Comparative Biodistributions of Yttrium- and Indium-Labeled Monoclonal Antibody B72.3 in Athymic Mice Bearing Human Colon Carcinoma Xenografts

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The biodistribution of yttrium- and indium-labeled monoclonal antibody (MAb) B72.3 IgG using three different chelate conjugates (SCN-Bz-EDTA, CA-DTPA, and SCN-Bz-DTPA) was compared in athymic mice bearing LS-174T tumors. The ^{88}Y-SCN-Bz-DTPA-B72.3 yielded 40% ID/g at 5–7 days in the tumors, while the ^{88}Y-SCN-Bz-EDTA-B72.3 and ^{88}Y-CA-DTPA-B72.3 showed only 6–8 %ID/g. The yttrium uptake in the bone with the SCN-Bz-EDTA and CA-DTPA conjugated IgG was over 14 and 11 %ID/g, respectively, while ^{88}Y-SCN-Bz-DTPA-B72.3 showed only 3 %ID/g. In contrast, the ^{111}In-labeled B72.3 uptake in the bone with all three chelate conjugates was 2–3 %ID/g. The differences in yttrium- versus indium-labeled MAb biodistributions demonstrate the difficulty in using ^{111}In-labeled MAbs to predict the biodistribution and dosimetry of ^{90}Y-labeled MAbs unless chelate conjugates such as SCN-Bz-DTPA are used.

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The use of antibodies for the treatment of cancer has been investigated for a long time (*1*). The development of monoclonal antibodies (MAbs) (*2*) directed against tumor-associated antigens (TAAs) has been instrumental in addressing the problems of antibody specificity, antibody heterogeneity, and availability. Many studies have been conducted in order to investigate the therapeutic potential of MAbs either alone or as carriers of therapeutic agents (*3–18*).

One major concern in using MAbs for therapy is the antigenic heterogeneity of solid tumors. It is well established that not all tumor cells in a given mass express the same pattern of many tumor-associated antigens (*19*). Some cells that express large quantities of a given TAA may be adjacent to a number of cells that do not express the antigen at detectable levels. Radionuclides that decay resulting in particle emissions (alpha, beta, or secondary electrons), can be linked to MAbs and kill cells over many cell diameters delivering a toxic dose to all cells in a tumor mass, and therefore, the antigenic heterogeneity that is observed in solid tumors may no longer be the limiting factor.

To date, iodine-131 (^{131}I) has been the radionuclide of choice, but radioiodinated MAb may be unstable in vivo (*20,21*). The ^{131}I (emitting a gamma-radiation component of 364 keV) bound to the MAb that is in circulation contributes to a radiation exposure to the entire body that affects normal tissues such as the bone marrow. This may limit the dose of this radionuclide that could be injected for therapeutic purposes.

A wide spectrum of radionuclides may be considered for use and may prove to be more efficient in tumor eradication. Radionuclides that have such potential include scandium-47, copper-67, and rhenium-188 that emit both beta and gamma rays; yttrium-90 (^{90}Y), which is pure beta emitter; as well as alpha emitters such as lead-212, bismuth-212, or astatine-211 (*22–24*). Labeling techniques using bifunctional chelates now permit proteins to be labeled with many of these radiometals. Chelating agents such as ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaa-

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cetic acid (DTPA) are most commonly used. The chelates themselves may be chemically modified to provide a method to link them to proteins. Examples are the cyclic and mixed anhydrides of DTPA (*25,26*). Alternatively, linking groups may be incorporated in the chelate molecule by synthesis as was done by the use of the isothiocyanatobenzyl group (SCN-Bz) covalently linked to the carbon backbone of EDTA or DTPA (*27,28*).

Many groups have proposed the use of the ^{90}Y linked to MAbs as a suitable therapeutic radionuclide (*15–17,22,29,30*). Determination of the biodistribution via external imaging of a pure beta emitter such as ^{90}Y is difficult because the gamma cameras will detect only the Bremsstrahlung radiation. The use of ^{111}In-labeled MAb (using the identical chelate) to trace the biodistribution of the yttrium has been proposed (*29*) despite the differences in indium and yttrium chemistry. Indium complexes are inherently inert, while yttrium complexes are more reactive in part because of the differences in coordination number (CN) (yttrium 7–9; indium 6) and ionic radius (r_i = 90 pm for Y^{+3}; r_i = 80 pm for In^{+3}). Thus the water exchange rate, so important for determining metal complex formation and dissociation rates, is measured for indium (+3) to be 4×10^4 sec^{-1} (*31*), while that of yttrium has been determined as 1.3×10^7 sec^{-1} (*32*), nearly three orders of magnitude difference in reactivity. Similarly, the metal exchange rate of indium (+3) in EDTA complexes is 1×10^{-6} sec^{-1} (*33,34*), while that of yttrium (+3) is 87 sec^{-1} (*35*). Such vast differences in reactivity should be expected to be reflected in the relative stability of the complexes in vivo.

Biodistribution studies using a pure beta emitter is difficult because of the need to fully solubilize the tissues before beta counting. Hnatowich et al. reported a good correlation between liquid scintillation and gamma scintillation counting of murine tissues (*36*). We have found that the efficiency of Bremsstrahlung counting is very low and Cerenkov counting has proven to be unreliable. In order to overcome these problems we have, therefore, used ^{88}Y which emits several high-energy gamma rays and thus can be used to accurately evaluate the biodistribution of yttrium-labeled MAbs.

In the present study we used the B72.3 MAb, a murine IgG_1, generated in our laboratory by immunization of mice with membrane-enriched fraction of a human carcinoma metastasis (*37*). It reacts with high specificity to a high molecular weight glycoprotein (termed TAG-72) (*38*) found in many epithelial-derived cancers, including colon, ovarian, breast, lung, pancreatic, and gastric malignancies (*39–41*). Little or no reactivity with normal adult tissues, with the exception of secretory endometrium, has been observed (*41*). We investigated the biodistribution of yttrium and indium radiometals in athymic mice bearing human colon carcinoma xenografts (LS-174T) (*42*) using three different chelate conjugates (SCN-Bz-EDTA, CA-DTPA, and SCN-Bz-DTPA with maximum CN of 6, 7, and 8, respectively) coupled with MAb B72.3 IgG and labeled with either ^{88}Y or ^{111}In.

MATERIALS AND METHODS

Monoclonal Antibody B72.3

B72.3 IgG was purified from ascitic fluid by ammonium sulfate precipitation followed by ion-exchange chromatography using diethylaminoethyl cellulose (DE-52, Whatman, Clifton, NJ). The antibodies were eluted by a 10 m*M* to 100 m*M* sodium phosphate (pH 7.5) gradient and column fractions were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (*43,44*). Appropriate fractions, with purity of over 95% of the protein being IgG, were pooled and dialyzed against phosphate buffered saline (PBS). The concentration was determined by the method of Lowry (*45*) using a bovine serum albumin (BSA) standard.

Conjugation of Chelates to B72.3

Carbon-14- (^{14}C) labeled chelates were synthesized and linked to MAb B72.3 by previously reported methods (*28*). Specific activities were determined by scintillation counting of known concentrations of ligand as measured by weight or from uv absorbance spectra. The average number of chelates per antibody was determined as previously described (*28*). A study of the dependence of the number of chelates linked to antibody versus the immunoreactivity in radioimmunoassays after labeling with ^{111}In demonstrated that increased numbers of chelates per molecule led to decreased immunoreactivity (*46*). Thus for comparative tissue distribution studies, an average of 0.5 ± 0.1 chelates were attached to each B72.3 IgG molecule.

Radiolabeling of B72.3 IgG Chelate Conjugates

B72.3 IgG was radiolabeled with either ^{88}Y or ^{111}In. The MAb was labeled after the radiometal antibody solution was adjusted to pH 4.2 in order to achieve adequate labeling with the ^{88}Y. Typically, 50 μl of ^{88}Y (yttrium-88, Oak Ridge National Laboratories, Oak Ridge; TN) (specific activity of ≈1Ci/mg; contaminants Fe ≈20ppm, Zn ≈8ppm, Al ≈100ppm, Na ≈50 ppm and Ca ≈175ppm) or ^{111}In [Indium-111 (NEZ-152, DuPont Company, Boston, MA], specific activity of >50 Ci/mg; total metal contaminants of ≈10 ppm in 0.1*M* HCl, 500 μCi) was adjusted to pH 4 with 11 μl of 2*M* sodium acetate solution. Next, 200 μl of chelate conjugated MAb (in 20 m*M* MES, 150 m*M* NaCl [pH 6.2], 1 mg B72.3 IgG) was titrated to pH 4.5 by the addition of 2*M* HCl; pH was monitored with a glass microelectrode.

The protein and radiometal solutions were mixed to form a solution at pH 4.2 and allowed to react. After 20 min, 10 μl of sodium acetate and 2μl of 0.01M EDTA was added. After 5 min, the sample was run on a TSK 4000 size-exclusion column using 20 m*M* MES and 150 m*M* NaCl, and the protein fraction was collected. Approximately 80% of the protein was recovered with labeling yields of 70% for ^{88}Y using the CA-DTPA and SCN-Bz-EDTA chelate conjugates and 15% for the SCN-Bz-DTPA chelate conjugate. This difference in yields is primarily due to kinetic differences in the reaction

of yttrium with the SCN-Bz-DTPA as compared with the two other chelate conjugates. The labeling yields with ^{111}In was in excess of 90% using all three chelate conjugates. The lower labeling yields of ^{88}Y versus ^{111}In was primarily the result of the low specific activity of the ^{88}Y; however, all radiolabeled MAb preparations had approximately the same amount of metal substituted chelates (0.05/IgG molecule).

Cell Lines

The LS-174T human colon carcinoma cell line (*42*) was obtained from Dr. P. Noguchi (Bureau of Biologics, FDA, Bethesda, MD) and was grown in Eagle's minimum essential medium supplemented with 1×L-glutamine, 1×nonessential amino acids, 50 μg/ml Gentamicin and 10% heat-inactivated fetal bovine serum. The cell line was subpassaged weekly at a 1:5 or 1:10 split ratio. Cells were removed from the culture flasks with 0.1% trypsin containing 0.5 m*M* EDTA and washed twice in growth medium without serum prior to inoculation into mice.

Radioimmunoassays

The immunoreactivity of the B72.3 chelate conjugates was assessed in a competition RIA using tumor extracts. MAb chelate conjugates were serially diluted in 1% BSA in PBS and added to plates containing 1 μg/well of an extract of a LS-174T xenograft. Following a 6-hr incubation at 4°C, 25 μl of ^{125}I-B72.3 was added to each well and incubated at 4°C overnight. The plates were washed and counted as described above. The percent inhibition was compared to unconjugated MAb which served as a standard.

The immunoreactivity of each radiolabeled B72.3-chelate preparation was assessed using a solid-phase radioimmunoassay (RIA) employing an extract of a colon cancer xenograft (LS-174T) as a positive control and an extract of human melanoma xenograft (A-375) as a negative control (*47*). Twenty micrograms (in 50 μl of PBS) of the tumor extracts were added to each well of 96-well microtiter polyvinyl plates and allowed to dry. The microtiter plates were treated with 100 μl of 5% of BSA for 1 hr at 37°C in order to minimize nonspecific protein absorption. The BSA was removed, and varying amounts of radiolabeled antibody (in 50 μl) were added. Following an overnight incubation at 4°C, the unbound immunoglobulin was removed by washing the plates with 1% BSA in PBS. The bound radioactivity was detected by cutting the individual wells from the plate and measuring the radioactivity in a well-type NaI gamma counter. All the ^{111}In-MAb preparations bound equally to the extracts containing the TAG-72 antigen (≈30%). Approximately 13% of the ^{88}Y-MAb preparations bound to the TAG-72 antigen. The lower percentage of binding of the ^{88}Y-MAbs is a result of their lower specific activity which necessitated the use of 10–20-fold more MAb in the solid-phase RIA resulting in the decreased binding of the radiometal labeled MAb.

SDS-Polyacrylamide Gel Electrophoresis

Each radiolabeled B72.3 chelate preparation was analyzed for purity and integrity by SDS-PAGE. The labeled antibodies were evaluated with and without disruption by beta-mercaptoethanol to determine the size of the IgG after labeling. The gels were run according to the method of Laemmli (*48*) using a 10% polyacrylamide gel (5 × 8 cm) with a stacking gel of 3% acrylamide. Radiolabeled antibodies were detected by autoradiography (XAR X-ray film, Kodak, Rochester, NY) using X-ray film and intensifying screens (Lightning-Plus, DuPont, Wilmington, DE) at −70°C.

Tumor Growth in Athymic Mice

Female athymic mice (nu/nu), obtained from the Frederick Cancer Research Facility at 4–6 wk of age, were injected subcutaneously on the back with 1×10^6 LS-174T cells (0.2 ml). Animals were utilized for biodistribution studies ~2 wk postinoculation when the animals had tumors measuring between 0.5–0.8 cm in maximal diameter.

Biodistribution Studies

Tumor-bearing mice were injected via the tail vein with ~0.5 μCi/mouse (≈30 μg) of ^{88}Y-B72.3 IgG or 2.5 μCi/mouse (≈1.4 μg) of ^{111}In-B72.3 IgG labeled using each of the three chelate conjugates. The difference in the amount of MAb injected is a result of the relatively low specific activity of the ^{88}Y. We have previously shown that injection of up to 500 μg of B72.3 IgG in athymic mice bearing LS-174T xenografts (≤200 mg) did not alter the biodistribution of the radiolabeled antibody that was concomitantly administered. In these studies no differences in tumor uptake, plasma clearance or normal tissue distribution were observed. Mice (four per data point) were killed, tumor and all the major organs were collected and wet-weighed using an analytic balance, then counted in a gamma-scintillation counter. The percentage of the injected dose per gram (%ID/g) for each organ was determined and tissue to blood ratios and radiolocalization indices (%ID/g in tumor divided by the %ID/g in the normal tissues) were calculated. In order to further evaluate the radioactivity found in the bone, femurs from each mouse were collected and the bone marrow was washed out using 1 ml of saline solution. The percentage of the radioactivity found in the cortical bone was then determined.

Furthermore, in order to establish the biodistribution of the yttrium and indium radiometals, athymic mice bearing LS-174T tumors were injected i.v. with ~0.5 μCi of [^{88}Y] acetate or 5 μCi [^{111}In]citrate and killed at various times.

RESULTS

Biodistribution of [^{88}Y]Acetate

Athymic mice bearing a human colon carcinoma xenograft, LS-174T, were injected with ^{88}Y-acetate to determine the biodistribution of the yttrium, should it be released in vivo from the chelate-MAb complex. At 24 hr postinjection of the [^{88}Y]acetate only 0.1% of the %ID/g remained in the blood (Table 1). The ^{88}Y that remained in the body was found primarily in the liver, kidneys, and the bone. The activity cleared from both the liver and kidney was only one-third of the 24-hr level remaining in these tissues at 7 days. The yttrium uptake in the bone, ~20 %ID/g at 24 hr, continued to rise at 48 hrs and then significant levels (80% of the activity found at 24 hr) remained in the bone at 7 days. This demonstrates the slow clearance of the yttrium from the bone and the need to carefully evaluate the level of radioactivity found in the bone after injection of yttrium-labeled MAbs. Studies were also performed

TABLE 1
Biodistribution of [^{88}Y]Acetate and [^{111}In]Citrate Administered i.v. in Athymic Mice Bearing Human Colon Carcinoma Xenografts*

	[^{88}Y]acetate			[^{111}In]citrate		
Tissue	24	48	168	24	48	168
Tumor	2.71	2.43	1.63	6.49	5.23	2.47
Blood	0.11	0.06	0.05	3.65	0.98	0.23
Liver	9.16	7.76	3.20	9.26	10.79	12.22
Spleen	1.58	1.74	1.05	4.76	5.06	6.77
Kidney	13.58	10.54	5.52	36.35	25.37	13.69
Lung	1.72	1.48	0.96	4.58	3.26	2.62
Heart	1.08	0.88	0.51	2.56	1.77	1.97
Intestine	0.86	0.56	0.22	8.13	5.44	1.15
Vertebrae	18.56	21.83	16.05	5.71	4.93	3.71
Pelvis	17.56	21.86	14.47	5.96	5.57	3.60
Femur	22.78	27.96	16.35	7.04	5.83	4.52

* Athymic mice bearing the LS-174T human colon carcinoma xenograft were injected i.v. with ~ 0.5 μCi of [^{88}Y]acetate or 5 μCi of [^{111}In]citrate and killed at the indicated time (hr). The results are expressed as %ID/g.

with i.p. administered [^{88}Y]acetate with similar results to that observed with the i.v. administered [^{88}Y]acetate.

The biodistribution of the [^{88}Y]acetate contrasts markedly with the distribution of [^{111}In]citrate. After injection of [^{111}In]citrate, the indium was found primarily in the kidney, liver, and spleen with lower levels in the bone (Table 1). Approximately 6 %ID/g of the indium was measured in the bone at 24 hr compared with the 20 %ID/g of the yttrium found in the bone at that time. A larger proportion of the injected ^{111}In, as compared with the ^{88}Y, was found in the liver and spleen. While the kidneys of the mice injected with the [^{111}In]citrate retained the highest percentage of the injected dose per gram of tissue, ~25 %ID/g at 48 hr postinjection, the liver retained the greatest amount of indium (10.9 %ID/organ) having ~1.5 times more indium than the kidneys (7.1 %ID/organ).

Chelation and Radiolabeling of B72.3 IgG

MAb B72.3 IgG was purified from ascitic fluid by using ion-exchange and gel filtration chromatography as described previously (*43*). The IgG was analyzed by SDS-polyacrylamide gel electrophoresis and by high performance liquid chromatography (HPLC) and shown to be over 95% IgG. The IgG was modified by the addition of either SCN-Bz-EDTA, CA-DTPA, or SCN-Bz-DTPA chelate conjugates as described in Materials and Methods. The ratio of chelate to MAb was kept at <1 to minimize the loss of immunoreactivity of the IgG that is found at higher chelate substitution ratios (*46*). An average of 0.5 ± 0.1 chelates per IgG molecule was therefore used. All preparations were tested in a competitive RIA to assess the effect of the addition of the chelate; no loss of immunoreactivity was observed.

The B72.3 IgG modified by the three different chelate conjugates was then radiolabeled with ^{111}In or ^{88}Y under conditions that minimized the loss of immunoreactivity and maintained the integrity of the labeled complex. This was achieved by maintaining the pH of the labeling solution above 4.2, thus preventing extensive aggregation of the immunoglobulin. Similarly, the number of chelates linked to the antibody was carefully monitored in order to maintain the immunoreactivity of the MAb.

All labeled preparations were assayed for immunoreactivity in a solid-phase RIA. They were able to bind to an extract that contained the TAG-72 antigen recognized by B72.3 with minimal background to extracts that do not have the TAG-72 antigen. The integrity of the IgG was assessed by SDS-polyacrylamide gel electrophoresis. The samples were subjected to electrophoresis with and without the addition of beta-mercaptoethanol and the resulting gels were autoradiographed. The ^{111}In and ^{88}Y was associated with the IgG molecule and could be found on both the heavy and light chains. The radiolabeled preparations were also examined by HPLC using size exclusion columns in the presence of 0.1 m*M* EDTA and the radiolabel was shown to be associated with the IgG fraction.

Biodistribution of Radiolabeled B72.3 IgG

Athymic mice bearing the LS-174T human colon carcinoma xenograft were injected with ^{111}In- or ^{88}Y-labeled B72.3 IgG that was modified using either the SCN-Bz-EDTA, CA-DTPA, or SCN-Bz-DTPA chelate conjugates. Approximately 2.5 μCi of ^{111}In- or 0.5 μCi of ^{88}Y-labeled B72.3 IgG was injected i.v. in mice bearing subcutaneous tumors of ~500 mg in weight. The mice were killed at various times up to 7 days postinjection of the labeled MAb. The ^{111}In-labeled B72.3 cleared from the blood with ~16, 14, and 18 %ID/g remaining in circulation at 24 hr with the SCN-Bz-EDTA, CA-DTPA, and SCN-Bz-DTPA chelate conjugates, respectively (Fig. 1A, C, and E), dropping to 7, 3, and 6 %ID/g, respectively, at 7 days. At 48 hr, ~25 %ID/g were found in the tumors of the mice injected with B72.3 IgG linked to both the SCN-Bz coupled chelates. This level stayed constant over the 7-day period of study. The mice injected with the ^{111}In-CA-DTPA-B72.3 localized ~19 %ID/g in the tumor at 24–48 hr which then dropped to ~13 %ID/g at 5 days. No accumulation of the ^{111}In was observed in the bone (Fig. 1) over the period of study (1–7 days). Approximately 2.5 %ID/g of bone was found at 24 hr in mice injected with B72.3 conjugated to all three chelates; this level dropped to ~1 %ID/g at 7 days (Fig. 1).

Significant differences were observed in the biodistribution of the ^{88}Y-labeled B72.3 IgG modified by the SCN-Bz-EDTA, CA-DTPA, and SCN-Bz-DTPA chelate conjugates. The yttrium coupled to B72.3 using the SCN-Bz-EDTA or CA-DTPA chelate conjugates

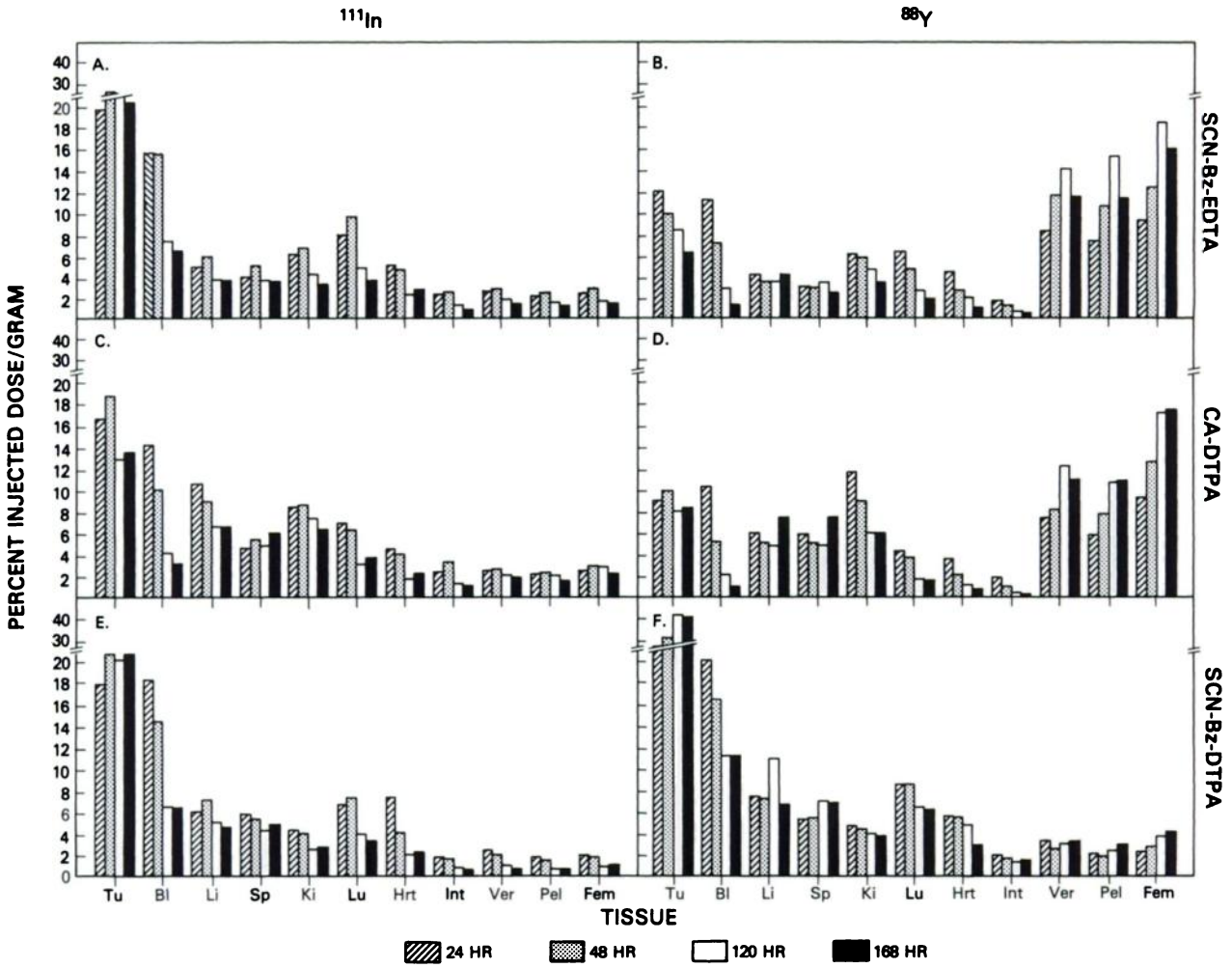

FIGURE 1

Biodistribution of B72.3 IgG labeled with ^{111}In or ^{88}Y in tumor bearing mice. Athymic mice bearing LS-174T, human colon carcinoma xenografts, were injected i.v. with ~ 2.5 μCi of ^{111}In- (Panels A, C, and E) or 0.5 μCi of ^{88}Y-labeled (Panels B, D, and F) B72.3 IgG. The MAb was modified with either SCN-Bz-EDTA (Panels A and B), CA-DTPA (Panels C and D), or SCN-Bz-DTPA (Panels E and F) chelate conjugates. Mice (four per group) were killed at 24 hr (▨), 48 hr (▩), 120 hr (□) or 168 hr (■) postinjection of the labeled MAbs. Tissues were wet weighted and the %ID/g determined. The s.e.m. was determined for each tissue and was <4% of the average values for most of the tissues. Tu, tumor; Bl, blood; Li, liver; Sp, spleen; Ki, kidneys; Lu, lungs; Hrt, heart; Int, intestines; Ver, vertebrae; Pel, pelvis; Fem, femur.

cleared the blood rapidly with only ~1.2 %ID/g remaining at 7 days postinjection (Fig. 1B and D). The ^{88}Y-SCN-Bz-DTPA-B72.3 showed a slower blood clearance with 11 %ID/g remaining in the blood at 7 days (Fig. 1F). The accumulation of yttrium in the tumor exhibited an inverse correlation with the blood clearance. The mice injected with the SCN-Bz-DTPA conjugated B72.3 had higher levels of ^{88}Y in the tumors with ~40 %ID/g at 5–7 days postinjection of the MAb in contrast to only 6–8 %ID/g in the tumor of the mice injected with the SCN-Bz-EDTA or CA-DTPA conjugated B72.3

The most important differences among the three chelates coupled to the B72.3 IgG were in the radionuclide uptake in the bone. The level of the ^{88}Y rose as a function of time in the bones of the mice injected with the SCN-Bz-EDTA and CA-DTPA conjugated IgG from ~8 and 7 %ID/g at 24 hr to over 14 and 11 %ID/g, respectively, in both the vertebrae and the pelvis at 5 days postinjection of the labeled MAbs. Slightly higher levels were observed in the femurs of all the groups of mice. Mice injected with the ^{88}Y-SCN-Bz-DTPA-B72.3 showed considerably lower levels of yttrium in the bone with only 3 %ID/g at 5 days postinjection.

Studies were undertaken to determine whether the radioactivity found in the bone was in the bone marrow or in the cortical bone. The bone marrow was removed from the femurs by extensive washing. At 24 hr postinjection of ^{88}Y-B72.3 IgG modified by the SCN-Bz-EDTA and CA-DTPA chelate conjugates these studies demonstrated that over 90% of the radioactivity was associated with the cortical bone. The percentage of the

^{88}Y in the cortical bone increased as a function of time; 97% of the total bone radioactivity was found in the cortical bone at 7 days. The majority of the radioactivity in the bone of mice injected with ^{88}Y-labeled B72.3 modified by the SCN-Bz-DTPA chelate conjugate, however, was associated with the bone marrow at 24 hr postinjection of the MAb. As the ^{88}Y-SCN-Bz-DTPA conjugated MAb cleared the blood pool the activity in the bone marrow decreased; therefore, the percentage of the remaining activity found in the cortical bone increased with time to ~80% at 7 days. Note, however, that the total activity in the bone for the ^{88}Y-SCN-Bz-DTPA-B72.3 was only 3.6 %ID/g at 7 days.

Tissue to Blood Ratios of ^{88}Y- and ^{111}In-labeled B72.3 IgG

Differences in the tissue localization between the ^{88}Y and ^{111}In radionuclides coupled by the three chelate conjugates may be a result of specific uptake by the tissues of the radiometal or differences in blood clearance of the radiometal. A significant proportion of the activity in the majority of the tissues is accounted for by the blood associated activity in those tissues. If there were differences in the blood-pool associated radioactivity there would be a corresponding change in the radioactivity in the normal tissues. As was noted above and shown in Figure 1, there were substantial differences in the %ID/g in the blood with the ^{88}Y-labeled B72.3 IgG using the SCN-Bz-EDTA, CA-DTPA chelate conjugates as compared with the SCN-Bz-DTPA chelate conjugate. In order to minimize the effect of the blood-pool differences, tissue-to-blood ratios were determined. If the activity in the tissue was simply a result of the blood-pool activity, then the tissue-to-blood ratio would remain constant with time. If there was a specific tissue accumulation of the radiometal, then the tissue-to-blood ratio will rise as a function of time.

The tumor-to-blood ratios increased with time with all the radiometal-chelate conjugate combinations (Fig. 2). The mice injected with the ^{111}In-labeled B72.3 showed an increase in the liver, spleen, and kidney to blood ratios as a function of time. This was most notably seen with the CA-DTPA (Fig. 2C) where the rise in the tissue-to-blood ratios was a result of both a faster decrease in the blood levels with a corresponding increase in the tissue uptake of the ^{111}In. The ^{88}Y-labeled B72.3 IgG using the SCN-Bz-EDTA and CA-DTPA chelate conjugates showed an increase in the tissue-to-blood ratios in the majority of tissues (Fig. 2B and D). This was most evident with the bone samples where the bone-to-blood ratio rose to ~9:1 for the vertebral column and pelvis and 12:1 and 15:1 for the femur with the yttrium labeled SCN-Bz-EDTA and CA-DTPA chelate conjugates.

The tissue-to-blood ratios were very different with the ^{88}Y-labeled B72.3 using the SCN-Bz-DTPA chelate conjugate. In no normal organ did the tissue-to-blood ratio rise to over 1 (Fig. 2F). The highest ratios were seen in the liver and spleen where the ratios to blood at 7 days were 0.6:1; this is in contrast to liver and spleen to blood ratios of 3:1 and 2:1, and 7:1 and 4:1 for the ^{88}Y-labeled MAb conjugated to the SCN-Bz-EDTA and CA-DTPA chelates, respectively. A major contrast between the SCN-Bz-DTPA and the other two chelate conjugates was also seen in the bone-to-blood ratios. They were very low when B72.3 was labeled with ^{88}Y using the SCN-BZ-DTPA chelate conjugate (Fig. 2F). There was only a slight increase in the bone-to-blood ratio with time rising from ~0.12:1 to 0.32:1 from 24 hr to 7 days postinjection of the labeled MAb.

Comparison of Biodistribution of the ^{88}Y- and the ^{111}In-labeled B72.3 IgG

The tissue distribution of the ^{111}In and the ^{88}Y radionuclides were compared after injection of the two metals coupled to B72.3 IgG using the SCN-Bz-EDTA, CA-DTPA, and SCN-Bz-DTPA chelate conjugates. The ratio of the tissue-to-blood ratios was determined for each of the chelate conjugates to normalize for the differences in the blood clearance of the ^{88}Y- and ^{111}In-labeled B72.3. As can be seen in Figure 3, Panels A and B, there are major differences in the biodistribution of the ^{88}Y and ^{111}In-label in mice injected with the SCN-Bz-EDTA and CA-DTPA chelate conjugates. While both radionuclides show an increase in the tissue-to-blood ratios as a function of time (Fig. 2), the rate of increase in the tissue-to-blood ratios of the yttrium, however, is faster than that of the indium in virtually all the tissues (Fig. 3). This is especially notable in the liver, spleen, and kidney where up to six times higher ratios were seen with the ^{88}Y-labeled MAb as compared to the ^{111}In-labeled MAb using the SCN-Bz-EDTA chelate conjugate (Fig. 3A). Approximately 2–3 times higher liver-, spleen-, and kidney-to-blood ratios were found with the ^{88}Y-labeled B72.3 as compared to the ^{111}In-labeled MAb (Fig. 3B). The major differences between the ^{88}Y- and ^{111}In-labeled MAbs using both the SCN-Bz-EDTA and CA-DTPA chelate conjugates were in the bone to blood ratios (Fig. 3A and B). Both the SCN-Bz-EDTA and CA-DTPA chelate conjugate labeled MAb preparations gave high ^{88}Y to ^{111}In ratios of the bone-to-blood ratios; up to 23:1 for the CA-DTPA chelate conjugate and up to 57:1 for the SCN-Bz-EDTA chelate conjugate.

The B72.3 IgG labeled with the SCN-Bz-DTPA chelate conjugate yielded very different ratios from those observed with the two other chelate conjugates. The ratio of the tissue-to-blood ratios was essentially 1 (1.01 ± 0.02) for the majority of tissues (all except bone) at all time points (Fig. 3C). The ratio of the bone-to-blood ratios rose from 1.01 at 24 hr to 1.70 at 7 days, showing a much smaller accumulation of the ^{88}Y in the bone.

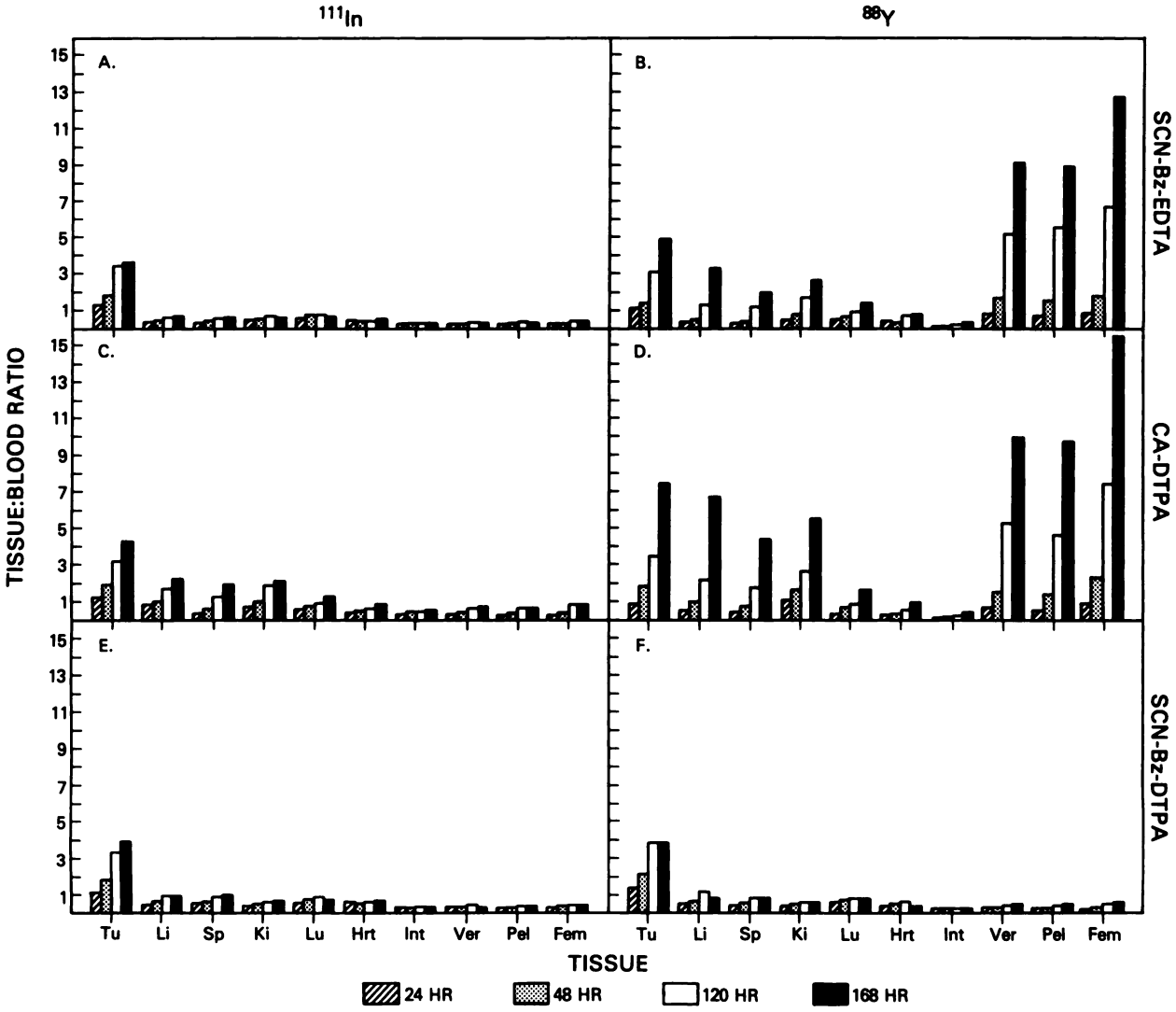

FIGURE 2

Tissue-to-blood ratios of mice injected with B72.3 IgG labeled with ^{111}In or ^{88}Y. The percent injected dose in the tissues were divided by the percent of the injected dose found in the blood. Athymic mice bearing LS-174T, human colon carcinoma xenografts, were injected i.v. with ~ 2.5 μCi of ^{111}In- (Panels A, C, and E) or 0.5 μCi of ^{88}Y-labeled (Panels B, D, and F) B72.3 IgG. The MAb was modified with either SCN-Bz-EDTA (Panels A and B), CA-DTPA (Panels C and D) or SCN-Bz-DTPA (Panels E and F) chelate conjugates. Mice (four per group) were killed at 24 hr (▨), 48 hr (■), 120 hr (□) or 168 hr (■) postinjection of the labeled MAbs. The s.e.m. was determined for the uptake in each tissue and was <4% of the average values for most of the tissues.

Radiolocalization Indices of the ^{88}Y-labeled B72.3 IgG

While all three ^{88}Y-labeled chelate conjugates localize the tumor (Fig. 1A, C, and E), the utility of yttrium-labeled MAbs as therapeutic agents depends on the amount of radioactivity localizing in the tumor as compared with normal organs. Radiolocalization indices (RI, %ID/g in the tumor divided by the %ID/g in the normal tissues) were calculated for the ^{88}Y-labeled B72.3 using the SCN-Bz-EDTA, CA-DTPA, and SCN-Bz-DTPA chelate conjugates. In mice injected with ^{88}Y-B72.3 modified by the SCN-Bz-EDTA chelate conjugate the RIs of the major organs, e.g., liver and spleen, decreased as a function of time (Fig. 4A). Similarly, the RIs of the bone decreased to ~0.5 at 5 days and 7 days. We found that while the tumor-to-liver and spleen ratios observed with the ^{88}Y-CA-DTPA-B72.3 were relatively constant over the 7-day period, the tumor-to-bone ratios decreased to ~0.75 at 7 days postinjection of the ^{88}Y-labeled MAb (Fig. 4B).

In contrast to the RIs observed with the SCN-Bz-EDTA and CA-DTPA conjugated B72.3, the ^{88}Y-SCN-Bz-DTPA-B72.3 showed an improvement with time in all the organs (Fig. 4C). The tumor-to-liver and spleen ratios increased from 3.2 and 4.4, respectively, at 24 hr to ~5.8 at 7 days. The tumor-to-bone ratios increased from 9.2 at 24 hr to 11.65 at 7 days.

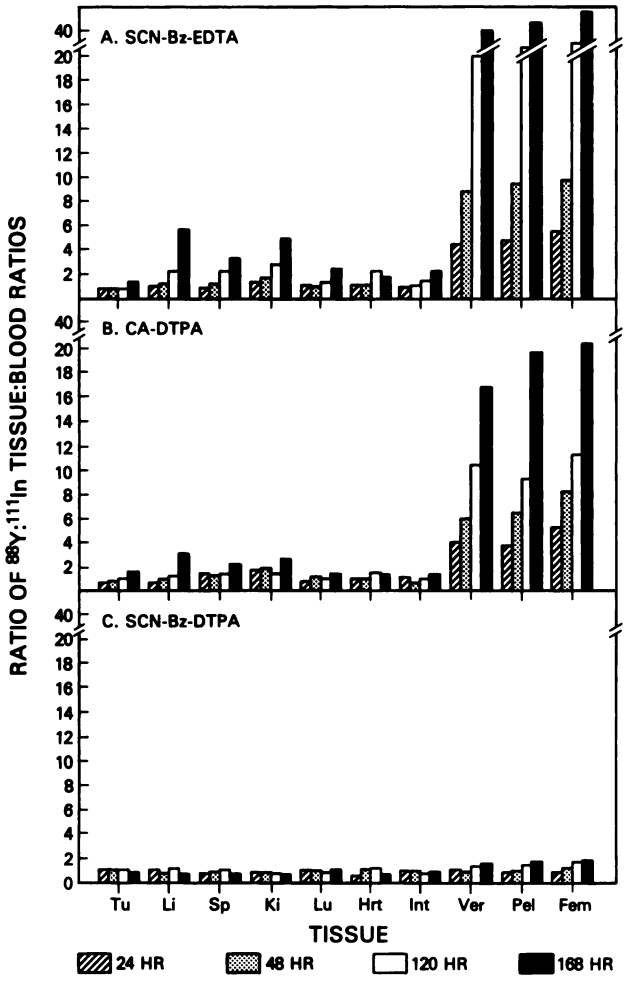

FIGURE 3
Comparison of the biodistribution of B72.3 IgG labeled with ^{88}Y versus ^{111}In in tumor-bearing mice. The tissue-to-blood ratios obtained with ^{88}Y-labeled B72.3 IgG were divided by the tissue-to-blood ratios obtained with ^{111}In-labeled B72.3 IgG. The MAb was modified with either SCN-Bz-EDTA (Panel A), CA-DTPA (Panel B), or SCN-Bz-DTPA (Panel C) chelate conjugates. Mice (four per group) were killed at 24 hr (▨), 48 hr (■), 120 hr (□), or 168 hr (■) postinjection of the labeled MAbs. The s.e.m. was determined for the uptake in each tissue and was <4% of the average values for most of the tissues.

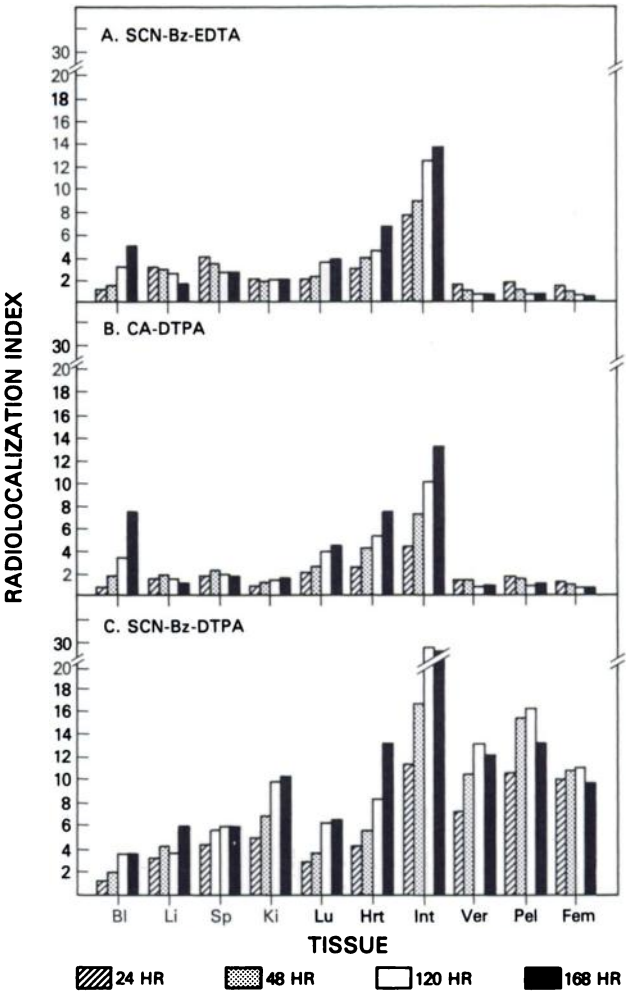

FIGURE 4
Radiolocalization indices of B72.3 IgG labeled with ^{88}Y in tumor bearing mice. The radiolocalization indices (%ID/g in tumor divided by the %ID/g in tissue) were determined for the mice injected with ^{88}Y-labeled B72.3 IgG modified with either SCN-Bz-EDTA (Panel A), CA-DTPA (Panel B), or SCN-Bz-DTPA (Panel C) chelate conjugates. Mice (four per group) were killed at 24 hr (▨), 48 hr (■), 120 hr (□), or 168 hr (■) postinjection of the labeled MAbs. The s.e.m. was determined for the uptake in each tissue and was < 4% of the average values for most of the tissues.

DISCUSSION

Recent interest has focused on the application of radiolabeled MAbs directed against tumor-associated antigens as in vivo therapeutic reagents. The development of labeling techniques that yield covalent linkage of chelates to proteins now permits the attachment of many different radiometals to antibodies (*25–28*). Several radionuclides have been considered to be potentially useful for radioimmunotherapy. Yttrium-90 has been selected as one of particular interest for therapeutic applications after conjugation to MAbs (*15–17,22,29,30*); it can be complexed with several chelate groups such as DTPA and EDTA which may be linked to proteins using a variety of methodologies (*25–28*). Yttrium-90, a pure beta emitter, has a suitable half-life of 64 hr that is long enough to permit efficient tumor uptake, but short enough to minimize toxicity to organs involved in the catabolism of the MAb chelate conjugate. The ^{90}Y decay yields high energy beta emission ($E_{\beta max}$ = 2.3 MeV, $E_{\beta ave}$ = 0.937 MeV) that results in the deposition of 90% of the energy of the beta particle within 5 mm of the point of disintegration. This should effectively kill tumor cells several cell diameters away from the source of emission. The fact that ^{90}Y is a pure beta emitter makes it difficult to estimate the radiation dose to a given tissue because the radioactivity accumulated in tissues cannot be accurately detected by

external imaging nor can it be accurately quantitated using gamma or beta scintillation counters. To overcome this problem, the gamma- emitting ^{111}In has been proposed as a tracer for yttrium in biodistribution studies (*29*).

The aim of our study was to compare the biodistribution of yttrium- and indium-labeled MAb B72.3 that have been modified with three different chelate conjugates (SCN-Bz-EDTA, CA-DTPA, and SCN-Bz-DTPA) to determine if the ^{111}In can be used to accurately predict the ^{90}Y localization and therefore, allow accurate dosimetric calculations. For this study, the gamma-emitting yttrium radionuclide ^{88}Y was chosen in order to facilitate the biodistribution analysis employing a well-type gamma counter. Athymic mice bearing LS-174T human colon carcinoma xenografts were injected via tail vein with MAb B72.3 IgG modified by the three different chelate conjugates and labeled either with ^{88}Y or ^{111}In. The uptake in the tumor was highest with ^{88}Y-SCN-Bz-DTPA-MAb (40 %ID/g at 5–7 days postinjection, Fig. 1F) compared with the two other chelate conjugates, SCN-Bz-EDTA and CA-DTPA (only 6–8 %ID/g, Fig. 1B and D). This is a result, in part, of the rapid drop in the blood levels for the ^{88}Y-labeled SCN-Bz-EDTA and CA-DTPA conjugated MAbs in contrast to the ^{88}Y-SCN-Bz-DTPA-B72.3 (Fig. 1).

B72.3 modified with SCN-Bz-DTPA or SCN-Bz-EDTA and labeled with ^{111}In showed a different distribution with ~25 %ID/g in the tumor; this activity remained constant over the 7-day period of study (Fig. 1E and A). The tumor uptake for ^{111}In-CA-DTPA, in contrast, rose to 19 %ID/g after 48 hr and then dropped to 13 %ID/g at 5 days (Fig. 1C). B72.3 IgG modified by all three chelate conjugates and labeled with ^{111}In cleared from the blood at similar rates (Fig. 1). Tissue-to-blood ratios increased as a function of time for the mice that were injected with the ^{111}In-labeled MAb (Fig. 2A, C, and E). This was a result of the decrease of activity in the blood and a progressive accumulation in the liver, spleen, and kidney. This was most notable with the CA-DTPA conjugated MAb where the rise in the tissue-to-blood ratios was a result of a faster decrease in the blood levels (Fig. 1C).

The ^{88}Y-SCN-Bz-EDTA chelate conjugate showed liver-, spleen-, and kidney-to-blood ratios of 3.2:1, 1.9:1, and 2.6:1, respectively (Fig. 2B). Similar high liver-, spleen-, and kidney-to-blood ratios of 6.8:1, 4.4:1, and 5.6:1 were seen using the ^{88}Y-CA-DTPA-B72.3 (Fig. 2D). In contrast, only a 0.6:1 ratio was seen in liver and spleen and 0.4:1 in kidney for ^{88}Y-SCN-Bz-DTPA-B72.3 indicating that this complex did not accumulate in these tissues (Fig. 2F). These observed differences in the tissue distribution of the three MAb chelate conjugates may be a result of differences in: (a) the stability and subsequent release of yttrium from the circulating MAb chelate conjugates; (b) the release of the ^{88}Y-chelate conjugate from the MAb in circulation; (c) the clearance from circulation of the MAb as a result of the modification of the MAb by the chelate conjugates; or (d) differential release from the hepatocytes of the various metal chelate conjugates after processing of the MAb chelate conjugate. The differences in the tissue uptake of the three MAb chelate conjugates is consistent with the release of the yttrium from the circulating MAb chelate conjugates as evidenced by the high uptake in the bone, liver, spleen, and kidney that is similar to the biodistribution that is seen after injection of yttrium acetate (Table 1).

Since the yttrium tends to accumulate in the bone in its uncomplexed form (see Table 1) and result in bone marrow toxicity as reported by Anderson-Berg et al. (*16*) and Sharkey et al. (*17*) in their therapeutic studies with ^{90}Y, particular attention has been paid to the evaluation of the yttrium uptake in the bone of the mice injected with the chelate conjugated B72.3. The level of the ^{88}Y rose as a function of time in the bones of mice injected with the SCN-Bz-EDTA and CA-DTPA-B72.3 with over 14 and 11 %ID/g found in bone at 5 days, respectively (Fig. 1B and D). This was probably caused by the deposition of free yttrium released from chelate-conjugated antibody. In fact, the levels of radioactivity in the bones of these animals are comparable to those observed in animals given injections of [^{88}Y]acetate. Free yttrium has been previously shown to deposit on the surfaces of the mineral fraction of bone in contact with the circulation (*49*). In contrast, the mice injected with ^{88}Y-SCN-Bz-DTPA-B72.3 showed significantly lower levels with only 3 %ID/g in the bone (Fig. 1F) indicating a better stability of this complex in vivo, with little apparent leakage into circulation of the yttrium with its subsequent deposition into the bone. The bone uptake for ^{111}In-labeled B72.3 using all three chelate conjugates resulted in only 2–3 %ID/g (Fig. 1A, C, and E), similar to that found with the ^{88}Y-SCN-Bz-DTPA-B72.3.

The differences between yttrium and indium biodistribution after administration of labeled MAbs, as shown in this study, illustrates the difficulties in using ^{111}In-labeled MAbs to predict the biodistribution and dosimetry of ^{90}Y-labeled MAbs. Although a given chelate conjugate may give a favorable biodistribution with ^{111}In, the biodistribution of yttrium in that chelate conjugate may be very different. This study also demonstrates the importance of using the appropriate chelate for attaching yttrium and indium to the MAb. As demonstrated in this study (Fig. 3) with the use of a chelate such as SCN-Bz-DTPA one can minimize loss of the radiometal from the chelate and obtain similar biodistributions using both indium and yttrium. The low levels of bone uptake of the ^{88}Y-SCN-Bz-DTPA-B72.3 are especially important in minimizing marrow toxicity. The high tumor-to-bone ratios suggest the

potential utility of ^{90}Y for radioimmunotherapy using this type of chelate conjugate.

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