Evaluation of the Serum Stability and In Vivo Biodistribution of CHX-DTPA and Other Ligands for Yttrium Labeling of Monoclonal Antibodies

Luigi Camera, Seigo Kinuya, Kayhan Garmestani, Chuanchu Wu, Martin W. Brechbiel, Lee H. Pai, Thomas J. McMurry, Otto A. Gansow, Ira Pastan, Chang H. Paik and Jorge A. Carrasquillo

Department of Nuclear Medicine, Warren G. Magnuson Clinical Center; Chemistry Section, Radiation Oncology Branch and Laboratory of Molecular Biology, National Cancer Institute; National Institutes of Health, Bethesda, Maryland

Serum stability and in vivo biodistribution of both A and B isomers of the 2-(p-isothiocyanatobenzyl) (p-SCN-Bz)-cyclohexyl-diethylenetriaminepentaacetic acid ligand (CHX-DTPA), a recently developed backbone-substituted derivative of DTPA, were evaluated and compared to those of 2-(p-SCN-Bz)-6-methyl-DTPA (1B4M-DTPA) and 2-(p-SCN-Bz)-1,4,7,10-tetraazacyclododecane tetra-acetic acid (2B-DOTA). **Methods:** Stability of ^{88}Y-labeled ligands (0.1 μM) was evaluated in serum for up to 17 days. For biodistribution, ligands were conjugated to monoclonal antibody (Mab) B3, a murine IgG1k, and labeled with ^{88}Y at 0.1–0.3 mCi/mg. Nontumor-bearing nude mice were injected intravenously with 1–2 μCi/4–10 μg of ^{88}Y-labeled B3-conjugates and killed at 6 hr and daily up to 168 hr postinjection. Indium-111-(1B4M)-B3 was co-injected in all mice as internal control. **Results:** Serum stability of ^{88}Y-DOTA failed to show any significant release of activity, whereas pseudo-first-order dissociation rate constants of 3.97×10^{-3}, 2.54×10^{-3} and 1.46×10^{-2} (day^{-1}) were calculated for ^{88}Y-1B4M, ^{88}Y-CHX-A and ^{88}Y-CHX-B, respectively. Accordingly, cortical bone uptake of ^{88}Y was significantly higher for all DTPA-derivative chelates than for DOTA. **Conclusions:** While none of the DTPA-derivative chelates could challenge DOTA in its ability to hold the radioyttrium, significant differences were observed in the kinetic inertness of the A and B isomers of CHX, indicating that the CHX-B ligand is not as suitable for ^{90}Y-labeling of Mabs.

Key Words: yttrium-88; ligands; monoclonal antibodies; biodistribution; serum stability

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Preclinical studies using radiolabeled monoclonal antibodies (Mabs) for radioimmunotherapy have established their potential as anti-tumor agents (*1–11*) and led to various clinical trials (*12–17*). While ^{131}I has been the most widely used isotope in radioimmunotherapy, limitations in its physical and biological characteristics have prompted the evaluation of several alternative radionuclides including beta-emitters such as ^{90}Y (*4–6*), ^{67}Cu (*7*), ^{186}Re (*8*), ^{177}Lu (*9*) and alpha-emitters such as ^{212}Bi (*10*) and ^{211}At (*11*). Among these, ^{90}Y has been the most extensively studied because of its good chelation properties (*18*), ready availability from a ^{90}Sr/^{90}Y generator (*19*) and its physical characteristics (*20*). These include a 64.1-hr half-life and a pure beta-emission of intermediate energy (E_{Max} = 2.28 MeV) that have resulted in higher dose rates and greater total dose delivered to tumor sites when compared to ^{131}I-labeled Mabs (*21*). Furthermore, the considerable path length in tissues of its beta-particles (r_{95} = 5.9 mm), represents a major advantage in solid tumors where penetration of antibody molecules is usually poor (*22*).

Because of the similarities in coordination chemistry of ^{111}In (*23*) and ^{90}Y (*24*), initial attempts to label Mabs with ^{90}Y have also utilized the cyclic anhydride of the diethylenetriaminepentaacetic acid (CA-DTPA) (*25*) as the chelating agent. In both experimental (*4–6*) and clinical settings (*15–17*), these studies have been hampered by dose-limiting myelotoxicity mainly resulting from in vivo instability of the ^{90}Y-chelate complex and subsequent deposition of the released ^{90}Y in the bone (*26*).

As a result of advances in bifunctional chelate technology (*27*), several preclinical studies have indicated that isothiocyanatobenzyl-derivatives of DTPA (SCN-Bz-DTPA) have greater stability than CA-DTPA, resulting in less bone uptake of ^{90}Y (*28–31*). More recently, the 1,4,7,10-tetraazacyclododecane tetra-acetic acid (DOTA), a macrocyclic ligand, has been shown to further reduce bone accumulation of ^{90}Y (*32,33*). However, immunogenicity to DOTA has been reported in patients (*34*) prompting further evaluation of other ligands.

In this present study, we investigated both in vitro and in vivo stability of the 2-(p-SCN-Bz)-cyclohexyl-DTPA ligand (CHX-DTPA), a recently developed backbone-substituted derivative of DTPA (*35*). Both A and B isomers of CHX-DTPA were evaluated and compared to 2-(p-SCN-Bz)-6-methyl-DTPA (1B4M-DTPA), previously described (*36*) and 2-(p-SCN-Bz)-DOTA (2B-DOTA) (*37*). Ligands were conjugated to Mab B3, a murine IgG1k previously

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For correspondence and reprints contact: Jorge A. Carrasquillo, MD, Dept. of Nuclear Medicine, National Institutes of Health, Bldg 10/Rm 1C-401, Bethesda, MD 20892.

described (*38*), and labeled with ^{88}Y, which emits several gamma-rays of high energy, to avoid the need to fully solubilize tissues for beta-counting and to correct for quench.

Biodistribution of ^{88}Y-labeled B3-conjugates was evaluated in nontumor-bearing nude mice up to 168 hr. Indium-111-(1B4M)-B3 was co-injected in all mice as internal control. Serum stability of ^{88}Y-labeled ligands was evaluated under physiological conditions up to 17 days.

MATERIALS AND METHODS

Monoclonal Antibody

The isolation and characterization of Mab B3 are described in detail elsewhere (*38*). Briefly, Mab B3 is a murine IgG1k obtained by immunizing BALB-c mice with trypsin-treated MCF-7 breast carcinoma cells. B3 was purified from serum-free culture media by ammonium sulfate precipitation and gel-filtration chromatography. Mab B3 reacts with a carbohydrate epitope found on the Le^y and polyfucosylated Le^x antigens. This epitope is present on a large number of glycoproteins and is abundantly and uniformly expressed by most carcinomas (*38*).

Conjugation of Chelates to Mab B3

Mab B3 was concentrated to ~5 mg/ml and dialyzed against 1 liter of 0.10 *M* HEPES, 0.15 *M* NaCl at pH 8.5 for 6 hr. The antibody preparation was then conjugated to one of the following ligands (Fig. 1): either the A or B isomer of the 2-(p-SCN-Bz)-cyclohexyl-DTPA (CHX-DTPA or CHX), a recently developed backbone-substituted derivative of DTPA (*35*); 2-(p-SCN-Bz)-6-methyl-DTPA (1B4M-DTPA or 1B4M), previously described (*36*) and 2-(p-SCN-Bz)-1,4,7,10-tetraazacyclododecane tetra-acetic acid (2B-DOTA or DOTA) which was prepared according to the method of McMurry et al. (*37*) (Fig. 1).

All ligands were labeled with ^{14}C as previously reported (*35–37*) via linkage methods that have been well described (*39*).

The average number of chelates per molecule of antibody was ~0.6 for all ligands as determined by scintillation counting of ^{14}C.

Radiolabeling of Mab B3-Chelate Conjugates

Carrier-free ^{88}Y (Los Alamos National Laboratory, Los Alamos, NM) was purified from metal contaminants by ion-exchange chromatography. Briefly, ^{88}Y stock solution (in 0.5 *M* HCl) was mixed with an equal volume of concentrated HNO_3 and heated to dryness. The ^{88}Y-activity was dissolved in 2.0 *M* HNO_3 and passed through a RE.Spec resin (Eichrom Industries Inc., Darien, IL) equilibrated with 2.0 *M* HNO_3. The eluant was heated to dryness and the ^{88}Y redissolved in 0.1 *M* HNO_3. Radiolabeling with ^{88}Y was performed at ~0.3 mCi/mg for 1B4M-B3, CHX-A-B3 and CHX-B-B3. 1B4M-B3 was labeled with ^{111}In at ~3 mCi/mg. Briefly, either ^{88}Y (1 mCi in 0.1 *M* HNO_3) or $^{111}InCl_3$ (Amersham Co., IL; 5 mCi, 0.04 *M* HCl) was adjusted to pH 4.2 with 1 *M* sodium acetate and placed in a polypropylene vial. Typically, 200 μl of antibody solution (5 mg/ml, pH 7) were added and allowed to react at 22°C for 30 min (pH 5.5). The reaction was quenched by addition of 10 μl of 2 *M* sodium acetate and 2–4 μl of 0.1 *M* EDTA to scavenge any free radionuclide.

Yttrium-88-labeling of DOTA-B3 was performed at ~0.12 mCi/mg. The reaction mixture (pH 5.5) was heated at 35°C for 2 hr and then quenched by addition of 10 μl of 1 *M* sodium acetate followed by 2–4 μl of 0.1 *M* EDTA.

Radiolabeled antibody preparations were purified by high-performance liquid chromatography (HPLC) using a TSK 3000 size-exclusion column and 20 m*M* MES elution buffer (pH 6.2) at 1.0 ml/min flow rate. Labeling yields were 70%–85% for ^{88}Y-(1B4M)-B3, ^{88}Y-CHX-A)-B3 and ^{88}Y-(CHX-B)-B3, 58% for ^{88}Y-(DOTA)-B3 and 75% for ^{111}In-(1B4M)-B3.

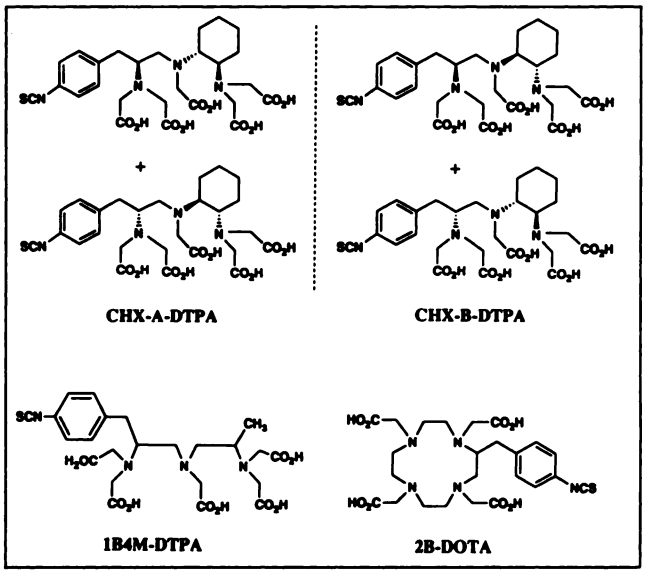

FIGURE 1. Schematic representation of the chemical structure of the bifunctional ligands evaluated in this study. Both CHX-A-DTPA and CHX-B-DTPA were obtained by stereoselective synthesis and exist as a pair of enantiomers. Whereas 1B4M-DTPA and CHX-DTPA are backbone-substituted derivatives of DTPA, DOTA is a macrocyclic ligand. All ligands have an isothiocyanate linker for covalent linkage to the immunoglobulin.

Serum Stability

The p-nitrobenzyl derivatives of 1B4M, CHX-A, CHX-B and DOTA were labeled with ^{88}Y and their stability in serum was evaluated up to 17 days. Briefly, 25-μl aliquots of each stock ligand solutions (4.0×10^{-4} *M* in Chelex-treated water) were mixed with ^{88}Y (60 μCi in 0.1 *M* HNO_3) and the pH was adjusted to 6 with 3 *M* ammonium acetate. The reaction mixture was incubated at 60°C for either 10 min (DTPA) or 60 min (DOTA). The ^{88}Y-labeled ligands were separated from uncomplexed ^{88}Y by a Chelex-100 resin column (3 mm × 20 mm), equilibrated with 0.02 *M* MES buffer (pH 6.8). Yttrium-88-labeled ligands were eluted from the resin with 0.5 ml MES buffer. In every case, labeling efficiencies exceeded 90%.

Aliquots (100 μl) of each ^{88}Y-labeled ligand stock solution (1.0×10^{-5} *M*) were then mixed with 9.9 ml of Fetal Bovine Serum (Hyclone Lab. Inc., Logan, UT) to give a final concentration of 1.0×10^{-7} *M*. The mixture was passed through a sterile 0.22-μm filter (Millipore Co., Bedford, MA) and aliquoted (1 ml) in 10 sterile culture tubes. Serum samples were incubated at 37°C (pH 7.3 ± 0.1) in a CO_2-enriched atmosphere (5% CO_2).

At each time point, the percentage of ^{88}Y activity dissociated from the ligands was assessed by paper chromatography (Whatman No. 1) using 0.2 *M* ammonium acetate (pH 6.8) as the mobile phase. Serum aliquots (15 μl) were spotted on the chromatography paper and allowed to run 10–12 cm from the origin.

All ^{88}Y-labeled ligands showed Rf values ~1, whereas in control experiments performed with ^{88}Y-acetate, less than 1% of the activity was found in the top half of the paper (Rf = >0.5).

Biodistribution Study

Female athymic nude mice (nu/nu) (4–5 wk old, 15–20 g) were obtained from Harlan Sprague Dawley, Frederick, MD. Nontumor-bearing animals were given intravenous injections with 1–2 μCi/mouse of one of the following radiolabeled preparations: ^{88}Y-(1B4M)-B3 (4.2 μg), ^{88}Y-(CHX-A)-B3 (8 μg), ^{88}Y-(CHX-B)-B3 (4.2 μg) and ^{88}Y-(DOTA)-B3 (10 μg). Indium-111-(1B4M)-B3 (7–10 μCi/2–4 μg) was co-injected in all animals as an internal control.

Groups of five animals were then killed by exsanguination at 6 hr and daily up to 168 hr after injection. All major organs were taken, blotted dry with gauze, weighed on an analytical balance and counted in a well-type gamma-counter (Packard Instrument Company, Downers Grove, IL) along with blood aliquots. Counting of the carcasses was also performed to obtain whole-body clearance. Counts were corrected for decay and crossover. Under our counting conditions, ^{111}In had a 0.2% and ^{88}Y a 12.8% spill-over into each other's window.

The percentage of the injected dose per gram of tissue (%ID/g) was then calculated for each organ and normalized to a 20-g mouse. Tissue-to-blood ratios were also determined.

Bone-Washing Procedure

To determine uptake of ^{88}Y in cortical bones, extensive bone washing was performed. Both femurs and portions of the spine were collected, broken in small pieces and counted to determine uptake in the whole bone. The bone fragments were first washed in 5 ml saline to remove blood-associated activity, then incubated for 4 hr at 37°C in 5 ml PBS/10% SDS to extract bone marrow-associated activity. Fractional bone uptake was obtained by separately counting saline, PBS/10% SDS and bone fragments to determine blood-associated activity, bone marrow-associated activity and cortical bone uptake, respectively. Counts were expressed as percentage of the total activity in the bone.

To validate this procedure, a separate experiment was carried out using female athymic nude mice (4–5 wk old) intravenously injected with one of the following: ^{131}I-albumin (Merck Frosst Inc., Kirkland, Canada) (5 μCi) as a plasma tracer; ^{111}In-transferrin (5 μCi) as a bone-marrow tracer and ^{88}Y-citrate (1.5 μCi) as a bone tracer.

Indium-111-transferrin was obtained by incubating mouse serum with $^{111}InCl_3$ at 22°C for 15 min. Groups of five animals were then killed by exsanguination at either 2 hr (^{131}I-albumin) or 24 hr postinjection. All major organs were taken, weighed and counted. Both femurs and the spine were collected and processed as previously described. Fractional bone uptake was then determined.

Quality Control and In Vivo Stability

Prior to injection into mice, all radiolabeled-B3 preparations were analyzed by HPLC (Waters 510, Millipore Corporation, Milford, MA) using TSK 2000 and 4000 size-exclusion columns connected in series. The columns were eluted with 0.067 *M* PBS, 0.1 *M* KCl buffer (pH 6.8) with a flow rate of 0.5 ml/min. Radiochemical purity of ^{111}In-transferrin was also analyzed by HPLC using ^{125}I-apotransferrin (Calbiochem, San Diego, CA) as a standard.

Serum samples (100 μl) from all groups of animals were analyzed at all time points to evaluate the in vivo stability of radiolabeled B3-conjugates. Fractions were collected and counted in a gamma-counter.

Paper chromatography (Whatman No. 1) was performed using normal saline as eluant to assess the presence of colloids in the ^{88}Y-citrate and ^{111}In-transferrin solutions.

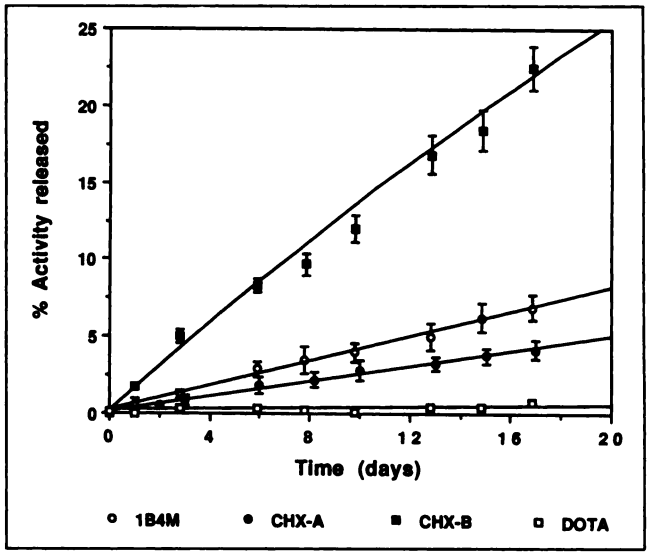

FIGURE 2. Serum stability of ^{88}Y-labeled ligands was evaluated under physiological conditions up to 17 days. The percentage of ^{88}Y-activity released from the ligands at day 0, 1, 3, 6, 8, 10, 13, 15 and 17 is plotted as a function of time. Each data point represents the average of three experiments. Data points were fitted to an exponential curve as appropriate for a pseudo first-order chemical kinetic. Data are reported as mean ± 1 s.d.

Statistical Analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA).

RESULTS

Serum Stability

Serum stability of ^{88}Y-labeled ligands is shown in Figure 2. While the loss of ^{88}Y from p-nitrobenzyl-DOTA over 17 days was very small (0.5% ± 0.06%, mean ± 1 s.d.), substantial release of activity was observed from all DTPA-derivative chelates. However, the percentage of activity dissociated at 17 days was significantly higher for the p-nitrobenzyl-CHX-B ligand (22.4% ± 1.4%) than for p-nitrobenzyl-1B4M (6.6% ± 0.8%) and -CHX-A (4.2% ± 0.6%) ($p < 0.0001$) (Fig. 2).

Dissociation rate constants were obtained by fitting data points to an exponential curve as appropriate for pseudo first-order chemical kinetics. Pseudo first-order rate constants (k) were 3.97×10^{-3}, 2.54×10^{-3} and 1.46×10^{-2} day^{-1} for p-nitrobenzyl-1B4M, -CHX-A and -CHX-B, respectively. The rate constant for p-nitrobenzyl-DOTA could not be accurately determined ($R^2 = 0.3$), but was less than 4×10^{-4}.

Quality Control and In Vivo Stability

Radiochemical purity of all radiolabeled B3 preparations and ^{111}In-transferrin was ~98% as determined by HPLC. The analysis of serum samples from all groups of animals showed that all the radioactivity recovered was in the IgG peak as determined by gamma-counting of the HPLC fractions.

No colloids were formed in either the ^{88}Y-citrate or the

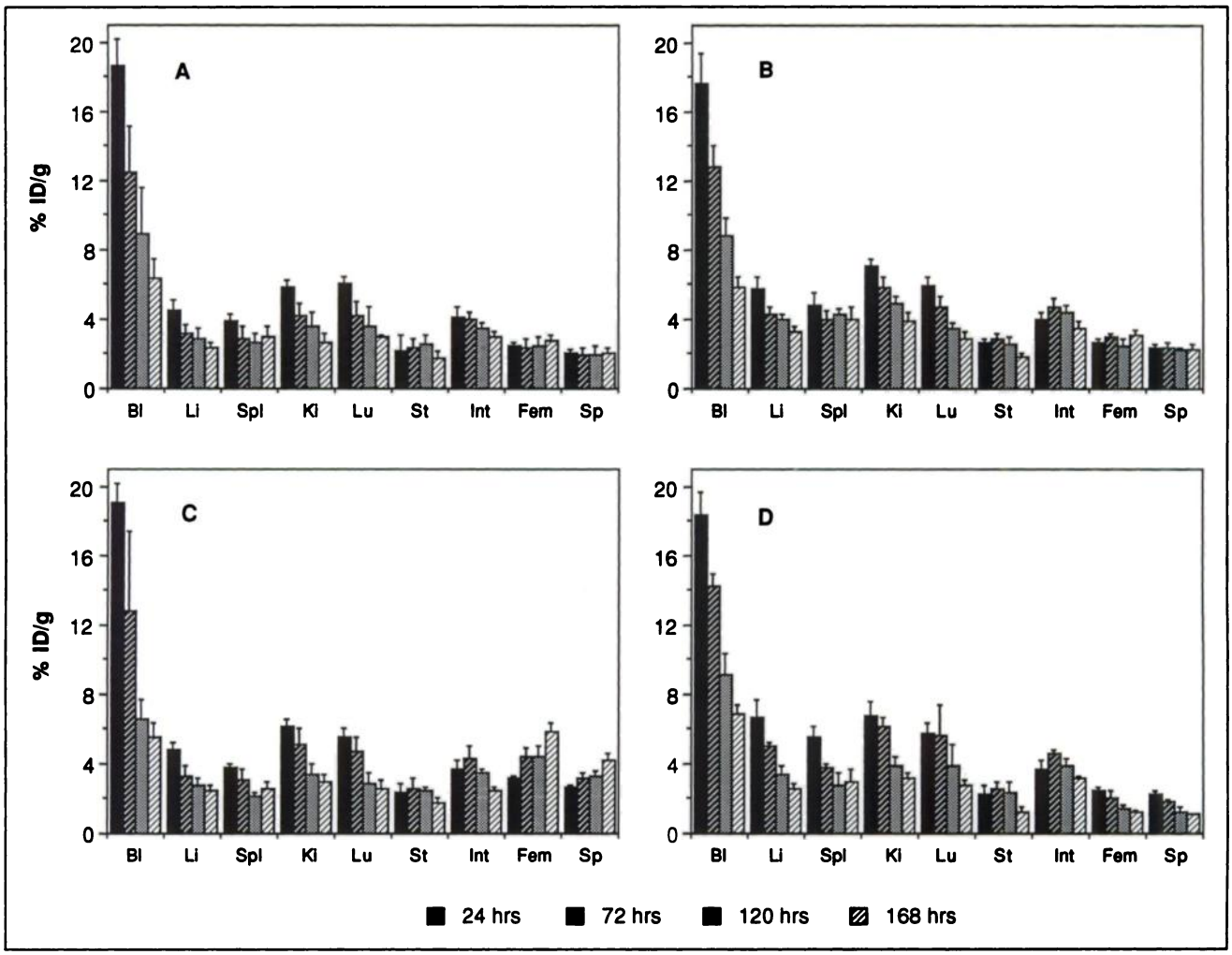

FIGURE 3. Biodistribution of ^{88}Y-labeled B3-conjugates in nontumor-bearing mice. Animals were killed in groups of five at 6 hr and daily up to 168 hr postinjection. The percentage of the injected dose per gram of tissue (%ID/g) in all major organs and in blood at 24, 72, 120 and 168 hr is shown for ^{88}Y-(1B4M)-B3 (A), ^{88}Y-(CHX-A)-B3 (B), ^{88}Y-(CHX-B)-B3 (C) and ^{88}Y-(DOTA)-B3 (D). Bl = Blood; Li = Liver; Spl = Spleen; Ki = Kidney; Lu = Lung; St = Stomach; Int = Intestine (small and large bowel); Fem = Femur; Sp = Spine. Uptake in the whole femur and spine is shown. Data are reported as mean ± 1 s.d.

^{111}In-transferrin solutions as determined by paper chromatography.

Biodistribution Study

All four groups of animals showed a similar biodistribution of ^{111}In-(1B4M)-B3 which was coinjected in all mice to document any variability among groups.

Conversely, significant differences were observed in the biodistribution of the four ^{88}Y-labeled B3-conjugates (Fig. 3). All ^{88}Y-labeled B3-conjugates showed a similar blood clearance; this resulted in 6.3% ± 1.0% ID/g in the blood at 168 hr for ^{88}Y-(1B4M)-B3 and 5.8% ± 0.6%, 5.5% ± 0.8% and 6.8% ± 0.5% ID/g for ^{88}Y-(CHX-A)-B3, ^{88}Y-(CHX-B)-B3 and ^{88}Y-(DOTA)-B3, respectively ($p > 0.05$).

At all time points, ^{88}Y-(CHX-A)-B3 showed higher retention in the liver, spleen and kidney (Fig. 3) which resulted in significantly higher tissue-to-blood ratios in these organs at 168 hr (Table 1).

However, the most significant differences were observed in bone uptake. All DTPA-derivative chelates showed significantly higher bone uptake than ^{88}Y-(DOTA)-B3, with 2.8% ± 0.2%, 3.0% ± 0.3%, 5.8% ± 0.5%, 1.24% ± 0.07% ID/g in the whole femur at 168 hr for ^{88}Y-(1B4M)-B3, ^{88}Y-(CHX-A)-B3, ^{88}Y-(CHX-B)-B3, ^{88}Y-(DOTA)-B3 and 2.0% ± 0.3%, 2.23% ± 0.2%, 4.2% ± 0.4%, 1.07% ± 0.06% ID/g in the whole spine at 168 hr, respectively ($p < 0.0001$) (Fig. 3).

Fractional bone uptake was also significantly different. The percentage of total bone activity recovered in the bone shaft after washing was significantly lower for ^{88}Y-(DOTA)-B3 than for the other three ^{88}Y-labeled B3-conjugates at all time points, with 42.3% ± 5.0% in the shaft of the femur at 168 hr compared to 82.5% ± 2.0%, 81.0% ± 2.2% and 90.2% ± 1.6% for ^{88}Y-(1B4M)-B3, ^{88}Y-(CHX-A)-B3 and ^{88}Y-(CHX-B)-B3, respectively ($p < 0.0001$). As a result, while accretion of ^{88}Y in the cortical bone increased with time for all DTPA-derivative chelates, ^{88}Y-

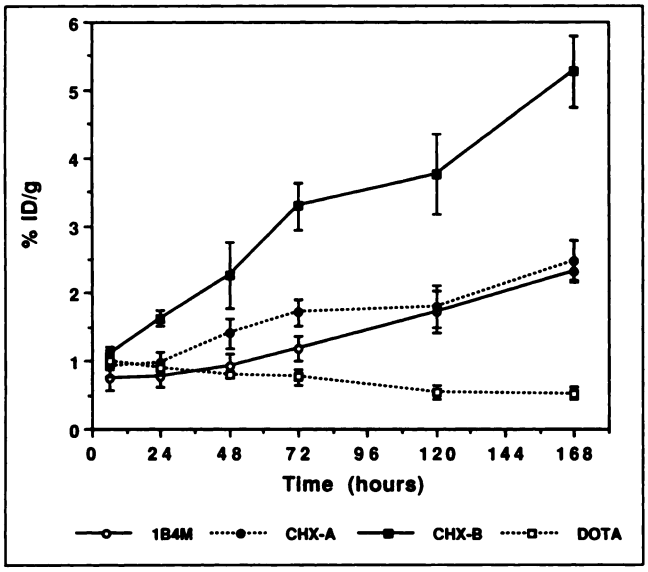

FIGURE 4. Cortical bone uptake of ^{88}Y in the femurs at 6, 24, 48, 72, 120 and 168 hr is shown for all ^{88}Y-labeled B3-conjugates. Similar results were observed in the spine. Femurs were processed as described in Materials and Methods. Data are reported as mean ± 1 s.d.

(DOTA)-B3 showed decreasing cortical bone uptake with only 0.5% ± 0.08% ID/g at 168 hr (Fig. 4). Both 1B4M and CHX-A, however, showed significantly lower cortical bone uptake than CHX-B with 2.4% ± 0.3%, 2.3% ± 0.1% and 5.2% ± 0.5% ID/g in the cortical bone of the femur at 168 hr, respectively ($p < 0.0001$) (Fig. 4).

All DTPA-derivative chelates showed significantly higher femur-to-blood and spine-to-blood ratios than ^{88}Y-(DOTA)-B3. However, both ^{88}Y-(1B4M)-B3 and ^{88}Y-(CHX-A)-B3 had lower ratios than ^{88}Y-(CHX-B)-B3 (Table 1).

Whole-body clearance was slower for ^{88}Y-(CHX-A)-B3 ($T_{1/2}$ = 153 ± 4 hr) and ^{88}Y-(CHX-B)-B3 ($T_{1/2}$ = 142 ± 25 hr) than for ^{88}Y-(1B4M)-B3 ($T_{1/2}$ = 125 ± 18 hr) and ^{88}Y-(DOTA)-B3 ($T_{1/2}$ = 124 ± 7 hr); however, the whole-body retention at 168 hr was similar for all ^{88}Y-labeled B3-conjugates with 40.7% ± 4.5%, 44.8% ± 3.9%, 42.5% ± 3.0% and 39.1% ± 2.7% ID for ^{88}Y-(1B4M)-B3, ^{88}Y-(CHX-A)-B3, ^{88}Y-(CHX-B)-B3 and ^{88}Y-(DOTA)-B3, respectively ($p > 0.05$).

Validation Study

The biodistribution of ^{131}I-albumin, ^{111}In-transferrin and ^{88}Y-citrate was evaluated at either 2 hr (^{131}I-albumin) or 24 hr (^{111}In-transferrin and ^{88}Y-citrate) postinjection. At 2 hr, 17.3% ± 1.2% ID/g of ^{131}I-albumin was still circulating in the blood compared to 1.3% ± 0.1% and 0.01% ± 0.005% ID/g for ^{111}In-transferrin and ^{88}Y-citrate, respectively. Uptake in the whole femur was 2.6% ± 0.2% ID/g for ^{131}I-albumin, 7.7% ± 0.7% ID/g for ^{111}In-transferrin and 15.5% ± 1.8% ID/g for ^{88}Y-citrate.

Fractional bone uptake of ^{131}I-albumin, ^{111}In-transferrin and ^{88}Y-citrate are reported in Table 2.

DISCUSSION

A major concern with ^{90}Y-labeled Mabs is the selection of a chelate with sufficient thermodynamic and kinetic stability to prevent in vivo loss of the radiometal, since dissociated ^{90}Y(III) rapidly accumulates in the bones (*26*), delivering an undesired amount of radiation to the radiosensitive marrow.

In this study we investigated both in vitro and in vivo stability of the A and B isomers of CHX-DTPA (*35*) and compared them to those of 1B4M-DTPA (*36*) and DOTA (*37*).

In vitro serum stability of ^{88}Y-labeled ligands was evaluated up to 17 days. The study showed that none of the DTPA-derivative chelates could challenge DOTA in its ability to hold the radiometal. The loss of ^{88}Y from p-nitrobenzyl-DOTA was so little that a pseudo first-order rate constant could not be accurately determined. This finding is in agreement with previous observations (*40*) and

TABLE 1
Biodistribution of ^{88}Y-Labeled B3-Conjugates in Nontumor-Bearing Mice: Tissue-to-Blood Ratios*

Organ	^{88}Y-(1B4M)-B3	^{88}Y-(CHX-A)-B3	^{88}Y-(CHX-B)-B3	^{88}Y-(DOTA)-B3	p†
Liver	0.366 ± 0.022	0.568 ± 0.038	0.439 ± 0.028	0.380 ± 0.025	<0.001
Spleen	0.469 ± 0.068	0.677 ± 0.066	0.463 ± 0.091	0.445 ± 0.106	<0.001
Kidney	0.420 ± 0.027	0.669 ± 0.014	0.548 ± 0.039	0.471 ± 0.060	<0.001
Lung	0.454 ± 0.070	0.493 ± 0.018	0.467 ± 0.050	0.406 ± 0.044	ns
Stomach	0.279 ± 0.068	0.326 ± 0.053	0.318 ± 0.108	0.183 ± 0.030	ns
Intestine‡	0.461 ± 0.054	0.585 ± 0.116	0.451 ± 0.072	0.458 ± 0.084	ns
Femur	0.445 ± 0.048	0.525 ± 0.082	1.066 ± 0.141	0.182 ± 0.015	<0.0001
Spine	0.326 ± 0.015	0.381 ± 0.030	0.774 ± 0.091	0.158 ± 0.011	<0.0001

*Tissue-to-blood ratios were calculated by dividing the percentage of the injected dose per gram (%ID/g) in each organ by the %ID/g in blood at 168 hr. Data at 24, 72 and 120 hr are omitted for simplification. Data are reported as mean ± 1 s.d.

†Statistical analysis was performed using analysis of variance with a two-tailed F test ($\alpha = 0.01$, $n = 5$).

‡Small and large bowel.

TABLE 2
Fractional Bone Uptake of ^{131}I-Albumin, ^{111}In-Transferrin and ^{88}Y-Citrate

Fraction	^{131}I-albumin	^{111}In-transferrin	^{88}Y-citrate
Saline (%)	86.4 ± 0.65	7.65 ± 1.43	0.3 ± 0.05
SDS (%)	3.3 ± 0.15	8.37 ± 0.73	0.4 ± 0.36
Bone (%)	10.3 ± 0.6	83.9 ± 2.11	99.2 ± 0.34

*Fractional bone uptake was determined by separately counting saline, PBS-10% SDS and bone. Counts are expressed as percentage of the total activity. Data are reported as mean ± 1 s.d.

clearly demonstrates the superiority of DOTA over the DTPA-derivative chelates tested in this study. Within this latter group, however, significant differences were observed. The percentage of ^{88}Y activity released was significantly higher for the p-nitrobenzyl-CHX-B ligand than for both p-nitrobenzyl-CHX-A and -1B4M (Fig. 2). Hence, the thermodynamic stability of the two ^{88}Y-CHX complexes is significantly affected by the absolute configuration of the three stereochemical centers of the ligands (Fig. 1). This is probably the result of subtle unfavorable steric hindrance present in one configuration (B enantiomer) but not in the other (A enantiomer).

The incorporation of ^{88}Y into the DOTA-B3 conjugate was higher than that reported by Deshpande et al. (*32*), probably as a result of our labeling method which was performed at 35°C for 2 hr. While we did not evaluate the immunoreactivity of ^{88}Y-(DOTA)-B3 in this study, no protein damage was expected to occur at this temperature. However, the slow rate of complexation of yttrium with DOTA represents a well-known drawback which may prevent its widespread use in the clinical setting (*41*).

The similarities in the blood clearance of the four ^{88}Y-labeled B3-conjugates suggests that the differences in their biodistribution were a result of differential handling of the yttrium-chelate complexes after the metabolic processing of the antibody.

Although the reason for the higher retention of ^{88}Y-(CHX-A)-B3 in the liver, spleen and kidney is not known, it was not related to inter-animal variability since similar differences were not observed in the biodistribution of ^{111}In-(1B4M)-B3.

However, the most striking differences were observed in bone uptake. Both the %ID/g in the whole bones and the fraction recovered in the shafts after washing were significantly lower for ^{88}Y-(DOTA)-B3 than for the other ^{88}Y-labeled B3-conjugates. Even though our bone washing procedure could discriminate plasma-associated activity from bone marrow-associated activity, it could not differentiate between bone marrow-associated and cortical bone activity (Table 2). However, this was not a major limitation since free yttrium is a bone seeker and its target is represented by the periosteal and endosteal bone (*26*).

In mice injected with ^{88}Y-(DOTA)-B3, cortical bone uptake at 168 hr was five times less than that observed for both ^{88}Y-(1B4M)-B3 and ^{88}Y-(CHX-A)-B3 and ten times less than that of ^{88}Y-(CHX-B)-B3 (Fig. 4). While these findings mirrored those of the in vitro serum stability study (Fig. 2), differences in the in vivo stability of the ^{88}Y-labeled B3-conjugates were not suggested by our HPLC analysis of serum samples which failed to detect species other than intact IgG. This does not represent an unusual finding and it is probably due to the fact that free yttrium is rapidly taken up by the bone and/or is retained by HPLC columns (*19,42*).

We have previously reported biodistribution and imaging characteristics of ^{111}In-(CHX-B)-B3 in tumor-bearing mice (*43,44*). While these studies have indicated that the CHX-B is a suitable chelate for ^{111}In-labeling of Mabs, the present study clearly showed that the CHX-B ligand does not retain ^{88}Y as well as ^{111}In. This is likely due to differences in the effective ionic radius (r_i = 90 pm for ^{88}Y(III); r_i = 80 pm for ^{111}In(III)) (*45*) and in the bonding interactions (*46*) of the two radionuclides. These differences determine different water exchange rates and ultimately affect the stability of the metal-chelate complex (*47*).

The cortical bone uptake observed in mice injected with ^{88}Y-(1B4M)-B3 and ^{88}Y-(DOTA)-B3 is similar to that found by other investigators using the 1B4M (*29,31*) or DOTA ligand (*33*).

Both ^{88}Y-(CHX-A)-B3 and ^{88}Y-(CHX-B)-B3 exhibited a slower whole-body clearance than ^{88}Y-(1B4M)-B3 and ^{88}Y-(DOTA)-B3. In the case of ^{88}Y-(CHX-B)-B3, this was likely related to the higher bone accumulation of ^{88}Y while for ^{88}Y-(CHX-A)-B3 it was probably the result of higher retention in liver, spleen and kidney. For both radioimmunoconjugates, however, the whole-body retention at 168 hr was more but not significantly higher than that of ^{88}Y-(1B4M)-B3 and ^{88}Y-(DOTA)-B3. This was probably due to the small fraction of the total body weight represented by these target organs (*48*).

CONCLUSIONS

This study confirms that bone uptake of radioyttrium can be significantly reduced by using the macrocyclic ligand DOTA (*32,33*).

Both the serum stability and the biodistribution study showed that none of the DTPA-derivative chelates could challenge DOTA in its ability to hold the yttrium. However, unfavorable kinetics of complexation with yttrium (*41*) and possible immunogenicity (*34*) may limit the widespread use of DOTA in the clinical setting. Therefore, evaluation of new ligands should be pursued.

Although some differences in biodistribution were seen between ^{88}Y-(1B4M)-B3 and ^{88}Y-(CHX-A)-B3, both chelates showed similar bone accumulation of ^{88}Y. Interestingly, significant differences were observed in the biodistribution and in the kinetic inertness of the A and B isomers of CHX, indicating that the CHX-B ligand is not an acceptable ligand for yttrium labeling of Mabs.

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Condensed from
15 Years Ago:

Thallium-201 Myocardial Imaging: A Comparison of the Redistribution and Rest Images

James L. Ritchie, Peter C. Albro, James H. Caldwell, Gene B. Trobaugh and Glen W. Hamilton
University of Washington School of Medicine, Seattle Veterans Administration Medical Center, Seattle, Washington

Forty-one patients with chest pain and suspected coronary artery disease underwent ^{201}Tl myocardial imaging, performed immediately following maximal treadmill exercise, also at "redistribution" 4-5 hr after exercise, and at rest 1 wk later. All had coronary angiography. All images in seven patients without coronary artery disease were normal. Twenty-seven of the 34 (79%) patients with coronary artery disease had new, exercise-induced image defects. The redistribution and rest images were identical in 15/27 (56%) patients (complete redistribution). In 10/27 (37%) patients with exercise-induced defects, some redistribution occurred, but defect size on the redistribution image was larger than that on the rest images (incomplete redistribution). In 2/27 (7%) patients with exercise-induced defects, redistribution was absent. The presence of prior myocardial infarction, regional abnormalities of left ventricular contraction or the severity of coronary stenoses did not correlate with the presence or absence of redistribution. Overall image quality between the two studies was similar, although image collection times for the redistribution study were prolonged.

We conclude that some redistribution (complete or incomplete) occurs in most patients with exercise-induced image defects. When both fixed and reversible perfusion defects are present, defect size was often larger in the redistribution image and may thus overestimate the extent of prior myocardial infarction.

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