Biodistribution and Radiation Dose Estimates for Yttrium- and Iodine-labeled Monoclonal Antibody IgG and Fragments in Nude Mice Bearing Human Colonic Tumor Xenografts

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ABSTRACT

An anti-carcinoembryonic antigen murine monoclonal antibody designated NP-4, and its F(ab')2 and Fab' fragments, were coupled to the 1:1 mixture of 1-iso-thiocyanato-benzyl-3-methyl- and 1-methyl-3-iso-thiocyanato-benzyl-dithiolenetrimipentaacetic acid chelate and labeled with 111In or 90Y. Biodistribution studies in nude mice bearing a human colon tumor xenograft were performed with these labeled conjugates, and comparisons were made to unconjugated NP-4 IgG and fragments labeled with 131I. Regardless of the labeling method, higher tumor uptake was found with the intact IgG than with the fragments, but due to faster blood clearance, tumor/blood ratios were higher for the fragments than for the IgG. Tumor uptake for the radiometal-labeled NP-4 was generally higher than the 111In-labeled NP-4. Tumor/nontumor ratios for the liver, kidney, and spleen were higher for the 111In- and 90Y-labeled NP-4 IgG than the respective radiometal-labeled fragments, but tumor/nontumor ratios for the 131I-labeled NP-4 fragments were higher than the 111In-labeled NP-4 IgG. Radiometal uptake in the kidney was approximately 8 and 150 times higher than the 131I-NP-4 F(ab')2 and Fab', respectively, and the clearance of radiometal activity in the kidneys was approximately 10 times slower than the radioiodide. Quantiative of 90Y or 111In activity in the femur showed 3–5% for the IgG and F(ab')2; and only 1–2% for the Fab'. The amount of radioactivity in the femur remained constant over time, and between 60 and 100% of the 90Y activity remained after flushing the core of the femur with saline, whereas 50–70% of the 111In and only 25–30% of the 131I activity remained after flushing. Radiation dose estimates derived from these studies suggest that at the maximal tolerated dose 131I-NP-4 IgG would deliver 5.9 times the dose to the tumor as 90Y-labeled NP-4 IgG. 90Y-labeled fragments would not be useful due to higher doses to the kidneys than to the tumor. However, with 111In-labeled IgG and fragments there is greater flexibility to permit tumoricidal doses without excessive toxicity to the normal tissues.

INTRODUCTION

Antitumor antibodies labeled with radionuclides have been shown to inhibit, and in some cases even cure, human tumor xenografts grown in animals (1–5). RAIT has also been attempted in man with some reports of tumor regression (6–10). Although encouraging, these early RAIT studies in man have not yet made a significant impact on the treatment of cancer. Since these clinical trials have been phase I/II studies involving advanced cases of radioresistant tumors, such as colorectal or hepatocellular carcinomas, and melanoma, it is not surprising that the efficacy seen in animal models has not been translated directly to humans. Indeed, we have shown that, even in animals, it is more difficult to treat well-established colonic cancer xenografts than smaller tumors (2), suggesting that there may be limitations to the amount of tumor burden that can be treated by RAIT. The limitation of RAIT will depend on many factors, of which the MAbs, radionuclide, and tumor type are a few examples. Currently, the most promising therapeutic results have been reported in patients with more radiosensitive lymphomas (9).

Although there are many factors that will contribute to the successful application of RAIT, there is considerable interest in the choice of radionuclides. The first generation isotope used for therapy has been 131I (11). This isotope is readily available, inexpensive, and can be conjugated to antibodies easily, and its distribution in tumor and tissue can be monitored by external scintigraphy. However, the maximal range of nonpenetrating /-emissions from 131I is only 2.4 mm, whereas the range of 90Y is 12 mm. 90Y has a 64-h half-life and can be produced from a reusable 90Sr generator, and antibodies can be labeled using DTPA types of chelates. For these reasons, 90Y has become the second generation radionuclide for RAIT studies. Animal studies have shown that 90Y-labeled antibodies can inhibit tumor growth, but there has been concern over the potential for excessive myelotoxicity due to the deposition of 90Y in the bone (12, 13). Bone marrow toxicity has lead some investigators to explore localized therapy in the peritoneal cavity in an attempt to reduce the accessibility of the 90Y-labeled antibody to the bloodstream and thereby reduce its contact with the marrow (14). Despite this regional delivery approach, Stewart et al. (15) have predicted that no more than 20 mCi of 90Y can be administered safely as a single i.p. injection.

We previously examined the biodistribution and therapy of an anti-CEA murine MAb labeled with 90Y in nude mice bearing a human colonic tumor xenograft (12). With a single i.v. injection of the 90Y-labeled antibody, tumor growth could be inhibited, but not prevented, because we were limited in the dose we could inject due to marrow toxicity. Whole body autoradiography showed substantial uptake in all bones. Recent reports by Brechbiel et al. (16) and Esteban et al. (17) have indicated that MAbs conjugated to DTPA using the CA-DTPA had more liver uptake and less tumor uptake than the same MAb conjugated to DTPA using an ITC-DTPA derivative. The coupling of DTPA to MAbs via the ITC-benzyl-DTPA allows more of the carboxyl groups to participate in the binding of radiometals than the CA-DTPA, thereby providing greater stability for the chelated metal. In addition, the monofunctional binding of the ITC-DTPA eliminates the possibility of intramolecular cross-linking to the MAB that may occur with the bifunctional CA-DTPA (16). We found that conjugation of an anti-CEA MAb to an ITC-DTPA type of chelate improves tumor uptake and reduces liver and bone deposition of 90Y. In this report, we have taken one of these chelates, the MX-DTPA,
and conjugated it to an anti-CEA MAb, NP-4 IgG, or its F(ab′)_2 and Fab′ fragments. Biodistribution studies were performed with both 111In and 88Y to determine how well 111In may predict the uptake of 88Y-labeled antibody. 88Y was used in place of 90Y because, as a γ-emitting radionuclide, it is easier to quantitate in tissues than 88Y. We also compared these results to the biodistribution of 111In-labeled NP-4 IgG and its fragments. The biodistribution data were used to provide radiation dose estimates to the tumor and normal tissues in an attempt to determine which radionuclide would be best for I.V. RAIT applications and which vehicle [IgG, F(ab′)_2, or Fab′] would be best suited to target radioactivity to the tumor.

**MATERIALS AND METHODS**

**Preparation and Conjugation of NP-4 and Its Fragments.** NP-4 IgG was isolated from mouse ascites by Protein A and ion exchange chromatography, and its purity was checked by immunoelectrophoresis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing. F(ab′)_2 was prepared by pepsin digestion and purified by Protein A chromatography. Fab′ fragments were generated from the F(ab′)_2 by reduction with cysteine and alkylation with iodoacetamide and purified by molecular sieve chromatography. The purity of the fragments was tested using the same procedures as the IgG.

The synthesis of the MX-DTPA and its conjugation and labeling to NP-4 IgG and fragments were all done in the laboratories of Dr. Otto Gansow. The final number of DTPA molecules/MAb was determined by using a trace quantity of 14C-labeled MX-DTPA in the coupling procedure, according to the method described by Brechbiel et al. (16). By this method, the conjugates were determined to have 1.0, 0.7, and 0.3 chelates/NP-4 IgG, F(ab′)_2, and Fab′, respectively.

**Radiolabeling and Quality Assurance.** Radiiodination with 131I purchased from New England Nuclear (Boston, MA) was by the chloramine-T reaction, as described previously (2). Radiolabeling with 111In and 88Y was performed as described earlier (16). The labeled products were purified by HPLC and shipped at 4°C by overnight express mail for injection into animals on the following day. Quality assurance assays were conducted on each antibody preparation on the day of injection. These assays included immunoactivity measurements, molecular size determination by HPLC, and measuring unbound radioactivity by procedures described earlier (16). For the blood, 9 values were obtained by extrapolation of the values reported by Hui and Poston (20). For the bone, a cylindrical shape with the dimensions of 2-mm diameter x 10-mm length was used to generate an S value as follows: the apparent β-absorption coefficient (μ) in cm²/g was first determined according to (21):

\[ \mu = \frac{18.6}{(\Sigma_0 - 0.036)^{1/2}} \]

where, \( \Sigma_0 \) is the maximum energy of the β-ray spectrum in MeV. This was multiplied by the diameter of the cylinder (d) and then multiplied by 1.9 g/cm³, the density of bone. An absorbed fraction (φ) of 0.45 was then obtained for this particular value of \( \mu d \) (22). The S value was then calculated to be 14.9 rads/μCi-h according to the relationship \( S = \Delta \mu m \), where A is the mean energy emitted/unit of cumulated activity and \( m \) is the mass of the cylinder in g (23). Using a similar procedure, a bone S value of 4.9 rads/μCi-h was obtained for 131I.

All statistics were made by a one-way analysis of variance using a two-tailed F test (24).

**RESULTS**

Biodistribution of Radiiodinated and Radiometal-labeled NP-4 IgG and Its Fragments. Fig. 1 shows the comparison of the tumor uptake of NP-4 IgG and fragments labeled with 90Y, 111In, or 131I. Maximal tumor accretion occurred by Day 3 for the radiolabeled NP-4 IgG. Unlike IgG, maximal tumor accretion of the fragments was seen by Day 1 for the radiometal-labeled and radiiodinated NP-4. F(ab′)_2 fragments maintained their level in the tumor for 2–3 days, whereas the tumor uptake of Fab′ fragments declined continually. The tumor uptake of the 88Y- and 131I-labeled F(ab′)_2 was similar on Day 1, but the amount of 131I-NP-4 Fab′ progressively decreased at a faster rate, so that by Day 7 there was almost 6 times more 88Y-NP-4 Fab′ in the tumor than the 131I-labeled antibody. The tumor uptake of the 111In-NP-4 F(ab′)_2 was slightly higher than the 88Y-labeled antibody on Days 1 and 3, but they were identical by Day 7. Tumor accretion of the radiometal-labeled Fab′ fragments was between 2 and 8 times higher than the 131I-Fab′, but the rate at which the radiolabeled Fab′ was removed from the tumor was about the same.

No significant differences were seen in the percentage of radioactivity in the blood over time between the radiometal-labeled or radiiodinated antibodies. However, the fragments...
Biodistribution and Dosimetry for $^{131}$I versus $^{88}$Y-IgG or Fragments

Fig. 1. Percentage of uptake/g of tumor for $^{131}$I-, $^{111}$In-, or $^{88}$Y-labeled NP-4 IgG, F(ab')2, and Fab' fragments. ID, injected dose; bars, SD.

Days Post Antibody Injection

Tumor Blood Ratio

Days Post Antibody Injection

Table 1 Tumor/nontumor ratios for $^{88}$Y- and $^{131}$I-labeled NP-4 fragments

<table>
<thead>
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<th>Days post-injection</th>
<th>Organ</th>
<th>$^{131}$I</th>
<th>$^{88}$Y</th>
</tr>
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<tbody>
<tr>
<td>F(ab')2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Liver</td>
<td>7.1 ± 1.9</td>
<td>1.5 ± 0.4</td>
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<tr>
<td></td>
<td>Spleen</td>
<td>6.7 ± 2.7</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>3.7 ± 1.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>3.0 ± 0.7</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>3</td>
<td>Liver</td>
<td>26.6 ± 6.6</td>
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<td>Spleen</td>
<td>27.4 ± 7.6</td>
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<td>Kidney</td>
<td>14.2 ± 3.6</td>
<td>0.4 ± 0.1</td>
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<td>Fab'</td>
<td></td>
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<td>13.6 ± 2.7</td>
<td>2.7 ± 1.2</td>
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<td>Lung</td>
<td>15.9 ± 6.1</td>
<td>4.6 ± 1.0</td>
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* Mean ± SD (n = 4–8).

were cleared more rapidly than the IgG. For example, on Day 1, the percentage of injected dose/g of blood for the radiolabeled IgG was between 16 and 21% and by Day 7 was reduced to 6.0–8.9%. For the F(ab')2 fragments, on Day 1 there was 2.5–5.8%/g in the blood, and by Day 7 this was reduced to 0.05–0.12%. The percentage of injected dose/g in the blood with the Fab' fragments was between 0.2 and 0.4 on Day 1 and by Day 3 had been reduced to 0.03–0.09%. The faster blood clearance of the fragments resulted in improved tumor/blood ratios for the fragments in comparison to the intact IgG (Fig. 2). Tumor blood ratios for the $^{111}$In-NP-4 IgG were slightly higher than for $^{131}$I-NP-4 IgG on Day 7, reflecting the higher tumor uptake of the $^{111}$In-NP-4 IgG on this day. Overall, the tumor/blood ratios for the radiometal-labeled fragments were higher than for $^{131}$I-labeled fragments. The higher tumor/blood ratios for the $^{88}$Y-NP-4 fragments, in comparison to the $^{111}$In-NP-4 fragments on Days 3 and 7 [Fab' and F(ab')2, respectively], were due to differences in blood concentration on these days [0.05 ± 0.02 (SD) versus 0.12 ± 0.01% of injected dose/g for $^{88}$Y and $^{111}$In-NP-4 F(ab')2, on Day 7 and 0.03 ± 0.01 versus 0.09 ± 0.03% of injected dose/g for $^{88}$Y and $^{111}$In-NP-4 Fab' on Day 3].

Tumors/nontumor ratios for the kidney, liver, lung, and spleen are presented in Fig. 3 for the NP-4 IgG labeled with $^{131}$I, $^{88}$Y, and $^{111}$In. Significantly improved tumor/liver and spleen ratios were found only on Day 7 for the $^{131}$I-NP-4 IgG in comparison to the radiometal-labeled IgG. $^{131}$I-labeled fragments had significantly higher tumor/nontumor ratios than the $^{88}$Y-labeled NP-4 fragments on all days tested (Table 1). For the most part, tumor/nontumor ratios for the liver, spleen, and lungs for the $^{88}$Y-labeled F(ab')2, and Fab' were similar, ranging from about 2 to 8. Tumor/kidney ratios were significantly higher for the $^{88}$Y-NP-4 F(ab')2, in comparison to the $^{88}$Y-NP-4 Fab', but there was still 2–4-fold more activity in the kidney.
than in the tumor. Tumor/kidney ratios for $^{111}$I-fragments were never less than 1, increased over time, and were 40 to almost 200 times higher than $^{88}$Y-labeled F(ab')$_2$ and Fab', respectively.

The increased uptake of $^{88}$Y- in comparison to $^{111}$I-labeled NP-4 IgG and fragments in the normal tissues is shown in Fig. 4, using the liver and kidneys as representative tissues. For radioiodinated NP-4 IgG, 5% of the injected dose/g was in the liver on Day 1, which decreased to 1.5% by Day 7. For the $^{88}$Y-NP-4 IgG, the activity in the liver remained between 5 and 7% throughout this entire period. Liver uptake of the $^{88}$Y-NP-4 F(ab')$_2$ was not significantly less than intact IgG on Days 1–7, but for the $^{111}$I-NP-4 F(ab')$_2$, activity in the liver was 1.5% on Day 1 and decreased to 0.04% on Day 7. Liver uptake of the $^{88}$Y-Fab' fragments was reduced only 2–3-fold in comparison to the IgG and F(ab')$_2$, but by Day 1, liver uptake of $^{111}$I NP-4 Fab' was 10 to 40 times less than the amount seen with $^{111}$I-labeled F(ab')$_2$ or IgG, respectively. The difference between the uptake of radioiodinated and radiometal-labeled fragments in normal tissues was most striking in the kidneys. The amount of activity in the kidney for $^{111}$I-NP-4 IgG was similar to the amount of $^{88}$Y-labeled NP-4 IgG for the first 3 days, but by Day 7 the $^{111}$I activity decreased to a level 2-fold lower than the $^{88}$Y-NP-4 IgG. This is in sharp contrast to the tremendous retention of radiometal-labeled fragments in the kidney. The percentage of injected dose/g in the kidney for the $^{111}$I-NP-4 IgG was 3% on Day 1 and decreased to 0.5% by Day 3. For $^{111}$I-NP-4 Fab', the percentage decreased from 1.9 to 0.1% on Days 1 and 3. With the $^{88}$Y-NP-4 F(ab')$_2$, the percentage of uptake in the kidney started at 50% on Day 1 and reduced to 28% by Day 3. The $^{88}$Y-NP-4 Fab' initially was 180%, reducing to 74% by Day 3. Thus, the level of $^{88}$Y activity in kidney for the fragments was reduced only 2-fold from Days 1 to 3, but radioiodinated fragments were removed from the kidney at a significantly faster rate.

Since $^{88}$Y is known for its accretion in bone (25), we used $^{88}$Y to quantitate the amount of activity in the femur. In addition, we compared the bone data for $^{111}$In and $^{88}$Y to determine whether $^{111}$In may be used to predict $^{88}$Y uptake. Of the two femurs removed from each animal, one was washed with saline. Therefore, the estimates provided here clearly do not discriminate completely the amount of activity in the cortical portion of the bone versus the marrow but rather represent a partial removal of activity in the red marrow.

Radiation Dose Estimates. Estimates of radiation doses are given in Table 2 with normalization of the tissue doses to a constant tumor dose. In order to give 5000 rads to the GW-39 tumor, 0.29 mCi of $^{111}$I-NP-4 IgG and 0.34 mCi of $^{88}$Y-NP-4 IgG would be required, but 2.5–10 times less $^{88}$Y-NP-4 fragments would be required than $^{111}$I-NP-4 fragments. The bone marrow is the dose-limiting organ with radiolabeled IgG, as shown by the high dose to the bone when compared to the tumor. At equal tumor doses, $^{90}$Y-NP-4 IgG or fragments would deliver a higher dose to the normal tissues than $^{111}$I-NP-4 IgG. For $^{90}$Y-labeled NP-4 F(ab')$_2$, the kidney may be the dose-limiting organ, but for $^{111}$I-NP-4 F(ab')$_2$, the blood, i.e., the marrow, remains the dose-limiting organ. With Fab' fragments, the kidneys become the dose-limiting organ for both $^{111}$I- and $^{88}$Y-labeled NP-4 Fab'. However, if the higher dose allowance to the kidney as compared to the marrow is considered (by external beam, sublethal dose to the marrow is 200 versus 1500 rads for the kidney (26); marrow toxicity may remain the dose-limiting organ for the $^{111}$I-labeled Fab', but the kidneys would probably remain the dose-limiting site for the $^{90}$Y-labeled Fab'.

DISCUSSION

Many factors need to be considered when developing radio-labeled antibodies for tumor therapy. The choice of radio-nuclides has only recently been added to these factors due to the advances in radiopharmaceutical chemistry, $^{111}$In has been the radiopharmaceutical principally used in these studies for almost 30 years, but antibodies have since been labeled with isotopes such as $^{90}$Y, $^{188}$Re, $^{186}$Re, $^{67}$Cu, $^{211}$Bi, $^{203}$At, and $^{109}$Pd (12, 13, 27–33). Although the chemistry involved in labeling antibodies with these nuclides has advanced, the availability, purity, and cost of some of these isotopes remains the most formidable obstacle to the further development of this research. Of the nuclides listed above, $^{90}$Y has surfaced as the first to breach these obstacles. We must now carefully examine whether $^{90}$Y is a suitable radionuclide for RAIT and whether it will be superior to $^{111}$In.

The studies described herein have compared the biodistribution of an anti-CEA MAb as an intact IgG to its F(ab')$_2$ and Fab' fragments labeled with either $^{88}$Y or $^{111}$I. $^{88}$Y was used instead of $^{90}$Y to facilitate the quantitation of radioactivity in tissues. We also included $^{111}$In-labeled NP-4 IgG and fragments to determine whether $^{111}$In may be used to predict $^{90}$Y distribution. If $^{90}$Y-labeled NP-4 were to be used clinically, $^{111}$In may
be used to estimate the distribution pattern of 90Y for calculations of radiation doses. For example, Leichner et al. (34) have used the distribution of 111In-labeled anti-ferritin antibody to predict radiation doses to normal tissues of 90Y-anti-ferritin antibody, because they claim to have observed comparable distribution patterns in animal and human tissues. However, Hnatowich et al. (35) showed that 111In and 90Y-labeled antibodies do not distribute identically in all tissues, especially in the bone. In this study, the percentage of 99mTc- or 111In-labeled NP-4 in the bone was similar, but there were differences in the total amount of 111In and 99mTc activity remaining in the bones after the marrow was partially removed by washing. Radiiodinated NP-4 IgG was shown to have a similar percentage of uptake in the bone on Days 1 and 3, but there was a steady decline in the 111In activity, such that by Day 7 there was significantly less 111In activity in the bone than that seen with 99mTc or 111In. Although the procedure used to wash the marrow from the bones was incomplete, it was apparent that less 99mTc activity could be removed than 111In or 111In. It is this additional amount of yttrium activity retained by the bone that would escalate the actual marrow dose in comparison to estimates derived from 111In-labeled antibody. Thus, because more precise measures of dose calculations for 90Y in the bone are lacking, careful correlation of myelotoxicity to estimated doses derived from 111In will be required for future clinical applications of 90Y-labeled antibody.

We have shown that 90Y-labeled antibodies also have the additional problem of bone accretion (12) that probably occurs as a consequence of the metabolic processing of the 90Y-labeled antibody in the serum and tissues. Bone uptake of 90Y acetate was demonstrated recently by Anderson-Berg et al. (13), but the molecular form of 90Y incorporated in the bone from animals given 90Y-labeled antibodies is unknown. It is uncertain whether chelates with a higher affinity to bind 90Y will solve the problem of 90Y uptake in the bone. Chelates that could bind 90Y more tightly may prevent leaching of 90Y from the antibody chelate in the serum, but the radioantibody will still be processed by many tissues in the body. It is this added metabolic processing by the tissues and the subsequent sequestration or release of the 90Y that new chelation technology must also address. Ward et al. (36) have shown that it is difficult to remove radiometals from the tissues by the administration of chelates once they have been internalized. However, Stewart et al. (15) reported a higher percentage of 90Y excreted in the urine of patients given 90Y-labeled antibody with i.v. EDTA than without EDTA. Thus, clinical interventions that may alter the metabolic processing of the 90Y-labeled antibody or its metabolites may play an important role in the future of radiometal-labeled antibody applications.

One reason for the uptake of 99mTc-NP-4 IgG in the bone may have been the increased time spent in the circulation. Thus, it was interesting that, despite the more rapid removal of the F(ab')2 from the blood, there was just as much accumulation of 88Y in the bone with the F(ab')2 as the IgG. This may suggest that the binding of 90Y to the F(ab')2 was not as strong as to the IgG. Radionuclides without the bone-seeking ability of 90Y may provide a more reasonable alternative for RAIT by reducing the impact of at least one obstacle, namely, bone deposition. In this regard, the principal candidate would be isotopes of rhenium (186Re or 188Re).

Radiation dose estimates provided in Table 2 were normalized to an equal rad dose to the tumor for either 90Y- or 111In-NP-4 IgG or its fragments in order to evaluate normal tissue doses at doses of potentially equal tumoricidal activity. Since the clearance rate of radioactivity in the blood has been suggested as a means of predicting marrow toxicity (37, 38), and in our clinical experience, marrow toxicity has correlated well with bone marrow doses determined from blood clearance studies (38), further normalization based on an equal blood dose may reflect the expected tumor dose at equal myelotoxic levels. Both blood and bone doses for 90Y-NP-4 IgG were about 2-fold higher than that calculated for 111In-NP-4 IgG. Preliminary therapy trials with 111In- and 90Y-labeled NP-4 IgG in this same tumor model have indicated an MTD dose (dose at which 100% of the animals survive with less than 20% weight loss) of 0.25 mCi for 111In-labeled NP-4 IgG and between 0.05 and 0.1 mCi for 90Y-NP-4 IgG. Normalizing the tumor doses at the MTD for each radiolabeled NP-4 IgG, we find that the dose to the tumor for 90Y- and 111In-labeled NP-4 IgG would be 1470 rads (0.1 mCi) and 4310 rads, respectively. Thus, at the assumed MTD, 111In-NP-4 IgG would provide as much as a 5.9-fold higher dose to the tumor than 90Y-NP-4 IgG. It should be emphasized that these dose calculations are based on 0.1-g tumors. As the tumor size increases, the difference between 90Y- and 111In-labeled MAb decreases such that in a 10-g tumor there is only a 1.8-fold difference. Thus, 90Y-labeled MAbS may have an advantage over 111In-labeled MAb in patients with bulky disease.

In the nude mouse, doses at the MTD to the blood and bone, respectively, for either 90Y (0.05–0.1 mCi) or 111In (0.25 mCi) would be 1878 and 225 rads for 111In-NP-4 IgG and 593–1186 and 89-178 rads for 90Y-NP-4 IgG. The discrepancy between dose estimates for the blood and bone and the observed toxicity with 111In- and 90Y-labeled NP-4 IgG may reflect inaccuracies in the calculations using the current medical internal radiation dose approximations. Although we have tried to account for several variables in our dose calculations (i.e., using newly calculated S values for small tissues and assuming a cylindrical shape for the bone rather than a sphere), it is clear that further evaluation of radiation dose estimates coupled with direct comparisons of tumoricidal activity of both 111In- and 90Y-NP-4 IgG and the MTD for each must be pursued.

Our biodistribution studies have confirmed several of the

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<th>Tissue</th>
<th>IgG</th>
<th>90Y</th>
<th>F(ab')2</th>
<th>IgG</th>
<th>90Y</th>
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<tr>
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<td>Bone</td>
<td>261</td>
<td>604</td>
<td>ND</td>
<td>1,141</td>
<td>ND</td>
<td>1,044</td>
</tr>
<tr>
<td>Blood</td>
<td>2,178</td>
<td>4,036</td>
<td>854</td>
<td>947</td>
<td>703</td>
<td>324</td>
</tr>
<tr>
<td>Kidney</td>
<td>1,109</td>
<td>1,244</td>
<td>701</td>
<td>14,514</td>
<td>1,246</td>
<td>123,860</td>
</tr>
<tr>
<td>Liver</td>
<td>907</td>
<td>2,461</td>
<td>374</td>
<td>3,885</td>
<td>445</td>
<td>3,036</td>
</tr>
<tr>
<td>Lung</td>
<td>1,383</td>
<td>1,649</td>
<td>809</td>
<td>952</td>
<td>904</td>
<td>1,117</td>
</tr>
<tr>
<td>Spleen</td>
<td>649</td>
<td>1,410</td>
<td>386</td>
<td>1,849</td>
<td>380</td>
<td>1,652</td>
</tr>
</tbody>
</table>

* Dose estimates for 90Y-labeled antibody was based on 90Y-labeled antibody biodistribution.
* Values in parentheses are the mCi of each that would be required to give a 5000-rad dose to the GW-39 tumor.
* ND, not determined.
observations already reported by others, namely, higher uptake of radiometal-labeled antibody than radiiodine immunonjugates in normal tissues, especially in the kidney, when antibody fragments have been used (39-42). Of course, quantitative differences in tumor uptake and other distribution properties between our study and others can be related to many other factors, most notably different tumor models, antibodies, and labeling procedures. Nevertheless, the increased uptake in normal tissues and the retention in the kidney have been observed universally. Above all, the kidney uptake is the most difficult problem to resolve if radiometal-labeled fragments are to be used for RAIT. If this is a general problem for all radiometal-labeled antibodies, fragments may not be a practical approach for RAIT with this type of radionuclide. Experience with external beam irradiation has shown that a tumor/kidney dose ratio of 2.5 is necessary to expect a therapeutic advantage (26). Although the dose relationship between the tumor and normal organs may be different for radiolabeled antibodies in comparison to external beam therapy, either a reduction in kidney uptake or an increase in tumor targeting may be necessary before considering 90Y-labeled fragments. In contrast, the bone marrow remains the dose-limiting organ for 131I-labeled F(ab')2. This allows for the consideration of fractionated schedules for 131I-F(ab'). We have shown that 3 injections of 2 mCi of 131I-NP-4 F(ab')2 given over 6 days was equally tumoricidal as a single 2-mCi injection of 131I-NP-4 IgG, but there was significantly less toxicity to the animals given the fractionated dose of fragments (43). This indicates that the doses of the fragments could be escalated further and thereby increase the tumoricidal ability of this treatment in comparison to a single injection of 131I-NP-4 IgG. Indeed, in hamsters we have escalated 131I-labeled F(ab')2 doses to 12 mCi given over 6 days without significant loss in body weight of the hamsters.5 Multiple injections of 131I-NP-4 IgG given below the MTD have not been tested, but predictions from biodistribution studies indicate that multiple treatment of fractionated doses of fragments may have an advantage over multiple treatments of intact IgG. If multiple injections of either an IgG or a fragment is considered in patients, the need for developing ways to circumvent a human anti-mouse antibody response will be prominent.

In summary, despite the more therapeutically efficacious energy emissions of 90Y, antibodies labeled with 131I may have equal to or even better therapeutic prospects than 90Y-labeled antibody, primarily due to the increased toxicity of 90Y-labeled antibody brought on by the prolonged and higher retention of 90Y in the normal organs, especially the bone. It is becoming increasingly apparent that fractionated dose schedules for 131I-labeled fragments will improve the tumoricidal effects while minimizing the toxicity to normal tissues, yet antibody fragments labeled with 90Y or other radioisotopes may increase the risk of renal toxicity in addition to myelotoxicity. These conclusions are based on i.v. injected NP-4 and may not be true for other MAbS or injections that may allow for a higher proportion of the injected antibody to concentrate in the tumor in comparison to the normal tissues. Intrahepatic or i.p. injections may serve this function. However, there have been reports of dose-limiting toxicity for these applications (14, 15, 44), and for the treatment of disseminated disease, 131I-labeled antibodies may be the isotope of choice. Improvements in chelation technology or the development of techniques to modify 90Y accretion in the bone or other tissues may also improve the prospects for use of 90Y in RAIT. Isotopes other than 131I or 90Y, such as 188Re or 67Cu, are also worthy of evaluation in RAIT studies.

ACKNOWLEDGMENTS

We thank Drs. O. A. Gansow and M. W. Brechbiel for the preparation and labeling of the MAb-DTPA conjugates, R. Aninipit for technical assistance, and C. Ballance for the preparation of the manuscript.

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Biodistribution and Radiation Dose Estimates for Yttrium- and Iodine-labeled Monoclonal Antibody IgG and Fragments in Nude Mice Bearing Human Colonic Tumor Xenografts


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