Enhanced Tumor Localization and in Vivo Stability of a Monoclonal Antibody Radiiodinated Using N-Succinimidyl 3-(Tri-n-butylstannyl)benzoate

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ABSTRACT

Loss of radionuclide after in vivo administration of labeled monoclonal antibodies (MAbs) to cancer patients is a likely cause of the low levels of tumor uptake of MAb which have been observed. In this study, we have evaluated the utility of N-succinimidyl 3-(tri-n-butylstanny1)benzoate (ATE) for the radiiodination of 81C6, a MAb reactive with the extracellular matrix antigen tenasin associated with gliomas and other tumors. In vitro binding properties of MAb labeled via ATE were slightly better than those of the Iodogen preparations. Paired-label studies were performed in athymic mice bearing s.c. D-54 MG xenografts and injected with both 81C6 labeled with 125I using the ATE method and 131I using the Iodogen method. These studies demonstrated that use of the ATE method (a) decreased thyroid uptake by 40- to 100-fold, suggesting a lower rate of dehalogenation compared to MAb labeled using Iodogen; (b) increased tumor uptake by as much as a factor of 4 at Day 1 to more than 12-fold at Day 8; and (c) resulted in superior tumor-to-normal-tissue dose ratios. The specificity of MAb uptake was investigated in a paired-labeled study comparing the distribution of 81C6 and isotype-matched control 45.6, both labeled using the ATE procedure. Localization indices for tumor ranged between 6 at Day 1 to 34 at Day 7, values considerably higher than those reported previously for 81C6 and 45.6 radiiodinated using a conventional method (chloramine T). These results demonstrate that the ATE method may be a valuable approach for labeling MAbs with iodine nuclides.

INTRODUCTION

Although the concept of exploiting MAbs to deliver diagnostic and therapeutic nuclides to cancers has been actively pursued for more than a decade, radiolabeled MAbs have yet to make a significant impact on the clinical management of cancer patients. A major problem has been that tumor uptake and retention of radionuclide has been low, on the order of 0.001% of injected dose per gram in most clinical studies (1). A compounding difficulty has been the accumulation of nuclides in normal tissues, with stomach and liver being the organs with the highest degree of uptake for radioiodinated (2, 3) and 131I-labeled (4, 5) MAbs, respectively. Physiological (6–8), pharmacological (9–11), and immunological factors (12–14) contribute to the suboptimal delivery of labeled antibodies to tumors and, in some cases, to normal tissue uptake of nuclides. Moreover, limitations in antibody labeling methodology, particularly with regard to resultant in vivo stability and immuno-reactivity, also must be addressed if the diagnostic and therapeutic potential of radiolabeled MAbs is to be realized.

For both preclinical and clinical investigations, utilization of iodine radionuclides as MAb labels offers certain advantages (15). However, after administration in vivo, radiiodinated MAbs frequently undergo deiodination, resulting in stomach and thyroid uptake of radioiodine (2, 3) and decreased availability of labeled MAbs for tumor binding. In addition, in animal models where tumor size represents a significant fraction of total body weight, it appears that increased loss of label from radioiodinated MAbs occurs as a consequence of tumor binding (16–18). Although binding-mediated dehalogenation would be difficult to observe in clinical studies, it seems likely that this phenomenon could contribute to the low levels of tumor uptake which have been observed.

The structural similarity between the iodotyrosines created in conventional protein iodinations and thyroid hormones, compounds known to undergo extensive enzymatic dehalogenation, is presumed to be a major cause of the loss of radioiodine from MAbs in vivo (19). To circumvent this problem, we developed a method that decreases the structural similarity of the labeling site on the protein to these enzymatically degradable substrates by avoiding substitution of the halogen ortho to a hydroxyl group on an aromatic ring. In this procedure, radiiodination of ATE is used to generate a labeled acylation reagent which is then reacted with the protein (20). Paired-label studies have demonstrated that use of the ATE method decreased the in vivo loss of label from proteins by a factor of 10 to 100 compared to those labeled using a conventional method (20, 21). Using the F(ab′)2 fragment of OC 125, an IgG, MAb reactive with a membrane and circulating antigen associated with ovarian carcinoma, as a model system, 27–49% higher tumor uptake of radioiodine was observed for MAb fragment labeled using the ATE reagent (21).

Because of our interest in using radioiodinated MAbs for the treatment of gliomas (22, 23), the present study was performed to determine the potential advantage of using ATE for labeling 81C6, an IgG2A MAb reactive with the glioma-associated extracellular matrix antigen tenasin (24). These experiments represent the first evaluation of the ATE method for labeling a whole MAb. Since clearance of intact MAbs from the body is slower than F(ab′)2 fragments (2), use of the ATE reagent for labeling a whole immunoglobulin could provide even greater improvements in tumor uptake, particularly at longer times after administration.

MATERIALS AND METHODS

Monoclonal Antibodies. MAb 81C6 is of the IgG2A isotype and defines an epitope of the extracellular matrix antigen tenasin, which is present in primary gliomas, multiple human glioma cell lines, and glioma xenografts, but not in normal brain (24, 25). In some experiments MAb 45.6, an immunoglobulin IgG3 with no known specificity derived from the myeloma cell line 45.6TG 17.1 (24), was used as a nonspecific control. Poor localization of 45.6 MAb labeled using chloramine T has been demonstrated previously (22, 23). Purification of MAbs from D-54 MG human glioma xenografts hybridoma culture supernatant was accomplished using a Protein A-Sepharose 4B column. MAb purity was determined using high-pressure liquid chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Antibody Labeling using Iodogen. A variation of the original Iodogen method (26) was used. 81C6 IgG (200 μg in 220 μl of 100 mM phosphate buffer, pH 7.4) was added to sodium [131]iodide (Dupont New England...
Antibody Labeling using ATE. The ATE reagent was synthesized as described (20) and utilized to radioiodinate MAb 81C6 and 45.6 using a variation of our previously reported method (21). In two experiments, the ATE method was used to label 81C6 with $^{125}$I (Dupont New England Nuclear). In the third, the ATE method was used to label 81C6 with $^{131}$I and to label 45.6 with $^{125}$I. In this procedure, 1% acetic acid in CHCl$_3$ was added to a 1-ml glass vial containing the radioiodine activity to adjust the pH to 5–5.5. Then, 15 µl of 9% t-butyldihyroperoxide (13 µmol) in CHCl$_3$ and 10 µl of 0.1 M ATE in CHCl$_3$ were added, diluted with an additional 50 µl of CHCl$_3$ and reacted for 30 min at room temperature. The reaction mixture was added to a silica Sep-Pak cartridge (Millipore) and eluted sequentially with 30 ml of hexane, 25 ml of 8% ethyl acetate in hexane and 3 ml of 30% ethyl acetate in hexane. $^3$-$^5$[Iodobenzoate was then eluted in an additional 15 ml of 30% ethyl acetate in hexane, concentrated by rotary evaporation and evaporated to dryness in a 1-ml glass vial. The MAb (200 µg in 50 µl of pH 8.5 borate buffer) was added to the residue and reacted at room temperature for 20 min. The reaction was stopped by addition of 300 µl of 200 mM glycine in borate buffer. A Sephadex G-25 column was used to purify the radioiodinated MAb. Trichloroacetic acid precipitability of MAb labeled using ATE ranged between 97% and 99%. All preparations were eluted as a single peak on size exclusion-high pressure liquid chromatography, with a retention time identical to that of intact IgG.

Immunoreactivity Assessment. The binding characteristics of 81C6 after radioiodination via the ATE and iodogen methods were evaluated using immunoreactivity and affinity assays. For the immunoreactivity assay, 50–100 ng of each labeled MAb preparation was incubated overnight at 4°C with homogenates of both D-54 MG human glioma tumor and normal rat liver in triplicate. After three washes with 1% bovine serum albumin in phosphate buffered saline, the supernatants and precipitates were counted for $^{125}$I or $^{131}$I activity using an automated gamma counter. Specific binding is defined as the percentage binding to D-54 MG tumor minus the percentage bound to liver. The affinity constants for the radioiodinated 81C6 preparations were determined using the antigen-positive human glioma line U251 MG clone 3 and the antigen-negative 2T human osteogenic sarcoma cell line. Serial dilutions of each labeled 81C6 preparation were added in quadruplicate to 96-well plates containing $5 \times 10^4$ cells/well and incubated overnight at 4°C. Affinity constants were determined from Scatchard plots of the binding data generated and analyzed using a computer program (27).

Biodistribution Measurements. Athymic mice (BALB/c, nu/nu) were obtained from a colony maintained at the Duke University Animal Isolation Facility and were injected in the right flank with 50 µl of D-54 MG tumor homogenate. Tissue distribution studies were begun when the average tumor volume was approximately 250 mm$^3$. In the first two experiments, mice were injected i.p. with 2–3 µg of 81C6 labeled with ATE and $1–2 \mu$g of 81C6 labeled with $^{131}$I using iodogen. In Experiment 1, groups of five mice were sacrificed by ether overdose at 1, 3, 4, 6, and 8 days after injection for paired-label biodistribution analysis. In the second experiment, groups of animals were studied at 1, 4, and 8 h and at 1, 2, 4, 5, 6, 7, and 8 days. In Experiment 3, mice received 3 µg of both $^{125}$I-labeled 81C6 and $^{131}$I-labeled 45.6, both radiiodinated using ATE. Animals were sacrificed at the same time intervals as Experiment 2, except that the 8-day group was omitted. Animals were dissected; tissues of interest were weighed and assayed for both $^{125}$I and $^{131}$I activity using the dual-channel gamma counter. A correction was applied for crossover of $^{131}$I activity into the $^{125}$I counting window. Results were expressed as percentage of the injected dose of MAb per gram tissue and as the ratio of nuclide in tumor per gram to that in each normal tissue per gram.

Mechanism of ATE Action. Although MABs labeled using ATE are probably less susceptible to dehalogenation because of the microenvironment of the radioiodine on the MAb, it is also possible that alterations in MAb structure created by the addition of m-iodobenzoic acid to lysines could render the MAB itself less likely to undergo deiodination in vitro. To investigate this possibility, 81C6 IgG was labeled with $^{131}$I using iodogen as described above. The MAb was also mock labeled using ATE and cold iodide at a substitution level of 2–3 iodines per MAb and then labeled with $^{131}$I using the iodogen method. Six BALB/c mice were injected with both preparations, sacrificed at 48 h, and the percentage injected dose of $^{125}$I and $^{131}$I localized in the thyroid was also determined.

RESULTS

Antibody Labeling. Yields for labeling 81C6 IgG with $^{131}$I ranged between 60% and 90% using the iodogen method, resulting in a labeled MAb with a specific activity of 6–7 µCi/µg (equivalent to a substitution level of about 0.3 iodine atoms per antibody molecule). Using the ATE method, N-succinimidyl 3-$^{125}$I or $^{131}$Iiodobenzoate was generated in 70–90% yield and 45–60% of this intermediate could be coupled to 81C6 following a 20 min reaction. The specific activity of 81C6 labeled using the ATE method ranged between 3 and 5 µCi/µg, also equivalent to an average substitution level of 0.3 iodines per antibody molecule. These results and those described below for evaluation of immunoreactivity are representative of more than ten preparations of 81C6 radioiodinated using ATE.

Experiment 1. In vitro binding studies were performed in order to compare the immunoreactivity of 81C6 radioiodinated by the two methods. The specific binding of 81C6 labeled using ATE was 68 ± 2% versus 57 ± 4% for MAb labeled using iodogen. Scatchard analyses of the binding data revealed that the affinity constant for 81C6 labeled using the ATE reagent, (5.4 ± 0.3) $\times 10^5$ M$^{-1}$, was significantly higher than that of 81C6 labeled using iodogen, (1.1 ± 0.1) $\times 10^5$ M$^{-1}$.

Pair-label biodistribution studies were performed in athymic mice bearing s.c. D-54 MG tumors to compare the inertness to dehalogenation and tumor retention of 81C6 IgG labeled with $^{131}$I using ATE and $^{131}$I using iodogen. Protein-associated activity, determined by trichloroacetic acid precipitation, was 97% for both preparations. As shown in Fig. 1, use of the ATE method reduced thyroid uptake of radioiodine to ≤0.1% of the injected dose, levels significantly less (P < 0.0001) than those observed for MAb labeled using iodogen (2.0–6.3%).

Twenty-four hours after injection, the tumor uptake of 81C6 labeled using ATE had reached 30.8 ± 6.7% ID/g, a value more than four times greater than that for MAb labeled using iodogen (7.0 ± 1.4% ID/g). At 8 days, the tumor delivery advantage for the ATE preparation had reached a factor of 12.6. Because of the rapid growth of the D-54 MG line, average tumor weight increased from 0.24 ± 0.13 g at Day 1 to 2.4 ± 0.4 g at Day 8. Integration of the area under the tumor uptake curves revealed that the cumulative activity in D-54 MG xenografts per mCi $^{131}$I was 28,500 µCi-h for 81C6 labeled via ATE, compared to

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\[ I^\text{131I} \] for MAb labeled with \[ I^\text{125I} \] using ATE and with \[ I^\text{131I} \] using Iodogen.

Tumor-to-normal-tissue ratios following injection of \( 81C6 \) radioiodinated using ATE were significantly higher than those for MAb labeled using Iodogen with the tumor specificity advantage increasing with time (Fig. 3A). Differences were most striking for tissues of the gastrointestinal tract as well as liver and spleen. Eight days after injection of the \( 81C6 \) ATE preparation, tumor-to-tissue ratios had reached 95, 131, 298, 77, 197, 30, and 807 to 1 for liver, spleen, stomach, kidneys, intestines, blood, and brain, respectively.

Experiment 2. In the second experiment, the specific binding of radioiodinated \( 81C6 \) prepared using ATE, 79 ± 2%, again was higher than \( 81C6 \) labeled using Iodogen, 68 ± 3%; however, the affinity constants for both MAbs were nearly identical, (4.1 ± 0.3) \( \times 10^8 \) \( \text{M}^{-1} \). Thyroid uptake of radioiodine was 40 to 100 times lower for the ATE labeled MAb in animals sacrificed between 1 and 8 days, with 4- to 16-fold differences observed from 1 to 8 h. The tissue distribution of radioiodine following the injection of \( 81C6 \) labeled with \( I^\text{131I} \) using the ATE reagent and with \( 125I \) using Iodogen is shown in Table 1. (Data for other time points is available upon request.) In general, when differences were observed, uptake of radioiodine in normal tissues was higher for ATE-labeled MAb from 1 h to 4 days, with the reverse behavior observed from 5 to 8 days. For example, no significant differences were observed in liver at 1, 3, 4, and 8 days while lower liver uptake for Iodogen-labeled \( 81C6 \) was seen at 8 h, 1 day, and 2 days (\( P < 0.02-0.01 \)). In contrast, liver uptake of ATE-labeled \( 81C6 \) was significantly lower than the Iodogen-labeled MAb at 5, 6, 7, and 8 days (\( P < 0.01-0.001 \)). Average tumor weights increased from 0.52 ± 0.10 g at 8 h to 3.7 ± 0.7 g at 8 days. For \( 81C6 \) labeled using ATE, tumor localization was greater than 20 %ID/g from 8 to 48 h (maximum 24.8 ± 3.5 %ID/g at 48 h). In contrast, tumor uptake of MAb labeled using Iodogen was maximal at 8 h (12.1 ± 2.3 %ID/g and decreased to 6.7 ± 1.3 %ID/g at 48 h). By 8 days, the tumor delivery advantage for ATE-labeled MAb reached a factor of 4.6. As shown in Fig. 2B, use of the ATE method for \( ^{131}\text{I} \) labeling of \( 81C6 \) IgG would result in a threefold greater cumulative activity in these tumors. As shown in Fig. 3B, tumor-to-normal-tissue ratios were comparable to those observed in the first experiment. Again, tumor-to-normal-tissue ratios for \( 81C6 \) labeled using the ATE method were superior at all time points to those for MAb labeled using Iodogen with

\[ ATE \quad \text{and} \quad 81C6 \quad \text{and} \quad 45.6 \quad \text{and} \quad \text{using} \quad ATE \quad \text{reagent} \]

\[ \begin{align*}
\text{ATE} & \quad 58,500 \text{ Ci-h} \\
\text{Iodogen} & \quad 5,100 \text{ Ci-h}
\end{align*} \]

\[ \begin{align*}
\text{ATE} & \quad 22,900 \text{ Ci-h} \\
\text{Iodogen} & \quad 7,200 \text{ Ci-h}
\end{align*} \]

\[ \begin{align*}
\text{ATE} & \quad 24,350 \text{ Ci-h} \\
45.6 & \quad 5,300 \text{ Ci-h}
\end{align*} \]

Fig. 2. Cumulative activity in s.c. D-54 MG xenografts estimated for a dose of 1 mCi \( 81C6 \) labeled by the ATE and Iodogen methods. Activity in Ci-h calculated from %ID/g biodistribution data and area-under-the-curve obtained by trapezoid integration method. A, Experiment 1; B, Experiment 2; C, Experiment 3. Dashed lines, fit of the shape of the activity curve from B to obtain estimate for Ci-h over a 0 to 8-day interval.
the difference increasing with time.

Experiment 3. In order to investigate the specificity of MAb uptake, a paired-label study was performed in which 81C6 was labeled with $^{131}$I, and 45.6, a nonspecific isotype-matched control MAb, was labeled with $^{125}$I, both using the ATE method. Specific binding for the 81C6 preparation was 58 ± 6%. The mean tumor weights in this experiment increased from 0.057 ± 0.034 g at 1 h to 0.84 ± 0.41 g at 7 days. Uptake of $^{131}$I-labeled 81C6 in D-54 s.c. tumors reached 23.1 ± 2.3 %ID/g by 8 h and increased to a maximum of 32.5 ± 5.3 %ID/g at 48 h. In contrast, accumulation of $^{125}$I-labeled 45.6 reached a maximum level of 7.5 ± 1.0 %ID/g at 8 h and declined to approximately half that value by 48 h. A cumulative activity in tumor of 24,350 μCi-h per hypothetical 1-mCi dose of $^{131}$I was calculated for 81C6, compared to 3,300 μCi-h for 45.6 control.

The specificity of 81C6 localization after radiohalogenation using the ATE reagent was examined by calculating the localization index, defined as the ratio of specific 81C6 MAb in tissue to nonspecific 45.6 MAb in tissue, normalized to simultaneous blood levels. Localization indices for tumor as well as tissue to nonspecific 45.6 MAb in tissue, normalized to simultaneous blood levels. Localization indices for tumor as well as normal tissues ranged between 0.6 and 1.7 at all time points. For radioiodinated MAbs, increased catabolism of label as a consequence of binding to tumor might be reflected by greater thyroid uptake of activity following injection of specific compared to control MAb. As shown in Table 2, thyroid accumulation following 81C6 administration was higher than that resultant from 45.6 injection, with a statistically significant difference seen at seven time points ($P < 0.25$ to $P < 0.1$). To minimize the contribution of uncertainty related to variations in individual animals and tissue sampling, these data were also analyzed by calculating the 81C6/45.6 thyroid uptake ratio for each animal and then determining the mean value of this ratio. With the exception of the 5-day group, significantly higher thyroid uptake ($P < 0.0001$ to $P < 0.025$) was observed for $^{131}$I-labeled 81C6, with the difference ranging from a factor of 1.76 ± 0.22 at 1 h to 2.61 ± 0.64 at 2 days.

Mechanism of ATE Action. In order to determine whether derivitization of MABs with m-iodobenzoic acid via the ATE reagent could also decrease the dehalogenation of conventionally radioiodinated MABs, the Iodogen method was used to label 81C6 MAb both before and after mock iodination of the MAb using ATE. In this paired-label study, no significant difference in thyroid uptake of radioiodine was observed between the two preparations (Iodogen, 4.9 ± 0.7%; cold ATE iodination followed by Iodogen labeling, 5.1 ± 1.9%).

Radiation Dosimetry. Tissue distribution data obtained from Experiments 2 and 3 were used to calculate the radiation doses which would result following the administration of 1 mCi of $^{131}$I-labeled MABs (Tables 3 and 4). In Experiment 2, an estimated 9,877 rads would be delivered to these D-54 MG s.c. tumors from 81C6 labeled with $^{131}$I using the ATE method, a value more than three times greater than for MAb labeled using Iodogen (Table 3). Radiation doses received by normal tissues for ATE labeled MAb ranged from 66 rads for brain to 2,476 rads for blood pool, values quite similar to those calculated for MAb labeled using Iodogen. In Table 4, radiation absorbed doses for $^{131}$I-labeled 81C6 and 45.6, both radiohalogenated using the ATE reagent, are compared. Radiation dose to tumor from 1 mCi of $^{131}$I-labeled 81C6 was estimated as 10,502 rads compared to 1,423 rads for nonspecific 45.6 MAb. Normal tissue radiation doses for 81C6 MAb ranged from 66 rads for brain to 2,476 rads for blood pool, values quite similar to those calculated for MAb labeled using Iodogen. In Table 4, radiation absorbed doses for $^{131}$I-labeled 81C6 and 45.6, both radiohalogenated using the ATE reagent, are compared. Radiation dose to tumor from 1 mCi of $^{131}$I-labeled 81C6 was estimated as 10,502 rads compared to 1,423 rads for nonspecific 45.6 MAb. Normal tissue radiation doses for 81C6 MAb ranged from 66 rads for brain to 2,359 rads for blood. For $^{131}$I-labeled 45.6, 50–100% higher radiation absorbed doses were calculated for most normal tissues.

**DISCUSSION**

Perhaps the most significant impediment to widespread clinical application of labeled MABs is the fact that the amount of nuclide retained in tumor is quite low, rendering detection of malignancies in the face of normal tissue background problematic and, in general, limiting radiation doses to tumor below therapeutic levels. Although utilization of more ideal MABs and pharmacologically advantageous routes of administration (9, 28) may help increase concentration of MABs in tumor, a critical problem which must be addressed is the loss of radioiodinated MAbs from the MBP prior to (and possibly as a consequence of) localization in tumor.

For radioiodinated MABs, it is generally assumed that loss of radioiodine from these proteins following in vivo administration can be attributed to the action of dehalogenases normally involved in the metabolism of thyroid hormones (19). Hypothesizing that decreasing the structural similarity between the MAB iodination site and these enzymatically degradable com-
pounds would increase the retention of iodine on the MAb in vivo, we developed a new approach for MAb labeling which utilizes ATE as an iodination intermediate (20). Although it is unlikely, modification of MAbs using ATE could also render the MAb itself less susceptible to dehalogenation via an indirect effect, such as steric interference with deiodinases. The results of this study indicate that modification of a MAB using ATE does not increase the in vivo stability of MAB labeled using a conventional radioiodination method and thus supports the hypothesis that it is the nature of the iodination site which is responsible for the inertness to dehalogenation imparted to MAbs radioiodinated using ATE.

Because of the proclivity of free iodide for the thyroid, accumulation of radiiodine activity in this tissue is often used as an indicator of dehalogenation. However, because the small
size of the mouse thyroid can lead to errors in organ removal, paired-label studies are essential in order to compare the in vivo stability of different labeled preparations. Labeling 81C6 using the ATE reagent reduced thyroid uptake of radioiodine to levels 40–100 times less than those observed in the same mice for MAb labeled by the iodogen method. In a previous paired-label study, we reported that use of the ATE method for labeling a murine MAb F(ab')2 fragment reduced thyroid accumulation by 40–100 times less than those observed in the same mice for MAbs labeled using the iodogen method. In addition, decreased loss of label from MAbs in the circulation decreases the activity which is available for binding to tumor. However, an additional problem which must be considered is the catabolism of labeled MAbs as a consequence of binding to the tumor. Loss of label from MAbs in the circulation decreases the activity which is available for binding to tumor. However, an additional problem which must be considered is the catabolism of labeled MAbs as a consequence of binding to the tumor.

Results of several investigations using MAbs radioiodinated using standard methods suggest that the dehalogenation of tumor-directed MAbs in animals bearing tumors is greater than that in animals without tumors (16–18). In addition, decreased loss of label from control MAbs in tumor-bearing animals has also been observed (16–18). In the present paired-label study comparing the biodistribution of 81C6 and 45.6 control MAbs, both labeled using the ATE method, thyroid uptake related to the tumor-directed MAb was twice as high as from nonspecific MAb. However, the thyroid uptake differential was only about 0.1% of the injected dose, suggesting the possibility that, in some cases, MAbs labeled using the ATE method may be less susceptible to tumor-mediated catabolism.

The most important aspect of this investigation is the demonstration that utilization of the ATE method for the radioiodination of 81C6 MAb increases the tumor uptake of radioactivity considerably without increasing the retention of label in normal tissues. It might be expected that rendering a MAb more inert to dehalogenation might increase the concentration of activity in tissues, particularly the liver, a tissue known to contain deiodinases (30). Although a small but significant increase in liver uptake was observed for 81C6 labeled using ATE at 8–48 h, the opposite trend was seen at later time points, presumably reflecting the rapid clearance of m-iodobenzoic acid, a catabolite of MAbs labeled using the ATE reagent, from the body (21).

A potential problem with using liver homogenates to determine the nonspecific binding of radioiodinated MAbs is underestimation of binding due to dehalogenation. Thus it might be expected that due to differences in susceptibility to dehalogenation, the nonspecific binding for MAb labeled using iodogen would be lower than that for MAb labeled using ATE, resulting in an overestimate of the specific binding for iodogen-labeled 81C6. This problem was avoided by running the binding assay at 4°C, a temperature at which deiodinase activity is drastically inhibited (30). Indeed, no differences in liver homogenate binding between 81C6 radioiodinated via the two methods has been observed in either these or other immunoreactivity assays.

In the first experiment, the immunoreactivity, in terms of both specific binding and affinity, was higher than MAb labeled via iodogen. The tumor delivery advantage was observed to increase from 4.4-fold at Day 1 to more than 12-fold at Day 8. In the second experiment, performed with preparations of identical affinity but with slightly higher specific binding for MAbs labeled via ATE, the tumor delivery advantage for 81C6 labeled using ATE ranged between 2.7 and 4.6. We infer that increased retention of label in tumor reflects the greater in vivo stability of the carbon-iodine bond in MAb labeled using the ATE method. Moreover, these results are in agreement with previous reports which emphasize the influence of MAb affinity on tumor uptake (21, 31, 32).

A recent report has described a conceptually similar approach for MAb labeling through the conjugation of PIP to MAbs (33). In studies performed in separate groups of mice receiving MAbs labeled using PIP and chloramine T, lower uptake in the neck region was reported for animals receiving MAb labeled using PIP. However, the tumor uptake of an intact MAb, an F(ab')2 fragment, and a Fab fragment labeled using PIP were all lower than when the chloramine T method was used. It is possible that differences between our results and this report are related to differences in MAb catabolism. However, our previous studies have shown that purification of the iodinated intermediate is essential for maintaining MAb affinity (21), and the PIP method does not include an isolation of the PIP reagent from excess stannyl precursor.

Use of a more optimal MAb-labeling method might provide a more accurate indication of the specificity of MAb localization since loss of label from MAb bound to tumor cells could decrease the apparent localization index, particularly at longer time points. In previous studies using 81C6 and 45.6 labeled using the chloramine T method, the localization index in s.c. D-54 MG glioma xenografts ranged from about 10 to 15 at Days 5 to 7, respectively (34, 35). Using the same MAbs labeled using the ATE method, localization indices were more than twice as high, ranging between 28 and 34 during the same time interval. Thus, the intrinsic in vivo specificity of 81C6 MAb may have been underestimated in previous studies.

Of critical importance for radioimmunotherapy is the ability to increase radiation dose to tumor while maintaining radiation dose to normal tissues at acceptable levels. In the D-54 MG xenograft model, use of the ATE method for labeling 81C6 MAb increased the dose to tumor by more than a factor of three over MAb labeled using a conventional method. Previous studies have shown that rapid growth of D-54 MG xenografts occurs at tracer versus therapeutic 131I levels, resulting in 35–52% underestimates in tumor dose; thus the tumor doses delivered per mCi in a therapeutic evaluation of 81C6 labeled with 131I using the ATE method are likely to be even higher than those described herein. Doses to normal tissues from the two 81C6 preparations were quite similar. Tumor-to-normal-tissue dose ratios for ATE-labeled 81C6 ranged from 4:1 for blood to more than 10:1 for most other tissues.

In summary, these results suggest that the ATE method is a valuable approach for the radioiodination of monoclonal anti-

Table 4  Radiation absorbed dose to mice bearing D-54 MG s.c. tumors after administration of 1 mCi of 131I-labeled 81C6 or 45.6

<table>
<thead>
<tr>
<th>Tissue</th>
<th>131I-81C6</th>
<th>131I-45.6</th>
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</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>10,502</td>
<td>1,423</td>
</tr>
<tr>
<td>Brain</td>
<td>57</td>
<td>84</td>
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<tr>
<td>Liver</td>
<td>949</td>
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<td>1,712</td>
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<tr>
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bodies. We have demonstrated that use of the ATE method for labeling 81C6 MAb resulted in increased tumor uptake and retention of activity and yielded significantly higher tumor-to-normal-tissue dose ratios. Experiments are in progress to evaluate the therapeutic potential of MABS radiolabeled using the ATE reagent.

REFERENCES


Enhanced Tumor Localization and in Vivo Stability of a Monoclonal Antibody Radioiodinated Using N-Succinimidyl 3-(Tri-n-butylstannyl)benzoate


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