Predictive Physiologically Based Pharmacokinetic Model for Antibody-Directed Enzyme Prodrug Therapy

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ABSTRACT:

Antibody-directed enzyme prodrug therapy (ADEPT) using anti-TAG-72 antibody and geldanamycin (GA) prodrug were validated in vitro. To understand the complexity and to explore optimal therapeutic regimens for ADEPT in vivo, a physiologically based pharmacokinetic model (PBPK) is applied to analyze each anatomical component/organ. The baseline model predicts that active drug tumor/plasma exposure (AUC) ratio is 2-fold, although antibody-enzyme conjugates (AbE) are distributed into tumors up to 9-fold higher than in plasma. However, the active drug tumor/plasma AUC ratio can be increased up to 100-fold when AbE are depleted from plasma. Similarly, the active drug tumor/plasma AUC ratio can be increased from 2- to 6-fold when the intrinsic clearance of AbE is accelerated by 10-fold. Several sensitive parameters are identified: 1) increasing flow inside tumor (J_tumor) significantly increases active drug tumor/plasma AUC ratio; 2) increasing permeability of prodrug (from range 1.4x10^{-6} to 1.4x10^{-4} cm/s) increases active drug tumor/plasma AUC ratio significantly, whereas active drug permeability enhancement (from range 5x10^{-4} to 5x10^{-2} cm/s) has minimal effect; 3) decreasing E_{max} and increasing EC_{50} for converting prodrug to active drug increase tumor/plasma AUC ratio for active drug. The PBPK model predicts that the optimal dosing interval between AbE and prodrug administration is 5 days, the optimal AbE dose is 0.1 B_{max} and the optimal dose for GA prodrug is 60 mg/kg. The current PBPK model successfully identifies sensitive parameters and predicts an optimal dosing regimen for ADEPT.

ADEPT was proposed by Dr. Bagshawe over two decades ago (Bagshawe, 1987; Bagshawe et al., 1988). ADEPT is a two-step process. In the first step, a drug-activating enzyme is targeted to the tumors by a tumor-targeting antibody (via AbE); in the second step, a nontoxic prodrug is administered systemically and converted to the active drug with high tumor concentration by the localized enzyme. However, the prodrug remains inactive in normal tissues (without drug-activating enzyme) and, thus, decreases its nonspecific toxicity. ADEPT provides many advantages (Bagshawe et al., 1999), including 1) amplification: each localized AbE molecule converts a larger number of nontoxic prodrugs to potent active drugs and increases the tumor active drug concentration; 2) by-stander effect: the locally activated drug molecules with high lipophilicity can diffuse into the cancer cells regardless of the heterogeneous antigen expression, and the by-stander effect addresses the issue of poor tumor penetration of AbE; and 3) AbE do not need to be internalized into each cancer cell for prodrug activation.

This is a rather complex therapeutic regimen considering the following facts (Xu and McLeod, 2001). First, at least two components or steps are indicated in ADEPT, (i.e., AbE should be specifically delivered to the desired tumor site and the nontoxic prodrug should be activated in the target site by the predelivered enzyme). Increasing therapeutic steps always makes it harder to control and predict the outcome, so better understanding of the therapy itself and optimal design are necessary. Second, high expression level of antigen in tumors but low level in normal tissues is required for AbE tumor targeting. Third, AbE might not penetrate into the target tissues. Fourth, elimination of the biological molecules (AbE) might be nonlinear. Fifth, the prodrug needs to be enzyme-specifically activated with minimal nonspecific activation. Large difference of cytotoxicity and other properties between the prodrug and activated drug is desired. For example, low permeability to the cell membrane is preferred for the prodrug, whereas high permeability is preferred for the activated drug (Syrigos and Epenetos, 1999).

Because of the complexity of ADEPT, it would be ideal to predict the therapeutic outcome before the large-scale lengthy and costly preclinical and clinical studies are undertaken. Application of pharmacokinetics (PK) and pharmacodynamics (PD) studies has been shown to guide and expedite drug development (Galluppi et al., 2001). The urgency of PK/PD modeling is clearly demonstrated in U.S. Food and Drug Administration guidance (http://www.fda.gov/cber/gdlns/poppharm.htm). It is without question that using mathematical modeling and deconstructing the complex system into elementary components will help us understand the complex ADEPT and optimize the therapeutic regimen. Few articles have been published that used the mathematical modeling method to provide insight and guidance for ADEPT therapy (Yuan et al., 1991; Baxter and Jain, 1996; Varner, 2005). However, these studies assumed simple compartment model...
for AbE, prodrug, and active drug. Although informative, the compartment model is very limited in its ability to describe the pharmacokinetic properties of biological molecules. PBPK has been proposed to predict the pharmacokinetics of antibodies itself (Baxter et al., 1994; Baxter et al., 1995; Friedrich et al., 2002; Ferl et al., 2005). PBPK approach is more physiologically relevant and allows for integration of all anatomical compartments together as they are in the biological system. This will enable the accurate prediction of the system change with each compartment variation. The current study intends to apply PBPK modeling to simulate and predict the outcome of ADEPT. PBPK modeling will be able to incorporate special features of ADEPT components, such as convection, which is more likely to contribute to the AbE distribution process. FcRn, which is important for antibody recycling and elimination, and tumor size, which might change significantly during the ADEPT course.

In our previous study, we used anti-TAG-72 (tumor-associated glycoprotein 72) antibody (HuCC49ΔCH2) to deliver a drug-activating enzyme for GA-prodrug activation in ADEPT (Cheng et al., 2005; Fang et al., 2006). It was shown that HuCC49ΔCH2-β-galactosidase conjugates were highly specific and enzymatically active. The conjugates were demonstrated to activate the GA prodrug and reduce the IC50 of prodrug to 1 μM from 25 μM against LS174T, colorectal cancer cells with high levels of TAG-72 expression. Several important factors contribute to the advantages of using the anti-TAG-72 antibody and GA prodrug. First, the TAG-72 antigen is primarily expressed in tumors with almost no detectable expression in normal tissues. Second, the humanized anti-TAG-72 antibody (HuCC49ΔCH2) was used for tumor detection in human clinical trials and showed no immunological response. Third, HuCC49ΔCH2 was shown to localize in the extracellular matrix. The large amount of antigen in the extracellular space may trap a large amount of AbE (Knox RJ, 1999). Unlike other antibodies against tumor cell membrane antigen, HuCC49ΔCH2 may avoid the problem of internalization of AbE for lysosomal degradation. Fourth, the GA prodrug with a sugar moiety is more polar and less membrane permeable than the activated drug with high lipophilicity (Tietze et al., 2002).

The current study aims to use the PBPK approach to understand the complexity of ADEPT therapy and to guide AbE selection, AbE dose regimen (dose and dosing interval before prodrug administration), prodrug design, and prodrug dose. The objectives are the following: 1) to build three levels of PBPK model for AbE, prodrug, and active drug and to provide a quantitative basis to carry out ADEPT therapy in vivo; 2) to identify the sensitive factors or parameters controlling the ADEPT process including antigen expression in tumor tissues, antibody affinity to antigen, clearance and depletion of AbE, and prodrug and active drug permeability; and 3) dosing regimen optimization for AbE and prodrug administration to achieve maximal active drug concentration in tumors and minimal active drug in blood and normal tissues.

Materials and Methods

Model Development. This model was built based on the LS174T colorectal cancer xenograft mouse model. The model was developed to study the distribution and targeting of AbE and the activation of prodrug to active drug based on previously published models (Baxter et al., 1994; Baxter et al., 1995; Ferl et al., 2005). A whole-body physiologically based model is used in which the major organs of the mice are represented as compartments. Each compartment is connected in an anatomical manner by the systemic and lymphatic circulation (Fig. 1). The organ compartments are then further subdivided into vascular and extravascular subcompartments.

The detailed PBPK description of AbE is adapted from a previous model for antibody against colorectal tumor antigen (Ferl et al., 2005). Since effective AbE do not affect the antigen-antibody binding process and AbE have a similar molecular size range as the antibody, it is hypothesized that AbE distribute into tissues under the same driving forces as antibody alone. The main transport processes in the AbE include the following: 1) AbE transport across capillary walls through convection and diffusion; 2) AbE reversibly and specifically bind to tumor antigens with saturation; 3) AbE are eliminated through the liver; and 4) in skin and muscle, FcRn-unbound AbE undergo endosomal degradation, whereas FcRn-bound AbE are protected and recycled back to vascular circulation. FcRn sites can be extended to other organs where FcRn receptors are expressed in the model. In the current model, FcRn receptors are added mainly in skin and muscle based on findings by Dr. Ferl et al. (2005) that a good fitting between experimental data and simulated results can be obtained when FcRn sites are considered only in skin and muscle.

The main transport processes for prodrug and active drug model include the following: 1) transport of prodrug and active drug is diffusion-limited across the endothelial wall of vascular and extravascular spaces; 2) the metabolism of prodrug and active drug is predominantly through liver; 3) the connection among AbE, prodrug, and active drug is that the predisposed AbE convert prodrug to active drug in each specified organ subcompartment.

The tumor mass was found to be highly variable based on the previous studies. Thus, a variable tumor mass submodel was adopted to reflect the real tumor physiology (Ferl et al., 2005). This phenomenon was also confirmed in rapidly growing LS174T xenograft model based on our own experience. The model nomenclature is given in Appendix A and mathematical equations accounting for the mass balance are presented in Appendix B, respectively.

Model Baseline Parameters. All the widely accepted physiological parameters for mice are from previous publications (Baxter et al., 1995; Baxter and Jain, 1996; Ferl et al., 2005). These physiological parameters are summarized in Appendix A. For AbE-related parameters, the baseline values of all parameters are obtained or estimated from the results of Ferl et al. (2005). Considering that the values of these constants may vary with different antibodies and selected enzyme and tumor cell lines, we arbitrarily chose baseline values based on literature values and our own experimental results for simulation studies. For prodrug and active drug, most parameters are from the literature and our own experimental data for GA prodrug (Fang et al., 2006). The effective permeability coefficients (P_a) of prodrug and active drug are assumed to be very different based on the high hydrophilic prodrug moiety and hydrophobic properties for active drug. This assumption is based on the fact that active drug needs to be more permeable than prodrug in order to penetrate into the tumor tissues in ADEPT system. All baseline values used are summarized in Table 1.

Time and Dosing Schedules of Simulation Studies. In the sensitivity analysis, the time interval between antibody-enzyme conjugate (AbE) and prodrug is fixed at 3 days based on the following rationale. First, antibody alone distributed into tumor tissues at the highest level at 3 days after antibody injection in the previous publication (Ferl et al., 2005). Second, this fixed interval allows us to study the effect of other sensitive parameters separately (such as antigen expression levels, AbE affinity to antigen, clearance and
Depletion of AbE from Plasma on Day 3, when the Prodrug Is Injected, Dramatically Increased Tumor/Plasma Concentration Ratio of the Active Drug. It was reported previously that tumor/plasma ratio of AbE exceeded 10,000:1 in a clinical trial study if a second antibody against AbE conjugate was applied to increase AbE clearance from plasma (Napier et al., 2000). This high ratio of tumor/plasma or tumor/normal tissues for AbE level provided a favorable environment for prodrug activation in tumors in theory. However, it is not clear how this high tumor/plasma ratio of AbE will affect the active drug concentration in tumors and tumor/plasma ratio. Therefore, we used the PBPK model to validate the effect on the active drug concentration in the tumor and plasma by removing AbE from the plasma by a secondary clearing antibody. On day 3, when prodrug is injected into the system, the AbE concentration in the plasma is set to 0 to reflect the removal. As shown in Fig. 3A, active drug concentration in the plasma is dramatically reduced but not to zero when AbE is in the plasma is removed. This is different from the previous study in which no conversion of prodrug to active drug was assumed in the plasma and, thus, the plasma concentration of the active drug is set close to zero (Yuan et al., 1991). Our simulated result is considered more reasonable since AbE, prodrug, and active drug can be directly collected into the circulation from organs such as liver, kidney, lung, and tumor physiologically. Our PBPK model in this study can account this phenomenon: the plasma active drug concentration peak time is delayed when AbE is removed, indicating that the active drug in other organs can “leak” back into the plasma.

In addition, the active drug concentration in the tumor remains relatively unchanged after AbE is removed from plasma (Fig. 3B). This observation could be explained as in the previous study (Yuan et al., 1991). Prodrug conversion in the plasma will be decreased when AbE is removed from the plasma, resulting in a higher prodrug concentration systemically; thus, tumor prodrug conversion will be increased because of the high tumor prodrug concentration. The transport from the tumor to other sites may increase due to the concentration difference of active drug, but the accelerated prodrug conversion to active drug compensates this transfer. Thus, the overall net change of the active drug concentration in the tumor is relatively small when AbE is depleted from plasma. Considering the dramatically reduced plasma concentration and almost unchanged tumor concentration, the tumor/plasma concentration ratio is significantly increased for the active drug. The ratio is increased up to 100-fold compared with 2-fold based on the baseline parameters (Fig. 3C).

Acceleration of AbE Intrinsic Clearance Significantly Increased Tumor/Plasma AUC Ratio of the Active Drug. Although a second antibody can deplete AbE from the plasma and increase the tumor/plasma ratio of active drug concentration, the second antibody may cause immune response and make ADEPT harder to control. Therefore, another approach is to increase AbE intrinsic clearance to accelerate removal of AbE from blood circulation (and normal tissues/organ) and reduce residual conjugate activity. We have studied an engineered humanized antibody HuCC49ΔCH2 with clearance of $1.5 \times 10^{-3}$ ml/min (Fang et al., 2007). The high clearance of this humanized antibody was due to the deletion of CH2 domain and glycosylation site.

However, increasing the clearance of AbE might also reduce the uptake and retention of AbE in tumor tissues since the remained AbE inside the body is removed. Therefore, a delicate balance must be established in order to maximize the active tumor retention and tumor/plasma ratio of AbE. PKPB model was used to simulate the effect of increasing AbE clearance on active drug concentration in tumors and plasma and their ratios. As predicted, increased AbE clearance from circulation leads to

### Table 1: Baseline values of the model of AbE, prodrug, and active drug

<table>
<thead>
<tr>
<th>Constants</th>
<th>Baseline Values</th>
<th>Reference and/or Explanations</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{up}$</td>
<td>1</td>
<td>Simplify the model</td>
</tr>
<tr>
<td>$f_{up}$</td>
<td>1</td>
<td>Simplify the model</td>
</tr>
<tr>
<td>$K_p$</td>
<td>1</td>
<td>Simplify the model</td>
</tr>
<tr>
<td>$K_p$</td>
<td>1</td>
<td>Simplify the model</td>
</tr>
<tr>
<td>$MW_{prodrug}$</td>
<td>700</td>
<td>Estimation</td>
</tr>
<tr>
<td>$P_{drug}$ (cm/s)</td>
<td>$1.4 \times 10^{-5}$</td>
<td>Yuan et al., 1991; sucrose’s $P$ value</td>
</tr>
<tr>
<td>$P_{w,prodrug}$ (cm/s)</td>
<td>$5 \times 10^{-3}$</td>
<td>Chosen to be close to lipophilic compounds, such as isopropyl alcohol</td>
</tr>
<tr>
<td>$E_{max}$ (1/min)</td>
<td>6000</td>
<td>Yuan et al., 1991; based on our only experiments</td>
</tr>
<tr>
<td>$EC_{so_{prodrug}}$ (μM)</td>
<td>200</td>
<td>Yuan et al., 1991; based on our only experiments</td>
</tr>
<tr>
<td>Surface area in muscle (SA/μm$^2$)</td>
<td>1.24</td>
<td>Estimation</td>
</tr>
<tr>
<td>$PS_{tumor}/PS_{muscle}$</td>
<td>10</td>
<td>Baxter et al., 1994</td>
</tr>
<tr>
<td>$PS_{liver}/PS_{muscle}$</td>
<td>10</td>
<td>Baxter et al., 1994</td>
</tr>
<tr>
<td>$PS_{organ}/PS_{muscle}$ (organs other than liver and tumor)</td>
<td>1</td>
<td>Baxter et al., 1994</td>
</tr>
<tr>
<td>$Cl_{fast,prodrug}$ (ml/min)</td>
<td>1</td>
<td>Estimation</td>
</tr>
<tr>
<td>$Cl_{fast,drug}$ (ml/min)</td>
<td>1</td>
<td>Estimation</td>
</tr>
<tr>
<td>Dose$_{\text{AbE}}$ (mol)</td>
<td>$3 \times 10^{-11}$</td>
<td>Based on results from Yuan et al. (1991)</td>
</tr>
<tr>
<td>Interval between AbE and prodrug (days)</td>
<td>3</td>
<td>Based on results from Yuan et al. (1991)</td>
</tr>
</tbody>
</table>

### Results

**AbE, Prodrug, and Active Drug Distribution with the Baseline Parameter Assumption.** We first analyzed the distribution of AbE, prodrug, and active drug with the baseline physiological, biological, and physical parameters (Fig. 2). As indicated in Fig. 2, A and B, the tumor/plasma AbE ratio rises rapidly before day 4, followed by a slowly rising phase up to day 8. Therefore, the selected baseline interval between AbE and prodrug injection is 3 days.

The prodrug distribution is rather nonselective between tumor and normal tissues. Prodrug has a much higher concentration in the plasma than in the tumor (Fig. 2C). However, the active drug, which is converted from prodrug by the AbE, distributes in the tumor at a higher level than in the plasma. The tumor/plasma concentration or AUC ratio for the active drug is between 1.5 and 2 based on the baseline parameters (Fig. 2D).

### Model Simulation and Simulation Criteria.

Three factors are critical for ADEPT to succeed: 1) active drug tumor/plasma exposure (AUC) ratio, 2) minimum effective active drug tumor AUC, and 3) the absolute tolerable plasma AUC for active drug. As indicated in Varner’s study (Varner, 2005), tumor-to-plasma ratio of active drug concentration or AUC is favorable environment for prodrug activation in tumors in theory. Although a second antibody against AbE conjugate was applied to increase AbE clearance from plasma (Napier et al., 2000). This high ratio of tumor/plasma or tumor/normal tissues for AbE level provided a favorable environment for prodrug activation in tumors in theory.
reduced AbE concentration (or distribution) in tumor and plasma (Fig.
4, A and B). However, the AUC ratio of active drug in tumor/plasma
is significantly increased when intrinsic clearance of AbE is increased
from $1.5 \times 10^{-5}$ to $1.5 \times 10^{-3}$ ml/min (Fig. 4C). It is worth noting
the nonlinear distribution of AbE in tumor in the PBPK model. AbE
distribute into tumor or other tissues in a nonlinear manner because of
the capacity-limited binding process. Because of the distribution equi-
librium between tumor (or other tissues) and circulation, the nonlinear
distribution of AbE in tumor (or other organs) causes nonlinear
removal from circulation. Consequently, when intrinsic clearance of
AbE is increased from $1.5 \times 10^{-5}$ to $1.5 \times 10^{-3}$ ml/min, AbE
concentration in the plasma is reduced to a small extent (Fig. 4B).
However, when clearance is increased from $1.5 \times 10^{-4}$ to $1.5 \times 10^{-3}$
ml/min, the AbE concentration in the plasma decreased significantly
(Fig. 4B). Therefore, the nonlinear distribution may have a “buffering”
effect when AbE clearance is increased from $1.5 \times 10^{-5}$ to $1.5 \times 10^{-4}$
ml/min, whereas this “buffering” effect is saturated when AbE
clearance is further increased to $1.5 \times 10^{-3}$ ml/min. Overall, the
active drug tumor/plasma AUC ratio increases up to over 6-fold when
AbE clearance is increased to $1.5 \times 10^{-3}$ ml/min.

Parameter Sensitivity Analysis in PBPK Modeling of ADEPT.
For complex ADEPT therapy, it is beneficial to know the effect of
each unknown or estimated parameter on the model for the dose
regimen optimization. These parameters include AbE enzymatic ac-
tivity, AbE binding affinity to antigen, permeability of prodrug and
active drug, and tumor physiological conditions. Since AUC is a more
relevant parameter to measure the therapeutic efficacy of active drug
in the tumor, tumor/plasma AUC ratio for the active drug was used in
the following study.

As shown in Fig. 5, a decrease of the enzymatic activity ($E_{\text{max}}$) of
AbE for prodrug conversion rate from baseline 6000 min$^{-1}$ to 600
min$^{-1}$, the tumor/plasma AUC ratio of active drug increases from 2-
to 3-fold. Further decrease of $E_{\text{max}}$ has minimal effect (Fig. 5A). This
is consistent with the previous finding that the prodrug concentra-
tions in both tumor and plasma are constant when $E_{\text{max}}$ is very low, thus the
prodrug conversion is limited by the AbE concentration in the indi-
vidual organ (Yuan et al., 1991). Consequently, initial reduction of
$E_{\text{max}}$ leads to increased tumor/plasma AUC ratio, whereas further
reduction of $E_{\text{max}}$ has no effect. However, the effect of $EC_{50}$ on the
tumor/plasma AUC ratio of active drug is opposite that of $E_{\text{max}}$. Initial
increase of $EC_{50}$ causes increase of active drug tumor/plasma AUC
ratio, whereas further increase has minimal effect (Fig. 5B).

The binding affinity of AbE is determined by on- and off-rate
constants. Given the parameters set in Table 1, the enhancement of the
enhancement of the off-rate leads to a decrease of active drug tumor/plasma AUC ratio. This suggests that a medium range on- and off-rate constant of AbE is preferred. Outside this sensitive range of on- or off-rate constants, AbE binding to the antigen has little effect, and other factors may dominate (Green et al., 2001). Specifically, there are two plateaus (i.e., lower affinity and higher affinity) where on- and off-rate have little effect. The parameters in Table 1, which are the actual affinity characteristics of the IgG antibody, set the model into the higher affinity plateau end.

To best optimize ADEPT, the permeability of active drug should be larger than that of prodrug since it is desired that active drug can penetrate into the tumors in depth. To best define the permeability range of the prodrug and active drug, we tested the sensitivity of permeability in this PBPK model.

For the defined prodrug permeability range (1.4 \times 10^{-6} \text{ cm/s} to 1.4 \times 10^{-4} \text{ cm/s}), increasing the prodrug permeability has a significant effect on the active drug tumor/plasma AUC ratio (Fig. 5, C and D). As predicted, increasing the prodrug’s permeability coefficient from 1.4 \times 10^{-6} \text{ cm/s} to 1.4 \times 10^{-4} \text{ cm/s} consistently increases the active drug tumor/plasma AUC ratio, especially in the first 10 min. This can be explained by the fact that enhancement of the prodrug permeability can increase the penetration of prodrug into the organs and tumor and increase the prodrug concentration in the organs and tumor, thus reducing the prodrug concentration in the plasma. As a consequence, the prodrug conversion is increased in the tumor while it is decreased in the plasma. Therefore, a high prodrug permeability should be preferred in the allowed permeability range (1.4 \times 10^{-6} \text{ cm/s} to 1.4 \times 10^{-4} \text{ cm/s}). However, for the defined active drug permeability range (5 \times 10^{-4} \text{ cm/s} to 5 \times 10^{-2} \text{ cm/s}), enhancement of the permeability coefficient beyond 5 \times 10^{-3} \text{ cm/s} has little effect. This may be due to the fact that significantly high permeability of the active drug can increase the leak of active drug from tumor to plasma, and thus active drug tumor/plasma AUC ratio cannot be further enhanced. Therefore, there is no need to increase active drug permeability to beyond 5 \times 10^{-3} \text{ cm/s}.

Tumor physiology may also significantly affect the outcome of ADEPT therapy. Fluid recirculation flow rate between vascular and interstitial compartment of tumor ($J_{iso, tumor}$) is a parameter to evaluate pressure inside the tumor. When the pressure inside the tumor is very high, $J_{iso, tumor}$ is very small. On the other hand, when the tumor is reasonably small and the inside pressure is still low, the blood supply to the tumor is fairly large and $J_{iso, tumor}$ is much bigger. As seen in Fig. 5E, the enhancement of $J_{iso, tumor}$ can significantly increase active drug tumor/plasma AUC ratio. This suggests that the PBPK model is valid in predicting that smaller tumors have much better therapeutic outcome after ADEPT treatment.

**The Determination of Time Interval between AbE and Prodrug Administration.** The PBPK model showed that active drug tumor/plasma AUC ratio increases as a result of increasing the AbE and prodrug dosing interval up to 5 days. When the interval is longer than 5 days, the AUC ratio decreases with increasing interval time (Fig. 6). Thus, the most optimal dosing interval of AbE and prodrug is 5 days in the current study. This is to the contrary of the previous finding that increasing the dosing interval between AbE and prodrug administration may increase active drug tumor/plasma concentration ratio consistently (Yuan et al., 1991). It was shown in the previous study that the concentration ratio increased from under 1 (interval = 0 day) to above 10 (interval = 14 days). The current PBPK model is believed to provide more accurate prediction. In the baseline parameters, the trapping of the active drug by the tumor cells was not considered ($K_p = 1$). Thus, as discussed previously, the only way to increase the active drug tumor/plasma AUC or concentration ratio is to increase the difference of the prodrug conversion (through AbE) between the
tumor and plasma (Yuan et al., 1991). Meanwhile, the absolute tumor AbE concentration is also very critical. The actual tumor AbE concentration cannot be too low; otherwise, the effective tumor prodrug conversion is so slow that the advantage is diminished even though the tumor/plasma AbE concentration ratio might be high. The best dosing interval should be balanced between the tumor/plasma AbE concentration ratio and the absolute tumor AbE concentration.

As shown, the tumor localized AbE is highest around day 4 (Fig. 2A), and the tumor/plasma AbE concentration ratio is continuously increasing beyond day 4 after AbE injection (Fig. 2B). Since the absolute concentration of AbE in tumor becomes lower after day 5, it is reasonable to expect that an optimal dosing interval of 5 days between AbE and prodrug is predicted by the current PBPK model.

**ADEPT Based on the Most Optimal Parameters.** The most optimal parameters from the sensitivity analysis replace the baseline values in the developed PBPK model for ADEPT. The combination of increased AbE clearance (1.5 \times 10^{-3} \text{ ml/min}) and prolonged interval (Tau = 5 days) gives the highest tumor/plasma AUC ratio of the active drug. Thus, the model with these two newly identified parameters is the selected optimal PBPK model of ADEPT system. Although there are other sensible parameters, replacement of them into the optimal system will have only minimal effect. As shown in Fig. 7A, tumor/plasma AUC ratio for the active drug is increased up to 10-fold in the optimal model compared with 2-fold using the baseline parameters. In addition, the absolute tumor concentration for the active drug is above the preset IC_{50} (1 \mu M), and the plasma active drug concentration is below 1 \mu M (Fig. 7B).

**AbE and Prodrug Dose Selection Based on the Optimal PBPK Model.** The optimal ADEPT should satisfy the following criteria: 1) tumor/plasma AUC ratio of the active drug is at least 10-fold and 2) active drug concentration in tumor is higher than 1 \mu M and lower than 1 \mu M in plasma. Therefore, the optimal PBPK model is used to select the optimal doses of AbE and prodrug considering the dosing interval of AbE and prodrug is 5 days.

The model shows that increasing AbE dose from $1 \times 10^{-11}$ mols (0.1 $B_{\text{max}}$) to $1 \times 10^{-10}$ mols (1 $B_{\text{max}}$) reduces the tumor/plasma AUC ratio of active drug and increases both tumor and plasma active drug concentrations above 1 \mu M. On the other hand, reducing AbE dose from $1 \times 10^{-11}$ mols (0.1 $B_{\text{max}}$) to $1 \times 10^{-12}$ mols (0.01 $B_{\text{max}}$) increases the tumor/plasma AUC ratio, but tumor active drug concentration is below 1 \mu M. Since 1 \mu M is the required concentration for active drug to achieve efficacy against cancer cells, the optimal dose for AbE is $1 \times 10^{-11}$ mols (0.1 $B_{\text{max}}$) (Fig. 8, A–C).

Changing prodrug dose has little effect on the tumor/plasma AUC ratio of active drug. The optimal dose of 60 mg/kg is selected since it can maintain tumor active drug concentration above 1 \mu M and plasma active drug concentration below 1 \mu M (Fig. 8, D–F).

**Discussion**

Irrespective of the expected complexity, ADEPT therapy has been widely tested by many research groups. Previous studies established systemic approaches to screen effective ADEPT therapeutic regimen from in vitro to in vivo and further to human clinical trials. In the early clinical trials from 1997 to 2000, limited colorectal cancer patients were enrolled in the study (Martin et al., 1997; Napier et al., 2000). A5B7 F(\text{ab}')_2 antibody (against carcinoembryonic antigen) conjugated to carboxypeptidase G2 was used in the clinical study. To accelerate the clearance of AbE from blood circulation, a clearing antibody SB43-gal (against the active site of carboxypeptidase G2) was applied. The high tumor/plasma ratio of AbE (exceeding 10,000:1) provided an advantage for prodrug administration around 50 h. Benzoic acid mustard-glutamate, which can be converted to the

![Fig. 4. The effect of AbE clearance acceleration on the AbE and active drug distribution in the tumor and plasma. A, tumor AbE concentration at different clearance. B, plasma AbE concentration at different clearance. C, active drug tumor/plasma AUC ratio with changing AbE clearance.](aspetjournals.org)
alkylating agent, was the selected prodrug. However, although there was evidence of tumor response by the active drug, the dosing-limiting myelosuppression was observed for the majority of the patients. This myelosuppression was attributed to the presence of active drug in the plasma since prodrug itself was not shown to cause toxicity in their previous study. It was suggested that active drug in the circulation was the result of "leakback" from tumor given that no active enzyme was found in plasma.

In 2002, a new phase I clinical trial using a new prodrug and galactosylated AbE was undertaken (Francis et al., 2002). The galac-
tosylated AbE was expected to be cleared faster from circulation without the help of a second clearing antibody. The elegantly designed prodrug was converted to highly potent active drug with a short half-life, which was expected to prevent the leakback of active drug to the circulation observed in the previous clinical trial. The result of this clinical trial was rather unexpected. Inadequate tumor localization of AbE (median tumor/normal tissue ratios are less than 1) was observed. Therefore, selective prodrug conversion in the tumor tissue was not observed for almost all the patients except one. The study concluded that each component (or step) of ADEPT must be strictly monitored in order to achieve the optimal therapeutic outcome. AbE with more efficient tumor localization and less immunogenicity was suggested if second clearing antibody was not used.

One critical study was published in 2005 (Sharma et al., 2005). In this study, a recombinant genetic fusion protein, composed of a single-chain Fv antibody and an enzyme, was expressed in *Pichia pastoris*, and a glycosylated protein with branched mannose resulted. The study successfully confirmed the hypothesis in two morphologically different human colon carcinoma xenografts (LS174T and SW1222) that mannosylated fusion protein’s clearance from the circulation was accelerated (rapid clearance from plasma within 6 h), via mannose receptors, without inducing toxicity. Also, the high AbE tumor-to-plasma ratios of 1400:1 and 339:1 were observed for LS174T and SW1222 models, respectively. In 2006, the clinical trial study result was published (Mayer et al., 2006). Eighty-fold dose escalation from the starting dose of prodrug was carried out, and the therapeutic benefit was evaluated.

Although ADEPT was demonstrated to have great potential, improvement on the optimal conditions for better outcome is necessary. From the long development journey, it was suggested that efficient approach to expedite the development process was desired. The use of large molecular proteins (such as AbE) introduces much more complexity to predict the outcome of ADEPT. The PK/PD properties of therapeutic proteins, such as monoclonal antibodies, are far more complicated than small molecular weight therapeutic agents. Specifically, the absorption is very variable, thus most antibodies on the market are administered intravenously and subcutaneously; the distribution is often mediated by convection and receptor-mediated transport; there is capacity-limited binding between antigen and antibody; and the elimination is usually expected to be nonlinear. Due to the expected complexity, the best approach to predict PK properties of biological therapeutic agents is PBPK. Many advantages are provided by PBPK modeling: 1) it allows integration of many physiological, physical, and biological parameters and translation of them into the in vivo therapeutic efficacy prediction; 2) it estimates the tissue-specific concentration changes as the function of time; 3) it predicts the kinetic changes by variations of physiological parameters, and thus 4) it enables the dose regimen scale-up from animal models to future clinical trials (Zhu et al., 1997).

In our current study, we successfully constructed PBPK simulation models for AbE, prodrug, and active drug. All three molecules’ pharmacokinetics were interconnected through prodrug activation to active drug by the pretargeted AbE. We believe this PBPK model is advantageous compared with the previously published compartmental model in the following aspects. First, the current PBPK model is more physiologically relevant. Our PBPK model integrates all the tissues/organs into the system as they are in the biological body. Thus they reflect the real biological system much better than the compartmental model. For example, the previous compartmental model does not consider the flow collection back from normal organs into the circulation, whereas this is captured in the current PBPK model (Yuan et al., 1991). Indeed, this phenomenon is confirmed by the previously published clinical trial study, the leakback of active drug from regular organs or tumor to the circulation was the main reason for the observed toxicity (Napier et al., 2000). If PBPK model was introduced before this clinical study, the leakback issue would be captured and understood better. Second, the model captures and predicts individual organ/tissue concentration-time response based on each organ’s char-

![FIG. 6. Active drug tumor/plasma AUC ratio when prodrug is injected at days 1, 3, 5, and 8 after AbE injection. Time interval of 5 gives the highest tumor/plasma AUC ratio of active drug.](image)

![FIG. 7. ADEPT based on the most optimal parameters. Active drug tumor/plasma AUC ratio is depicted in (A), and absolute active drug concentration in the tumor (solid line) and plasma (dotted line) are represented in (B).](image)
acteristics. For example, the skin and muscle are unique compared with other organs in the current study since FcRn binding sites were found to be in these organs. It will be difficult for previous compartmental models to incorporate those unique features. Thus the current PBPK model is much more flexible in this aspect.

It is also important to note that further refinement of the current PBPK model is necessary. For example, active drug trapping in the tissues is not considered; however, it is more likely that the active drug can be trapped in the tumor cells. It was previously reported that the active metabolite of 17-AG was trapped in the tumor more than the parent compound 17-AAG itself (Xu et al., 2003). It is reasonable to expect that active drug would behave similarly since active drug

![Graphs showing dose selection for AbE (A–C) and prodrug (D–F).]
may bind to its target inside the tumor cells. Consequently, the active
drug tumor/plasma AUC ratio might be even higher in the real
situation. In addition, FcRn binding sites can be extended to other
organs in addition to skin and muscle. In the current PBPK model,
only skin and muscle are considered to contain FcRn binding sites
according to a previous study (Ferl et al., 2005).

In our previous study, we successfully synthesized GA prodrugs
suitable for ADEPT therapy (Cheng et al., 2005; Fang et al., 2006). In
addition, humanized anti-TAG-72 antibody HuCC492CH2 and β-ga-
lactosidase were chemically conjugated and tested for specific act-
ivation of GA prodrugs. We expect the current PBPK modeling study
will shed light on our future ADEPT experiment design and further
therapeutic outcome.

Appendix A: Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qorgan</td>
<td>Plasma flow rate of each organ (ml/min).</td>
</tr>
<tr>
<td>Jorgan</td>
<td>Lymph flow rate of each organ (ml/min/g).</td>
</tr>
<tr>
<td>Jiso,organ</td>
<td>Fluid recirculation flow rate for each organ (flow rate through large pore to the interstitial space for L = 0; ml/min/g).</td>
</tr>
<tr>
<td>Massorgan</td>
<td>Organ weight (gram).</td>
</tr>
<tr>
<td>Vorgan</td>
<td>Vascular space of each organ (ml).</td>
</tr>
<tr>
<td>Vinter,organ</td>
<td>Interstitial space of each organ (ml).</td>
</tr>
<tr>
<td>Vorgan</td>
<td>Total volume of each organ (ml).</td>
</tr>
<tr>
<td>σ1, σ2</td>
<td>Osmotic reflection coefficient for large and small pores, respectively.</td>
</tr>
<tr>
<td>αs, αe</td>
<td>Fraction of extravasation via large and small pores, respectively.</td>
</tr>
<tr>
<td>PSorgan</td>
<td>Permeability-surface area product for each organ (ml/min) for large and small pores, respectively.</td>
</tr>
<tr>
<td>PeLorgan, PeSorgan</td>
<td>Péclet number, ratio of convection to diffusion across large and small pores [J/(1 − σ)/(PS)].</td>
</tr>
<tr>
<td>Jcorgan</td>
<td>Transcapillary fluid flow rate (vascular–interstitial) for each organ (ml/min) via large and small pores, respectively.</td>
</tr>
<tr>
<td>Bmax</td>
<td>Maximum tumor-associated antigen concentration inside tumor tissue (mol/ml).</td>
</tr>
<tr>
<td>KCSEA</td>
<td>Association rate constants for the binding of antibodies (ml/mol/min).</td>
</tr>
<tr>
<td>KDSEA</td>
<td>Dissociation rate constants for the binding of antibodies (1/min).</td>
</tr>
<tr>
<td>FCNRntot</td>
<td>Total FcRn binding capacity in the skin and muscle (mol/ml).</td>
</tr>
<tr>
<td>KNFcRn</td>
<td>Nonspecific internalization rate constant of free antibody into endosome for binding to FcRn sites (1/min).</td>
</tr>
<tr>
<td>FcRnR</td>
<td>Recycling rate of FcRn bound antibody back to vascular space (1/min).</td>
</tr>
<tr>
<td>FCNR</td>
<td>Binding constant for IgG-FcRn interaction (ml/mol/min).</td>
</tr>
<tr>
<td>KdFcRn</td>
<td>Dissociation constant for IgG-FcRn interaction (1/min).</td>
</tr>
<tr>
<td>KdegFcRn</td>
<td>Degradation rate constant of unbound endosomal IgG (1/min).</td>
</tr>
<tr>
<td>CIkeLiver</td>
<td>Clearance of antibody-enzyme conjugate (AbE) from liver (ml/min).</td>
</tr>
<tr>
<td>CIAb</td>
<td>AbE concentration in plasma (mol/ml).</td>
</tr>
<tr>
<td>Ciorgan</td>
<td>AbE concentration in the vascular space of each organ (mol/ml).</td>
</tr>
<tr>
<td>Clorgan</td>
<td>Free AbE concentration in the interstitial space of each organ (mol/ml).</td>
</tr>
</tbody>
</table>

Appendix B: Mathematical Model and Governing Equations

The mass balance equations for the pharmacokinetic model describe
the circulation of antibody–enzyme conjugate (AbE), prodrug, and
active drug throughout the body of a 20-g nude mouse (Fig. 1). For
large molecular weight molecules, AbE, each organ (except tumor,
skin, and muscle) is further divided into two subcompartments,
the vascular space and extravascular space. Nonspecific binding is not
considered in those organs. A specific, saturable, and reversible binding
compartment is added in the extravascular space in skin and muscle tissues.
Inside all these organs or tissues, the net flux of AbE across the
capillary between vascular and extravascular fluid is determined by
the two-pore mechanism proposed by Rippe and Haraldsson (1987).

For small molecules, prodrug, and active drug, only vascular and
extravascular spaces were considered. In each subcompartment, the
predispensed conjugate will convert the exit prodrug to the active
drug. In tumor tissue, both free and bound AbE will convert existing
prodrug to the active drug. In skin and muscle, the predelivered
endosome free and bound AbE will convert the prodrug in the vas-
ular space to the active drug.

B.1: Mass Balance Equation for Antibody-Enzyme Conjugate.

B.1.1: Mass balance equation for plasma. According to the circulation
scheme illustrated, the mass balance equation for the plasma com-
partiment is
There is an additional constraint on the volumetric flow rates as

\[ Q_{\text{long}} = Q_{\text{liver}} - L + Q_{\text{kidney}} - L_{\text{kidney}} + Q_{\text{tumor}} - L_{\text{tumor}} + Q_{\text{skin}} - L_{\text{skin}} + Q_{\text{muscle}} - L + Q_{\text{bone}} - L_{\text{bone}} + Q_{\text{heart}} - L_{\text{heart}} \]

**B.1.2: Mass balance equation for noneliminating organs.**

Vascular space:

\[
J_{L,\text{organ}} = J_{I,\text{organ}} + \alpha_{L} J_{\text{organ}}
\]

\[
J_{S,\text{organ}} = J_{I,\text{organ}} + \alpha_{S} J_{\text{organ}}
\]

\[
V_{v,\text{organ}} \left( \frac{dC_{v,\text{organ}}}{dt} \right) = Q_{\text{organ}} C_{v,\text{organ}} - (Q_{\text{organ}} - L_{\text{organ}}) C_{v,\text{organ}} - J_{L,\text{organ}}
\]

\[
\times (1 - \sigma_{L}) C_{v,\text{organ}} - PS_{L,\text{organ}} (C_{v,\text{organ}} - C_{I,\text{organ}}) \frac{Pe_{L,\text{organ}}}{exp(Pe_{L,\text{organ}})} - 1
\]

\[
- J_{S,\text{organ}} (1 - \sigma_{S}) C_{v,\text{organ}} - PS_{S,\text{organ}}
\]

\[
\times (C_{v,\text{organ}} - C_{I,\text{organ}}) \frac{Pe_{S,\text{organ}}}{exp(Pe_{S,\text{organ}})} - 1
\]

Interstitial space:

\[
V_{I,\text{organ}} \left( \frac{dC_{I,\text{organ}}}{dt} \right) = J_{L,\text{organ}} (1 - \sigma_{L}) C_{I,\text{organ}} + PS_{L,\text{organ}} (C_{I,\text{organ}} - C_{I,\text{organ}})
\]

\[
\times \frac{Pe_{L,\text{organ}}}{exp(Pe_{L,\text{organ}})} + J_{S,\text{organ}} (1 - \sigma_{S}) C_{I,\text{organ}} + PS_{S,\text{organ}}
\]

\[
\times (C_{I,\text{organ}} - C_{I,\text{organ}}) \frac{Pe_{S,\text{organ}}}{exp(Pe_{S,\text{organ}})} - 1
\]

**B.1.3: Mass balance equation for tumor.**

Vascular space:

\[
V_{v,\text{tumor}} \left( \frac{dC_{v,\text{tumor}}}{dt} \right) = Q_{\text{tumor}} C_{v,\text{tumor}} - (Q_{\text{tumor}} - L_{\text{tumor}}) C_{v,\text{tumor}} - J_{L,\text{tumor}}
\]

\[
\times (1 - \sigma_{L}) C_{v,\text{tumor}} - PS_{L,\text{tumor}} (C_{v,\text{tumor}} - C_{I,\text{tumor}}) \frac{Pe_{L,\text{tumor}}}{exp(Pe_{L,\text{tumor}})} - 1
\]

\[
- J_{S,\text{tumor}} (1 - \sigma_{S}) C_{v,\text{tumor}} - PS_{S,\text{tumor}}
\]

\[
\times (C_{v,\text{tumor}} - C_{I,\text{tumor}}) \frac{Pe_{S,\text{tumor}}}{exp(Pe_{S,\text{tumor}})} - 1
\]

Interstitial free concentration:

\[
V_{I,\text{tumor}} \left( \frac{dC_{I,\text{tumor}}}{dt} \right) = J_{L,\text{tumor}} (1 - \sigma_{L}) C_{I,\text{tumor}} + PS_{L,\text{tumor}}
\]

\[
\times (C_{v,\text{tumor}} - C_{I,\text{tumor}}) \frac{Pe_{L,\text{tumor}}}{exp(Pe_{L,\text{tumor}})} - 1
\]

\[
+ J_{S,\text{tumor}} (1 - \sigma_{S}) C_{I,\text{tumor}} + PS_{S,\text{tumor}}
\]

\[
\times (C_{v,\text{tumor}} - C_{I,\text{tumor}}) \frac{Pe_{S,\text{tumor}}}{exp(Pe_{S,\text{tumor}})} - 1
\]

Interstitial bound concentration:

\[
V_{I,\text{tumor}} \left( \frac{dC_{I,\text{tumor}}}{dt} \right) = J_{L,\text{tumor}} (1 - \sigma_{L}) C_{I,\text{tumor}} + PS_{L,\text{tumor}}
\]

**B.1.4: Mass balance equation for skin (muscle is similar).**

Vascular space:

\[
V_{v,\text{skin}} \left( \frac{dC_{v,\text{skin}}}{dt} \right) = Q_{\text{skin}} C_{v,\text{skin}} - (Q_{\text{skin}} - L_{\text{skin}}) C_{v,\text{skin}} - J_{L,\text{skin}}
\]

\[
\times (1 - \sigma_{L}) C_{v,\text{skin}} - PS_{L,\text{skin}} (C_{v,\text{skin}} - C_{I,\text{skin}}) \frac{Pe_{L,\text{skin}}}{exp(Pe_{L,\text{skin}})} - 1
\]

\[
- J_{S,\text{skin}} (1 - \sigma_{S}) C_{v,\text{skin}} - PS_{S,\text{skin}}
\]

\[
\times (C_{v,\text{skin}} - C_{I,\text{skin}}) \frac{Pe_{S,\text{skin}}}{exp(Pe_{S,\text{skin}})} - 1
\]

The endosome is assumed to be the continuous phase of vascular space, so the whole endosome concentration is equal to the vascular concentration for the conjugate. Thus:

\[
C_{\text{tot,endo,skin}} = C_{v,\text{skin}} = \frac{A_{e,\text{skin}} + A_{b,\text{skin}}}{V_{\text{endo,skin}}}
\]

\[
V_{\text{endo,skin}} = \frac{A_{e,\text{skin}} + A_{b,\text{skin}}}{C_{v,\text{skin}}}
\]

Interstitial free concentration:

\[
V_{t,\text{skin}} \left( \frac{dC_{t,\text{skin}}}{dt} \right) = J_{L,\text{skin}} (1 - \sigma_{L}) C_{t,\text{skin}} + PS_{L,\text{skin}} (C_{t,\text{skin}} - C_{I,\text{skin}})
\]

\[
\times \frac{Pe_{L,\text{skin}}}{exp(Pe_{L,\text{skin}})} - 1
\]

\[
- J_{S,\text{skin}} (1 - \sigma_{S}) C_{t,\text{skin}} + PS_{S,\text{skin}}
\]

\[
\times (C_{v,\text{skin}} - C_{I,\text{skin}}) \frac{Pe_{S,\text{skin}}}{exp(Pe_{S,\text{skin}})} - 1
\]

Endosome free concentration:

\[
V_{e,\text{endo,skin}} \left( \frac{dC_{e,\text{skin}}}{dt} \right) = K_{\text{int,FeR}(C_{v,\text{skin}} - C_{\text{skin}} - K_{\text{deg,FeR}} C_{e,\text{skin}} V_{\text{endo,skin}})
\]

\[
- K_{\text{on,FeR}} (C_{e,\text{skin}} - C_{\text{skin}} C_{e,\text{skin}} V_{\text{endo,skin}})
\]

\[
+ K_{\text{off,FeR}} C_{e,\text{skin}} V_{\text{endo,skin}}
\]
Endosome bound concentration:

\[
V_{\text{endo,skin}} \left( \frac{dC_{\text{eb,skin}}}{dt} \right) = K_{\text{FcRn}}f_{\text{Fcrn}} - C_{\text{eb,skin}}C_{\text{cf,skin}}V_{\text{endo,skin}} - K_{\text{def}}FcRnC_{\text{eb,skin}}V_{\text{endo,skin}} - K_{\text{sec}}FcRnC_{\text{eb,skin}}V_{\text{endo,skin}}
\]

B.1.5: Mass balance equation for liver.

Vascular space:

\[
V_{\text{v,liver}} \left( \frac{dC_{\text{v,liver}}}{dt} \right) = (Q_{\text{v,liver}} + L_{\text{spleen}} - Q_{\text{pl}} - Q_{\text{spleen}})C_{\text{pl}} + (Q_{\text{pl}} - L_{\text{pl}})C_{\text{pl}} + (Q_{\text{spleen}} - L_{\text{spleen}})C_{\text{spleen}} - (Q_{\text{v,liver}} - L_{\text{v,liver}})C_{\text{v,liver}} - J_{\text{L,liver}}(1 - \sigma_{I})C_{\text{v,liver}} - PS_{\text{L,liver}}C_{\text{v,liver}} - C_{\text{l,liver}}\frac{P_{\text{e,liver}}}{\exp(P_{\text{e,liver}}) - 1}
\]

\[
- J_{\text{L,liver}}(1 - \sigma_{I})C_{\text{v,liver}} - PS_{\text{K,liver}}C_{\text{v,liver}} - C_{\text{l,organ}}\frac{P_{\text{e,organ}}}{\exp(P_{\text{e,organ}}) - 1} - CL_{\text{deg,Liver}}C_{\text{v,liver}}
\]

Interstitial space:

\[
V_{\text{i,organ}} \left( \frac{dC_{\text{i,organ}}}{dt} \right) = J_{\text{L,organ}}(1 - \sigma_{I})C_{\text{v,organ}} + PS_{\text{L,organ}}(C_{\text{i,organ}} - C_{\text{v,organ}})\frac{P_{\text{e,organ}}}{\exp(P_{\text{e,organ}}) - 1} + J_{\text{S,organ}}(1 - \sigma_{S})C_{\text{v,organ}} + PS_{\text{S,organ}}(C_{\text{i,organ}} - C_{\text{v,organ}})\times\frac{P_{\text{e,organ}}}{\exp(P_{\text{e,organ}}) - 1} - L_{\text{organ}}C_{\text{i,organ}}
\]

In each organ, the total (or average) concentration is the weighted average of the concentrations within each subcompartment

\[
C_{\text{tot}} = C_{\text{v,organ}}V_{\text{v,organ}} + C_{\text{i,organ}}V_{\text{i,organ}} + (C_{\text{eb,organ}} + C_{\text{cf,organ}})V_{\text{endo,organ}}
\]

or for skin and muscle

\[
C_{\text{tot}} = C_{\text{v,organ}}V_{\text{v,organ}} + C_{\text{i,organ}}V_{\text{i,organ}} + (C_{\text{eb,organ}} + C_{\text{cf,organ}})V_{\text{endo,skin}}
\]

B.2: Mass Balance Equation for Prodrug and Active Drug

Diffusion-limited model is assumed for both prodrug and active drug. Liver is considered as the only eliminating organ.

Prodrug in vascular space:

\[
V_{\text{v,organ}} \left( \frac{dC_{\text{prodrug,organ}}}{dt} \right) = Q_{\text{organ}}(C_{\text{pl}} - C_{\text{v,prodrug,organ}}) - PS_{\text{prodrug,organ}}(C_{\text{v,prodrug,organ}} - C_{\text{i,prodrug,organ}}) - E_{\text{max,prodrug}}C_{\text{i,organ}}V_{\text{i,organ}}f_{\text{up,prodrug}}C_{\text{i,prodrug,organ}}\times\frac{E_{\text{C50,prodrug}} + f_{\text{up,prodrug}}C_{\text{i,prodrug,organ}}}{E_{\text{C50,prodrug}} + f_{\text{up,prodrug}}C_{\text{i,prodrug,organ}}}
\]

Active drug in vascular space:

\[
V_{\text{v,organ}} \left( \frac{dC_{\text{drug,organ}}}{dt} \right) = Q_{\text{organ}}C_{\text{pl,drug}} - C_{\text{v,drug,organ}} - PS_{\text{drug,organ}}(C_{\text{v,drug,organ}} - C_{\text{i,drug,organ}}) + EC_{\text{50,drug}} + f_{\text{up,drug}}C_{\text{i,drug,organ}}\times\frac{EC_{\text{50,drug}} + f_{\text{up,drug}}C_{\text{i,drug,organ}}}{EC_{\text{50,drug}} + f_{\text{up,drug}}C_{\text{i,drug,organ}}}
\]

Prodrug in the interstitial space:

\[
V_{\text{i,organ}} \left( \frac{dC_{\text{prodrug,organ}}}{dt} \right) = PS_{\text{prodrug,organ}}(C_{\text{v,prodrug,organ}} - C_{\text{i,prodrug,organ}}) + E_{\text{max,prodrug}}C_{\text{i,organ}}V_{\text{i,organ}}f_{\text{up,prodrug}}C_{\text{i,prodrug,organ}}\times\frac{EC_{\text{50,prodrug}} + f_{\text{up,prodrug}}C_{\text{i,prodrug,organ}}}{EC_{\text{50,prodrug}} + f_{\text{up,prodrug}}C_{\text{i,prodrug,organ}}}
\]

Active drug in the interstitial space:

\[
V_{\text{i,organ}} \left( \frac{dC_{\text{drug,organ}}}{dt} \right) = PS_{\text{drug,organ}}(C_{\text{v,drug,organ}} - C_{\text{i,drug,organ}}) + E_{\text{max,drug}}C_{\text{i,organ}}V_{\text{i,organ}}f_{\text{up,drug}}C_{\text{i,drug,organ}}\times\frac{EC_{\text{50,drug}} + f_{\text{up,drug}}C_{\text{i,drug,organ}}}{EC_{\text{50,drug}} + f_{\text{up,drug}}C_{\text{i,drug,organ}}}
\]

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