Pharmacokinetic Analysis of Two-Step Approaches Using Bifunctional and Enzyme-conjugated Antibodies

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ABSTRACT

Bifunctional antibodies (BFA) and enzyme-conjugated antibodies (ECA) can be used to preferentially deliver a hapten or drug to tumor sites for diagnosis and therapy. We present here a simple pharmacokinetic model for the above two systems by considering only two compartments, the plasma and tumor. The models predict that the longer the time delay between the BFA and hapten or between the ECA and prodrug injections, the higher the tumor-plasma concentration ratio of the hapten or drug. In addition, multiple injections of the hapten or prodrug is predicted to give a more uniform concentration of the hapten or drug in both the tumor and plasma than bolus injection. We suggest that, initially, the most effective dose of BFA should be selected and then the hapten concentration chosen accordingly. The decrease of the ECA injection dose would increase the tumor-plasma concentration ratio of the drug and yet decrease the tumor concentration of the drug. In clinical application of the ECA system, consideration of ECA dose should be balanced between the tumor concentration and the tumor-plasma concentration ratio of the drug. The dose of the prodrug injection is suggested to be equal to the required toxic concentration of the drug in the tumor. There are several ways to improve the tumor-plasma concentration ratio of the hapten or drug, such as changing the binding kinetics of the antibody to tumor or the hapten to BFA and removing the antibody from the plasma before the injection of the hapten or prodrug. One notable difference between the BFA and ECA approaches is that there is an upper limit for maximum hapten concentration in the former, and hence, from the point of drug delivery alone the latter approach is presumably superior. The limitations of the models and therapeutic implications are also discussed.

INTRODUCTION

MAbs1 alone or linked to radionuclides, drugs, toxins, enzymes, growth factors, or effector cells offer a promising approach to the treatment of solid tumors. Their strengths include their high degree of specificity for tumor-associated antigens and the fact that exchange vessels and interstitium of tumors have a "leaky" structure. Their clinical limitations, however, result from their inadequate uptake and heterogeneous distribution in tumors. The physiological barriers responsible for the poor delivery in tumors include heterogeneous blood supply, interstitial hypertension, antigen distribution, and the long distances that MAbs have to traverse in the interstitium (1).

One strategy to overcome these physiological barriers is to use low molecular weight agents with a high degree of specificity. Two recently introduced approaches satisfy this requirement: the use of low molecular weight haptens (chelates or a molecule recognized by an antibody) with BFAs (2, 3) and the use of low molecular weight prodrugs with ECAS (4–6). In these two-step approaches, BFA (or ECA) is injected into a patient, permitted to bind to antigenic sites in the tumor, and then cleared from normal tissues. After an appropriate time interval, a radionuclide attached to a low molecular weight hapten (or a prodrug) is injected into the patient. The advantage of using the low molecular weight hapten (or the prodrug) is their rapid movement in the tumor and clearance from the body. Since BFAs or ECAs may not be severely toxic to normal tissues, they can be injected at much higher doses than an antibody bound to radionuclide or cytotoxic agents. While the higher dose might not solve the problem of heterogeneous distribution, it would increase the absolute concentration of antibody in the tumor and bind to a larger fraction of antigenic sites in a tumor. Obviously, the BFA/ECA in nontarget tissues and blood has to be cleared to reduce side effects when the hapten/prodrug is injected.

The current BFA-hapten and ECA-prodrug systems may not be optimal. There is an urgent need to develop a pharmacokinetic framework for determining the doses and time schedule of the injection of these agents. In particular, the questions that need to be answered are: What should be the optimal dose and schedule of BFA/ECA injection? At what time after antibody injection should the hapten/prodrug be injected? What should be the optimal dose and schedule of hapten/prodrug injection? How sensitive are the systems to variations in affinity constants, hapten, drug, and prodrug pharmacological properties and hapten or prodrug excretion rates? What are the optimal molecular weights of BFA/ECA and hapten/prodrug? What is the effect of antigen concentration on the delivery of radionuclides/drugs to the tumor? While these questions can be answered by doing a large series of carefully designed experiments, considerable insight into the pharmacokinetics may be obtained by using an appropriate mathematical model.

Currently, there are several lumped (7–9) and distributed (10–15) parameter models to describe the pharmacokinetics of MAbs. However, to date there are no models for the two-step approaches using antibodies and haptens or prodrugs. Therefore, the purpose of this work was to develop a simple pharmacokinetic model to answer the questions raised earlier.

MATERIALS AND METHODS

Model Development

Model for BFA-Hapten System. In order to simplify the problem and yet give some initial results to guide further experiments, the BFA-hapten system in this paper was simulated by a nonlinear two-compartment model, as shown in Fig. 1. The major purpose of this model is to investigate the effects of time schedule and dose of BFA and hapten injections and the values of kinetic constants on the total concentration of hapten in the tumor and the concentration ratio of hapten between the tumor and plasma. In this preliminary lumped model we neglected the heterogeneous distribution of molecules and blood vessels in the tumor and the transport of the hapten into tumor cells. The importance of considering interstitial transport was discussed in detail by Baxter and Jain (10–14). The present analysis is useful for micrometastases and cancer detection and treatment for which the
average hapten or drug concentration is important. This assumption will be discussed further in “Model Limitations” and a forthcoming paper. We assumed that the tumor compartment is too small to affect the plasma concentrations of BFA and hapten. This assumption is valid for small tumors. The plasma concentrations of BFA and hapten in our model were therefore determined by their clearance through the kidney and the transport of BFA and hapten between the plasma and normal tissues. Since the concentration of BFA or hapten in normal tissues is not considered in this initial model, the transport of BFA and hapten molecules into the normal tissues and the kidney were combined, and the concentration of BFA and hapten in the plasma was thus determined by a one-compartment model. The lumped rate constants and free hapten clearances through the normal tissues and the kidney were calculated from the experimentally measured half-life of the corresponding molecules in the plasma, respectively, as shown in Table 1. In addition, we assumed that the binding of the BFA to tumor antigen and the binding of the hapten to BFA are independent of each other, and there is no difference between the binding of hapten to BFA in the tumor and plasma.

Model for ECA-Prodrug System. The model for the ECA-prodrug system is similar to that for the BFA-hapten system, as shown in Fig. 2. We also assumed that the conversion of prodrug to its active form is a typical Michaelis-Menten enzyme-catalyzed reaction. Similar to the model for the BFA-hapten system, the transport of ECA, prodrug, and drug to the normal tissues and kidney was combined, and the plasma concentrations of ECA, prodrug, and drug were assumed to be unaffected by the tumor compartment. The same binding affinity was chosen for BFA and ECA to tumor antigen. We assumed that the binding of ECA to tumor has no effect on the rate of prodrug conversion, that there is no difference in the kinetic constants of prodrug conversion between the tumor and plasma, and that there is no nonspecific conversion of the prodrug.

Governing Equations and Model Parameters

In order to further simplify the problem, we assumed that the binding and dissociation reactions in the above two models are very fast compared to clearance and extravasation rates, so that the ratio of free to bound species is merely a function of the total concentration. In addition, we assumed that the permeabilities of the vessel wall and the lumped rate constants of plasma clearance of BFA, BFA-hapten complex, and ECA-prodrug systems are the same (see also “Appendix B”). The permeabilities of the vessel wall to hapten, prodrug and drug were chosen to be equal to sucrose and the lumped rate constants of plasma clearance of prodrug and drug were assumed to be the same as that of hapten. Based on all the assumptions above, the governing equations for the models of BFA-hapten and ECA-prodrug systems were obtained, as shown in “Appendix B.”

The baseline values of all the model parameters were obtained from experimental results in the literature (Table 1). The value of these constants may vary with different antibodies (IgG, M, 150,000), tumor cell lines, hapten, prodrugs and drugs. In this preliminary work, we arbitrarily chose one set as the baseline values based on literature values and perturbed them in later simulations. The maximum binding capacity of the tumor, B0, is defined as the total number of specific antibody-binding sites on a tumor cell surface multiplied by the volume of the tumor cells/liter tissue and divided by the volume of the tumor cell and the Avogadro constant. The effective permeability coefficient (Peff) of the antibody in solid tumor was assumed to be 1/10 of the value measured by Gerlowski and Jain (22) as in the previous studies (10–14), due to the increased tumor interstitial pressure which reduces transcapillary filtration. For small molecules (e.g., hapten or drug), the diffusive transport is dominant in both tumor and normal tissues. Therefore, Peff and P0 were chosen to be the same as the measured effective permeability of sucrose.

The baseline time schedules for both BFA-hapten and ECA-prodrug systems are the same. The hapten or prodrug was given in one of the following ways: (a) a bolus injection; (b) one injection/day for 4 days; and (c) keeping a constant concentration of total hapten in the plasma. In each case, the hapten or prodrug was given starting on the third day after the antibody injection.

The injection doses for BFA, ECA, hapten, and prodrug are given in the form of plasma concentration increase after each injection. In this work, we assumed that the BFA was IgG (M, 150,000), although Fab′ (M, 50,000) and F(ab′)2 (M, 100,000) are also used as BFA/ECA for tumor targeting (16). The baseline dose of antibody injection (A) for both systems was chosen to be 10−4 M. The baseline dose of a single
bolus hapten injection was chosen to be $10^{-7}$ M, which is of the same order as the maximum binding capacity $B$ of the tumor. The baseline dose of a single bolus prodrug injection $L_{BFA}$ was chosen to be $10^{-4}$ M, which is within the experimental range of Senter et al. (6). For multiple injections, given once/day on 4 successive days, the dose of each hapten or prodrug injection is equal to $1/4$ of bolus injection. For the case of constant concentration of the total hapten in the plasma (i.e., $[H]_{P}$ is constant), we assumed that the hapten is injected such that $[H]_{P}$ is maintained at $10^{-9}$ or $10^{-10}$ M.

The governing equations for both BFA-hapten and ECA-prodrug systems were solved numerically by using the computer program for the Bulirsch-Stoer extrapolation method in the IMSL software package (23) (see also “Appendix B”). The data presented in Figs. 3 through 12 are either either these numerical solutions (e.g., the drug concentration in tumor $[D]_{T}$) or the ratios of these solutions (e.g., the tumor-plasma concentration ratio of drug, $[D]_{T}/[D]_{P}$).

RESULTS

BFA-Hapten System. As a result of the quasi-steady state assumption for the binding and dissociation reactions, the fraction of free-total hapten concentration, or ratio of bound:free species, is a known function of the total concentration. These ratios and typical values for the baseline parameters are given in “Appendix B.” An asymptotic solution for antibody concentration valid for a constant bound:free ratio is also given in “Appendix B,” Equation B18.

The effect of BFA injection dose on the BFA concentration in the tumor and plasma is shown in Fig. 3. The BFA concentration in the plasma is proportional to the dose of BFA injection at any time and decreases exponentially to zero with time. When the dose of BFA injection is lower than the maximum binding capacity $B_{T}$ of the tumor (cases 2 and 3 in Fig. 3), the concentration of the BFA in the tumor is lower than $B_{T}$, for up to 2 weeks and depends significantly on the dose of BFA. The decrease of the BFA concentration in the tumor is very slow, and the estimated time constant for this process is about 23 days for our baseline values (Table 2). This is due to the high affinity binding of BFA to the tumor antigen and the low permeability of the vessel wall to BFA transport. The concentration of BFA in the tumor could be higher than the initial plasma concentration of BFA (Fig. 3), because of the binding of BFA to the tumor antigen.

The behavior of the hapten concentration in the tumor $[H]_{T}$, depends on whether or not the initial concentration of the hapten in the tumor is higher than the BFA concentration in the tumor, as shown in Fig. 4. For the baseline initial value of $[H]_{T}$, which is higher than $[A]_{T}$, $[H]_{T}$ will decrease to the level of $[A]_{T}$ at about 1 day after the hapten injection, when its decrease is slowed down by the binding of the hapten to the BFA in the tumor (cases 1 and 2 in Fig. 4, solid lines). When the initial $[H]_{T}$ is less than $[A]_{T}$, there is no rapid decrease phase for $[H]_{T}$, and $[H]_{T}$ is always less than $[A]_{T}$ (cases 1 and 2 in Fig. 4, dashed lines). Thus, the lower dose of hapten injection will lead to lower tumor concentration of the hapten, but it may lead to a higher tumor:plasma concentration ratio of the hapten (see below). In contrast to the bolus injection, keeping a constant level of the hapten in the plasma will lead to a uniform increase in the hapten concentration in the tumor. The total AUC, which represents the hapten availability to the tumor (24), of the multiple injection is about 40% greater than the bolus injection as shown in Table 3, and the time delay of hapten injection has little effect on the AUC of hapten in the tumor.

An important factor that the model tried to predict was the
total concentration ratio of the hapten between the tumor and plasma \([H]_T/[H]_P\). A higher concentration ratio is desired for both tumor detection and therapy. Many factors could affect \([H]_T/[H]_P\). The increases of the time interval between the injections of BFA and hapten, the values of binding affinities \(K_a\) and \(K_a\) for BFA-tumor antigen and hapten-BFA, respectively, the maximum binding capacity of the tumor \(B_0\), the effective permeability of the vessel wall to BFA (\(P_a\)), and the rate constant of hapten clearance (\(k_d\)) from the plasma will increase the concentration ratio \([H]_T/[H]_P\), as shown in Table 2. However, \([H]_T/[H]_P\) will increase as the effective permeability coefficient of the vessel wall to the hapten (\(P_h\)) is decreased (Fig. 5F), since the hapten would be retained longer in the tumor while being cleared from the plasma. A discussion of these results is given in the next section. The effects of the injection doses of both BFA and hapten were investigated and are shown in Fig. 6. Three days after BFA injection, the hapten was injected. The value of \([H]_T/[H]_P\) was calculated 1 day after hapten injection. Based on the model parameters chosen, the optimal dose of BFA injection, which is defined here as the dose that will lead to the maximum \([H]_T/[H]_P\) 1 day after hapten injection, is of the order of \(10^{-8} \text{ M}\), which is about 10% of the maximum binding capacity of the tumor. The decrease of \(I_{tr}\) may increase \([H]_T/[H]_P\), but it will decrease \([H]_T\) (Fig. 4). If the dose of the hapten is much higher than the concentration of the BFA in the tumor, e.g., \(I_{tr} = 10^{-4} \text{ m}\), then it will take at least 2 days for \([H]_T/[H]_P\) to be significantly higher than unity (results not shown). This is because it will take time for \([H]_T\) to decrease to the level of \([A]_T\), which is required for \([H]_T/[H]_P\), greater than unity. The results in Fig. 6 suggest an equal dose of hapten and BFA to be optimal. In order to increase \([H]_T/[H]_P\) and yet keep \([H]_T\) high, Goodwin et al. (25) suggested that, before the injection of hapten, the BFA should be removed from the plasma (e.g., by injection of unlabeled hapten, etc.). If there is no binding of hapten to the BFA in the plasma, the hapten molecules would be cleared very rapidly from the plasma, resulting in a decreased concentration of the hapten in the plasma and the increased concentration ratio of the hapten \([H]_T/[H]_P\) (Fig. 7).

ECA-Prodrug System. The concentration distributions of ECA in both the tumor and plasma are identical to that of BFA in the tumor and plasma, respectively, because the rates of antibody binding/clearance are independent of hapten concentration. Therefore, the concentration distribution of ECA in both the tumor and plasma is also shown by Fig. 3. The drug concentration in the tumor decreases exponentially with time (Fig. 8) and is almost proportional to the dose of prodrug injection (results not shown). This is because the dose of prodrug is much higher than the concentration of ECA and no binding of drug in the tumor is involved. The total AUC of the drug in the tumor is almost the same for both the bolus and multiple injections as shown in Table 3. The time delay between ECA and prodrug injections has little effect on the AUC of the drug in the tumor.

One of the major advantages of the ECA-prodrug system, compared to the direct injection of the active drug into the body for tumor therapy, is that the concentration ratio of drug between the tumor and plasma \([D]_T/[D]_P\) can be higher than unity. This enables the drug to have a greater effect on the tumor while reducing the side effects of the drug in normal tissues. In this initial model, the trapping of the drug by the tumor cells was not considered. Thus, the only mechanism to increase the tumor:plasma concentration ratio of the drug \([D]_T/[D]_P\) was to increase the difference of the prodrug conversion between the tumor and plasma. After most of the prodrug molecules have been converted to active drug, the concentration ratio of the drug \([D]_T/[D]_P\) would approach unity, as shown in Fig. 9, because the drug molecules are too small so that they could easily cross the vessel wall (the time constant is 0.1 h as shown in Table 2). Factors that could increase \([D]_T/[D]_P\) were studied. The increase of the time interval between the ECA and prodrug injections could increase \([D]_T/[D]_P\) (Fig. 9A), because the concentration ratio of ECA between the tumor and plasma was increased (Fig. 3). When the rate constant \(k_e\) of the prodrug conversion is high, almost all the prodrug in both tumor and plasma is converted into the drug within a very short time (in a few minutes), and the drug in the tumor is almost in equilibrium with that in the plasma during this time. Thus, \([D]_T/[D]_P\) is close to unity. When \(k_e\) is very low, the prodrug conversion is so slow that the prodrug concentrations in both tumor and plasma are almost constant. The rate of prodrug conversion in this case is limited by the ECA concentration which is much higher in the tumor than in the plasma. Therefore, as \(k_e\) decreases, \([D]_T/[D]_P\) increases (Fig. 9B). The effect of \(K_m\) on \([D]_T/[D]_P\) is opposite to that of \(k_e\). As \(K_m\) is decreased, the rate of prodrug conversion is increased and, thus, \([D]_T/[D]_P\) is decreased (Fig. 9C). The molecular weight of prodrug or drug is one of the factors that will determine the vascular permeability to this molecule. Fig. 9D shows that the concentration ratio \([D]_T/[D]_P\) increases more rapidly as \(P_D\) is increased. \([D]_T\) also increases with \(P_D\), because \([D]_T\) is independent of \(P_D\). The dependence of \([D]_T/[D]_P\) on \(P_D\) is only for a short time period (<1 h) until the tumor and plasma concentration levels equilibrate. The injection dose of ECA is important when the ECA-prodrug system is used for tumor therapy. The results of our model reveal that the decrease of the ECA injection dose would potentiate the effect of ECA on the tumor (Fig. 9E) and decrease the concentration of the drug in the tumor (Fig. 10). However, the decrease of the tumor concentration of the drug is insignificant.

We also investigated the effect of the removal of ECA from the plasma on the concentration ratio of the drug \([D]_T/[D]_P\) and the concentration of the drug in the tumor \([D]_T\) in a way similar to that already described for the BFA-hapten system. If the ECA is removed from the plasma before the injection of the prodrug, theoretically, there is no conversion of the prodrug in the plasma and the concentration of the drug in the plasma \([D]_P\) is equal to zero, because we assumed that the tumor compartment is too small to affect the concentrations of ECA, prodrug, and drug in the plasma. Therefore, \([D]_T/[D]_P\) theoretically becomes infinity but, in practice, a very large number. The concentration of the drug in the tumor \([D]_T\) will decrease after the removal of ECA from the plasma, because of the increased concentration difference across the vessel wall in the tumor. However, as shown in Fig. 11 the decrease of \([D]_T\) is
Fig. 5. Effect of (A) the time interval $r$ between the BFA and hapten injections, (B) the binding affinity $K_a$ of BFA to tumor antigen, (C) the binding affinity $K_a2$ of hapten to BFA, (D) the maximum binding capacity $B_i$ of the tumor, (E) the vessel permeability $P_a$ to BFA, (F) the vessel permeability $P_h$ to hapten, and (G) the plasma clearance constant $k_h$ for hapten on the tumor-plasma concentration ratio of hapten $[H]_T/[H]_p$. A bolus injection of hapten ($I_H = 10^{-7} M$) was given with different time delay $r$ (A) or $r = 3$ days (B-G), and then $[H]_T/[H]_p$ was calculated.
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Fig. 6. Effect of the injection doses of hapten $I_H$ and BFA $I_A$ on the tumor:plasma concentration ratio of the hapten $[H]_{T}/[H]_{P}$. The hapten was given as a bolus injection 3 days after BFA injection, and $[H]_{T}/[H]_{P}$ was calculated 1 day after hapten injection.

Fig. 7. Effect of BFA removal from the plasma on the tumor:plasma concentration ratio of the hapten. On day 3 after BFA injection ($I_A = 10^{-8} \text{ M}$), the BFA was either removed or remained in the plasma and a bolus injection of hapten ($I_H = 10^{-7} \text{ M}$) was given; then, $[H]_{T}/[H]_{P}$ was calculated.

DISCUSSION

The present models for BFA-hapten and ECA-prodrug systems are the first in the literature for the mathematical simulation of two-step approaches using antibodies. Several initial results were obtained from this simulation, which focused on the effects of time schedule and doses of BFA/ECA, hapten, and prodrug injections, the binding kinetics, and the vascular permeabilities on the tumor concentration and tumor:plasma concentration ratio of hapten or drug. Other factors, such as the heterogeneous distributions of BFA/ECA, hapten and drug in the tumor, the binding and metabolism of hapten, prodrug, and drug by tumor cells, and pharmacological factors that affect the biodistribution of drugs, were not considered in the present models (see “Model Development” and “Model Limitations”).

It is useful to consider the relative time scales for the many processes in this system. Table 2 gives approximate equations and time constants for plasma clearance, extravasation, reaction, and diffusion. Interstitial diffusion is rapid over intercapillary distances but extremely slow (weeks to months) over a 1 cm distance. For the baseline parameters given in Table 1, the conversion of prodrug to drug is very rapid in tumors saturated with antibody. The filtration or absorption of free small molecules by blood vessels is also very rapid, achieving a quasi-steady state within minutes. The time scales for extravasation and clearance of the antibody are much greater. However, it should be noted that the effective rate of hapten leaving the tumor is greatly reduced when a large fraction is bound to BFA in the tumor. From this table we see that the production of drug should not be limited by a slow reaction rate but, rather, by undesirable conversion in plasma or normal tissue.

Time Schedule of Injection

Because the time scale for the concentration decrease of BFA/ECA in the tumor is of the order of several weeks, and the time constant for plasma clearance of BFA/ECA is several days (Table 2), the present models suggest that the longer the time interval $\tau$ between the BFA and hapten or between the ECA and prodrug injections the higher the tumor:plasma concentration ratio of hapten or drug. However, the increase of the time interval $\tau$ between the two injections may decrease the total hapten or drug concentration in the tumor. For example, after 3 days of hapten injection, $[H]_{T}/[H]_{P} = 0.963 \times 10^{-8} \text{ M}$ for $\tau = 4$ days and $0.192 \times 10^{-8} \text{ M}$ for $\tau = 14$ days, respectively. The decrease in drug concentration, $[D]_{T}$, due to the change in $\tau$ is insignificant. The reason for the decreased tumor concentration of the hapten or drug is that the concentration of the antibody in the tumor will decrease with time (Fig. 3). The time delay has an insignificant effect on AUC of both hapten and drug in the tumor (Table 3). In addition, bolus and multiple injections are compared in this paper (Figs. 4 and 8). For short time simulation, the concentration of hapten or drug in the tumor obtained by bolus injection is higher than that by multiple injections, because the former injects more hapten or prodrug at one time than the latter. However, for longer time simulation, the multiple injection results in a relatively higher concentration of hapten or drug in the tumor. Clinically, if cancer cells can be killed within a short time period (<1 day), the bolus injection of hapten or prodrug would be suggested for tumor therapy by this simulation. Otherwise, the multiple injection should be used. For tumor imaging, the bolus injection would obviously be a better choice, because the tumor needs to be observed for a short duration.

**Fig. 8.** Effect of prodrug injection schedule on the drug concentration in the tumor. Solid line, $I_{Dpr} = 10^{-4} \text{ M}$ (bolus injection); dashed line, $I_{Dpr} = 0.25 \times 10^{-4} \text{ M}$ (multiple injections). The time delay $\tau = 3$ days.
Dose of Injection

The purpose of the specific delivery of hapten or prodrug to the tumor by using BFA or ECA is to increase the absolute tumor concentration and the tumor:plasma concentration ratio of the hapten or drug. However, the mechanism for the BFA-hapten system is different from that for the ECA-prodrug system in this regard.

For the BFA-hapten system, the increase of the total concentration of hapten in the tumor \([H]_T\) and the tumor:plasma concentration ratio of the hapten \([H]_T/[H]_P\) is achieved by increasing the binding of the hapten to BFA and the tumor:plasma concentration ratio of the BFA. Therefore, if the concentrations of the hapten in both tumor and plasma are much higher than that of BFA, the advantage of the BFA-hapten system will be lost, because most of the hapten molecules in both the tumor and plasma are not bound to BFA. Thus, a higher local concentration of the hapten in the tumor \((|H|_T \gg B)\) is required. In this case, the dose of the hapten should be slightly higher than the required concentration of the hapten in the tumor. Generally, the optimal dose of hapten can be defined as the dose which leads to a maximum tumor:plasma concentration ratio of hapten while keeping the tumor concentration of the hapten of the same order of magnitude as the BFA concentration in the tumor. Thus, the optimal dose of the hapten depends on the concentration of the BFA in the tumor. We define the optimal dose of BFA injection as the one that could maximize the tumor:plasma concentration ratio of the hapten. The results in Fig. 6 suggest that the optimal dose of the BFA is of the order of \(10^{-5} \text{ M}\), which is about 10% of the maximum binding capacity \(B\) of the tumor. This optimal value of BFA dose depends on the baseline values of the model parameters. Our simulations shown in Figs. 4 and 6 suggest...
decreased. The key question here is whether the decrease of the order to increase \[D_r/D_p\] the tumor-plasma concentration ratio at 10 min postinjection. These results suggest that \[D_r/D_p\] amount. For example, increasing the initial dose by a factor of 3, \[A_v/A_l\] will be increased, if the dose of ECA injection is increased. As shown in Fig. 3, \[A_v/A_l\] will be increased, if the dose of ECA injection is decreased. The key question here is whether the decrease of the ECA injection dose \(I_E\) will significantly decrease the concentration of the drug in the tumor \(D_T\). The results shown in Fig. 10 indicate that the decrease of \(D_T\) is insignificant when \(I_E\) is decreased from \(10^{-7}\) to \(10^{-9}\) M. This is because the smaller ECA concentration \(10^{-9}\) M is sufficient to rapidly convert most of the prodrug to drug. If the optimal dose of ECA is defined as the dose that will lead to the initial concentration of the drug in the tumor to be of the same order as prodrug concentration in the tumor and a maximum of the tumor-plasma concentration ratio of the drug, then these results suggest that the optimal dose of ECA injection is of the order of \(10^{-9}\) M, which is equal to 1% of the maximum binding capacity \(B\) of the tumor. This, of course, is not a general conclusion, because it depends on the values of our model parameters. Generally, the optimal dose of ECA should have \(A_v/A_l\) near \(B\) while maintaining high tumor-plasma concentration ratio of the drug. If only the tumor concentration and the tumor-plasma concentration ratio of the drug are considered in selecting the optimal dose of prodrug, there is no limitation on the dose of prodrug injection. Therefore, we suggest that it would be better to give a slightly higher dose of the prodrug than the required local toxic concentration of the drug in the tumor.

Improvement of Tumor:Plasma Concentration Ratio

There are several ways to improve the concentration ratio of hapten or drug between the tumor and plasma. We have shown that the increase of the time between the two injections will increase the tumor-plasma concentration ratio of both the hapten (Fig. 5A) and the drug (Fig. 9A), because in this case the tumor-plasma concentration ratio of BFA/ECA is increased (Fig. 3). In addition, changes of the binding kinetics of the BFA/ECA to tumor antigen and the hapten to BFA and the conversion rate of the prodrug to the active drug could also improve the tumor-plasma concentration ratio of the hapten or drug.

The removal of BFA/ECA from the plasma before injection of the hapten or prodrug is another method suggested by Goodwin et al. (25) to improve the tumor-plasma concentration ratio of the hapten or drug. Based on the simulation in this paper, this approach is more efficient for the ECA-prodrug system than for the BFA-hapten system. For the BFA-hapten system, if the BFA is removed from the plasma before the injection of the hapten, the binding of the hapten to BFA occurs only in the tumor. Therefore, the hapten in the plasma will be quickly cleared from the body, and the total tumor-plasma concentration ratio of the hapten (BFA-bound plus free hapten) is increased compared with that if the BFA is not removed from the plasma (Fig. 7). For the ECA-prodrug system, the removal of ECA from the plasma before the injection of the prodrug will cause the tumor-plasma concentration ratio of the drug to be very high and has an insignificant effect on the concentration of the drug in the tumor (Fig. 11), because there is no prodrug conversion in the plasma, \(D_T = 0\). Actually, \(D_T\) is not equal to zero but very small. The mechanism for this difference is as follows. If the ECA in the plasma is not removed before the injection of the prodrug, the prodrug concentrations in both tumor and plasma will decrease very rapidly to zero as shown by the solid lines in Fig. 12, due to the rapid conversion of the prodrug to the active drug. However, if the ECA is removed from the plasma before the injection of prodrug, the decay of the prodrug in the plasma will be controlled by the clearance of the prodrug through the kidney and normal tissues, instead of the prodrug conversion to the active drug. Therefore, the decay of the prodrug concentration in the plasma will be slowed down when the ECA is removed from the plasma. The slow decay of plasma concentration of prodrug will further result in the slow
causes the increase in the transport of the drug from the tumor to the plasma compartment. The conversion of prodrug to drug inside the cell, and the mode of action of the small molecule. For toxins this uptake is essential for killing cells, while being of less importance for radionuclides. In addition, the prodrug itself may be toxic to normal cells to a lesser degree.

5. The choice of 19 days for calculating the AUC is arbitrary, and therefore, the conclusion may be different if a different end point for integration were chosen. For drug, if the end point for integration is at least 1 day after the prodrug injection, then it does not affect the value of AUC very much, because we assumed that there was no binding of the drug in the tumor which results in an exponential decrease in the concentration of the drug in both the tumor and plasma.

6. A serious limitation is that the heterogeneous nature of tumors was not considered explicitly in these models. As pointed out by Jain (1), the heterogeneity of tumor properties and transport is a major problem in drug delivery to solid tumors. It has been suggested that nonuniform interstitial pressure and blood vessel distribution are important factors leading to reduced and nonuniform uptake of macromolecules (10–14). In particular, elevated interstitial pressure in the center of large solid tumors may reduce the effective permeability coefficient by a factor of 100, while central necrosis will severely reduce the surface area available for exchange. When combined with the slow rate of diffusion over large distances in the interstitium, little material may reach the tumor center. In these situations, the use of parameters (permeability coefficient, blood vessel surface area, antigen density) corresponding to the necrotic center of the tumor will give the “worst case” concentrations. Qualitatively, this would require an increase in the antibody dosage and a longer delay before small molecule (e.g., hapten or prodrug) injection. We plan to modify the distributed parameter model of Baxter and Jain (10–14) for the two-step approaches considered here.

Model Limitations

The physical situation being modeled has been simplified to gain insight into the rational basis of two-step approaches and to guide future modeling and experimentation in this area. However, there are a number of limitations which must be considered.

1. Our model assumes that the volume of distribution is the same for large and small molecules by considering only one plasma compartment. The conversion of prodrug to drug in normal tissues was not included. A nonspecific degradation pathway may exist for some prodrug systems, and significant drug production may occur in certain normal tissues. A more complete model would include normal tissues as separate compartments, which would lead to results that may be quantitatively different from, but qualitatively similar to, our present results.

2. The convective transport of macromolecules is not included in our present model. This would lead to asymmetric transport across the vessel walls. Our future work on distributed parameter models would take this into account formally.

3. Our model assumes instantaneous reversible binding for antibodies. In reality there is a finite rate at which the antibody will bind to the cancer cells or hapten, although for most antibody systems this will be much faster than the rate of extravasation or plasma clearance.

4. The uptake and metabolism of antibody, hapten, prodrug, and drug by tumor cells or other tissues were not considered in the present analysis. The effect of these processes depends upon the turnover rate of surface antigens, the fate of the molecule inside the cell, and the mode of action of the small molecule. For toxins this uptake is essential for killing cells, while being of less importance for radionuclides. In addition, the prodrug itself may be toxic to normal cells to a lesser degree.

Conclusions and Implications

Guidelines for Two-Step Approaches

Based on the results of these simple models we propose the following preliminary guidelines for the rational development of two-step approaches.

Determination and Modification of System Parameters. The physiological stage of the tumor should be the first consideration. Is it a small relatively homogeneous tumor or a large heterogeneous tumor with elevated pressure? What is the state of vascularization in the tumor? What type of antigens are expressed and in what quantity? Answering these questions will determine the parameters such as vascular surface area, vascular permeability, and antigen concentration. If it is possible, these parameters must be modified (e.g., increase antigen expression and permeability, reduce pressure, etc.).

Mode of Injection. A constant plasma concentration over an extended time period may lead to a more uniform increase of the tumor concentration and tumor:plasma concentration ratio of the hapten, compared with just giving a bolus injection. The multiple injections will give intermediate results. Although keeping a constant plasma concentration of the hapten in the plasma might cause a relatively higher concentration of the hapten in the normal tissue for a long period, the side effect of hapten might be insignificant, if its level is lower than a thresh-
old value. For tumors of the lymph node or peritoneum, local injection modes (s.c. or i.p.) may be better.

Time Delay between First and Second Phases. Next, the time delay between injection of the antibody and smaller molecule should be chosen. This interval may be constrained by practical limitations because of tumor size, concerns for the patient, or adherence to accepted protocols. Otherwise, a time may be chosen to obtain a high antibody concentration in the tumor with a high tumor:plasma ratio. The concentration and tumor:plasma concentration ratio can be determined numerically with our model. Based on the simulation, it is better to choose the time delay of several days (especially with large $K_{a1}$). Clearing the antibody from the plasma will be useful in increasing the tumor:plasma concentration ratio.

Binding Affinity. As discussed by Baxter and Jain (13, 14) it is best to use the highest possible binding affinity between the antibody and tumor associated antigen which does not lead to inhomogeneities on a microscopic level ($K_{a1} > 10^{5}$ A leads to perivascular distribution, which may not be a problem for cancer detection but may be a problem for chemotherapeutic agents). A large binding affinity between BFA and the hapten would lead to increased tumor:plasma concentration ratios and a longer residence time in the tumor but would decrease the interstitial diffusion rate.

Conversion Rate. We assumed that the conversion rate of prodrug to drug was very rapid. The reaction rate will remain greater than the rate of extravasation for $k_d[A]_p/(K_m + [D_p]) > V/(P_n S)$. Thus, reducing the rate of unwanted nonspecific reactions in plasma and normal tissue and reducing the toxicity of the prodrug are more important factors than obtaining the fastest possible reaction rate, $k_d$.

Dose of Antibody. The optimal antibody dose depends on many factors, including the mode of injection, time delay before small drug delivery, and binding affinity. However, the general objective is to achieve a relatively high tumor concentration while maintaining a high tumor:plasma ratio. This is realized by maintaining the total tumor concentration at a level just below saturation (antigen concentration) while allowing time for the antibody to be cleared from the plasma. The model may be used to calculate what initial dose will yield a nearly saturated tumor (as in Fig. 3). As a rough guideline, for an antibody with a large binding affinity ($K_{a1}B > 10$), an initial plasma concentration $\frac{V_m}{V}$ of $B$ is sufficient.

Dose of Small Molecule. For the BFA approach the tumor:plasma concentration ratio of the hapten will be highest with a hapten concentration below the total BFA concentration in the tumor. For a single injection (or small number of multiple injections) the dose should yield an initial plasma concentration of hapten to be approximately the same as $[A]_p$, assuming a high affinity for the BFA ($K_{a1}[A]_p > 10$). Keeping a constant concentration of the hapten in the plasma will lead to a uniform increase of both the tumor concentration and tumor:plasma concentration ratio of the hapten. For the ECA approach, the tumor:plasma ratio of the drug is not a strong function of the dose of prodrug. The prodrug dose should yield an initial plasma concentration at or slightly above the toxic concentration for the drug against the cancer cells.

Implications

The mathematical models developed in this paper have predicted some interesting results which might be used to guide future experiments and modeling. We have shown that the tumor:plasma ratio for hapten may increase without too great a decrease in the absolute hapten concentration by waiting for a sufficient period after BFA injection. The BFA strategy was found to be limited in that significant tumor:plasma ratios could be obtained only for doses smaller than the antibody concentration, while the optimal dose for the ECA strategy may be much higher. Taking active steps to remove ECA from the plasma, or allowing sufficient time for plasma clearance, was seen to be an important factor in increasing the effectiveness and localization of the drug.

We have also suggested a rational approach for the selection of the proper drug dosage and scheduling for two-step approaches. This strategy highlights the need for more quantitative data concerning the transport properties of drugs in the body and in tumors. It may prove to be useful to have pretreatment nontherapeutic injections to determine key parameters for optimizing drug delivery.

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APPENDICES

Appendix A: Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>BFA or ECA</td>
</tr>
<tr>
<td>$B$</td>
<td>Free binding sites in the tumor</td>
</tr>
<tr>
<td>$BA$</td>
<td>Tumor-bound BFA or ECA</td>
</tr>
<tr>
<td>$H$</td>
<td>Hapten</td>
</tr>
<tr>
<td>$AH$</td>
<td>BFA-hapten complex</td>
</tr>
<tr>
<td>$BAH$</td>
<td>Tumor-bound BFA-hapten complex</td>
</tr>
<tr>
<td>$D_p$</td>
<td>Prodrug</td>
</tr>
<tr>
<td>$D$</td>
<td>Drug</td>
</tr>
<tr>
<td>$B_t$</td>
<td>Maximum binding capacity of tumor</td>
</tr>
<tr>
<td>$P_f$</td>
<td>Effective permeability coefficient of the vessel wall in the tumor</td>
</tr>
<tr>
<td>$k_r$</td>
<td>Rate constant of plasma clearance for $F(F$ will represent $A, AH, H, D_p, and D)$</td>
</tr>
<tr>
<td>$S/V$</td>
<td>Surface area/unit volume for transport in the tumor</td>
</tr>
<tr>
<td>$k_i, k_{-1}$</td>
<td>Association and dissociation rate constants of BFA/ECA to tumor antigen</td>
</tr>
<tr>
<td>$k_2, k_{-2}$</td>
<td>Association and dissociation rate constants of hapten to BFA</td>
</tr>
<tr>
<td>$k_3, k_{-3}$</td>
<td>Association and dissociation rate constants of prodrug to enzyme</td>
</tr>
<tr>
<td>$k_d$</td>
<td>Rate constant of prodrug conversion</td>
</tr>
<tr>
<td>$k_{a1}$</td>
<td>Binding affinity ($=k_{d1}/k_{-1}$)</td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td>Binding affinity ($=k_{d2}/k_{-2}$)</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Michaelis constant</td>
</tr>
<tr>
<td>$I_0$</td>
<td>Injection dose of $S(S$ will represent $A, H, and D_p) at t = t_0$</td>
</tr>
<tr>
<td>$\delta$</td>
<td>dirac delta function</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Ratio of immobile antibody to mobile antibody</td>
</tr>
<tr>
<td>$\psi$</td>
<td>Ratio of hapten associated antibody to hapten-free antibody</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Ratio of complexed to free hapten</td>
</tr>
<tr>
<td>$D_a$</td>
<td>Diffusion coefficient of antibody in the tumor</td>
</tr>
<tr>
<td>$D_H$</td>
<td>Diffusion coefficient of hapten in the tumor</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Time interval between the BFA and hapten or between ECA and prodrug injections</td>
</tr>
</tbody>
</table>

Subscripts

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T$</td>
<td>Concentration in the tumor</td>
</tr>
<tr>
<td>$P$</td>
<td>Concentration in the plasma</td>
</tr>
<tr>
<td>$T_t$</td>
<td>Total concentration in the tumor</td>
</tr>
<tr>
<td>$P_t$</td>
<td>Total concentration in the plasma</td>
</tr>
</tbody>
</table>
Appendix B

Governing Equations for BFA-Hapten System. Based on the general kinetic theory and the law of conservation of mass for each component in the model, the governing equations for the BFA-hapten system at quasi-equilibrium state were obtained as follows.

\[
\frac{d[A]}{dt} = \frac{P_s S}{V} - (k a_l + k_{A+}(AH) + [AH]_r - [A]_f) \tag{B1}
\]

\[
\frac{d[H]}{dt} = \frac{P_s S}{V} ([H]_r - [H]_T) + \frac{P_s S}{V} ([AH]_r - [AH]_T) \tag{B2}
\]

\[
\frac{d[A]}{dt} = -k a_l[A]_l + \sum_I I_{AF}(t - t_l) \tag{B3}
\]

\[
\frac{d[H]}{dt} = -k a_l[A]_l + k_{H+}(AH) + \sum_I I_{HF}(t - t_l) \tag{B4}
\]

\[
[B]_T + [BA]_T + [BAH]_T = B, \tag{B5}
\]

\[
K_{a+} = \frac{k_{1+}}{k_{-1+}} = \frac{[BA]_T}{[B]_T[A]} = \frac{[BAH]_T}{[B]_T[A][H]_T} \tag{B6}
\]

\[
K_{a-} = \frac{k_{2-}}{k_{-2-}} = \frac{[BAH]_T}{[B]_T[H]_T} = \frac{[AH]_T}{[A]_T[H]_T} \tag{B7}
\]

where \([\cdot]_T\) and \([\cdot]_P\) represent the concentration of each component in the tumor and plasma, respectively, \(S/V\) is the surface area/unit volume for transport in the tumor (12), \(\delta\) is the dirac delta function, and

\[
[A]_T = [A]_T + [BA]_T + [BAH]_T + [AH]_T \tag{B8}
\]

\[
[H]_T = [H]_T + [BAH]_T + [AH]_T \tag{B9}
\]

\[
[A]_P = [A]_P + [AH]_P \tag{B10}
\]

\[
[H]_P = [H]_P + [AH]_P \tag{B11}
\]

In addition, we have assumed that \(k_{AH} = k_A\) and \(P_{AH} = P_A\), respectively. At time \(t = t_j\) the BFA or hapten was injected, and the doses of injections \(I_{AF}\) and \(I_{HF}\) were given in the form of plasma concentration increase of the BFA and hapten after each injection, respectively. From Equations B5 through B11, we obtained

\[
[H]_T = \sqrt{[1 + K_{a+}[A]_l - [H]_T] + 4K_{a+}[H]_T} \tag{B12}
\]

\[
[AH]_T = [H]_T - [H]_T \tag{B13}
\]

\[
[B]_T = \frac{[1 + K_{a+}[A]_l - [H]_T]}{2K_{a+}} + \sqrt{[1 + K_{a+}[A]_l - [H]_T]^2 + 4K_{a+}[H]_T} \tag{B14}
\]

\[
[\cdot]_T - \frac{[I_{AF}]}{V(1 + \theta)} - \frac{k_AV}{P_A S} \tag{B21}
\]

Substituting Equations B12 through B17 into B1 through B4, we obtained a set of nonlinear ordinary differential equations for \([A]_T\), \([H]_T\), \([A]_P\), and \([H]_P\). They were solved simultaneously by using IMSL software package (23).

The ratios of bound to free species may be defined as follows:

\[
\theta = \frac{[BA]_T + [BAH]_T}{[A]_T} = \frac{[BAH]_T}{[A]_T} \tag{B15}
\]

\[
\phi = \frac{[AH]_T + [BAH]_T}{[H]_T} = K_{a+}(A) + [BA]_T \tag{B16}
\]

\[
\Psi = \frac{[AH]_T + [BAH]_T}{[A]_T + [BA]_T} = \frac{[BAH]_T}{[A]_T + [BA]_T} \tag{B17}
\]

These ratios may be combined to determine the concentration of any species in the tumor:

\[
\frac{[A]_T}{[A]_n} = \frac{1}{1 + \theta + \Psi} \tag{B18}
\]

\[
\frac{[H]_T}{[H]_n} = \frac{1}{1 + \theta + \Psi} \tag{B19}
\]

\[
\frac{[BA]_T}{[A]_n} = \frac{\phi}{1 + \theta + \Psi} \tag{B20}
\]

From B18 and B19, we have

\[
\phi = \frac{K_{a+}[A]_n}{I + \Psi} \tag{B21}
\]

For the baseline values of parameters used, the values for \(\theta\), \(\Psi\), and \(\phi\) are: \(\theta \approx 21.6\), i.e., most of hapten is bound to BFA, if we assume \([B]_T \approx B\); \(\Psi \approx 430\) for \([H]_T\) of \(10^{-3}\) m and \(4.3\) for \([H]_T\) of \(10^{-4}\) m, thus the fraction of BFA binding a hapten changes greatly with time; \(\phi \approx 0.35\) for \([H]_T\) of \(10^{-3}\) m and \(28.6\) for \([H]_T\) of \(10^{-4}\) m, if \([A]_n \approx 0.5 B\). These indicate that most of hapten is free, when \([H]_T \approx [A]_n\), and bound to BFA, when \([H]_T < K_{a+}[A]_n\). In addition, \(\phi_{max} = K_{a+}(A)_{max} \approx 303\) if \((A)_{max} \approx K_{a+}(A)_{max}\).

If the parameters and times of interest are such that the ratio of immobile to mobile antibody (\(\theta\)) is constant (true for \([A]_n \approx [A]_T\) or \([A]_n \approx [A]_T\)), then the analytical solution for the total antibody concentration in the tumor is

\[
[A]_T = \exp(-k_A t) - \frac{1 - \exp(-\frac{P_s S}{V(1 + \theta)} t)}{1 + \theta} \tag{B22}
\]
which is obtained by assuming \( \theta = \text{constant} \) and solving Equations B1 and B3. For a bolus injection, \([A]_p = [A]_n (t = 0)\).

**Governing Equations for ECA-Prodrug System.** Similar to the equations for the BFA-hapten system, the governing equations for ECA-prodrug system were as follows:

\[
\frac{d[A]}{dt} = \frac{P_S}{V} ([A]_p - [A]_r) \tag{B22}
\]

\[
\frac{d[D]_r}{dt} = -k_d[A]_r[D]_r + \frac{P_S}{V} ([D]_p - [D]_r) \tag{B23}
\]

\[
\frac{d[D]}{dt} = k_d[A]_n[D]_r + \frac{P_S}{V} ([D]_p - [D]_r) \tag{B24}
\]

\[
\frac{d[A]_f}{dt} = -k_4[A]_f + \sum I_\Delta \delta(t - t_j) \tag{B25}
\]

\[
\frac{d[D]_f}{dt} = -k_0[D]_f + k_4[A]_n[D]_r + \sum I_{q\delta}(t - t_j) \tag{B26}
\]

\[
[B]_r + [BA]_r = B, \tag{B28}
\]

\[
K_{e_1} = \frac{k_1}{k_2} = \frac{[B]_r[A]_r}{[B][A]_f}, \tag{B29}
\]

\[
K_m = k_4 + k_3 \tag{B30}
\]

where \([.]_r \) and \([.]_p \) represent the concentration of each component in the tumor and plasma, respectively, \(S/V\) and \(\delta\) are the same as that in the model for BFA-hapten system, and

\([A]_n = [A]_r + [BA]_r \) (total antibody concentration in the tumor) \( (B31)\)

In addition, we have assumed that \(k_{o\text{pr}} = k_0 = k_{H} \) and \(P_{o\text{pr}} = P_o = P_{H} \), respectively. At time \(t = t_j\) the ECA or prodrug are injected, and the doses of injections \(I_\Delta\) and \(I_{q\delta}\) are given in the form of plasma concentration increases of ECA and prodrug after each injection, respectively. From Equations B28, B29, and B31, we have

\[
[A]_r = \left[ 1 + K_{s_1}(B - [A]_r) \right] + \sqrt{[1 + K_{s_1}(B - [A]_r)^2 + 4K_{s_1}[A]_r]} \tag{B32} \]

Substituting Equation B32 into B22 and combining with Equations B23 through B30, we obtained a set of nonlinear ordinary differential equations for \([A]_n, [D]_r, [D]_f, [A]_f, [D]_p, \) and \([D]_r\). They were solved simultaneously by using IMSL software package (23).

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Pharmacokinetic Analysis of Two-Step Approaches Using Bifunctional and Enzyme-conjugated Antibodies

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


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