Optimized Conditions for Chelation of Yttrium-90-DOTA Immunoconjugates

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Radioimmunotherapy (RIT) with ^{90}Y-labeled immunoconjugates has shown promise in clinical trials. The macrocyclic chelating agent 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (DOTA) binds ^{90}Y with extraordinary stability, minimizing the toxicity of ^{90}Y-DOTA immunoconjugates arising from loss of ^{90}Y to bone. However, reported ^{90}Y-DOTA immunoconjugate product yields have been typically only ≤50%. Improved yields are needed for RIT with ^{90}Y-DOTA immunoconjugates to be practical. **Methods:** (S) 2-[p-(bromoacetamido)benzyl]-DOTA (BAD) was conjugated to the monoclonal antibody Lym-1 via 2-iminothiolane (2IT). The immunoconjugate product, 2IT-BAD-Lym-1, was labeled in excess yttrium in various buffers over a range of concentrations and pH. Kinetic studies were performed in selected buffers to estimate radiolabeling reaction times under prospective radiopharmacy labeling conditions. The effect of temperature on reaction kinetics was examined. Optimal radiolabeling conditions were identified and used in eight radiolabeling experiments with 2IT-BAD-Lym-1 and a second immunoconjugate, DOTA-peptide-chimeric L6, with 248–492 MBq (6.7–13.3 mCi) of ^{90}Y. **Results:** Ammonium acetate buffer (0.5 M) was associated with the highest uptake of yttrium. On the basis of kinetic data, the time required to chelate 94% of ^{90}Y (four half-times) under prospective radiopharmacy labeling conditions in 0.5 M ammonium acetate was 17–148 min at pH 6.5, but it was only 1–10 min at pH 7.5. Raising the reaction temperature from 25°C to 37°C markedly increased the chelation rate. Optimal radiolabeling conditions were identified as: 30-min reaction time, 0.5 M ammonium acetate buffer, pH 7–7.5 and 37°C. In eight labeling experiments under optimal conditions, a mean product yield (± s.d.) of 91% ± 8% was achieved, comparable to iodination yields. The specific activity of final products was 74–130 MBq (2.0–3.5 mCi) of ^{90}Y per mg of monoclonal antibody. The immunoreactivity of ^{90}Y-labeled immunoconjugates was 100% ± 11%. **Conclusion:** The optimization of ^{90}Y-DOTA chelation conditions represents an important advance in ^{90}Y RIT because it facilitates the dependable and cost-effective preparation of ^{90}Y-DOTA pharmaceuticals.

Key Words: yttrium-90; chelate; 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid; radioimmunotherapy

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Yttrium-90 is a useful radionuclide for radioimmunotherapy (RIT), due to its energetic beta emissions (E_{max} = 2.3 MeV), ready availability at moderate cost and absence of γ emissions, allowing outpatient RIT. Its half-life of 64 hr is comparable to the uptake and residence time of many antibodies on the tumor (1). Clinical trials of RIT using ^{90}Y-labeled monoclonal antibodies (MoAbs) have been encouraging (2–6), resulting in clinically measurable responses, even in patients with advanced therapy-resistant breast cancer (5,6). Multimodality therapy using ^{90}Y RIT has been examined in preclinical studies and clinical trials (7,8). In an exceptionally promising study of combination ^{90}Y RIT/paclitaxel therapy, high rates of response

and cure of an aggressive human breast cancer (HBT 3477) were achieved in the xenografted mouse model (9).

Numerous chelating agents for ^{90}Y have been investigated for RIT, primarily derivatives of the acyclic chelator diethylenetriaminepentaacetic acid (DTPA) (10–13) and the macrocyclic chelator 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (DOTA) (13–15). Yttrium complexes of the acyclic chelators are not as kinetically stable as those of DOTA (15,16). The extraordinary stability of the yttrium-DOTA complex minimizes the loss of ^{90}Y to bone, a target organ of the radionuclide (17). Comparatively, DTPA, conjugated to MoAbs, chelates yttrium readily, often in yields of ≥90%, whereas radiolabeling yields of ^{90}Y-DOTA immunoconjugates of 50% are typical (13). Improved yields are needed to reduce the cost of therapy with ^{90}Y-DOTA immunoconjugates and to minimize the exposure of radiolabeling personnel to radiation.

In a study of the ^{90}Y chelation properties of DOTA, Stimmel et al. (13) observed low chelation efficiency at DOTA concentrations of ≤10 μM; however, in conventional radiolabeling procedures, in which DOTA is conjugated to MoAb and ^{90}Y is subsequently added, the concentration of DOTA is ≤1 μM. To circumvent this obstacle, Li et al. (18) developed the prelabeling method, in which a bifunctional DOTA chelating agent at a concentration of ≥10 mM is radiolabeled with ^{90}Y, followed by purification of the ^{90}Y-DOTA chelate and conjugation to MoAb. The prelabeling system minimizes perturbation of the MoAb's biological properties because little or no unlabeled DOTA is conjugated to MoAb, virtually eliminating multiply conjugated MoAbs; however, overall radiolabeling yields of ≤40% have been reported. Another strategy to improve ^{90}Y-DOTA labeling yields is to attach multiple DOTA groups to a dendrimeric linker, which is, in turn, conjugated to MoAb at a single site (19).

An ideal MoAb radiolabeling scheme has the characteristics of a kit procedure, i.e., speed, simplicity, dependability and the production of material of consistently excellent quality. The pre-eminence of ^{131}I for RIT is due largely to such practical considerations. A prospective scheme in which MoAbs, conjugated to DOTA at a low chelator-to-antibody ratio, are readily labeled with ^{90}Y in high yield, meets these criteria. This scheme challenges the assumption that DOTA at a low concentration cannot be labeled with ^{90}Y with high efficiency. This study was undertaken to determine conditions under which DOTA immunoconjugates can be labeled with ^{90}Y rapidly and in high yield. The conditions examined were chosen to emulate prospective radiopharmacy labeling conditions: innocuous reagents to assure patient safety, moderate pH to avoid degradation of the MoAb conjugate and a labeling time of ≤1 hr to minimize autoradiolysis (20). Subsequently, radiolabelings were performed under conditions identified as optimal, product yields were evaluated and the ^{90}Y-labeled immunoconjugates were examined for structural and functional integrity. Two immunoconjugates using different MoAbs conjugated to different de-
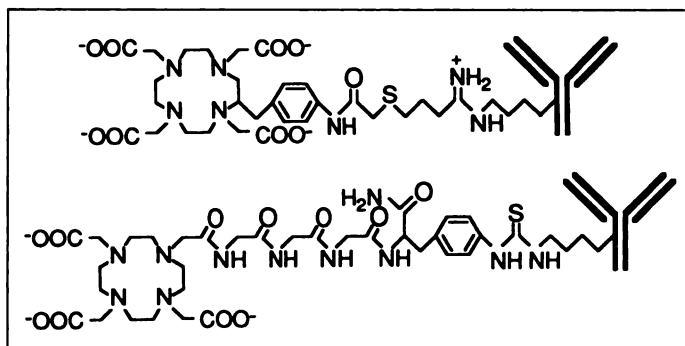
FIGURE 1. DOTA-MoAb conjugates 2IT-BAD-Lym-1, with four free carboxyls in chelating moiety (upper), and DOTA-peptide-ChL6, with three free carboxyls (lower). Yttrium-90 radiolabeling of both immunoconjugates was examined to test general applicability of method.

rivatives of DOTA, with three and four free carboxyl arms (Fig. 1), were radiolabeled to examine the general applicability of the radiolabeling procedure.

MATERIALS AND METHODS

Antibodies

Lym-1 (Techniclone International, Santa Ana, CA) is an anti-lymphoma mouse IgG2a MoAb (21). Chimeric L6 (ChL6; Bristol-Meyers Squibb, Seattle, WA) is an antiadenocarcinoma MoAb in which the constant domains C-G2a and C-κ of the mouse MoAb have been replaced by the human C-G1 and C-κ (22).

General Laboratory and Analytical Techniques

Pure water (18 MΩ cm^{-1}) was used throughout. When metal-free conditions were needed, glassware was washed with a mixed acid solution (concentrated sulfuric acid and nitric acid, 1:1 v/v) and thoroughly rinsed with deionized distilled water. Plasticware was either purchased metal-free or washed with 3 M HCl and thoroughly rinsed.

High-performance liquid chromatography (HPLC) (Beckman 332; Beckman, San Ramon, CA) was performed using a 7.8 × 300-mm molecular sieving column (Phenomenex SEC S3000; Phenomenex, Torrance, CA) eluted in 0.1 M sodium phosphate, 0.1 M potassium sulfate and 0.025% (w/v) sodium azide (pH 7.1) at 1.0 ml/min. Radiolabeled MoAbs were detected by ultraviolet (UV) absorbance at 280 nm (Beckman 166 detector) and radioactivity (Beckman 170 detector). Cellulose acetate electrophoresis (CAE) (Gelman Sciences, Inc., Ann Arbor, MI) was performed using 0.05 M sodium barbital buffer (pH 8.6). Samples were electrophoresed at 300 V for 11 min and 45 min. At 11 min, free chelates were resolved from immunoconjugates. At 45 min, monomeric immunoconjugates were resolved from aggregated species. Thin-layer chromatography (TLC) was run on plastic-backed silica gel plates (EM Science, Cherry Hill, NJ) using a solution of 10% (w/v) ammonium acetate and methanol (1:1 v/v) as the eluant. In this system, protein conjugates and unchelated yttrium remain at the origin, whereas free chelates migrate to R_f 0.4–0.8. The immunoreactivity of ^{90}Y-labeled ChL6 was assessed by HBT-3477 cell binding radioimmunoreactive assay (RIA) (23,24), and that of ^{90}Y-labeled Lym-1 was assessed by solid-phase RIA against partially purified Raji cell homogenates, as described previously (25,26).

Radiation Counting

Carrier-added analytical samples were counted in a well counter (Pharmacia LKB 1282; Pharmacia Biotech, Inc., Piscataway, NJ). High-activity samples were measured in an ionization chamber dose calibrator (Capintec CRC-12; Capintec, Inc., Pittsburgh, PA) calibrated for containers and volumes using ^{90}Y standards. To

calculate the activity of ^{90}Y, samples were measured at a dose calibrator setting of 048, and the readings were multiplied by 10, in accordance with current National Institute of Standards and Technology convention (27,28).

Immunoconjugates

The bifunctional chelating agent (S)–2-[p-(bromoacetamido)-benzyl]-DOTA (BAD) was prepared as described previously (15,29) and conjugated to Lym-1 via 2-iminothiolane (2IT) (Sigma Chemical Co., St. Louis, MO) (29). 2-Iminothiolane, BAD and Lym-1 were combined in 0.1 M tetramethylammonium phosphate (pH 9.0) to final concentrations of 1.3, 2.5 and 0.1 mM, respectively. The conjugation solution was incubated for 45 min at 37°C. 2IT-BAD-Lym-1 (Fig. 1) was purified and transferred to 0.1 M ammonium acetate (pH 5.5) by G-25 molecular sieving chromatography (Sigma). The mean number of DOTA chelating groups conjugated per antibody was determined to be 2.8 by cobalt metal binding assay (26). The immunoconjugate was divided into 1-ml aliquots (11.7 mg), frozen in liquid nitrogen and stored at −70°C until immediately before use.

The bifunctional chelating agent DOTA-glycylglycylglycyl-L-p-isothiocyanatophenylalanine amide (DOTA-peptide-ITC) was prepared as described previously (30) and conjugated to ChL6. DOTA-peptide-ITC and ChL6 were combined in 0.1 M tetramethylammonium phosphate (pH 9.0) to final concentrations of 1.0 and 0.3 mM, respectively. The conjugation solution was incubated for 45 min at 37°C. DOTA-peptide-ChL6 (Fig. 1) was purified and transferred to 0.1 M ammonium acetate (pH 5.5) by centrifuged column gel filtration (31). The mean number of DOTA chelating groups conjugated per antibody was determined to be 1.3 by cobalt metal binding assay. The immunoconjugate was divided into 0.1-ml aliquots (5 mg), frozen in liquid nitrogen and stored at −70°C until immediately before use.

Radionuclides

Yttrium-88 in 0.1 M HCl (Los Alamos National Laboratory, Los Alamos, NM), with a half-life of 108 days, was used for the radiolabeling buffer survey and kinetics study described below, to allow sufficient time to perform preliminary studies needed to determine appropriate experimental conditions. Yttrium-90 in 0.05 M HCl (Pacific Northwest National Laboratory, Richland, WA) was used for the other experiments.

Radiolabeling Buffer Survey

Buffers were prepared under metal-free conditions by bubbling ammonia gas into aqueous solutions of acetic acid (J.T. Baker, Phillipsburg, NJ), citric acid (Fisher Scientific, Pittsburgh, PA), aspartic acid (Sigma) and glutamic acid (Sigma). To prepare carrier-added ^{88}Y, 1.0 M yttrium chloride in 0.001 M HCl was prepared gravimetrically from yttrium chloride hexahydrate (Aldrich, Milwaukee, WI) and added to trace dried ^{88}Y. 2-Iminothiolane-BAD-Lym-1 was transferred to water by centrifuged column gel filtration. Carrier-added ^{88}Y and 2IT-BAD-Lym-1 were added to ammonium acetate (pH 5.0, 6.0 and 7.0), ammonium aspartate (pH 6.5, 7.5 and 8.5), ammonium citrate (pH 5.0, 6.5 and 8.0) and ammonium glutamate (pH 6.5, 7.5 and 8.5), such that final concentrations of DOTA chelating groups and yttrium (III) were 13 and 71 μM, respectively, and the final concentrations of buffers were 0.5 and 2.0 M. Triplicate samples were prepared for each buffer at every concentration and pH. The chelation solutions were incubated for 1 hr at room temperature. Yttrium-2IT-BAD-Lym-1 was purified and transferred to 0.2 M ammonium citrate (pH 6.5) by centrifuged column gel filtration. DTPA (Sigma; 0.05 M in 0.2 M ammonium citrate, pH 6.5) was added to a final concentration of 12.5 mM to scavenge nonspecifically bound yttrium (III). After 60 min, the conjugate was again purified by centrifuged column gel

TABLE 1
Radiolabeling Experiments with Yttrium-90-2IT-BAD-Lym-1 and Yttrium-90-DOTA-Peptide-ChL6 Under Optimized Conditions

Immunoconjugate	Initial ^{90}Y (MBq)	Initial immunoconjugate (mg)	Volume of radiolabeling solution (ml)	Purified ^{90}Y-immunoconjugate recovered (MBq)
2IT-BAD-Lym-1	474	4.0	0.25	392
	366	3.6	0.24	296
	396	3.0	0.16	389
DOTA-peptide-ChL6	481	4.0	0.23	474
	492	5.0	0.13	470
	307	3.0	0.24	248
	248	2.0	0.05	244
	411	5.0	0.25	370

filtration. Samples were counted in a well counter and compared with a 1-nmol carrier-added ^{88}Y counting standard to determine the concentration of yttrium (III). Samples were assayed by UV spectroscopy (280 nm) to determine the concentration of Lym-1, using a E_{280} of 224,430 $M^{-1} \cdot cm^{-1}$ ($E_{280}^{1\%}$ = 14.4, using mol wt 155,000) (26). These data were used to calculate the mean number of yttrium (III) ions bound per molecule of 2IT-BAD-Lym-1. As a control, a single assay in which unmodified Lym-1 was substituted for 2IT-BAD-Lym-1 was performed at every buffer pH and concentration.

Kinetic Studies

Yttrium-88 in 0.5 M HCl was dried under an infrared lamp and then dissolved in 1.0 M ammonium acetate (pH 4.5, 5.5, 6.5, 7.5 and 8.25). 2IT-BAD-Lym-1 was transferred to water by centrifuged column gel filtration, diluted in water to desired concentrations of chelating agent and added to buffered ^{88}Y. The final concentrations of DOTA, ^{88}Y and ammonium acetate buffer in the chelation solutions were 10 μM, 1.5 MBq/ml (= 33 nM) and 0.5 M, respectively. Duplicate solutions were prepared for each conjugate at every pH. The solutions were incubated in a water bath at 20°C. Aliquots were removed from the chelation solutions at measured time points. To scavenge nonspecifically bound ^{88}Y, DTPA (0.02 M in 0.5 M ammonium acetate, pH 5.5–9.0) was added to the aliquots to a final concentration of 10 mM and a final pH of 5.0–6.1. In previous experiments using unbuffered DTPA, ^{88}Y was not completely chelated at a pH of >6.5 (unpublished data). The DTPA-challenged solutions were incubated for 20 min and then examined by TLC. Controls in which unmodified Lym-1 was substituted for 2IT-BAD-Lym-1 were performed at every pH.

Because these experiments were conducted under pseudo-first-order kinetic conditions, that is, the concentration of DOTA was much greater than that of ^{88}Y, the second-order rate constant k may be determined as follows. The second-order rate equation for the chelation of metal M by chelating agent C is:

$$dMC/dt = k \, [M] \, [C] \qquad \text{Eq. 1}$$

or

$$dMC/dt = k \, ([M]_o - [MC]) \, ([C]_o - [MC]), \qquad \text{Eq. 2}$$

where k is the second-order rate constant ($M^{-1} \cdot sec^{-1}$). In a pseudo-first-order reaction where $[C]_o \gg [M]_o$, then $[C]_o - [MC] \approx [C]_o$, simplifying Equation 2 to:

$$dMC/dt = k \, ([M]_o - [MC]) \, [C]_o. \qquad \text{Eq. 3}$$

Therefore:

$$dMC/dt = k_{obs} \, ([M]_o - [MC]), \qquad \text{Eq. 4}$$

where:

$$k_{obs} = k \, [C]_o. \qquad \text{Eq. 5}$$

The integrated form of Equation 4 is:

$$\ln \, ([M/[M]_o) = - \, k_{obs} t. \qquad \text{Eq. 6}$$

So when the log of the fraction of metal not chelated is plotted against time, the pseudo-first-order rate constant k_{obs} is given by the negative of the slope, and the second-order rate constant k is given by dividing k_{obs} by $[C]_o$.

Radiolabeling Temperature

Yttrium-90 (74 MBq in 1 μl of 0.05 M HCl) was buffered in 100 μl of 4 M ammonium acetate (pH 7.3). 2-Iminothiolane-BAD-Lym-1 (0.5 mg in 900 μl of 0.1 M ammonium acetate, pH 5) was added. The final pH was 7.0. The sample was divided into two aliquots incubated at room temperature (~25°C) and in a 37°C water bath. Samples of the chelation reaction solutions (5 μl) were removed after 5, 15, 30 and 60 min. DTPA (10 μl; 10 mM in 0.5 M ammonium acetate, pH 5) was added immediately to each sample. The DTPA-challenged solutions were incubated at room temperature for 15 min and then examined by TLC. Controls in which unmodified Lym-1 was substituted for 2IT-BAD-Lym-1 were performed.

Yttrium-90 Radiolabeling

Radiolabeling experiments with ^{90}Y-2IT-BAD-Lym-1 and ^{90}Y-DOTA-peptide-ChL6 were performed under optimized conditions. Reagent quantities are provided in Table 1. To maximize the concentration of DOTA chelating groups, 2IT-BAD-Lym-1 was concentrated to 30 mg/ml and transferred to 0.4 M ammonium acetate (pH 7) by dilution in the desired buffer and reconcentration (Centriprep; Amicon, Beverly, MA); the concentration of DOTA-peptide-ChL6 was 50 mg/ml. The following procedure was used for all radiolabelings. Yttrium-90 in 0.05 M HCl was buffered in 4 M ammonium acetate (pH 7.3) and then 2IT-BAD-Lym-1 or DOTA-peptide-ChL6 in 0.1 M ammonium acetate (pH 5) were added. The final concentration of ammonium acetate was 0.4–0.5 M, and the final pH was 7. Radiolabeling solutions were incubated at 37°C for 30 min DTPA (0.1 M) in 0.5 M ammonium acetate (pH 5) was added to a final concentration of 10 mM. The DTPA-challenged radiolabeling solution was incubated at room temperature for 15 min and then purified and transferred to saline by G-25 molecular sieving chromatography or centrifuged column gel filtration. The radioimmunoconjugates were examined by HPLC, CAE and RIA.

RESULTS

Radiolabeling Buffer Survey

The maximum uptake of yttrium (III) ion by 2IT-BAD-Lym-1 occurred in 0.5 M ammonium acetate (pH 7.0) in which 100% of metal binding sites were occupied (Fig. 2). The relatively high concentrations of buffer were chosen to avoid hydrolysis of yttrium ion to unchelatable yttrium hydroxide.
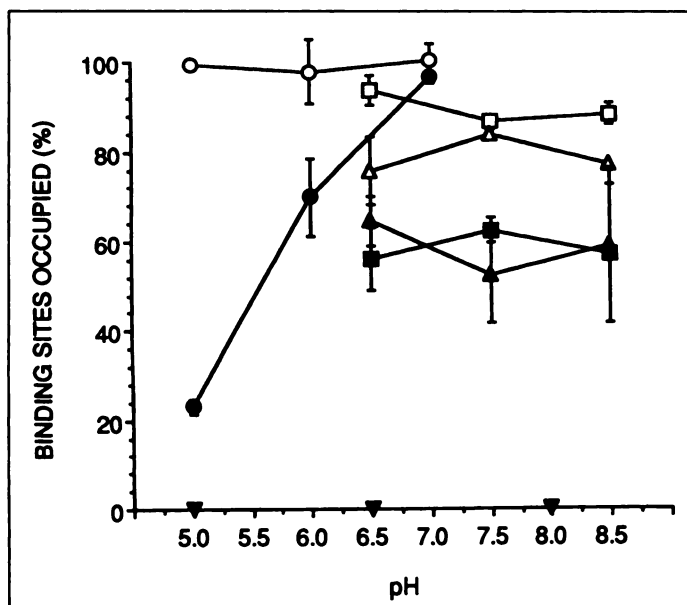
FIGURE 2. Survey of buffers for yttrium-DOTA chelation. Excess yttrium (III) ion was added to 2IT-BAD-Lym-1 in ammonium acetate, 0.5 M (○) and 2.0 M (●); ammonium aspartate, 0.5 M (□) and 2.0 M (■); ammonium glutamate, 0.5 M (△) and 2.0 M (▲); and ammonium citrate, 0.5 M (▽) and 2.0 M (▼). Points for 0.5 M ammonium citrate are obscured by those for 2.0 M ammonium citrate. Data points = means of three measurements; bars = s.d. Ammonium acetate (0.5 M) was associated with most extensive chelation.

The uptake of ^{90}Y by immunoconjugate was greater in 0.5 M buffer than it was in 2.0 M buffer among all buffers surveyed, except citrate, in which there was no uptake at either concentration. Moderate ranges of pH were selected as prospective radiopharmacy labeling conditions to avoid damage to the MoAb conjugate. Over the ranges surveyed, pH had little effect on uptake except in the case of 2.0 M acetate, where higher pH favored ^{90}Y binding. No uptake was observed among the unmodified Lym-1 controls.

Kinetic Study

The chelation of ^{88}Y-2IT-BAD-Lym-1 was faster at higher pH (Fig. 3A). The data generated straight lines on semilog plots (Fig. 3B), from which k_{obs} values were determined according to Equation 6. The second-order rate constants were calculated in turn, according to Equation 5, to be 0.29, 3.1, 3.2, 47 and 221 $M^{-1} \cdot sec^{-1}$ in 0.5 M ammonium acetate at pH 4.5, 5.5, 6.5, 7.5 and 8.25, respectively. All ^{88}Y was scavenged by DTPA in the unmodified Lym-1 controls.

Radiolabeling Temperature

The concentration of DOTA used in the study had been calculated such that the half-time for uptake of ^{90}Y at pH 7 at room temperature would be ~30 min, based on the results of the kinetic study. At room temperature, 62% of ^{90}Y was chelated in 30 min; comparatively, at 37°C, 89% of ^{90}Y was chelated in only 5 min (Fig. 4).

Yttrium-90 Radiolabeling

Under optimized conditions, the yield of initial ^{90}Y recovered as purified ^{90}Y-DOTA immunoconjugate (± s.d.) was 91% ± 8% (Table 1). The specific activity of final products was 74–130 MBq ^{90}Y per mg of MoAb. By HPLC, the percentage of ^{90}Y associated with immunoconjugate was 98% ± 2.5%. The percentage of ^{90}Y-labeled immunoconjugates in aggregated form was 2.9% ± 2.5%. The immunoreactivity of ^{90}Y-labeled immunoconjugates was 100% ± 11% relative to unmodified MoAb.
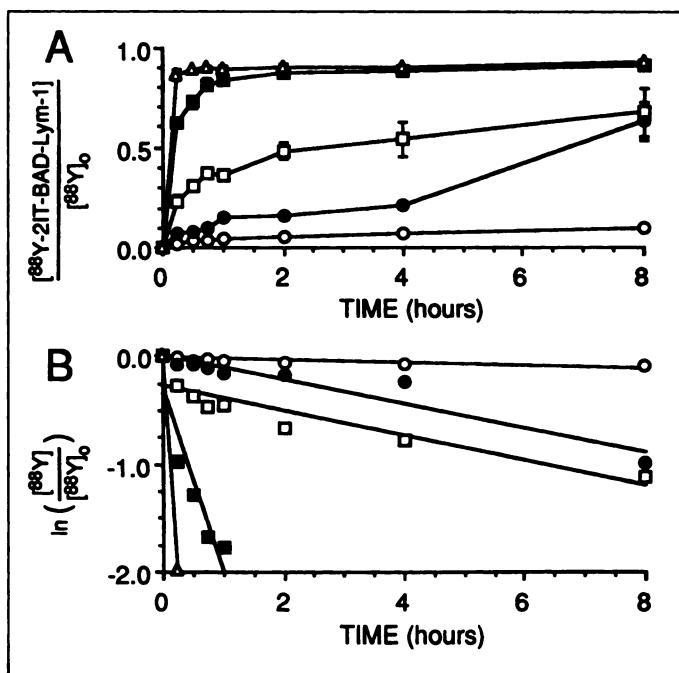

FIGURE 3. Radiolabeling of ^{88}Y-2IT-BAD-Lym-1 in 0.5 M ammonium acetate at pH 4.5 (○), 5.5 (●), 6.5 (□), 7.5 (■) and 8.25 (△). (A) Higher pH was associated with faster uptake of ^{88}Y. Data points = means of two measurements; bars = range. (B) Excellent fitting of data to log function was observed. Log function was used to determine pseudo-first-order rate constants for chelation of reaction; these, in turn, were used to estimate radiolabeling reaction times under radiopharmacy labeling conditions.

DISCUSSION

This study was undertaken to determine the optimal conditions to radiolabel ^{90}Y-DOTA immunoconjugates rapidly and in high yield. The survey of radiolabeling buffers was conducted under conditions of excess yttrium (III) relative to DOTA metal binding groups, to determine in which buffer all DOTA groups are most readily accessible to yttrium ion. Under the assay conditions, all DOTA groups were occupied by yttrium (III) in 0.5 M ammonium acetate, over the pH range of 5.0–7.0. However, in a radiopharmaceutical labeling with ^{90}Y, the concentration of yttrium ion is much lower; hence, the
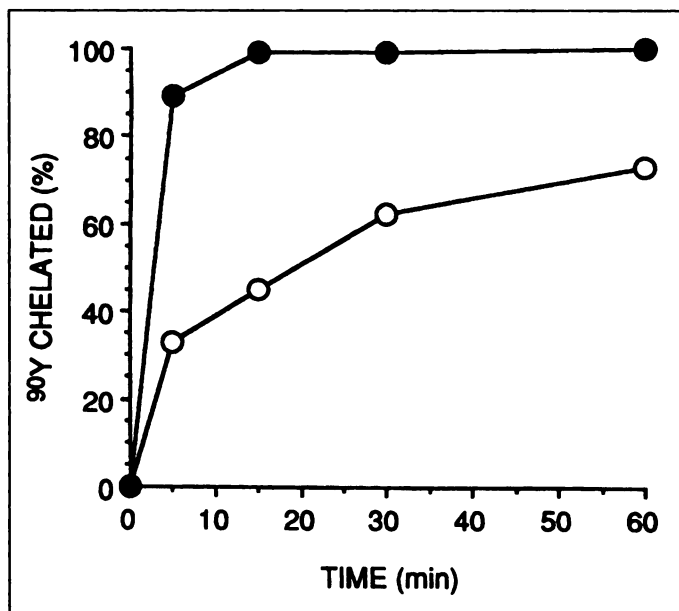

FIGURE 4. Radiolabeling of ^{90}Y-2IT-BAD-Lym-1 under similar conditions at room temperature (○) and 37°C (●). Uptake of ^{90}Y was faster at 37°C.

TABLE 2
Half-Times for Uptake of Yttrium-90 by DOTA-MoAb
Immunoconjugate in 0.5 M Ammonium Acetate Buffer Under
Prospective Radiopharmacy Labeling Conditions*

pH	Half-time (min)
4.5	46–410
5.5	4.3–38
6.5	4.2–37
7.5	0.28–2.5
8.25	0.06–0.53

*Radiopharmacy labeling conditions are: 15–45 mg/ml DOTA-MoAb, one to three DOTA chelating groups conjugated per MoAb molecule and 1.5–11 GBq/ml ^{90}Y.

uptake of ^{90}Y would not necessarily be equivalent over the range of pH. Therefore, a kinetic study was performed, which showed that, in 0.5 M ammonium acetate, slightly alkaline pH was associated with markedly faster ^{90}Y-DOTA chelation kinetics.

By knowing the reagent concentrations for ^{90}Y radiopharmaceutical labelings, the time required for chelation of ^{90}Y can be calculated from the kinetic constants obtained in this study. Prospective radiopharmacy labeling conditions are: 15–45 mg/ml DOTA-MoAb conjugate; one to three DOTA chelating groups conjugated per MoAb molecule (below a level associated with functional impairment of the MoAb) (26); and 1.5–22 GBq/ml ^{90}Y, i.e., 100–250 MBq per mg of DOTA-MoAb conjugate (6). Therefore, the ranges of concentration of DOTA and ^{90}Y in the radiolabeling reaction solution will be 97–870 μM and 0.83–6.1 μM, respectively. From these data, half-times for the chelation of ^{90}Y in 0.5 M ammonium acetate were calculated (Table 2). The time required to chelate 94% of ^{90}Y (four half-times) was 17–148 min at pH 6.5, but it was only 1–10 min at pH 7.5; one unit of pH conferred a significant practical advantage in terms of ^{90}Y-DOTA radiolabeling time and yield. At pH 8.25, chelation was even faster, but buffering capacity was low, which would make it difficult to reproducibly achieve a final pH of 8.25 under radiopharmacy labeling conditions. For this reason, pH 8.25 was not used for subsequent radiolabelings.

To investigate other means to increase the speed and yield of ^{90}Y-DOTA chelation, the effect of temperature was examined. At 37°C, the rate of ^{90}Y-DOTA chelation was significantly enhanced compared to room temperature (Fig. 4).

On the basis of the above experiments, the optimized conditions for ^{90}Y radiolabeling of DOTA-MoAb conjugates were determined to be: 0.5 M ammonium acetate buffer, pH 7.0–7.5 and 37°C. The kinetic data indicated that quantitative uptake of ^{90}Y could be achieved within 30 min. Radiolabelings were conducted under optimized conditions, using the conjugates 2IT-BAD-Lym-1 and DOTA-peptide-ChL6, with four and three free carboxyl arms, respectively (Fig. 1). A mean product yield (± s.d.) of 91% ± 8% was obtained (Table 1), comparable to yields achieved with ^{90}Y-DTPA immunoconjugates and ^{131}I-MoAbs (32). The specific activity of final products was 74–130 MBq ^{90}Y per mg of MoAb. Despite the high levels of radioactivity in the radiolabeling mixture, radiolysis of the MoAb immunoconjugates was minimal as measured by HPLC, CAE and RIA; the final products exceeded rigorous quality assurance standards for clinical radiopharmaceuticals (5,33).

Currently, ^{90}Y-DOTA chelation is generally performed in 0.1 M ammonium acetate, pH 5–6 (13,15,17). Because we inves-

tigated higher pH, we increased the buffer concentration to prevent the hydrolysis of yttrium ion to unchelatable yttrium hydroxide. As discussed above, raising the pH by two units to 7.5 was shown to increase the kinetic constant by two orders of magnitude, to allow the rapid chelation of ^{90}Y-DOTA at low concentrations of DOTA. Prolonged storage of ^{90}Y in buffer before radiolabeling is not recommended; however, no hydrolysis problems were observed in this study.

Compared with other strategies for increasing the radiolabeling yield of ^{90}Y-DOTA immunoconjugates, the optimization of ^{90}Y-DOTA chelation kinetics eliminates the need to raise the concentration of DOTA by conjugating multiple DOTAs to MoAb via a dendrimeric macromolecule. Similarly, there is no need to radiolabel concentrated bifunctional DOTA for subsequent conjugation to MoAb, as in prelabeling, and the simpler radiolabeling scheme used in this study is more amenable to development as a kit procedure. Overall, preparation of ^{90}Y-labeled MoAbs as described in this study is comparable to ^{131}I iodination in simplicity, yield and product quality. The optimization of ^{90}Y-DOTA chelation represents an important advance in ^{90}Y RIT by facilitating the dependable and routine preparation of ^{90}Y-DOTA immunopharmaceuticals (34).

CONCLUSION

To make ^{90}Y-DOTA immunoconjugates more practical for RIT, optimal radiolabeling conditions were determined. These conditions resulted in rapid labeling, to minimize autoradiolysis and radiation exposure to personnel; high yields, to reduce cost; and high specific activity and excellent product quality, suitable for pharmaceutical use. Yield, ease of labeling and product quality were, in fact, comparable to those achieved with ^{131}I iodination. The optimization of ^{90}Y-DOTA chelation conditions will facilitate the cost-effective, dependable preparation of ^{90}Y-DOTA immunopharmaceuticals.

REFERENCES

1. DeNardo GL, DeNardo SJ. Perspectives on the future of radioimmunodiagnosis and radioimmunotherapy of cancer. In: Burchiel SW, Rhodes BA, eds. *Radioimmunoimaging and radioimmunotherapy.* New York: Elsevier Science Publishing Co., Inc.; 1983:41–62.
2. White CA, Halpern SE, Parker BA, et al. Radioimmunotherapy of relapsed B-cell lymphoma with yttrium 90 anti-idiotype monoclonal antibodies. *Blood* 1996;87:3640–3649.
3. Maraveyas A, Snook D, Hird V, et al. Pharmacokinetics and toxicity of an yttrium-90-CITC-DTPA-HMFG1 radioimmunoconjugate for intraperitoneal radioimmunotherapy of ovarian cancer. *Cancer* 1994;73:1067–1075.
4. Herpst JM, Klein JL, Leichner PK, Quadri SM, Vriesendorp HM. Survival of patients with resistant Hodgkin's disease after polyclonal yttrium 90-labeled antiferritin treatment. *J Clin Oncol* 1995;13:2394–2400.
5. DeNardo SJ, Richman CM, Goldstein DS, Shen S, Salako QA, Kukis DL. Yttrium-90/Indium-111DOTA-peptide-chimeric L6: pharmacokinetics, dosimetry and initial results in patients with incurable breast cancer. *Anticancer Res* 1997;17:1735–1744.
6. DeNardo SJ, Kramer EL, O'Donnell RT, et al. Radioimmunotherapy for breast cancer using indium-111/yttrium-90 BrE-3: results of a Phase I clinical trial. *J Nucl Med* 1997;38:1180–1185.
7. Remmenga SW, Colcher D, Gansow O, Pippen CG, Raubitschek A. Continuous infusion chemotherapy as a radiation-enhancing agent for yttrium-90-radiolabeled monoclonal antibody therapy of a human tumor xenograft. *Gynecol Oncol* 1994;55:115–122.

8. Bierman PJ, Vose JM, Leichner PK, et al. Yttrium 90-labeled antiferritin followed by high-dose chemotherapy and autologous bone marrow transplantation for poor-prognosis Hodgkin's disease. *J Clin Oncol* 1993;11:698–703.

9. DeNardo SJ, Kukis DL, Kroger LA, et al. Synergy of Taxol and radioimmunotherapy with yttrium-90-labeled chimeric L6 antibody: efficacy and toxicity in breast cancer xenografts. *Proc Natl Acad Sci USA* 1997;94:4000–4004.

10. Ali MS, Quadri SM. Maleimido derivatives of diethylenetriaminepentaacetic acid and triethylenetetraaminehexaacetic acid: their synthesis and potential for specific conjugation with biomolecules. *Bioconjug Chem* 1996;7:576–583.

11. Mardirossian G, Wu C, Hnatowich DJ. The stability in liver homogenates of indium-111 and yttrium-90 attached to antibody via two popular chelators. *Nucl Med Biol* 1993;20:65–74.

12. Kozak RW, Raubitschek A, Mirzadeh S, et al. Nature of the bifunctional chelating agent used for radioimmunotherapy with yttrium-90 monoclonal antibodies: critical factors in determining in vivo survival and organ toxicity. *Cancer Res* 1989;49:2639–2644.

13. Stimmel JB, Stockstill ME, Kull FC. Yttrium-90 chelation properties of tetraazatetraacetic acid macrocycles, diethylenetriaminepentaacetic acid analogues, and a novel terpyridine acyclic chelator. *Bioconjug Chem* 1995;6:219–225.

14. Schott ME, Schlom J, Siler K, et al. Biodistribution and preclinical radioimmunotherapy studies using radiolanthanide-labeled immunoconjugates. *Cancer* 1994; 73(suppl):993–998.

15. Moi MK, Meares CF, DeNardo SJ. The peptide way to macrocyclic bifunctional chelating agents: synthesis of 2-(p-nitrobenzyl)-1,4,7,10-tetraazacyclododecane-N′-N′,N′′,N′′′-tetraacetic acid and study of its yttrium (III) complex. *J Am Chem Soc* 1988;110:6266–6267.

16. Harrison A, Walker CA, Parker D, et al. The in vivo release of ⁹⁰Y from cyclic and acyclic ligand-antibody conjugates. *Int J Radiat Appl Instrum B* 1991;18:469–476.

17. DeNardo GL, Kroger LA, DeNardo SJ, et al. Comparative toxicity studies of yttrium-90 MX-DTPA and 2-IT-BAD conjugated monoclonal antibody (BrE-3). *Cancer* 1994;73(suppl):1012–1022.

18. Li M, Meares CF, Zhong GR, Miers L, Xiong CY, DeNardo SJ. Labeling monoclonal antibodies with ⁹⁰yttrium- and ¹¹¹indium-DOTA chelates: a simple and efficient method. *Bioconjug Chem* 1994;5:101–104.

19. Wu C, Brechbiel MW, Kozak RW, Gansow OA. Metal-chelate-dendrimer-antibody constructs for use in radioimmunotherapy and imaging. *Bioorg Med Chem* 1994;4:449–454.

20. Chakrabarti MC, Le N, Paik CH, De Graff WG, Carrasquillo JA. Prevention of radiolysis of monoclonal antibody during labeling. *J Nucl Med* 1996;37:1384–1388.

21. Epstein AL, Marder RJ, Winter JN, et al. Two new monoclonal antibodies, Lym-1 and Lym-2, reactive with human-B-lymphocytes and derived tumors, with immunodiagnostic and immunotherapeutic potential. *Cancer Res* 1987;47:830–840.

22. Liu AY, Robinson RR, Hellström KE, Murray EDJ, Chang CP, Hellström I. Chimeric mouse-human IgG1 antibody that can mediate lysis of cancer cells. *Proc Natl Acad Sci USA* 1987;84:3439–3443.

23. Beaumier PL, Neuzil D, Yang HM, et al. Immunoreactivity assay for labeled anti-melanoma monoclonal antibodies. *J Nucl Med* 1986;27:824–828.

24. DeNardo SJ, Zhong G-R, Salako Q, Li M, DeNardo GL, Meares CF. Pharmacokinetics of chimeric L6 conjugated to ¹¹¹In and ⁹⁰Y DOTA-peptide in tumor bearing mice. *J Nucl Med* 1995;36:829–836.

25. DeNardo SJ, Peng J-S, DeNardo GL, Mills SL. Immunochemical aspects of monoclonal antibodies important for radiopharmaceutical development. *Nucl Med Biol* 1986;13:303–310.

26. Kukis DL, DeNardo GL, DeNardo SJ, et al. Effect of the extent of chelate substitution on the immunoreactivity and biodistribution of 2IT-BAT-Lym-1 immunoconjugates. *Cancer Res* 1995;55:878–884.

27. Coursey BM, Calhoun JM, Cessna JT. Radioassays of yttrium-90 used in nuclear medicine. *Nucl Med Biol* 1993;20:693–699.

28. Salako QA, DeNardo SJ. Radioassay of yttrium-90 radiation using the radionuclide dose calibrator. *J Nucl Med* 1997;38:723–726.

29. McCall MJ, Diril H, Meares CF. Simplified method for conjugating macrocyclic bifunctional chelating agents to antibodies via 2-iminothiolane. *Bioconjug Chem* 1990;1:222–226.

30. Li M, Meares CF. Synthesis, metal chelate stability studies, and enzyme digestion of a peptide-linked DOTA derivative and its corresponding radiolabeled immunoconjugates. *Bioconjug Chem* 1993;4:275–283.

31. Meares CF, McCall MJ, Reardan DT, Goodwin DA, Diamanti CI, McTigue M. Conjugation of antibodies with bifunctional chelating agents: isothiocyanate and bromoacetamide reagents, methods of analysis, and subsequent addition of metal ions. *Anal Biochem* 1984;142:68–78.

32. DeNardo GL, Mills SL, Kukis DL, Anderson V, DeNardo SJ. Review of a four-year experience iodinating monoclonal antibodies with therapeutic amounts of I-131. *Antibody Immunoconj Radiopharm* 1995;8:1–10.

33. DeNardo SJ, DeNardo GL, Peng JS. Pharmaceutical quality control of radiolabeled monoclonal antibodies and their fragments. *Am J Physiol Imag* 1989;4:39–44.

34. Vriesendorp HM, Quadri SM, Jaeckle KA, Freedman RS, Cromeens DM. Proposal for translational analysis and development of clinical radiolabeled immunoglobulin therapy. *Radiother Oncol* 1996;41:151–161.

Quantifying the Radiation Dosage to Individual Skeletal Lesions Treated with Samarium-153-EDTMP

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Samarium-153-ethylenediaminetetramethylenephosphonate (EDTMP) is used in the treatment of painful skeletal lesions. This study attempted to quantify the radiation dosage to individual lesions on both the macroscopic and microscopic level. **Methods:** A gamma camera-based quantification technique was adapted and refined for ¹⁵³Sm. The accuracy of the technique was determined by using a realistic phantom. The activity and volume of lesions as well as normal bone were determined and used to estimate the radiation dosages to these regions. Two patients died of unrelated causes shortly after receiving ¹⁵³Sm-EDTMP. This made it possible to compare the gamma camera results with direct measurements. It also allowed for autoradiographic examination of the lesions. Finally, the microscopic radiation dosages were estimated. **Results:** The phantom study indicated that the quantification technique was off, on average, by 4.1% (s.d. = 8.1%). The absolute activity concentration of trabecular bone was found to be ~0.22 MBq/g, and that of cortical bone was found to be ~0.1 MBq/g, regardless of the dosage administered. The corresponding concentrations for lesions were between 3 and 7 times higher than that of normal bone, with no apparent ceiling. From these results, the macroscopic radiation dosage could be estimated. The dosage to normal bone varied between 0.9 and 3.9 cGy · kg/MBq, and that of the lesions varied between 5.2 and 27.1 cGy · kg/MBq. The autopsy results confirmed that the gamma camera technique was accurate. The autoradiography showed clearly that the activity was associated with the surface of the bone. From these findings, the microscopic radiation dosage distribution was estimated for cortical and trabecular bone as well as osteoblastic lesions. The variation in the microscopic dosage compared to the macroscopic dosage was quite large. Microscopic dosages, when compared to the macroscopic dosages, were as high as 965% and as low as 14.9%. **Conclusion:** The techniques used have been proven to be accurate. The activity in normal bone may be at a ceiling value for all the administered doses, which could explain the small variation. This is not true for the lesions. The large variation in dosages on a microscopic scale, combined with the ceiling in normal bone, may explain the lower than expected toxicity and relatively quick relapse of the patients.

Key Words: samarium-153-EDTMP; quantification; radiation dosimetry

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Samarium-153-ethylenediaminetetramethylenephosphonate (EDTMP) has been used with good results in the palliation of