Effects of Radiolabeling Monoclonal Antibodies with a Residualizing Iodine Radiolabel on the Accretion of Radioisotope in Tumors

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ABSTRACT

The effect of using a "residualizing" iodine radiolabel, dilactitol-iodo-tyramine, for radioimmunolocalization of antibodies to tumors was investigated. This tracer is designed to be lysosomally trapped after catabolism of the labeled antibody, mAbs RS7 and RS11 were used for in vivo and in vitro studies on the uptake and retention of radioisotope into tumor cells. Both are murine IgG1 mAbs with pan-carcinoma reactivity, which react with integral membrane glycoproteins. mAb RS7 has been shown to be relatively rapidly catabolized by the antigen-bearing cell line Calu-3, whereas RS11 is catabolized more slowly in the same cells. An 111In- or 131I-pentaacetic acid; ITC-Bz, /i-isothiocyanatobenzyl; %ID/g, percentage of injected dose/g. Thus, only mAbs internalized via clathrin-dependent endocytosis (coated pits) will be internalized efficiently within 1–2 h. Most mAbs are probably internalized by the non-clathrin-dependent pathway, which is much slower (reviewed in Ref. 3). Similar results have been obtained with carcinomas of various histological types, astrocytomas, and melanomas (1, 2).

Once the antibody is catabolized, which occurs within lysosomes, the fate of the radiolabeled catabolic product becomes a key factor. It is known that iodotyrosine rapidly exits from the lysosome and the cell after its generation after catabolism of conventionally iodinated proteins (4–6). However, other radiolabels are trapped within lysosomes, essentially because of their inability to cross the lysosomal membrane. Iodinated radiolabels have been designed to be lysosomally trapped by taking advantage of their linkage to nonmetabolizable disaccharides (reviewed in Ref. 7). It might be expected that such radiolabels would accumulate within tumor cells to a greater extent than conventional iodine labels, and such results have been demonstrated in vitro (4, 8, 9). However, in vivo results with such residualizing labels have not, to date, been impressive, partly because of accretion in certain normal tissues. The cellobiose-tyramine label was tested by the methods of Ali et al. (9, 10) using an anti-Thy-1.1 mAb and a mouse T-cell lymphoma, but only slightly increased tumor accretion was demonstrated. Demignot et al. (11), using radioiodine-labeled DLT mAb 791T/36 and the human sarcoma 791T, found increased tumor accretion of the DLT label, relative to chloramine T, but also increased accretion in many normal tissues, and there was not a large improvement in tumor/nontumor ratios. However, it seemed worthwhile to perform additional experiments with the DLT label for several reasons. The only direct comparison of the two residualizing labels suggested that DLT might be superior to cellobiose-tyramine (12). The DLT label was tested only with mAb 791T/36, which appears to localize within tumors primarily in the stroma adjacent to the tumor, rather than on the tumor cell surface (13). Clearly, the advantage of residualizing labels should best be seen with the use of mAbs that bind to the cell surface and are catabolized.

In previous studies of radioimmunodetection and radioimmunotherapy, we have utilized mAbs RS7 and RS11, which react with integral membrane proteins on the surface of lung carcinoma cells, and other carcinomas (14–17). RS7 was shown to be catabolized relatively rapidly (18), and RS11, an antibody to EGP-2, is catabolized more slowly at a rate similar to that for most mAbs binding to the cell surface (2). Although we have not directly examined the mode of endocytosis involved, it is likely that RS11 internalization is via noncoated vesicles, as discussed previously (1). The faster catabolism of RS7 is compatible with either a coated or noncoated vesicle pathway (19). This experimental system seemed useful for investigating the advantage of residualizing radiolabels. We anticipated that DLT would be most useful with the rapidly catabolized mAb and be less of an advantage with the slowly catabolized mAb. However, our results demonstrated that the use of DLT provided a large advantage with both mAbs.
MATERIALS AND METHODS

mAbs, Cell Lines, and Radiolabeling. The production and initial charac-
terization of RS7 and RS11 (also referred to as RS7-3G11 and RS11-51, re-
spectively) have been described previously (14, 18). Ag8 (American Type
Culture Collection, Rockville, MD), an irrelevant mouse myeloma IgGl de-
ignated P3 × As Ag8, was used as a negative control antibody in this study.
The antibodies were purified from ascites fluid by passage through a protein
A-immunosorbent column.

Calu-3, a human adenocarcinoma of the lung cell line, was purchased from
the American Type Culture Collection. The cells were grown as monolayers in
RPMI 1640 (JRH Biosciences, Lenexa, KS) supplemented with 5% fetal
bovine serum, 5% horse serum, 100 units/ml penicillin, 100 µg/ml streptomyc-
cin, and 2 mM L-glutamine. The cells were routinely passed after detachment
with trypsin-0.2% EDTA.

mAbs were radiolabeled with 131I or 125I (New England Nuclear, North
Billericia, MA) by the chloramine-T method (20) or via dillactitol-tyramine
by the method of Strobol et al. (12). Briefly, 5 nmol DLT in 25 µl of 0.5 m sodium
phosphate buffer (pH 7.6) were added to 0.5 ml polypropylene vials (number
72.730; Sarstedt, Pennsauken, NJ), which had been coated with 60 µg lodogen
(Pierce Chemical Co., Rockford, IL) evaporated from 150 µl dichloromethane.
Five mCi 131I, diluted to 50 µl in the same buffer, were added and incubated
30 min at room temperature. After transfer to a clean vial, 4 units of galactose
oxidase (Sigma Chemical Co., St. Louis, MO) were added in 5 µl of the same
buffer and incubated 45 min at 37°C. After addition of 0.25 mg mAb in 15–50
µl oxidase (Sigma Chemical Co., St. Louis, MO) were added in 5 µl of the same
buffer. The mixtures were vortexed and then incubated at 37°C for 1 h.
To prepare the mAbs for injection, 50 µl of each radiolabeled sample was
added to a column of the desired size, which had been coated with 60 µg of
labeled antibodies were injected i.v., via the lateral tail vein, into the tumor-bearing
animals. Details on the quantities of radioisotope injected are indicated in
"Results" for each study. The animals were sacrificed at the times indicated,
and the radioactivity in the tumor, liver, spleen, kidneys, lungs, small and large
intestines, muscle, bone (whole femur), and blood was determined after cor-
rection for physical decay in a γ-scintillation counter. Results are given as the
mean ± SD of four to five animals/group.

Radiation dose estimates were determined by first integrating the trapezoi-
dal regions (for tumors) or exponential regions (for normal tissues) defined by
the time activity data (corrected for physical decay). Trapezoidal integration
was also used for estimation of the bone and spleen dose in the yttrium study
because the correlation coefficient was <0.9 for the exponential fit for these
tissues. To generate conservative dose estimates that avoid overestimation of
the tumor cumulative dose and underestimation of the cumulative dose in the
normal tissues calculated with the use of trapezoidal integration, a zero time
value of zero is assumed for the trapezoidal fit of the tumor; for the normal
tissues, the zero time value is estimated by extrapolating the line described
by the first data pair. For the other tissues, the zero time point was extrapolated
according to the exponential curve. The resulting integral for each organ is
corrected to CgY/mCi with the use of S values appropriate for isotopic and
organ weight. These S values are calculated for each isotope by assuming
uniformly distributed activity in small unit-density spheres (25).

RESULTS

Retention and Processing of mAbs on Calu-3 Cells in Culture.
Calu-3, an adenocarcinoma of the lung cell line, was used as a model
system for evaluating the retention and cellular processing of radio-
labeled mAbs by tumor target cells with the use of mAbs RS7 and
RS11. Comparisons were made between the binding and processing of
the two mAbs radioiodinated by either the conventional chloramine-T
procedure or with the use of the residualizing dillactitol-
triamine. The mAbs were also labeled with 111In with the use of
ITC-Bz-DTPA. The results of the processing studies of the three
radioconjugates of each of these mAbs are summarized in Table 1.

The data indicate a difference in the retention of the two mAbs
when radioiodinated by the conventional chloramine-T procedure.
The processing of 125I-labeled RS7 was faster than 125I-labeled RS11,
consistent with the results of earlier studies on the relative rates of
internalization of the two mAbs (2, 18). This was especially apparent
at 21 h when only 22.9 ± 0.8% (SD) of initially bound RS7 remained
bound to the cells, whereas with RS11, 47.9 ± 2.4% remained. When
the residualizing labels were used, the percentage of radiolabel re-
tained by the cells increased substantially. With 125I-labeled DLT and
111In-labeled mAbs, approximately 60% of initially bound cpm re-
mained at 21 h. Nonspecific binding averaged 4.8% (ranging from 2.4
7.8%). Both 125I-labeled DLT and
111In-labeling yielded approxi-
mately equal retention of the labels with either mAb RS7 or RSI 1,
indicating that the rate of antibody internalization and catabolism does
not affect the cellular retention of the radiolabel when residualizing
labels are used.

In Vitro Antibody Retention Experiments. As described previously in
detail (1), confluent cells in 96-well plates were incubated with 5 × 106 cpm
of labeled antibody for 2 h at 37°C, then washed four times to remove unbound
labeled antibody. Tissue culture medium (0.2 ml) was added and incubation
was continued for various times as indicated in "Results." Under these
conditions, the cells remain healthy and continue to divide for at least 3 days. At
desired times, 0.1 ml of media was collected, and after additional washing,
the cells were solubilized with 2.0 M NaOH. After determining the cpm in
the culture medium, iodinated samples were precipitated with 5 ml cold 10%
trichloroacetic acid, and the iodine-labeled samples were precipitated with 5
ml methanol with the use of 1.0 mg of bovine IgG as a carrier protein, and
the precipitate was collected by centrifugation. Nonspecific antibody binding
was determined in each experiment by adding a large excess of unlabeled antibody
to control wells.

In some experiments, the incubation with radiolabeled mAbs was continued
for 3 days, in a total volume of 0.2 ml, with other conditions being the same
as described above. At various times, cells were washed 4 times, and the
cell-bound cpm was determined. In these experiments, >90% of the binding
was antigen specific, as shown by inhibition with a large excess of unlabeled
mAb, at 50 µg/ml, which was also present continuously.

In Vivo Biodistribution Studies. Tumors were propagated in female nu/nu
mice (Harlan Sprague-Dawley, Indianapolis, IN) at 6–8 weeks of age by s.c.
injection of 1 × 106 washed Calu-3 cells, which had been propagated in
tissue culture. The mice were used for in vivo biodistribution studies approx-
imately 3 weeks after the injection of cells when tumors reached a weight of
approximately 0.2 g (generally in the range of 0.1–0.4 g). Radiiodinated
antibodies were injected i.v., via the lateral tail vein, into the tumor-bearing

Table 1 Processing of radioconjugates by Calu-3 cells

<table>
<thead>
<tr>
<th>mAb</th>
<th>Label</th>
<th>4 h</th>
<th>21 h</th>
<th>45 h</th>
<th>69 h</th>
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<tr>
<td>RS7</td>
<td>125I-chloramine-T</td>
<td>79.28 ± 2.69</td>
<td>22.91 ± 0.82</td>
<td>9.93 ± 0.98</td>
<td>4.70%</td>
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<td>RS7</td>
<td>125I-labeled DLT</td>
<td>74.37 ± 9.03</td>
<td>64.90 ± 6.00</td>
<td>52.57 ± 0.25</td>
<td>45.39 ± 2.33</td>
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<td>111In</td>
<td>77.00 ± 11.35</td>
<td>60.11 ± 18.20</td>
<td>49.11 ± 12.16</td>
<td>42.72 ± 11.77</td>
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</tr>
<tr>
<td>RS11</td>
<td>125I-chloramine-T</td>
<td>85.38 ± 2.70</td>
<td>47.86 ± 2.39</td>
<td>33.27 ± 7.09</td>
<td>16.49%</td>
</tr>
<tr>
<td>RS11</td>
<td>125I-labeled DLT</td>
<td>80.27 ± 6.03</td>
<td>58.64 ± 6.39</td>
<td>48.48 ± 5.22</td>
<td>41.65 ± 5.37</td>
</tr>
<tr>
<td>111In</td>
<td>82.51 ± 7.90</td>
<td>67.03 ± 13.90</td>
<td>56.28 ± 8.84</td>
<td>51.30 ± 8.08</td>
<td></td>
</tr>
</tbody>
</table>

* "No SD is reported because this time point was not determined in the second experiment.
cells were incubated for up to 3 days in tissue culture medium containing a mixture of SDS of triplicates were all $0.381 \pm \sigma$ of the total cpm/well for RS11 and so. 11% for RS7. At varying times, cells were washed and collected and the percentage cpm specifically bound was determined. Nonspecific binding was determined by including a large excess of unlabeled mAb in control wells, and the specific binding was calculated by subtraction. At varying times, cells were washed and collected and the percentage cpm specifically bound was determined. Nonspecific binding was determined by including a large excess of unlabeled mAb in control wells, and the specific binding was calculated by subtraction.

![Graph](image1.png)

Fig. 1. Accumulation of radioisotopes in Calu-3 tumor cells in vitro. Adherent tumor cells were incubated for up to 3 days in tissue culture medium containing a mixture of mAbs labeled with either $^{125}\text{I}$ (A) or $^{111}\text{In}$ (B). The Ab was either RS11 (A) or RS7 (B). At varying times, cells were washed and collected and the percentage cpm specifically bound was determined. Nonspecific binding was determined by including a large excess of unlabeled mAb in control wells, and the specific binding was calculated by subtraction. SDS of triplicates were all $\leq 0.38\%$ of the total cpm/well for RS11, and $\leq 0.11\%$ for RS7. C, from the same experiment, the value plotted is (% $^{111}\text{In}$ specifically bound)/(%$^{125}\text{I}$ specifically bound). The increase in this ratio with time indicates greater retention of indium in comparison with iodine. (A), RS7; (V), RS11. Similar results were obtained in a second experiment.

As reported previously (1), a relatively small fraction of bound antibody was released intact from the cells. The majority of released cpm was found in a degraded form (trichloroacetic acid or methanol soluble fraction; data not shown). At 3 days, the percentage of bound antibody released in intact form (i.e., not catabolized) varied from 10 to 27% of the initially bound antibody, depending on the conjugate. In general, this value was reached within the first day, in contrast to the release of degradation products, which continued to increase during the 3-day time course.

**In Vitro Experiments Using a Prolonged Antibody Incubation.** In **vivo**, an antibody remains at high levels in the circulation for several days, although it gradually decreases due to clearance from the blood. To more closely mimic the **vivo** situation, **vitro** experiments were performed in which the cells were incubated with the mAbs continuously for up to 3 days. This protocol can potentially result in the accumulation of large amounts of radioactivity/cell. At various times, wells were harvested to determine the accumulation of cell-bound cpm. A double label study was performed comparing the residualizing label, $^{111}\text{In}$, with conventionally conjugated $^{125}\text{I}$ in the same wells. To ensure that uptake was via antigen-specific binding, and not due to fluid-phase uptake of mAb present in the medium, control wells contained a large excess of unlabeled mAb; this was effective at inhibiting mAb uptake by >90%. The results, shown in Fig. 1, demonstrate that accumulation of $^{125}\text{I}$ increased to 2 to 24 h but reached a plateau after 24 h. In contrast, $^{111}\text{In}$ continued to accumulate for 3 days, the duration of the experiment, with both mAbs. Note, however, that the advantage of the residualizing label, although clearly seen, is less pronounced in this experiment than it was in the earlier **vitro** experiments. This can be attributed to the fact that $^{125}\text{I}$ uptake increases markedly from 2 to 24 h, probably due to cell multiplication, which makes the retention of $^{111}\text{In}$ less prominent.

The ratio of the percentage of specifically bound cpm of the two radioisotopes (%$^{111}\text{In}$ specifically bound)/(%$^{125}\text{I}$ specifically bound) is presented in Fig. 1C. This graph shows that the advantage of the residualizing label is considerably greater for RS7 than it is for RS11 because the ratio increases by 2.4-fold with RS7 and only 1.2-fold for RS11, from 2 to 72 h. Therefore, this experiment also suggests that residualizing labels should produce more of an advantage with RS7 than with RS11.

**In Vivo Studies.** A comparison of the **vivo** targeting of mAbs RS7 and RS11 conjugated with the conventional and residualizing radiolabels was performed in nude mice bearing xenografts of Calu-3. An unreactive mAb, Ag8, was included as a control. These biodistribution studies were performed to assess whether the increases in tumor cell retention observed in **vitro** when the residualizing labels ($^{131}\text{I}$-labeled DLT and radiometal) were used translate into higher accretion of radiolabel at the target site in vivo. In addition, the contribution of the rate of mAb internalization and catabolism to radioisotope accretion and tumor:nontumor ratios was investigated by comparing the results obtained with the use of the two mAbs. The results are summarized in Fig. 2. Marked improvements in the tumor accretion of radiolabel were seen with the use of the $^{131}\text{I}$-labeled DLT- or $^{86}\text{Y}$-labeled mAbs in comparison to the conventional $^{131}\text{I}$-labeled antibodies. For example, at day 7 the %ID/g in tumor was $5.54 \pm 1.47\%$ (SD), $38.06 \pm 8.04\%$, and $43.18 \pm 19.50\%$ for the
The results of these studies are shown in more detail in Figs. 3 and 4, which show the percentage of the injected dose of radiolabel/g of normal tissue and the tumor:non-tumor ratios for these tissues for the RS7 studies over the 14-day time course. Similar results were observed with the radiolabeled RS11 (data not shown). As seen in Fig. 3, the % ID/g of radioisotopes were relatively low for all three labels in the normal tissues. This indicates that although the 131I-labeled DLT and 88Y-labeled RS7 are accumulating in the tumor to levels approximately 7–8 times greater than those observed with the use of conventional 131I-labeled mAbs (Fig. 2), the accretion in normal tissues is not changed as much by the use of the residualizing labels. The effect of increased tumor accretion without matching increases in normal tissue accretion is the generation of increases in tumor:non-tumor ratios (Fig. 4). Tumor:liver ratios at day 7 are 3.40 ± 0.69 (SD) with 131I-(conventionally)labeled RS7 compared to 16.26 ± 4.12 with 131I-labeled DLT-RS7 and 15.49 ± 4.66 with the use of 88Y-labeled RS7. Maximal tumor:non-tumor ratios were seen in the muscle with 90.64 ± 25.49 and 84.00 ± 31.62 observed at day 7 after injection of 131I-labeled DLT-RS7 and 88Y-labeled RS7, respectively, compared to 11.60 ± 2.42 with the use of conventional 131I-labeled RS7. At day 7, the lowest ratios were seen in the blood, at 0.87 ± 0.19, 8.84 ± 1.86, and 8.05 ± 2.73, for the conventional 131I-labeled, 131I-labeled DLT, and 88Y-labeled RS7, respectively.

The increases over time in the tumor:non-tumor ratios were similar for the 131I-labeled DLT and 88Y-labeled mAbs, the only exception being bone, where the tumor:non-tumor ratio decreases after day 3 with the 88Y label but not with the 131I-labeled DLT-antibodies. This most likely results from the known bone-seeking properties of the yttrium, which has become dissociated from the chelator. The accretion of yttrium by bone seen here is only significantly different from that of the iodine labels at the latest time point (14 days after injection) and would not contribute a significant radiation dose to the bone when 90Y is used therapeutically, due to the short physical half-life (64 h) of 90Y.

Residualizing labels will accumulate not only in tumor cells to which the antibody binds but also in any normal tissues that are responsible for antibody clearance and catabolism. The organs most active in IgG catabolism/g of tissue are the liver, spleen, and possibly the kidney (26). Although the kidney was noted to have increased accretion of IgG (or its degradation products), the results are difficult to interpret because the kidney may accumulate degradation products that are generated in other organs (26). Differences in the accretion of the radiolabels in various normal organs are addressed in Fig. 5, which shows the accumulation of 131I-labeled DLT-RS7 in various organs in the Calu-3-bearing nude mice, in comparison to RS7 labeled conventionally with 131I. Elevated localization ratios, defined as (%ID/g 131I-labeled DLT mAb)/(%ID/g conventional 131I-labeled mAb), are seen in the liver, spleen, and kidney, as expected (26). Although the liver, spleen, and kidney accrete more radioiodine with 131I-labeled DLT than with the conventional 131I label, the increases in the ratios are not as large as those observed in the Calu-3 tumor. Thus, the tumor:non-tumor ratios are increased even for these organs. Fig. 5 suggests that 131I-labeled DLT slowly accumulates to a small extent in the bone because the localization ratio increased from day 7 to day 14.
RESIDUALIZING RADIOLABELS ON mAbs

Fig. 5. Relative accumulation of $^{131}$I-labeled DLT-RS7 in tumor and normal mouse tissues. The value plotted is the (% injected dose/g of tissue with $^{131}$I-labeled DLT-RS7)/(% injected dose/g of tissue with conventional $^{131}$I-labeled-RS7), to show the preferential accumulation of DLT in certain tissues. For tissues that do not retain either label preferentially, the ratio will be 1.0. The ratios are derived from the data presented in Figs. 2 and 3. Int., intestine.

Similar results were observed in the other experiments described here, and also in experiments utilizing normal BALB/c mice (data not shown). This accretion is likely to be due to the macrophage content of the bone marrow.

The possible contribution of the rate of mAb internalization and catabolism to radioisotope accretion and tumor:nontumor ratios was investigated by directly comparing the results obtained with the use of the two mAbs. Tumor:liver ratios of the mAbs are shown in Fig. 6. No significant differences in tumor:nontumor ratios were observed between RS7 and RS11 with the $^{131}$I-labeled DLT or $^{88}$Y labels. With the conventional $^{131}$I-labeled mAbs, at day 14 after injection, the tumor:liver ratio with RS7 was lower than that with RS11. This difference, which was also seen in other normal tissues, is due to faster normal blood clearance of $^{131}$I-RS11 rather than increased tumor accretion. Thus, the marked advantage obtained with residualizing labels is seen equally well with both mAbs.

Dosimetry. Cumulative absorbed radiation doses were calculated from the biodistribution data of the above studies (Table 2). $^{88}$Y was used in the biodistribution experiments in lieu of $^{131}$I, which would be used therapeutically. This substitution was made because the $\gamma$ emissions of $^{88}$Y allow counting of the tissues in the $\gamma$ counter. Mean cumulative absorbed doses were computed for the $^{88}$Y data because the distribution of the two isotopes of yttrium can be assumed to be the same. To compare these studies, the absorbed doses were normalized to a blood dose of 1500 cGy, a level which has been observed to be approximately the maximal tolerated dose in this system. The data are summarized in Table 3. The administered doses of these conjugates necessary to achieve a 1500-cGy absorbed dose to blood are 242 µCi conventionally $^{131}$I-labeled-RS7, 368 µCi $^{131}$I-labeled DLT-RS7, and 170 µCi $^{88}$Y-labeled RS7. Although the max-

![Fig. 6. Comparison of tumor/liver ratios for radiolabeled RS7, RS11, and Ag8 in nude mice bearing Calu-3 tumor xenografts. Ratios are calculated with the use of data derived from the animals presented in Fig. 2. •, RS7; ○, RS11; △, Ag8.](image-url)

### Table 2 Mean cumulative absorbed dose in tissues

<table>
<thead>
<tr>
<th>Dose to tissue (cGy/µCi)</th>
<th>$^{131}$I-labeled DLT mAb</th>
<th>$^{131}$I-labeled CT mAb</th>
<th>$^{90}$Y-labeled DTPA mAb</th>
</tr>
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<tbody>
<tr>
<td>RS7</td>
<td>Calu-3</td>
<td>4561</td>
<td>24051</td>
</tr>
<tr>
<td>Blood</td>
<td>6208</td>
<td>4077</td>
<td>8844</td>
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<tr>
<td>Bone</td>
<td>949</td>
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<td>2141</td>
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<tr>
<td>Kidney</td>
<td>1559</td>
<td>2829</td>
<td>3005</td>
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<tr>
<td>Large intestine</td>
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<td>1048</td>
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<tr>
<td>Liver</td>
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<tr>
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<tr>
<td>Spleen</td>
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<td>3217</td>
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</table>

*a, CT labeling performed with the use of the conventional chloramine-T procedure.
b Calculated from $^{88}$Y-labeled DTPA-RS11 biodistribution data.
Table 3 Mean cumulative absorbed dose normalized to 1500 cGy dose to blood

| Dose to tissue (cGy) | 131I-labeled CT\(^{+}\) mAb | 131I-labeled DLT mAb | 90Y-labeled DTPA mAb
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>RS7</td>
<td>(242 μCi)(^{a})</td>
<td>(368 μCi)</td>
<td>(170 μCi)(^{b})</td>
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<tr>
<td>Calu-3</td>
<td>1,102</td>
<td>8,849</td>
<td>5,138</td>
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<tr>
<td>Blood</td>
<td>1,500</td>
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<tr>
<td>Bone</td>
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<td>669</td>
<td>539</td>
</tr>
<tr>
<td>Muscle</td>
<td>119</td>
<td>128</td>
<td>125</td>
</tr>
<tr>
<td>Small intestine</td>
<td>207</td>
<td>347</td>
<td>228</td>
</tr>
<tr>
<td>Spleen</td>
<td>319</td>
<td>844</td>
<td>526</td>
</tr>
<tr>
<td>RS11</td>
<td>(297 μCi)(^{c})</td>
<td>(436 μCi)</td>
<td>(150 μCi)(^{d})</td>
</tr>
<tr>
<td>Calu-3</td>
<td>1,250</td>
<td>10,905</td>
<td>4,283</td>
</tr>
<tr>
<td>Blood</td>
<td>1,500</td>
<td>1,500</td>
<td>1,500</td>
</tr>
<tr>
<td>Bone</td>
<td>223</td>
<td>369</td>
<td>342</td>
</tr>
<tr>
<td>Kidney</td>
<td>526</td>
<td>1,459</td>
<td>496</td>
</tr>
<tr>
<td>Large intestine</td>
<td>166</td>
<td>318</td>
<td>177</td>
</tr>
<tr>
<td>Liver</td>
<td>673</td>
<td>1,667</td>
<td>832</td>
</tr>
<tr>
<td>Lungs</td>
<td>647</td>
<td>741</td>
<td>648</td>
</tr>
<tr>
<td>Muscle</td>
<td>143</td>
<td>208</td>
<td>120</td>
</tr>
<tr>
<td>Small intestine</td>
<td>195</td>
<td>349</td>
<td>221</td>
</tr>
<tr>
<td>Spleen</td>
<td>317</td>
<td>1,041</td>
<td>481</td>
</tr>
</tbody>
</table>

\(^{a}\) CT, labeling performed with the use of the conventional chloramine-T procedure.  
\(^{b}\) Calculated from \(^{90}\)Y-labeled DTPA-RS11 biodistribution data.  
\(^{c}\) Numbers in parentheses, the administered dose necessary to achieve 1500 cGy absorbed dose to blood.

IMAL tolerated doses using \(^{131}\)I-labeled DLT mAbs have not yet been determined, the levels calculated for the conventionally iodinated and \(^{90}\)Y-labeled RS7 are comparable to previous experimentally determined values for \(^{131}\)I- (27) and \(^{90}\)Y-labeled mAbs.\(^{4}\) Dosimetry calculations performed on the biodistribution data show that increases of approximately 8- and 4-fold in absorbed dose to tumor can be expected to be achieved with the use of \(^{131}\)I-labeled DLT- and \(^{90}\)Y-labeled mAbs, respectively, compared to the conventional \(^{131}\)I. These results were similar for both RS7 and RS11. Comparing \(^{131}\)I-labeled DLT with \(^{90}\)Y, an increase of about 2-fold in absorbed dose to tumor is observed with DLT, due primarily to the longer half-life of \(^{131}\)I in comparison to \(^{90}\)Y. Increased doses to the normal tissues, which had increased accretion of the residualizing labels (i.e., liver, spleen, and kidney) were also observed. However, the doses to these organs remain below levels expected to cause toxicity.

**DISCUSSION**

We report here that a residualizing label such as DLT can provide a great improvement in tumor:non-tumor localization ratios in comparison with a conventional iodine label. Improvement is marked not only for tissues such as lung, muscle, and intestinal, which are not major sites of normal antibody catabolism, but also for the liver, spleen, and kidney, where DLT tends to accumulate. This effect was observed with use of the lung adenocarcinoma cell line, Calu-3, but was less dramatic with a second carcinoma, LoVo.\(^{5}\) Results published previously with DLT (11) were more similar to the LoVo results than were the Calu-3 results, in that relatively high accretion in liver, spleen, and kidney were observed. We cannot currently explain these differences, and additional experimentation in this direction is in progress. Clearly, the rate of mAb uptake in tumors and/or the rate of mAb catabolism is significant variables that must be taken into consideration. At present, we speculate that tumors may differ signif-

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\(^{4}\) R. M. Sharkey, R. D. Blumenthal, G. Y. Wong, G. L. Griffiths, and D. M. Goldenberg. Radioimmunotherapy with equitoxic doses of \(^{131}\)I- \(^{90}\)Y- and \(^{188}\)Re-labeled monoclonal antibody in human colon tumor xenograft models, manuscript in preparation.

\(^{5}\) M. J. Mattes and R. D. Blumenthal, unpublished data.

The relationship between radiometals and DLT must be considered because both labels increase the cellular retention of radioisotope. It was recently reported that radiometals bound to strong chelators such as ITC-Bz-DTPA are trapped within cells after catabolism of the antibodies to which they were conjugated (8) and that they are trapped within lysosomes (28). Although no radiolabel appears to be trapped permanently within lysosomes (7, 8), the retention of DLT and ITC-Bz-DTPA seemed to be very similar in a direct comparison (8). Thus, it could be predicted that these two labels would produce very similar antibody localization results, and this is confirmed in the present study. It has been widely accepted that the difference in tumor localization between radiometals and a conventional iodine label is primarily due to deiodination. However, as discussed previously in detail (8), experimental evidence points directly to an explanation based on differences in the processing of the catabolic products rather than deiodination.

Because chelated radiometals have been utilized in a large number of investigations and sometimes have been compared with a conventional iodine label, such results can potentially provide additional insight into the advantage of residualizing labels. However, this analysis is complicated by two factors: (a) most earlier studies used relatively unstable chelators, mainly the cyclic anhydride or mixed anhydride of DTPA. These chelators release a substantial amount of free metal, which then binds to transferrin and is taken up by the liver (29). Work with these chelators did demonstrate increased accumulation in tumors, relative to an iodine label (30, 31), but in regard to normal tissue uptake the results obtained with these unstable chelators are difficult or impossible to compare with our experiments; and (b) a considerable variety of the newer, stronger metal chelates have been developed (29, 32, 33) with some significant differences between them. Although all chelators may release very small amounts of free metal, they may differ in other characteristics, including lysosomal retention. In view of these considerations, only a few comparisons of strong metal chelators with iodine labels are clearly relevant to the present study.

Sharkey et al. (34) compared ITC-Bz-DTPA with iodine using an ant carcinoembryonic antigen mAb. Their results demonstrated increased tumor accretion of the metal, but there was also increased normal tissue accretion, and dosimetry calculations indicated that yttrium was probably inferior to iodine for purposes of radioimmunotherapy. The most extensively characterized mAb tumor system has been mAb B72.3 and the LS174T colon carcinoma, in which a wide variety of benzyl-DTPA derivatives have been tested (35, 36). In this case, it was reported that there was no significant difference in biodistribution between a strong metal chelator and iodine. This result can perhaps be attributed to the fact that the B72.3 antigen is not a cell surface antigen but rather a secreted mucin, and that the mAb is, therefore, not internalized and catabolized. This consideration may also apply to the results with carcinoembryonic antigen antibodies (34) because the carcinoembryonic antigen occurs in both secreted and membrane-bound forms (37). In this context, we note that different benzyl-DTPA derivatives may vary in their level of lysosomal retention, but this of course can be seen only with mAbs that are efficiently internalized and degraded. Stein et al. (15) compared iodine and indium using mAb RS7 on ME180 cervical carcinoma tumors in a system essentially identical to that used here except for the different target cell. In that study, indium produced higher absolute tumor accretion and higher tumor:non-tumor ratios for some organs but lower ratios for the liver and spleen. In summary, although the use
of indium does generally result in increased tumor accretion, the dramatic difference in tumor:non-tumor ratios described here has not been described in other experimental systems. Thus, the advantage of residualizing labels will depend on the particular mAb and on the particular tumor target.

Although DLT and benzyl-DTPA seem to be very similar in terms of retention within lysosomes, significant differences between these labels remain. One difference is in the accretion of yttrium by bone. Such bone accretion can occur only after the metal escapes from the chelator; therefore, it is relatively minor when strong chelators such as benzyl-DTPA are used. Some release of yttrium does still occur, and this material appears to be efficiently taken up by bone. Sharkey et al. (34) concluded that bone accretion was an obstacle to the use of benzyl-DTPA chelates of yttrium. This factor, therefore, represents an advantage of DLT over yttrium. The release of yttrium can be reduced with the use of even stronger chelators, namely the macrocycles (32), but such chelators are inconvenient to use because radiolabeling is less efficient. Our data indicate that bone accretion is probably not entirely due to uptake of free metal but also due to accumulation of residualizing labels in the bone. Such accumulation was seen with DLT, although at a level much less than in liver, spleen, and kidney. This accumulation is unlikely to be due to uptake in the bone itself, and we tentatively attribute this to uptake by bone marrow macrophages. It would be expected that benzyl-DTPA- yttrium would similarly accumulate in bone. Thus, bone accretion can occur by two distinct mechanisms. However, it should be emphasized that the level of bone marrow accretion of the residualizing labels is very low and, therefore, unlikely to represent a toxicity problem.

The major difference between yttrium and iodine, however, is seen not in the tumor:non-tumor ratios but only in the dosimetry and is due to the difference in decay half-life. Thus, an approximately 2-fold difference in radiation delivered to the tumor is seen with estimated equitoxic doses. It is apparent that, due to relatively slow uptake and accumulation of the radiolabel within the tumors, a 3-day radiation half-life is not long enough to take full advantage of the retention of the label at the tumor site. There are few if any other residualizing radiolabels with relatively long half-lives (1–2 weeks) that have been utilized. One potential candidate is 177Lu (38). However, it cannot be assumed that lutetium or other metals will behave identically to the benzyl-DTPA chelate of indium or yttrium, and results may depend on the particular chelator used.

Although we expected that the advantage of a residualizing label would be greater for a rapidly catabolized mAb, RS7, than it would be for a slowly catabolized mAb, RS11, such a difference was not seen. In retrospect, this is perhaps not surprising. The difference in the rate of catabolism of these two mAbs (seen in vitro) is only approximately 2-fold, which perhaps is a small difference compared to the other processes that are involved. Although we attempted to modify our in vitro experiment to more closely simulate the in vivo situation, by keeping the labeled mAb in the medium for 2–3 days, these results did not appear to more closely parallel the in vivo data. This discrepancy may be due to differences in the physiological state of the cells when grown in nude mice or in tissue culture. For example, differences in the rate of membrane turnover would have major effects on the rate of antibody processing. Because RS11 has consistently been observed to be processed in a manner similar to the majority of “typical” membrane-reactive mAbs on a variety of cell lines (2), we expect that most mAbs reacting with the surface of Calu-3 would be processed similarly.

Additional use of DLT for radioimmunotherapy is complicated partly by relatively low conjugation efficiency. The efficiency of protein iodination obtained in these studies, 3–6%, is sufficient for radioimmunodetection experiments in mice and for in vitro experiments but not for clinical studies. However, we have recently improved the efficiency to approximately 33%, simply by increasing the concentration of mAb used by a factor of 20. Additional improvements in efficiency are under investigation.

In summary, the prolonged retention of 131I-labeled DLT mAbs in tumor cells, the 8-day half-life of the 131I, and the relatively low levels of accretion in normal tissues combine to make radioiodinated DLT an adduct of great potential for radioimmunotherapy. The effectiveness of this methodology appears not to be limited by whether a mAb is rapidly catabolized by antigen-expressing cells and should be of general applicability.

ACKNOWLEDGMENTS

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REFERENCES

RESIDUALIZING RADIOLABELS ON mAbs


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