How the Enhanced Permeability and Retention (EPR) effect regulates tumor accumulation of liposomal doxorubicin in three murine models: optimizing delivery efficiency.

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Supplementary Information

Modeling

Two compartment model. The time-dependent concentration of a drug in circulation is often analyzed using a two-compartment pharmacokinetic model, with a central compartment (vasculature and highly perfused tissue) and a peripheral tissue compartment. This model works well when a drug is intravenously injected and subsequently follows a bi-exponential decay.[1] The first order distribution rate constants between the two compartments, k_p and k_d, in addition to an elimination rate constant, k_{el}, describe the drug behavior (Figure S1). The rate constant k_{el} represents all elimination pathways and includes clearance by the kidneys and the mononuclear phagocyte system (MPS).

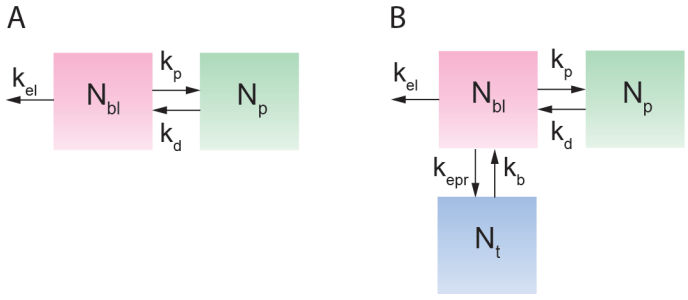

Figure S1. Schematic illustration of pharmacokinetic models. (A) Two compartment pharmacokinetic model with first order rate constants describing exchange between vascular (blood) compartment and peripheral tissue (k_p and k_d) and elimination (k_{el}). (B) Three compartment model with an additional tumor compartment. To account for exchange into and out of this tumor compartment, the first order rate constants k_{epr} and k_b are defined, respectively.

Evaluation of mass balance for each compartment yields the following equations for this system:

$$V_b \frac{dc_b}{dt} = V_p k_d c_p - V_b k_p c_b - V_b k_{el} c_b \quad (1)$$

$$V_p \frac{dc_p}{dt} = V_b k_p c_b - V_p k_d c_p \quad (2)$$

where V_b is the volume of blood (typically 5 - 6 L), V_p is the volume of the peripheral compartment, c_b is the concentration in blood, c_p is the concentration in the peripheral compartment, k_p is the rate constant for transport from the blood into the peripheral tissue, k_d is the rate constant for transport from the peripheral tissue back into circulation, and k_{el} is the rate constant for elimination.

These are represented by the second order differential equation:

$$\frac{d^2 c_b}{dt^2} + A\frac{dc_b}{dt} + Bc_b = 0 \quad (3)$$

The solutions to equations (2) and (3) are of the form:

$$C_b(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (4)$$

where A and B describe the y-intercept for the distribution and elimination phases, respectively, and α and β describe the half-lives of distribution and elimination, respectively. From mass balance, A+B is equal to the initial dose (at time 0). The parameters A, B, α, and β can then be determined by fitting to experimental data. By solving the mass balance equations, it is possible to relate these terms to the physiologically relevant rate constants k_{el}, k_p, and k_d:

$$A + B = Dose \quad (5)$$

$$k_d = \frac{A\beta + B\alpha}{A + B} \quad (6)$$

$$\alpha\beta = k_{el} k_d \quad (7)$$

$$\alpha + \beta = k_d + k_{el} + k_p \quad (8)$$

Three compartment model. In applying pharmacokinetic models of drug delivery to solid tumors, conventional models incorporate tumor accumulation in the elimination rate constant. To evaluate tumor accumulation we have developed a three-compartment model with the addition of a tumor compartment (Figure S1) that allows for quantitative evaluation of the EPR effect on pharmacokinetics.[2]

The rate equations for the three-compartment model (Figure S1) are:

$$V_b \frac{dc_b}{dt} = V_p k_d c_p - V_b k_p c_b + V_t k_b c_t - V_b k_{epr} c_b - V_b k_{el} c_b \tag{9}$$

$$V_p \frac{dc_p}{dt} = V_b k_p c_b - V_p k_d c_p \tag{10}$$

$$V_t \frac{dc_t}{dt} = V_b k_{epr} c_b - V_t k_b c_t \tag{11}$$

where V_t is the tumor volume, c_t is the concentration in the tumor, k_{epr} is the rate constant for transport into the tumor compartment from the blood, and k_b represents transport from the tumor compartment back into circulation. The drug concentration in the blood and the tumor compartment are represented by the third order differential equations:

$$\frac{d^3 c_b}{dt^3} + A\frac{d^2 c_b}{dt^2} + B\frac{dc_b}{dt} + Cc_b = 0 \tag{12}$$

$$\frac{d^3 c_t}{dt^3} + A\frac{d^2 c_t}{dt^2} + B\frac{dc_t}{dt} + Cc_t = 0 \tag{13}$$

The general solutions to the concentration in blood (Equation 14) and in the tumor (Equation 15) are of the form:

$$c_b(t) = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \tag{14}$$

$$c_t(t) = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \tag{15}$$

Similarly, the sum of the constants A, B, and C in the blood are constrained to the initial dose, and using the differential equations from the mass balance, we can relate A, B, and C to the rate constants.

$$A = k_p + k_d + k_{el} + k_{epr} + k_b \tag{16}$$

$$B = k_b k_p + k_b k_{el} + k_{epr} k_d + k_d k_b + k_d k_{el} \tag{17}$$

$$C = k_b k_d k_{el} \tag{18}$$

Further evaluation yields the complex solutions for α, β, and γ:

$$\alpha = Q - \frac{1}{Q} - \frac{A}{3} \tag{19}$$

$$\beta = \frac{-\frac{A^2}{9} + \frac{B}{3}}{2Q} - \frac{A}{3} - \frac{Q}{2} - \frac{\sqrt{3}\left(\frac{-\frac{A^2}{9} + \frac{B}{3}}{Q} + Q\right)i}{2} \tag{20}$$

$$\gamma = \frac{-\frac{A^2}{9} + \frac{B}{3}}{2Q} - \frac{A}{3} - \frac{Q}{2} + \frac{\sqrt{3}\left(\frac{-\frac{A^2}{9} + \frac{B}{3}}{Q} + Q\right)i}{2} \tag{21}$$

where the Q is given by:

$$Q = \left[\frac{AB}{6} - \frac{C}{2} + \sqrt{\left(-\frac{A^2}{9} + \frac{B}{3}\right)^3 + \left(\frac{A^3}{27} - \frac{AB}{6} + \frac{C}{2}\right)^2} - \frac{A^3}{27}\right]^{1/3} \tag{22}$$

Analysis of *in vivo* data

In order to elucidate rate constants describing drug distribution and tumor accumulation, we first utilize the two-compartment model for tumor-free mice to obtain values for k_p, k_d and k_{el}. It can be assumed that k_p, and k_d remain constant for experiments in tumor-bearing mice on administration of the same drug at the same concentration. Using this assumption, there are three unknown rate constants for tumor-bearing mice: k_{el}, k_{epr} and k_b. Fits of experimental data to the mass balance equations were performed using Matlab. The rate constants were determined by an error minimization method. For each data point, the error was determined to be the

difference between the experimentally obtained value and the predicted value and was then normalized to the average experimental value for both pharmacokinetic and tumor accumulation data. The normalized error for all data points, including both pharmacokinetic and tumor accumulation data, was then added together to yield a total normalized error. For iterative values of k_{epr} and k_b, we determined the total normalized error, and the instance of minimum error provides the best fit and yields rate constants to evaluate liposome distribution.

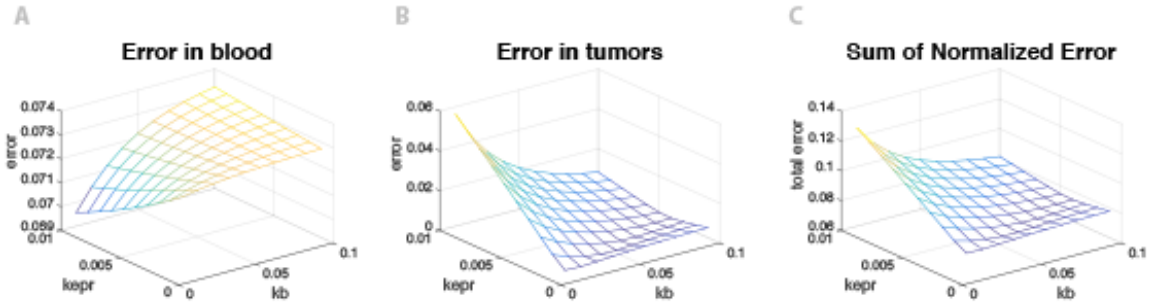

Figure S1. Optimization of fits to 3-compartment model. To optimize fits of the 3-compartment model to our data, the errors are normalized to concentration in (A) blood or (B) tumor. (C) These values are summed to get the total normalized error that is minimized to provide the best values for k_{epr} and k_b. A similar process was completed to find the best value for k_{el}.

References

1. Saltzman, W. M., *Drug delivery: engineering principles for drug therapy*. (Oxford University Press, Oxford ; New York, 2001).

2. Wong, A. D.; Ye, M.; Ulmschneider, M. B.; Searson, P. C. Quantitative Analysis of the Enhanced Permeation and Retention (EPR) Effect. *PLoS One*. **2015**, 10, e0123461.