Using a Single Parameter to Describe Time–Activity Curves

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Abstract

Time–activity uptake curves [$u(t)$ in % injected dose per gram of tissue] may be described by different—often complicated—functional forms. Because of the need to readily compare sequences of engineered radiopharmaceuticals, it is efficient to use mean residence time (MRT) as a one-parameter descriptor. In applying this computation to a sequence of five cognate anti-carcinoembryonic antigen (CEA) antibodies, it was found that the intact form had the longest MRT in the blood with the other four cognates having values less by approximately a factor of 10 or more. This difference probably follows from the lack of an intact Fc segment on the latter engineered molecules. MRT values for a sequence of six scFv-Fc engineered fragments demonstrated that the double mutant had the shortest blood residence time—30-fold less compared with the wild type. Whereas it is not possible to directly apply the MRT to nonbolus (tumor or organ) curves, a residence time (τ) may be assigned using the uptake function. Using τ , it was found that the intact (natural) form of the anti-CEA cognate set had the longest time at the tumor site in the human xenograft model in nude mice. The MRT and τ concept are proposed to also allow comparison of possible relative blood and tissue exposures, respectively, for cognate sets of unlabeled engineered antibodies used to treat malignancies although no data are yet available in the literature for this application.

Key words: blood curve representation, mean residence time, protein engineering, unlabeled antibodies

Introduction

A basic outcome of a radiopharmaceutical-based animal biodistribution or clinical study is a set of time–activity curves. These outputs are corrected for radiodecay of the label so that the tabulated results, usually called uptakes (u), are technically pharmacokinetic (PK) distributions of the agent. In the following, uptakes may be considered to be in units of percent injected dose/g (%ID/g) as functions of time (t). In this study, dose may be in MBq for labeled or mg for unlabeled materials.

One important topic in agent engineering is the comparison of a given organ's $u(t)$ when the targeting entity is modified at the molecular level. An example is an engineered sequence of antibody cognates that bind using the same attachment site to a particular tumor-associated molecule. Any comparison is inherently difficult as there are many possible representations of the outcome data so that

the multiple $u(t)$ functions may be of very different mathematical formats. Probably, the most common representation is a sum of exponential terms as follows:

$$u(t) = \sum_{i=1}^N A_i \exp(-k_i t) \quad (1)$$

However, other functions, such as gamma variates¹ and cubic splines,² may also be used. If one opts for Equation (1) for every curve, most uptakes can be represented by two exponentials, that is, $N=2$. In this simple case, the user would need to compare one set of four parameters (A_1 , A_2 , k_1 , and k_2) with various other quartets. Even if the rate constants (k_i) were identical for all agents, their relative weights (A_i) may be dissimilar enough such that a simple relationship is not possible. If one agent were represented by a lower order (e.g., $N=1$) or a nonexponential function, this evaluation becomes even more problematic as the parameters of the two models would themselves not be comparable.

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Similar difficulties arise if one tries to compare uptakes across organs subjected to a single radiotracer. To find where the agent is preferentially spending time, the analyst needs to compare multiple—perhaps dissimilar curves. Again, these functions may not be of the same type; for example, an Akaike analysis³ may imply a lower order set of exponentials in one organ, whereas higher order results are needed for other tissues.

Materials and Methods

A direct method to expedite both agent comparison and organ comparison relative exposures is to implement a one-parameter representation of the entire $u(t)$ uptake curve. This strategy would result in a single time value analogous to the half-life of a radionuclide label. Possibly, the earliest such single-parameter representation is the Zierler's mean residence time (MRT)⁴ for a bolus injection of a radiolabeled agent into an organ as follows:

$$MRT(bolus) = \int_0^{\infty} u(t)dt / u(0) \quad (2)$$

Notice that the area under the curve of the uptake function is the numerator and the initial tissue uptake $u(0)$ is the denominator. Meier and Zierler⁴ used this format to represent mean transit times across an organ; since we have a bolus injection, the transit and residence times are the same.

Results

Anti-carcinoembryonic antigen cognate results

Because of access issues, it is generally not possible to provide bolus inputs into tissues in an intact, living animal. One exception is the blood compartment whereby a rapid injection into the venous system acts as a good approximation to an arterial bolus. Table 1 gives the blood results in a LS174T xenograft-bearing nude mouse model for a set of five cognate cT84.66 monoclonal antibodies (mAbs) against the carcinoembryonic antigen (CEA).⁵ For each cognate, the antigen recognition site was kept the same. In this case, all five proteins required biexponential fits to their respective blood curves, which were used as the $u(t)$ function.

Persistence of the blood curve is a *sine qua non* for tumor targeting. This follows from the fact that lesions are a small fraction of the total body mass. For the 100–200-mg tumors used in our analysis of 20 g mice, the lesion size was <1.0% of the total accessible tissue. Thus, any potential tumor

tracer must stay in the blood for an extended interval to accumulate in significant amounts at the lesion. A magic bullet that goes directly to the tumor and bypasses all normal tissues cannot exist. Any realistic agent must circulate and pass through the entire anatomy multiple times before the targeting mechanism can manifest itself.

Using data from the table, we find that the intact mAb had the largest MRT, and correspondingly, the largest relative blood exposure. This 150 kDa form had an MRT for blood that was approximately one order of magnitude larger than any of the other four engineered cognates. Generally, the MRT—and thus the relative exposure—increased with increasing molecular weight.

scFv-Fc engineering

A second example of the application of MRT can be found in analysis of a sequence of six scFv-Fc anti-CEA antibodies (104 kDa) engineered to have variable blood residence times.⁶ Table 2 gives the results in normal BALB/c mice for these agents as various amino acids in the Fc segment were changed. Again, a clear distinction was found between the wild-type and five modified forms with the shortest blood MRT, and least relative exposure, occurring with the double mutant H310A/H435Q. As in the case of the five cT84.66 cognates, the recognition site was identical for all six of these scFv-Fc antibodies to CEA.

Discussion

Analysis of uptake data is made complicated by the use of various mathematical forms to represent the information. Even with a simple two-exponential function for the blood curves, there are four parameters that must be compared between one agent and another. Determining which of the several potential agents for a given target is superior generally requires that a single parameter be used in lieu of multiple combinations of numerical values. From historical precedents, one may select a number of quantities that have been termed MRTs. In the above, we suggest using a traditional formula from nuclear medicine to provide this model-independent comparison parameter for bolus injections—the MRT.

This parameter uses the uptake curve's time derivative as the differential probability that a given structure will clear in the time dt . By selecting a blood criterion for a cognate antibody set, one can find the optimal mAb for that outcome. For example, we may wish to maximize MRT so as to increase the likelihood of tumor targeting. An important reason for these MRT differences between the intact

TABLE 1. COMPARISON OF MRT FOR FIVE COGNATE cT84.66 PROTEINS (MABS) IN THE BLOOD OF TUMOR-BEARING NUDE MICE

Protein	MW (kDa)	$A_1 \pm SE$ (%ID/g)	$A_2 \pm SE$ (%ID/g)	$k_1 \pm SE$ (hour)	$k_2 \pm SE$ (hour)	$MRT \pm SE$ (hour)
scFv	25	14.4 ± 0.13	3.77 ± 0.12	4.7 ± 0.16	0.21 ± 0.01	1.16 ± 0.01
Diabody	50	33.0 ± 1.31	6.44 ± 1.24	2.76 ± 0.22	0.24 ± 0.06	0.99 ± 0.001
Minibody	80	18.8 ± 5.29	30 ± 5.38	1.05 ± 0.41	0.13 ± 0.03	5.06 ± 0.70
F(ab') ₂	120	12.7 ± 2.28	26.4 ± 2.15	1.41 ± 1.06	0.11 ± 0.01	6.61 ± 0.36
Intact	150	15.4 ± 2.67	22.9 ± 2.32	0.19 ± 0.07	0.01 ± 0.002	59.34 ± 2.43

SE, standard error of the mean; MRT, mean residence time; mAbs, monoclonal antibodies.

TABLE 2. COMPARISON OF SIX scFv-Fc-ENGINEERED ANTI-CEA ANTIBODIES IN BLOOD OF BALB/C MICE

<i>scFv-Fc protein (104 kDa)</i>	<i>MRT (hour)</i>
Wild type	207
H435R	70.4
H435Q	44.4
O253A	31.4
H310A	16.5
H310A/H435Q	7.00

antibody and its modified four cognates is probably the lack of an intact Fc segment in the latter cases. Using the MRT computation, removal of the antibodies' intact Fc segment by molecular engineering has reduced the blood residence time by approximately one order of magnitude.

Similar results occur when we evaluated six scFv-Fc fragments that have their respective Fc segments manipulated through single or double amino acid changes so as to cause blood circulation times to decrease. It was found that the double mutant (H310A/H435Q) had the minimal MRT—~30-fold less than the wild type. Again, the MRT allows a simple comparison of the agents' blood exposure within this category. The pharmacist can thus quantitatively evaluate progress in achieving blood curve changes by simply looking at the MRT values for a sequence of one or two amino acid exchanges in the Fc segment. Simultaneously, the relative importance of one amino acid change versus another can be expressed numerically after bolus administration into blood.

In the above, we are limited to blood data due to the bolus constraint in Zierler's determination of MRT. It would be efficient if one could also investigate the time spent at selected nonblood sites using an analogous single-parameter analysis. Equation (2) cannot be used for two reasons. First, the tissue injection cannot generally be a bolus function. Moreover, $u(0)$ would be zero so that the result of Equation (2) becomes undefined. In such cases, following the MIRD schema,⁷ we propose to assess a labeled agent's exposure at the tumor or other sites using the residence time (τ) function as follows:

$$\tau(\text{tissue}) = m/100 \int_0^{\infty} u(t) \exp(-\lambda t) dt \quad (3)$$

where m is the tissue's mass. In effect, we correct uptake (%ID/g) for the percentage factor and tissue size. Recall that $u(t)$ is a PK variable so that the decay factor [$\exp(-\lambda t)$] is needed to obtain the amount (e.g., in MBq) of activity at the tissue site. If the mAb were unlabeled, the decay factor is not required and the amount of material would be in mg. Table 3 gives residence times for the five anti-CEA T84.66 cognates. It is seen that the intact mAb gave the best tumor residence time with the other four cognates having reduced tumor exposure values due to their correspondingly smaller blood MRT values as noted above. A decay factor appropriate for ¹³¹I was used in this table. As with the blood analysis, the single-chain cognate had the lowest τ value by about two orders of magnitude as compared with the intact version.

TABLE 3. TUMOR RESIDENCE TIMES (τ) FOR FIVE COGNATE CT84.66 PROTEINS

<i>Protein</i>	<i>Residence time at LS174T tumor (hour)</i>
scFv	0.037
Diabody	0.50
Minibody	1.76
F(ab') ₂	1.35
Intact	7.74

Conclusions

A simple one-parameter method (MRT) is suggested to evaluate blood curves from a sequence of engineered antibodies. Such comparisons are used to distinguish various anti-CEA proteins as antibody structures or amino acid residues are changed. In this study, our approach has had the same primary motivational factor as indicated by Yamaoka et al.'s original PK proposal⁸—which used a different definition of MRT—to use a moment-based approach to make comparisons more readily and eliminate reference to specific mathematical formats in the analysis.

If tumor curves are analyzed using a single parameter, we propose the use of the residence time (τ) instead of the MRT. This necessity follows from the requirement of the bolus injection for MRT evaluation—a constraint that cannot generally be satisfied in an intact animal (or patient). As in the case of MRT, we apply this single parameter to a sequence of cognates whereby the targeting aspect of the molecule is unchanged; for example, in our example, the CEA recognition site.

We have analyzed published examples of radiolabeled anti-CEA mAbs. This restriction is due to the lack of other cognate sequences in the literature. In the future, it may be possible to apply blood MRT and tumor τ assessment to both labeled and cold antibodies. Two unlabeled anti-CD20 intact antibodies that effect B-cell lymphoma regression have been reported.^{9,10} No literature results are yet available for engineered versions of either anti-CD20 protein. If structural changes were eventually applied to these agents, MRT in blood would allow a relative ranking of antibody exposures in the resultant cognate sequences. In such cases, the pharmacologist could determine the effectiveness of each amino acid variation by documenting the corresponding MRT change in the blood. As indicated for labeled proteins, the τ analysis may also be expected to be applicable to tumor-targeting effectiveness of the members of the resultant cognate class. In this case, the residence time τ would be indicative of the length of time at the tumor site, and hence, the relative effectiveness of that engineered agent for imaging or therapy. As with the labeled moieties, we assume that the engineering does not disturb the antigen recognition site on these various engineered unlabeled proteins.

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Disclosure Statement

No competing financial interests exist.

References

1. Davenport R. Derivation of the gamma-variate relationship for tracer dilution curves. *J Nucl Med* 1983;24:945.
2. Wang Y, Wahba G, Gu C, et al. Using smoothing spline anova to examine the relation of risk factors to the incidence and progression of diabetic retinopathy. *Stat Med* 1997;16:1357.
3. Kletting P, Kull T, Reske SN, et al. Comparing time activity curves using the Akaike information criterion. *Phys Med Biol* 2009;54:N501.
4. Meier P, Zierler KL. On the theory of the indicator-dilution method for measurement of blood flow and volume. *J Appl Physiol* 1954;6:731.
5. Williams LE, Wu AM, Yazaki PJ, et al. Numerical selection of optimal tumor imaging agents with application to engineered antibodies. *Cancer Biother Radiopharm* 2001;16:25.
6. Kenanova V, Olafsen T, Crow DM, et al. Tailoring the pharmacokinetics and positron emission tomography imaging properties of anti-carcinoembryonic antigen single-chain Fv-Fc antibody fragments. *Cancer Res* 2005;65:622.
7. Snyder WS. "S," Absorbed Dose Per Unit Cumulated Activity for Selected Radionuclides and Organs. New York: Society of Nuclear Medicine, 1975.
8. Yamaoka K, Nakagawa T, Uno T. Statistical moments in pharmacokinetics. *J Pharmacokinet Biopharm* 1978;6:547.
9. Maloney DG, Grillo-Lopez AJ, White CA, et al. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* 1997;90:2188.
10. Kaminski MS, Estes J, Zasadny KR, et al. Radioimmunotherapy with iodine (131)I tositumomab for relapsed or refractory B-cell non-Hodgkin lymphoma: Updated results and long-term follow-up of the University of Michigan experience. *Blood* 2000;96:1259.