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ABSTRACT

Objective: The principles of pinhole SPECT imaging are well known, however, multipinhole SPECT was only recently introduced for small animal imaging mainly to improve resolution and sensitivity. In this study, biodistributions of the ¹¹¹In labeled antibody CC49 in the LS174T mouse model under varying conditions have been determined by necropsy and clear images were obtained with Bioscan's HiSPECT multipinhole imager.

Methods: After CC49 was conjugated with p-SCN-DTPA and radiolabeled with ¹¹¹In, the accumulations of the antibody were measured in normal organs and tumor of mice bearing tumors between 0.1-1.5 g, at dosages between 10-200 µg, and over a period of 96 h. To confirm the biodistribution results by sacrifice, a mouse with a 0.4 g tumor having received ¹¹¹In-CC49 was imaged at 48 h. The acquired data were reconstructed using a commercial software.

Results: Tumor accumulations increased steadily until about 50 h and decreased slowly thereafter in part due to tumor growth. For a tumor of about 1 g, the accumulation was about 20 %ID/g. Neither the tumor nor the normal tissue accumulations in %ID/g were influenced by the antibody over the dosage range considered. Since tumor accumulations decreased with increasing size almost exponentially, the most important determinant of tumor accumulation was tumor size. In addition, the images clearly showed heterogeneous radioactivity distribution in the tumor.

Conclusion: The tumor accumulations of the CC49 antibody were quantitatively determined with varying tumor size, antibody dosage, and timing. The multipinhole SPECT imaging showed an inhomogeneous intratumor distribution. This information will be useful in future investigations of both pretargeting and direct-targeting studies with this antibody.

INTRODUCTION

We have recently developed a semiempirical model capable of predicting the biodistributions of effector in pretargeted mice under the variance of dosage and timing in contrast to determining them by the tedious trial-and-error approach. We believe this strategy is also useful in future clinical trial in the optimization of pretargeting conditions especially for tumor therapy since suboptimal conditions may impose unnecessary radiation burden on the normal tissues and may limit the maximum tolerable dose (MTD).

In this semiempirical model, quantitative information about the in vivo behavior of the pretargeting antibody is required. Although it is possible to obtain this information by necropsy of mice in the preclinical investigation, non-invasive imaging approach has to be used in the clinical study.

PET with ¹⁸F is an accurate and non-invasive way to determine the biodistribution of the pretargeting antibody, but the short half life of 110 minutes is often a disadvantage. As an alternative, conventional SPECT is attractive despite the lower resolution, since an array of gamma-emitting nuclides with suitable half-lives are available. Pinhole SPECT imaging may address the resolution issue but with the disadvantage of low sensitivity. Only recently, multipinhole SPECT has been introduced for small animal imaging with improved sensitivity and this approach is in principle also applicable for human imaging.

In preparing for preclinical tumor pretargeting on a LS174T mouse model using the antiTAG-72 antibody CC49, we report herein on the biodistribution data of the CC49 with the variations of tumor size, the dosages, and the time post injection as well as the 3D image by small animal multipinhole SPECT.

MATERIALS AND METHODS

The CC49 was custom produced from its hybridoma. The base sequences of MORF and its complement (cMORF) were previously described¹. The p-SCN-Benzyl-DTPA was from Macrocyclics. The P-4 resin was from Bio-Rad Laboratories and the Sephadex G-100 resin was from Pharmacia Biotech. The ¹¹¹InCl₃ was from Perkin Elmer Life Science Inc. All other chemicals were reagent grade and used without purification.

Radiolabeling CC49 with ¹¹¹In

The ¹¹¹In labeling of CC49 was achieved as previously described¹. The conjugation of DTPA to the CC49 was by mixing a CC49 solution with a p-SCN-Benzyl-DTPA solution in pH 9.3 NaCO₃-NaHCO₃ at a DTPA/antibody molar ratio of 55. After 15-24 h, the mixture was purified on a 0.7×20 cm Sephadex G-100 open column.

Tumor model and biodistribution

For tumor induction, 10⁶ LS174T cells were injected into the left thigh of each Swiss NIH nude mouse. The animals were used on day 14 when the tumors were about 1.0 g for the dosage study, at day 10 for the timing study, and at day 9-15 for the tumor size study. At sacrifice under anesthesia, samples of blood and other organs were removed, weighted, and counted in a NaI(Tl) well counter along with a standard. Blood and muscle were assumed to constitute 7 % and 40 % of body weight respectively. The tumored thigh was excised and the skin and as much of the muscle and bone as possible were removed before counting. The radioactivity therein was attributed to the tumor since the radioactivity levels in bone and muscle were negligible. After counting, the tumor mass was dissected to isolate the residual bone and muscle so that their weights could be subtracted for the net tumor weight.

Influence of dosage, targeting time, and tumor size

For the study of the influence of antibody dosage, six groups of mice (N=4) were used with each group receiving 20, 40, 80, 120, 160, or 200 µg of ¹¹¹In labeled CC49 (12 µCi) per mouse. After 48 h, the mice were sacrificed for biodistribution by exsanguination via heart puncture under anesthesia.

For the study of different targeting time, five groups of mice (N=4) were used. Each mouse received 30 µg (17 µCi) of ¹¹¹In labeled CC49 and the mice were sacrificed at 10.8, 24, 48, 72, or 96 h

For the study of the influence of tumor size, 20 mice were used and, starting from day 9, four mice per day were given 30 µg (24 µCi at day 9) of ¹¹¹In labeled CC49 until all the mice were used. Each mouse was sacrificed 48 h for biodistribution. Data from each mouse was treated individually rather than grouped as above.

Tumor accumulation by HiSPECT multipinhole SPECT imaging

A tumored mouse received 110 µg (932 µCi) ¹¹¹In labeled antibody and was imaged at 48 h on a multipinhole SPECT imaging system constructed according to the design by Schramm et al². The three heads of a PRISM 3000 gamma camera (Philips Med.) were fitted with HiSPECT hardware: three lead collimators with exchangeable tungsten six-pinhole aperture plates. The subject mouse was anesthetized and placed on a flat Lucite extension clamped to the headrest of the gamma camera bed. The pinhole apertures were on a circular orbit with a radius of 3.7 cm. Data acquisition was done for 20 min at 36 degree intervals for each head through 360 degrees. The data were transferred to a personal computer and reconstructed using commercial software (Bioscan, Washington DC).

RESULTS

Influence of antibody dosage on tumor accumulations

As shown in Fig 1, the absolute accumulations of labeled CC49 in mouse LS174T tumor and blood increase linearly with dosage. Similar results are obtained in other normal organs. Both the linear increasing absolute tumor accumulation and the constant percent tumor accumulation of antibody with dosage implies that, over the dosage range, the tumor antigen are not saturated by antibody nor its accessibility to antibody compromised by the formation of antibody-antigens complexes.

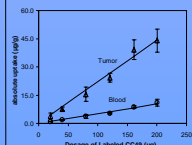


Fig 1. Absolute accumulations of ¹¹¹In labeled CC49 48 h after intravenous administration.

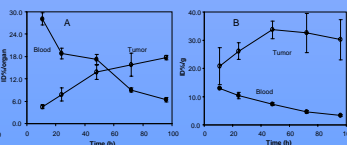


Fig 2. Tumor accumulation and blood level of ¹¹¹In labeled CC49 at different times (A) in %ID/organ and (B) in %ID/g.

Influence of targeting time on tumor accumulations

Tumor accumulation is a value depending on the time of determination. Fig 2 shows how the radiolabeled antibody clears from blood and accumulates into tumor with time. The tumor curve in panel A shows that tumor accumulation almost levels off after 48 h and, in panel B, tumor accumulation in %ID/g slightly declines after 48 h because of tumor growth. Therefore, the tumor accumulation curve was established at 48 h.

Influence of tumor size on the accumulation of antibody in tumor

Tumor size strongly influences the tumor accumulations. As a consequence, variance in the average tumor size between two groups of animals may increase the uncertainty of the comparison results and therefore the tumor size influence should be corrected. The tumor growth in the above timing study is shown in Fig 3A. However, we have observed that the tumor growth can be variable over time but once the tumors have grown to a palpable size, the growth appears to be constant at an average of about 0.1 g/day in this tumor model. This relationship will be useful in correcting the influence of tumor growth in future prediction models. In addition, as shown in Fig 3B, the tumor size (g) correlates almost linearly with the product of width and thickness of the tumor thigh (cm²).

The relationship of tumor accumulation of antibody with tumor size at 48 h is shown in Fig 4. The tumor accumulation in %ID/g is decreasing with tumor size but not linearly. As the tumor is getting bigger, the influence of tumor size and accumulation decreases.

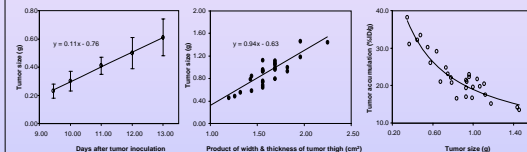


Fig 3. Increasing tumor size with time (A) and its correlation with the product of tumor width and thickness (B).

Fig 4. The relationship of tumor accumulation with tumor size

multipinhole SPECT image

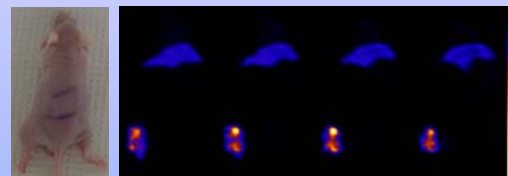


Fig 5. Four coronal slices of the multipinhole 3D images of a tumored mouse received ¹¹¹In-DTPA-Benzyl-CC49 48 h earlier and also the photo of the mice.

Fig 5 show four 1.2 mm thick coronal slices of a mouse that received ¹¹¹In labeled CC49 48 h earlier. The radioactivity is localized mainly in tumor and liver, while the radioactivity levels in other organs are much lower. This image confirms the biodistribution obtained by necropsy (in %ID/g) : tumor 23.9, liver 9.21, heart 1.46, kidney 3.70, lung 2.22, spleen 3.23, salivary gland 1.02, muscle 0.48, and blood 2.98. In addition, the image clearly shows the inhomogeneous distribution of radioactivity in tumor. A photo of the mouse is given as reference.

CONCLUSIONS

Pharmacokinetic behavior of ¹¹¹In labeled CC49 in tumored mice and the influence on tumor accumulation of the antibody dosage and tumor size have been determined. The image clearly shows the intratumor inhomogeneous distribution of radioactivity.

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