Pharmacokinetic Analysis of the Perivascular Distribution of Bifunctional Antibodies and Haptens: Comparison with Experimental Data

Laurence T. Baxter, Fan Yuan, and Rakesh K. Jain

Edwin L. Steele Laboratory, Department of Radiation Oncology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114

Abstract

A mathematical model is developed to describe the concentration profiles around individual tumor blood vessels for two-step approaches to cancer treatment. The model incorporates plasma pharmacokinetics, interstitial diffusion, reversible binding between antibody and hapten and between antibody and tumor-associated antigens, and physiological parameters to evaluate present experimental approaches and to suggest new guidelines for the effective use of two-step approaches. Results show considerable interaction between the binding kinetics, initial drug doses, and antigen density, with optimal parameter ranges depending on the desired goal: treatment or detection. The hapten concentration in tumors was found to be nonuniform because of specific binding to antibodies. While binding of the hapten to the bifunctional antibody is necessary for improved retention, too large a binding affinity may lead to very poor penetration of the hapten into regions far away from blood vessels. The time delay between antibody and hapten injection was found to be an important parameter. Longer time delays were found to be advantageous, subject to constraints such as internalization of the antibody and tumor growth during treatment. A proper combination of initial doses for the two species was also seen to be crucial for maximum effectiveness. Comparison of the model with the experimental data of Le Doussal et al. (Cancer Res., 51: 6650-6655, 1991) and Stickney et al. (Cancer Res., 50: 3445-3452, 1990) suggests two novel, yet testable, hypotheses: (a) the early pharmacokinetics of low molecular weight agents can have an important effect on later concentrations using two-step approaches; and (b) metabolism may play an important role in reducing concentrations in the tumor and tumor:plasma concentration ratios. These results should help in the effective design of two-step strategies.

Introduction

Two-step approaches to cancer detection and treatment have been developed which seek to combine the selectivity of certain high molecular weight agents (i.e., antibodies and their fragments) with the superior tissue penetration and rapid plasma clearance of low molecular weight agents. These two-step approaches include BFA\(^2\) and haptens (1-3), enzyme-conjugated antibodies (4, 5), and other antibody-based localization schemes (6, 7). The concept is to preinject a macromolecule which binds specifically within the tumor, using a high dose and waiting long enough to achieve good localization. Later, a second molecule of low molecular weight is injected. It should selectively be taken up (or converted into an active agent) where the macromolecule concentration is high, presumably in the tumor. In these approaches there are many physiological and kinetic parameters which influence uptake and distribution of the active agent.

Although these two-step approaches have shown significant progress, current strategies may be suboptimal. There is a definite need for mathematical modeling of two-step approaches to design experiments, to guide therapies, to understand the factors involved in biodistribution, and to identify the key parameters which limit or enhance delivery. One postulated advantage of using haptens in conjunction with antibodies is better penetration into tumor tissue, yet this has not been evaluated for two-step approaches. We have previously developed distributed parameter models which investigate different aspects of antibody penetration in tumors, on both macroscopic (8) and microscopic scales (9). We have also developed a lumped parameter compartmental model for the uptake of haptens and produgs in two-step therapies (10). The present investigation builds upon our previous models to study for the first time the concentration profiles of both antibody and hapten around individual tumor blood vessels in two-step approaches. Yuan et al. (10) presented guidelines for improving two-step approaches based on a compartmental model, but the lack of spatial concentration profiles was an acknowledged limitation which has now been overcome. The comparison of our model results with the experimental data of Le Doussal et al. (1) and Stickney et al. (2) offers novel insights into the role of plasma pharmacokinetics and antigen internalization in two-step therapies.

Model Development

The tissue is modeled as a Krogh cylinder (11) in which a microvessel of radius \(r\) is surrounded by tissue of radius \(R\). A three-compartment model is used to describe plasma pharmacokinetics. Diffusion-reaction equations are written to describe the extravascular concentrations as a function of both time and radial position (Appendix B). A permeability boundary condition governs the transvascular flux, and there is no flux condition due to symmetry between surrounding Krogh cylinders. These differential equations (in both tissue and plasma) were solved simultaneously using a numerical finite difference method.

Parameter Values. The baseline parameters chosen for the simulations are given in Table 1. The model BFA-hapten system is based on the work of Le Doussal et al. (1) for \(^{111}\text{In}\)-labeled hapten (In-DTPA) with a BFA made up of the G7A5 Fab' against human melanoma and the 734 clone of an anti-indium-DTPA antibody. A time delay (\(r\)) of 24 h between BFA injection and hapten injection was reported to work well for detection with good T:P ratios for hapten achievable after 3 h. Initial doses used in Ref. 1 were \(2 \mu g\) of BFA and 1.5 pmol of hapten, typical for detection purposes. Our choice of \(2.0 \times 10^{-5} M\) for \(C_p^{\text{HA}}\) is based on this antibody dose, while the value of \(1.0 \times 10^{-7} M\) for \(C_p^{\text{HA}}\) is much higher and more typical of treatment. For the BFA, we have fit the human pharmacokinetic data of Stickney et al. (2) for F(ab')\(_2\). For the hapten we have used the pharmacokinetic data of Houston et al. (12) for free \(^{111}\text{In}\)-DTPA in human patients. Cellular uptake of the antibody (with rapid internalization of surface antigen and metabolism of the antibody) may play an important role (13, 14). The relevance of...
this process as well as the corresponding rate constant would vary greatly from system to system. To study the effect of antigen internalization on hapten distribution in two-step therapies, we have included a simulation with \( k_9 = 1.9 \text{ day}^{-1} \) (as used in Ref. 14).

### Results and Discussion

#### Baseline Simulations

Fig. 1 shows the solution to the distributed parameter model for our baseline case. The extravascular concentration is plotted as a function of time and distance from the center of the blood vessel. The total concentrations of BFA (Fig. 1A) and hapten (Fig. 1B) have been plotted, inasmuch as these would be the only relevant values for treatment or detection using radiolabeled agents. In general the profiles are steep initially, becoming more uniform with time as material diffuses away from the blood vessel. When the plasma concentration of free species drops below the free extravascular concentration, material begins to be reabsorbed into the bloodstream from the tissue, and concentration levels thereafter decrease with time. Extravascular binding helps retard this reabsorption process and maintain high concentration levels for a longer time period. Free hapten is present throughout the Krogh cylinder, even in regions where little antibody is present, but the distribution of bound hapten mimics the BFA distribution. These results indicate a trade-off between high specificity and uniformity of distribution.

A number of indices were calculated to help compare different simulations. The average T:P ratio is a measure of how the solutes localize in the tissue and is an important parameter for tumor detection. \(<C>\) is the sum of the concentrations for each species in various forms, averaged over the volume of the cylinder. A related quantity, the percentage of i.d./g, may be calculated as

\[
\% \text{ Injected dose/g tissue} = \frac{<C> / Cp^0}{\text{plasma volume} \times \text{tissue density}} \times 100%
\]

For cancer treatment, the total dose received is often measured by the AUC, or its ratio with the plasma AUC, the TI. To help understand the role of binding in the two-step approach we have also calculated the SAT, which is the total BFA-hapten complex concentration divided by the total BFA (for hapten-BFA binding), or the total BFA concentration divided by the tumor antigen density (for BFA-TAA binding). SR is the average total concentration in the tumor for a binding species divided by the concentration of an equivalent species which does not bind at all. UNI is a measure of the spatial uniformity of the distribution which is simply the volume around the blood vessel in which one-half of the BFA or hapten has distributed, divided by the volume required for a uniform distribution (i.e., one-half the total volume). UNI is a function of time, equal to zero when all the material is just outside blood vessel, and equal to 1 for uniform distribution. The importance of this parameter depends on the mode of detection or treatment. Lumped or compartmental models (such as in Ref. 10) are not capable of calculating this index. For cancer detection, high (and early) T:P and SR are important, while successful treatment also requires sufficiently high absolute concentrations throughout the tumor. These analytical indices for all of our simulations are given in Table 2.

#### Sensitivity Analysis

Since there is a wide range in the choice of doses, affinities, and other parameters, we have carried out simulations to show how the drug distributions change with different sets of parameters. A close examination of Table 2 reveals some of the key points of these perturbation simulations. The reported values are obtained 24 h after hapten injection, except for the AUC and TI which are calculated 2 weeks postinjection (as an approximation of the total dose received). For the baseline parameter values used, the T:P ratio, \(<C>\), UNI, AUC, and TI are not significantly improved for the hapten compared to the BFA, at 24 h after hapten injection. The SR is, however, much higher at that time, since most of the nonbinding hapten has been cleared from the bloodstream. For a system without antigen internalization, T:P and SR continue to increase with time, while the average hapten concentration in the tumor reaches a peak approximately 30 min after injection (for the baseline parameters). Many of the results of these sensitivity simulations are the same as in our previous compartmental model of two-step approaches (10). In particular, the earlier model showed that antibody doses greater than those needed for saturation may reduce SR and T:P and that clearing antibody from the bloodstream, longer time delays, and proper choice of binding

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**Table 1 Baseline model parameters**

<table>
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<th>Symbol</th>
<th>Definition</th>
<th>(Fab)_2 BFA</th>
<th>Hapten</th>
</tr>
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<tr>
<td>( D )</td>
<td>Diffusion coefficient</td>
<td>2.0 x 10^{-4} (18)^a</td>
<td>4.3 x 10^{-4} (19)^p</td>
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<tr>
<td>( P )</td>
<td>Permeability coefficient</td>
<td>9.0 x 10^{-7} (18)^a</td>
<td>1.0 x 10^{-4} (20)^p</td>
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<tr>
<td>( k_9 )</td>
<td>Association rate constant</td>
<td>4.33 x 10^{14} (14)</td>
<td>8.6 x 10^{12} (25)</td>
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<tr>
<td>( k_i )</td>
<td>Dissociation rate constant</td>
<td>2.0 x 10^{-8} = ( k_{ij}/K_{a1} )</td>
<td>2.0 x 10^{-3} (1)</td>
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<tr>
<td>( K_a )</td>
<td>Binding affinity</td>
<td>2.1 x 10^4 (1)</td>
<td>4.3 x 10^3 (1)</td>
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<tr>
<td>( k_9 )</td>
<td>Metabolism/internalization rate constant</td>
<td>0 (2.17 x 10^{-3})^f</td>
<td>0.0 (n.a.)^d</td>
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<tr>
<td>( k_0 )</td>
<td>Initial plasma concentration</td>
<td>2.0 x 10^{-8} (1)</td>
<td>n.a.</td>
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<tr>
<td>( C_F )</td>
<td>Pharmacokinetic parameters for free species</td>
<td>0.76 (2)</td>
<td>0.69 (12)</td>
</tr>
<tr>
<td>( \lambda_1 )</td>
<td>Pharmacokinetic parameters for free species</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>( \lambda_2 )</td>
<td>Pharmacokinetic parameters for free species</td>
<td>4.44 x 10^{-3} (ZCE-025/CHA-255)</td>
<td>0.588 (11^2In-DTPA)</td>
</tr>
<tr>
<td>( \lambda_3 )</td>
<td>Pharmacokinetic parameters for free species</td>
<td>4.10 x 10^{-4}</td>
<td>3.34 x 10^{-2}</td>
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<tr>
<td>( \rho )</td>
<td>Blood vessel radius</td>
<td>10</td>
<td>(9, 18)</td>
</tr>
<tr>
<td>( R )</td>
<td>Intercapillary half-distance</td>
<td>100</td>
<td>(9, 18)</td>
</tr>
<tr>
<td>( \tau )</td>
<td>Time delay between BFA and hapten injections</td>
<td>24</td>
<td>(1)</td>
</tr>
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</table>

*a* Scaled for molecular weight by correlation for tumor diffusivity, \( D \sim (M_t)^{-1.14} \) (8, 19).

*b* Scaled for molecular weight from sucrose, 342, to a 600 M, hapten (2) by correlation for aqueous diffusivity, \( D_{aq} \sim (M_t)^{-0.478} \) (19).

*c* Baseline value is zero, but the simulation which includes metabolism uses a value of \( 2.17 \times 10^{-11} \text{s}^{-1} \) (14).

*d* n.a., not applicable.

*Le Doussal et al. (1) reported 3.4 x 10^2 sites/cell, taking 2.83 x 10^3 cells/liter (assumed in Ref. 14), \( C_F = 1.6 \times 10^{-7} \text{M} \).
parameters can lead to greater uptake and SR values. Similar to results in Ref. 10, multiple injections of hapten (4 injections spaced 1 day apart, each one-fourth of total dose) led to approximately 40% increases in the AUC and TI, and a similar increase in T:P (results not shown).

Tumor Vasculature. An advantage of this distributed model is that the effect of the PS/V product can be analyzed by separating it into the components of vascular permeability and vascular density (or S/V, reflected by a change in the intercapillary distance or vessel radius). It showed that the two are not equivalent on a microscopic scale. With the present model, increasing the permeability increased all analytical indices (Table 2). A Krogh cylinder of smaller radius (i.e., high density of blood vessels indicating high S/V) also increased concentration levels, SR, etc., and resulted in a more uniform profile (due to a smaller distance). A simulation with 10-fold lower effective capillary distance or vessel radius. It showed that the two are not equivalent on a microscopic scale. With the present model, increasing the permeability increased all analytical indices (Table 2). A Krogh cylinder of smaller radius (i.e., high density of blood vessels indicating high S/V) also increased concentration levels, SR, etc., and resulted in a more uniform profile (due to a smaller distance). A simulation with 10-fold lower effective permeability or decreased Krogh cylinder radius shows the extent of the error introduced by compartmental models in situations of high affinity binding. In the case of high affinity binding, the average extravascular concentration is much less than the concentration at the wall, and the compartmental model overestimates the uptake. This is seen in the fact that the average BFA concentrations in the high vascular density simulation are essentially 10 times the baseline, while the more heterogeneous high permeability simulation shows less than a 6-fold increase. The difference between an increase in permeability as opposed to vascular density is greatest in a high binding affinity situation.

Clearing BFA. Clearing the antibody from the plasma before hapten injection results in very high T:P ratio for the antibody and improved T:P for the hapten by decreasing the amount of hapten trapped in the bloodstream. At the same time, there is little change in the TI or spatial distribution of hapten, while the SAT and the SR actually decrease (due also to quicker elimination from the bloodstream). The combination of a longer delay with BFA clearance is more effective with IgG (results not shown).

Initial Doses. If the antibody dose is increased by a factor of 10 (which may be possible only for an unlabeled antibody), the concentration and SAT in the tumor increase, the drug distributions are more uniform, and a TI of 15 is obtained for hapten. For a 100-fold increase in $C_p A$ (results not shown) the increase in BFA concentration in the tumor was not proportional to the increase in dose, and the T:P hapten ratios decreased as hapten was trapped in the plasma. Similar results are seen when high concentrations of BFA and hapten are used together. When the hapten dose alone is increased by a factor of 10, the increase in its average concentration in tumor is approximately 2-fold, but the TI and SR are sharply reduced.

Binding Kinetics. The choice of binding parameters plays an important role in determining uptake and distribution. It should be noted that AUC and TI for nonbinding hapten are within an order of magnitude of those in the baseline binding case. The difference is the distribution of BFA and hapten within the tissue cylinder; the profiles are very heterogeneous, with one-half of all material found within 7.5% of the volume surrounding the blood vessel, while the distribution of free hapten is uniform. Table 2 shows that increasing the antigen density also makes the spatial distribution very nonuniform. Increasing the BFA-hapten binding affinity has very similar results in this range of parameters. However, increasing this binding affinity by a factor of 100 results in trapping of hapten in the plasma, with the AUC and TI decreased below baseline, decreasing the effectiveness of the two-step approach (results not shown).

Time Delay between Injections and Antigen Internalization. An important parameter which can be controlled is the time delay between BFA and hapten injection. Twenty-four h is insufficient time for the antibody to penetrate throughout the tumor tissue, and the average concentration in the tumor is still below the initial plasma concentration. Since the time scale for the BFA clearance from the bloodstream is 2–3 days (especially for an IgG molecule), a $\tau$ of 1 or 2 weeks is needed to significantly reduce the plasma concentration (unless the BFA is cleared artificially). Increasing $\tau$ from 1 day to 2 weeks increased T:P 40-fold for both BFA and hapten, with higher concentration levels, AUC, TI, and a more uniform distribution throughout the Krogh cylinder. Of course there are practical constraints in a clinical setting including the tumor growth during treatment. In addition, internalization of the antibody by the cells and, to a lesser extent, antigen shedding, can severely

![Fig. 1. Model baseline simulation. Extravascular concentration profiles are shown for the baseline simulation for total antibody (in all forms, free and bound) (A) as a function of time ($t = -24$ to $+48$ h, with arrow at $t = 0$ denoting time of hapten injection) and distance from the center of the blood vessel ($r = 10$ to 100 $\mu$m). B, total hapten ($r = 0$ to $48$ h).]
limit the gains received by long time delays. The effect of antibody metabolism or antigen internalization was completely detrimental to diagnostic or therapeutic goals. All analytical indices were decreased compared to the baseline and in some cases even worse than for a nonbinding system without antigen.

Table 2 Analytical indices normalized with respect to baseline

<table>
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<tr>
<th>Case</th>
<th>T:P</th>
<th>BFA</th>
<th>Hap</th>
<th>&lt;C&gt;</th>
<th>SAT</th>
<th>UNI</th>
<th>SR</th>
<th>AUC</th>
<th>TI</th>
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<td>Baseline</td>
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<td></td>
<td>8.27</td>
<td>10.8</td>
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<td>0.25</td>
<td>0.26</td>
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<td>Clear BFA from blood</td>
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<td>1.07</td>
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<td>0.13</td>
<td>0.14</td>
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<td>1 wk between BFA and hapten</td>
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<td>IgG (instead of F(ab')2)</td>
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<tr>
<td>Metabolism (kM = 1.87 day⁻¹)</td>
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<td>Nonbinding BFA and hapten</td>
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Indices shown are spatial averages, for time = 24 h post-hapten (Hap) injection (which was 24 h after BFA injection), except where otherwise noted.

AUC and TI calculated at 2 weeks after hapten injection, to more closely approximate the total doses received.

Comparison with Experimental Data

The baseline simulations and parameter perturbations have been compared to the experimental data of Stickney and colleagues (2) with our model results. We have also compared our model to the experimental data of Le Doussal et al. (1). These investigators coinject 3 µg of BFA as a carrier along with the hapten (8 pmol 111In-EOTUBE) 24 h after the original BFA injection, to minimize the very rapid initial clearance phase of free hapten. No plasma concentration data were available for free hapten or BFA; therefore the pharmacokinetic rate constants given above for Le Doussal's data were used again. The presence of the carrier leads to a strong dependence of the hapten plasma concentration on the BFA pharmacokinetics, since the BFA and hapten are in equilibrium before the injection is made. The T:P ratios obtained with this simulation are compared with experimental data in Fig. 2D. Since these data were collected over a longer, 5-day period, metabolism was incorporated as an adjustable parameter which served to decrease concentration values at later times. Fig. 2D shows the T:P data of Stickney et al. (2) with our model results for no metabolism and for metabolism rate constants near 0.001 min⁻¹. The T:P at day 5 is over 5000 without metabolism but is reduced to 5 with kM = 0.001 min⁻¹.

**Role of Plasma Pharmacokinetics.** Using the baseline values for pharmacokinetic parameters, the plasma concentrations approach equilibrium values very quickly, within 1 min after injection. Yet the plasma concentration data of Le Doussal et al. (1) could not be explained by equilibrium kinetics, which predicted much higher plasma concentrations than those seen experimentally. Within the first minute the free hapten took a finite time to mix within the plasma and to associate with the BFA. This allowed the free hapten concentration to drop before reaching steady state. This effect is most pronounced for
smaller binding affinities ($K_{22}$) and slow pharmacokinetic rate constants. Using a biexponential function (as reported in Ref. 1) instead of a triexponential led to a very poor fit between the plasma model and the data and yielded exaggerated values for the percentage of i.d./g in tumor at all time periods. It is not surprising that this initial clearance phase could be important. The time scale for BFA-hapten association is approximately 1 s ($=\ln 2/C_1/K_{22}$). The first-phase time scale was 1.2 min ($=\ln 2/\lambda_1$) in humans (12), but the physiological time scale for clearances in mice is approximately one-eighth that in humans [body weight$^{-0.25}$ (15)], which would be 9 s. The plasma mixing time scale has been estimated to be 12 s (16) in rabbits and would be smaller in mice. Initial loss of hapten from the bloodstream before complete mixing or binding is consistent with observations that hapten injected without a carrier is very rapidly eliminated from the plasma. Such an early phase effect would make the percentage of i.d./g dependent on processes occurring in the first min after injection. Fortunately, in a human system the binding is just as rapid while clearance times are longer, reducing the magnitude of this effect. Fig. 2A also suggests the existence of a physiological compartment with a very slow decay rate, only evident at later times. The plasma concentrations are very small after 24 h, and their lack of relevance in single-step approaches with haptens might explain why long-term, slow-decay rates have not been reported. However, the presence of BFA for binding and the longer persistence times may lead to a significant contribution to the AUC, which should not be neglected.

**Implications and Limitations**

It should be emphasized that the difference between effective detection and treatment is not simply an increase in dose. Rather, the whole set of physiological, kinetic, and scheduling parameters should be optimized for the desired use of the two-step procedure. If the primary goal is detection the advantages of the two-step approach are the greater T:P and SR obtained compared to that for BFA or hapten alone. In addition these
peak ratios occur much more rapidly with the hapten than with antibody alone. Nonuniform distributions are not a problem in this case, and the highest possible BFA-TAA binding affinities and antigen densities should be sought. For effective treatment, where absolute concentration levels and the time-averaged doses are important and a more uniform distribution is desired, different parameter values must be chosen, with higher doses, somewhat lower BFA-TAA affinities, and increased \( r \) and \( P \). In both cases the BFA-hapten binding affinity must be chosen with regard to plasma trapping and the need for uniformity on a local scale. Targeting of antigens which have a low internalization rate and the development of antibodies which have a weak immunogenic response and slow metabolism are important areas of research. Knowledge that a specific BFA will not be internalized much over a long time period would allow for the enhanced hapten localization possible with a time delay greater than the typical 1–2 days.

The advantage of this two-step method versus that of antibody alone appears to be marginal in two respects: (a) the perivascular penetration is hindered because of rapid binding with the heterogeneously distributed BFA; (b) the TI for hapten in a two-step treatment is not significantly better than for BFA alone (if an equivalent dose of radiolabeled BFA could be given). Currently the dose of radiolabeled antibody alone is limited by toxicity in normal tissues. A major advantage of the two-step approach is the higher dose of (unlabeled) BFA which is possible and which may allow greater tumor uptake and penetration. Other definitive advantages of the BFA-hapten approach are as follows: (a) the SR are much greater for hapten than for BFA; (b) peak T:P ratios occur very quickly; (c) the hapten clears from the bloodstream much more quickly, which may be useful for many radioactive agents; (d) hapten is expected to penetrate more effectively into necrotic or avascular regions; and (e) repeated or continuous hapten injections may be given, as an immune response is less likely for low molecular weight agents.

The following limitations remain in the present model: (a) there is a need for a whole body pharmacokinetic model for two-step approaches to allow for scaling between species, to incorporate sensitive organs of the body, and to provide needed information on early times; and (b) the present model does not address issues of heterogeneities within the tumor on a macroscopic scale (8); the effectiveness of the BFA/hapten system must be addressed in the light of necrosis, variable blood supply, and elevated interstitial pressure (as suggested by the sensitivity of the present results to the vascular permeability coefficient and vascular density).

**Acknowledgments**

We thank D. Mackensen and J. M. Le Doussal for their help.

**Appendix A: Nomenclature**

\[ A_i = \text{Extravascular concentration, mols/liter, where } i = 1, 2, 3 \text{ for the mobile species free BFA, free hapten, BFA-hapten complex, and } i = 4, 5, 6 \text{ for the immobile species, TAA-BFA complex, TAA-BFA-hapten complex, and the available TAA bindings sites.} \]

\[ A_i^0 (t = 0) = \text{Total (initial) concentration of binding sites in the extravascular space} \]

\[ C_{ij} = \text{Concentration of the free species above } i = 1, 2, 3, \text{ mols/liter, where } j = 1, 2, 3 \text{ represents the plasma and the well perfused and poorly perfused peripheral compart-} \]

ments, respectively. \( C_{p1}^0, C_{p2}^0 \) are synonymous for initial concentrations \( C_{1i}^0 \)and \( C_{2i}^0 \)

\[ D_i = \text{Interstitial diffusion coefficient for the mobile species } (i = 1, 2, 3), \text{ cm}^2/\text{s} \]

\[ k_r = \text{Elimination (metabolism) rate constant for species bound to TAA, } (A_4 \text{ and } A_5), \text{ s}^{-1} \]

\[ k_{1i}, k_{2i} = \text{Forward binding rate constant for BFA-TAA and BFA-hapten, respectively, } m^{-1}s^{-1} \]

\[ k_{1i}, k_{2i} = \text{Reverse (dissociation) rate constant for BFA-TAA and BFA-hapten, s}^{-1} \]

\[ K_{a1}, K_{a2} = \text{Binding affinity for BFA-TAA (}= k_{1f}/k_{1u}) \text{ and BFA-hapten (}= k_{2f}/k_{2u}), \text{ respectively} \]

\[ P_i = \text{Effective vascular permeability coefficient for the mobile species } (i = 1, 2, 3), \text{ cm/s} \]

\[ r = \text{Radial position (distance from center of blood vessel), } \mu \text{m} \]

\[ R = \text{one-half of mean intercapillary distance (also radius of Krogh cylinder), } \mu \text{m} \]

\[ S/V = \text{Surface area per unit volume for transcapillary exchange, cm}^{-1} \]

\[ t = \text{Time, min} \]

\[ \alpha_i, \lambda_i = \text{Pharmacokinetic constants for free species in plasma } (i = 1, 2, 3), \text{ min}^{-1} \]

\[ \rho = \text{Blood vessel radius, } \mu \text{m} \]

\[ \tau = \text{Time interval between the BFA and hapten injections, h} \]

**Appendix B: Model Equations**

Five extravascular species are considered in the model. We assume that transport of BFA and its binding to the TAA is not changed when complexed with hapten (a simplifying but not fundamental assumption). The equations for unsteady-state diffusion and reaction for the extravascular species are:

\[ \frac{\partial A_1}{\partial t} = D_1 \nabla^2 A_1 - k_{1i} A_1 A_6 + k_{1i} A_4 - k_{2i} A_4 A_2 + k_{2i} A_3 \]

\[ \frac{\partial A_2}{\partial t} = D_2 \nabla^2 A_2 - k_{2i} A_2 (A_1 + A_6) + k_{2i} (A_3 + A_5) \]

\[ \frac{\partial A_3}{\partial t} = D_3 \nabla^2 A_3 - k_{1i} A_3 A_6 + k_{1i} A_3 + k_{2i} A_4 A_2 - k_{2i} A_3 \]

\[ \frac{\partial A_4}{\partial t} = k_{1i} A_4 A_6 - k_{1i} A_4 - k_{2i} A_4 A_2 + k_{2i} A_5 - k_{4i} A_4 \]

\[ \frac{\partial A_5}{\partial t} = k_{1i} A_5 A_6 - k_{1i} A_5 + k_{2i} A_4 A_2 - k_{2i} A_5 - k_{4i} A_5 \]

where \( \nabla^2 \) is the Laplacian operator in cylindrical coordinates. Metabolism (antigen internalization) is treated as a first-order process which eliminates BFA and BFA-hapten bound to the TAA (antigen, however, is renewed immediately). In addition the number of free binding sites on the tumor cells, \( A_6 \), equals \( A_6^0 - A_4 - A_5 \).

There is a no-flux boundary condition for mobile species at the surface of the cylinder \( (r = R) \) because of symmetry with surrounding identical Krogh cylinders:

\[ \frac{\partial A_i}{\partial r} \bigg|_{r=R} = 0 \quad (i = 1, 2, 3) \]
between hapten and BFA. In the present study a three-compartment model is used for hapten (and two for BFA):

\[
\frac{dC_i}{dt} = \sum_{j=1}^{3} \left[ k_{ij}(C_{ji} - C_{ij}) \right] - \phi_i(k_{3j}C_{ji} - k_{3j}C_{ji}) - k_{10}/C_{1i} \quad (i = 1, 2, 3)
\]

where \(\phi_i = 1\) for \(i = 1, 2\), and \(\phi_i = -1\) for \(i = 3\); \(j\) represents the three compartments, with \(k_{kj}\) representing the transfer coefficient of species \(i\) from compartment \(j\) to \(k\), and \(k_{10}\) the rate constant for elimination from the plasma (e.g., urine). These compartmental transfer coefficients may be related to experimental data for the plasma concentration \((C_{1i})\) of free species (17).

\[
\frac{C_{1i}}{C_{10}} = a_1\exp(-\lambda_1 t) + a_2\exp(-\lambda_2 t) + (1 - a_1 - a_2)\exp(-\lambda_3 t) \quad (i = 1, 2, 3)
\]

References

Pharmacokinetic Analysis of the Perivascular Distribution of Bifunctional Antibodies and Haptens: Comparison with Experimental Data

Laurence T. Baxter, Fan Yuan and Rakesh K. Jain


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