New Method for the Chelation of Indium-111 to Monoclonal Antibodies: Biodistribution and Imaging of Athymic Mice Bearing Human Colon Carcinoma Xenografts

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B72.3, a murine monoclonal antibody (MAb) that reacts with 85% of human colon carcinomas as well as other epithelial neoplasias, was labeled with ^{111}In using four chelating agents: 1-(p-isothiocyanatobenzyl)-DTPA (SCN-Bz-DTPA), isobutylcarboxycarbonic anhydride (MA-DTPA), cyclic anhydride (CA-DTPA), and 1-(p-isothiocyanatobenzyl)-ethylenediaminetetraacetic acid (SCN-Bz-EDTA). Comparative biodistribution and imaging studies were performed in athymic mice bearing human colon carcinoma xenografts (LS-174T). Tumor uptake of radiolabel was very similar between the chelates (30% ID/g) and tumors were identified in scintigraphic images with all the chelate-antibody complexes. The uptake by normal organs, especially the liver, was greater for MA-DTPA, CA-DTPA, and SCN-Bz-EDTA chelate-B72.3 IgG (1.3:1 to 2.5:1) in comparison to that found with the B72.3-SCN-Bz-DTPA (~5:1) and abdominal organ, and uptake was very prominent on imaging with these chelate-MAb complexes but was virtually absent in the mice injected with B72.3-SCN-Bz-DTPA. Purification of the MAb-chelate complex by Sephadex G-50 chromatography followed by HPLC using a TSK-3000 column provided better subsequent biodistribution and also resulted in clearer images as compared to MAb chelate complexes purified by less rigorous purification protocols. We conclude that the ^{111}In-SCN-Bz-DTPA complex is superior, at least when bound to MAb B72.3, to other chelate-complexes currently in use.

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Most of the initial efforts in the use of radiolabeled MAbs as radiopharmaceuticals for the localization of occult malignant tumors were focused in the iodides as the radionuclides of choice. These radionuclides have enabled successful targeting of tumors, both in experimental models and in patients with a variety of diseases (*1,2*). The high-energy beta (toxicity considerations) and higher than optimal energy gamma emissions (inefficient for gamma imaging) as well as the apparent in vivo dehalogenation (*3,4*) may restrict the utility of iodine-131 in clinical trials. Strong chelating groups can be covalently attached to proteins and labeled with radioactive metals. Indium-111 (^{111}In) with its lower energy gamma and lack of beta emissions is a better choice of radionuclide for radiolocalization studies. The different labeling methodologies currently available to link chelates to proteins, using the cyclic anhydride (CA) (*5*) and mixed anhydride (MA) (*6*) derivatives of DTPA (diethylenetriaminepentaacetic acid), form protein-diethylenetriaminetetraacetic acid (DTTA) complexes. When ^{111}In is placed in these chelates, the resulting complexes are kinetically and thermodynamically unstable in vivo (*7*). Release of the ^{111}In from the conjugates produces a continuous leakage of radionuclide into the blood resulting in transport through transferrin to the liver and other organs of the reticuloendothelial system. This liver uptake (*8–11*) poses a significant problem that drastically limits the utility of ^{111}In in clinical trials since many carcinomas metastas-

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ize to the liver. 1-(p-isothiocyanatobenzyl)diethylenetriaminepentaacetic acid (SCN-Bz-DTPA) is a new bifunctional chelate recently described (*12*). Indium-111 is more stable in vivo in the SCN-Bz-DTPA chelate because the chelate remains as DTPA when complexed with proteins. Since ^{111}In requires a high coordination number and since it is known to form kinetically labile hydroxyl complexes, we prepared the chelate that remains as intact DTPA and have shown that the in vivo release of the metal is slow in comparison to DTTA and EDTA derivatives. Presumably the additional coordination sites compete with hydroxyl ions to retard dissociation.

Monoclonal antibody (MAb) B72.3 is a murine IgG_1 that reacts with a high molecular weight glycoprotein (designated TAG-72) (*13*) expressed in ~85% of colon carcinomas, 70% of breast carcinomas, and 95% of ovarian carcinomas (*14–17*). Its expression in normal adult tissues is negligible. We have previously shown that iodine-125 (^{125}I) and ^{131}I-labeled B72.3 IgG can selectively bind human colon carcinomas xenografts in athymic mice (*1,18*) and have recently extended these studies into clinical trials in patients with metastatic colorectal cancer (*19*).

We have linked B72.3 IgG to the new chelating agent (SCN-Bz-DTPA) and labeled the conjugate with ^{111}In for radiolocalization studies in an attempt to overcome the limitations of available methodologies. We report here biodistribution and imaging studies in athymic mice bearing human colon carcinoma xenografts (LS-i74T) and have compared this new MAb-chelate complex with other MAb-chelate complexes that are currently being used.

MATERIALS AND METHODS

Monoclonal Antibody B72.3

Mouse MAb B72.3 is reactive with a membrane glycoprotein of $>10^6$ daltons present in common human epithelial malignancies such as colon, breast, and ovary. Its generation by immunizing BALB/c mice with a membrane enriched fraction of a human breast tumor metastasis and reactivities have been previously described (*14–17*). B72.3, an IgG_1, was purified from ascitic fluid by precipitation using 50% ammonium sulfate and further purification was performed by ion-exchange chromatography (DE52) and size exclusion chromatography. Details on the purification protocol have been previously described (*1*).

Coupling of Chelates to Antibody B72.3

MA-DTPA. A modification of the method of Krejcarek and Tucker (*6*) was used to link MA-DTPA to B72.3. Carbon-14-(^{14}C) labeled DTPA was employed at various input concentrations, ranging from 100 to 300 moles per mole of IgG to enable the quantitation of the number of chelates bound per molecule of IgG. Input ratios of 100:1 provided a final ratio of ~1:1 with 100% retention of the MAb immunoreactivity.

The MAb-chelate complex was dialyzed in metal-free buffers to purify protein from unreacted ligand. The first dialysis was against 50 m*M* citrate (pH 5.5), 0.15*M* NaCl with 0.1 m*M* ascorbic acid. The next two dialyses were performed with the same solution without the ascorbate. The final dialysis was done against 0.02*M* [2-(N-morpholino)ethanesulfonic acid] (MES, pH 5.9), 0.15*M* NaCl.

CA-DTPA. Carbon-14-DTPA dianhydride was prepared by the method of Eckelman (*20*) and used at a 2–16:1 molar ratio to MAb. The final chelate:antibody ratio also was ~1:1 when a 2:1 initial input was used. Purification of protein from unreacted ligand was performed by sequential dialyses as described above.

Isothiocyanate chelates (EDTA and DTPA). Carbon-14-SCN-Bz-EDTA and SCN-Bz-DTPA chelates were synthesized with isothiocyanate linkage groups on the backbone of the chelate as previously described (*12,21*). The chelates were reacted for 2 hr at various molar ratios 0.5–12:1 of chelate to MAb. Final ratios of ~1:1 were obtained when the input was 1–2:1 and 3:1 for SCN-Bz-EDTA and SCN-Bz-DTPA, respectively. The protein was purified from unreacted chelate by sequential dialyses as described above.

Determination of Average Number of Chelates Linked to an Antibody Molecule

Quantification of the average chelate to antibody ratio was accomplished by liquid scintillation counting of the [^{14}C] chelate and measurement of protein concentration using the method of Lowry et al. (*22*). A measured volume was counted and corrected for quench and efficiency, to determine the amount of ^{14}C present in the protein solution. The activity present was corrected for specific activity of the chelate and volume thus yielding the amount of chelate present. The ratio of ligand concentration to protein concentration provided the number of chelates per antibody molecule.

Indium-111 Labeling

26.4 μl of 2*M* HCl was added to a solution of 60 μl ^{111}In (3 mCi, 0.05*M* HCl*). After 5 min, the solution was adjusted to pH 4.2 with 26 μl 1*M* sodium acetate. B72.3 IgG (200 μl at 3 mg/ml in 20 m*M* MES, 0.15*M* NaCl, pH 6.0) was immediately added and allowed to react for 30 min before being purified as described below. Protein concentration was then measured and ^{111}In activity determined using a dose calibrator.† Specific activities ranged from 3–5 mCi/mg, but higher values (30 mCi/mg) were obtained using more ^{111}In and less protein while keeping reaction solution volumes as small as possible.

Purfication of ^{111}In-Labeled B72.3

Four protocols were employed in order to test the importance of the purification method for obtaining good tissue distribution and tumor images with ^{111}In-labeled B72.3. All four protocols were employed to purify a single labeling of SCN-Bz-DTPA chelate-IgG complex that was labeled with ^{111}In. Tissue biodistribution and scanning studies were then performed.

EDTA "chase" method. Indium-111-B72.3 was purified according to the general method as suggested by Goodwin et al. (*23*). Indium-111-labeled antibody solution was added to 1 ml of a solution containing 0.1 m*M* EDTA, 0.1*M* citrate (pH 5) and allowed to sit for 1 hr prior to use. Samples were diluted with PBS just before injection into mice.

Size exclusion column chromatography. Sephadex G-50

(fine) was swollen overnight in phosphate buffered saline and a 1 cm × 8 cm column prepared. The ^{111}In-B72.3 labeling solution was chromatographed and 0.5 ml aliquots were collected. The protein peak (tubes 5, 6) containing ~2 mCi ^{111}In was pooled for use.

HPLC purification. Protein B72.3 labeled with ^{111}In was injected onto a 7.5 mm × 30 cm TSK 3000 column using a LKB Model 700 HPLC equipped with uv and radiometric detectors. The column was run at a flow rate of 1 ml/min using phosphate buffered saline. Protein aggregates cleanly separated at a retention time of 6.5 min., while labeled IgG appeared at 9.5 min. Aggregate peaks usually accounted for ~10% of both protein and ^{111}In label. Specific activity was usually ~3–6 mCi/mg of recovered IgG (80%) which was collected and pooled from three 0.5-ml fractions.

Gel column plus HPLC purification. The pooled eluant of the G-50 column separation described above was further purified by injection onto the TSK-3000 HPLC column of 0.5 ml protein solution. The column was eluted with PBS and collection of protein was performed as described above. Both aggregate and IgG peaks were detected. Usually ~30% of activity was lost in this final purification. This methodology was used to purify all the B72.3-chelate complexes in the studies comparing the various chelates.

After purification, each antibody preparation was assayed for retention of immunoreactivity by solid phase RIA against human tumor extracts and checked for purity by SDS-polyacrylamide electrophoresis before use for imaging and/or tissue distribution studies.

Radioiodination

Radioiodination of B72.3 IgG was performed using the Iodogen method. Forty micrograms of B72.3 was adjusted to 0.1*M* sodium phosphate buffer (pH 7.2) and added to a glass tube coated with 20 μg of Iodogen, followed by the addition of 0.5 mCi Na^{125}I. After a 2-min incubation, the free ^{125}I was separated by Sephadex G-25 (10 ml column) chromatography. This protocol provided iodinated IgG with specific activities of 5 to 15 μCi/μg with up to 60% of the input ^{125}I bound to the protein.

Competition Radioimmunoassay

The immunoreactivity of B72.3 IgG-chelate preparations was assessed by a competition solid-phase radioimmunoassay (RIA) using an extract of a colon carcinoma xenograft (LS-174T) at 1 μg per well. Antibody samples were serially diluted in 1% BSA in PBS and added to the plates in triplicate (in 25 μl). Following a 6 hr incubation at 4°C, 25 μl of ^{125}I-B72.3 (50,000 cpm) was added to each well, and incubated for an additional 18 hr at 4°C. The plates were then washed with 1% BSA in PBS. The bound ^{125}I-B72.3 was detected by cutting individual wells from the plate and measuring the radioactivity in a gamma counter. The percent inhibition, as compared to a control buffer sample, was determined and plotted versus the protein concentration. Unmodified MAb B72.3 IgG (i.e., without chelate) served as a standard.

Cell Lines

The LS-174T cell line (*24*) was obtained from the American Type Culture Collection and was grown in Eagle's minimum essential medium with nonessential amino acids supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). The A375 human melanoma cell line was obtained from Dr. S. Aaronson (National Cancer Institute). The A375 cell lines were grown in Dulbecco's modified Eagle's medium with the same supplements as the LS-174T cell line. The cells were removed from culture flasks with 0.1% trypsin containing 0.5 m*M* EDTA and washed twice in growth medium without serum before inoculation into animals. All cell lines were tested for the presence of Mycoplasma species and were negative.

Tumor Growth in Athymic Mice

Female athymic mice (nu/nu) were obtained ~4 wk of age.‡ One week later, mice were inoculated s.c. (0.1 ml/mouse) with the human colon carcinoma cell line, LS-174T (4×10^5 cells/animal), or with the human melanoma cell line, A375 (4×10^6 cells/animal). Tumors measuring between 0.5 and 0.8 cm in maximal diameter were present at ~2–3 wk postinoculation of the cells, at which time the animals were used for biodistribution and scanning studies.

Biodistribution Studies

Tumor-bearing mice were injected through the tail vein with ~5 μCi/mouse of ^{111}In-B72.3 labeled with each of the four different chelates and the ^{111}In-B72.3-SCN-Bz-DTPA purified using the various methods as outlined above. A group of selected animals were co-injected with ^{125}I-labeled B72.3. Mice (approximately four per group) were killed at 8, 24, 48, and 72 hr postinjection of the label. Blood, tumor, and various organs were immediately collected, wet-weighed on an analytical balance and counted in a gamma counter together with standards prepared from the injected material. Calculations of percent of injected dose/g (% ID/g) for each organ were determined, and tumor-to-tissue ratio calculated.

Groups of mice bearing A375 tumors (human melanoma cell line), negative for the TAG-72 antigen, were also included as a B72.3 negative control for the biodistribution studies.

Imaging Studies

Animals injected with each ^{111}In-MAb-chelate and ^{111}In-B72.3-SCN-Bz-DTPA purified by the various methodologies were used for scintigraphic studies. The mice were anesthetized with Avertin (4 mg/mouse) and scanned with a gamma camera equipped with a 0.25-in. aperture pinhole collimator set at 8 cm from the animal. Images were taken at 8, 24, 48, and 72 hr postinjection. No background subtraction or image enhancement was performed. After the last imaging session, the animals were killed and biodistribution studies were performed.

RESULTS

Chelation of B72.3 IgG

B72.3 IgG was incubated with four chelates, MA-DTPA, CA-DTPA, SCN-Bz-DTPA, and SCN-Bz-EDTA, at different molar ratios to determine the maximum number of chelates that could be bound to each IgG molecule without loss of its immunoreactivity. Immunoreactivity was determined, after the chelate-antibody complexes were exhaustively dialyzed, using a competition radioimmunoassay. The antibody-chelate immunoreactivity varied significantly with the molar ratio of chelate to antibody in the linkage reaction.

The MAb-chelate retained 100% of the immunoreactivity when compared to unmodified IgG when ratios of ~1:1 were used for all the chelates (Fig. 1). Attempts to increase the molar ratio chelate/antibody resulted in a decrease in immunoreactivity of B72.3 (Fig. 1). The use of chelate/antibody molar ratios of >3:1 resulted in the loss of over 50% of the antibody's immunoreactivity (*12*). All the biodistribution studies were performed, therefore, with the preparations yielding chelate:IgG ratios of ~1:1 that retained 100% of the immunoreactivity of the antibody.

The radiolabeled B72.3 IgG was subjected to SDS-polyacrylamide gel electrophoresis before use in vivo. As shown in Figure 2, B72.3 IgG when labeled with ^{125}I or ^{111}In yielded a similar profile when run under non-reducing conditions. The multiple bands at ~150,000 daltons are due to the microheterogeneity of the B72.3 IgG molecule. Differences were observed, however, when the chelates were subjected to beta-mercaptoethanol and heating in order to reduce the IgG molecule to its heavy and light chains. B72.3 labeled using MA-DTPA or CA-DTPA chelates did not retain the ^{111}In radionuclide (Fig. 2). The B72.3-SCN-Bz-DTPA and SCN-Bz-EDTA complexes retained the ^{111}In even under the reducing conditions thus demonstrating the increased stability of the ^{111}In in these chelates.

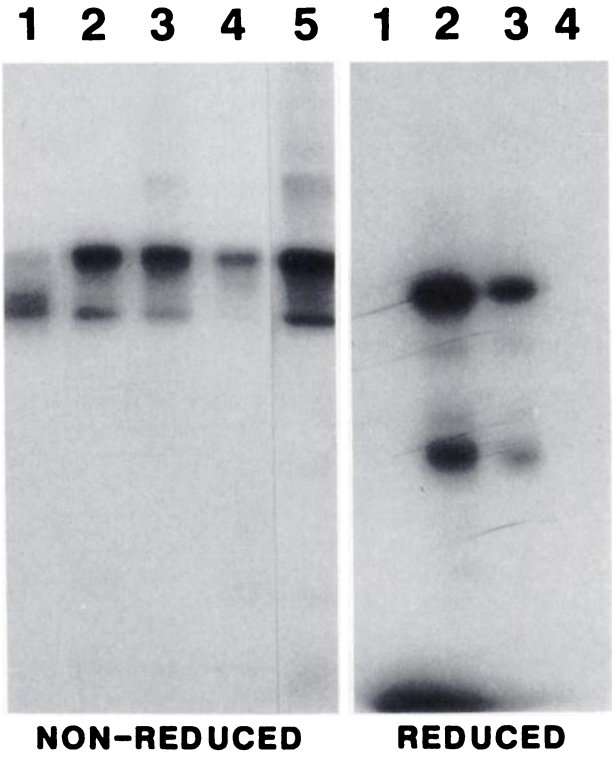

FIGURE 2
SDS-polyacrylamide gel electrophoresis of B72.3 IgG after ^{111}In labeling with four different chelates, under nonreduced and reduced conditions: CA-DTPA (lane 1), SCN-Bz-DTPA (lane 2), SCN-Bz-EDTA (lane 3), MA-DTPA (lane 4). Iodine-125-labeled B72.3 IgG (lane 5) is included for comparison.

Biodistribution

Athymic mice bearing the human colon carcinoma line (LS-174T) were injected with ^{111}In bound to B72.3 IgG using all four chelates linkers. Mice were killed at various times and the radioactivity in each organ was determined. The percent ID/g activity present in the tumors rose with time to approximately 30% by 72 hr postinjection in the groups of animals injected with ^{111}In-B72.3-MA-DTPA, ^{111}In-B72.3-SCN-Bz-EDTA, and ^{111}In-B72.3-SCN-Bz-DTPA, while only 20% of the activity was in the tumor with ^{111}In-B72.3 using CA-DTPA chelate. As seen in Table 1, the accumulation of the label in the vital organs was significantly higher in

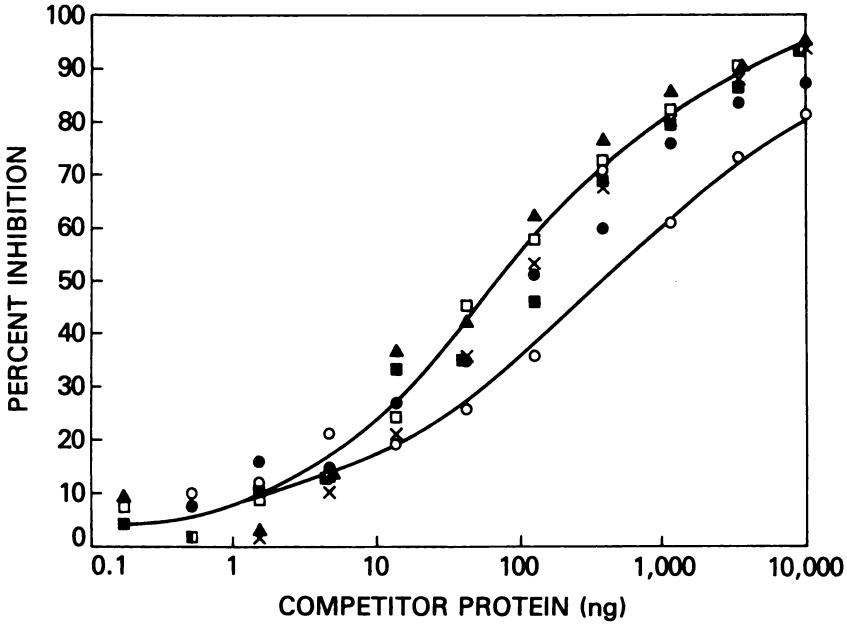

FIGURE 1
Immunoreactivity of B72.3 chelate conjugates. The immunoreactivity of B72.3 IgG after linkage to the different chelates at various ratios of chelates per antibody was determined by competition radioimmunoassays as described in Materials and Methods. Antibody-chelate conjugates were tested at ~1:1 molar ratios for CA-DTPA (□), MA-DTPA (▲), and SCN-Bz-EDTA (■); SCN-Bz-DTPA was tested at 1:1 and 2:1 (●, ○) molar ratios. Unmodified B72.3 IgG was used as a control (X).

the groups of animals injected with B72.3 complexed with CA-DTPA, MA-DTPA and SCN-Bz-EDTA chelates than in the ^{111}In-B72.3-SCN-Bz-DTPA group. It is of particular importance that the liver accumulation rose with time to 13–15% of ID/g by 72 hr when ^{111}In-B72.3 labeled with CA-DTPA, MA-DTPA, and SCN-Bz-EDTA chelates were used. When ^{111}In-B72.3-SCN-B_2-DTPA was used the maximal level of activity in the liver was 9% ID/g at 24 hr which then decreased to 6% over the next 2 days; similar levels were observed in the other organs. There was, therefore, no preferential uptake in the liver when the SCN-Bz-DTPA chelate was used to label B72.3 with ^{111}In. Tumor to organ ratios also demonstrated a clear difference in the biodistribution of ^{111}In when the different chelates were used. Indium-111-B72.3-CA-DTPA showed the least favorable biodistribution with tumor to liver ratios of only 1.3:1 by 72 hr. Indium-111-B72.3 labeled using MA-DTPA and SCN-Bz-EDTA chelates provided slightly better ratios of 1.9:1 and 2.5:1 as seen in Figure 3. These values were inferior to those observed when the SCN-Bz-DTPA chelate was used, where the tumor to liver, spleen, kidney, and lung ratios rose over time reaching greater than 4.8:1 by 72 hr. High tumor to tissue ratios were indicative of the ability to detect tumors by gamma camera scanning.

A group of mice were co-injected with B72.3 labeled with ^{111}In using SCN-Bz-DTPA ligand and ^{125}I-B72.3 in order to compare the biodistribution of the MAb when labeled with both radionuclides. As seen in Figure 4 during the first 72 hr the tumor to organ ratios were very similar for both radionuclides. After that time these ratios kept rising with ^{125}I-B72.3, while the ratios for ^{111}In-B72.3-SCN-Bz-DTPA did not show any improvement with time. These results show that the ^{111}In-B72.3-SCN-Bz-DTPA is as good a MAb-radionuclide conjugate as ^{125}I-B72.3 during the initial 3 days postinjection, and since the $T_{½}$ of ^{111}In is 2.8 days, optimal gamma scanning should proceed or approximate 72 hr post MAb-chelate administration.

Effect of Purification on Biodistribution

Since the best biodistribution results were observed using the SCN-Bz-DTPA chelate, studies on the effects of purification were performed using that chelate-antibody complex. B72.3-SCN-Bz-DTPA was labeled with

TABLE 1
Biodistribution in Athymic Mice Bearing LS-174T Tumors of ^{111}In-Labeled B72.3 After Chelation Using Four Different Ligands

Ligand	Tissue	Time (hr) 8	24	48	72
CA-DTPA	Blood	22.86 (0.26)*	16.74 (0.12)	10.67 (0.03)	7.91 (0.23)
	Tumor	7.38 (0.30)	12.72 (0.09)	18.97 (0.09)	20.38 (0.40)
	Liver	14.81 (0.14)	15.67 (0.25)	17.20 (0.09)	15.81 (0.30)
	Spleen	10.09 (0.20)	9.92 (0.16)	9.14 (0.04)	14.36 (0.32)
	Kidney	16.06 (0.00)	17.63 (0.11)	17.14 (0.08)	19.33 (0.56)
	Lung	13.35 (0.21)	11.43 (0.10)	6.30 (0.03)	7.03 (0.23)
MA-DTPA	Blood	19.40 (0.24)	17.36 (0.26)	13.33 (0.17)	10.71 (0.05)
	Tumor	15.43 (0.15)	16.50 (0.38)	31.51 (0.32)	29.70 (0.61)
	Liver	10.22 (0.07)	11.19 (0.05)	11.59 (0.06)	12.97 (0.18)
	Spleen	6.70 (0.03)	6.78 (0.05)	7.63 (0.01)	8.92 (0.09)
	Kidney	12.63 (0.11)	17.94 (0.31)	17.52 (0.27)	15.78 (0.33)
	Lung	10.06 (0:12)	9.45 (0.16)	7.40 (0.07)	5.84 (0.06)
SCN-Bz-EDTA	Blood	22.82 (0.05)	18.77 (0.32)	19.82 (0.62)	14.75 (0.45)
	Tumor	18.88 (0.49)	15.77 (0.78)	20.82 (0.05)	28.51 (0.62)
	Liver	6.80 (0.03)	8.13 (0.61)	14.26 (0.15)	15.85 (0.27)
	Spleen	5.69 (0.03)	7.52 (0.20)	11.68 (0.15)	9.12 (0.15)
	Kidney	9.33 (0.05)	14.14 (0.16)	16.91 (0.18)	13.03 (0.24)
	Lung	12.16 (0.03)	11.00 (0.23)	9.19 (0.53)	6.34 (0.27)
SCN-Bz-DTPA	Blood	21.82 (0.21)	17.08 (0.34)	18.37 (0.28)	12.01 (0.13)
	Tumor	10.25 (0.22)	13.17 (0.77)	25.94 (1.01)	29.80 (0.40)
	Liver	7.91 (0.08)	9.15 (0.06)	9.09 (0.07)	6.65 (0.10)
	Spleen	5.53 (0.03)	6.18 (0.02)	5.03 (0.09)	6.52 (0.02)
	Kidney	7.45 (0.08)	9.31 (0.06)	8.99 (0.08)	6.47 (0.04)
	Lung	12.25 (0.24)	13.13 (1.46)	9.94 (0.12)	6.92 (0.08)

* % ID/g (average of three to four animals injected with 5 μCi of ^{111}In-B72.3-chelate complex); the standard error of the mean is given in brackets.

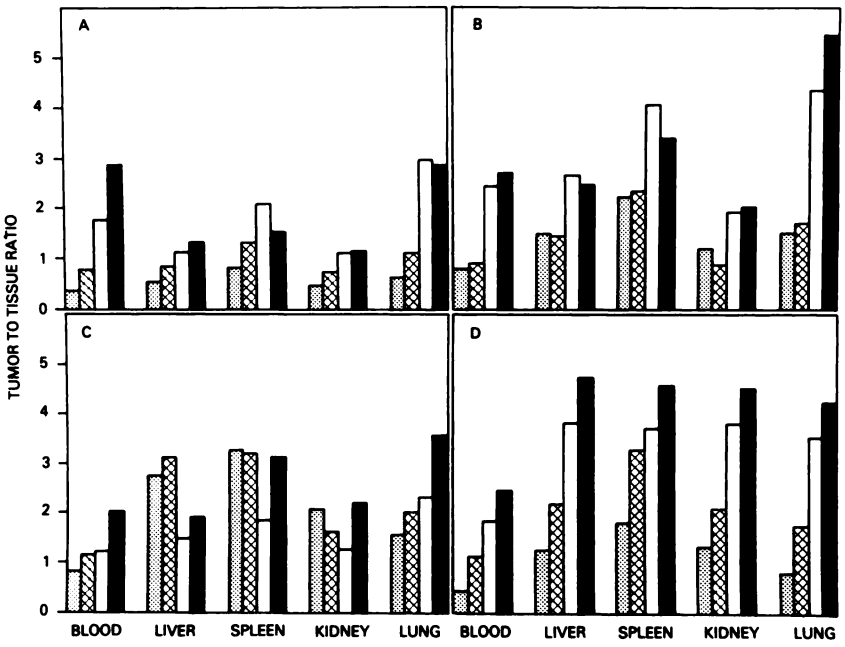

FIGURE 3
Effect of the different chelates in the biodistribution of ^{111}In-labeled B72.3 IgG. Athymic mice, bearing LS-174T tumors were injected i.v. with 5 μCi of ^{111}In-B72.3 after the coupling was done with CA-DTPA (panel A), MA-DTPA (panel B), SCN-Bz-EDTA, (panel C), and SCN-Bz-DTPA (panel D) as described in Material and Methods. Mice (3–4 per group) were killed at 8 hr (□), 24 hr (■), 48 hr (□) and 72 hr (■). Tumor-to-organ ratios were determined. The standard error of the mean was determined for each tissue and was <10% of the average value for most of the data points.

^{111}In and divided into multiple aliquots and purified by Sephadex G-50 chromatography, high performance liquid chromatography (HPLC) using a TSK-3000 column, or a combination of both methods. An aliquot was also treated with excess EDTA to bind any unincorporated ^{111}In. When the conjugates were treated with excess EDTA (chase method) the tumor to organs ratios were the poorest, with tumor to liver ratios of <1.6:1 (Fig. 4A) at 72 hr postinjection of the MAb-radionuclide conjugate, while tumor to liver ratios of 4.6:1 were obtained when the Ab-chelate was purified by HPLC. This demonstrates that the simple addition of excess free EDTA to the ^{111}In-antibody-chelate solution was not sufficient to bind all the free and adventitiously bound ^{111}In and have it excreted as an ^{111}In-chelate complex. Other purification methods are, therefore, required if one wants to avoid residual free metal localizing the abdominal organs. As seen in Figure 4, Sephadex G-50 chromatography produced slightly better results than the "chase" method (Fig. 4B), but was inferior to the HPLC purified Ab-chelate. The combination of Sephadex G-50 separation and HPLC, however, provided the best results with tumor to liver ratios of 5:1 (Fig. 4C), i.e., it was clearly superior to those obtained with the "chase" or G-50 column methods: 1.6:1 and 2:1, respectively.

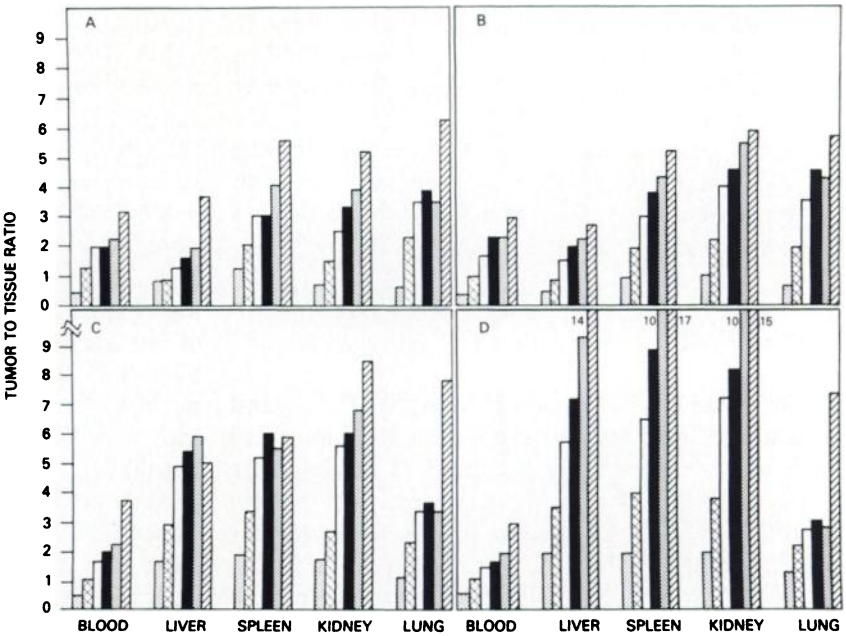

FIGURE 4
Effect of purification protocols of ^{111}In-B72.3-SCN-Bz-DTPA on its biodistribution in athymic mice. Athymic mice bearing LS-174T carcinomas were injected with 5 μCi of ^{111}In-B72.3-SCN-Bz-DTPA purified following the protocols described in Material and Methods: "Chase" (panel A), G-50 (panel B), G-50 + HPLC (panel C). Mice were killed at 8 hr (□), 24 hr (▩), 48 hr (□), 72 hr (■), 96 hr (□), and 168 hr (▨), and tumor-to-organ ratios were determined. A group of mice was coinjected with ^{125}I-B72.3-IgG (panel D). The standard error of the mean was determined for each tissue and was <10% of the average value for most of the data points.

Animals bearing TAG-72 antigen negative A375 melanoma tumors were used as negative controls and were injected similarly with ^{111}In-labeled B72.3 using the four different chelates, as well as with ^{111}In-B72.3-SCN-Bz-DTPA after purification by the four different protocols. Biodistribution studies showed a lack of specific uptake of the radiolabeled MAb by the melanomas as demonstrated by tumor to organs ratios of <1:1 with different chelates or purification methods. The percentage of injected dose per gram in the tumors was <2%. The amount of the ^{111}In-B72.3 found in the normal organs was similar to that found in mice bearing the LS-174T colon carcinoma xenograft. This lack of localization of ^{111}In-B72.3 in the TAG-72 negative tumors further demonstrates the specificity of the B72.3 localization in the LS-174T colon carcinomas.

Imaging Analyses

Athymic mice bearing LS-174T tumors were injected with ^{111}In-B72.3 using all four chelate-antibody complexes. The tumor was evident in images obtained after 24 hr postinjection. At this time the differences between the four chelates were visibly evident, reflecting the tumor to organ ratios obtained in the biodistribution studies. The B72.3 labeled with ^{111}In using CA-DTPA chelate showed the poorest tumor specific uptake. Indium-111-B72.3-MA-DTPA and ^{111}In-B72.3-SCN-Bz-EDTA provided slightly better images due to the less intense abdominal background activity. The images of the four different chelates at 72 hr postinjection are shown in Figure 5. The ^{111}In-B72.3 uptake by the abdominal organs, primarily the liver, is less intense with the SCN-Bz-DTPA chelate when compared with the other chelates. When examining the time course images of ^{111}In-B72.3-SCN-Bz-DTPA and ^{111}In-B72.3-MA-DTPA (Fig. 6) it can be appreciated that the high percent ID/g accumulated in the tumor with ^{111}In-B72.3-SCN-Bz-DTPA, together with higher tumor to liver, spleen and kidney ratios, made possible the best images of the tumor with minimal uptake in the liver and other organs.

The control animals bearing A375 tumors showed no specific uptake by the tumor using all four MAb chelates, but the CA, MA, and EDTA MAb-chelates complexes had significant liver uptake.

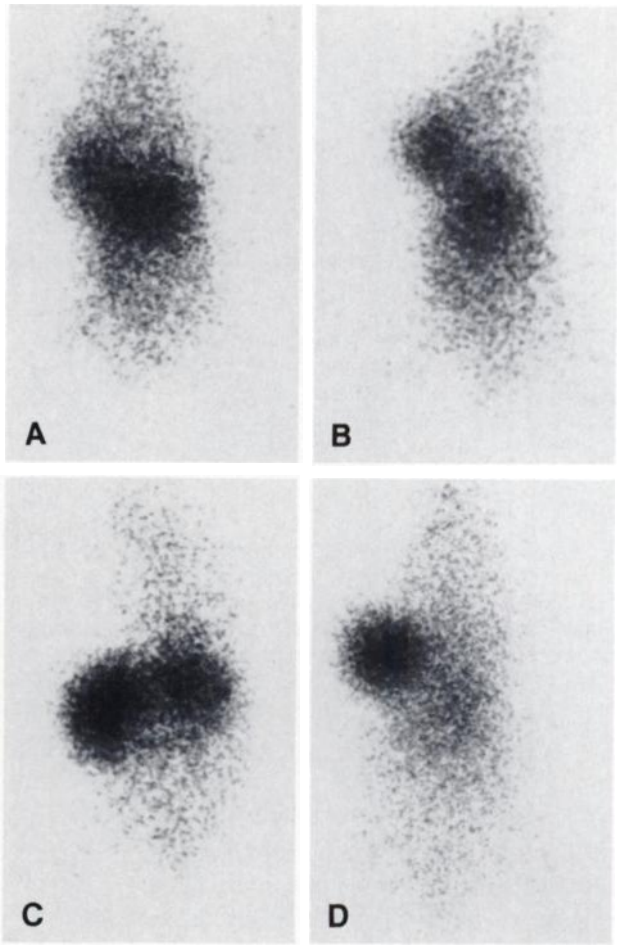

FIGURE 5
Comparison of the scintiscan images of nude mice bearing LS-174T tumors using chelates CA-DTPA (panel A), MA-DTPA (panel B), SCN-Bz-EDTA (panel C) and SCN-Bz-DTPA (panel D). Athymic mice bearing 100–200 mg tumors were injected with 5 μCi of ^{111}In-labeled B72.3 IgG and scanned 72 hr later.

DISCUSSION

The availability of numerous MAbs generated against a variety of immunogens present on the tumor cell surface, together with the feasibility of linking these MAbs to a variety of radionuclides without significantly altering the immunoreactivity of the antibody has opened the potential for a new era in the management of human malignancies. There are many groups that have successfully labeled MAb with iodide radionuclides and used them for localization of malignant tumors both in experimental models and clinical trials (*1*,*2*). Iodine-131 is not the best radionuclide, however, for localization purposes due to its undesirable physical properties (strong beta and high energy gamma emissions) and the potential of in vivo dehalogenation of some MAbs. Indium-111 may be a more suitable isotope for radiolocalization studies. Its gamma energies of 173 and 247 keV, the lack of beta emission and the 67 hr half-life are some of the advantages of this radionuclide. It should be pointed out, however, that contrary to previously published reports (*3*,*4*), some iodinated MAbs show no evidence of significant dehalogenation (*1*) (or dehalogenation whose effect cannot be overcome by the use of SSKI). On the other hand, iodination of some MAbs may result in the loss of immunoreactivity of the Ig molecule, either due to the effects of the iodination reaction mixture or to the

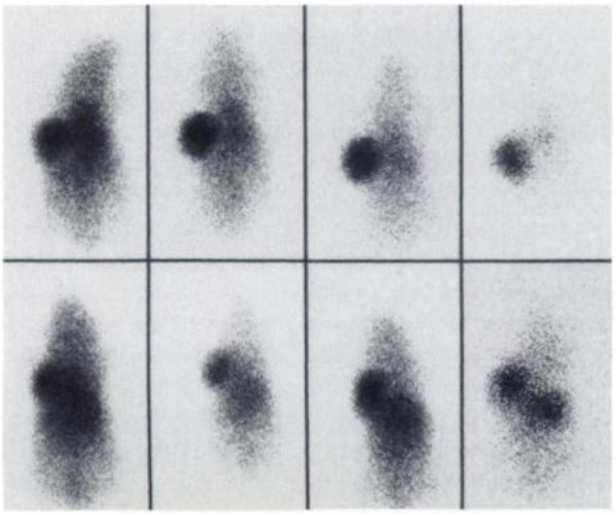

FIGURE 6
Scintiscan images of athymic mice bearing LS-174T tumor after injection of B72.3 IgG labeled with ^{111}In using two chelates: SCN-Bz-DTPA (upper panels) and MA-DTPA (lower panels). Athymic mice bearing 100–200 mg tumors were injected with 5 μCi of ^{111}In-B72.3 and scanned at 8 hr, 24 hr, 48 hr, and 72 hr postinjection.

labeling of tyrosine in the Ig binding site. In these cases, ^{111}In-chelate-MAb is obviously more useful.

Since bifunctional chelating agents were used for linking proteins to metals, the in vivo instability encountered with the direct labeling method has been overcome. After the first bifunctional chelating agent EDTA was prepared in 1975, many others have been used to prepare radiometal labeled proteins. Mixed anhydrides (MA) (*25,26*) and cyclic anhydrides (CA) of DTPA (*27–29*) conjugates are most commonly used due to the efficiency, stability, and simplicity of the method as well as their long storage potential. The labeling methodologies use one of the DPTA sidearms to link to the protein, therefore chelate is a DTTA. DTTA-In complexes are inherently less stable then DTPA-In complexes. Many of the in vivo studies performed with those chelates also provided good tumor uptake of the label. There is, however, a major drawback with these chelates in the high nonspecific uptake of the metal by the liver (believed to be mediated through serum transferrin) (*8–11,25*). Since the liver is the organ where most of the common epithelial tumors metastasize, a significant uptake of radiolabel in that organ will obscure the images of possible metastases, therefore restricting the utility of ^{111}In-MAb in many clinical trials. Rodwell et al. (*31*) have recently described a method for the attachment of DTPA to the oligosaccharide moiety of the IgG molecule using a peptide. The addition of the peptide to the DTPA molecule may add additional coordination sites and thus help stabilize the In in the chelate.

Athymic mice bearing LS-174T human colon carcinoma xenografts were injected through tail vein with ^{111}In-B72.3-SCN-Bz-DTPA. The uptake by the tumor rose four-fold over the first 2 days reaching 30% ID/g at 72 hr. The blood uptake dropped over the same period while the rest of the organs remained relatively constant with liver uptake values in the range of 9 to 6% ID/g from 8 to 72 hr. Tumor to organ ratios rose uniformly over 72 hr time period and consequently the images taken over that period became progressively clearer with most of the activity present in the tumor and decreasing abdominal activity over time. This demonstrates that MAb-SCN-Bz-DTPA complex forms a very stable bond with the radionuclide in vivo without apparent leakage into the blood with subsequent transport by transferrin into the liver.

We compared the above results with those obtained in parallel studies performed with three different MAb-chelates: CA-DTPA and MA-DTPA, two of the more popular bifunctional chelates and SCN-Bz-EDTA. Accumulation of the MAb-chelate conjugate in the tumor similar to that observed with ^{111}In-B72.3-SCN-Bz-DTPA was noted with the ^{111}In-B72.3-MA-DTPA and less deposition was seen with the ^{111}In-B72.3-CA-DTPA. However, the percent ID per gram found in all the organs and particularly the liver was much higher with these chelates. Consequently, the tumor-to-organ ratios, similar to those reported in previous studies (*25, 26*), never rose to the values obtained by ^{111}In-B72.3-SCN-Bz-DTPA. This was reflected in the scintigraphic images by a more prominent background activity in the abdominal organs, mostly in the liver, that obscured the tumor image. Indium-111-B72.3-SCN-Bz-EDTA also proved to be an unstable preparation with tumor-to-liver ratio rising for the first 24 hr, and dropping thereafter. The ^{111}In apparently leaked out of thermodynamically less stable chelate and accumulated in the liver, impairing the tumor images.

We have also systematically studied four different purification methods of ^{111}In-B72.3-SCN-Bz-DTPA to determine if a cleaner conjugate could improve the biodistribution results and consequently the scintigraphic images. Our best results were obtained after the conjugate was purified by passage through a G-50 chromatography column and HPLC through a TSK-3000 column yielding tumor to liver and spleen ratios of 5:1. The currently used method of adding excess free EDTA provided the worst biodistribution (i.e., tumor to liver and spleen ratios of <2:1). Therefore, other purification methods should be considered if one wants to minimize the deposition of free-radionuclide in abdominal organs. The biodistribution studies using ^{125}I and ^{111}In-labeled B72.3 co-injected in the same colon carcinoma tumor bearing mice, proved that the uptake of the MAb by the tumor and organs was very similar with both radionuclides. Tumor to organ ratios were virtually identical up to 72 hr postinjection, tumor to organ

ratios continued to improve with the ^{125}I-labeled B72.3 while the ^{111}In-labeled antibody showed no further improvement.

The results reported here show that with the new method for linking DTPA to MAbs, we have minimized the problem of high liver uptake of radionuclide. The method is simple and efficient and may prove to be of use in clinical trials for detection of primary tumors and metastases to liver and other abdominal organs. We have also demonstrated an advantage in extensively purifying the MAb-chelate conjugate prior to in vivo use in order to optimize the scintiscan images.

NOTES

* DuPont Company, No. Billerica, MA.

† Capintec, Ramsey, NJ.

‡ Charles River, Inc., Kingston or the Frederick Cancer Research Facility.

The gamma camera used in these studies was a Raytheon Step 1/Step 2 large field-of-view camera equipped with a 0.25-in. aperture pinhole collimator.

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