factor in Sheep

Parameter (units)	Full model <sup>a</sup>	CV%	Equilibrium model	CV%
$k_{nsb}$ (h <sup>-1</sup> )	0.338	4.6	0.389	5.2
$k_{el}$ (h <sup>-1</sup> )	1.54	3.3	1.49	5.0
$V_c$ (mL/kg)	53.2	5.8	51.2	7.5
$k_{on}$ (nM <sup>-1</sup> h <sup>-1</sup> )	11.3	15.5	N/A	N/A
$K_D$ (nM)	0.1	— <sup>b</sup>	1.22	34.6
$k_{int}$ (h <sup>-1</sup> )	2.05	14.7	3.16	17.6
$k_{deg}$ (h <sup>-1</sup> )	0.566	14.3	0.670	21.7
$R_{totss1}$ (nM)	5.30 <sup>c</sup>	10.8	8.19	20.0
$R_{totss2}$ (nM)	1.09 <sup>c</sup>	21.9	1.42	26.5

CV, Coefficient of variation; N/A, not applicable.

<sup>a</sup> Values are from Segrave *et al.* (11).

<sup>b</sup> Fixed value.

<sup>c</sup> Parameter was originally modeled in amount terms (nmol/kg) and was converted to concentration units (nM) by dividing by  $V_c$ .

concentration ( $C_{char}$ ) may be calculated for verification. In contrast to prior equilibrium nonlinear protein-binding models, the kinetics of the pharmacological target may be included in the model, thereby removing the need to assume that total target density remains constant with drug exposure. This new quasi-equilibrium TMDD model retains the potential for exploiting the time course of pharmacological target occupancy in subsequent pharmacodynamic analyses of drug effects.

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## APPENDIX A

### Quasi-equilibrium equations for the TMDD model

Equations (7a) and (7b) define the variables  $C_{tot}$  and  $R_{tot}$  as the concentration sums of free and bound complexes. To derive the differential equations describing them, similar operations should be performed on the equations for the TMDD model. Adding Eq. (1) to Eq. (4) will result in:

$$dC_{tot}/dt = In(t) - k_{int}RC - (k_{el} + k_{pt})C + k_{tp} \cdot A_T/V_c \quad (A1)$$

The RC variable can be calculated from Eq. (7a), such that:

$$RC = C_{tot} - C \quad (A2)$$

After substituting Eq. (A2) for RC in Eq. (A1), it can be rearranged to Eq. (8). Similarly, to obtain the differential equation for  $R_{tot}$ , one needs to add Eqs. (3) and (4) and eliminate the variables RC and R using Eq. (A2):

$$R = R_{tot} - RC = R_{tot} - (C_{tot} - C) \quad (A3)$$

This procedure results in differential equations for  $C_{tot}$  and  $R_{tot}$  that contain the variable  $C$  which in turn can be calculated from the quasi-equilibrium Eq. (6) after substituting Eqs. (A2) and (A3) for RC and R:

$$\frac{C[R_{tot} - (C_{tot} - C)]}{C_{tot} - C} = K_D \quad (A4)$$

Equation (A4) can be multiplied side by side by the denominator of the left-hand side and rearranged to the quadratic equation for  $C$ :

$$C^2 - (C_{tot} - R_{tot} - K_D)C - K_D C_{tot} = 0 \quad (A5)$$

Because  $K_D > 0$  and  $C_{tot} \geq 0$ , this equation has exactly one nonnegative solution:

$$C = 1/2 \left[ (C_{tot} - R_{tot} - K_D) + \sqrt{(C_{tot} - R_{tot} - K_D)^2 + 4K_D C_{tot}} \right] \quad (A6)$$

which is Eq. (11). Equation (A6) is algebraically equivalent to Eq. (A4) under assumption  $C \geq 0$ . The differential equation for  $A_T$  in the equilibrium model is identical to Eq. (2).

## APPENDIX B

### Steady-state equations for the quasi-equilibrium TMDD model

To derive the steady-state equations for the quasi-equilibrium model, we will assume that the input rate is constant [ $In(t) \equiv In$ ] and the nonspecific tissue-binding site ( $A_T$ ) is present ( $k_{tp}$ ,  $k_{pt} > 0$ ). The derivations will hold for receptor turnover with the synthesis rate  $k_{syn} > 0$  and degradation or internalization ( $k_{int} + k_{deg} > 0$ ). Also, we consider the model with at least one clearance mechanism ( $k_{el} + k_{int} > 0$ ) and the binding process always present ( $K_D > 0$ ). At steady state, there is no change in the model variables, and therefore, the time derivatives in Eqs. (8)–(10) can be set to 0 resulting in a system of four algebraic equations with four unknowns ( $C_{ss}$ ,  $A_{Tss}$ ,  $C_{totss}$ , and  $R_{totss}$ ):

$$0 = In - k_{int} C_{totss} - (k_{el} + k_{pt} - k_{int}) C_{ss} + k_{tp} \cdot A_{Tss}/V_c \quad (B1)$$

$$0 = k_{pt} C_{ss} V_c - k_{tp} A_{Tss} \quad (B2)$$

$$0 = k_{syn} - (k_{int} - k_{deg})(C_{totss} - C_{ss}) - k_{deg} R_{totss} \quad (B3)$$

$$C_{ss} = 1/2 \left[ (C_{totss} - R_{totss} - K_D) + \sqrt{(C_{totss} - R_{totss} - K_D)^2 + 4K_D C_{totss}} \right] \quad (B4)$$

Equation (B4) is equivalent to the equilibrium Eq. (6) that can be rewritten as follows:

$$C_{totss} = C_{ss} + R_{totss} C_{ss} / (K_D + C_{ss}) \quad (B5)$$

where the relationships defined by Eqs. (7a) and (7b) have been exploited. Equation (B2) allows one to express  $A_{TSS}$  as a function of  $C_{SS}$ :

$$A_{TSS} = k_{pt} V_{ss} \cdot C_{ss} / k_{tp} \quad (B6)$$

Dividing Eq. (B2) by  $V_c$  and adding it to Eq. (B1), we cancel off the  $A_T$  term and obtain:

$$k_{int}(C_{totss} - C_{ss}) + k_{el} C_{ss} = In \quad (B7)$$

If  $In = 0$ , and because  $C_{totss} \geq C_{ss}$  and  $k_{syn} > 0$ , then Eqs. (B3), (B5), and (B7) imply that  $k_{deg} > 0$ , and:

$$C_{ss} = C_{totss} = 0 \text{ and } R_{totss} = k_{syn} / k_{deg} \quad (B8)$$

Later, we will assume that  $In > 0$ . Let us first consider the case where receptors are eliminated through the turnover process ( $k_{deg} > 0$ ). Equation (B5) can be used to calculate  $C_{totss} - C_{ss}$  and substitute for this term in Eq. (B3) yielding:

$$R_{totss} = \frac{k_{syn}(K_D + C_{ss})}{k_{int}C_{ss} + k_{deg}K_D} \quad (B9)$$

The only unknown now is  $C_{ss}$ . If  $k_{int} = 0$ , then  $C_{ss}$  can be easily calculated from Eq. (B7):

$$C_{ss} = In / k_{el} \quad (B10)$$

If  $k_{int} > 0$ , then calculation of the steady states becomes more troublesome.  $C_{ss}$  can be determined from Eq. (B3) if Eq. (B5) is used to replace  $R_{totss}$  and Eq. (B7) to substitute for  $C_{totss} - C_{ss}$  resulting in the following equation for  $C_{ss}$ :

$$0 = k_{syn} - (k_{int} - k_{deg})(In - k_{el}C_{ss}) / k_{int} - k_{deg} \times (In - k_{el}C_{ss})(K_D + C_{ss}) / (k_{int}C_{ss}) \quad (B11)$$

After multiplying both sides of Eq. (B11) by  $k_{int}C_{ss}$ , it can be rearranged to the equation for  $C_{ss}$ :

$$k_{el}k_{int}C_{ss}^2 + [k_{int}(k_{syn} - In) + k_{deg}k_{el}K_D]C_{ss} - k_{deg}K_DIn = 0 \quad (B12)$$

If  $k_{el} = 0$ , then the positive solution exists only if  $k_{syn} > In$ :

$$C_{ss} = \frac{k_{deg}K_DIn}{(k_{syn} - In)k_{int}} \quad (B13)$$

If  $k_{el} > 0$ , then the positive solution is:

$$C_{ss} = \frac{Ink_{int} - k_{deg}k_{el}K_D - k_{syn}k_{int} + \sqrt{(Ink_{int} - k_{deg}k_{el}K_D - k_{syn}k_{int})^2 + 4k_{el}k_{int}k_{deg}InK_D}}{2k_{el}k_{int}} \quad (B14)$$

Let us now consider the case  $k_{deg} = 0$ . Then the only clearance mechanism for bound receptors is the internalization process. If  $k_{int} > 0$ , then Eq. (B3) implies:

$$C_{totss} - C_{ss} = k_{syn} / k_{int} \quad (B15)$$

and from Eq. (B7), it follows that for  $k_{el} > 0$  and  $In > k_{syn}$ :

$$C_{ss} = (In - k_{syn}) / k_{el} \quad (B16)$$

Table II. Steady-State Solutions for the Quasi-EquilibriumTMDD Model<sup>a</sup>

$k_{int}$	$k_{deg}$	$k_{el}$	Condition	$C_{ss}$	$A_{TSS}$	$C_{totss}$	$R_{totss}$
+	+	+	None	$\frac{k_{pt}V_c C_{ss}}{k_{tp}}$	$\frac{In - (k_{int} - k_{el})C_{ss}}{k_{int}}$	$\frac{k_{syn}(K_D + C_{ss})}{k_{int}C_{ss} + k_{deg}K_D}$	
+	+	0	$In < k_{syn}$	$\frac{k_{pt}V_c C_{ss}}{k_{tp}}$	$C_{ss} + \frac{In}{k_{int}}$	$\frac{k_{syn}(K_D + C_{ss})}{k_{int}C_{ss} + k_{deg}K_D}$	$\frac{k_{syn}(k_{syn} - In) + Ink_{deg}}{k_{int}k_{deg}}$
+	0	+	$In > k_{syn}$	$\frac{k_{pt}V_c C_{ss}}{k_{tp}}$	$C_{ss} + \frac{k_{syn}}{k_{int}}$	$C_{ss} + \frac{k_{syn}}{k_{int}}$	$\frac{k_{syn}(K_D + C_{ss})}{k_{int}C_{ss} + k_{deg}K_D}$
0	+	+	None	$\frac{k_{pt}V_c C_{ss}}{k_{tp}}$	$C_{ss} + \frac{k_{syn}C_{ss}}{k_{deg}K_D}$	$C_{ss} + \frac{k_{syn}C_{ss}}{k_{deg}K_D}$	$\frac{k_{syn}(K_D + C_{ss})}{k_{int}C_{ss} + k_{deg}K_D}$
+	0	0	$In = k_{syn}, R_{tot} - C_{tot} = A_T / V_c = \text{constant}$	$\frac{k_{pt}V_c C_{ss}}{k_{tp}}$	$C_{ss} + \frac{k_{syn}}{k_{int}}$	$C_{ss} + \frac{k_{syn}}{k_{int}}$	$\frac{In}{k_{int}} + C_{ss} \left(1 + \frac{k_{pt}}{k_{tp}}\right) + \text{constant}$
+ / 0	+	+ / 0	$In = 0$	0	0	0	$k_{syn} / k_{deg}$

<sup>a</sup> Assuming  $k_{pt} > 0$ ,  $k_{pr} > 0$ ,  $k_{syn} > 0$ ,  $k_{int} + k_{el} > 0$ , and  $k_{int} + k_{deg} > 0$ . For some cases, the steady-state solutions can be obtained under certain conditions. Also, they might not be unique or even not exist. In the case  $k_{el} = k_{deg} = 0$ , the model variables are linearly dependent and the constant can be selected from additional conditions (e.g., predose initial values) resulting in the steady-state dependent on such conditions.

$R_{\text{totss}}$  can be determined from Eq. (B8). If  $k_{\text{cl}} = 0$ , then after adding Eqs. (B3) and (B7), one can conclude that  $k_{\text{syn}} = \text{In}$ , thus implying:

$$R_{\text{tot}} - C_{\text{tot}} - A_T/V_c = \text{constant} \quad (\text{B17})$$

Hence,

$$R_{\text{totss}} = C_{\text{totss}} + k_{\text{pt}} \cdot C_{\text{ss}}/k_{\text{tp}} + \text{constant} \quad (\text{B18})$$

Inserting  $R_{\text{totss}}$  from Eq. (B18) and  $C_{\text{totss}}$  described by Eq. (B15) into Eq. (B5) yields an equation for  $C_{\text{ss}}$ :

$$k_{\text{syn}}/k_{\text{int}} = (C_{\text{ss}} + k_{\text{syn}}/k_{\text{int}} + k_{\text{pt}} \cdot C_{\text{ss}}/k_{\text{tp}} + \text{constant}) \times C_{\text{ss}}/(K_D + C_{\text{ss}}) \quad (\text{B19})$$

which can be transformed to the following quadratic equation:

$$(1 + k_{\text{pt}}/k_{\text{tp}})C_{\text{ss}}^2 + \text{constant} \cdot C_{\text{ss}} - K_D k_{\text{syn}}/k_{\text{int}} = 0 \quad (\text{B20})$$

Equation (B21) has only one nonnegative solution:

$$C_{\text{ss}} = \frac{1}{2\left(1 + \frac{k_{\text{pt}}}{k_{\text{tp}}}\right)} \left( -\text{constant} + \sqrt{\text{constant}^2 + 4 \frac{k_{\text{syn}}K_D}{k_{\text{int}}} \left(1 + \frac{k_{\text{pt}}}{k_{\text{tp}}}\right)} \right) \quad (\text{B21})$$

This completes derivations of the steady-state solutions for the quasi-equilibrium model, which are summarized in Table II.

## APPENDIX C

### Derivation of Wagner nonlinear tissue binding [Eq. (16)]

For derivation of Eq. (16), the best form of the equilibrium Eq. (6) is:

$$C_{\text{tot}} = C + R_{\text{tot}}C/(K_D + C) \quad (\text{C1})$$

where  $R_{\text{tot}}$  is constant. One can differentiate both sides of Eq. (C1) and obtain:

$$\frac{dC_{\text{tot}}}{dt} = \frac{dC}{dt} + \frac{R_{\text{tot}}K_D}{(K_D + C)^2} \frac{dC}{dt} \quad (\text{C2})$$

Equation (8), with  $k_{\text{int}} = 0$ , can be substituted in the left-hand side of Eq. (C2) resulting in:

$$\begin{aligned} \text{In}(t) - (k_{\text{cl}} + k_{\text{pt}})C + k_{\text{tp}} \cdot A_T/V_c \\ = \left( 1 + \frac{R_{\text{tot}}K_D}{(K_D + C)^2} \right) \frac{dC}{dt} \end{aligned} \quad (\text{C3})$$

Finally, one can solve Eq. (C3) for  $dC/dt$  and arrive at Eq. (16).

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