Similarities and Differences in ^{111}In- and ^{90}Y-Labeled 1B4M-DTPA AntiTac Monoclonal Antibody Distribution

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Monoclonal antibodies (MoAb) labeled with ^{90}Y are being used for radioimmunotherapy. Because ^{90}Y is a beta emitter, quantitative information from imaging is suboptimal. With the concept of a "matched pair" of isotopes, ^{111}In is used as a surrogate marker for ^{90}Y. We evaluated the differences in biodistribution between ^{111}In- and ^{90}Y-labeled murine antiTac MoAb directed against the IL-2Rα receptor. **Methods:** The antiTac was conjugated to the 2-(4-isothiocyanatobenzyl)-6-methyl-diethylenetriamine pentaacetic acid (1B4M-DTPA, also known as MX-DTPA). Nine patients with adult T-cell leukemia were treated. Patients received approximately 185 MBq (5 mCi) ^{111}In-labeled antiTac for imaging and 185–555 MBq (5–15 mCi) ^{90}Y-labeled antiTac for therapy. The immunoreactivity of ^{111}In-labeled antiTac was 90% ± 6%, whereas for ^{90}Y-labeled antiTac, it was 74% ± 12%. **Results:** The differences in blood and plasma kinetics of the two isotopes were small. The area underneath the blood radioactivity curve was 1.91 percentage ± 0.58 percentage injected dose (%ID) × h/mL for ^{111}In and 1.86% ± 0.64 %ID × h/mL for ^{90}Y. Urinary excretion of ^{90}Y was significantly greater than that of ^{111}In in the first 24 h (P = 0.001), but later, the excretion of ^{111}In was significantly greater (P = 0.001 to P = 0.04). Core biopsies of bone marrow showed a mean of 0.0029 ± 0.0012 %ID/g for ^{111}In, whereas the ^{90}Y concentration was 0.0049 ± 0.0021 %ID/g. Analyses of activity bound to circulating cells showed concentrations of 500–30,000 molecules of antiTac per cell. When cell-bound activity was corrected for immunoreactive fraction, the ratio of ^{111}In to ^{90}Y in circulating cells was 1.11 ± 0.17. Three biopsies of tumor-involved skin showed ratios of ^{111}In to ^{90}Y of 0.7, 0.9 and 1.1. **Conclusion:** This study shows that differences typically ranging from 10% to 15% exist in the biodistribution between ^{111}In- and ^{90}Y-labeled antiTac. Thus, it appears that ^{111}In can be used as a surrogate marker for ^{90}Y when labeling antiTac with the 1B4M chelate, although underestimates of the bone marrow radiation dose should be anticipated.

Key Words: ^{111}In; ^{90}Y; radioimmunotherapy; monoclonal antibody; pharmacokinetics

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The use of radiolabeled monoclonal antibodies (MoAb) for radioimmunotherapy of lymphoma and leukemia has had encouraging results (*1*). Most studies have used ^{131}I-labeled MoAb (*1*). Advantages of ^{131}I are its availability, the ease of labeling techniques, such as chloramine T or iodogen, and its gamma ray emissions, which can be imaged, allowing for biodistribution studies before and after therapy. Nevertheless, ^{131}I has some limitations, including rapid dehalogenation (*2*) and emission of high-energy gamma rays, which impose certain radiation safety constraints.

As an alternative to ^{131}I for radioimmunotherapy, ^{90}Y has been evaluated (*3–10*) because of its ready availability from a ^{90}Sr/^{90}Y generator (*11*) and its physical and biologic characteristics. Although ^{90}Y has favorable characteristics for therapy ($t_{1/2}$ = 64 h; pure beta emission [E_{max} = 2.28 MeV]), the lack of gamma ray emission makes it suboptimal for imaging and assessing biodistribution (*12*). To trace the biodistribution of ^{90}Y, ^{111}In has been used as a surrogate marker because it has similar coordination chemistry (*13,14*) and metabolic handling (*15,16*). Preclinical studies have shown the importance of chelate selection in determining the stability of ^{90}Y radioconjugates. First-generation chelates, such as cyclic or mixed anhydride of DTPA, have shown major differences in the rate of release of these isotopes in solution (*17,18*) and in preclinical animal models (*9*). Newer chelates have greater in vitro and in vivo stability (*19–21*). Nevertheless, even with improved chelates, some differences between ^{111}In and ^{90}Y have been observed (*21,22*). Although several animal studies have compared the differences in biodistribution, few studies have evaluated these differences in humans, and none has presented comprehensive biodistribution data in circulation and other tissues (*6,8,10,23–25*). In this report, we analyze the pharmacokinetics and biodistribution of the antiTac labeled with ^{111}In and ^{90}Y via the 1B4M chelate (also known as MX-DTPA) (*20*) in patients undergoing radioimmunotherapy for adult T-cell leukemia (ATL). We have previously reported on other aspects of this phase 1 clinical trial (*7*).

MATERIALS AND METHODS

AntiTac Monoclonal Antibody

AntiTac is a murine IgG2a MoAb that recognizes the IL-2Rα receptor. It was produced as previously described (*26,27*). This

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MoAb was purified to 99% IgG from mouse ascites as assessed by high-pressure liquid chromatography (HPLC) and sodium dodecylsulfate-polyacrylamide gel electrophoresis.

Radiolabeling

The antiTac preparation was conjugated to 2-(4-isothiocyantobenzyl)-6-methyl-diethylenetriamine pentaacetic acid (1B4M-DTPA) (*20*). Radiolabeling was performed with pharmaceutical grade ^{111}In (Dupont NEN, Wilmington, DE; Medi-Physics Inc., Arlington Heights, IL) for imaging and/or pharmaceutical grade ^{90}Y for therapy (Westinghouse-Hanford Co., Richland, WA; Dupont NEN; Medi-Physics Inc.). In brief, 1.0–1.2 mg conjugated antiTac was put into a polypropylene vial that served as the reaction vessel. For ^{111}In, 351.5–1302.4 MBq (9.5–35.2 mCi) were added to the reaction vessel and allowed to react for 30–60 min. For ^{90}Y labeling, the starting amount of radioactivity and antibody dose depended on the number of patients to be injected. Typically, 481–4218 MBq (13–114 mCi) ^{90}Y were incubated with 1.2–4.8 mg conjugate. Excess DTPA was then added to the incubation mixtures to form complexes with unreacted ionic isotope. The antiTac-bound fraction was separated by preparative size-exclusion HPLC (*7*). Purification resulted in a final product with >99% antibody-bound ^{111}In or ^{90}Y. Purity was determined by instant thin-layer chromatography that used silica gel-impregnated glass fiber sheets (2:2:1, 10% ammonium formate in water/methanol/0.2 *M* citric acid) and paper chromatography that used saline solvent and Whatmann no. 1 paper pretreated with 5% human serum albumin. The final product was filtered with a sterile 0.22-μm low-protein-binding filter (Millex-GV; Millipore Inc., Bedford, MA). The specific activities of the ^{111}In-labeled antiTac doses (n = 15) ranged from 133.2 to 802.9 MBq/mg (3.6–21.7 mCi/mg) (318.2 ± 214.6 MBq [8.6 ± 5.8 mCi/mg]). The specific activities of the ^{90}Y-labeled antiTac doses (n = 38) ranged from 125.8 to 788.1 MBq/mg (3.4–21.3 mCi/mg) (440.3 ± 185 MBq [11.9 ± 5.0 mCi/mg]). All products passed sterility and pyrogen testing. The ^{111}In-labeled products were injected within 72 h of preparation: 9 were injected the day of labeling, 5 within 24 h and 1 within 72 h. Of 38 ^{90}Y-labeled antiTac doses, 28 were injected the same day of labeling, whereas all other products were injected the next day (~20 h). Those products injected the day after labeling were retested before injection and showed similar protein-bound radioactivity. The immunoreactivity of the radiolabeled products was tested by using a modification of the cell-binding assay described by Lindmo et al. (*28*). In brief, HUT 102, a Tac-positive cell line, was used at antigen excess. To determine whether there was a relationship between the drop in immunoreactivity and the radiation dose to the antibody solution during labeling and storage, the correlation coefficient between radiation dose received and the immunoreactivity was determined for all ^{90}Y-labeled antibodies. With the MIRD technique (*29*), the radiation dose to the antibody solution was calculated. In brief, the cumulative activity received by the antibody solution was calculated by determining the amount of ^{90}Y in the solution and the elapsed time of autoirradiation and correcting for the volume of the antibody solution (μCi × h/g). This cumulative activity was then multiplied by the mean energy emitted for unit cumulative activity for ^{90}Y (1.99 g × rad)/(μCi × h).

Patients and Treatment Schedule

Nine patients with histologically confirmed HTLV-1-associated ATL were studied (Table 1). Their ages ranged from 24 to 61 y (mean 43 y). These patients were classified as having acute ATL (*5*) or chronic ATL (*4*) according to the Japanese Lymphoma Study Group criteria (*30*). Inclusion criteria were (a) expression of Tac antigen (IL-2Rα) on at least 10% of peripheral white blood cell count, lymph node or dermal T cells; (b) no evidence of human antimouse antibodies (HAMA); and (c) no cytotoxic chemotherapy or radiation therapy for at least 4 wk before antibody treatment. Portions of this phase 1 trial detailing toxicity and clinical response to treatment have been previously published (*7*). The Intramural Review Board for Human Research of the National Cancer Institute approved this study, and each patient gave informed consent.

Each group of three patients was scheduled to receive escalating doses of ^{90}Y-labeled antiTac every 6 wk, if tolerated (Table 1). The initial ^{90}Y dose was 185 MBq (5 mCi) and was escalated in every three patients by 185 MBq (5 mCi) if no dose-limiting toxicity was observed. A total of 38 ^{90}Y-labeled antiTac treatments were administered. No intrapatient ^{90}Y dose escalation was performed. The patients received a co-infusion of ^{111}In-labeled antiTac mixed and injected simultaneously with the ^{90}Y-labeled antiTac on up to three occasions for imaging purposes. Two of the patients received ^{90}Y-labeled antiTac therapy without any doses of ^{111}In-labeled antiTac. A total of 14 imaging doses were administered with a mean

TABLE 1
Patient Characteristics

Patient no.	Type of ATL	Age	Sex	Race	Doses ^{111} In-labeled antiTac Total (per cycle) mCi	Doses ^{90}Y-labeled antiTac Total (per cycle) mCi	Soluble IL-2Rα (U/mL)	WBC/μL
1	Chronic	42	F	B	8.8 (3.8, 5)	45 (5, 5, 5, 5, 5, 5, 5, 5, 5)	4026	14600
2	Acute	32	F	H	14.6 (4.6, 5, 5)	20 (5, 5, 5, 5)	47141	23800
3	Acute	24	F	B	1.5 (1.5)	5 (5)	2750	6400
4	Acute	55	M	B	10 (5, 5)	20 (10, 5, 5)	57626	20100
5	Acute	34	F	B	9.3 (4.3, 5)	45 (10, 10, 10, 10, 5)	2950	11200
6	Chronic	44	M	B	12 (5, 5, 2)	66 (10, 10, 10, 6, 10, 10, 10)	2113	6900
7	Chronic	38	F	B	10 (5, 5)	50 (15, 15, 10, 5, 5)	2938	32600
8	Chronic	61	F	B	None	20 (15, 5)	7596	37200
9	Acute	54	F	B	None	25 (15, 10)	2097	6500

ATL = acute T-cell leukemia; WBC = white blood cell count; B = black; H = Hispanic.
Portions of this table have been previously published (*7*).

of 160.2 MBq (4.33 mCi). In all instances, a total of 10 mg of antiTac was infused by adding unlabeled antiTac to the mixture of labeled antibody in enough quantity to bring the total to 10 mg. The antibody was infused over about 2 h. Patients with less than grade 3 hematologic toxicity were eligible for retreatment with the same dose of ^{90}Y if they had no evidence of disease progression and remained HAMA negative. The patients were retreated at 6 wk or when blood cell counts returned to an acceptable level. Although we aimed to re-treat patients with their initial ^{90}Y dose, in some patients who were receiving repeated 370–555 MBq (10 or 15 mCi) doses, hematologic toxicity necessitated that we decrease subsequent doses to 185–370 MBq (5–10 mCi) ^{90}Y (Table 1).

Levels of soluble IL-2Rα (sIL-2Rα) were determined by using a previously described ELISA technique (*31*). Values >502 U/mL are considered abnormal. Patients were monitored for presence of HAMA before initial treatment and before each subsequent dose by using a two-arm capture ELISA technique. All patients who were HAMA positive were excluded from further treatment (*7*).

Pharmacokinetics

Intravascular kinetics were determined by counting ^{111}In or ^{90}Y radioactivity in blood and in plasma aliquots obtained at the following times after the end of infusion: 5 min (T0), 30 min, 1 h, 2 h, 6 h, 12 h, 24 h and daily up to 7 d after the end of the infusion. The percentage injected dose (%ID) per milliliter was obtained by comparing the counts to a standard of the ID. The plasma and blood volumes were estimated at each time of treatment by using a nomogram based on body surface area. With the latter-estimated volumes and the %ID per milliliter, the total %ID in the blood and plasma volume were estimated. Because the infusion time was short compared with the disposition half-life ($t_{1/2}$), the intravascular data were treated similar to an intravenous bolus. The %ID per milliliter of blood or plasma was fitted to a biexponential curve to obtain both the α- and β-phase $t_{1/2}$ by using a least-squares fit algorithm (SigmaPlot; Jandel Scientific, Duarte, CA). Conventional pharmacokinetic parameters were then derived (*32*). The areas underneath the blood or plasma curves (AUC) were calculated in two steps. First, the AUC from the end of antibody infusion (T0) to 168 h was obtained by trapezoidal integration of the decay-corrected blood and plasma data; then, the terminal AUC was estimated by using the terminal clearance rate to extrapolate from the activity retained at the last measured time point. With this data, we then estimated additional pharmacokinetic parameters, including volume of distribution of central compartment (Vc), volume of distribution at steady state (Vss) and mean residence time (MRT) (*32*). Serial 24-h urine collections were obtained for up to 96 h so that we could compare the urinary excretion of the two tracers. Whole-body clearance of ^{111}In was determined from the imaging data (see later discussion).

Cell-Bound Radioactivity

The number of antibody molecules delivered in vivo to the circulating cells was determined. The blood was sampled at the end of the infusion and 2 h after the infusion in 27 of the 38 treatments. The lymphocytes in ~5 mL blood were separated by using per cell gradient centrifugation (LSM; Organon Teknika Corp., Durham, NC). In brief, 1 part blood was diluted in 2 parts phosphate-buffered saline (PBS) without calcium or magnesium at room temperature. The mononuclear layer was then removed in accordance with the manufacturer's instructions and was washed twice with 10 mL PBS without calcium or magnesium. Cells were then resuspended in 3–5 mL, counted and processed before gamma and beta counting. Typically, this resulted in >98% viable cells. The cells were then solubilized, bleached and counted as described later for patient samples. The percentage of the injected dose in the cell aliquot was then divided by the total cell count, and the number of molecules per mononuclear cell was then estimated. The estimates from the first treatment were used to determine the mean number of antibody molecules per cell. Because the separation included all mononuclear cells, this estimate represents a lower limit.

Imaging

Scintillation camera images were first recorded up to six times with a large-field-of-view gamma camera within 2 h of the end of the infusion and daily for up to 6 or 7 d. Analog and digital images of anterior and posterior whole-body as well as spot views (5 min per image) were recorded.

Patients receiving ^{90}Y underwent bremsstrahlung imaging. Images were acquired with the same gamma camera by using a medium- or high-energy collimator and a 90% window centered at 100 keV. Whole-body retention measurements were made by obtaining the geometric mean counts from patients imaged with ^{111}In or ^{90}Y (when given alone) and comparing the serial imaging data to the initial geometric mean counts obtained shortly after the radiolabeled antibody was administered.

Counting Methods

Dual-isotope counting of ^{111}In and ^{90}Y was performed in the same samples. The ^{111}In-labeled gamma ray peaks were counted in a gamma counter using a 100- to 500-keV energy setting. Because ^{90}Y is counted with <4% efficiency in a gamma counter, Cerenkov counting in a beta counter was also used. Because Cerenkov counting is sensitive to quench and geometry, all samples were processed in a similar and reproducible manner. Samples of blood, plasma and urine were first treated with 0.5 mL sodium dodecylsulfate (SDS) at 56°C, followed by bleaching with 0.4 mL 30% hydrogen peroxide to minimize quenching. The counts in the samples were referred back to a standard of the injected dose. When the same total counts from the standard were counted in blood or plasma, the ^{111}In counts in blood were 1.00 ± 0.01 of those detected in plasma, whereas the ^{90}Y counts in blood were 0.89 ± 0.02 of those detected in plasma. Therefore, to mimic the quenching observed in the patient samples, standards were mixed with 0.1 mL of the patient's baseline blood or plasma. These standards underwent the same processing and resulted in similar quench as the patient samples. All samples were then brought up to a volume of ~11 mL with distilled water. Beta counting was performed by using an energy range of 0–200 keV (A4530D Packard, Downers Grove, IL). The counts obtained in the gamma and beta counters were corrected for cross-talk and decay.

Uptake in Tissues

Six patients underwent bone marrow biopsy of the posterior iliac spine. Four biopsies were performed 7 d after therapy and two at 8 d after initial therapy. The biopsy core was weighed on an analytical balance and was put in a conical tube with 10 mL PBS for 1 h. The core was broken with a jagged-edged glass rod. This was centrifuged for 10 min at 640*g*, and the supernatant was removed and counted (saline fraction). The pelleted core was broken with a jagged-edged glass rod and was then mixed with 0.5 mL 10% SDS. The core was heated to 56°C for 30 min in an attempt to remove any cell-bound activity. After the sample cooled, 0.4 mL 30% hydrogen peroxide was added as bleach and the mixture was incubated at 56°C for 1 h to bleach the sample. Ten

milliliters of distilled water were added, and the sample was again centrifuged for 10 min. The supernatant was separated for counting (SDS fraction). Perchloric acid (0.2 mL) was then added to the remaining bone chips, and the mixture was incubated at 56°C until the bone was dissolved (bone fraction). This sample was again treated with hydrogen peroxide as previously described. After cooling, the sample was transferred to a counting vial with 10 mL distilled water. All samples were then counted in the gamma and beta counters with the appropriate decay and cross-talk corrections.

One patient from this phase 1 trial and two additional patients with ATL subsequently treated with similar doses of ^{111}In- and ^{90}Y-labeled antiTac underwent punch biopsies of the skin. These biopsies were solubilized in perchloric acid, were bleached and were counted as previously described in the gamma and beta counters.

Statistics

To compare independent data, we used ^{111}In and ^{90}Y patient data obtained from the initial dual-injection study. Paired *t* test or Wilcoxon signed-rank test (when data were not normally distributed) were performed to assess the differences in biodistribution between the two radiolabels. Pearson's correlation coefficient was used to evaluate the relationship between the two parameters.

RESULTS

Cell-Binding Assay

The ^{111}In-labeled antiTac had a mean immunoreactivity of 90% ± 6%, whereas the mean immunoreactivity of the ^{90}Y-labeled antiTac was 74% ± 12% (Mann-Whitney test, $P < 0.001$). The total doses delivered to the ^{90}Y antiTac during the labeling process ranged from 60 to 1500 Gy (mean = 370 Gy). The immunoreactivity values were inversely correlated with the radiation doses delivered to the solution during the labeling process (Pearson correlation, $r = -0.72$; $P = 0.000002$). The drop in immunoreactivity correlated better with the total dose to the solution than with the specific activity at which the antibody was labeled (Pearson correlation, $r = -0.54$; $P = 0.0009$).

Pharmacokinetics

The pharmacokinetic parameters derived from the blood and plasma counting data were compared in the initial study of seven patients who underwent dual-isotope injections (Table 2). These patients had an estimated mean plasma volume of 2572 ± 355 mL and an estimated mean blood volume of 4173 ± 674 mL, based on their heights and weights. Although the estimated values from ^{111}In and ^{90}Y for the central compartments were well correlated for both plasma and blood (Pearson correlation coefficient, $r > 0.98$; $P < 0.00001$), the numbers derived from the ^{90}Y counting showed slightly higher estimates than from the ^{111}In data (Table 2). To compare all the pharmacokinetic parameters estimated from the ^{111}In and ^{90}Y paired studies, we subtracted those derived from ^{111}In from those derived from ^{90}Y. The results were biased in one direction, suggesting that ^{90}Y cleared faster than ^{111}In from the blood (Fig. 1).

Although the sIL-2Rα levels were elevated in these patients and various degrees of complex formation were documented (data not shown), it did not appear to affect blood or plasma clearance because circulating IL-2Rα levels did not show a good correlation with the AUC or the %ID retained in the blood pool at the end of infusion (Pearson correlation coefficient, $r = -0.55$ and $r = -0.50$, respectively; $P > 0.13$).

The estimated amount of radioactivity remaining in the plasma at the end of infusion in the patients receiving both ^{111}In- and ^{90}Y-labeled antiTac ($n = 7$) was 81% ± 22% for ^{111}In and 79% ± 23% for ^{90}Y. Although these differences were small, they were significant with the paired *t* test ($P = 0.001$). The estimated radioactivity remaining in the blood volume of these patients at the end of infusion was 82% ± 22% for ^{111}In and 79% ± 22% for ^{90}Y (paired *t* test, $P = 0.009$). The mean plasma and blood time-activity curves for all patients receiving both ^{111}In- and ^{90}Y-labeled antiTac showed small differences (Fig. 2).

The urinary excretion of the two radioisotopes was determined from the first study of each patient who received both ^{111}In and ^{90}Y ($n = 7$) (Table 3). The urinary excretion of ^{111}In in the first 24 h was greater than that of ^{90}Y, but this pattern later reversed (Table 3). The same pattern was always seen in the repeat coinfusion studies (data not shown). The median whole-body clearance determined from

TABLE 2
Pharmacokinetic Parameters

	^{111}In in blood	^{90}Y in blood	*P*	^{111}In in plasma	^{90}Y in plasma	*P*
AUC (%ID × h/mL)	1.91 ± 0.58	1.86 ± 0.64	0.405*	1.75 ± 1.0	1.71 ± 1.82	0.297*
$t_{1/2}$ alpha (h)	3.57 ± 1.99	3.51 ± 1.69	0.745*	3.09 ± 1.63	3.04 ± 1.52	0.835†
$t_{1/2}$ beta (h)	50.01 ± 11.40	54.01 ± 13.90	0.054*	47.29 ± 11.90	51.80 ± 14.33	0.078*
MRT (h)	53.5 ± 14.60	56.77 ± 17.14	0.469*	50.51 ± 16.75	49.47 ± 17.00	0.728†
Vc (mL)	5460 ± 1656	5711 ± 1929	0.2†	3337 ± 981	3450 ± 1076	0.036†
Vss (mL)	6571 ± 2700	7549 ± 3326	0.013†	4221 ± 1684	4671 ± 1985	0.017†

*Wilcoxon signed-rank test (for data not normally distributed).
†Paired *t* test.
AUC = areas under blood or plasma curves; %ID = percentage injected dose; MRT = mean residence time; Vc = volume of distribution of central compartment; Vss = volume of distribution at steady state.

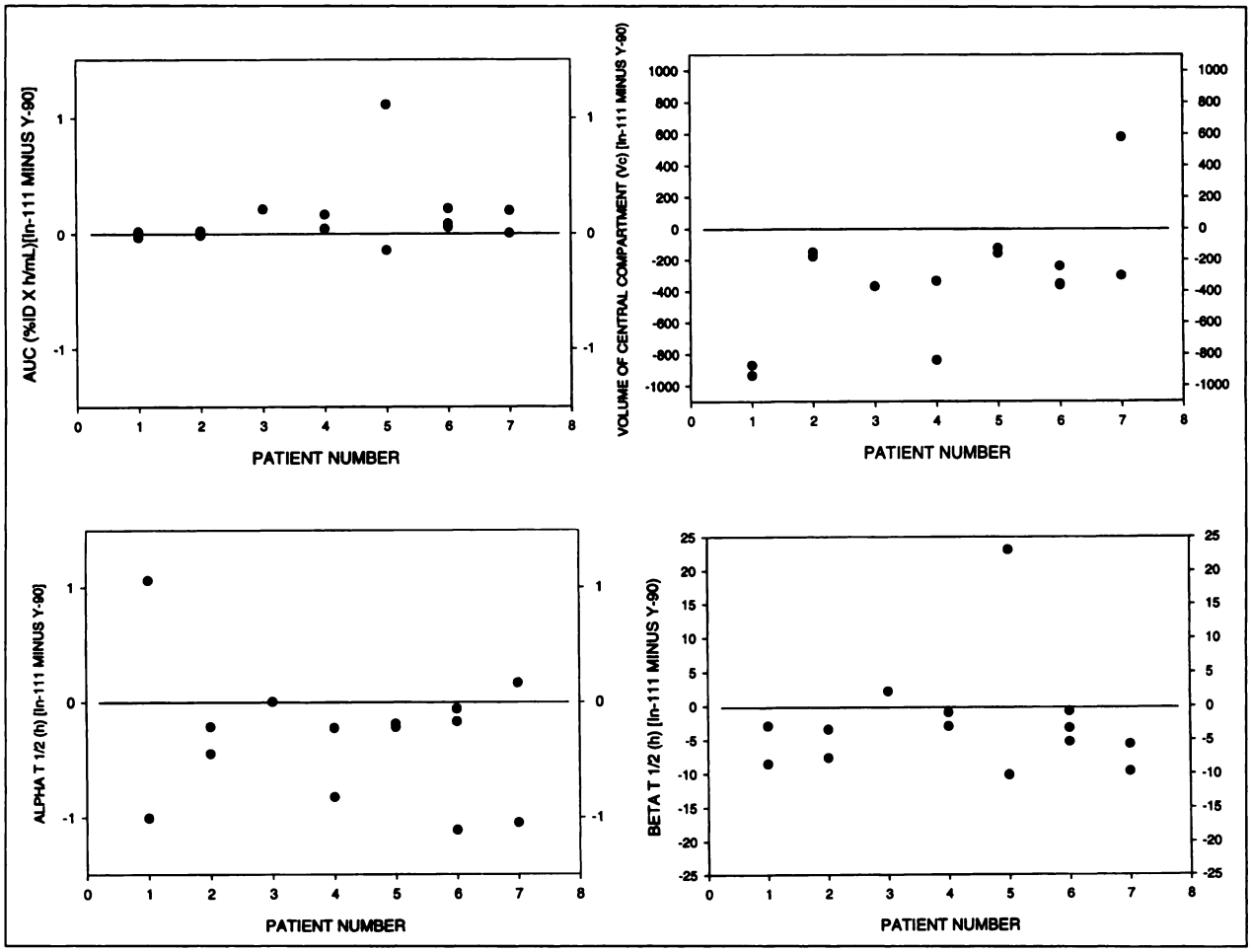

FIGURE 1. Comparison of pharmacokinetic parameters from all 14 studies in which patients were co-injected with ^{111}In- and ^{90}Y-labeled antiTac. Pharmacokinetic parameter derived from ^{90}Y data was subtracted from that derived from patients' corresponding ^{111}In data. The horizontal line in each plot is at zero and indicates that parameters are identical. Any deviation point above the line indicates that parameter derived from ^{111}In was greater than that from ^{90}Y. Parameters compared are as follows: left upper panel, area underneath the curve (AUC); right upper panel, volume of the central compartment (Vc); left lower panel, α $t_{1/2}$; right lower panel, β $t_{1/2}$.

the initial paired ^{111}In and ^{90}Y antiTac studies in seven patients based on urinary excretion of ^{111}In was 314 h, whereas that based on ^{90}Y urinary excretion was 450 h (Wilcoxon signed-rank test, $P = 0.016$). In the initial 24 h after tracer administration, a trend toward higher ^{90}Y excretion was seen when the initial 24-h urinary excretion from all data from ^{111}In and ^{90}Y were compared (Fig. 3).

The whole-body half-life based on the whole-body gamma scans obtained serially from the first ^{111}In study was 219 ± 45 h, whereas the half-life based on urinary excretion was 282 ± 94 h (n = 7 studies; paired *t* test, $P = 0.038$). There was a good correlation between whole-body clearance based on ^{111}In whole-body scans and urine (Pearson correlation coefficient, r = 0.8; $P = 0.001$). The ^{90}Y whole-body retention of patients receiving ^{90}Y antiTac alone, based on imaging showed >100% retention at 24 h or later, based on gamma camera whole-body imaging, thus indicating that these measurements were not valid for ^{90}Y bremsstrahlung imaging. A representative spot image from ^{111}In- and ^{90}Y-labeled antiTac is shown in Figure 4. The ^{90}Y images have low resolution, do not show clear outlines of the organ borders and showed no or minimal localization in tumor. The ^{111}In spot images obtained during the initial study showed excellent localization in sites known to be involved with disease. Although some excretion into bowel was seen, this was not a major route of excretion.

Cell-Bound Activity

The calculated number of antiTac molecules per cell, based on the total number of circulating mononuclear cells during the patient's first (n = 4) or second (n = 2) treatment, averaged ~11,000 (range 500–30,000). The estimates of the number of molecules of antiTac per circulating mononuclear cell were always higher (1.37 ± 0.30 times) when based on the ^{111}In-labeled antiTac counting versus the ^{90}Y-labeled antiTac data (n = 6) (paired *t* test, $P = 0.006$). When the cell-bound values of ^{111}In and ^{90}Y were corrected by their respective immunoreactive fractions, the values were only

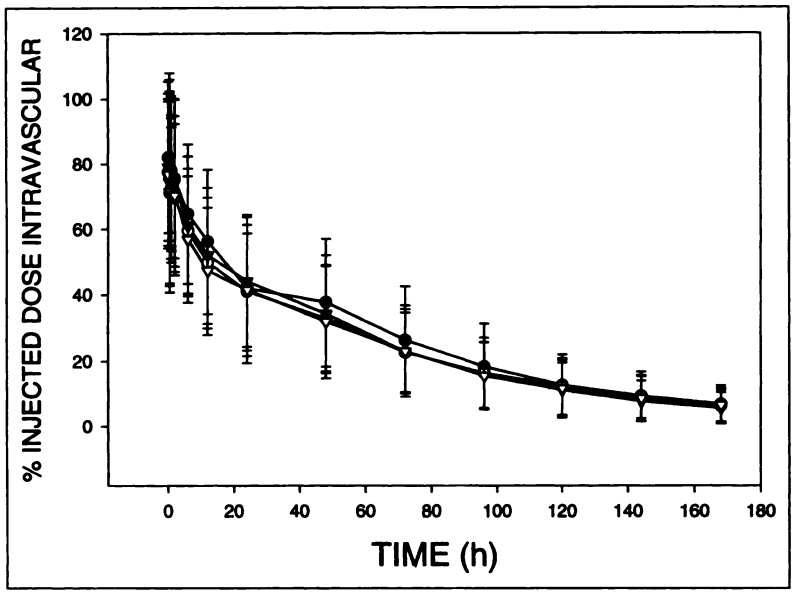

FIGURE 2. Percentage of injected dose of ^{111}In-labeled antiTac in blood or plasma volume was determined (see Materials and Methods section). The ^{111}In and ^{90}Y intravascular retention is very similar, although small differences were seen. These findings also indicate that most radioactivity was in plasma rather than cell bound. Clearance of ^{111}In in plasma (●), ^{90}Y in plasma (○), ^{111}In in blood (▼) and ^{90}Y in blood (▽) is plotted (mean ± SD).

1.14 ± 0.22 times higher, and the differences between ^{111}In and ^{90}Y estimates were not significantly different (paired *t* test, $P = 0.26$). Most radiolabeled antiTac was in plasma rather than was cell bound (Fig. 2).

Bone Marrow and Skin Biopsies

The mean concentration of ^{111}In in untreated bone marrow was 0.0031 ± 0.0012 %ID/g. Very little radioactivity was lost during the processing of the bone marrow into the three fractions (saline, SDS and bone). When the amounts in the processed fractions were added together, the total ^{111}In activity was 0.0029 ± 0.0012 %ID/g, indicating that >94% of the ^{111}In was recovered. The mean ^{90}Y concentration in the untreated bone marrow was 0.0034 ± 0.0014 %ID/g. In contrast to the case of ^{111}In, the sum of the ^{90}Y radioactivity in the processed samples was much higher than in the nonprocessed marrow, showing 0.00494 ± 0.0021 %ID/g. The amount of ^{90}Y in the processed bone marrow was significantly higher than that of ^{111}In (paired *t* test, $P = 0.0042$). The distribution of ^{111}In and ^{90}Y differed among the processed fractions (saline, SDS and bone) (Fig. 5). The respective mean percentages of the activity in the bone marrow in the saline wash, SDS wash and bone wash were 36%, 36% and 29% for ^{111}In and were 8%, 21% and 72% for ^{90}Y.

Skin biopsies were quantified in three patients. The ^{111}In-labeled antiTac showed a mean of 0.0038 %ID/g (0.0016, 0.0074 and 0.0024 %ID/g). ^{90}Y-labeled antiTac showed a mean of 0.0039 %ID/g (0.0014, 0.0088 and 0.0015 %ID/g). This represented a mean of 1.21 times more activity of ^{111}In than of ^{90}Y. When the concentrations in the biopsies were normalized by the immunoreactive fraction of antibody administered, the ^{111}In concentrations were 0.0017, 0.0084 and 0.0027 %ID/g, whereas ^{90}Y concentrations were 0.0017, 0.0117 and 0.0026 %ID/g, which represented a mean of 0.9 times more activity of ^{111}In than of ^{90}Y.

TABLE 3
Daily Urinary Excretion of ^{111}In and ^{90}Y After Co-infusion of ^{111}In- and ^{90}Y-Labeled AntiTac

Time (h)	Paired studies		
	^{111}In %ID excreted	^{90}Y %ID excreted	Paired *t* test
0–24	4.4 ± 1.4	8.5 ± 4.2	$P = 0.001$
24–48	5.1 ± 3.7	3.4 ± 2.0	$P = 0.04$
48–96	6.8 ± 3.9	3.1 ± 2.1	$P = 0.002$
96–120	5.9 ± 2.1	2.2 ± 0.8	$P = 0.001$

%ID = percentage injected dose.

DISCUSSION

Biodistribution studies are included with radioimmunotherapy trials to determine whether there is satisfactory tumor uptake and to quantify the radiation in tumor and normal organs. For antibodies labeled with pure beta emitters, it is more difficult to obtain this information. As has been suggested previously, in this study we used ^{111}In as a surrogate marker for ^{90}Y (*15,19,21,33*). We have clearly shown some significant yet small differences between these two isotopes. Studies of first-generation chelates showed large differences in stability of ^{111}In- and ^{90}Y-labeled MoAb (*17,19,22*), whereas second- and third-generation chelates have been shown to be more stable in vitro (*19,22*). In this study, we used 1B4M-DTPA chelate, which shows only minor differences in biodistribution between ^{111}In and ^{90}Y in preclinical studies (*21,34*). As in those preclinical trials, the differences in the intravascular kinetics in our study were small and generally not statistically significant (Table 2), although a comparison from all studies showed a trend toward faster clearance of ^{90}Y (Fig. 1). As expected, the major differences were in bone accumulation. Large differences were also observed in urinary excretion. These

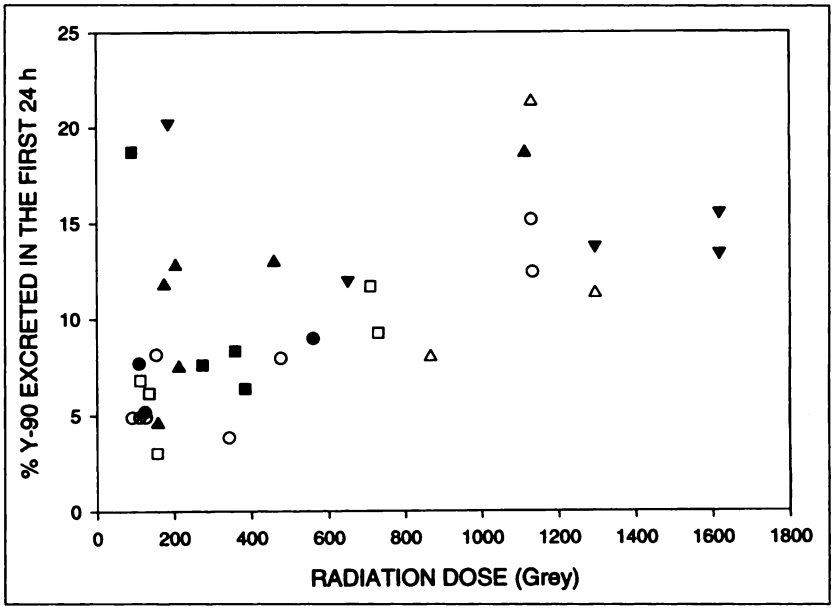

FIGURE 3. Percentage ID of ^{90}Y excreted in urine in first 24 h compared with radiation dose received by ^{90}Y-labeled antibody during labeling and before administration. Data from 34 studies are included. Each symbol represents different patient.

probably reflect an excretion of metabolites of the ^{111}In and ^{90}Y and may reflect greater accumulation of ^{90}Y in bone. Although other studies have used chelates with ^{111}In as surrogates for ^{90}Y, details are limited on the differences in clearance, tissue uptake and pharmacokinetics between these isotopes (*25*). Preclinical studies with macrocycles have shown greater stability of ^{90}Y than of ^{111}In, with less bone accumulation of ^{90}Y than of ^{111}In (*21*), therefore indicating that our findings are likely specific to the chelate used.

The immunoreactivity of the ^{90}Y antiTac was significantly lower than that of the ^{111}In-labeled antiTac. This finding was secondary to radiolytic damage during the labeling, because doses up to 100,000 rads were delivered to the antibody solution during the labeling and storage process. The faster urinary excretion of ^{90}Y than of ^{111}In observed in the first 24 h after administration is also consistent with radiolytic damage. In addition, when differences in immunoreactivity were considered, the differences in skin uptake and cell targeting in the circulation between the ^{111}In and ^{90}Y decreased typically to within ~10%. Several studies have documented radiolytic damage and decreased immunoreactivity when high radiation doses were delivered to the MoAb (*35,36*). Our previous experience with ^{111}In-labeled T101 MoAb showed that radiation doses of 80,000–160,000 rads resulted in a mean drop of 15% to 35%, respectively (*36*). Prompted by recent studies, we are now evaluating the use of radioprotectants, not only after purification, but also during the labeling (*36*).

Large differences in the urinary excretion of the two tracers were observed. Although initially (0–24 h) the ^{90}Y

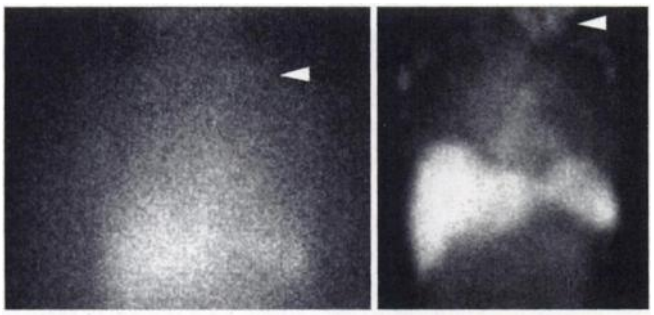

FIGURE 4. Left panel shows an image obtained in ^{111}In window 48 h after 185 MBq (5 mCi) injection of ^{111}In-labeled antiTac (co-injected with 185 MBq [5 mCi] ^{90}Y antiTac). Right panel shows image obtained 48 h after second therapy with 185 MBq (5 mCi) ^{90}Y-labeled antiTac alone. ^{90}Y image was acquired with medium-energy collimator. Although patient had had some tumor response from her first treatment, some residual tumor was still present in her left supraclavicular region (arrowhead). ^{90}Y image has poor resolution.

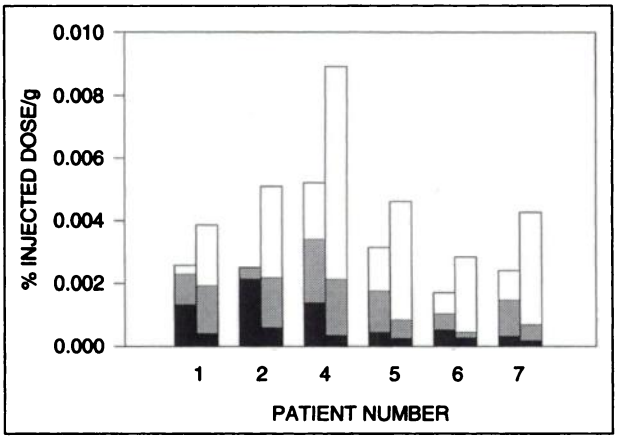

FIGURE 5. Bone marrow concentrations of ^{111}In and ^{90}Y were determined. Bone marrow biopsies were processed as described in Materials and Methods section. Radioactivity in bone marrow from ^{111}In-labeled antiTac is shown in left columns and that from ^{90}Y is shown in right columns. Radioactivity in following fractions separated are shown: bone fraction (white bar), SDS fraction (shaded bar) and intravascular fraction (black bar).

excretion was greater than that of ^{111}In, at later times there was greater retention of ^{90}Y. The initial faster clearance of ^{90}Y is probably due to the rapid catabolism of the antiTac that was damaged by radiation during labeling and storage. Similar findings of higher excretion of ^{90}Y in the first 24 h after injection have been previously reported with isothiocyantobenzyl DTPA (*8,33*). Review of ^{90}Y images showed no obvious excretion of ^{90}Y into bowel that would suggest preferential excretion through this route for ^{90}Y. As expected from preclinical trials, the concentration of ^{90}Y was higher than that of ^{111}In in the bone marrow, mainly in the bone wash (Fig. 5) (*37*). This higher amount of ^{90}Y in the bone marrow could result in a radiation dose to bone up to 1.7 times higher than would be expected from ^{111}In. Because the blood and plasma clearance of ^{111}In and ^{90}Y were similar and the urinary excretion products have low molecular weights (data not shown), these findings in urine represent differences in handling of catabolic products of the radiolabeled antibody. Unfortunately, in this study we could not address the fate of ^{90}Y in the major organs or lymph nodes. Because these are major sites of catabolism, it is possible that once catabolism occurs there is a preferential release of ^{90}Y from the chelate that we did not detect in the urine, because bone uptake would have rapidly occurred; alternatively, these organs may retain ^{90}Y longer than ^{111}In.

Measuring ^{111}In and ^{90}Y in the same specimen is complicated. Although counting the high energy peaks of ^{111}In and the bremsstrahlung radiation of ^{90}Y together is possible, the low efficiency of ^{90}Y makes errors due to cross-talk significant. The gamma counting and Cerenkov counting method we used were reliable and consistent. Although, because Cerenkov counting was affected by quench, it required meticulous preparation of counting standards to mimic the precise conditions of the patient specimens. In the case of bone marrow, counting was even more problematic. Although ^{111}In counting was easily performed with little loss of radioactivity as result of processing, ^{90}Y counting required processing of the bone to detect all radioactivity present. When processed, the ^{90}Y counts in the sample resulted in 1.49 times higher counts than the nonprocessed sample, indicating a higher efficiency of counting, which was perhaps related to higher Cerenkov generation when the bone was dissolved. Animal studies validating our bone-washing method suggest that it does not underestimate the fraction of ^{90}Y in the bone, but it may overestimate the amount of ^{111}In in the bone fraction (*22*).

Although the imaging aspects were the focus of this article, this study did show that bremsstrahlung imaging could give a gross idea of tracer distribution with visualization of large organs such as liver, spleen, blood pool or large tumor sites. Nevertheless, because of the limited resolution of bremsstrahlung imaging and the difficulties in clearly outlining borders, this study showed that targeting could not be assessed adequately by ^{90}Y bremsstrahlung gamma camera imaging (Fig. 4). Although quantitation of pure beta emitters has been described in the literature, those studies have consisted of well-defined phantoms. No patient images or data have been analyzed (*38*). Our studies show that imaging of bremsstrahlung results in low-resolution images with ill-defined borders. Therefore, given the technical difficulties already inherent in quantitation from gamma emitters, it is unlikely that these lower resolution bremsstrahlung images will provide adequate quantitative information. Attempts at obtaining ^{111}In whole-body clearance data from serial images were successful in this study with a correlation coefficient of $r = 0.8$ between ^{111}In imaging and urine measurements; some difference observed between urinary estimates and gamma camera estimates may have been related to the difficulties of obtaining complete urinary collections. In contrast, geometric mean data from bremsstrahlung whole-body images gave spurious results, with higher estimates of whole-body retention at 24 h and beyond. These findings are likely related to the redistribution of tracer outside of the vasculature and to the varying efficiency of bremsstrahlung generation and attenuation.

CONCLUSION

Differences in biodistribution were seen between ^{111}In- and ^{90}Y-labeled antiTac in circulating cells, skin, bone and whole-body retention, whereas little difference was observed in the circulation. These differences were small, typically 10% to 15%, particularly when the differences in immunoreactivity were considered. Thus, it appears that ^{111}In can be used as a surrogate marker for ^{90}Y when labeling antiTac, although underestimates of the bone marrow radiation dose should be anticipated.

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