Pretargeting of Renal Cell Carcinoma: Improved Tumor Targeting with a Bivalent Chelate


ABSTRACT

Radiolabeled monoclonal antibodies (mAbs) can target tumors selectively. Sustained activity levels in nontarget tissues limit their application. Pretargeting approaches using bispecific mAbs (bsmAbs) or the biotin-avidin interaction have been proposed to improve tumor:nontumor ratios. Pretargeting a tumor and simultaneously administering the radioactivity as a low molecular weight ligand fundamentally changes the pharmacokinetics of the radiolabel. In previous studies, we have shown successful radioimmunotargeting of diethylenetriaminepentaacetic acid (DTPA) labeled with indium-111 to renal cell carcinoma (RCC) after pretargeting in nude mice. In this study, we aimed to optimize further a pretargeting strategy in nude mice with RCC xenografts based on a bispecific antibody (bsmAb) against tumor-associated antigens,

INTRODUCTION

mAbs3 against tumor-associated antigens can be used to target tumors selectively. Labeled with γ-emitters, antibodies can be used to visualize tumors scintigraphically, and labeled with β-emitters, antibodies potentially can guide cytotoxic radiation doses to tumors. RIT has produced durable responses in the majority of patients with refractory B-cell lymphomas (1, 2) and in patients with other hematological malignancies (3). RIT studies in patients with solid tumors have been less successful (for review see Ref. 4), mainly due to the relatively low uptake of the radiolabeled antibody in the tumor as compared with its sustained levels in the circulation. Numerous approaches have been explored to reduce the uptake of radiolabeled antibodies in the nontumor tissues, such as: (a) use of antibody fragments, (b) use of anti-antibodies, and (c) extracorporeal removal of the circulating antibody. Although each of these approaches have been applied successfully in animal models, a major improvement of the efficacy of RIT cancer has not yet been accomplished. The main limitation of these approaches is that the reduction of the radiation dose to the nontarget tissues is accomplished by a reduction of the radiation dose to the tumor.

More than 10 years ago, a new approach to targeting tumors with antibodies and radionuclides was proposed (5). In this so-called “pretargeting” approach, the antitumor antibody and the radionuclide are administered separately. In the first step, the unlabeled antitumor antibody is administered. This vehicle targets or, rather, pretargets the tumor, and the circulating antitumor antibody is allowed to clear from the circulation. Thereafter, the radionuclide is administered as a low molecular weight ligand. Several pretargeting procedures have been developed that depend on different interactions between the first and second injectates. Pretargeting approaches based on either the biotin-avidin interaction or the use of bispecific antibodies have been developed. These approaches fundamentally change the pharmacokinetics of the radionuclide: because the radionuclide is administered as a fast-clearing agent, it either localizes in the tumor or is rapidly excreted via the kidney in the urine.

In a previous study, we developed and optimized a bsmAb-based pretargeting approach in nude mice with RCC xenografts (6). In these studies, the human RCC xenografts (NU-12) in nude mice were pretargeted by i.v. injection of G250 × DTPA bsmAb, 3 days before injection of 111In-DTPA. Uptake of the radiolabel in the tumor decreased from 7% ID/g at 1 h p.i. of 111In-DTPA to 0.5% ID/g at 48 h p.i. Because the 111In-DTPA cleared even faster from the nontumor tissues, very high tumor:nontumor ratios were obtained: the tumor: blood ratio exceeded 500 at 24 h p.i.

In nude mice with A375 melanoma tumors, Le Doussal et al. (7) improved tumor targeting of 111In-DTPA by using a bivalent hapten (111In-DTPA-Tyr-Lys-DTPA-111In). The bivalent DTPA accreted more efficiently in the tumor (3.5% ID/g, 24 h p.i.) than monovalent 111In-DTPA (2.8% ID/g, 24 h p.i.). It was suggested that, at the tumor cell surface, the bivalent hapten is bound by two bispecific antibodies, resulting in more avid binding of the radiolabeled hapten. Subsequently, another chemically produced bispecific antibody preparation (anti-CEA Fab’ × anti-DTPA Fab’) has been produced to use this approach for targeting CEA-expressing tumors. In nude mice with colon carcinoma xenografts this pretargeting approach (anti-CEA × anti-DTPA-In bsmAb in combination with 131I-labeled-dITPA-In) resulted in significantly enhanced tumor growth inhibition as compared with treatment with 131I-labeled anti-CEA antibodies (8, 9), indicating that the pretargeting approach can be applied for RIT. Currently, this approach is being tested as a therapeutic modality in patients with CEA-expressing tumors (10).

Here, we report on the further optimization of the pretargeting approach for RCC in nude mice using a tetrapeptide that was bivalently substituted with DTPA.

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5The abbreviations used are: mAb, monoclonal antibody; RIT, radioimmunotherapy; bsmAb, bispecific mAb; RCC, renal cell carcinoma; DTPA, diethylenetriaminepentaacetic acid; ID, injected dose; p.i., postinjection; CEA, carcinoembryonic antigen; TLC, thin layer chromatography; DOTA, 1,4,7,10-tetraazacyclododecane-N,N,N’,N’’,N’’’-tetraacetic acid.

[111In-DTPA-Tyr-Lys-DTPA-111In]. The bivalent DTPA accreted more rapidly in the tumor (78% ID/g, 4 h p.i.) and was virtually completely retained in the tumor during several subsequent injections. Clearance of the 111In-dITPA from the blood and kidneys was rapid and complete without the need to clear the bsmAb from the blood, probably due to the relative lability of the univalent bsmAb-dITPA complexes in the blood. As a result, with this two-step pretargeting approach tumor:blood ratios increased up to values as high as 3500 at 72 h p.i. High doses of diDTPA could be targeted preferentially to the tumor, indicating that this approach could also be used for radioimmunotherapy. Tumors could be imaged up to 1 week p.i. of 50 µCi of 111In-diDTPA. Quantitative analysis of the images confirmed the biodistribution data and indicated that, at 20 h p.i., 50 ± 15% of the whole-body activity was localized in the tumor.

In conclusion, these studies indicate that the use of bivalent chelates can very effectively optimize two-step targeting of tumors with bsmAbs. Our data indicate that this approach could optimize radioimmunotherapy.
MATERIALS AND METHODS

mAbs

The anti-RCC mAb G250 (IgG1) is directed against the RCC-associated antigen G250. This antigen is expressed in 75% of all RCCs, whereas expression in normal tissues is restricted to gastric mucosal cells and cells of the biliary ducts (11). The gene encoding the G250 antigen has been cloned (12) and is identical to the gene encoding the MN antigen (13). The affinity of G250 for the G250 antigenic determinant on SK-RC-52 cells is 0.25 nM (14). The production and characterization of the anti-DTPA-In mAb DTIn1 has been described previously (15). The affinity of DTIn1 (IgG2a) for 111In-DTPA is 0.23 nM (1), as determined by Scatchard analysis. The production and characterization of the bispecific anti-RCC × anti-DTPA mAb G250 × DTIn1 has been described (15).

Synthesis of Bivalent Chelate

The tetrapeptide Phe-Lys-Tyr-Lys (FKYK) was synthesized using a solid-phase peptide synthesizer. The NH₂-terminus of the peptide was acetylated, whereas the ε-amino groups of both lysine residues were substituted with DTPA by reaction with monoactivated DTPA (Aldrich Chemical Co., Milwaukee, WI). Electrospray mass spectrometry (Mass Consortium Corp., San Diego, CA) revealed a molecular weight of 1376.5, which was in agreement with the calculated value.

Radiolabeling

Monovalent 111In-DTPA. Forty µl of 111InCl₃ (Mallinkrodt, Petten, the Netherlands) was added to 150 µl of 0.5 M sodium acetate (pH 6.1) and 10 µl of DTPA [5.3 × 10⁻³ m in 0.5 m sodium acetate (pH 6.1)] and incubated for 60 min at room temperature. Complexing of 111In was assessed by ITLC on silica strips (Gelman Sciences, Ann Arbor, MI). The radiochemical purity exceeded 95%, as determined by ITLC. The immunoreactive fractions of the G250 antibody were obtained and stored digitally in a 256 x 256 matrix. The scintigraphic results were analyzed by drawing regions of interest over the tumor and over the whole animal. The percentage of the residual activity in the tumor (tumor:whole body ratio) was calculated at each time point.

RESULTS

Radiochemical Purity and Immunoreactivity. The radiochemical purity of the 111In-labeled preparations used in all experiments (111In-DTPA, 111In-diDTPA, and 111In-DTPA-G250) exceeded 95%, as determined by ITLC. The immunoreactive fractions of the G250 × DTIn1 bsAb and of 111In-DTPA-G250, prepared under conditions of infinite antigen excess, as determined on SK-RC-52 cells under conditions of infinite antigen excess (19), were 78 and 69%, respectively.

Biodistribution Studies

The murine G250 antibody (IgG1) was conjugated with 1-(p-isothiocyanatobenzyl)-DTPA and labeled with indium-111 as described previously (specific activity, 0.5 µCi/pmol; Ref. 16). The radiochemical purity was determined using ITLC on silica strips (Gelman Sciences, Ann Arbor, MI) using 0.15 M citrate buffer (pH 6.0) as the mobile phase.

Dose Finding of Bivalent and Monovalent 111In-DTPA

Mice with s.c. NU-12 tumors (0.04–0.2 g) were injected with 100 pmol of G250 × DTIn1. After 3 days, 111In-diDTPA or 111In-DTPA (0.7–700 pmol) was injected. Biodistribution of the radiolabel was determined after 1 h as described above.

Tumor Retention of 111In-diDTPA

Mice with s.c. NU-12 tumors (0.04–0.2 g) were primed with 100 pmol of G250 × DTIn1 bsAb. Three days later, 7 pmol of 111In-diDTPA or 70 pmol of 111In-DTPA (20 µCi per mouse) were injected. Biodistribution of the radiolabel was determined after 1, 4, 24, 48, and 72 h, as described above. As a control, a group of mice (n = 5) was primed with 100 pmol of the parental G250 antibody. Three days later, these mice received 111In-labeled diDTPA. One h after administration of the radiolabel, these mice were killed, and their tissues were analyzed. As a reference, a set of 25 mice received 15 pmol of 111In-DTPA-G250 (10 µCi per mouse). Groups of five mice were killed and dissected at 1, 4, 24, 48, and 72 h p.i.

Imaging Studies

Mice with s.c. NU-12 xenografts (0.3–0.6 g) were injected with 100 pmol of G250 × DTIn1 bsAb. Three days later, mice received 50 µCi (7 pmol) of 111In-diDTPA i.v. Another group of mice received 50 µCi (20 pmol) of 111In-DTPA-G250. Mice were anesthetized (halothane-nitrous oxide) and were placed prone on a single-headed gamma camera equipped with a parallel-hole medium-energy collimator (Siemens Orbiter; Siemens Inc., Hoffmann Estate, IL). Mice were imaged at 1 min and 1, 2, 4, 24, 48, 72, 96, and 168 h after injection. Images were obtained and stored digitally in a 256 x 256 matrix. The scintigraphic results were analyzed by drawing regions of interest over the tumor and over the whole animal. The percentage of the residual activity in the tumor (tumor:whole body ratio) was calculated at each time point.

Statistical Analysis

All values are given as means ± SD. Statistical analysis of tissue distribution data were performed using one-way ANOVA. For multiple comparisons, the Bonferroni correction was applied. The level of significance was set at P < 0.05.
diDTPA can be labeled with relatively high activity doses (up to 100 µCi of indium-111) that can be used for radioimmunodetection.

**Tumor Retention of 111In-diDTPA.** The biodistribution of the optimal 111In-diDTPA dose (7 pmol) was studied between 1 and 72 h p.i. to determine the retention of the radiolabel in tumor. The results of these experiments are summarized in Table 1 and Fig. 2. Again, rapid and high accumulation of the 111In-diDTPA in the tumor was observed (53 ± 16% ID/g at 1 h p.i. and 78 ± 41% ID/g at 4 h p.i.). More importantly, the radiolabel was virtually completely retained in the tumor for several days (tumor uptake: 93 ± 41% ID/g, 72 h p.i.; Fig. 2A). Variance of the tumor uptake values was relatively high, due to the hyperbolic nature of the relationship between tumor uptake and tumor size.

The bivalent chelate cleared from the blood and all other normal tissues very efficiently, and consequently, extremely high tumor:non-tumor ratios were obtained (tumor:blood ratios exceeded 1000 from 48 h p.i. on). Tumor retention of 111In-diDTPA was not observed when the tumor was pretargeted with parental G250 antibody: when compared with pretargeting with G250 × DTIn1 bsmAb, tumor uptake was significantly lower [0.13% ID/g ± 0.14% ID/g (data not shown) versus 52.9 ± 15.8% ID/g, 1 h p.i. (P < 0.0001)].

For comparison, the biodistribution of monovalent 111In-DTPA was studied in this model at the same time points. The 111In-DTPA did not accumulate as efficiently in the tumor as the bivalent chelate: tumor uptake of 111In-DTPA did not exceed 8% ID/g at any time point (Table 1 and Fig. 2B).

G250 labeled directly with indium-111 via DTPA was used as a reference in these experiments (Table 1 and Fig. 2C). One-step tumor targeting in this nude mouse model with 111In-DTPA-G250 was not as efficient as tumor targeting in the same range as tumor uptake of 111In-diDTPA after pretargeting (105 ± 24% ID/g versus 93 ± 42% ID/g, 72 h p.i.), whereas

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**Table 1. Biodistribution of 111In-diDTPA and 111In-DTPA after pretargeting with G250 × DTPA bsmAb in athymic mice with s.c. NU-12 tumors.**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>6.53 ± 0.82</td>
<td>1.44 ± 0.43</td>
<td>0.30 ± 0.03</td>
<td>0.10 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.88 ± 0.17</td>
<td>0.20 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.10 ± 0.01</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Tumor</td>
<td>7.55 ± 1.52</td>
<td>2.24 ± 0.66</td>
<td>1.27 ± 0.23</td>
<td>0.90 ± 0.12</td>
<td>0.87 ± 0.11</td>
</tr>
<tr>
<td>Liver</td>
<td>1.29 ± 0.35</td>
<td>0.51 ± 0.14</td>
<td>0.52 ± 0.15</td>
<td>0.54 ± 0.17</td>
<td>0.52 ± 0.16</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.50 ± 0.29</td>
<td>2.41 ± 0.20</td>
<td>2.17 ± 0.09</td>
<td>1.73 ± 0.11</td>
<td>1.30 ± 0.08</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.25 ± 0.15</td>
<td>0.70 ± 0.06</td>
<td>0.68 ± 0.05</td>
<td>0.63 ± 0.06</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>Lung</td>
<td>2.77 ± 0.28</td>
<td>0.83 ± 0.10</td>
<td>0.41 ± 0.05</td>
<td>0.36 ± 0.08</td>
<td>0.36 ± 0.06</td>
</tr>
<tr>
<td>Intestine</td>
<td>1.06 ± 0.10</td>
<td>0.66 ± 0.08</td>
<td>0.79 ± 0.15</td>
<td>0.79 ± 0.07</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>Femur</td>
<td>0.96 ± 0.25</td>
<td>0.24 ± 0.04</td>
<td>0.29 ± 0.17</td>
<td>0.32 ± 0.04</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>Tumor/blood</td>
<td>1.16 ± 0.22</td>
<td>1.89 ± 1.46</td>
<td>4.16 ± 0.48</td>
<td>8.95 ± 0.77</td>
<td>25.4 ± 11.1</td>
</tr>
</tbody>
</table>

**111In-DTPA-G250**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>25.4 ± 1.11</td>
<td>17.4 ± 0.32</td>
<td>12.2 ± 1.12</td>
<td>11.6 ± 1.82</td>
<td>10.8 ± 0.37</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.65 ± 0.04</td>
<td>0.52 ± 0.02</td>
<td>0.87 ± 0.04</td>
<td>0.79 ± 0.15</td>
<td>0.80 ± 0.05</td>
</tr>
<tr>
<td>Tumor</td>
<td>6.41 ± 1.15</td>
<td>14.2 ± 0.90</td>
<td>49.3 ± 10.6</td>
<td>95.0 ± 19.2</td>
<td>105 ± 24.2</td>
</tr>
<tr>
<td>Liver</td>
<td>8.20 ± 0.55</td>
<td>5.32 ± 1.63</td>
<td>7.30 ± 0.25</td>
<td>6.18 ± 0.36</td>
<td>6.18 ± 0.31</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.72 ± 0.16</td>
<td>5.92 ± 0.46</td>
<td>4.04 ± 0.39</td>
<td>3.19 ± 0.66</td>
<td>2.93 ± 0.03</td>
</tr>
<tr>
<td>Spleen</td>
<td>4.16 ± 0.28</td>
<td>3.04 ± 0.52</td>
<td>2.96 ± 0.54</td>
<td>3.71 ± 0.26</td>
<td>3.01 ± 0.05</td>
</tr>
<tr>
<td>Lung</td>
<td>14.8 ± 1.35</td>
<td>8.56 ± 0.65</td>
<td>5.17 ± 0.45</td>
<td>5.45 ± 1.15</td>
<td>5.41 ± 0.50</td>
</tr>
<tr>
<td>Intestine</td>
<td>2.49 ± 0.12</td>
<td>1.56 ± 0.12</td>
<td>1.39 ± 0.06</td>
<td>1.29 ± 0.30</td>
<td>1.13 ± 0.09</td>
</tr>
<tr>
<td>Femur</td>
<td>1.77 ± 0.65</td>
<td>0.77 ± 0.10</td>
<td>0.70 ± 0.06</td>
<td>0.75 ± 0.15</td>
<td>0.75 ± 0.12</td>
</tr>
<tr>
<td>Tumor/blood</td>
<td>0.25 ± 0.05</td>
<td>0.82 ± 0.41</td>
<td>4.02 ± 0.50</td>
<td>8.16 ± 1.69</td>
<td>9.82 ± 2.59</td>
</tr>
</tbody>
</table>

* Mice received 100 pmol of G250 × DTIn1 bsmAb i.v. on day −3. On day 0, mice were injected with either 20 µCi of 111In-DTPA (7 pmol) or 20 µCi of 111In-diDTPA (7 pmol) i.v. and were killed at 1, 4, 24, 48, and 72 h p.i. of the radiolabeled preparation (five mice per group). An extra set of mice with NU-12 tumors received 7.5 µCi of the directly labeled antibody 111In-DTPA-G250 (15 pmol) and were killed and analyzed at the same time points.
tumor-blood ratios obtained with the directly labeled G250 antibody were much lower (9.8 ± 2.6 versus 3490 ± 1930, 72 h p.i.).

**Scintigraphic Imaging.** Imaging of the mice with NU-12 tumors that received 50 μCi of 111In-diDTPA following pretargeting (100 pmol of G250 × DTIn1, day −3) confirmed the rapid tumor accrual of the 111In-labeled bivalent chelate as well as the effective retention of the radiolabel in the tumor (Fig. 3). Tumors were visualized as early as 1 h p.i. of the bivalent chelate. On the images acquired 1 h and 4 h p.i., some activity in the bladder was observed, reflecting the renal excretion of 111In-diDTPA. The images obtained at later time points (>20 h p.i.) illustrate that there was no retention of the radiolabel in any normal tissue. Quantitative analysis of the images allowed the quantitation of the whole-body excretion as well as calculation of the tumor:whole-body ratio (Fig. 4). Whole-body excretion of the radiolabel was rapid: >80% of the activity was excreted within the first 24 h (Fig. 4A). In addition, from 20 h p.i. on, the major fraction of the whole-body activity was localized in the tumor (Fig. 4B).

**DISCUSSION**

Pretargeting approaches aim to reduce the radioactivity (and thus the radiation dose) in nontarget tissues. As compared with the use of directly labeled antibodies, two-step (and multistep) tumor targeting should allow more rapid and distinct visualization of tumors and/or more effective RIT. Using the G250 × DTIn1 bispecific antibody in nude mice with human RCC xenografts, we demonstrated that targeting of RCC tumors with this pretargeting approach improved dramatically when a bivalent chelate was used instead of a monovalent chelate.

As reported in previous pretargeting studies, accurate dosing of the radiolabeled hapten was very critical (6, 7): optimal tumor uptake of the 111In-DTPA and 111In-diDTPA was achieved at lower doses (≤7 pmol). Assuming that the blood level of the bsmAb at 3 days p.i. is 12% ID/g, this would mean that the molar bsmAb:diDTPA ratio in the circulation should not exceed 1:0.3. At the 7-pmol dose, a tremendous effect of the bivalency of the hapten was observed in the nude mouse model: tumor uptake at 1 h p.i. increased almost 10-fold from 9% ID/g (with 111In-DTPA) to 80% ID/g (with 111In-diDTPA). Most importantly, retention of the radiolabel in the tumor also improved considerably: 2 days after injection, the 111In-diDTPA tumor uptake was still 75 ± 18% ID/g compared with 0.9 ± 0.1% ID/g for 111In-DTPA. The reasons for this major improvement of tumor uptake are not yet understood. In other studies in nude mouse models, some improvement of tumor uptake was obtained when a bivalent chelate was used, but these improvements were not as pronounced as in the model system that was used in our studies. LeDoussal et al. (7) reported that indium-111 tumor uptake increased from 2.8 to 3.5% ID/g when switching from monovalent DTPA to bivalent DTPA in mice with A375 melanomas. Similarly, Goodwin et al. (20) reported that uptake of DOTAs88Y was 1.7% ID/g, whereas 4.4% ID/g was found when diDOTA–88Y was used. A variety of factors that could be responsible for this major improvement. (a) The tumor localizaton of the G250 antibody is relatively high. At later time points, the directly labeled G250 antibody also reveals relatively high tumor uptake (105% ID/g, 72 h p.i.). Similarly, in several RCC patients focal tumor uptake in primary RCC exceeded 0.1% ID/g (14), which is higher than the tumor uptake of most other antitumor antibodies. (b) The affinity of the DTIn1 antibody is high; however, it is in the same range as the affinity reported for other antichelate antibodies (7, 20–22). (c) Most pretargeting studies have used bispecific antibodies that were produced by chemically coupling two Fab’ fragments to a F(ab’)2 construct; the G250 × DTIn1 bsxAAb is a biologically produced IgG molecule. (d) In other studies, bivalent constructs other than the Ac-Phe-Lys(DTPA)-Tyr-Lys(DTPA)-NH2 bivalent chelate were used (23–25). Currently, we are investigating the effects of each of these factors on the pretargeting of tumors in preclinical studies. This knowledge will allow us to determine the applicability of this approach in other tumors using other radiolabeled chelates and/or other bivalent constructs.

The almost complete retention of the 111In-diDTPA at the tumor site is remarkable. It has been pointed out by LeDoussal et al. (26) that 111In-diDTPA can be bound at the tumor cell surface (bivalently) by two bsmAbs. It has been hypothesized that this so-called “affinity enhancement system” facilitates the high retention of the radiolabel in the tumor. However, directly labeled antibodies, which also bind to a tumor cell
bivalently, generally show a much lower tumor retention than the bivalent chelate, especially at low blood levels (27). This would suggest that additional mechanisms cause the high tumor retention of the radiolabel. One could speculate that the bivalently bound chelate is internalized after tumor cell binding and that the good tumor retention is mainly due to intracellular retention of the indium-111 radiolabel because it has been shown that Lys-DTPA-111 In can be trapped intracellularly in the lysosomes (28). However, there is no indication that the G250 antibody-antigen complex is internalized by the NU-12 cells (29).

Alternative pretargeting approaches based on the avid interaction between biotin and avidin have also been developed. In initial efforts, the tumor was pretargeted with biotinylated antibodies, followed by the injection of radiolabeled avidin (30). The most successful protocols now use three subsequent injections (31, 32). The pancarcinoma antibody NR-LU 10 has been used in a three-step pretargeting approach: in the first step the tumor is pretargeted with an antibody-avidin conjugate. To remove the circulating antibody-avidin conjugate from the blood, unlabeled galactosylated biotinylated albumin is injected. In the third step, the chelated radionuclide (biotin-DOTA-111 In for diagnosis or biotin-DOTA-90 Y for therapy) is administered (31). Paganelli et al. (32) have tested a similar biotin-avidin-based pretargeting approach for the treatment of cancer in patients with glioma. In their approach, the tumor is pretargeted with a biotinylated antitensin antibody. The circulating antibody is cleared from the blood using avidin. Subsequently, the tumor is avidinized by injection of streptavidin. In the final step, the radiolabeled 90 Y-biotin-DOTA is injected (32). Initial clinical results are promising. However, the immunogenicity of (strept)avidin hampers multiple dosing in these approaches. In these approaches, injection of a serum-clearing agent (galactosylated albumin or avidin) as a second step is necessary to prevent binding of the radiolabeled chelate to the targeting vehicle in the circulation. In our approach, the targeting vehicle (i.e., the bsMAb) does not have to be cleared from the circulation because the bsMAb-diDTPA complexes formed in the circulation are relatively labile. Due to the lability of these complexes, the 111 In-diDTPA is rapidly cleared via the
kidneys. Apparently, the bivalent complexes formed at the tumor site are more stable.

The pharmacokinetics of the radiolabel as shown in the biodistribution and imaging studies (rapid tumor accretion, high tumor retention, and rapid clearance from nontumor tissues) suggests that this can be used for pre RT. However, in previous studies, we have shown that the DTIn1 antibody displays a very high metal selectivity; the affinity of the antibody for DTPA loaded with other metals than indium-111 is at least a 100-fold lower. As a result, the G250 × DTIn1 bsmAb does not trap 131I-DTPA or 131I-DOTA chelates in vivo. Recently, a series of high affinity anti-Y-DOTA and anti-Cu-DOTA antibodies have been developed (22). Production of bispecific antibodies based on these anti-DOTA antibodies could make this approach suitable for RT. Alternatively, β-emitters or even α-emitters could be coupled the Phe-Lys-(DTPA-In)-Tyr-Lys-(DTPA-In) core because this can be used in combination with the present G250 × DTIn1 bsmAb. Analogously, Gautherot and colleagues have labeled their dDTIna-In-Tyr-Lys hapten with 131I and used it for RT (9, 10).

In summary, these studies in nude mice indicate that the use of a bivalent chelate can very effectively optimize two-step targeting of RCC tumors with the G250 × DTIn1 bsmAb. Our data indicate that this approach could improve RT.

ACKNOWLEDGMENTS

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