Yttrium-90-Labeled Monoclonal Antibody for Therapy: Labeling by a New Macrocyclic Bifunctional Chelating Agent

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Yttrium-90 (^{90}Y) is a promising radiometal for therapy of cancer due to its high-energy beta emission and a physical half-life of 2.67 days. Bifunctional chelating agents based on DTPA cyclic anhydride or EDTA do not form Y(III) complexes that are stable under physiologic conditions. A new macrocyclic bifunctional chelating agent based on 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (DOTA) forms a stable Y(III) complex. It was converted to *p*-bromoacetamidobenzyl-DOTA (BAD), and conjugated to monoclonal antibody Lym-1 via 2-iminothiolane, either as the free ligand or as the ^{88}Y chelate. Stability studies of Lym-1-2IT-BAD-^{88}Y in human serum in vitro showed no measurable loss of Y(III) from the ligand over a 25-day period. In Raji-tumored mice, tumor uptake was 16.8% of the injected dose per gram of tissue on Day 3. The bone uptake was 2.0, 3.6, and 2.1% injected dose per gram of tissue on Day 1, 3, and 5, respectively. The biodistribution of the control ^{88}Y-citrate demonstrated continuous increase in bone uptake from 13.8% injected dose per gram on Day 1 to 24.9% injected dose per gram on Day 4.

J Nucl Med 1990; 31:473-479

Yttrium-90 (^{90}Y) is considered a suitable radionuclide for radioimmunotherapy (*1–4*). It can be produced easily by a ^{90}Sr/^{90}Y generator, has a physical half-life of 2.7 days, and is a pure, high-energy beta emitter useful for therapy in solid tumors. Hnatowich et al. (*5, 6*) chelated ^{90}Y with diethylenetriaminepentaacetic acid (DTPA) conjugated to monoclonal antibody by an anhydride method and studied its behavior in patients on therapy protocols. Bone marrow suppression in these patients suggested that significant amounts of ^{90}Y were incorporated into the bone. Animal experiments indicated that this may be due to the loss of ^{90}Y from the DTPA chelate in vivo (*7–10*). Recent studies of benzyl-DTPA based bifunctional chelating agents, however, showed that the loss of Y(III) in vitro in human serum was much slower than with unsubstituted DTPA (*11*) and that Y(III) loss in vivo from those chelates can be substantially decreased by the addition of methyl groups on the DTPA backbone (*12*).

We have chosen an alternate approach to bind Y(III) and have synthesized a macrocyclic bifunctional chelating agent: 2-p-nitrobenzyl-1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (*p*-nitrobenzyl-DOTA), in our laboratory for stably binding Y(III) (*11*). Serum stability studies of the Y(III) chelate of *p*-nitrobenzyl-DOTA showed no loss of yttrium to any serum component under physiologic conditions during 18 days in human serum.

In the present study we labeled an anti-B cell monoclonal antibody, Lym-1, with ^{88}Y, using this new bifunctional chelating agent. We used ^{88}Y for laboratory study instead of ^{90}Y due to its long half-life and gamma-emitting properties. The biodistribution of labeled Lym-1 (Lym-1-2IT-BAD-^{88}Y) was performed in Raji-tumored nude mice with special emphasis given to obtaining reproducible data for bone uptake. The biodistribution of ^{88}Y-citrate was studied for comparison.

MATERIALS AND METHODS

The bifunctional chelating agent *p*-nitrobenzyl-DOTA was prepared according to the method of Moi et al. (*11*). The nitro group was reduced to an amino group and was acylated with bromoacetyl bromide by the methods described previously (*13*). 2-Iminothiolane was obtained from Pierce (Rockford, IL). Yttrium-88 was obtained from Los Alamos National Laboratory (Los Alamos, NM) as ^{88}Y-chloride in 12 *M* HCl.

Analytical Techniques

Thin-Layer Chromatography. Thin-layer chromatography (TLC) of the samples was performed using plastic-backed silica-coated TLC plates (EM Science, DC-Plastikfolien kieselgel 60 F254, Cherry Hill, NJ). The mobile phase was a 1:1 mixture of equal volumes 10% (w/v) aqueous ammonium

Received Aug. 1, 1989; revision accepted Dec. 4, 1989.

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acetate and methanol. The developed TLCs were scanned for radioactivity using a radioactive gel scanner (AMBIS systems, San Diego, CA) or cut at Rf 0.3 and counted in a gamma well-counter.

High-Performance Liquid Chromatography (HPLC). HPLC of the radiopharmaceuticals was performed using a Beckman HPLC system, Beckman Instruments, Inc., Palo Alto, CA, with a TSK-3000 gel filtration column. The radioactivity of the effluent was continuously determined by a flow-through radioisotope detector (Beckman Model 170).

Cellulose Acetate Electrophoresis. Cellulose acetate electrophoresis (CAE) of the radiopharmaceuticals was performed using a Gelman electrophoresis unit (Gelman Sciences, Inc., Ann Arbor, MI). The CAEs were scanned for radioactivity using the AMBIS gel scanner.

Purity of Yttrium-88

Yttrium-88 (760 μCi) was dried in a metal-free vial using a heat block and a gentle flow of nitrogen gas. To the dry ^{88}Y, 7.0 μl of 0.1 *M* tetramethylammonium acetate (TMA), pH 5, was added. An aliquot of 0.1 μl of this solution was mixed with 10 μl of TMA and was divided into two 5.0-μl aliquots. To one of the aliquots, 5.0 μl of 0.1 *M* ethylenediaminetetraacetic acid (EDTA), pH 7 was added. After 10 min TLC was performed on these two solutions. The results showed that ^{88}Y could be completely chelated by EDTA and that the unchelated ^{88}Y control remained at the origin.

Preparation of BAD-^{88}Y Complex

To the remaining 6.9 μl solution of ^{88}Y, 4.0 μl of 11.4 m*M* *p*-bromoacetamidobenzyl-DOTA (BAD) was added and mixed well. The pH was increased to 6.0 by using dilute TMA at pH 9. TLC was performed at 30, 60, and 150 min after incubation at room temperature. At 150 min, 75% of the yttrium was incorporated into the macrocycle.

Separation of BAD-^{88}Y complex from Free Yttrium-88

A 0.25-ml column of DTPA modified P2 resin (compliments of Prof. T. G. Wensel, Baylor University) was prepared by using a 1.0-ml tuberculin syringe cut at 0.5 ml. The column was washed with metal-free water until the pH of the effluent was 6. The ^{88}Y reaction mixture was loaded on this column, eluted with water and one-drop fractions were collected. The radioactive fractions were pooled together and TLC was performed on this solution.

Preparation of Lym-1-2IT-BAD-^{88}Y

The method by which chelate was conjugated to antibody is outlined in Figure 1. One-hundred microliters of Lym-1 (Damon Biotech, Needham Heights, MA, lot # 3-171-860818, 16.2 mg/ml in 0.1 *M* sodium phosphate buffer pH 8.3) and 2.5 μl of *p*-mercaptoethanol were added to 50 μl of 25 m*M* 2-iminothiolane prepared in 50 m*M* triethanolamine at pH 8.5. The mixture was incubated for 1.0 hr at 4°C. The resulting conjugate, Lym-1-2IT, was separated by centrifuged column-gel filtration using Sephadex G-50-80 in 0.1 *M* sodium phosphate buffer at pH 8.3 (*14*). For the murine biodistribution studies, the purified BAD-^{88}Y obtained above (330 μCi, 0.0264 μmol BAD in 250 μl, chelate to antibody ratio 2.5) and 40 μl of 0.1 *M* EDTA was added to the Lym-1-2IT, and the pH was maintained at 8 to 8.5. EDTA was included here to chelate any tracers of transition metals that might catalyze oxidation of thiol groups on Lym-1-2IT. Nitrogen gas was gently blown over the solution, the vial was capped and the solution incubated at 4°C overnight. To alkylate the remaining sulfhydryl groups, 2.0 mg of iodoacetamide was added and the solution was incubated for 30 min.

The ^{88}Y-labeled protein was separated from the small molecular weight BAD-^{88}Y, and the buffer changed to phosphate buffered saline, pH 7.4, by centrifuged column-gel filtration chromatography.

For studies on yttrium labeling of the antibody-chelator conjugate rather than the chelator alone, BAD without yttrium was conjugated to the Lym-1-2IT in the same manner. The conjugated Lym-1-2IT-BAD (25 μ*M* antibody, conjugated with 100 μ*M* DOTA groups) which had not been prelabeled with ^{88}Y was incubated 2 hr at pH 4.4 with carrier-free ^{88}Y in 0.1 *M* ammonium acetate buffer. TLC evaluation was performed as follows to assess the percent of ^{88}Y incorporation. Aliquots taken at 10-min intervals were made 10 μ*M* in DTPA, incubated 10 min, and applied to the TLC plate. Lym-1-2IT-BAD-^{88}Y smeared from the origin to relative mobility (Rm) 0.3; DTPA-^{88}Y migrated to Rm 0.6 without smearing. TLC plates were cut and counted in the gamma counter.

Quality Assessment of the Radiopharmaceutical

Twenty-five microliters of the radiopharmaceutical was diluted to 110 μl with normal saline, pH 7.4. Two 10-μl aliquots were placed in two separate vials, and 10 μl of 0.1 *M* EDTA was added to one of the aliquots and incubated at room temperature for 30 min. These solutions were compared by TLC. The radiopharmaceutical for use in serum stability and murine biodistribution also was evaluated by CAE, HPLC, and solid-phase radioimmunoassay (*15*).

Stability Studies

Human serum was prepared by allowing blood collected from a healthy volunteer to clot 1 hr at room temperature in a closed tube. The sample was centrifuged and the supernatant serum was transferred to sterile plastic culture tubes. It was then incubated overnight at 37°C in a humidified 5% carbon dioxide, 95% air atmosphere. The pH of an aliquot was measured as 7.4 before the addition of Lym-1-2IT-BAD-^{88}Y, and maintained at pH 7.4 ± 0.1 throughout the experiment.

Prior to this addition, the labeled antibody was challenged with 1.0 m*M* *p*-nitrobenzyl-DTPA, then isolated and transferred from phosphate buffer to water by centrifuged column-gel filtration; this solution was examined by TLC. In duplicate, 147 μl of labeled antibody was then added to 2.0 ml of serum to achieve final Lym-1 and DOTA concentrations of 80.2 n*M* and 84.2 n*M*, respectively. The serum solutions were then returned to incubate at 37°C in a CO_2-enriched atmosphere.

After the introduction of the labeled antibody, 20-μl aliquots of the serum were analyzed by polyacrylamide gel electrophoresis at daily intervals for 25 days. Six percent polyacrylamide native protein slab gels were used in this study; electrophoresis using a 10% gel was also performed at 25 days. The tris/HCl buffered gels were of pH 8.5 and the tris/glycine tank buffer, pH 8.9 (*16*). Bromophenol blue solution in adjacent lanes marked the dye front. The relative mobilities of serum proteins were determined by electrophoresis and staining of Lym-1-2IT-BAD-^{88}Y, human serum albumin (HSA) (purchased from Worthington Biochemical, Freehold,

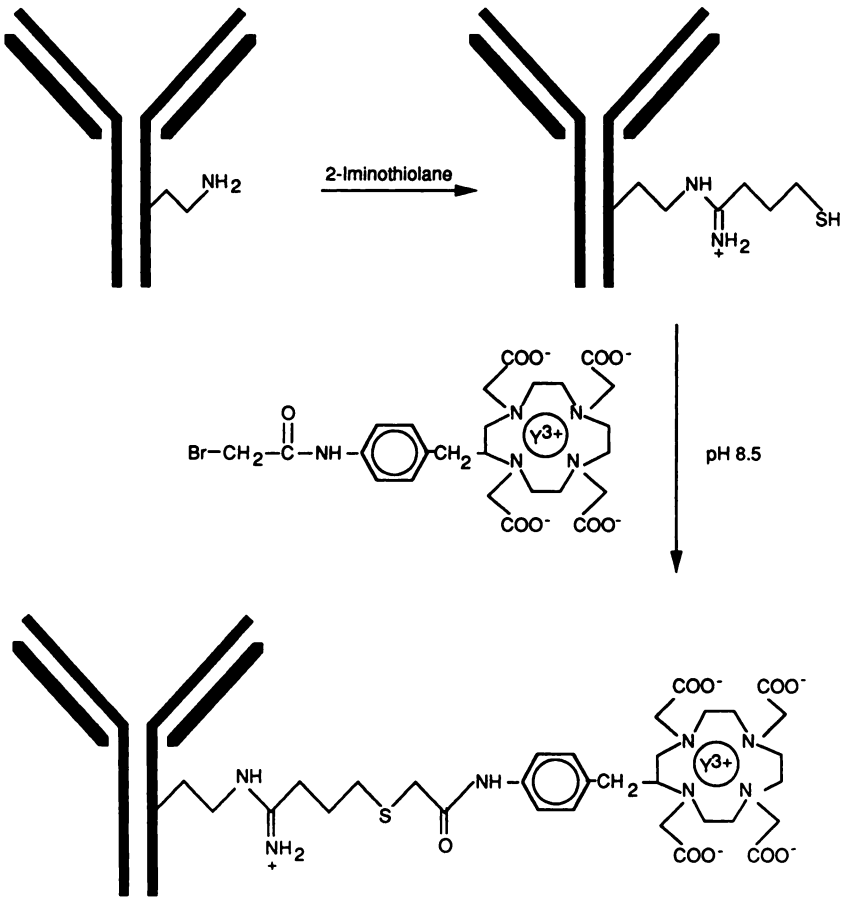

FIGURE 1
Scheme of labeling the monoclonal antibody with ^{88}Y using the bifunctional chelating agent p-bromoacetamidobenzyl-DOTA. As described in Materials and Methods, yttrium may also be added last.

NJ), and human serum transferrin (purchased from Sigma Chemical, St. Louis, MO). The gels were dried, cut into pieces, and counted on a Beckman 310 Gamma counter using a window of 0–1.84 MeV.

Mouse Biodistribution Study

Athymic nude mice (3–4 wk old) were obtained from the Harlan Sprague Company (Indianapolis, IN) or the Simonson Company (Gilroy, CA). The mice were treated with 400 rad whole-body irradiation 3 days prior to subcutaneous injection of 5×10^6 Raji cells. After 3 wk, each of 22 mice with tumors (0.05–0.86 g) were given 20 μg (2.7 ± 0.3 μCi) of labeled radiopharmaceutical, Lym-1-2IT-BAD-^{88}Y, by tail vein injection and the biodistribution was studied using a dual-probe for counting whole-body retention and a gamma well counter for counting blood and organs (*14*). To measure the activity in the bone, the femurs were cleanly dissected from killed mice and the marrow was removed using a 25-gauge needle on a 1.0-ml syringe. The bone was thoroughly washed with water to remove any trace of marrow or blood and dried at 40°C, weighed and counted with the standards. In addition, 12 nontumored nude mice were injected with ^{88}Y-citrate in 0.05 *M* ammonium citrate buffer at pH 7.4 and biodistribution was studied in the same manner as described above. Statistical calculations to obtain standard deviation for organ uptake were done by using the data from at least four mice at each time point.

RESULTS

Conjugation and Labeling

Lym-1-2IT-BAD-^{88}Y for the murine biodistribution and serum stability studies was prepared by a method that assured that all the ^{88}Y was in the chelate and none was bound to other sites on the antibody, by prechelating the ^{88}Y prior to conjugating it to the antibody. In 2.5 hr, 75% of the ^{88}Y was chelated by the macrocyclic bifunctional chelating agent, BAD. The TLC of the effluent in the separation of BAD-^{88}Y from unchelated ^{88}Y, showed no radioactivity at the origin, indicating that all the free ^{88}Y had been retained by the purification column. After reaction with monoclonal antibody Lym-1, the conjugation yield for the preparation of Lym-1-2IT-BAD-^{88}Y was 42%. The average number of chelates conjugated per antibody was 1.05 and the specific activity of the labeled antibody was 138 μCi/mg.

Chelation of carrier-free ^{88}Y-88 by 100 μM antibody-bound DOTA groups (four chelates per antibody) occurred with a half-life of 2 hr at pH 4.4, 21°C in 0.1 *M* ammonium acetate. Further experiments to determine optimal conditions are in progress.

Quality Assessment of the Radiopharmaceutical

The TLC of the radiopharmaceutial Lym-1-2IT-BAD-^{88}Y used for mouse biodistributions showed a single radioactive spot at the origin. When challenged with 5 m*M* EDTA, the TLC of this radiopharmaceutical did not change, indicating the absence of any unchelated ^{88}Y. The HPLC of Lym-1-2IT-BAD-^{88}Y demonstrated that 87% chromatographed as the 150 Kd molecular weight monomer and 13% as a dimer (300 Kd). CAE showed the migration of radioactivity between 0 and 1.5 cm from the origin at 11 min, suggesting the absence of any free BAD-^{88}Y, which moves to 0.3 cm in this time. The 45-min CAE showed 90% of the radioactivity migrated at 3.5 cm from the origin and 10% remained at the origin. The radioactivity at the origin was thought to be the dimer present in the radiopharmaceutical as demonstrated by HPLC. The solid-phase assay for immunoreactivity of Lym-1-2IT-BAD-^{88}Y showed 42% bound, as compared to 43%–44% of the standard ^{125}I-labeled Lym-1 preparation (chloramine-T:Ab = 1:10, I:Ab = 1:10). The standard ^{125}I-Lym-1 preparation binds 80% in live-cell assay (*15*) and 43%–44% on solid-phase assay. Thus, the immunoreactivity of the Lym-1-2IT-BAD-^{88}Y is 95%–96% relative to the ^{125}I standard preparation by the simple solid-phase assay.

Serum Stability Study

An aliquot of Lym-1-2IT-BAD-^{88}Y was challenged with *p*-Nitrobenzyl-DTPA, and the TLC of this solution showed no indication of the formation of nitrobenzyl-DTPA-^{88}Y.

Relative mobility of the labeled antibody, transferrin, albumin, and free chelate (not bound to antibody) were found to be 0.13, 0.49, 0.93, and 1.0, respectively, in the 6-percent gel; 0.06, 0.18, 0.36, and 1.0, respectively, in the 10-percent gel.

Counts in 6-percent gels from Rm 0.0–0.4 were demonstrated to be Lym-1-2IT-BAD-^{88}Y. A small decline in the fraction of total counts in this region over time, measured at 0.2% per day, was offset by a rise in the fraction of total counts in the Rm 0.9–1.1 region. This corresponds in 6-percent gels both to albumin and free chelate, but in 10-percent gels, only to free chelates. On a 10-percent gel, these counts again appear in the Rm 0.9–1.1 region, showing that the counts arose not from transcomplexation of ^{88}Y to albumin, but rather from the hydrolysis of the bond linking the chelate to the antibody.

The sum of counts from Rf 0.0–0.4 and 0.9–1.1 were divided by total counts to determine the fraction of total counts attributable to intact chelated ^{88}Y. There was no measurable loss of chelated ^{88}Y over the period of study; a least-squares fit of the data showed a rate of change of −0.03%/day, but the 95-percent confidence limits range from +0.03% to −0.08% per day (Fig. 2). Therefore, it cannot be stated with confidence that any ^{88}Y was lost from the chelate during the 25-day period in serum.

Mouse Study

The whole-body clearance for the conjugate was slow, with a biologic half-life >5 days. This was comparable to the biologic half-life of ^{88}Y-citrate. (Fig. 3). The blood-clearance half-life of Lym-2IT-BAD-^{88}Y was 3 hr. The localization of the radioactivity in the liver showed a slow increase until 2 days after injection and decreased thereafter (Fig. 4A). The mice injected with ^{88}Y-citrate showed continuous decrease in the liver levels from 8.2% I.D./g on Day 1 to 2.7% I.D./g on Day 4 (Fig. 4B). With the conjugate Lym-1-2IT-BAD-^{88}Y, the kidney had 16.3% I.D./g uptake on Day 1 and decreased continuously to 12.1% I.D./g on Day 3. During this time, there was <1.3% I.D./g radioactivity in the intestinal contents for this conjugate and the ^{88}Y-citrate. This suggests that the urinary system is the excretory pathway of the yttrium and Lym-1-2IT-BAD-yttrium metabolites. The mean tumor uptake for Lym-1-2IT-BAD-^{88}Y was 8.2% ± 2.9% I.D./g (n = 6) on Day 1; it peaked at 17.5% ± 7.6% (n = 6) on Day 2 and decreased to 14.5% ± 2.3% (n = 5) on Day 5.

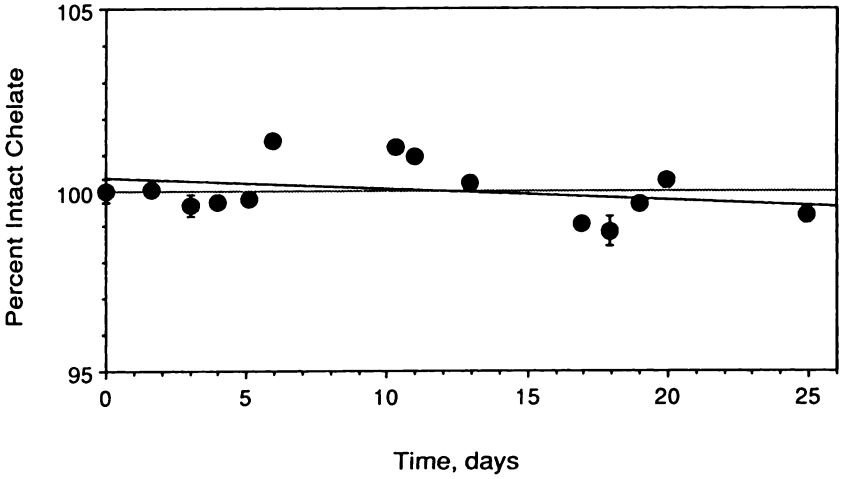

FIGURE 2
In vitro human serum stability study of Lym-1-2IT-BAD-Y-88 over 25 days shows no measurable decomposition of metal chelate. While the time plot of percent total radionuclide chelated suggests a rate of loss marginally greater than zero, 95% confidence limits of the rate of change range from +0.03% to −0.08% per day. Some values are greater than 100% because all data have been adjusted, relative to the value at t = 0. All values represent the average of two measurements, whose range is displayed by error bars when it exceeds the height of the dot.

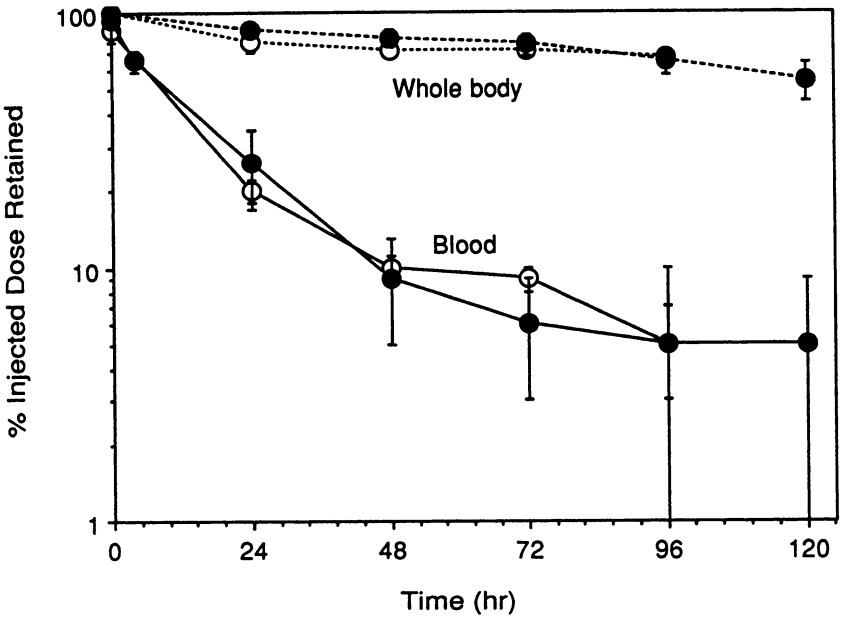

FIGURE 3
Whole-body (dotted lines) and blood- (solid lines) retention curves for Lym-1-2IT-BAD-Y-88 (0) and Citrate-Y-88 (O) from murine biodistribution studies. Both whole-body and blood-retention curves for these two radiopharmaceuticals were similar. For whole body, the number of animals included was 16, 4, 6, 6, and 6 on days 1, 2, 3, 4, and 5 respectively. For blood, the number of animals included was 16, 6, 5, 8, 14, 3, 4, 4, and 4 at time points 5 min, 1 hr, 3 hr, 6 hr, 1 day, 2 day, 3 day, 4 day, and 5 day respectively.

The deposition of ^{88}Y in the bone of the mice injected with ^{88}Y-citrate increased continuously from 13.8% I.D./g on Day 1 to 24.9% I.D./g on Day 4 (Fig. 4B). The mice injected with Lym-1-2IT-BAD-^{88}Y had 2.0 ± 1.9, 3.6 ± 2.1, and 2.1 ± 0.5 I.D./g uptake in the bone 1, 3, and 5 days postinjection, respectively.

DISCUSSION

Labeling of monoclonal antibodies with ^{90}Y for radioimmunotherapy requires the use of a suitable bifunctional chelating agent (BCA). The Y(III) chelate of this bifunctional chelating agent should be stable in vivo and should not lose the yttrium to competing metal-binding ligands on proteins in blood or other organs. In addition, the covalent binding of the BCA should not alter the monoclonal antibody structure or its tumor-targeting ability.

Some DTPA- and EDTA-based bifunctional chelating agents that are currently in use do not form Y(III) complexes that are stable under physiologic conditions. Based on our experience with a macrocyclic bifunctional chelating agent for copper-67- (^{67}Cu) labeled monoclonal antibodies (*14*), we developed another ma-

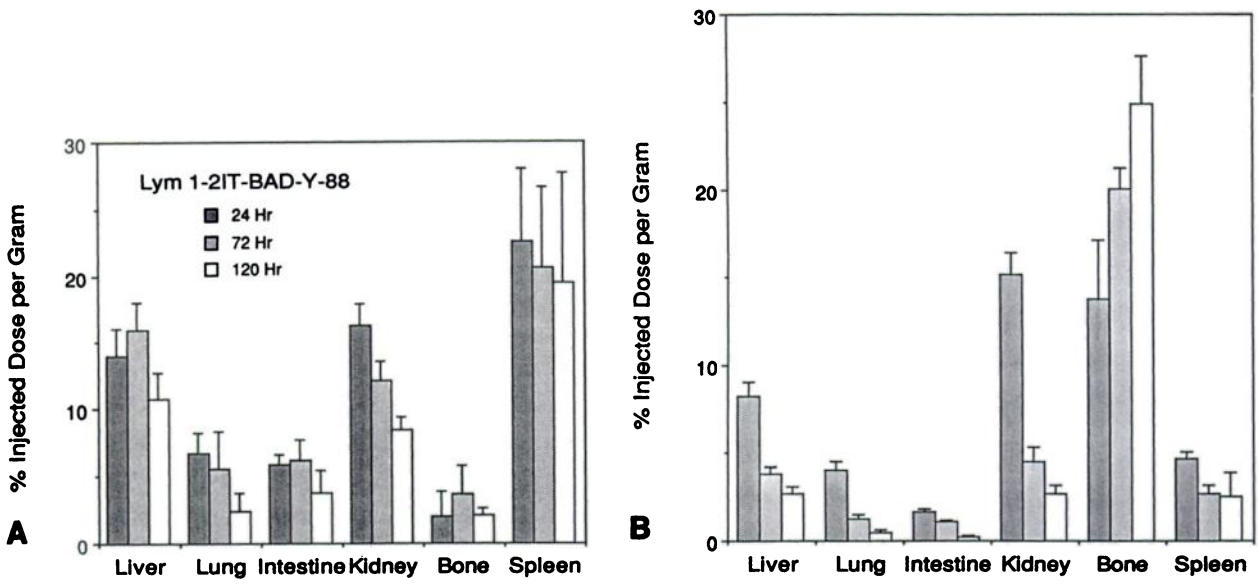

FIGURE 4
(A) The organ distribution of Lym-1-2IT-BAD-^{88}Y in mice at 24 hr (■), 72 hr (■) and 120 hr (□) postinjection. Note the bone uptake of 2.2% injected dose per gram 120 hr after injection. Data from six animals were used for each time point. (B) The organ distribution of ^{88}Y-citrate in mice at 24 hr (■), 72 hr (■) and 96 hr (□) postinjection. The bone uptake increased with time. For each time point, data were acquired from four animals.

crocyclic bifunctional chelating agent *p*-nitrobenzyl-DOTA, which is suitable for ^{90}Y (*11*). The Y(III) chelate of this BCA showed a total loss of Y(III) of <05.% to any serum protein during incubation in the serum for 18 days (*11*). This loss of Y(III) was so small that the rate of loss could not be calculated.

When Y(III) chloride is injected into a mammal, the yttrium is deposited in the bone (*9,10*). Transchelation of ^{90}Y from a labeled antibody would similarly result in the deposition of ^{90}Y in bone, thus, increasing the radiation dose to the bone and closely associated marrow. Our intention in the present study was to determine the in vivo stability of the Y(III) complex of DOTA conjugated to a monoclonal antibody. To do this, it was necessary to prepare a radiopharmaceutical which could not contain any trace of free or nonspecifically bound Y(III). Therefore, the BAD-^{88}Y chelate was prepared, separated from unchelated Y (III), and then conjugated to the antibody instead of using the pharmaceutical prepared by conjugating BAD to the antibody and then labeling it with ^{88}Y. The latter method can be used in more routine procedures, now that the bone uptake of the authentic Lym-1-2IT-BAD-Y is established. We have demonstrated that yttrium chelation to the pre-conjugated antibody can occur with at least 50% yield in two hours of incubation. However, the conditions to optimize this chelation rate have not been determined. The radiopharmaceutical was also challenged with EDTA before injecting into the mice to confirm the absence of unchelated ^{88}Y.

The remarkable stability of Lym-1-2IT-BAD-^{88}Y in serum agrees with previous studies in which the rate of loss of ^{88}Y (III) from the small molecule *p*-nitrobenzyl-DOTA was too small to measure. In contrast, the pseudo-first-order rate constants for loss of ^{88}Y (III) from *p*-nitrobenzyl-DTPA and from DTPA-monobutylamide were found to be 0.6% and 27% per day, respectively (*11*). The stability of this macrocyclic chelate in serum appears to be superior to that of any metal chelate tested to date (*12,13*).

The whole-body clearance of Lym-1-2IT-BAD-^{88}Y and ^{88}Y-citrate from the mice were similarly slow. However, a considerable difference was observed in the deposition of the ^{88}Y in the organs. The ^{88}Y injected as yttrium citrate was increasingly deposited in the bone over the time of the study. In contrast, the bone uptake of the Lym-1-2IT-BAD-^{88}Y was 2.1% ± 0.5% I.D./g on Day 5—comparable to the bone uptake of 2.53% ± 0.29% I.D./g on Day 5 reported by Kozak et al. (*12*) using a backbone-substituted benzyl-DTPA-based bifunctional chelating agent. Our earlier mouse biodistribution studies using ^{67}Cu- and ^{111}In-labeled conjugates Lym-1-2IT-*p*-bromoacetamidobenzl-1,4,8,11-tetraaza-cyclotetra-decane-(BAT)^{67}Cu and Lym-1-CITC-^{111}In showed comparable ^{111}In and ^{67}Cu levels associated with the mineral bone (Table 1). The metabolism of copper and indium in the body does not lead to deposition of these radionuclides in the mineral bone (*17,18*); thus, this yttrium level in bone is considered a nonspecific tissue level. We, therefore, conclude that the DOTA macrocyclic bifunctional chelating agent forms an ^{88}Y complex that is stable in vivo. In contrast, reports by several investigators using the DTPA-anhydride method of chelating ^{90}Y to antibodies for cancer patient therapy suggested that there was significant ^{90}Y deposition in the bone (*5,19,20*).

The liver, kidney, and spleen levels of the conjugate decreased at a slower rate than observed in the case of ^{88}Y-citrate, and were similar to Lym-1-2IT-BAT-^{67}Cu (*14*). The higher spleen uptake in the case of Lym-1-2IT-BAD-^{88}Y is likely due to the presence of high molecular weight species in the radiopharmaceutical. Most of this dimeric form of the antibody is a result of the conjugation chemistry, particularly the amount of β-mercaptoethanol added in these experiments, although the unmodified antibody also shows a tendency to dimerize.

The new bifunctional chelating agent *p*-bromoacetamidobenzyl-DOTA and the antibody conjugate Lym-1-2IT-BAD form Y(III) complexes under physiologic

TABLE 1
Bone Uptake Data for Different Radiopharmaceuticals

Radiopharmaceutical	Day 1	Day 3	Day 5
^{88}Y	13.8 ± 3.4 (4)	20.1 ± 1.2 (4)	24.9 ± 2.7 (4)
Lym-1-2IT-BAD-^{88}Y	2.0 ± 1.9 (6)	3.9 ± 2.1 (6)	2.1 ± 0.5 (5)
Lym-1-2IT-BAT-^{67}Cu*	2.4 ± 0.4 (5)	3.7 ± 1.8 (5)	0.9 ± 0.3 (5)
Lym-1-CITC-^{111}In†	2.0 ± 1.1 (6)	2.1 ± 0.7 (6)	1.4 ± 0.5 (6)
Lym-1-^{125}I‡	2.2 ± 1.5 (3)	0.9 ± 0.1 (3)	0.5 ± 0.2 (3)

The marrow was removed from the bone as completely as possible. The bone uptake of Lym-1-2IT-BAD-^{88}Y is comparable to the bone uptake of ^{67}Cu, ^{111}In, or ^{125}I labeled Lym-1. Note the high bone uptake for the mice that received ^{88}Y-citrate. Data is expressed as % I.D./g. The values in the parentheses give the number of animals.

mean ± s.d.

* Ref. 14

† Adams GP, et al. *Cancer Res* 1989; 49:1707–1711.

‡ Unpublished results, DeNardo SJ.

conditions. Yttrium labeling of the chelate antibody conjugate has been performed with effective yields for therapy. The plasma stability studies and mouse biodistribution of the ^{88}Y-labeled monoclonal antibody, Lym-1, using this macrocyclic bifunctional chelating agent, showed evidence of a stable yttrium label with no measurable loss of Y(III) over 25 days in plasma, and little or no deposition of the yttrium in the bone. We believe clinical pharmacokinetic studies with this radiopharmaceutical, Lym-1-2IT-BAD-^{90}Y, are warranted, since yttrium as ^{90}Y in this stable pharmaceutical form may provide an effective tool in cancer therapy.

ACKNOWLEDGMENT

Supported in part by Department of Energy Grant DE FG03-84ER60233 (SJD), National Cancer Institute Grant CA 16861 (CFM), and National Cancer Institute Grant PO1 CA47829-02 (GLD).

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