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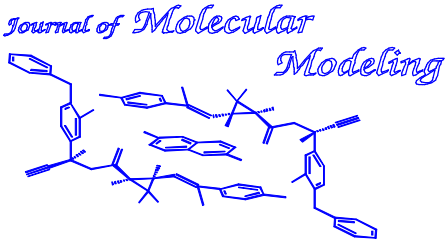

Simulation of Tumor-Specific Delivery of Radioligand Comparison of One Step, Two Step, and Genetic Transduction Systems

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Abstract

A mathematical model simulation was performed to estimate the amount of radioactivity in plasma, normal tissues, and tumor tissue through three delivery approaches: one step radiolabeled monoclonal antibody (MAb) CC49 i.v. bolus injection, two step method with biotin conjugated CC49 i.v. bolus injection followed 72 hours later by i.v. bolus radiolabeled streptavidin injection, and gene therapy method to express biotin on the tumor cell surface followed by i.v. bolus radiolabeled streptavidin injection. The mathematical model was built based on a system of ordinary differential equations consisting of inputs and outputs of model components in plasma, normal tissues, and tumor tissue. Through computer modeling, we calculated concentrations of each component for plasma, tumor and normal tissues at various time points. Radioactivity ratios of tumor to plasma and tumor to normal tissues increased with time. The increase of tumor to normal tissue ratios was much faster for the gene therapy approach than for single step and two step approaches, e.g., a ratio of 24.26 vs. 2.06 and 6.24 at 72 hours after radioligand injection. Radioactivity ratios predicted by the model varied with the amount of radioactivity injected and the time interval between injections. The model could be used to evaluate different radioimmunotherapy strategies and to predict radioactivity biodistribution using other receptor-ligand systems.

Keywords: Cancer, computer simulation, gene therapy, mathematical model, MAb.

Introduction

The use of radiation therapy has improved curative treatment for many tumors. However, this technique has practical limitations in regard to limited field of therapy, normal tissue toxicity, and radioresistance mechanisms. Considerable research efforts have been directed at ways to "target" radioactive isotopes to sites of malignant disease. Currently, the use of monoclonal antibodies (MAb) directed to "tumor-associated" antigens on cancer cells represents one approach to this problem which has had success in various animal model systems [1] and is the subject of considerable current phase I and II clinical trials in humans [2-10]. Such a strategy provides the ability to localize radioactive isotopes to multiple sites of disease with hopefully adequate amounts of radia-

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tion to produce an antitumor effect and/or radioimmune imaging for diagnostic purposes. A second emerging strategy is to use radioactively labeled peptides able to bind to receptor positive tumor cells [11, 12]. Research efforts which provide better radioactive isotope delivery systems and/or targeting strategies will enhance our ability to apply targeted radiation therapy to the human cancer problem. Currently, the following delivery systems and targeting strategies have been actively studied in various models.

1. Single step method. This is the traditional approach with direct application of radiolabeled MAb for radioimmunodetection and radioimmunotherapy of tumors [3-10].

2. Pretargeting radioimmunotherapy strategies (two or three step pretargeting methods]. The two step approach takes advantage of the high affinity receptor-ligand systems, such as avidin-biotin, by conjugating one of the pair to a MAb for targeting and the other member of the pair in a radioligand preparation [13-15]. In this way, the conjugated MAb is administered first to bind to tumor antigen and after a certain interval to allow for plasma clearance, radiolabeled ligand with high binding affinity to the MAb conjugate is administered. Systems using either streptavidin radioligand [16-20] or biotin radioligand [16,17,21-24] have been described. The three step approach uses an additional step through an unlabeled ligand chase to clear circulating antibody conjugates before the administration of radiolabeled ligand [18,25].

3. Gene therapy approach. Gene therapy techniques have been used to induce expression of high affinity receptors/ molecules on tumor cell surfaces [26-29] and optimal radioisotope-labeled ligands capable of being delivered to these receptors in tumors are being developed.

Successful application of radioimmunodetection and radioimmunotherapy of tumors has been affected by many problems. While laboratory experiments and clinical trials are underway to test these strategies, optimal delivery is complicated by a complex and variable schedule of MAb conjugate infusion. Questions regarding dosing, timing, affinity constants, and rate of metabolism still need to be answered. Computer modeling and simulation has been used to gain insight into the pharmacokinetics of various schedules, including pretargeting approaches. Mathematical models applying linear and nonlinear differential equations have been developed for this purpose. As more information from experimental and clinical trials is available and new strategies become possible, mathematical modeling and computer simulation can shed new light on the complexity of the pharmacokinetics and pharmacodynamics. In this report, we present results from a mathematical model simulation to compare the amount of radioactivity in plasma, normal tissues, and tumor tissue through three delivery approaches: single step radiolabeled MAb CC49 i.v. bolus injection, two step strategy with biotin conjugated CC49 i.v. bolus injection followed 72 hours later by i.v. bolus radiolabeled streptavidin injection, and gene therapy approach to express biotin on the tumor cell surface followed by i.v. bolus radiolabeled streptavidin injection.

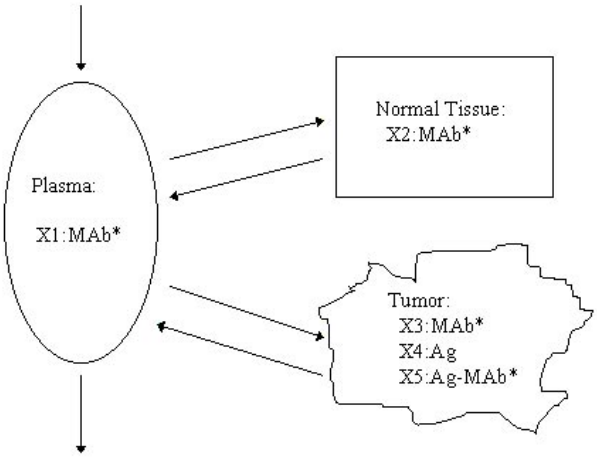

Figure 1. *Schematic representation of the one step method in which directly radiolabeled MAb (MAb*) is administered.*

Methods

Conceptual models were developed for three delivery approaches as depicted in Figures 1-3. The first approach is a single step directly radiolabeled antibody CC49 (MAb*) i.v. bolus injection to target antigen (Ag) in tumor tissue. The second approach is a typical two step strategy with biotin conjugated MAb (MAb-b) i.v. bolus injection to target antigen (Ag) in tumor tissue followed 72 hours later by i.v. bolus radiolabeled streptavidin (Av*) injection. The third approach is the gene therapy approach, where the biotin gene is delivered to the tumor cells and biotin is expressed on the cell surface (b). Radiolabeled streptavidin (Av*) is injected afterward.

Monoclonal antibodies

MAb CC49 is an IgG1 which was obtained by immunizing mice with purified TAG-72 [30]. Extensive experience has been gained in using this antibody in human trials. For this study, results from a phase II trial with ^{131}I-labeled CC49 at 10 mCi/mg in prostate cancer patients were used to provide pharmacokinetic values for simulation [6]. Plasma radioactivity time series data were statistically modeled for estimation of pharmacokinetical parameters of MAb CC49 for 15 patients in the clinical trial. The phamacokinetical analysis was based on linear compartment models that can also be represented by linear differential equations. Pharmacokinetical parameters, such as $T_{1/2}$, AUC, Vd_{ss}, Cl, and MRT, were calculated using a SAS nonlinear regression program

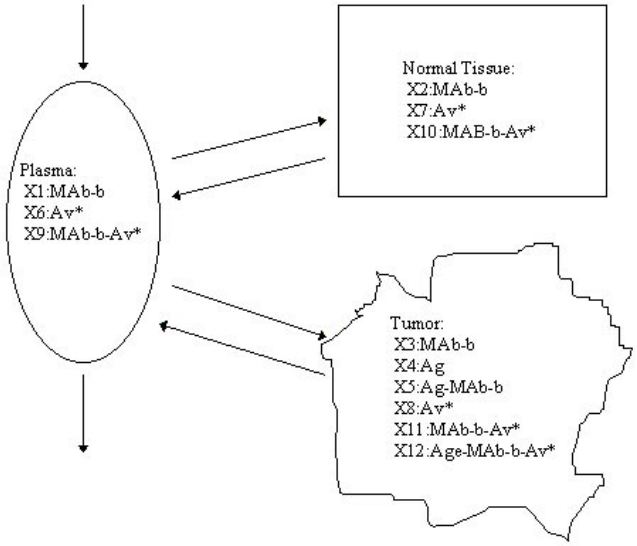

Figure 2. *Schematic representation of the two step method in which biotinylated MAb (MAb-b) is administered first. Radiolabeled streptavidin (Av*) is injected later with an interval such as 72 hours.*

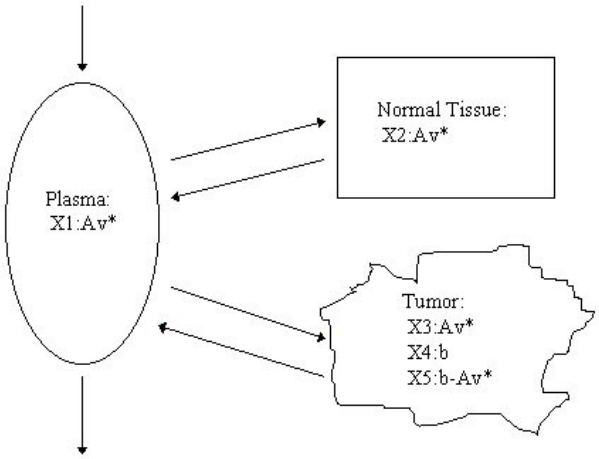

Figure 3. *Schematic representation of the gene therapy method in which biotin is expressed on the tumor cell surface through genetic transduction such as direct intra-tumor injection of a viral vector. Radiolabeled streptavidin (Av*) is then injected.*

(PROC NLIN) [31-32]. Rate constants derived from these data were used in the simulation.

Avidin-biotin system

The avidin-biotin system represents a high affinity binding system for possible tissue targeting. Avidin and its analogue, streptavidin, are tetrameric proteins with a high affinity biotin-binding site on each subunit [33], which has one million-fold greater affinity than that of most antigen-antibody interactions. Since the binding is rapid and, once formed, very stable, it is expected that avidin and the vitamin biotin found in low concentrations in tissues and plasma offer possibilities for tumor imaging and therapy. Streptavidin has been reported to show less non-specific binding to tissues and is more suitable for radioiodination because it contains more tyrosine residues per subunit [14, 15]. Biotin can be chemically attached to proteins by its carboxyl end while its binding to avidin remains unaffected. Most biomolecules can be biotinylated without significant loss of biological activity [34]. Thus, the avidin-biotin system offers a universally applicable technology for cross-linking and targeting of biomolecules that has been used extensively *in vitro* [34] and *in vivo* [16-24,35-36]. In this study, we chose the avidin-biotin system with biotinylated MAb (MAb-b) CC49 to bind target cells, followed by the administration of radiolabeled streptavidin (Av*).

Gene expression

Methods to genetically induce tumor cell membrane expression of the high affinity biotin-streptavidin system are under development to allow employment of the biotin-streptavidin system for delivery of radioligands to tumor cells. This would involve the derivation of fusion genes encoding chimeric proteins derived from the RSV-G viral glycoprotein and short peptides with binding specificity for either biotin or streptavidin. In this study, we chose the approach of expressing biotin on the tumor cell surface. Radiolabeled streptavidin was administered later.

Design and production of appropriate radioactive ligands

Radiolabeling with ^{131}I and ^{125}I of MAb (MAb*) or streptavidin (Av*) uses the standard Iodogen method. Radiolabeling with other radioisotopes, such as ^{90}Y, ^{186}Re, and ^{111}In can be accomplished through standard procedures with commercially supplied reagents.

Localization, imaging studies and dosimetry

Localization of the various radiolabeled ligands and level of their persistence over time can be quantified experimentally using described procedures [37-42]. The absolute amount or concentration of radioactivity is proportional to the amount of ligand labeled, such as in a ratio of 10 mCi/mg. In this

Table 1. *Values for the Model Parameters Obtained from Literature or Clinical Trial Data. [a]*

Parameter	Value
MAb* and MAb-b: radiolabeled or biotin-conjugated CC49	
k_{12}, rate constant from plasma to tissue	5.129×10^{-6} s^{-1}
k_{21}, rate constant from tissue to plasma	1.444×10^{-5} s^{-1}
k_{el}, rate constant from plasma to environment	3.089×10^{-6} s^{-1}
Av*: radiolabeled avidin or streptavidin	
k'_{12}, rate constant from plasma to tissue	1.875×10^{-4} s^{-1}
k'_{21}, rate constant from tissue to plasma	1.870×10^{-4} s^{-1}
k'_{el}, rate constant from plasma to environment	3.710×10^{-5} s^{-1}
MAb-b-Av*	
k''_{el}, rate constant from plasma to environment	6.250×10^{-6} s^{-1}
k_f, rate constant for binding of MAb or MAb-b to antigen	10 mM^{-1} s^{-1}
k_r, rate constant for dissociation of antibody/antigen complex	10^{-5} s^{-1}
k_{met}, rate constant for internalization of antigen and expressed biotin	0
k'_f, rate constant for binding of Av to MAb-b and b	7×10^4 $mM^{-1}s^{-1}$
k'_r, rate constant for dissociation of Av from MAb-b and b	9×10^{-8} s^{-1}
n, valence of the antibody/antigen binding	2
n', valence of MAb-b binding to Av	1
c_0, initial free antigen or genetically expressed biotin concentration	1 mM
c_{MAb0}, initial plasma concentration of MAb* or MAb-b	3.92 mM, 39.2 mM, 392 mM
c_{Av0}, initial plasma concentration of Av*	3.92 mM, 39.2 mM, 392 mM

[a] Rate constants for MAb CC49 were estimated from clinical trial data [6]. All other model parameters were chosen from literature reports from experimental studies [14,15,18,19].

study, plasma concentration, normal tissue and tumor tissue concentrations of radioactivity will be calculated as proportional to the radiolabeled ligand concentration but without specific unit.

Mathematical modeling

The mathematical model was built based on a system of ordinary differential equations consisting of inputs and outputs of model components, such as MAb, antigen, streptavidin, and antigen, antibody, biotin, and streptavidin complexes in plasma, normal tissue, and tumor tissue. Model parameters, such as rate constants, transport coefficients, and initial values were based on previous clinical trial data or literature reports from experimental and theoretical estimates [6,19]. Through computer modeling, we calculated concentrations of each component in each tissue. Total radioactivity was calculated for each tissue by combining concentrations of free radiolabeled ligand with complexed radiolabeled ligand. Ratios of radioactivities of tumor to plasma and tumor to normal tissue were calculated for each time point.

General assumptions. Based on pharmacokinetic studies, conceptual models were developed as shown in Figures 1-3. After i.v. injection, directly labeled MAb or biotinylated MAb CC49 was distributed in the plasma, normal tissues and tumor tissue. The transport between plasma and tissues and from plasma to environment was determined by the rate constants estimated from clinical trial data, k_{12}, k_{21}, and k_{el}, listed in Table 1. The MAb binds to antigen according to a rate constant, k_f and dissociates from antigen/antibody complexes according to a rate constant k_r. When radiolabeled streptavidin

Table 2. *Predicted Radioactivity Ratios from Computer Simulation with Injected Amount of 3.92mM of Reagents. [a]*

Index	Time after radiotherapy (h)	One step method Ratio	Two step method Ratio	Two step method Relative ratio [b]	Gene therapy method Ratio	Gene therapy method Relative ratio [b]
Tumor:	4	0.08	1.39	17.38	2.38	29.75
Plasma	24	0.52	6.01	11.56	4.28	8.23
	72	0.84	– [c]	–	26.08	31.05
Tumor:	4	1.11	2.24	2.02	2.12	1.91
Normal tissue	24	1.55	4.68	3.02	3.99	2.57
	72	2.06	6.24	3.03	24.26	11.78

[a] Same amount of MAb and streptavidin (3.92mM) was administered with an interval of 72 hr in the two step method. Radioactivity in tumor is 0.96 at 30 hr, 1.62 at 4 hr, and 1.74 at 4 hr after radiolabeled ligand injection for one step, two step and gene therapy method, respectively.

[b] Ratio of radioactivity ratios compared to one step method.

[c] Radioactivity in the plasma is completely eliminated.

was injected into the plasma, it distributes in the body according to rate constants, k'_{12}, k'_{21}, and k'_{el}. It also binds to biotin (MAb-b and b) with high affinity, k'_f and k'_r. The MAb-b-Av* complex was eliminated from plasma at a rate constant, k''_{el}. We assume that there is no internalization of antigen or cell surface biotin, and non-specific antigen or biotin levels outside of tumor tissue are ignored. Previous experience of fitting data with pharmacokinetic models indicates that linear compartmental models fit the plasma data quite well. Therefore, it is assumed that the distribution between plasma and tissue is according to linear first-order kinetics. The linear kinetics implies that transference (input and output) is at a rate proportional to the concentration or amount of ligand.

Mathematical formulation. The conceptual model outlined above was represented by a set of differential equations - state matrix [43,44]. For a set of linear first-order differential equations, the general form is:

$$\frac{dX_i}{dt} = \sum_{j=1}^{n} k_{j-i} X_j - \sum_{j=0}^{n} k_{i-j} X_i , \quad X_i(0) = c$$

Where X_i or X_j is the state variable representing the concentration of each component, i.e., Ag, b, MAb*, MAb-b, Av*, Ag-MAb*, Ag-MAb-b, MAb-b-Av*, Ag-MAb-b-Av*, and b-Av*, in compartment (plasma, normal tissue, and tumor tissue) i or j (i, j = 1,2,3). $X_i(0)$ is the initial concentration in compartment i. We specified that the initial concentration for antigen and genetically expressed biotin is 1 mM. The initial concentration for MAb* and MAb-b was specified as 3.92 mM with an increase of 10 times in the sensitivity analyses. The initial concentration for Av* in the two step method was specified to be equivalent to that of MAb-b. The choice of these initial values is for consistency with other investigators in mathematical modeling.

Numerical methods. A FORTRAN program, ADAPT II, was used for simulation on a DEC Alpha 3800s computer. The ADAPT II program uses the differential equation solver LSODA (Livermore Solver for Ordinary Differential equations with Automatic method switching for stiff and nonstiff problems), which uses variable order, variable step size formulations of Adam's method and Gear's method as the nonstiff and stiff equation solvers, respectively [45,46]. After the linear differential equations are defined by the state matrix and entered into the subroutine, the solution is obtained using the exponential of the matrix. This matrix exponential is approximated using an eigenvalue decomposition. The concentration of each component in each tissue is then calculated for each time point specified in the simulation.

Results

A series of computer simulations were performed based on a system of ordinary differential equations with specified rate constants for each model component, such as MAb, antigen, streptavidin, and antigen, antibody, biotin, and streptavidin complexes, as presented in Table 1. Through computer simulation, we calculated concentrations of each component in each tissue. Since our interest was in the total radioactivity in plasma, normal tissue, and tumor tissue, concentrations

Table 3. *Predicted Radioactivity Ratios from Computer Simulation with Injected Amount of 39.2mM of Reagents. [a]*

Index	Time after radiotherapy (h)	One step method Ratio	Two step method Ratio	Two step method Relative ratio [b]	Gene therapy method Ratio	Gene therapy method Relative ratio [b]
Tumor:	4	0.08	1.17	14.63	8.70	109
Plasma	24	0.34	3.24	9.53	3634	10688
	72	0.44	– [c]	–	–	
Tumor:	4	1.04	1.00	0.96	4.95	4.76
Normal tissue	24	1.06	1.00	0.94	2180	2057
	72	1.09	1.00	0.92	–	–

[a] Same amount of MAb and streptavidin (39.2mM) was administered with an interval of 72 hr in the two step method. Radioactivity in tumor is 6.71 at 30 hr, 12.50 at 4 hr, and 25.88 at 4 hr after radiolabeled ligand injection for one step, two step and gene therapy method, respectively.
[b] Ratio of radioactivity ratios compared to one step method.
[c] Radioactivity in the plasma is completely eliminated.

from radiolabeled components, such as free radiolabeled ligand and complexed radiolabeled ligand, were combined. Ratios of radioactivities of tumor to plasma and tumor to normal tissue were calculated for each time point. The rate constants of directly labeled MAb or biotinylated MAb CC49 by i.v. injection between plasma and tissues and from plasma to environment (k_{12}, k_{21}, and k_{el}) was estimated from clinical trial data [6]. The rate constants for MAb binding to antigen (k_f) and dissociation from antigen/antibody complexes (k_r) were from experimental data reported in the literature [19]. Radiolabeled streptavidin rate constants, k'_{12}, k'_{21}, and k'_{el} and its binding affinity to biotin, k'_f and k'_r were chosen from experimental data [14,15,18,19]. The MAb-b-Av* complex elimination rate constant, k''_{el}, was assumed as reported by Sung et al. [19].

Using phamacokinetical data from directly radiolabeled MAb CC49 in the single step approach, the ratio of tumor to plasma radioactivity increased from 0.08 at 4 hours to 0.52 at 24 hours and to 0.84 at 72 hours after radiolabeled antibody injection (Table 2). The corresponding ratios of tumor to normal tissue were 1.11, 1.55, and 2.06. By modeling the two step method, the ratio of tumor to plasma radioactivity increased from 1.39 at 4 hours to 6.01 at 24 hours after radiolabeled streptavidin injection (Table 2). The plasma radioactivity was completely eliminated after 36 hours. Ratios

Table 4. *Predicted Radioactivity Ratios from Computer Simulation with Injected Amount of 392mM of Reagents. [a]*

Index	Time after radiotherapy (h)	One step method Ratio	Two step method Ratio	Two step method Relative ratio [b]	Gene therapy method Ratio	Gene therapy method Relative ratio [b]
Tumor:	4	0.07	0.59	8.43	1.08	15.43
Plasma	24	0.32	0.75	2.34	1.09	3.41
	72	0.41	1.94	4.73	1.25	3.05
Tumor:	4	1.00	1.00	1.00	1.01	1.01
Normal tissue	24	1.01	1.01	1.00	1.02	1.01
	72	1.01	1.01	1.00	1.17	1.16

[a] Same amount of MAb and streptavidin (392mM) was administered with an interval of 72 h in the two step method. Peak radioactivity in tumor is 64.00 at 30 hr, 93.01 at 4 hr, and 108.39 at 4 h after radiolabeled ligand injection for one step, two step and gene therapy method, respectively.
[b] Ratio of radioactivity ratios compared to one step method.
[c] Radioactivity in the plasma is completely eliminated.

Table 5. *Predicted Radioactivity Ratios from Two Step Method with Varying Intervals. [a]*

Index	Time after radiotherapy (h)	Time Interval in Two Step Method (h) 24	48	72	96
Tumor:	4	0.67	1.08	1.39	1.82
Plasma	24	1.53	3.37	6.01	–
	72	13.71	– [b]	–	–
Tumor:	4	1.56	1.92	2.24	1.00
Normal tissue	24	2.40	3.49	4.68	1.00
	72	2.83	4.46	6.24	1.00

[a] Same amount of MAb and streptavidin (3.92mM) was administered with varying intervals of 24, 48, 72, and 96 h in the two step method. Radioactivity in tumor is 1.13, 1.44, 1.62, and 1.40, respectively at 4 h after radiolabeled ligand injection.

[b] Radioactivity in the plasma is completely eliminated.

of tumor to normal tissue were 2.24 at 4 hours, 4.68 at 24 hours, and 6.24 at 72 hours after radiolabeled streptavidin injection. By modeling the gene therapy approach, the ratio of tumor to plasma radioactivity increased from 2.38 at 4 hours to 4.28 at 24 hours and to 26.08 at 72 hours after radiolabeled streptavidin injection (Table 2). The corresponding ratios of tumor to normal tissue were 2.12, 3.99, and 24.26. The relative ratios of tumor to plasma radioactivity ratios were 11.56 to 17.38 comparing the two step method to the one step method, and 8.23 to 31.05 comparing the gene therapy method to the one step method. The relative ratios of tumor to normal tissue radioactivity ratios were about 2 to 3-fold when comparing the two step method to the one step method, and about 2 to 12-fold when comparing the gene therapy method to the one step method.

Radioactivity ratios and relative ratios were also calculated from the sensitivity analysis by varying the amount of MAb and radioligand. Tables 3 and 4 present results for the amount of 39.2mM and 392mM compared to the 3.92mM results in Table 1. For the one step method, the tumor to plasma and tumor to normal tissue radioactivity ratios decreased to less than 0.5 and about 1 relative to those at the 3.92mM dose. For the two step method, both tumor to plasma and tumor to normal tissue ratios decreased markedly as the

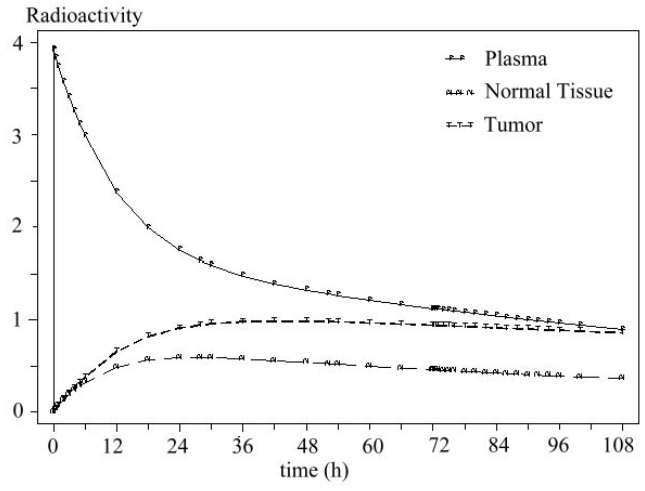

Figure 4. *Concentrations of radioactivity in plasma, normal tissues and tumor tissue in one step method following the injection of 3.92μM radiolabeled MAb.*

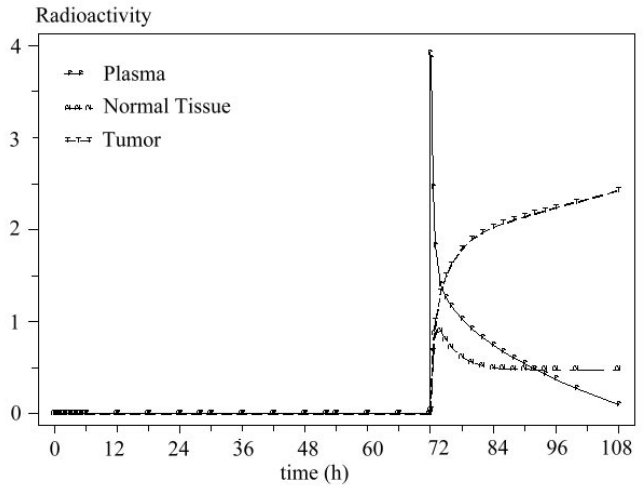

Figure 5. *Concentrations of radioactivity in plasma, normal tissues and tumor tissue in two step method with 3.92mM MAb-biotin (MAb-b) injection followed 72 hours later by injection of 3.92μM radiolabeled streptavidin.*

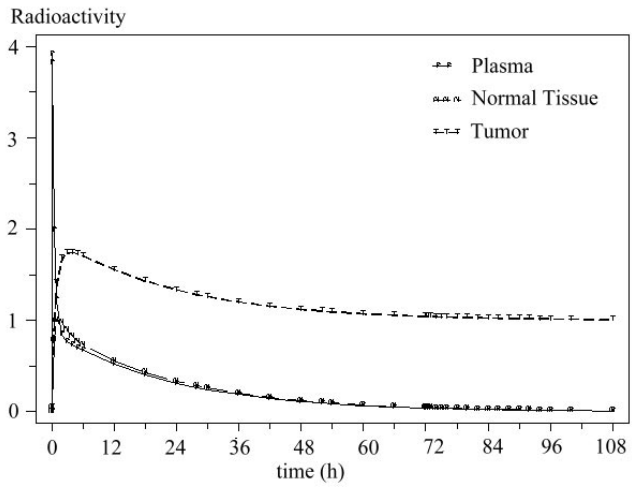

Figure 6. *Concentrations of radioactivity in plasma, normal tissues and tumor tissue in the gene therapy method following the injection of 3.92μM radiolabeled streptavidin.*

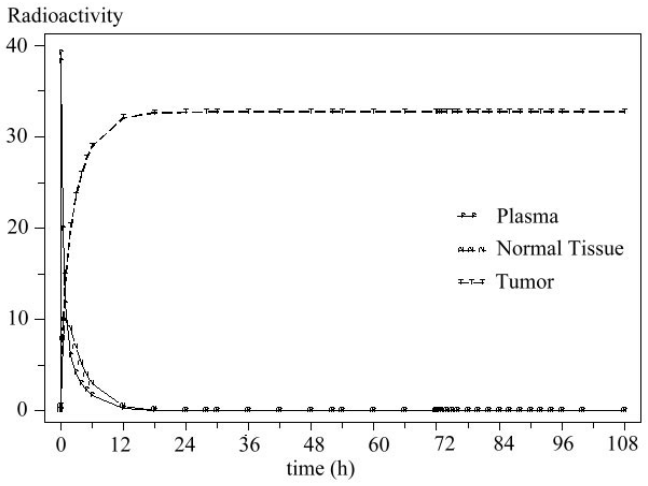

Figure 7. *Concentrations of radioactivity in plasma, normal tissues and tumor tissue in the gene therapy method following the injection of 39.2μM radiolabeled streptavidin.*

reagent concentrations increased. The gene therapy method results in increased ratios at the 39.2mM dose compared to the 3.92mM dose with marked increases of the ratios relative to the one step method.

Table 5 lists the ratios for the two step method with varying intervals between MAb-b and Av* injections. Both tumor to plasma and tumor to normal tissue ratios increased with longer intervals between 24 and 72 hours. However, the ratios decreased at the interval of 96 hours. This result indicates that an interval of about 72 hours is optimal.

Comparing the absolute concentrations of radioactivity (Figures 4-6) shows that a large amount of radioactivity remained in the plasma and the radioactivity concentration in plasma, tumor and normal tissues were not very different for a period of time in the one step method. In both the two step and gene therapy methods, plasma radioactivity disappearance was much faster due to the shorter half life of Av* compared to MAb*. The tumor concentrations were much higher than the plasma and normal tissue concentrations in both the two step method and the gene therapy method. The increase of tumor concentration was accompanied by lower levels of plasma and normal tissue concentrations in the gene therapy method than in the two step method. The increase of tumor concentration in the gene therapy method was substantial when the injected dose of radioligand increased from 3.92mM to 39.2mM (Figure 7).

Discussion

In directly radiolabeled MAb administration strategies, approaches have been taken to increase the tumor to plasma ratio by using low molecular weight fragments to achieve a lower plasma concentration than with intact antibodies. However, this is achieved at the cost of lower tumor uptake. To maximize targeting molecule (*e.g.* MAb) deposition into tumor sites while minimizing radioactive isotope exposure to the bone marrow, investigators have designed several strategies to separate these two components. One strategy has been to develop bifunctional MAb with one combining site for tumor and a second binding site for the radioactive ligand [47-49]. The bifunctional antibody is administered and allowed to circulate for several days (optimal tumor deposition) and then the radioligand is administered which has a rapid tissue distribution and short plasma half-life. This strategy allows tumor localization of the isotope to occur rapidly (matter of a few hours) with limited radiation dose to the bone marrow. The major limitation of this strategy has been the reduced affinity of the individual antigen combining sites (single rather than dual binding sites similar to Fab fragments), variable kinetics/distribution of the separate components making optimal schedules of therapy difficult to standardize. The two step pretargeting approach takes advantage of the high affinity avidin-biotin system by conjugating one of the pair to a MAb for targeting and the other member of the pair in a radioligand preparation. In this way, the conjugated MAb is administered first to bind to tumor antigen, and after a certain interval of clearance radiolabeled ligand with high binding affinity to the MAb conjugate is administered. These systems appear attractive in animal models and in radioimaging studies in humans. A major drawback of this system is that the high affinity binding of radioligand to MAb conjugates occurs with any residual MAb in the plasma or extravascular space resulting in a high level of background radiation. The use of an additional step [18,25] to clear circulating antibody conjugates improves the distribution of the radioligand but results in a complex and variable schedule of MAb conjugate infusion, plasma conjugate clearing reagent

administration, and radioligand infusion. Direct intra-tumor injection of genes has been employed in a variety of anti-cancer gene therapy strategies in human trials. In this regard, Plautz *et al.* have utilized a technique of *in situ* tumor transduction in pre-clinical and clinical trials with genes encoding alloantigens to achieve anti-tumor immunization [50]. In addition, direct intra-tumor injection of viral vectors has been carried out in pre-clinical and clinical trials to achieve toxin gene delivery for therapy of gliomas [51]. Thus, the technique of direct tumor transduction is a method which would allow implementation of a variety of anti-cancer gene therapy strategies such as the avidin-biotin system. Scheduling through this approach is much simpler.

Results from experimental models have demonstrated the advantage of the two step strategy over direct radiolabeled antibody administration. Khawli *et al*. have shown that treatment of tumor-bearing nude mice with biotinylated MAb can achieve a 1.3-2.6 fold increase of tumor localization ratios at 24 hours after injection of radiolabeled streptavidin in a two step pretargeting strategy with biotinylated MAb and radiolabeled streptavidin compared to directly labeled antibodies [18]. A similar magnitude of ratios has also been reported by other investigators [13]. While neither experimental nor mathematical modeling results have been reported so far for the gene therapy approach of radioimmunotherapy of cancer, pharmacokinetic comparisons and mathematical modeling have been performed with different two step pretargeting strategies [19-22]. Sung et al. have developed a pharmacokinetic model involving two step pretargeting [19]. The model describes three compartments: an avascular tumor nodule, such as a very early primary tumor or a micrometastasis; the normal tissue; and the plasma. The results indicate that the two step protocol yields an approximately 2- to 3-fold enhancement compared with the one step direct radiolabeled antibody administration method.

In this study we have compared the two step method with a one step method based on a three compartment model similar to that developed by Sung et al [19]. However, the tumor nodule is not avascular in our model. The vascular tumor tissue represents well established rather than early tumor, which is more commonly treated with radiation therapy. Nevertheless, our results comparing the two step method with a one step method give similar ratios of tumor to normal tissue radioactivity, such as 2.02 to 3.03-fold increases from 4 to 72 hours after radioligand injection, supporting the advantage of pretargeting approaches. Moreover, we modeled the gene therapy approach in this study. Although multicompartment, numerical models of cellular events in the pharmacokinetics of gene therapies have been developed [52], our modeling of the gene therapy approach for radioimmunotherapy is new. According to the simulation results, much greater tumor to plasma and tumor to normal tissue ratios can be achieved through the gene therapy approach, such as 31.05 and 11.78-fold increases at 72 hours compared to the one step method and markedly greater ratios can be achieved through dose increment.

It is noted that all modeling results are subject to the presumed parameter values as well as the model specified. Uncertainties exist in the representation of the model to the real physiological and pathological process associated with the different approaches. Many of the parameter values used in the simulation are derived from animal model experiments or are estimates from preliminary cell culture studies. Improvement in the modeling can be made when new information comes available from experimental studies. For example, in the gene therapy method, the dramatic degree of simulated localization reflects the absence of significant amounts of biotin in the plasma or extravascular space of normal tissues and the high affinity binding of radiolabeled streptavidin to biotin expressed in the tumor. However, the degree to which this strategy will approach these dramatic values will depend on the future success of *in vivo* genetic transduction and radioligand design. Published results from our cell culture studies have indicated that the approach of genetic transduction is promising (26). Information about the amount and disappearance of genetically expressed receptors will greatly enhance the prediction through computer simulation.

Sensitivity analyses in this study showed that optimal dosing or timing schedules could be achieved with changing parameter estimates. For MAb CC49, a murine MAb with a plasma half-life of about 40 hours from one compartment modeling of phase I clinical trial data, an interval of about two half-lives between the injections of MAb-b and Av* seems most beneficial. In conclusion, this study demonstrates that the two step method can achieve a 2 to 3-fold increase in tumor to normal tissue radioactivity ratios compared to a one step method. The gene therapy method can achieve even higher ratios, e.g., 1.91 to 11.78-fold from 4 to 72 hours after radioligand injection, and the strategy appears to be easier to schedule. Concentrations of radioactivity and the relative ratios can be calculated from modeling and optimal strategies regarding dosing and timing can be evaluated from simulation. As a tool, the computer simulation is useful in examining the performance of different therapeutic strategies as long as new pharmacokinetical information is incorporated into the model from experimental and clinical studies.

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