Predicted and Observed Effects of Antibody Affinity and Antigen Density on Monoclonal Antibody Uptake in Solid Tumors

Cynthia Sung, Ty R. Shockley, Paul F. Morrison, Harold F. Dvorak, Martin L. Yarmush, and Robert L. Dedrick

Chemical Engineering Section, Biomedical Engineering and Instrumentation Program, National Center for Research Resources, NIH, Bethesda, Maryland 20892

INTRODUCTION

Uptake of MAbs by solid tumors depends on factors governing the antibody-antigen interaction as well as on host-related factors such as plasma clearance and tumor physiology. Pharmacokinetic models offer theoretical descriptions of the interdependence of these various factors (1–6). The models can suggest experiments to test the relevance of these factors as well as provide ideas for strategies to improve MAb-based therapies. In this paper, we present a physiologically based, compartamental pharmacokinetic model which relates tumor uptake of a MAb to its plasma kinetics, capillary permeability-area product, and binding affinity and to the interstitial fluid flow rate, interstitial volume fraction, and concentration of antigen in the tumor. The purpose of developing this model was 2-fold. One was to simulate the effects on MAb uptake in solid tumors produced by changing the value of parameters believed to play an important role in transport and binding. By relating the transport and binding events to physiological processes and antibody-antigen interactions, we could examine theoretically the effect of parameters within a range of values representative of MAbs and test experimentally whether parameter changes would lead to model-predicted changes in MAb uptake. The other was to apply the model to analysis of experimental data in order to obtain quantitative estimates of the transport and binding constants in vivo systems. Fitting data to a mathematical model requires that the model be parsimonious in the number of unknown constants. Therefore, our approach was to develop as simple a model as possible, including only the predominant processes governing transport and binding while recognizing that some quantitative conclusions drawn from data fitting are limited by approximations in the model. Our pharmacokinetic model differs from previously published models (1, 3–5) in that the others include a more comprehensive range of processes that may affect uptake of MAbs in tumors. For the experimental conditions considered by us, however, the larger number of adjustable parameters and the complexity of the equations in those models makes them more cumbersome for data analysis. The model presented in this paper provides a useful exploratory tool for the experimentalist and aids in understanding how certain MAb and tumor properties affect the pharmacokinetics of MAbs.

Recently, we described the special case when MAb doses are low enough such that the binding sites in the tumor are far from being saturated (6). The low dose case allowed us to represent the ratio of free and bound drug in the interstitium as a constant. This has the advantage that the relevant differential equation is linear, making it easier to understand the mathematical relationship among the various parameters. Often, however, doses of interest are sufficiently high that the binding sites approach saturation (7–10). In the present paper, we extend our earlier work over a wider dose range by representing binding with the Langmuir adsorption isotherm for reversible, saturable binding. The differential equation which describes the interstitial MAb concentration no longer has an exact analytical solution but must be solved numerically. Below we show several model simulations on the effects of varying the affinity constant, $K_a$, or the concentration of binding sites, $B_0$. We also present experimental data on MAb uptake at high doses in solid melanoma xenografts using MAbs which vary in $K_a$ or $B_0$. The experimental results support predictions of the model that a very high affinity MAb does not necessarily attain measurably higher uptake in tumors compared to a moderate affinity MAb whereas increases in $B_0$ are more likely to yield higher MAb uptake.

Received 6/19/91; accepted 11/1/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Building 13, Room 3W13.

2 Present address: Baxter Healthcare Corp., Round Lake, IL 60073.

3 The abbreviations used are: MAb, monoclonal antibody; AUC, area under the concentration-time curve; CEA, carcinoembryonic antigen.
MATERIALS AND METHODS

Model Development. The plasma and total tumor concentrations of a MAb are experimentally measurable quantities in tumor uptake studies. The total interstitial tumor concentration (e.g. mol/g interstitial fluid) can be calculated from the total tumor concentration (mol/g tumor) by subtracting the amount of MAb which exists in the plasma space of the tumor and dividing the remainder by the fractional interstitial volume of the tumor, \( \phi \) (6, 11). The model represents the plasma and interstitial tumor spaces as compartments of uniform concentration (Fig. 1) and assumes that: (a) after a bolus i.v. injection, the plasma MAb concentration, \( C_p \), decreases biexponentially from an initial plasma concentration \( C_{p0} \) (Equation A); (b) MAb is transported from the plasma into the tumor unidirectionally at a rate equal to the product of the plasma-to-tissue transport constant, \( k \), and the coexisting plasma concentration; (c) MAb in the tumor interstitium rapidly equilibrates between free and bound forms according to the Langmuir adsorption isotherm (Equation B), where \( K_a \) is the MAb affinity constant and \( B_0 \) is the interstitial concentration of binding sites; and (d) loss of free MAb from the tumor occurs at a rate governed by a loss constant, \( L \), and the concentration of free antibody in the tumor. Physiological interpretations of \( k \) and \( L \) are that \( k \) represents the capillary permeability times the capillary surface area per g of tumor and that \( L \) represents the interstitial fluid flow per g of tumor, the mechanism for clearance of free interstitial proteins from the tumor. Assumptions b and d are combined in an ordinary differential equation that describes the overall rate of change of antibody concentration in the tumor interstitium (Equation D). Equation C is a mass balance which states that the sum of the bound and free interstitial concentrations, \( C_b \) and \( C_f \), respectively, is equal to the total interstitial concentration, \( C_t \).

**Plasma compartment:**

\[
\frac{C_t}{C_{p0}} = R e^{-kt} + (1-R) e^{-\lambda t} \quad \text{(A)}
\]

**Tissue compartment:**

\[
\frac{C_i}{C_t} = \frac{K_a B_0 C_t}{1 + K_a C_t} \quad \text{(B)}
\]

\[
C_i = C_b + C_f \quad \text{(C)}
\]

\[
\phi \frac{dC_i}{dt} = kC_p - LC_i \quad \text{(D)}
\]

We have modeled transcapillary transport as a unidirectional process because macromolecules the size of albumin and larger are transported into normal tissues mainly by hydrostatically driven convection and are cleared mainly by lymphatic drainage (12) rather than being transported back across the capillaries (13). Tumors lack a lymphatic network, but interstitial fluid similar in protein content to normal lymph is observed to exude from tumors (14). Thus, interstitial fluid flow in tumor probably serves a role similar to lymph flow in normal tissue as the main clearance mechanism for proteins. The assumption of unidirectional transcapillary transport, however, limits application of the model to large macromolecules; the model equations as written would not be appropriate for MAb fragments such as Fab or Fv. This assumption can easily be relaxed by including transcapillary tumor-to-plasma transport in Equation D, although at the cost of introducing another parameter.

The validity of the assumption that the tumor may be represented as a compartment of uniform concentration depends on many factors such as the homogeneity of tumor structure, intercapillary distances, MAb affinity, and dose. For example, the model is probably appropriate for small tumors but not for large tumors which have substantial pressure gradients or necrotic areas. The distributed model of MAb transport of Fujimori et al. (3) predicts that for an intercapillary distance of 170 \( \mu \)m, fairly uniform concentrations are obtained with MAb affinities less than \( 10^8 \) M\(^{-1}\) but that nonuniformities become more pronounced as MAb affinity is increased. Thus, for high affinity antibodies and large intercapillary distances, the number of binding sites that is functionally accessible to MAb may be only a fraction of the total. Dose also affects the homogeneity of MAb distribution: Matzku et al. (8) and Fenwick et al. (9) have shown that as dose is increased, the spatial distribution of MAb becomes increasingly uniform.

The assumption of rapid equilibrium was tested in Fujimori's model (3) which allows for nonequilibrium binding. It was found that for a MAb with 10\(^8\) M\(^{-1}\) affinity, when the forward rate constant is greater than \( 3 \times 10^4 \) M\(^{-1}\) s\(^{-1}\) as is the case for most MAbs (15, 16), the average tumor concentration is not affected. Thus, the equilibrium assumption is expected to apply for most MAbs. Measurement of binding will also depend upon the extent of internalization and metabolism of the MAb. For example, if a radiolabeled MAb is rapidly internalized and metabolized and the radioactive label is lost from the tumor as a small diffusible compound, or if the MAb binds to a shed antigen and the immune complex is a mobile species which can be cleared by interstitial fluid flow, less radioactivity would be found in the tumor than if such events were absent. Therefore, the model may underestimate the extent of binding when the MAb is directed against a secreted or rapidly metabolized antigen. Because of the difficulty in obtaining both binding and metabolic constants from fitting a model to time-averaging tumor concentration data, we chose not to include an explicit metabolic term. It is important to bear in mind, however, that interpretation of the model-fitted value for \( B_0 \) may be influenced by internalization and metabolic effects as well as the degree to which MAb is nonuniformly distributed. Hence, \( B_0 \) represents only an apparent binding site density.

**Computer Calculations.** Simulations were generated by solving the differential equation (Equation D) numerically with the condition that the tumor concentration is initially zero. A fourth-order Runge-Kutta algorithm in Mathematica (Wolfram Research, Inc., Champaign, IL) was used on a Macintosh Ilci. Fit of experimental data to the model was carried out using Gear's method (17) for numerical solution of the differential equation and a finite difference Levenberg-Marquardt algorithm (18) for performing a nonlinear weighted least-squares regression; the weighting factors were the variances in concentration at each time point. The computer program uses subroutines from the IMSL statistical and mathematical libraries (IMSL, Inc., Houston, TX). Intertissue concentrations for binding and nonbinding MAbs at various times after i.v. injection are input values for the computer program.

**Animal Model.** The animal model was the athymic mouse with s.c. solid human melanoma tumor M21 or SK-MEL-2 receiving an i.v. injection of 20 \( \mu \)g of \(^{125}\)I-labeled specific MAb and 20 \( \mu \)g of \(^{125}\)I-labeled nonspecific MAb. Details of the animal experiments and characteriza-

---

**Fig. 1.** Pharmacokinetic model of MAb transport from plasma to tissue. The transport of MAb from plasma to tissue is assumed to be unidirectional and governed by the capillary permeability-area product, \( k \), and the coexisting plasma concentration. In the tissue compartment, the MAb is assumed to rapidly distribute between free and bound compartments in accordance with the Langmuir equilibrium binding isotherm where \( K_a \) is the MAb affinity and \( B_0 \) is the concentration of binding sites. Unbound MAb is assumed to be transported out of the tissue at a rate equal to the convective flow of interstitial fluid in tumors or lymph flow in normal tissues, \( L \), times the coexisting interstitial unbound MAb concentration. This figure is similar to one appearing in Ref. 6 except that \( K_a \) and \( B_0 \) now are treatable as separate parameters.
Model Simulations. The pharmacokinetic equation (Equation D) can be solved numerically for given parameter values of the plasma kinetics \(C_{P0}, R, \lambda_1, \lambda_2\), tumor physiology \((k, L, \phi, \text{and MAb binding } K_a, B_0)\). To carry out the simulations, we used values that are characteristic of MAbs and tumor-associated antigens. For the plasma kinetics, we used data on the plasma clearance of an IgG monoclonal antibody (MAb 436) after i.v. injection into athymic mice \((11); R\) was set equal to 0.459; \(\lambda_1\) to 1.11 \(\times 10^{-2}\) min\(^{-1}\) and \(\lambda_2\) to 1.38 \(\times 10^{-4}\) min\(^{-1}\). We set \(k\) equal to 0.5 \(\mu\)l/min/g based on experiments reported by Colapinto et al. for a MAb given to mice bearing s.c. glioma xenografts \((19)\). \(L\) was set equal to 0.8 \(\mu\)l/min/g based on our experiments with s.c. TE671 rhabdomyosarcoma xenografts \((6), \phi\) a value which also is consistent with experiments by Nakagawa et al. \((20)\) with intracranial gliomas. A value of 0.243 was used for \(\phi\) based on experimental measurements in TE671 rhabdomyosarcoma \((6)\). We examined \(K_a\) values from \(10^6\) to \(10^{11}\) M\(^{-1}\) and \(B_0\) values from 3 to 3000 nM. This range of \(B_0\) corresponds to approximately 500 to 500,000 binding sites/cell \((6), \phi\) values representative of many tumor-associated antigens. The simulations examine the 120-h period following i.v. injection, covering a time frame typical of many MAb uptake experiments and clinical radioimmunoimaging protocols.

The simulation in Fig. 2 shows the behavior of \(C_p, C_t, C_b,\) and \(C_f\) after injection of MAb when the initial plasma concentration is 100 nM. Values of \(K_a\) and \(B_0\) were set at \(10^8\) M\(^{-1}\) and 100 nM, respectively. \(C_p\), initially 100 nM, decreases at first at a rapid rate followed by a period of slower decline (biexponential decay); \(C_t\) increases from an initially zero value. At early times, nearly all of the MAb in the tumor is bound. If one defines the period when \(C_t > 50\% B_0\) as “approaching saturation,” this period begins at approximately 7 h in this example.

The effect of antibody affinity on MAb uptake in solid tumors is examined in Fig. 3 where all other parameter values are fixed and the affinity is varied from \(10^6\) M\(^{-1}\) to \(10^{11}\) M\(^{-1}\). The initial plasma concentration was set equal to 100 nM, and \(B_0\) was fixed at 100 nM in Fig. 3A and 1000 nM in Fig. 3B. The model predicts that it is important to have a reasonably high affinity MAb \((K_a > 10^8\) M\(^{-1}\)) but also that, with fixed plasma and permeation kinetics, there is a limit to how high MAb uptake will increase with increasing \(K_a\). In these examples it appears that above an affinity of about \(10^8\) M\(^{-1}\), minimal increases in tumor uptake are achieved with increases in \(K_a\). Even in the limit of infinite MAb affinity, \(C_t\) is bounded finitely, and its
value depends only upon the plasma clearance and capillary permeability-area product when \( C_i \leq B_0 \) and additionally upon the interstitial fluid flow and antigen density when \( C_i > B_0 \) (see "Appendix").

For therapeutic applications, MAb doses typically are in the range approaching saturation of the binding sites in a tumor, whereas for imaging applications, nonsaturating doses usually provide better contrast between tumor and normal tissue. The response of the pharmacokinetic model to changes in \( K_a \) depends very much on the administered dose. Table 1 shows the maximum MAb concentration \( (C_{i,\text{max}}) \) attained in the interstitial fluid when \( K_a = 10^5, 10^6, 10^9, \) and infinity for initial plasma concentrations ranging from 1 to 300 nM. For a 20-g mouse, these values of \( C_{i,0} \) represent MAb doses ranging from approximately 0.15 to 45 \( \mu \)g; for a 70-kg human, they represent doses from approximately 0.45 to 130 mg. Values of \( B_0 \) from 10 to 1000 nM were examined. When the maximum interstitial MAb concentration at infinite affinity is less than \( B_0 \), the maximum is called the permeation limit (see "Appendix," Equation J). In \( C_{i,0} > 50\% \) \( B_0 \) or as the permeation limit is approached \( (C_{i,max} > C_{i,\text{max}} > 50\% \) \( B_0 \) or as the permeation limit is approached \( (C_{i,max} > 50\% \) permeation limit). From these calculations, it appears that increasing the antibody affinity from a moderate to a very high value will result in an increased MAb uptake only when lower doses are used.

Another example of the sensitivity of this model to MAb dose is illustrated in Fig. 4. In these simulations, \( K_a \) and \( B_0 \) were varied but the product \( K_a B_0 \) was held constant. At the lower dose \( (C_{i,0} = 1 \) nM; Fig. 4A), as long as the product \( K_a B_0 \) is fixed at 30, and \( K_a \) is varied from 10 to 1000 \( M^{-1} \). The values of \( C_{i,0} = 1 \) and 100 nm, correspond to MAb doses of approximately 0.45 and 45 mg, respectively, in humans and 0.15 and 15 \( \mu \)g, respectively, in mice. All other parameter values are the same as in Fig. 2. (A) At the lower \( C_{i,0} (1 \) nM), the total interstitial MAb concentration is not greatly affected by variations in \( K_a \) and \( B_0 \) as long as the product \( K_a B_0 \) is held constant until \( K_a \) rises above 100 \( M^{-1} \). (B) At the higher \( C_{i,0} (100 \) nM), a low affinity MAb which has a high concentration of binding sites in the tumor attains a higher interstitial MAb concentration than a high affinity MAb which has a low concentration of binding sites. The model simulations suggest that at higher doses, use of a moderate affinity MAb which has an abundant number of receptors in the targeted tissue is a better strategy for achieving higher tumor concentrations than use of a high affinity MAb which binds to an antigen expressed at low density.

### Table 1 Maximum interstitial MAb concentration after i.v. injection (nM)

<table>
<thead>
<tr>
<th>( C_{i,0} ) = 1 nM, ( B_0 = 10 ) nM</th>
<th>( K_a = 10^5 M^{-1} )</th>
<th>( K_a = 10^6 M^{-1} )</th>
<th>( K_a = 10^9 M^{-1} )</th>
<th>( K_a = \infty )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.8</td>
<td>4.5*</td>
<td>8.2*</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.9</td>
<td>3.9</td>
<td>6.7*</td>
<td>8.2*</td>
</tr>
<tr>
<td>100</td>
<td>1.9</td>
<td>5.3*</td>
<td>7.7*</td>
<td>8.2*</td>
</tr>
<tr>
<td>300</td>
<td>3.4</td>
<td>6.5*</td>
<td>7.8*</td>
<td>8.2*</td>
</tr>
<tr>
<td>1000</td>
<td>5.2*</td>
<td>7.4*</td>
<td>8.0*</td>
<td>8.2*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( C_{i,0} ) = 3 nM, ( B_0 = 10 ) nM</th>
<th>( K_a = 10^5 M^{-1} )</th>
<th>( K_a = 10^6 M^{-1} )</th>
<th>( K_a = 10^9 M^{-1} )</th>
<th>( K_a = \infty )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6</td>
<td>4.5</td>
<td>8.7</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.8</td>
<td>8.9</td>
<td>17.2</td>
<td>24.5*</td>
</tr>
<tr>
<td>100</td>
<td>5.7</td>
<td>15.0*</td>
<td>21.9*</td>
<td>24.5*</td>
</tr>
<tr>
<td>300</td>
<td>10.1</td>
<td>19.5*</td>
<td>23.4*</td>
<td>24.5*</td>
</tr>
<tr>
<td>1000</td>
<td>15.6*</td>
<td>22.3*</td>
<td>24.1*</td>
<td>24.5*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( C_{i,0} ) = 10 nM, ( B_0 = 10 ) nM</th>
<th>( K_a = 10^5 M^{-1} )</th>
<th>( K_a = 10^6 M^{-1} )</th>
<th>( K_a = 10^9 M^{-1} )</th>
<th>( K_a = \infty )</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>9.7</td>
<td>12.2</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>8.5</td>
<td>21.3</td>
<td>30.1</td>
<td>31.9</td>
</tr>
<tr>
<td>100</td>
<td>17.7</td>
<td>44.8*</td>
<td>67.8*</td>
<td>81.5*</td>
</tr>
<tr>
<td>300</td>
<td>32.5</td>
<td>63.4*</td>
<td>77.6*</td>
<td>81.5*</td>
</tr>
<tr>
<td>1000</td>
<td>51.5*</td>
<td>74.0*</td>
<td>80.2*</td>
<td>81.5*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( C_{i,0} ) = 30 nM, ( B_0 = 10 ) nM</th>
<th>( K_a = 10^5 M^{-1} )</th>
<th>( K_a = 10^6 M^{-1} )</th>
<th>( K_a = 10^9 M^{-1} )</th>
<th>( K_a = \infty )</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.2</td>
<td>17.4</td>
<td>18.4</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>21.2</td>
<td>34.1</td>
<td>37.4</td>
<td>37.8</td>
</tr>
<tr>
<td>100</td>
<td>44.9</td>
<td>87.6</td>
<td>103.1</td>
<td>123.3</td>
</tr>
<tr>
<td>300</td>
<td>88.9</td>
<td>172.8*</td>
<td>222.4*</td>
<td>244.5*</td>
</tr>
<tr>
<td>1000</td>
<td>150.3*</td>
<td>219.4*</td>
<td>239.9*</td>
<td>244.5*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( C_{i,0} ) = 100 nM, ( B_0 = 10 ) nM</th>
<th>( K_a = 10^5 M^{-1} )</th>
<th>( K_a = 10^6 M^{-1} )</th>
<th>( K_a = 10^9 M^{-1} )</th>
<th>( K_a = \infty )</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.8</td>
<td>39.1</td>
<td>39.4</td>
<td>39.5</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>50.6</td>
<td>57.5</td>
<td>58.5</td>
<td>58.6</td>
</tr>
<tr>
<td>100</td>
<td>97.2</td>
<td>121.9</td>
<td>125.6</td>
<td>126.1</td>
</tr>
<tr>
<td>300</td>
<td>213.1</td>
<td>303.1</td>
<td>316.8</td>
<td>318.8</td>
</tr>
<tr>
<td>1000</td>
<td>448.3*</td>
<td>678.7*</td>
<td>780.9*</td>
<td>815.2*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( C_{i,0} ) = 300 nM, ( B_0 = 10 ) nM</th>
<th>( K_a = 10^5 M^{-1} )</th>
<th>( K_a = 10^6 M^{-1} )</th>
<th>( K_a = 10^9 M^{-1} )</th>
<th>( K_a = \infty )</th>
</tr>
</thead>
<tbody>
<tr>
<td>98.2</td>
<td>99.2</td>
<td>99.4</td>
<td>99.4</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>115.0</td>
<td>118.3</td>
<td>118.3</td>
<td>118.4</td>
</tr>
<tr>
<td>100</td>
<td>174.3</td>
<td>184.2</td>
<td>185.4</td>
<td>185.6</td>
</tr>
<tr>
<td>300</td>
<td>341.2</td>
<td>372.3</td>
<td>377.7</td>
<td>378.2</td>
</tr>
<tr>
<td>1000</td>
<td>725.6*</td>
<td>1050.4</td>
<td>1052.7</td>
<td>1052.7</td>
</tr>
</tbody>
</table>

\* Approaching the permeation limit (>50% permeation-limited value).
\* Permeation limit (see "Appendix," Equation J).
THEORETICAL AND EXPERIMENTAL ANTIBODY PHARMACOKINETICS

...lated in Fig. 5 for $B_0$ varying from 10 to 3000 nm while $K_a$ and $C_{Po}$ are fixed at $10^4 M^{-1}$ and 100 nm, respectively. In this simulation, it is evident that tumor uptake of MAb is a very sensitive function of $B_0$ from 30 to 1000 nm, corresponding to approximately 5,000 to 200,000 binding sites/cell. The effects of increasing $B_0$ on the maximum $C_t$ at other values of $K_a$ and $C_{Po}$ are shown in Table 1. It can be seen that $C_{t, max}$ increases substantially with increasing $B_0$ until the permeation limit is approached. One is less likely to reach the permeation limit at higher doses, and therefore, for high dose applications, choosing an antigen which is abundantly expressed appears to be a very crucial factor for increasing MAb uptake. Since metabolism of bound MAb with subsequent release of the radioactive label lowers the apparent $B_0$, other possible strategies which can lead to an effective increase in apparent $B_0$ are to target an antigen which is slowly metabolized or internalized or to use a radioisotope or radiolabeling method which causes the metabolized label to be trapped in the cell or tissue (21-23). Targeting a slowly internalized antigen is suitable for radioimmunotherapy or radioimmunomaging where the lethal effects of isotope emissions do not require internalization but would not be appropriate for drug- or toxin-MAb conjugates where internalization is a key event for cell killing.

The pharmacological effect of a drug in a tissue is often related to the AUC. If the curves for $C_t$ in Fig. 5 are integrated, nonlinearities between the ratio of AUC for a target and non-target tissue and the corresponding ratio of antigen densities become evident. For example, suppose that an antigen A has a concentration of 100 and 10 nm in tumor and a normal tissue, respectively, whereas an antigen B has a concentration of 300 and 30 nm, respectively. Although the ratio of antigen density for tumor to normal tissue is 10 for both antigens, the AUC ratios for tumor to normal tissue for antigens A and B are 3.0 and 4.5, respectively, for the 120-h period following injection, and, if the integration is carried out to the time when all of the MAb is cleared from the tissues, the ratios are 3.9 and 7.0, respectively. Even if the concentration of antigen B in the normal tissue were 60 nm, yielding a ratio of 5 for tumor to normal antigen densities, the ratio of AUC of tumor to normal tissue for antigen B would still be slightly higher than that for antigen A for the 120-h period postinjection; over the period for complete clearance of MAb from the tissue, antigen B would have a tumor to normal AUC ratio of 4.4. The model thus predicts that when the therapeutic effect depends upon the total interstitial MAb concentration is a sensitive function of $B_0$ from 30 to 1000 nm. The model suggests that a critical determinant for achieving good localization of an antibody to a target tissue is the use of an antibody which binds to an antigen expressed abundantly in vivo.

...tions were approximately 120 nm. MAb 436 and 9.2.27 recognize 2 distinct melanoma-associated antigens with nearly the same density on M21 cells, but MAb 436 has an affinity of $3.8 \times 10^6 M^{-1}$ whereas MAb 9.2.27 has an affinity of $5 \times 10^6 M^{-1}$ (11). Despite having an affinity higher by more than 2 orders of magnitude, MAb 9.2.27 attained a slightly lower concentration in the tumor compared to MAb 436 over the 72-h period following i.v. injection (Fig. 6A). The slightly more rapid plasma clearance of MAb 9.2.27 compared to MAb 436 (11) can account almost entirely for the lower uptake of MAb 9.2.27.

Fig. 6B shows a comparison of MAb 436 and IND1, antibodies which have virtually identical plasma clearances and affinities for 2 distinct melanoma-associated antigens on SK-MEL-2 cells. However, MAb 436 has about 3 times more binding sites than MAb IND1, as measured by flow cytometry on cells taken directly from s.c. tumors (11). This 3-fold increase in binding density was associated with significantly higher uptake of MAb 436 compared to MAb IND1 (Fig. 6B). Figs. of the data to the pharmacokinetic model for all three specific MAb 436, 9.2.36, and IND1 and a nonspecific MAb (B72.3) were carried out for both M21 and SK-MEL-2 tumors in order to obtain quantitative estimates of the capillary permeability-area products, the interstitial fluid flow, and the apparent binding site densities. Fitted curves for the experimental data are drawn in Fig. 6. A detailed presentation of the results of the model analysis can be found in the accompanying paper (11).

DISCUSSION

The variety of MAb now available and the ease of generating new ones make it possible to give more deliberate consideration to the question of what properties should be selected for or designed into a MAb in order to optimize its uptake into a target tissue. The compartmental pharmacokinetic model presented in this paper provides a framework for examining some of the factors which are likely to affect tumor uptake of large macromolecules. The model employs the Langmuir isotherm (Equation B) to represent antibody-antigen binding and extends...
The transport and binding parameters in the model and $B$ represent least-squares fits of the experimental data to the pharmacokinetic model in athymic mice. (A) M21 tumor. MAbs 436 and 9.2.27 differ in $K_a$ by a factor of 130 ($K_a$ of $3.8 \times 10^8$ and $5 \times 10^{10} \text{M}^{-1}$, respectively) but have nearly the same $B$. A dose of 20 $\mu$g was administered; the average $\Delta P$ for animals receiving MAb 436 was 127 $\text{nm}$, for MAb 9.2.27, 115 $\text{nm}$. The pharmacokinetic model predicts that at this dose and these values of $B$, and assuming that the plasma kinetics of the antibodies are identical, an increase in binding affinity will result in only a marginally higher uptake in the tumor (see Fig. 3). The experimental data show that the higher affinity antibody MAb 9.2.27 attained a slightly lower tumor concentration than MAb 436. This can be largely attributed to the slightly lower plasma concentrations of MAb 9.2.27 compared to MAb 436 during the 72 h after injection [11]. (B) SK-MEL-2 tumor. MAbs 436 and INDI have identical plasma kinetics and nearly identical affinities ($3.9 \times 10^8$ and $3.3 \times 10^8 \text{M}^{-1}$, respectively), but $B$ for MAb 436 is 3 times that of MAb INDI, based on measurements on tumor cells obtained directly from s.c. tumors. The experimental data support the model prediction that higher antibody uptake will be attained in the tumor with the higher antigen density (see Fig. 5). The curves in both $A$ and $B$ represent least-squares fits of the experimental data to the pharmacokinetic model (see “Materials and Methods”). Model fits yielded quantitative estimates of the capillary permeability-area product, the apparent binding site concentration, and the interstitial fluid flow [11].

Our recently published pharmacokinetic model [6] to encompass a broad range of doses that might be used experimentally and clinically. The transport and binding parameters in the model are known for antibodies within a fairly well-defined range, and therefore simulations with numbers within this range can provide insight into which parameters will affect tissue concentrations. This paper examines some of the general features of the model and shows that the effects of binding affinity and antigen density on tumor uptake predicted by the model were observed in animal experiments.

The pharmacokinetic model predicts that the effect of increasing antibody affinity on MAb uptake in tumors is very dependent on dose. At doses which do not approach saturation of binding sites, the model predicts that increases in MAb affinity as high as $10^{11} \text{M}^{-1}$ can lead to substantial increases in tumor uptake of MAb when uptake is not permeation-limited. However, at doses high enough to approach saturating conditions and which are more typical for therapeutic applications, model simulations suggest that increasing the antibody affinity above $10^8 \text{M}^{-1}$ will not lead to significant further increases in MAb uptake. Matzku et al. have reported on tumor localization of MAbs in mice given 0.4–1 $\mu$g doses of MAb ($C_{p0}$ approximately 2–6 nm) [24]. Comparing two MAbs with affinities of $4.8 \times 10^8 \text{M}^{-1}$ and $1.6 \times 10^{10} \text{M}^{-1}$ with nearly the same density of binding sites (5000 sites/cell; $B_0$ approximately 30 $\text{nm}$), they found that the higher affinity MAb attained approximately twice as high a concentration in tumor, a result consistent with our model predictions for lower doses in non-permeation-limited cases (Table 1). Our experiments at 20-$\mu$g doses ($C_{p0}$ approximately 120 nm) comparing MAbs with affinities of $3.8 \times 10^8 \text{M}^{-1}$ and $5 \times 10^{10} \text{M}^{-1}$ but with similar numbers of binding sites showed that MAb uptake was not increased for the higher affinity MAb (Fig. 6A), a finding which supports the pharmacokinetic model prediction for higher doses. The model thus reconciles apparently conflicting data on the effect of MAb affinity on tumor uptake by taking into account the nonlinearities of antibody pharmacokinetics as a function of dose. This is an important result because it suggests that for high dose applications, it is not necessary to expend a large effort to find MAbs with extremely high affinity if moderate affinity MAbs for the target antigen are already available. Moreover, the pharmacokinetic model of Fujimori et al. [3] which examines the spatial distribution of a MAb in a volume of tissue surrounding a capillary provides a compelling rationale for avoiding very high affinity antibodies; simulations from their model indicate that very high affinity ($\geq 10^{10} \text{M}^{-1}$) greatly slows MAb penetration and leads to spatial nonuniformity.

The pharmacokinetic model predicts that tumor interstitial MAb concentrations are very sensitive to changes in binding site concentrations in a range characteristic of tumor-associated antigens when doses are high enough such that uptake is not perimeter limited. Our experiments with two MAbs of similar affinity but differing in antigen density in SK-MEL-2 tumor showed that a significantly higher tumor uptake was attained with the MAb which has approximately a 3-fold higher in vivo affinity but differing in antigen density in SK-MEL-2 tumor models differed in vascularity, capillary permeability, and fractional interstitial volume, factors which can also affect tumor uptake of MAbs, the sizable difference in antigen expression among the different tumor cells likely dominates the results of their experiments. Philben et al. [26] carried out experiments with a MAb to the CEA on four different tumors with different CEA concentrations. MAb uptake increased with increasing antigen content except for the highest CEA content tumor; 48 h after injection of MAb, the percentages of injected dose/g of tumor for tumors containing 0, $1 \times 10^3$, 2 $\times 10^3$, and $1.8 \times 10^4$ ng CEA/g were 1.4, 16.4, 51.1, and 29.5, respectively. The authors had evidence suggesting that the tumor containing the highest amount of CEA shed the CEA antigen more rapidly than the other tumors: CEA was secreted more rapidly by our model predictions for lower doses in non-permeation-limited cases (Table 1). Our experiments at 20-$\mu$g doses ($C_{p0}$ approximately 120 nm) comparing MAbs with affinities of $3.8 \times 10^8 \text{M}^{-1}$ and $5 \times 10^{10} \text{M}^{-1}$ but with similar numbers of binding sites showed that MAb uptake was not increased for the higher affinity MAb (Fig. 6A), a finding which supports the pharmacokinetic model prediction for higher doses. The model thus reconciles apparently conflicting data on the effect of MAb affinity on tumor uptake by taking into account the nonlinearities of antibody pharmacokinetics as a function of dose. This is an important result because it suggests that for high dose applications, it is not necessary to expend a large effort to find MAbs with extremely high affinity if moderate affinity MAbs for the target antigen are already available. Moreover, the pharmacokinetic model of Fujimori et al. [3] which examines the spatial distribution of a MAb in a volume of tissue surrounding a capillary provides a compelling rationale for avoiding very high affinity antibodies; simulations from their model indicate that very high affinity ($\geq 10^{10} \text{M}^{-1}$) greatly slows MAb penetration and leads to spatial nonuniformity.

The pharmacokinetic model predicts that tumor interstitial MAb concentrations are very sensitive to changes in binding site concentrations in a range characteristic of tumor-associated antigens when doses are high enough such that uptake is not permeation limited. Our experiments with two MAbs of similar affinity but differing in antigen density in SK-MEL-2 tumor showed that a significantly higher tumor uptake was attained with the MAb which has approximately a 3-fold higher in vivo affinity but differing in antigen density in SK-MEL-2 tumor showed that a significantly higher tumor uptake was attained with the MAb which has approximately a 3-fold higher in vivo antigen density (Fig. 6B). Goldenberg et al. [25] have found similar results with a MAb targeted to an antigen (epidermal growth factor receptor) expressed at different densities on three different tumor cell lines. The percentages of injected dose per g of tumor for tumors expressing $5 \times 10^3$, $3 \times 10^3$, and $2 \times 10^4$ receptors/cell were 0.9 (day 7), 4.2 (day 7), and 12.4 (day 8), respectively. While Goldenberg does not describe how the tumors differed in vascularity, capillary permeability, and fractional interstitial volume, factors which can also affect tumor uptake of MAbs, the sizable difference in antigen expression among the different tumor cells likely dominates the results of their experiments. Philben et al. [26] carried out experiments with a MAb to the CEA on four different tumors with different CEA concentrations. MAb uptake increased with increasing antigen content except for the highest CEA content tumor; 48 h after injection of MAb, the percentages of injected dose/g of tumor for tumors containing 0, $1 \times 10^3$, $2 \times 10^3$, and $1.8 \times 10^4$ ng CEA/g were 1.4, 16.4, 51.1, and 29.5, respectively. The authors had evidence suggesting that the tumor containing the highest amount of CEA shed the CEA antigen more rapidly than the other tumors: CEA was secreted more rapidly by cultured tumor cells with the highest CEA content compared to the second highest; and the liver, the organ which catabolizes
shed immune complexes, had the highest radioactivity in the animals with the tumor producing the highest levels of CEA. Boerman et al. conducted a study similar to ours wherein comparison was made between two MAbs of similar affinity but targeted to different antigens on the same cell. The in vitro antigen density differed by a factor of 3, but in contrast to our results, they found that MAb uptake was lower for the MAB to the more highly expressed antigen (27). They were not able to detect shedding of the antigen, but a possible explanation for their experiments is that the MAB directed to the more highly expressed antigen was more rapidly catabolized in the tumor compared to the other MAB, resulting in loss of the radioactive label from the tumor cells. Another possible explanation for their results is that antigen expression was different on cells in vitro compared to cells from cultures. We have observed differences in antigen expression on M21 and SK-MEL-2 cells in culture and on cells directly taken from in vivo tumors (11). Antigen expression was sometimes increased and sometimes decreased, by up to a factor of 3 in either direction, and we could not discern any factor which correlated with the magnitude or direction of the changes. While our experiments and many in the literature support the pharmacokinetic model prediction that higher antigen density will lead to higher tumor interstitial concentrations of MAB, it is evident that further experiments are needed to examine in vivo-in vitro antigen differences and the effect of MAB catabolism by the target cells on tumor uptake of MAbs.

The model clearly is a simplification of the many complex factors which govern MAB localization to a target tissue. For example, it does not contain any information on the spatial distribution of MAB within the tumor cell. If there are regions of tumor which are far from a capillary or are unperfused or necrotic, then the volume that is accessible to the MAB will be lower than the total interstitial volume of the tumor. Developing an accurate spatially distributed model, unfortunately, is confounded by the need to incorporate information on the distribution of capillaries, pressure gradients, existence of poorly perfused regions, etc., information which generally is not available or well characterized, although progress is being made in this area by several groups (5, 28). The advantage of the simple compartmental model presented in this paper and its limited number of adjustable parameters is that it allows the use of the model for data fitting when only the average MAB concentration in the interstitium is known at various times after injection of MAB. In the accompanying paper, we have demonstrated the application of the model to analysis of data on uptake of MAbs in solid melanoma tumors. Model-fitted values of Bo were consistently lower than values from direct measurements on cells removed from in vivo tumors, pointing to the possible existence of heterogeneous MAB distribution in the tumor tissue or MAB internalization and metabolism. On the other hand, values obtained for the capillary permeability-area product and interstitial fluid flow were consistent with histological characteristics of the tumors and with other published experiments on tumor physiology, demonstrating the utility of the model in characterizing quantitatively these transport parameters for MAbs in solid tumors.

ACKNOWLEDGMENTS

We wish to thank Drs. Kenji Fujimori and William van Osdol for performing many customized computer simulations using the PERC package to calculate effects of parameters in a distributed pharmacokinetic model. We also would like to acknowledge Dr. Richard J. Youle for critiquing the manuscript.

APPENDIX

Equations B and C may be combined to obtain an expression for CI in terms of C0. The result is Equation E.

\[ C_I = 0.5\left( -B_0 + C_1 - 1/K_3 \right) + \sqrt{(B_0 - C_1 + 1/K_3)^2 + 4C_1/K_4} \]  

(E)

In the limit as \( K_3 \to \infty \), Equation E shows that when \( C_1 < B_0 \), then \( C_I \to 0 \). The differential equation which describes the time-varying total interstitial concentration, Equation D, simplifies to

\[ \frac{dC_I}{dt} = kC_I \quad \text{for } 0 \leq t < t_0 \]  

(F)

where \( t_0 \) is the time required to attain complete saturation of the binding sites, \( i.e., C_I = B_0 \), and is given by Equation G.

\[ B_0 \phi = C_0(t) dt \]  

(G)

For times greater than \( t_0 \), \( i.e., C_I > B_0 \), Equation E shows that in the limit as \( K_3 \to \infty \), \( C_I \to C_0(C_1 - B_0) \). Equation D becomes Equation H with initial condition that \( C_I(t_0) = B_0 \).

\[ \frac{dC_I}{dt} = kC_I - L(C_I - B_0) \quad \text{for } t > t_0 \]  

(H)

Equations F and H have no functional dependence on \( K_3 \). Solution of these equations leads to values of \( C_I \) that are bounded over all time provided that \( C_I \) decreases with time, \( k \) and \( L \) are finite, and \( \phi \) is nonzero. \( C_I \) from Equations F and H represents the highest tissue level attainable by increasing \( K_3 \) to infinity.

For biexponentially decaying plasma kinetics (Equation A), solution of Equation F yields Equation I.

\[ C_I = \frac{kC_0}{\phi} \left[ \frac{R}{\lambda_1} \left( 1 - e^{-\lambda_1 t} \right) + \frac{1 - R}{\lambda_2} \left( 1 - e^{-\lambda_2 t} \right) \right] \]  

(I)

For certain combinations of values for \( k \), dose (related to \( C_0 \)), and plasma kinetic parameters \( (R, \lambda_1, \lambda_2) \), the permeation rate of MAB into tumor \( (kC_0) \) may be low enough such that even for an infinite affinity MAB, \( C_I \) is less than \( B_0 \) for all times. In such cases, the maximum interstitial MAB concentration is called the "permeation limit" and is given by Equation J, the asymptotic limit of Equation I.

\[ C_I = \frac{kC_0}{\phi} \left[ \frac{R}{\lambda_1} + \frac{1 - R}{\lambda_2} \right] \quad \text{(permeation limit)} \]  

(J)

Table 1 lists the maximum interstitial MAB concentrations for an infinite affinity MAB for a variety of doses and antigen densities. When this value equals the permeation limit, it is marked with Footnote b.

REFERENCES

Predicted and Observed Effects of Antibody Affinity and Antigen Density on Monoclonal Antibody Uptake in Solid Tumors

Cynthia Sung, Ty R. Shockley, Paul F. Morrison, et al.


Updated version  Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/52/2/377](http://cancerres.aacrjournals.org/content/52/2/377)

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.