Preclinical Assessments of 90Y-labeled C110 Anti-Carcinoembryonic Antigen Immunotoxin: A Therapeutic Immunoconjugate for Human Colon Cancer

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ABSTRACT

We have synthesized 90Y-labeled immunotoxin (IT) containing ricin A chain and C110 anti-carcinoembryonic antigen monoclonal antibody (MAb) to produce a therapeutic immunoconjugate for human colon cancer. The C110 IT was labeled with 90Y via a benzylisothiocyanate derivative of diethylenetriaminepentaacetic acid. The efficiency of 90Y labeling was consistently 90 to 98%, with a specific activity of about 1 μCi/μg. In vitro stability studies, more than 80% of 90Y remained bound to the C110 IT for up to 5 days after incubation. The percentage of binding of 90Y-labeled C110 IT to carcinoembryonic antigen-coated microbeads was 86%, indicating good retention of the initial immunoreactivity of the C110 MAb. In vivo protein synthesis inhibition assays, 90Y-labeled C110 IT was approximately 3.7-fold more toxic to the LS174T human colon carcinoma cell line than unmodified C110 IT and 1380-fold more toxic than 90Y-labeled C110 MAb. Biodistribution studies of 90Y-labeled C110 IT in LS174T tumor-bearing mice showed that, at 24 h following i.p. injection, high accumulation of radioactivity was seen in the i.p. tumor and liver and, thereafter, high accumulation in these tissues remained almost unchanged until up to 168 h, with percentage of injected dose/g ranging from 15 to 18% in the tumor and 10 to 15% in the liver. The radioactivity in the spleen and bone gradually increased with time and reached their highest levels (approximately 8% of injected dose/g) at 168 h. Estimation of absorbed radiation doses to the tissues showed that i.p. tumor would have received an approximately 1.5 to 7 times higher radiation dose than normal organs. In vivo therapeutic trials, 90Y-labeled C110 IT provided survival prolongation of LS174T tumor-bearing mice superior to that with either unmodified C110 IT or 90Y-labeled C110 MAb (p < 0.01; Mann-Whitney U test). These results indicate that 90Y-labeled C110 anti-carcinoembryonic antigen IT may be a potent therapeutic immunoconjugate for human colon cancer and that it may have direct relevance for i.p. treatment of peritoneal carcinomatosis from colon cancers.

INTRODUCTION

Targeted cancer therapy using immunoconjugates, MABs coupled to the various kinds of cytotoxic agents, is receiving intense scrutiny in current cancer therapy research. Among these immunoconjugates, ITs containing ricin A chain and MABs have shown selective cytotoxicity in vitro for target cells (1-10) and antitumor activity in vivo in tumor-bearing mice (11, 12). The cytotoxic activity of ITs is based upon the internalization of ricin A chain into the cytoplasm, which results in inhibition of ribosomal protein synthesis. Large molecules such as ITs must be internalized in a cancer cell to cause cell killing.

Thus, incomplete internalization of ITs in all of the cancer cells may limit the effectiveness of this form of therapy. Since MABs labeled with therapeutic radionuclides produce cytotoxic effects by their emitted high energy particles, internalization of the radionuclides is not necessary for radiolabeled MABs to produce their cytotoxic effects. In addition to the cytotoxic effects on labeled cells, adjacent cells with or without surface antigen expression also can be damaged. Therefore, radionlabeling of ITs with therapeutic radionuclides may augment the effectiveness of ITs and overcome the problem of ITs described above, thereby providing more potent reagents for cancer therapy than ITs or radiolabeled MABs alone. For this purpose, we have synthesized an IT containing ricin A chain and C110 anti-CEA MAb, further labeled with 90Y, which is one of the radionuclides being used in radioimmunotherapy (13).

In this paper, we describe our preclinical assessments of 90Y-labeled C110 anti-CEA IT, as compared with unmodified C110 IT and 90Y-labeled C110 MAB. The results obtained in this study indicate that 90Y-labeled C110 IT may be a potent therapeutic immunoconjugate for human colon cancer.

MATERIALS AND METHODS

Monoclonal Antibody and Immunotoxin. The C110 MAb (IgG1) directed against CEA was obtained from Abbott Laboratories (Abbott Park, IL) as a MAB-DTPA conjugate. The production of C110 MAB and its coupling to the benzylisothiocyanate derivative of DTPA (14) have been described by Sumerdon et al. (15). The average number of chelating groups incorporated into each antibody, determined by a 51Co binding assay (16), was 4 DTPA groups/antibody molecule.

C110 IT-DTPA conjugates were prepared in our laboratory by coupling native ricin A chain (Inland Laboratories, Austin, TX) to the C110 MAB-DTPA conjugate via disulfide linkage, as described previously (7, 8). Briefly, C110 MAB-DTPA conjugates were reacted with a 15- to 20-fold molar excess of N-succinimidyl-3(2-pyridyldithio)propionate reagent (Pharmacia, Piscataway, NJ) (17). The reaction proceeded at room temperature for 30 min, after which time reaction products were dialyzed against PBS for 16 h at 4°C. The N-succinimidyl-3(2-pyridyldithio)propionate-derivatized C110 MAB-DTPA conjugates were then reacted with a 5-fold molar excess of ricin A chain overnight at room temperature. The resulting C110 IT-DTPA conjugates were purified by gel filtration on a Sephadex G-200 column and then analyzed by sodium dodecyl sulfate-polycrylamide gel electrophoresis (18). The immunoreactivity of C110 IT-DTPA conjugate was assessed by an enzyme linked immunosorbent assay, and immunoreactivity comparable to that of the underivatized C110 MAB was shown (19, 20).

Labeling of C110 IT-DTPA and MAB-DTPA Conjugates with 90Y. The 90Y used in this study was obtained from a high activity 90Sr/90Y radionuclide generator developed in our laboratory. The construction of the generator, its elution, and the conversion of 90Y-EDETA to the acetate complex have been described by Hnatowich et al. (19, 20).

Before labeling with 90Y, the C110 IT-DTPA conjugate at 1 mg/ml was dialyzed against 4 liters of 6.0 mM DTPA-50 mM Na2CO3-0.9% NaCl-trace metal-free solution, pH 8.0, at 4°C, using a sulfur-free EDTA-treated cellulose dialysis tube (Spectrum, Los Angeles, CA), for 2 days. The conjugate was then dialyzed against 50 mM Na2CO3-0.9% NaCl.
NaCl-TMF solution, pH 8.0, at 4°C, to remove free DTPA. C110 IT-DTPA and MAB-DTPA conjugates were mixed with carrier-free 90Y-acetate and incubated at room temperature for 30 min. Labeling efficiency in the final samples was determined by ITLC (21). Briefly, 90Y-labeled C110 IT or MAB was incubated with 0.05 M DTPA, pH 6.0, at room temperature for 10 min. A 3-μl aliquot of each sample was spotted onto an ITLC-SG strip (1 x 8 cm; Gelman Sciences, Ann Arbor, MI). The ITLC strips were developed with 0.9% NaCl solution as solvent. In this system, protein-bound 90Y remains at the origin of the strip, whereas free 90Y moves with the solvent front. After the solvent moved to the top of the strip, the strip was dried, cut in half, and counted in a gamma counter (Packard Instrument, Downers Grove, IL), with the energy window set to 50 to 500 keV. The percentage of applied 90Y bound to the protein was calculated by comparing the net cpm of the origin section of the strip with that of both origin and solvent front sections.

To determine the in vitro stability of incorporated 90Y, 90Y-labeled C110 IT or MAB was incubated in human serum at 37°C. The percentage of 90Y radioactivity retained in the IT or MAB was measured by ITLC over a period of 5 days.

Immunoreactivity of 90Y-labeled C110 IT and MAB was determined by a binding assay using CEA-coated Sepharose beads (15). Briefly, a 50-μl aliquot of 90Y-labeled C110 IT or MAB, previously diluted with PBS to a conjugate concentration of 0.5 μg/ml, was added to 0.5 ml of a 50% (v/v) CEA-coated bead suspension in a 10- x 75-mm test tube. The test tube was sealed with Parafilm and the contents were mixed end-over-end at 30°C for 1 h. The test tube with mixed contents was then counted in a gamma counter. After counting, an additional 2.5 ml of PBS was added into the test tube and the tube was centrifuged to pellet the beads. The beads were washed by resuspension in 2.5 ml of PBS and resuspension, with this being repeated three times. The final bead pellet was resuspended in 0.5 ml of PBS and counted again in a gamma counter. The percentage of radiolabeled C110 IT or MAB bound to the CEA-coated beads was determined by comparing the net cpm of the test tube contents after washing with that before washing.

In Vitro Cytotoxicity Assay. Cytotoxicity was measured by inhibition of protein synthesis, as described previously (19). Briefly, LS174T human colon carcinoma cells (22), suspended in leucine-free minimum essential medium, were placed into a 96-well flat-bottomed microtiter plate (Dynatech Laboratories, Arlington, VA), 5 x 10⁴ cells in a final volume of 200 μl/well. The cells were then incubated with PBS alone (control) or with specific additions of 90Y-labeled IT (5.1 x 10⁻⁴ M, 5.1 x 10⁻⁵ M, 5.1 x 10⁻⁶ M, or 5.1 x 10⁻⁷ M) for 18 h at 37°C in 5% CO₂. Similar experiments were performed with the unmodified C110 IT and 90Y-labeled C110 MAB. The medium was then removed and replaced with leucine-free minimum essential medium supplemented with [³H]leucine (New England Nuclear, Boston, MA) at a concentration of 1.5 μCi/well in 200 μl. Following a 1-h incubation at 37°C in 5% CO₂, the microtiter plates were frozen for 4 weeks to allow for 90Y decay. Following a 10-min treatment with 0.05% trypsin-0.02% EDTA (100 μl/well), the cells were harvested onto glass fiber filters using a MASH II cell harvester (Microbiological Associates, Bethesda, MD) to remove any [³H]leucine that was not incorporated into cellular protein. The glass fiber discs were dried, and [³H]leucine incorporation was determined by liquid scintillation counting. The average cpm of the quadruplicate treated cell samples was calculated and compared with that of the untreated control cell samples. The results were expressed as the percentage of control response.

In enhancing studies with monensin (a carboxylic ionophore) (23, 24), the cells were preincubated with 10⁻⁷ M monensin (Calbiochem, La Jolla, CA) for 1 h and then treated with specific additions of 90Y-labeled C110 IT or unmodified C110 IT (1.6 x 10⁻⁷ M, 1.6 x 10⁻⁸ M, or 1.6 x 10⁻⁹ M). Incorporation of [³H]leucine into cellular protein was determined as described above.

Animal Tumor Model. Six-week-old female BALB/c nu/nu mice weighing 20 to 25 g (Charles River Biotechnology Services, Wilmington, MA) were first given i.p. injections of 1 ml of Pristane (2,6,10,14-tetramethylpentadecane) to induce ascites. Two weeks later, mice were given i.p. injections of 1.5 x 10⁴ LS174T cells in 0.5 ml of PBS. Approximately 10 to 14 days after injection of tumor cells, the mice developed i.p. solid tumors with malignant ascites. Transplantability of the tumor with this method was greater than 99%.

Biodistribution. To determine the biodistribution of 90Y-labeled C110 IT, the mice were given i.p. injections of 120 μCi of 90Y-labeled C110 IT (specific activity, 1 μCi/μg) 2 weeks after inoculation of tumor cells. At 6, 24, 72, 120, and 168 h after radiolabel injection, groups of mice (three mice/group) were euthanized by ether inhalation. Solid tumors in the i.p. cavity and selected organs were removed, weighed, and counted in a gamma counter, along with a standard of the injectate. Samples were counted while immersed completely in 1 ml of water, to establish a linear relationship between counting rate and tissue size (19). The radioactivity in each tissue was expressed as the percentage of ID/g of tissue. The values of percentage of ID/g were normalized to a body weight of 25 g by multiplying by body weight/25.

Furthermore, in order to compare the biodistributions of 90Y-labeled C110 IT and MAB, the mice were given i.p. injections of a known amount (120 μCi) of one of the 90Y-labeled immunoclonjugates. At 24, 72, and 120 h after radiolabel injection, groups of mice (two mice/group) were sacrificed by ether inhalation and frozen rapidly in liquid nitrogen. The mice were then processed for quantitative whole-body autoradiography, and videodensitometric measurements of radioactivity in tumor and selected organs were performed as described previously (25). The results were expressed as percentage of ID/g of tissue.

Radiation Dosimetry. The cumulative radiation doses absorbed by the tumor and major organs were calculated from the biodistribution data for 90Y-labeled C110 IT in LS174T tumor-bearing mice. Assuming that 90Y activity was uniformly distributed throughout the organs and that all of the β-decay energy emitted was absorbed in the source organs, absorbed radiation dose (D) is given by the equation for the uniform isotropic model in the Medical Internal Radiation Dose Pamphlet No. 1, Revised (26):

\[
D(\text{rad}) = \frac{\sum \Delta \alpha}{C}
\]

where C is the cumulated mean activity per unit mass in μCi·h/g and \(\Delta \alpha\) is the equilibrium absorbed dose constant in g·rad/μCi·h. The values of C for the tumor and major organs were calculated from the integration of time-activity curves, which were generated by fitting the biodistribution data to three-exponential retention curves using linear and nonlinear least squares regression techniques. The equilibrium absorbed dose constant for 90Y is 1.99 g·rad/μCi·h (27).

In Vivo Therapeutic Trials. Two weeks after inoculation of tumor cells, the mice were treated with (a) PBS (control), (b) unmodified C110 IT (80 μg), (c) 90Y-labeled C110 MAB (80 μg with specific activity of radiolabel of 1 μCi/μg), (d) 90Y-labeled C110 IT (80 μg with specific activity of radiolabe of 1 μCi/μg), or (e) a mixture of b and c. Each mouse received a 1-ml injection of immunoclonjugates or PBS i.p. once a week for 4 weeks. Deaths were monitored daily and recorded when they occurred. Statistical comparison of survival curves was accomplished by the log-rank (Mann-Whitney U) test.

RESULTS

Preparation of 90Y-labeled C110 IT. Analysis of C110 IT-DTPA conjugates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that one to three molecules of ricin A chain were coupled with one molecule of antibody and that the percentage of C110 MAB-DTPA conjugates coupled with ricin A chain was approximately 80%.

The efficiency of labeling of C110 IT-DTPA conjugates with 90Y was consistently 90 to 98%, resulting in a specific activity of about 1 μCi/μg. Similar labeling yields were obtained with 90Y-labeled C110 MAB.

The 90Y-labeled C110 IT was evaluated for its in vitro stability in human serum by ITLC (Fig. 1). After a 1-day incubation in human serum at 37°C, 93% of 90Y was retained in the IT; after a 5-day incubation, 80% of 90Y was still associated with the IT. These results were comparable to those for 90Y-labeled C110 MAB.
The immunoreactivity of 90Y-labeled C110 IT was compared with that of 90Y-labeled C110 MAb by a binding assay using CEA-coated microbeads. The percentage of binding of 90Y-labeled C110 IT and MAb under conditions of approximately infinite antigen excess was 86 and 98%, respectively, indicating excellent retention of the initial immunoreactivity of the C110 MAb during the process of preparing these immunoconjugates.

**In Vitro Cytotoxicity Assay.** Dose-response curves for inhibition of [3H]leucine incorporation in LS174T cells by 90Y-labeled C110 IT, unmodified C110 IT, and 90Y-labeled C110 MAb were shown in Fig. 2. Both 90Y-labeled C110 IT and unmodified C110 IT had comparable cytotoxic effects on the LS174T cell line and reduced [3H]leucine incorporation in the cellular protein with an IC50 of 2.1 x 10^{-6} M and 7.7 x 10^{-9} M, respectively. These cytotoxic effects were approximately 1380- and 380-fold more potent than that of 90Y-labeled C110 MAb (IC50, 2.9 x 10^{-6} M).

Dose-response curves for inhibition of [3H]leucine incorporation in LS174T cells by 90Y-labeled C110 IT and unmodified C110 IT in the presence of monensin are shown in Fig. 3. The cytotoxic effects of both ITs on the LS174T cell line were slightly enhanced by 10^{-7} M monensin; their IC50 values were 9.0 x 10^{-10} M and 1.7 x 10^{-9} M, respectively.

**Biodistribution.** The biodistribution of 90Y-labeled C110 IT at various times after i.p. injection into LS174T tumor-bearing mice is summarized in Table 1. The disappearance of radiolabel from the ascites was primarily due to its accumulation in the i.p. tumor and liver and its absorption into the blood at up to 24 h. At 24 h following injection, high accumulation of radiolabel was seen in the tumor and liver, approximately 15 and 10% ID/g, respectively. Thereafter, high accumulation of radiolabel in these tissues remained almost unchanged until up to 168 h. The radioactivity in the blood showed its peak level of 7.28% ID/g at 24 h and thereafter declined with time. The radioactivity levels in the spleen and bone, which were lower than that in the blood at 24 h, gradually increased with time and reached their highest values at 168 h (7.85 and 7.98% ID/g, respectively). The splenic mass in the animals sacrificed at 120 and 168 h decreased by 2- to 3-fold. The radioactivity in other tissues showed peak levels at 24 to 72 h and then decreased when blood radioactivity cleared after 120 h.

The comparative biodistributions of 90Y-labeled C110 IT and MAb in LS174T tumor-bearing mice following i.p. injection were determined by quantitative whole-body autoradiography (Table 2). Representative autoradiograms of the tumor-bearing mice are shown in Figs. 4 and 5. The accumulation of 90Y-labeled C110 IT in the liver was almost 1.5 times more than that of 90Y-labeled C110 MAb at all times. On the other hand, radioactivity of 90Y-labeled C110 IT in the blood decreased more rapidly than that of 90Y-labeled C110 MAb. In the other organs, as well as the tumor, no significant difference was seen between the biodistributions of 90Y-labeled C110 IT and MAb. In addition, the biodistribution of 90Y-labeled C110 IT determined by quantitative autoradiography was almost the same as that determined by tissue counting.

**Radiation Dose Estimates.** Estimates of radiation doses absorbed by the tumor and major organs in LS174T tumor-bearing mice given i.p. injections of 120 µCi of 90Y-labeled C110 IT and 90Y-labeled C110 MAb are summarized in Table 2. Representative autoradiograms of the tumor-bearing mice are shown in Figs. 4 and 5. The accumulation of 90Y-labeled C110 IT in the liver was almost 1.5 times more than that of 90Y-labeled C110 MAb at all times. On the other hand, radioactivity of 90Y-labeled C110 IT in the blood decreased more rapidly than that of 90Y-labeled C110 MAb. In the other organs, as well as the tumor, no significant difference was seen between the biodistributions of 90Y-labeled C110 IT and MAb. In addition, the biodistribution of 90Y-labeled C110 IT determined by quantitative autoradiography was almost the same as that determined by tissue counting.

**Table 1.** Biodistribution of 90Y-labeled C110 IT in LS174T tumor-bearing mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>6 h</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>10.34 ± 6.35</td>
<td>14.85 ± 7.88</td>
<td>18.04 ± 9.12</td>
<td>17.36 ± 5.47</td>
<td>14.28 ± 2.34</td>
</tr>
<tr>
<td>Heart</td>
<td>0.46 ± 0.22</td>
<td>2.31 ± 0.45</td>
<td>2.03 ± 0.37</td>
<td>1.85 ± 0.28</td>
<td>0.85 ± 0.14</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.93 ± 0.19</td>
<td>2.73 ± 0.32</td>
<td>4.46 ± 0.53</td>
<td>2.19 ± 0.25</td>
<td>1.00 ± 0.06</td>
</tr>
<tr>
<td>Liver</td>
<td>4.11 ± 0.84</td>
<td>9.72 ± 2.18</td>
<td>11.47 ± 1.87</td>
<td>14.78 ± 3.18</td>
<td>13.33 ± 1.37</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.62 ± 0.68</td>
<td>3.99 ± 2.14</td>
<td>5.31 ± 1.25</td>
<td>5.94 ± 1.79</td>
<td>7.85 ± 2.81</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.61 ± 0.47</td>
<td>4.67 ± 0.52</td>
<td>6.52 ± 0.70</td>
<td>3.68 ± 0.61</td>
<td>2.06 ± 0.33</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.37 ± 0.03</td>
<td>1.60 ± 0.37</td>
<td>1.48 ± 0.12</td>
<td>0.96 ± 0.14</td>
<td>0.72 ± 0.10</td>
</tr>
<tr>
<td>Bone</td>
<td>0.35 ± 0.06</td>
<td>2.91 ± 0.53</td>
<td>5.27 ± 1.54</td>
<td>6.80 ± 2.27</td>
<td>7.98 ± 1.98</td>
</tr>
<tr>
<td>Blood</td>
<td>1.89 ± 0.82</td>
<td>7.28 ± 5.37</td>
<td>4.93 ± 3.30</td>
<td>0.94 ± 0.41</td>
<td>0.67 ± 0.25</td>
</tr>
<tr>
<td>Ascites</td>
<td>29.01 ± 3.23</td>
<td>8.75 ± 4.71</td>
<td>3.48 ± 0.64</td>
<td>0.83 ± 0.21</td>
<td>0.36 ± 0.08</td>
</tr>
</tbody>
</table>

* Mean ± SD; n = 3.

**Table 2.** Radiation Dose Estimates

<table>
<thead>
<tr>
<th>Tissue</th>
<th>6 h</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>29.01 ± 3.23</td>
<td>8.75 ± 4.71</td>
<td>3.48 ± 0.64</td>
<td>0.83 ± 0.21</td>
<td>0.36 ± 0.08</td>
</tr>
</tbody>
</table>

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Table 2 Videodensitometrically determined biodistribution of \(^{90}\)Y-labeled C110 IT and MAb in LS174T tumor-bearing mice

Mice were inoculated i.p. with \(1.5 \times 10^7\) LS174T cells. Two weeks later, the mice were given i.p. injections of 120 \(\mu\)Ci of \(^{90}\)Y-labeled C110 IT or MAb (specific activity, 1 \(\mu\)Ci/\(\mu\)g). Radioactivity in tissues was determined by quantitative whole-body autoradiography and is expressed as the percentage of ID/g of tissue at 24, 72, and 120 h following radiolabel injection. The values for percentage of ID/g were normalized to a body weight of 25 g by multiplying by body weight/25. The tabulated values are geometric means of percentage of ID/g from two mice.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>(^{90})Y-labeled C110 IT</th>
<th>(^{90})Y-labeled C110 MAb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
</tr>
<tr>
<td>Tumor</td>
<td>13.4</td>
<td>16.2</td>
</tr>
<tr>
<td>Liver</td>
<td>9.9</td>
<td>12.8</td>
</tr>
<tr>
<td>Spleen</td>
<td>5.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>6.9</td>
<td>5.2</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Bone</td>
<td>4.9</td>
<td>7.6</td>
</tr>
<tr>
<td>Blood</td>
<td>6.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Fig. 5. Whole-body autoradiograms of LS174T tumor-bearing mice at 24 h (a), 72 h (b), and 120 h (c) following i.p. injection of \(^{90}\)Y-labeled C110 MAb. Dark areas correspond to high concentrations of radioactivity. The general radiographic patterns of \(^{90}\)Y-labeled C110 MAb were almost identical to those of \(^{90}\)Y-labeled C110 IT shown in Fig. 4. However, the radioactivity in the liver is relatively lower and that in the blood is relatively higher, as compared with \(^{90}\)Y-labeled C110 IT (see quantitative analysis in Table 2). Arrows, i.p. tumors; Bl, blood; L, liver; B, bone.

C110 IT are shown in Table 3. The doses represent the cumulative radiation doses to the tissues over 168 h and were calculated based on the biodistribution data shown in Table 1. The cumulative radiation dose to the i.p. tumor was calculated as 2780 rad, approximately 1.5 to 7 times greater than those to the normal organs, which range from a high of 1850 rad for the liver to a low of 400 rad for the lungs.

In Vivo Therapeutic Trials. The survival curves of LS174T tumor-bearing mice treated with PBS or various immunoconjugates are shown in Fig. 6. Significant prolongation of survival was achieved in all immunoconjugate-treated groups, in comparison to the control PBS group (\(P < 0.03\) for unmodified C110 IT and \(^{90}\)Y-labeled C110 MAb; \(P < 0.001\) for \(^{90}\)Y-labeled C110 IT and a mixture of unmodified C110 IT and \(^{90}\)Y-labeled C110 MAb). Moreover, \(^{90}\)Y-labeled C110 IT and a mixture of unmodified C110 IT and \(^{90}\)Y-labeled C110 MAb provided survival prolongation of LS174T tumor-bearing mice superior to that of either unmodified C110 IT or \(^{90}\)Y-labeled C110 MAb alone (\(P < 0.01\)). In contrast, survival of mice treated with \(^{90}\)Y-labeled C110 IT was equivalent to that produced by the mixture of unmodified C110 IT and \(^{90}\)Y-labeled C110 MAb. In immunoconjugate-treated groups, the cause of all animal deaths was massive progression of i.p. tumors and/or malignant ascites, and no animal deaths were attributable to treatment with the...
Y-Labeled Anti-CEA Immunotoxin

Table 3 Cumulative radiation doses to the tissues in LS174T tumor-bearing mice given i.p. injections of 120 µCi of 90Y-labeled C110 IT

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cumulative dose after 168 h (rad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>2780</td>
</tr>
<tr>
<td>Lungs</td>
<td>400</td>
</tr>
<tr>
<td>Liver</td>
<td>1850</td>
</tr>
<tr>
<td>Spleen</td>
<td>950</td>
</tr>
<tr>
<td>Kidney</td>
<td>730</td>
</tr>
<tr>
<td>Bone</td>
<td>910</td>
</tr>
</tbody>
</table>

The cumulative radiation doses to the tumor and major organs were calculated based on the tissue biodistribution data presented in Table 1.

Fig. 6. Survival curves of LS174T tumor-bearing mice following treatment with PBS or specific immunoconjugates. Groups of mice were inoculated i.p. with 1.5 x 10^7 LS174T cells. Two weeks later, different groups were treated i.p. with PBS (control) (a), unmodified C110 IT (80 µg) (b), 90Y-labeled C110 MAb (80 µg; specific activity of radiolabel, 1 µCi/µg) (c), 90Y-labeled C110 IT (80 µg; specific activity of radiolabel, 1 µCi/µg) (d), or a mixture of b and c (e), once a week for 4 weeks.

 designated doses of immunoconjugates or neutropenic infections resulting from myelosuppression by 90Y.

DISCUSSION

The C110 MAb used in this study is an IgG1 murine monoclonal antibody with a high affinity for human CEA. Since specific tumor localization of C110 MAb has been confirmed in the animal (15) and initial clinical studies, this antibody may be considered for its therapeutic application in colon cancer. Our preliminary studies have indicated that conjugates of the C110 MAb with either ricin A chain or 90Y prolonged the survival of nude mice bearing human colon cancer xenografts as i.p. tumors. Thus, we designed this study to assess an immunoconjugate containing both ricin A chain and 90Y on the same C110 MAb molecule as a more potent reagent for colon cancer therapy.

In in vitro PSI assays, 90Y-labeled C110 IT died of massive progression of i.p. tumors and/or malignant ascites. No animal deaths were attributable to treatment with the designated dose of 90Y-labeled C110 IT or neutropenic infections resulting from myelosuppression by 90Y. However, in the biodistribution study of 90Y-labeled C110 IT, spleenic hypoplasia was observed in animals at 120 and 168 h following i.p. injection. Thus, dose-related toxicity to the hematopoietic organs, which has been reported in treatments using 90Y-labeled MAb (30-33), should also be present in treatments using 90Y-labeled C110 IT, although we did not perform any histological examinations or hematological measurements in this study. Our observations that there were no animal deaths attributable to treatment might be due to the i.p. route of administration. Esteban and colleagues (31, 33) also have reported that i.p. injection of 90Y-labeled anti-CEA MAb (ZCE025) at up to 160 µCi did not cause any radiation-induced mortality, although histological examinations of all treated animals disclosed up to 85% reduction of their bone marrow population and splenic hypoplasia with fibrosis.

The biodistributions of 90Y-labeled C110 IT and MAb in LS174T tumor-bearing mice following i.p. injection were almost identical to each other in the majority of tissues (Table 3). Both immunoconjugates showed specific tumor localization. The only organ in which the biodistributions of 90Y-labeled C110 IT and MAb differed from each other was liver. The accumulation of 90Y-labeled C110 IT in the liver was almost 1.5 times more than that of 90Y-labeled C110 MAb at all times. This higher uptake of 90Y-labeled C110 IT in the liver is attributed to recognition of the mannose and fucose residues on the ricin A chain moiety of the IT by the receptors present on the parenchymal and nonparenchymal liver cells (34). However, chemical destruction of the mannose and fucose residues on the ricin A chain (i.e., deglycosylation) can reduce the liver uptake of ricin A chain (35, 36). In addition, mannose-containing blocking agents, such as ovalbumin, ovomucid, or manno-
syl-lysin, or excess d-mannose or fucose also can inhibit liver uptake of ricin A chain (37, 38). Therefore, the methods described above may reduce the greater uptake of $^{90}\text{Y}$-labeled C110 IT in the liver.

Whole-body autoradiograms of LS174T tumor-bearing mice in this study revealed that $^{90}\text{Y}$-labeled C110 IT and MAb injected i.p. were localize to the periphery of i.p. tumor foci, with little penetration to the center of the masses (Figs. 4 and 5). Microdosimetry within the tumor foci by videodensitometric analysis of the autoradiograms has shown that $^{90}\text{Y}$-labeled C110 IT and MAb injected i.p. into LS174T tumor-bearing mice may not penetrate into i.p. inoculated tumor foci to a great extent and that the degree of their penetration may not exceed about 1 mm from the tumor surface (data not shown).

In conclusion, immunoconjugates containing both biological toxin and therapeutic radionuclide on the same MAB molecules (i.e., radioimmunotoxins) may be more potent than those containing either agent alone. In this study, we have synthesized an immunoconjugate linked with ricin A chain to the C110 anti-CEA MAB, further labeled with $^{90}\text{Y}$. This immunoconjugate showed satisfactory efficiency of $^{90}\text{Y}$ labeling, retention of the initial immunoactivity, in vitro stability, and in vivo cytotoxicity. In vivo therapeutic trials, moreover, showed that $^{90}\text{Y}$-labeled C110 IT provided survival prolongation of the tumor-bearing mice superior to that of either unmodified C110 IT or $^{90}\text{Y}$-labeled C110 MAb alone. Therefore, if the greater uptake of this immunoconjugate by the liver could be eliminated using appropriate approaches, it may be a potent therapeutic immunoconjugate for human colon cancer.

REFERENCES


Preclinical Assessments of $^{90}$Y-labeled C110 Anti-Carcinoembryonic Antigen Immunotoxin: A Therapeutic Immunoconjugate for Human Colon Cancer

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