Supporting Information

Model-based Target Pharmacology Assessment (mTPA): An approach using PBPK/PD modeling and machine learning to design medicinal chemistry and DMPK strategies in early drug discovery

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S1 Generation of Virtual Compounds

A physiological based pharmacokinetic (PBPK) model can be parameterized directly from ADME properties, making it the ideal tool to understand their impact on dose. The parameters and ranges used for in silico PKPD experiments are defined in Table S1. A single set of 30,000 virtual compounds was generated, each described by the list of parameters in Table S1. Parameters were randomly sampled from either continuous uniform or log-uniform distributions. Log-uniform distributions were used in cases where the range spanned two or more log units to ensure representative sampling across the parameter space. Refer to Figure S1 for more details.

Parameter	Units	Distribution Type	Lower Limit	Upper Limit
IC50/EC50*	ng/mL	log-uniform	0.01	20
CL_{int}	mL/min/g Liver	log-uniform	1	100
fup	dimensionless	log-uniform	0.001	0.7
B:P	dimensionless	uniform	0.5	5
solubility	mg/mL	log-uniform	0.01	5
Peff	$x10^{-4}$ cm/s	log-uniform	0.01	5
Deff	$x10^{-6}$ cm/s	uniform	1.6	18
logP	dimensionless	uniform	0	6
AB class	dimensionless	discrete	1	6

Table S1. Parameters and distributions used for in silico experiments. *IC50 was used in direct response, indirect response, and precursor inhibition models while EC50 was used in the tumor cell death model. CL_{int} is unbound intrinsic clearance; fup is fraction unbound in plasma; B:P is blood-to-plasma ratio; Peff is effective jejunal permeability; Deff is effective diffusion coefficient; AB class is the acid/base classification.

The parameter "AB class" refers to the classification of the compound as a strong acid, strong base, weak acid, weak base, zwitterion, or neutral species at physiological pH. Each compound was randomly assigned an integer between 1 and 6 that represents an acid/base classification. After the AB class of the compound was defined, the acidic and basic pKa's of the compound were randomly sampled from a uniform distribution using the ranges listed in Table S2.

AB Class	Acidic pKa Lower Limt	Acidic pKa Upper Limit	Basic pKa Lower Limit	Basic pKa Upper Limit
strong acid	1	6.99	NA	NA
strong base	NA	NA	7	13
weak acid	7	13	NA	NA
weak base	NA	NA	1	6.99
neutral	NA	NA	NA	NA
zwitterion	1	6.99	7	13

Table S2. Acidic and basic pKa ranges for each compound class.

Figure S1 compares uniform and log-uniform distributions for a random variable ranging from 0.01 to 5, similar to those used for solubility and permeability. The median value for the uniform distribution is approximately 2.5, meaning 50% of the values are above 2.5. When considering the typical ranges for parameters like solubility and permeability, this is an unrealistic representation of the expected values. Alternatively, the median of the log-uniform distribution is a better representation of the expected range of values.

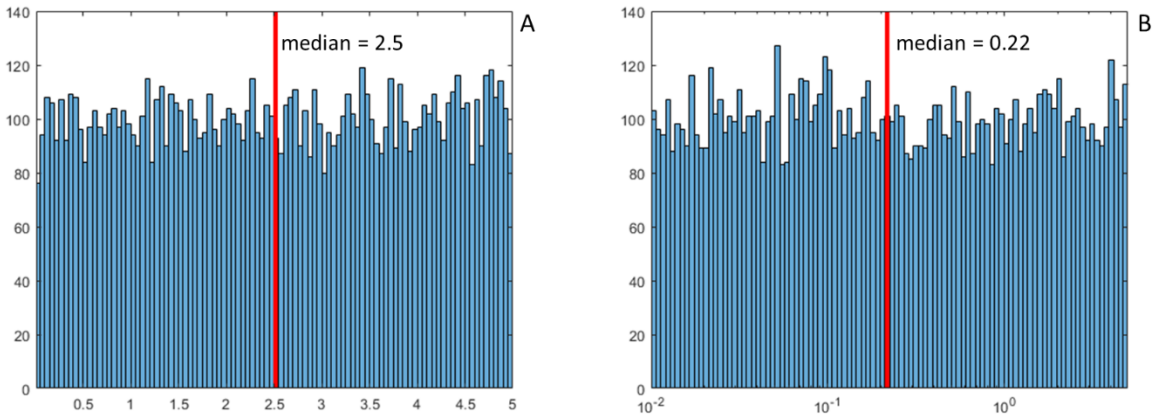

Figure S1. Comparison of (A) uniform and (B) log-uniform distributions with a range between 0.01 and 5. 10,000 random samples with 100 bins were used for each figure.

S2 Pharmacodynamic models

The four pharmacodynamic models used in this manuscript to demonstrate the utility of Target Pharmacology Assessment are as follows:

1. **Indirect Response model (with fast turnover)** – Drug inhibits the production of a biomolecule with a fat turnover time whose level is the intended pharmacological outcome. For extremely fast turnover time (i.e. seconds) this mathematically approximates **a direct response model** in which drug directly agonizes/antagonizes biomolecule whose activity is the intended PD outcome.
2. **Indirect Response model (with slow turnover)** - Drug Inhibits the production of a biomolecule with a slow turnover time whose level is the intended pharmacological outcome.
3. **Precursor Inhibition Model** - Drug inhibits the activation of a precursor to form the active moiety, whose level is the intended pharmacological outcome.
4. **Cell Death Response Model** - Drug promotes the death rate of tumor cells.

The pharmacodynamic responses of models 1 through 3 were simulated using the same set of equations that describe the activation of precursor (model 3) as below

$$\frac{dP}{dt} = k_{syn} - k_{act} \cdot \left(1 - \frac{I_{max} f_{uT} C_T}{IC_{50} + f_{uT} C_T}\right) \cdot P - k_{deg,P} \cdot P \qquad (S2.1)$$

$$\frac{dA}{dt} = k_{act} \cdot \left(1 - \frac{I_{max} f_{uT} C_T}{IC_{50} + f_{uT} C_T}\right) \cdot P - k_{deg,A} \cdot A \qquad (S2.2)$$

Where P and A are the level of precursor and active biomolecules, respectively, and the intended pharmacological outcome is represented by the level of A. The activation of the precursor to form the active biomolecule is inhibited by the unbound drug concentration in the target tissue, $f_{uT} C_T$ represented by a sigmodal inhibition function.

These equations are used to approximate the pharmacodynamic behavior of model 1 and model 2 (no precursors) by setting a very high baseline precursor level so that precursor level remains essentially constant throughout the simulation. So, for model 1 and 2, the above equations become

$$\frac{dA}{dt} \rightarrow k_{deg,A} \cdot A_0 \cdot \left(1 - \frac{I_{max} f_{uT} C_T}{IC_{50} + f_{uT} C_T}\right) - k_{deg,A} \cdot A \qquad (S2.3)$$

The parameters for model 1-3 used in the simulations were tabulated in the table below

Parameter	Description	Unit	Model 1	Model 2	Model 3
P_o	Baseline level of the precursor biomolecule	$dimensionless$	1000	1000	2
A_o	Baseline level of the active biomolecule	$dimensionless$	1	1	1
k_{syn}	Synthesis rate of the precursor biomolecule	$1/hour$	$(k_{deg,P} + k_{act}) * P_0$		
k_{act}	Activation rate constant	$1/hour$	$\frac{k_{deg,A} \cdot A_0}{P_0}$		
$k_{deg,P}$ ·	Degradation rate of the precursor biomolecule	$1/hour$	1000	1000	0.01
$k_{deg,A}$	Degradation rate of the active biomolecule	$1/hour$	10	0.01	0.1
I_{max}	Maximal inhibition of the activation rate	dimensionless	1	1	1
IC_{50}	Unbound target concentration achieving 50% inhibition	ng/mL	variable	variable	variable
Q	Tissue Blood Flow	mL/hour	140	140	140
V	Tissue Volume	mL	95.2	95.2	95.2

Table S3. Parameter for model 1-3.

The pharmacodynamic response for model 4 is represented by the following differential equation

$$\frac{dT}{dt} = \left(k_{gro} - k_{dea} \cdot \left(\frac{E_{max} f_{uT} C_T}{EC_{50} + f_{uT} C_T} \right) \right) \cdot T \qquad (S1.4)$$

$$\frac{dZ_1}{dt} = k_{dea} \cdot (T - Z_1)$$

$$\frac{dZ_2}{dt} = k_{dea} \cdot (Z_1 - Z_2)$$

$$\frac{dZ_3}{dt} = k_{dea} \cdot (Z_2 - Z_3)$$

$$T_{total} = T + Z_1 + Z_2 + Z_3$$

Where T represents the mass of proliferating tumor cells (proportional to the number of tumor cells), Z1, Z2, and Z3 represent various stages of cell damage that ultimately lead to cell death. The rate of proliferating tumor cell damage is promoted by the unbound drug concentration in the target tissue, $f_{uT}C_T$ represented by the sigmodal stimulatory function.

The parameters for model 4 used in the simulations are tabulated in the table below. Note in this case the tumor tissue was assumed to be well perfused.

Parameter	Description	Unit	Parameter values
T_o	Baseline level of the tumor cell mass	$gram$	1.3
k_{gro}	First order growth rate of the tumor cells	$1/hour$	0.01
k_{dea}	First order death rate of the tumor cells	$1/hour$	0.05
E_{max}	Maximal death rate	dimensionless	1
EC_{50}	Unbound target concentration achieving 50% death rate promotion	ng/mL	variable
Q	Tumor blood flow	mL/hour	35
V	Tumor volume	mL	1.3

Table S4. Parameters for model 4.

S3 Quantifying the distinction between fast and slow tissues in terms of physiological and ADME parameters

As mentioned in the main text, several factors such as tissue partitioning, permeability, and the action of active uptake or efflux transporters can result in prolonging tissue MRT over blood MRT. The impact of these factors can be estimated using the following equations (derived in Supplementary Materials 4),

$$AUC_{uT} = \left(\frac{\phi_b}{\phi_T}\right)\left(\frac{PSA_f}{PSA_r}\right) \cdot AUC_{ub} \qquad (S3.1)$$

$$MRT_T = MRT_b + \frac{V_v}{Q_T} + \left(\frac{\phi_b f_{ub} PSA_f}{\phi_T f_{uT} PSA_r}\right)\frac{V_T}{Q_T} + \frac{V_T}{\phi_T f_{uT} PSA_r}, \qquad (S3.2)$$

where V_v is the tissue-associated vascular volume (including rapidly equilibrating interstitial spaces), V_T is the tissue volume (intracellular and slowly equilibrating interstitial spaces), f_{ub} and f_{uT} are the unbound fractions in blood and tissue, respectively, ϕ_b and ϕ_T are the fraction of unbound drug which is unionized in blood and tissue, respectively, Q_T is the tissue blood flow, and PSA_f and PSA_r are the influx and efflux permeability-surface area products, respectively. (Note that $\phi_b f_{ub}$ is simply the fraction of blood compound, which is unbound and unionized, and similarly for tissue.)

Equation (S3.2) shows the extent that tissue MRT exceeds blood MRT depends on three terms. The first term, V_v/Q_T, is the time it takes for blood perfusion to a tissue to completely refresh the "vascular" volume of the tissue. The physiological interpretation of the "vascular volume" must be made with caution, as it depends on which membrane - capillary wall or the cell membrane - is the rate-limiting step in the drug's passage from capillary blood through the interstitial fluid to reach the intracellular fluid of the target tissue. In tissues such as muscle, fat, and CNS, whose endothelium cells form a continuous wall around the lumen of the capillary with tight junctions between cells, the capillary wall would be the rate limiting membrane, and in that case V_v refers to the volume of capillary space (typically 2-4% of the total tissue volume). However, in tissues with fenestrated capillaries - such as intestinal villi, endocrine glands, and kidney glomerular - or sinusoidal capillaries - such as liver, bone marrow, and spleen sinusoidal - the capillary wall is porous to allow small molecules to pass with relative ease. For these tissues, V_v would encompass both capillary space and the interstitial fluid, where drug concentration can equilibrate quickly, with plasma membrane being the barrier rate-limiting the drug from reaching the intracellular space.

The second term in the equation quantifies the contribution of blood perfusion to the tissue residence time. V_T/Q_T is the time it takes for blood flow to completely turn over the volume of tissue and is analogous to V_v/Q_T from the vascular space. The prefactors are just the blood-to-tissue partition coefficient, $K_B = (\phi_b f_{ub} PSA_f)/(\phi_T f_{uT} PSA_r)$, and amplify the contribution of

perfusion turnover time to the tissue resistant time. For compounds with low tissue partitioning and without tissue uptake, $K_B \approx 1$, and the contribution from this term is minimal. Uptake transport increases, while efflux transport reduces, tissue resident time.

The third term, $V_T/(\phi_T f_{uT} PSA_r)$, relates to the tissue residence time of a compound due to reverse permeability flux. For highly permeable compounds, this term's contribution is minimal. Remember, the membrane in question depends on whether capillary wall or the cell membrane is rate limiting the compound's crossing.

Despite its apparent complexity, Eq. (S3.2) predicts changes which are in accord with our intuition: fast tissues are those with rapid blood flow (relative to tissue volume) and little tissue accumulation, while slow tissues have relatively slow blood flow and/or large tissue accumulation.

The table below provides quantitative data on spleen, a prototypical fast tissue, and muscle, a prototypical slow tissue.

Parameters			**Spleen**	**Muscle**
Physiological and anatomical parameters	Blood Flow (mL/min)		150	538
	Tissue Volume (mL)		142	16,946
	Volume of vascular space (% of tissue volume)		23	3
	Surface area available for permeation SA/V_T (cm^2/g)		250	70
Contribution to tissue resident time (min) by various terms in Eqn 3.2	by perfusion turnover of "vascular" space V_v/Q_T (1)		0.5	0.9
	by perfusion turnover of tissue volume V_T/Q_T (2) (see note 2)	K_B=1	0.5	30.5 (≈0.5 hr)
		K_B=3	1.4	91.6 (≈1.5 hr)
		K_B=10	4.8	305.4 (≈5 hr)
	by membrane permeation, $V_T/(\phi_T f_{uT} PSA_r)$, assuming $f_{uT} = f_{ub} = 0.1$, $\phi_T = \phi_b = 1$, and $K_B = 1$ (3)	P=100x10^{-6} cm/s	6.7	23.8
		P=30x10^{-6} cm/s	22.2	79.4 (≈1 hr)
		P=10x10^{-6} cm/s	66.7 (≈1 hr)	238.1 (≈4 hr)

Table S5. Calculations of Mean Tissue Resident Time using spleen and muscle as examples of "fast" and "slow" tissues.

1. Unlike other terms, this delay is independent of the ADME properties of the drug, such as tissue partitioning.
2. Similar numbers can be obtained for fat, skin, or bone. The contribution of this term to the tissue residence time is amplified by K_B. For spleen, the amplification lead to negligible increase in tissue resident time. However, for muscle, an increase in K_B would significantly increase residence time (e.g. if $K_B = 10$, then this term would lead to a 5-hour slowdown in the tissue residence time, which becomes significant)
3. The surface area available for permeation is not available for all tissues. However, available literature data (Gersh and Still 1945, Pappenheimer, Renkin et al. 1951, Crone 1963, Casley-Smith, O'Donoghue et al. 1975) suggests that some tissues such as skin, muscle and fat all have small surface to tissue mass ratio (S/V) around 70 cm/g or less, while many other tissues, such as brain, kidney, liver, and lung all have large S/V ratio around 250 cm^2/g, and intestine has a value of 125 cm^2/g. For highly permeable compounds (e.g. $100x10^{-6}$ cm/s), this term adds relatively little to the lengthening of tissue residence time. The tissue residence time, however, is significantly prolonged for poorly permeable compounds (e.g. $10x10^{-6}$ cm/s), particularly for slow tissues such as muscle.

References

1. Casley-Smith, J., P. O'Donoghue and K. Crocker (1975). "The quantitative relationships between fenestrae in jejunal capillaries and connective tissue channels: proof of “tunnel-capillaries”." Microvascular research **9**(1): 78-100.

2. Crone, C. (1963). "The permeability of capillaries in various organs as determined by use of the ‘indicator diffusion’ method." Acta physiologica scandinavica **58**(4): 292-305.

3. Gersh, I. and M. A. Still (1945). "Blood vessels in fat tissue. Relation to problems of gas exchange." The Journal of experimental medicine **81**(2): 219-232.

4. Pappenheimer, J., E. Renkin and L. Borrero (1951). "Filtration, diffusion and molecular sieving through peripheral capillary membranes: a contribution to the pore theory of capillary permeability." American journal of physiology-legacy content **167**(1): 13-46.

S4 Relationship between blood and tissue drug concentrations in terms of physiological and ADME parameters

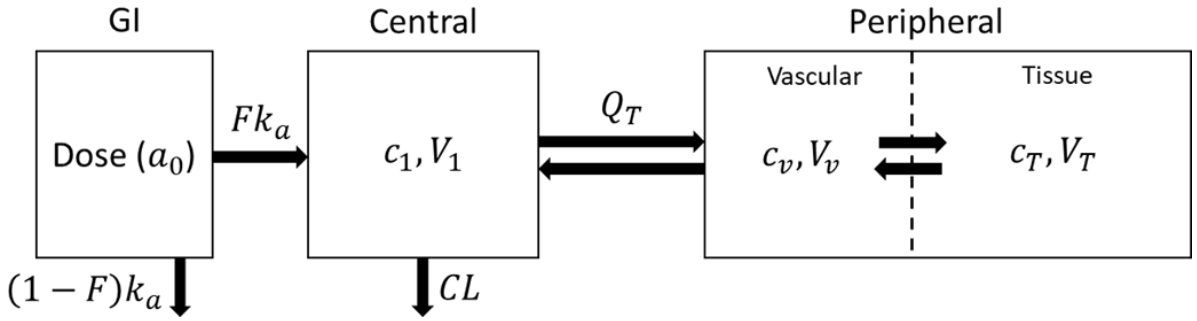

Figure S2. Schematic representation of the compartmental PK model investigated in this section. The model is a modified two-compartment model, where the peripheral compartment is divided into tissue-associated vascular space and tissue space (intracellular and slowly equilibrating interstitial spaces).

Here we investigate the relationship between the drug concentrations in blood and tissue in terms of physiological and ADME properties, in order to quantify how this relationship depends on tissue type and tissue partitioning. The outputs of this analysis are equations (1), (2), and (3) in the main text, which relate unbound tissue AUC to unbound blood AUC, and tissue MRT to blood MRT, respectively.

We consider a modified two-compartment model with a central compartment and a peripheral compartment (Figure S2), where the peripheral compartment is divided into two sub-compartments representing vascular and tissue spaces (the latter representing intra-cellular space and slowly equilibrating interstitial spaces). The drug is dosed in the gut and a fraction, F, is absorbed via a first-order process, with rate k_a, into the central compartment. Let the drug concentration in the ith compartment be c_i and the compartment volume be V_i, where $i = 1$ for the central compartment, $i = v$ for the vascular compartment, $i = T$ for the tissue compartment, and $i = \text{GI}$ for the gut compartment. Let $a_i = V_i c_i$ be the amount of drug in the ith compartment. Let the total peripheral volume be $V_2 = V_v + V_T$, the total amount of drug in the peripheral compartment be $a_2 = a_v + a_T$, and the peripheral concentration be $c_2 = a_2/V_2$. Then the kinetic equations are given by

$$\frac{da_1}{dt} = -CL\, c_1 - Q_T c_1 + Q_T c_v + Fk_a a_{GI} \qquad (S4.1)$$

$$\frac{da_v}{dt} = Q_T c_1 - Q_T c_v - \phi_b f_{ub} PSA_f c_v + \phi_T f_{uT} PSA_r c_T \qquad (S4.2)$$

$$\frac{da_T}{dt} = \phi_b f_{ub} PSA_f c_v - \phi_T f_{uT} PSA_r c_T \qquad (S4.3)$$

$$\frac{da_{GI}}{dt} = -k_a a_{GI} \qquad (S4.4)$$

where CL is the blood clearance in the central compartment, Q_T is blood flow to the peripheral compartment, f_{ub} and f_{uT} are the fraction unbound in blood and tissue, respectively, ϕ_b and ϕ_T are the fractions of unbound drug which are unionized in blood and tissue, respectively, and PSA_f and PSA_r are the permeability-surface area products for tissue influx and efflux, respectively. Note that $\phi_b f_{ub}$ is simply the fraction of blood compound which is both unbound and unionized (and similarly for tissue), and we have assumed only this fraction can cross the cell membrane.

Equation (S4.4) for the gut compartment is easy to solve and gives $a_{GI}(t) = a_0 e^{-k_a t}$, where a_0 is the total dose. The notation will be simpler if we work with amounts, a_i, instead of concentrations, and define the following five rates:

$$w_1 = \frac{\phi_b f_{ub} PSA_f}{V_v},\ w_2 = \frac{\phi_T f_{uT} PSA_r}{V_T},\ p_1 = \frac{Q_T}{V_1},\ p_2 = \frac{Q_T}{V_v},\ \text{and } k_{10} = \frac{CL}{V_1}. \qquad (S4.5)$$

Then the kinetic equations can be written as

$$\frac{da_1}{dt} = -(k_{10} + p_1)a_1 + p_2 a_v + F k_a a_0 e^{-k_a t} \qquad (S4.6)$$

$$\frac{da_v}{dt} = p_1 a_1 - (p_2 + w_1)a_v + w_2 a_T \qquad (S4.7)$$

$$\frac{da_T}{dt} = w_1 a_v - w_2 a_T, \qquad (S4.8)$$

with initial conditions $a_1(0) = 0,\ a_v(0) = 0$, and $a_T(0) = 0$.

Before going further, it is helpful to solve this system in the two extreme limits of a permeability limited tissue and a perfusion limited tissue.

Permeability Limited Tissue

In the permeability limited case the influx and efflux rates are much slower than the blood flow rate, that is, $PSA_f, PSA_r \ll Q_T$. In this case we expect $c_v \approx c_1$ (this will be confirmed from the exact solution below), and the solution in the central compartment is simply

$$c_1(t) = \frac{F a_0}{(V_1 + V_v)} \frac{k_a}{k_a - k'_{10}} \left(e^{-k'_{10} t} - e^{-k_a t}\right),$$

where $k'_{10} = CL/(V_1 + V_v)$ and we use $V_1 + V_v$ because the central and vascular compartments equilibrate so rapidly they should be treated as a single compartment (which gives rise to the condition $c_v \approx c_1$). In this approximation the equation for $c_T(t)$ is

$$\frac{dc_T}{dt} = \frac{\phi_b f_{ub} PSA_f}{V_T} \frac{F a_0}{(V_1 + V_v)} \frac{k_a}{k_a - k'_{10}} \left(e^{-k'_{10} t} - e^{-k_a t}\right) - \frac{\phi_T f_{uT} PSA_r}{V_T} c_T$$

which can be directly integrated after rewriting as

$$\frac{d}{dt}\left[c_T(t)\exp\left(\frac{\phi_T f_{uT} PSA_r}{V_T}t\right)\right] = \frac{\phi_b f_{ub} PSA_f}{V_T}\frac{F a_0}{(V_1+V_v)}\frac{k_a}{k_a - k'_{10}}\left[e^{\left(\frac{\phi_T f_{uT} PSA_r}{V_T} - k'_{10}\right)t} - e^{\left(\frac{\phi_T f_{uT} PSA_r}{V_T} - k_a\right)t}\right]$$

giving

$$c_T(t) = \frac{\phi_b f_{ub} PSA_f}{V_T}\frac{F a_0}{(V_1+V_v)}\frac{k_a}{k_a - k'_{10}}\left\{\frac{1}{k_a - \frac{\phi_T f_{uT} PSA_r}{V_T}}\left[e^{-k_a t} - e^{-\frac{\phi_T f_{uT} PSA_r}{V_T}t}\right] + \frac{1}{k'_{10} - \frac{\phi_T f_{uT} PSA_r}{V_T}}\left[e^{-\frac{\phi_T f_{uT} PSA_r}{V_T}t} - e^{-k'_{10}t}\right]\right\}.$$

Perfusion Limited Tissue

In this case the blood flow rate is much slower than the tissue influx and efflux rates ($PSA_f, PSA_r \gg Q_T$), and we expect the system to reduce to the conventional two-compartment PK model. We assume that the vascular and tissue spaces rapidly equilibrate, giving $\phi_b f_{ub} PSA_f c_v = \phi_T f_{uT} PSA_r c_T$. We are interested in the total peripheral concentration, $c_2(t) = \frac{V_v c_v(t) + V_T c_T(t)}{V_2} = \frac{1}{V_2}\left(V_v + V_T \frac{\phi_b f_{ub} PSA_f}{\phi_T f_{uT} PSA_r}\right) c_v(t)$. We define the tissue-to-blood partition coefficient to be

$$K_B = \frac{\phi_b f_{ub} PSA_f}{\phi_T f_{uT} PSA_r} \qquad (S4.9)$$

and a related coefficient $K'_B = (V_v + V_T K_B)/V_2$, giving $c_v(t) = c_T(t)/K'_B$. Note that in the usual two-compartment model the vascular space is assumed to take up a negligible fraction of the peripheral compartment, which means we are interested in the limit $V_v \to 0$, in which case $K'_B \to K_B$.

Returning to our kinetic equations, we add together the kinetic equations for a_v and a_T to get two equations describing the system in terms of a_2 and a_1,

$$\frac{da_1}{dt} = -CL\ c_1 - Q_T c_1 + Q_T c_v + F k_a a_0 e^{-k_a t}$$
$$\frac{da_2}{dt} = Q_T c_1 - Q_T c_v \quad .$$

Substituting c_2 for c_v gives

$$\frac{da_1}{dt} = -(CL + Q_T)c_1 + Q_T c_2/K'_B + F k_a a_0 e^{-k_a t}$$
$$\frac{da_2}{dt} = Q_T c_1 - Q_T c_2/K'_B \quad .$$

Note that the vascular concentration no longer appears in the kinetic equations, so we are free to take $V_v \to 0$, giving $K_B' \to K_B$ and $c_2 \to c_T$, and we can now write

$$\frac{da_1}{dt} = -(CL + Q_T)c_1 + \frac{Q_T c_T}{K_B} + Fk_a a_0 e^{-k_a t} \qquad (S4.10)$$

$$\frac{da_T}{dt} = Q_T c_1 - Q_T c_T / K_B. \qquad (S4.11)$$

Equations (S4.10) and (S4.11) are the kinetic equations for the conventional two-compartment PK model, where the peripheral compartment volume is identified as $V_2 = K_B V_T$. The solution of these equations is well-known and will not be repeated here.

General Solution

We now solve the kinetic equations for the complete system. Let $\tilde{a}_i(s)$ be the Laplace tranform of $a_i(t)$, and take the Laplace transforms of equations (S4.6) -(S4.8) to get

$$\tilde{a}_1 = -(k_{10} + p_1)\tilde{a}_1 + p_2\tilde{a}_v + \frac{Fk_a a_0}{s + k_a} \qquad (S4.12)$$

$$\tilde{a}_v = p_1\tilde{a}_1 - (p_2 + w_1)\tilde{a}_v + w_2\tilde{a}_T \qquad (S4.13)$$

$$\tilde{a}_T = w_1\tilde{a}_v - w_2\tilde{a}_T. \qquad (S4.14)$$

Equation (S4.14) gives

$$\tilde{a}_T(s) = \frac{w_1}{s + w_2}\tilde{a}_v(s), \qquad (S4.15)$$

and plugging (S4.15) into (S4.13) allows us to write $\tilde{a}_v$ in terms of $\tilde{a}_1$, giving

$$\tilde{a}_v(s) = \frac{p_1(s + w_2)}{s^2 + (w_1 + w_2 + p_2)s + p_2 w_2}\tilde{a}_1(s). \qquad (S4.16)$$

Finally, using (S4.16) in (S4.12) allows us to solve for $\tilde{a}_1$, giving

$$\tilde{a}_1(s) = \left(\frac{Fa_0 k_a}{s + k_a}\right)\left(\frac{s^2 + \lambda s + \kappa}{s^3 + \beta s^2 + \gamma s + \delta}\right), \qquad (S4.17)$$

where

$$\begin{aligned} \lambda &= w_1 + w_2 + p_2 \\ \kappa &= p_2 w_2 \\ \beta &= k_{10} + p_1 + p_2 + w_1 + w_2 \\ \gamma &= k_{10}(p_2 + w_1) + w_2(k_{10} + p_1) + p_1 w_1 + p_2 w_2 \\ \delta &= k_{10} p_2 w_2 \,. \end{aligned} \qquad (S4.18)$$

Let s_1, s_2, and s_3 be the roots of the cubic in the denominator of Eq. (S4.17), and define $r_i = -s_i$ for $i = 1,2,3$. Now factor the denominator and use partial fraction decomposition to get

$$\begin{aligned} \frac{\tilde{a}_1(s)}{Fa_0 k_a} &= \frac{1}{s + k_a}\left[\frac{s^2 + \lambda s + \kappa}{(s + r_1)(s + r_2)(s + r_3)}\right] \\ &= \frac{1}{s + k_a}\left[\frac{r_1^2 - \lambda r_1 + \kappa}{(r_2 - r_1)(r_3 - r_1)}\frac{1}{s + r_1} - \frac{r_2^2 - \lambda r_2 + \kappa}{(r_2 - r_1)(r_3 - r_2)}\frac{1}{s + r_2} + \frac{r_3^2 - \lambda r_3 + \kappa}{(r_3 - r_1)(r_3 - r_2)}\frac{1}{s + r_3}\right] \end{aligned}$$

$$= \frac{r_1^2 - \lambda r_1 + \kappa}{(r_2 - r_1)(r_3 - r_1)(k_a - r_1)}\left(\frac{1}{s + r_1} - \frac{1}{s + k_a}\right) - \frac{r_2^2 - \lambda r_2 + \kappa}{(r_2 - r_1)(r_3 - r_2)(k_a - r_2)}\left(\frac{1}{s + r_2} - \frac{1}{s + k_a}\right) + \frac{r_3^2 - \lambda r_3 + \kappa}{(r_3 - r_1)(r_3 - r_2)(k_a - r_3)}\left(\frac{1}{s + r_3} - \frac{1}{s + k_a}\right)$$

and take the inverse Laplace transform to get

$$a_1(t) = Fa_0k_a\left[\frac{r_1^2 - \lambda r_1 + \kappa}{(r_2 - r_1)(r_3 - r_1)(k_a - r_1)}\left(e^{-r_1 t} - e^{-k_a t}\right) - \frac{r_2^2 - \lambda r_2 + \kappa}{(r_2 - r_1)(r_3 - r_2)(k_a - r_2)}\left(e^{-r_2 t} - e^{-k_a t}\right) + \frac{r_3^2 - \lambda r_3 + \kappa}{(r_3 - r_1)(r_3 - r_2)(k_a - r_3)}\left(e^{-r_3 t} - e^{-k_a t}\right)\right]. \quad (S4.19)$$

To solve for $a_v(t)$, use the unfactored form of $\tilde{a}_1(s)$ to write

$$\begin{aligned}\tilde{a}_v(s) &= \left[\frac{p_1(s + w_2)}{s^2 + \lambda s + \kappa}\right]\left(\frac{Fa_0k_a}{s + k_a}\right)\left[\frac{s^2 + \lambda s + \kappa}{(s + r_1)(s + r_2)(s + r_3)}\right] \\ &= \frac{Fa_0k_ap_1}{s + k_a}\left[\frac{s + w_2}{(s + r_1)(s + r_2)(s + r_3)}\right] \\ &= Fa_0k_ap_1\left\{\frac{w_2 - r_1}{(r_2 - r_1)(r_3 - r_1)}\frac{1}{(s + k_a)(s + r_1)} + \frac{r_2 - w_2}{(r_2 - r_1)(r_3 - r_2)}\frac{1}{(s + k_a)(s + r_2)} + \frac{w_2 - r_3}{(r_3 - r_1)(r_3 - r_2)}\frac{1}{(s + k_a)(s + r_3)}\right\} \\ &= Fa_0k_ap_1\left\{\frac{w_2 - r_1}{(r_2 - r_1)(r_3 - r_1)}\frac{1}{(k_a - r_1)}\left[\frac{1}{s + r_1} - \frac{1}{s + k_a}\right] + \frac{r_2 - w_2}{(r_2 - r_1)(r_3 - r_2)}\frac{1}{(k_a - r_2)}\left[\frac{1}{s + r_2} - \frac{1}{s + k_a}\right] + \frac{w_2 - r_3}{(r_3 - r_1)(r_3 - r_2)}\frac{1}{(k_a - r_3)}\left[\frac{1}{s + r_3} - \frac{1}{s + k_a}\right]\right\},\end{aligned}$$

and invert the Laplace transform to get

$$a_v(t) = Fa_0p_1\left\{\frac{w_2 - r_1}{(r_2 - r_1)(r_3 - r_1)}\frac{k_a}{(k_a - r_1)}\left[e^{-r_1 t} - e^{-k_a t}\right] + \frac{r_2 - w_2}{(r_2 - r_1)(r_3 - r_2)}\frac{k_a}{(k_a - r_2)}\left[e^{-r_2 t} - e^{-k_a t}\right] + \frac{w_2 - r_3}{(r_3 - r_1)(r_3 - r_2)}\frac{k_a}{(k_a - r_3)}\left[e^{-r_3 t} - e^{-k_a t}\right]\right\}. \quad (S4.20)$$

To solve for $a_T(t)$, use the unfactored form of $\tilde{a}_v(s)$ to write

$$\begin{aligned}
\tilde{a}_T(s) &= \left(\frac{w_1}{s+w_2}\right)\left(\frac{Fa_0k_ap_1}{s+k_a}\right)\left[\frac{s+w_2}{(s+r_1)(s+r_2)(s+r_3)}\right] \\
&= \frac{Fa_0k_ap_1w_1}{s+k_a}\left[\frac{1}{(s+r_1)(s+r_2)(s+r_3)}\right] \\
&= Fa_0k_ap_1w_1\left[\frac{1}{(r_2-r_1)(r_3-r_1)}\frac{1}{(s+k_a)(s+r_1)} - \frac{1}{(r_2-r_1)(r_3-r_2)}\frac{1}{(s+k_a)(s+r_2)}\right. \\
&\qquad \left. + \frac{1}{(r_3-r_1)(r_3-r_2)}\frac{1}{(s+k_a)(s+r_3)}\right] \\
&= Fa_0k_ap_1w_1\left\{\frac{1}{(r_2-r_1)(r_3-r_1)}\frac{1}{(k_a-r_1)}\left[\frac{1}{s+r_1} - \frac{1}{s+k_a}\right]\right. \\
&\qquad - \frac{1}{(r_2-r_1)(r_3-r_2)}\frac{1}{(k_a-r_2)}\left[\frac{1}{s+r_2} - \frac{1}{s+k_a}\right] \\
&\qquad \left. + \frac{1}{(r_3-r_1)(r_3-r_2)}\frac{1}{(k_a-r_3)}\left[\frac{1}{s+r_3} - \frac{1}{s+k_a}\right]\right\},
\end{aligned}$$

and invert the Laplace transform to get

$$\begin{aligned}
a_T(t) = Fa_0p_1w_1\Bigg\{&\frac{1}{(r_2-r_1)(r_3-r_1)}\frac{k_a}{(k_a-r_1)}\left[e^{-r_1t} - e^{-k_at}\right] \\
&- \frac{1}{(r_2-r_1)(r_3-r_2)}\frac{k_a}{(k_a-r_2)}\left[e^{-r_2t} - e^{-k_at}\right] \\
&+ \frac{1}{(r_3-r_1)(r_3-r_2)}\frac{k_a}{(k_a-r_3)}\left[e^{-r_3t} - e^{-k_at}\right]\Bigg\}.
\end{aligned} \qquad (S4.21)$$

Finally, let's write down expressions for r_i. Let $\sigma = \beta^2 - 3\gamma$ and $\tau = (9\beta\gamma - 2\beta^3 - 27\delta)/2$, then

$$\begin{aligned}
r_1 &= \frac{\beta}{3} + \left(\frac{1-i\sqrt{3}}{2}\right)\frac{\sigma}{3\left(\tau+\sqrt{\tau^2-\sigma^3}\right)^{1/3}} + \left(\frac{1+i\sqrt{3}}{2}\right)\frac{\left(\tau+\sqrt{\tau^2-\sigma^3}\right)^{1/3}}{3} \\
r_2 &= \frac{\beta}{3} - \frac{\sigma}{3\left(\tau+\sqrt{\tau^2-\sigma^3}\right)^{1/3}} - \frac{\left(\tau+\sqrt{\tau^2-\sigma^3}\right)^{1/3}}{3} \\
r_3 &= \frac{\beta}{3} + \left(\frac{1+i\sqrt{3}}{2}\right)\frac{\sigma}{3\left(\tau+\sqrt{\tau^2-\sigma^3}\right)^{1/3}} + \left(\frac{1-i\sqrt{3}}{2}\right)\frac{\left(\tau+\sqrt{\tau^2-\sigma^3}\right)^{1/3}}{3}
\end{aligned} \qquad (S4.22)$$

and note that these rates are real and non-negative despite the presence of the imaginary unit.

Lastly, convert our solutions from amounts to concentrations to obtain

$$c_1(t) = \frac{Fa_0}{V_1}\left[\frac{r_1^2 - \lambda r_1 + \kappa}{(r_2 - r_1)(r_3 - r_1)}\frac{k_a}{(k_a - r_1)}\left(e^{-r_1 t} - e^{-k_a t}\right)\right.$$
$$- \frac{r_2^2 - \lambda r_2 + \kappa}{(r_2 - r_1)(r_3 - r_2)}\frac{k_a}{(k_a - r_2)}\left(e^{-r_2 t} - e^{-k_a t}\right)$$
$$\left. + \frac{r_3^2 - \lambda r_3 + \kappa}{(r_3 - r_1)(r_3 - r_2)}\frac{k_a}{(k_a - r_3)}\left(e^{-r_3 t} - e^{-k_a t}\right)\right], \qquad (S4.23)$$

$$c_v(t) = \frac{Fa_0 p_1}{V_v}\left[\frac{w_2 - r_1}{(r_2 - r_1)(r_3 - r_1)}\frac{k_a}{(k_a - r_1)}\left(e^{-r_1 t} - e^{-k_a t}\right)\right.$$
$$- \frac{w_2 - r_2}{(r_2 - r_1)(r_3 - r_2)}\frac{k_a}{(k_a - r_2)}\left(e^{-r_2 t} - e^{-k_a t}\right)$$
$$\left. + \frac{w_2 - r_3}{(r_3 - r_1)(r_3 - r_2)}\frac{k_a}{(k_a - r_3)}\left(e^{-r_3 t} - e^{-k_a t}\right)\right], \qquad (S4.24)$$

$$c_T(t) = \frac{Fa_0 p_1 w_1}{V_T}\left[\frac{1}{(r_2 - r_1)(r_3 - r_1)}\frac{k_a}{(k_a - r_1)}\left(e^{-r_1 t} - e^{-k_a t}\right)\right.$$
$$- \frac{1}{(r_2 - r_1)(r_3 - r_2)}\frac{k_a}{(k_a - r_2)}\left(e^{-r_2 t} - e^{-k_a t}\right)$$
$$\left. + \frac{1}{(r_3 - r_1)(r_3 - r_2)}\frac{k_a}{(k_a - r_3)}\left(e^{-r_3 t} - e^{-k_a t}\right)\right]. \qquad (S4.25)$$

AUC, AUMC, and Mean Residence Time

For compartment i, the AUC, AUMC, and MRT are related to the Laplace transform of the concentration by

$$AUC_i(\infty) = \tilde{c}_i(0) \qquad (S4.26)$$
$$AUMC_i(\infty) = -\tilde{c}_i'(0) \qquad (S4.27)$$
$$MRT_i = -\frac{\tilde{c}_i'(0)}{\tilde{c}_i(0)}. \qquad (S4.28)$$

Furthermore, if the Laplace tranforms of concentrations in two compartments, i and j, are related by $\tilde{c}_i(s) = F_{ij}(s)\tilde{c}_j(s)$, then we have

$$MRT_i = MRT_j - \frac{F_{ij}'(0)}{F_{ij}(0)}. \qquad (S4.29)$$

Here we have

$$\tilde{c}_1(s) = \left(\frac{Fa_0 k_a}{V_1}\right)\left[\frac{s^2 + \lambda s + \kappa}{(s + k_a)(s^3 + \beta s^2 + \gamma s + \delta)}\right] \qquad (S4.30)$$
$$\tilde{c}_T(s) = \left(\frac{Fa_0 k_a p_1 w_1}{V_T}\right)\frac{1}{(s + k_a)(s^3 + \beta s^2 + \gamma s + \delta)} \qquad (S4.31)$$
$$F(s) = \left(\frac{V_1 p_1 w_1}{V_T}\right)\frac{1}{s^2 + \lambda s + \kappa} \qquad (S4.32)$$

with derivatives

$$\tilde{c}_1'(s) = \left(\frac{Fa_0k_a}{V_1}\right)\left\{\frac{2s+\lambda}{(s+k_a)(s^3+\beta s^2+\gamma s+\delta)} - \frac{(s^2+\lambda s+\kappa)[(s^3+\beta s^2+\gamma s+\delta)+(s+k_a)(3s^2+2\beta s+\gamma)]}{[(s+k_a)(s^3+\beta s^2+\gamma s+\delta)]^2}\right\} \quad \text{(S4.33)}$$

$$F'(s) = -\left(\frac{V_1p_1w_1}{V_T}\right)\frac{2s+\lambda}{(s^2+\lambda s+\kappa)^2} \quad \text{(S4.34)}$$

$$\tilde{c}_T'(s) = F(s)\tilde{c}_1'(s) + F'(s)\tilde{c}_1(s). \quad (S4.35)$$

Evaluating these at $s = 0$ gives

$$\tilde{c}_1(0) = \frac{Fa_0}{CL}$$
$$\tilde{c}_T(0) = \frac{K_BFa_0}{CL}$$
$$F(0) = K_B$$
$$\tilde{c}_1'(0) = -\frac{Fa_0}{CL}\left[\frac{K_BV_T}{CL} + \frac{V_1}{CL} + \frac{1}{k_a} + \frac{V_v}{CL}\right]$$
$$F'(0) = -K_B\left[\frac{K_BV_T}{Q_T} + \frac{V_v}{Q_T} + \frac{V_T}{\phi_Tf_{uT}PSA_r}\right]$$
$$\tilde{c}_T'(0) = -\frac{K_BFa_0}{CL}\left[\frac{K_BV_T}{Q_T} + \frac{V_v}{Q_T} + \frac{V_T}{\phi_Tf_{uT}PSA_r} + \frac{K_BV_T}{CL} + \frac{V_1}{CL} + \frac{1}{k_a} + \frac{V_v}{CL}\right].$$

We can now directly read off

$$AUC_1 = \frac{Fa_0}{CL} \quad \text{(S4.36)}$$

$$AUC_T = \frac{K_BFa_0}{CL} \quad \text{(S4.37)}$$

$$AUMC_1 = \frac{Fa_0}{CL}\left[\frac{K_BV_T}{CL} + \frac{V_1}{CL} + \frac{1}{k_a} + \frac{V_v}{CL}\right] \quad \text{(S4.38)}$$

$$AUMC_T = \frac{K_BFa_0}{CL}\left[\frac{K_BV_T}{Q_T} + \frac{V_v}{Q_T} + \frac{V_T}{\phi_Tf_{uT}PSA_r} + \frac{K_BV_T}{CL} + \frac{V_1}{CL} + \frac{1}{k_a} + \frac{V_v}{CL}\right] \quad \text{(S4.39)}$$

$$MRT_1 = \frac{K_BV_T}{CL} + \frac{V_1}{CL} + \frac{1}{k_a} + \frac{V_v}{CL} \quad \text{(S4.40)}$$

$$MRT_T = \frac{K_BV_T}{Q_T} + \frac{V_v}{Q_T} + \frac{V_T}{\phi_Tf_{uT}PSA_r} + \frac{K_BV_T}{CL} + \frac{V_1}{CL} + \frac{1}{k_a} + \frac{V_v}{CL} \quad \text{(S4.41)}$$

$$MRT_T = MRT_1 + \frac{K_BV_T}{Q_T} + \frac{V_v}{Q_T} + \frac{V_T}{\phi_Tf_{uT}PSA_r} \quad (S4.42)$$

Equation (S4.42) appears in the main text as equation (3). To get main text equation (1), combine (S4.36) and (S4.37) to get

$$AUC_T = K_BAUC_1, \quad (S4.43)$$

and use $AUC_{uT} = f_{uT}AUC_T$, $AUC_{ub} = f_{ub}AUC_1$, and equation (S4.9) to write

$$AUC_{uT} = \left(\frac{\phi_b}{\phi_T}\right)\left(\frac{PSA_f}{PSA_r}\right)AUC_{ub}\,. \quad (S4.44)$$

The unbound tissue: blood partition coefficient, $K_{B,uu}$, is defined through $c_{uT} = K_{B,uu} c_{ub}$. Using $c_{u,i} = AUC_{u,i}/\tau$, where τ is the dose interval, and using Equation (S4.44), we immediately arrive at equations (1) and (2) in the main text.

S5 Compartmental model with fast and slow tissues

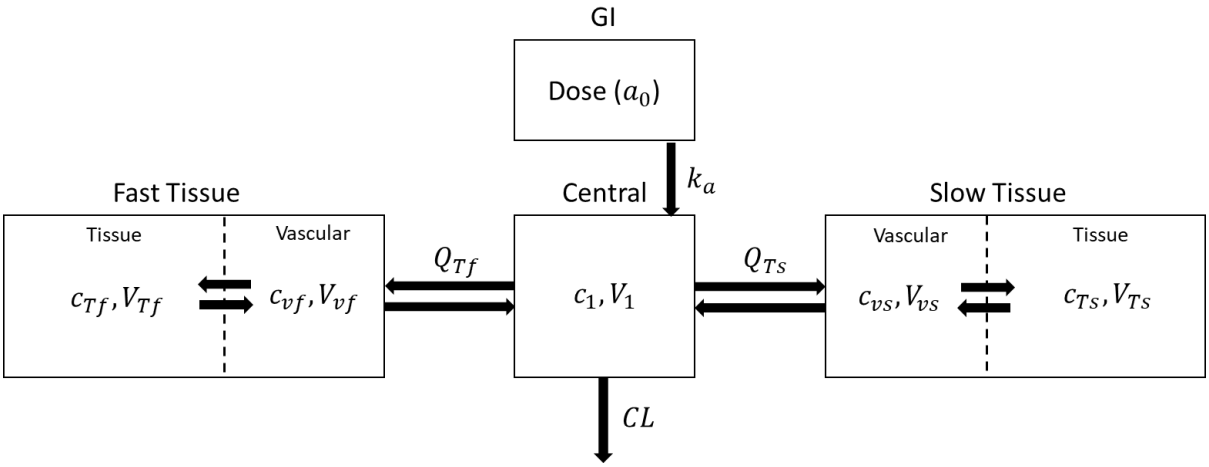

Figure S3. Diagram of the compartmental model described in this supplement.

In this section we describe the PK model with fast and slow tissues which was used to generate Figures 4 and 5 of the main text. The model consists of a central compartment, a GI compartment, and two peripheral compartments, each of which is divided into a tissue-associated vascular space (consisting of vascular space and rapidly equilibrating interstitial space) and a tissue compartment (Fig. S3). For simplicity we only consider a neutral compound. (For an ionizable compound simply replace all instances of f_{ub} and f_{ut} with the fraction that is both unbound and unionized.) The kinetic equations for this system are

$$\frac{da_1}{dt} = -(CL + Q_{Tf} + Q_{Ts})c_1 + Q_{Tf}\,c_{Tf} + Q_{Ts}\,c_{Ts} + k_a a_{GI} \quad (S1)$$

$$\frac{da_{vf}}{dt} = Q_{Tf}\,c_1 - Q_{Tf}c_{vf} - f_{ub}PSA_{ff}c_{vf} + f_{uTf}PSA_{rf}c_{Tf} \quad (S2)$$

$$\frac{da_{Tf}}{dt} = f_{ub}PSA_{ff}c_{vf} - f_{uTf}PSA_{rf}c_{Tf} \quad (S3)$$

$$\frac{da_{vs}}{dt} = Q_{Ts}c_1 - Q_{Ts}c_{vs} - f_{ub}PSA_{fs}c_{vs} + f_{uTs}PSA_{rs}c_{Ts} \quad (S4)$$

$$\frac{da_{Ts}}{dt} = f_{ub}PSA_{fs}c_{vs} - f_{uTs}PSA_{rs}c_{Ts} \quad (S5)$$

where a_1 is the amount of drug in the central compartment, a_{vi} is the amount of drug in the tissue-associated vascular space ($i = f$ for fast tissue, $i = s$ for slow tissue), a_{Ti} is the amount of drug in the tissue space ($i = f$ for fast, $i = s$ for slow), and the compartment volumes (V_1, V_{vf}, V_{Tf}, etc), tissue blood flow (Q_{Tf} and Q_{Ts}), and drug concentrations ($c_1 = a_1/V_1$, $c_{vf} = a_{vf}/V_{vf}$, etc) follow the same subscript convention. The drug is dosed orally and absorbed from the GI via a first-order process with rate k_a, and we have $a_{GI}(t) = a_0 e^{-k_a t}$ for the amount of drug in the GI compartment, where a_0 is the total amount of drug dosed (here we assume the fraction absorbed is 1). Drug is cleared via first-order clearance from the central compartment.

This system was constructed and solved in SimBiology (Matlab R2019b).

We denote the total tissue volume as $V_{tot,i}$ $(i = s, f)$ and let F_V be the fraction of vascular space, here assumed equal in the two tissues. Then we have $V_{vi} = F_V V_{tot,i}$ and $V_{Ti} = (1 - F_V)V_{tot,i}$. We used muscle as a prototypical slow tissue and spleen as a prototypical fast tissue – all other tissues were assumed to be extremely fast and grouped in with the central compartment. (In reality it is unlikely that muscle would be the only slow compartment, but the model is only being used for illustrative purposes and it is convenient to select a single fast and single slow tissue to parameterize the tissue compartments.)

All plots in Figures 4 and 5 were generated assuming the compound has a molecular weight of 500 g/mol and the subject received a PO dose of 10 mg/kg.

The baseline simulation (Figure 4A) was run with parameters listed in Table S6. Parameter changes used to generate Figures 4B, 4C, and all plots in Figure 5 are described in the figure captions in the main text.

Parameter	Value	Units
V_1	1000	ml/kg
$V_{tot,f}$	2.02	ml/kg
$V_{tot,s}$	242	ml/kg
F_V	0.27	dimensionless
Q_{Tf}	128.4	ml/hr/kg
Q_{Ts}	461.4	ml/hr/kg
CL	542.86 (a)	ml/hr/kg
PSA_{ff}	181.8 (b)	ml/hr/kg
PSA_{rf}	181.8 (c)	ml/hr/kg
PSA_{fs}	6089.4 (d)	ml/hr/kg
PSA_{rs}	6089.4 (c)	ml/hr/kg
k_a	10	hr^{-1}
f_{ub}	0.1	dimensionless
f_{uT}	0.1	dimensionless

Table S6. Baseline model parameters.

(a) CL was assumed to be 50% of liver blood flow.

(b) The permeability-surface area product for the fast tissue was obtained by using the capillary surface area of the spleen ($70\ \mathrm{cm}^2/(\mathrm{g\ tissue})$), the spleen volume ($2.02\ \mathrm{ml/kg}$), and assuming the drug has high permeability ($100 \times 10^{-6}\ \mathrm{cm/s}$).

(c) The baseline simulation assumes the tissue influx and efflux rates are identical, i.e., there are no active transport processes.

(d) The permeability-surface area product for the slow tissue was obtained using the capillary surface area of the muscle ($250\ \mathrm{cm}^2/(\mathrm{g\ tissue})$), the muscle volume ($242\ \mathrm{ml/kg}$), and assuming the drug has high permeability ($100 \times 10^{-6}\ \mathrm{cm/s}$).