Pharmacokinetics of ^{111}In- and ^{125}I-Labeled AntiTac Single-Chain Fv Recombinant Immunotoxin

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The use of immunotoxins for cancer therapy is an attractive strategy that exploits the targeting specificity of monoclonal antibodies and their fragments as well as the exquisite toxicity of the toxins. However, few studies of immunotoxins have evaluated their biodistribution in vivo. Previous studies have used ^{125}I for tracing immunotoxin biodistribution in mice. Because the immunotoxin works only when it is internalized and because of known problems with quick dehalogenation after internalization of antibodies, we decided to use ^{111}In, which has greater intracellular retention than iodine. **Methods:** To trace the in vivo pharmacokinetics of the immunotoxin in mice, we labeled the antiTac(Fv)-PE38 with ^{111}In and compared it with ^{125}I-labeled antiTac(Fv)-PE38. We successfully labeled antiTac(Fv)-PE38 with ^{111}In at up to 2.96 GBq/mg. A 3- to 4-fold decrease in cytotoxicity was observed for both radiolabeled preparations. We evaluated the internalization of ^{111}In- and ^{125}I-labeled antiTac(Fv)-PE38 into ATAC4 cells (Tac-positive) as well as their biodistribution and pharmacokinetics in vivo in mice. In addition, some mice receiving these reagents were co-infused with 30 mg L-lysine to inhibit renal accumulation. **Results:** Significantly more ^{111}In- than ^{125}I-labeled antiTac(Fv)-PE38 accumulated in the ATAC4 cells (20% versus 5% of initial surface-bound radioactivity; $P < 0.001$). In vivo, significantly more ^{111}In- than ^{125}I-labeled antiTac(Fv)-PE38 accumulated in the kidney (119 versus 31 percentage injected dose per gram [%ID/g]; $P < 0.001$). The tumor accumulation of ^{111}In-labeled antiTac(Fv)-PE38 at 96 h was 13-fold greater than that of ^{125}I-labeled antiTac(Fv)-PE38 (1.4 versus 0.1 %ID/g; $P < 0.001$). No antiTac(Fv)-PE38 was excreted into the urine in its intact form unless lysine was co-infused. Co-injected lysine reduced the renal accumulation of ^{111}In-labeled antiTac(Fv)-PE38 by 62%. **Conclusion:** We evaluated the biodistribution, pharmacokinetics, and catabolism of ^{111}In-labeled antiTac(Fv)-PE38 and found that it differed from ^{125}I-labeled antiTac(Fv)-PE38. These studies suggest that ^{111}In-labeled antiTac(Fv)-PE38 can be used to trace the fate of antiTac(Fv)-PE38 in humans.

Key Words: interleukin-2 receptor α subunit; immunotoxin; ^{111}In; ^{125}I; monoclonal antibody

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The use of immunotoxins for cancer therapy is an attractive strategy that exploits the targeting specificity of monoclonal antibodies and their fragments as well as the exquisite toxicity of the toxins (*1,2*). Several studies have shown successful therapeutic responses in preclinical models (*3–5*). Clinical trials showing responses have been performed using conjugates of monoclonal antibodies with toxins, such as RFB4-dgA or RFB4(Fab′)-dgA targeting CD22 (*6–8*); RFT5-SMPT-dgA targeting CD25 (*9*); IgG-HD37-dgA, B4-bRicin, and B43-PAP targeting CD19 (*10–12*); and B1-LysPE38 targeting Le^y (*13*). Recombinant fusion toxins have also shown responses in clinical trials, particularly DAB_{486}IL-2 and DAB_{389}IL-2 targeting high-affinity interleukin-2 receptor (IL-2R) (*14*). To date, clinical trials have shown limited therapeutic responses before reaching dose-limiting toxicity (*13*).

Numerous trials have characterized the in vivo biodistribution of radiolabeled antibodies because they lend themselves to pharmacokinetic analyses and imaging studies. In contrast, very few studies have evaluated the in vivo biodistribution of immunotoxins in animal models (*15,16*), and none have evaluated their biodistribution in humans. Imaging studies using radiolabeled immunotoxins could help clarify the basis of dose-limiting organ toxicities associated with immunotoxins and may provide a method to monitor strategies attempting to improve biodistribution and minimize toxicity.

AntiTac(Fv)-PE38 contains the Fv domains from the monoclonal antibody antiTac (*17*), which binds to the α subunit of IL-2R (IL-2Rα) (CD25, Tac, or p55) fused to PE38, a mutant form of *Pseudomonas* exotoxin that contains the translocation and adenosine diphosphate ribosylation domains (*1,18*). IL-2Rα is overexpressed by various hematologic malignancies of both B- and T-cell origin (*19*). We have previously shown the complete regression of IL-2Rα-positive tumor in animals given antiTac(Fv)-PE38 (*4,18*). Immunotoxin must be internalized to kill target cells (*1,18*). Previous studies evaluating the biodistribution of antiTac(Fv)-PE38 have used the ^{125}I-labeled compound (*15*), but dehalogenation of ^{125}I-

labeled proteins is known to be a problem. In the case of antibodies or immunotoxins that are internalized, this dehalogenation occurs quickly and results in release of the radioiodine from the cells (*20–23*). When this occurs, the ^{125}I no longer faithfully reflects the cells targeted by the radiolabeled antibody or immunotoxin. Our previous study showed that between 6 and 24 h, ^{125}I label in the plasma of mice becomes mostly dissociated from active immunotoxin (*15*). In contrast, when ^{111}In-labeled antibodies are internalized, the ^{111}In is not released from the cells or is released very slowly (*20,21*). Thus, in this study we evaluated the internalization, biodistribution, and pharmacokinetics of an ^{111}In-labeled antiTac(Fv)-PE38 and compared it with ^{125}I-labeled antiTac(Fv)-PE38.

MATERIALS AND METHODS

Fusion Protein of Immunotoxin with Single-Chain AntiTac Fv

AntiTac(Fv)-PE38 was derived from the antiTac IgG2a, a murine-derived monoclonal antibody that binds to IL-2Rα (*17*). Production of antiTac(Fv)-PE38 (63 kDa) has been described previously (*2,18*). Briefly, to make a single-chain Fv (scFv) molecule, the V_H and V_L domains were genetically connected with a $(Gly_4Ser)_3$ linker, and V_L was fused to truncated *Pseudomonas* exotoxin (PE38) (*2*). The plasmid was expressed in *Escherichia coli*. The proteins were recovered as cytosolic inclusion bodies and refolded. The refolded protein was purified by ion-exchange and size-exclusion chromatography. The antiTac(Fv)-PE38 was >98% pure as determined by size-exclusion high-performance liquid chromatography (HPLC) with an ultraviolet detector. The immunotoxin contains 13 lysines in the Fv portion, 3 lysines in the PE38 portion, 15 tyrosines in the Fv portion, and 11 tyrosines in the PE38 portion.

Radiolabeling

The Iodo-Gen (Pierce Chemical Co., Rockford, IL) method was used to label the antiTac(Fv)-PE38 with ^{125}I (*24*). Briefly, approximately 1–2 mCi sodium ^{125}I was added to 100 μg antiTac(Fv)-PE38 in 80 μL phosphate-buffered saline (PBS), pH 7.2, in a conical polypropylene vial coated with 10 μg Iodo-Gen. After incubation for 10 min at room temperature, the radiolabeled products were purified using a PD-10 column (Pharmacia, Uppsala, Sweden). The specific activity of the ^{125}I-labeled antiTac(Fv)-PE38 was 407–592 MBq/mg. The radiochemical purity of all radiolabeled products was >97% as confirmed by instant thin-layer chromatography (ITLC), analytical size-exclusion HPLC, and sodium dodecyl sulfate–polyacrylamide gel elctrophoresis (SDS-PAGE).

To label the immunotoxin with ^{111}In, we first conjugated it with the A″ stereoisomer of 2-(*p*-isothiocyanatobenzyl)-cyclohexyl-diethylenetriaminepentaacetic acid (CHX-A″) (*25,26*). Synthesis of the various stereoisomers is described elsewhere (*26,27*). Carrier-free $^{111}InCl_3$ was purchased from DuPont–NEN (Boston, MA). The antiTac(Fv)-PE38 was reacted with 50–200 molar excess of CHX-A″ in 300 mmol/L carbonate buffer, pH 9.5, for 45 min at room temperature. To remove excess nonincorporated CHX-A″, the mixture was purified using a D-salt column (Pierce Chemical Co.). The antiTac(Fv)-PE38-CHX-A″ conjugates were reacted with 37–74 MBq ^{111}In in 200 mmol/L sodium acetate, 20 mmol/L citrate buffer, pH 7.2, for 90 min at room temperature as described (*27–30*). To remove any nonincorporated free ^{111}In, 10 μL 0.1 mol/L diethylenetriaminepentaacetic acid (DTPA) was added and purified using a D-salt column. The specific activity of the ^{111}In-labeled antiTac(Fv)-PE38 was 0.41–2.96 GBq/mg. The radiochemical purity of all radiolabeled products was >97% as confirmed by ITLC, analytical size-exclusion HPLC, and SDS-PAGE.

Analysis of Labeling Compound with Furin Digestion

To determine the region of the immunotoxin that was radiolabeled, we used furin digestion, cutting the immunotoxin in the *Pseudomonas* toxin portion 27 amino acids from the junction with the Fv fragment. The digestion was performed by incubating 500 ng ^{125}I- or ^{111}In-labeled antiTac(Fv)-PE38 with 1 μL furin in 200 mmol/L sodium acetate and 1 mmol/L calcium chloride with 0.2% human serum albumin, pH 5.5, at 37°C for 24 h (*31–33*). The samples (20,000 cpm) were analyzed using SDS-PAGE under reducing and nonreducing denaturing conditions with a 4%–20% gradient polyacrylamide gel (Novex, San Diego, CA). The gels were quantitated on a phosphoimaging device (Fuji, Tokyo, Japan) and analyzed with MacBas software (Fuji).

Cell Lines and Animal Models

The immunoreactivity of all radiolabeled preparations was tested with a cell-binding assay that used the IL-2Rα-positive SP2/Tac cell line and the IL-2Rα-negative SP2/0 cell line (*34*). SP2/0 cells are from a nonimmunoglobulin-secreting murine myeloma line (CRL 1581; American Type Culture Collection, Rockville, MD) that does not express the IL-2Rα. The SP2/Tac is a genetically engineered cell line generated by transfecting SP2/0 cells with the gene that encodes for the IL-2Rα (*35*). The cells were provided by Dr. Thomas Waldmann of the Metabolism Branch, National Cancer Institute (Bethesda, MD). Both cell lines were grown in Dulbecco's modified Eagle's medium (GIBCO Laboratories, Grand Island, NY) containing 10% fetal calf serum (GIBCO Laboratories) and 0.03% L-glutamine at 37°C in 5% CO_2.

Tumor xenografts were generated with the ATAC4 cell line, which expresses the IL-2Rα. This cell line was generated by genetically transfecting the plasmid encoding IL-2Rα and a neomycin-resistant gene into the receptor-negative A431 cells (*36*). A431 is a human epidermoid carcinoma line that does not express IL-2Rα. Both cell lines were grown in RPMI 1640 medium (GIBCO Laboratories) containing 10% fetal calf serum (GIBCO Laboratories) and 0.03% L-glutamine at 37°C in 5% CO_2. Sometimes this medium was supplemented with 750 μg/mL sulfate Geneticin (GIBCO) to avoid loss of the Tac antigen expressing plasmid from the ATAC4 cells.

Animal studies were performed under an approved Institutional Animal Care and Use Committee Protocol. We used female athymic nude mice (*nu/nu*) that were 5–7 wk old and weighed 15–20 g (Harlan Sprague-Dawley, Frederick, MD). Tumor xenografts were established by subcutaneous inoculation of 3×10^6 ATAC4 and 5×10^6 A431 cells. Experiments on tumor-bearing mice were performed 10–11 d after implantation, when both ATAC4 and A431 tumors weighed a mean of 0.2 ± 0.1 g.

All mice were killed with CO_2 inhalation and exsanguinated by cardiac puncture before dissection. The organs were harvested, blot dried, and weighed on an analytical balance; the radioactivity was then counted in a γ counter, making appropriate corrections for spillover.

Immunoreactivity

Immunoreactivity was determined with a cell-binding assay as described (*24*). Aliquots of the ^{125}I- or ^{111}In-labeled antiTac(Fv)-PE38 (3 ng/100 μL) were incubated for 2 h at 4°C with 2×10^6 to 2×10^7 SP2/Tac cells. The cell-bound radioactive fraction was separated by centrifugation, and the radioactivity was counted in a γ counter. Nonspecific binding to the cells was examined under conditions of antibody excess (25 μg nonradiolabeled antiTac IgG antibody).

Amino Acid Preparations

To block renal accumulation of the antiTac(Fv)-PE38, we prepared a stock solution of L-lysine (270 mg/mL) in 0.1 mol/L phosphate buffer (PB), pH 7.5, using L-lysine monohydrochloride (Pierce Chemical Co.) and 1N NaOH. This solution was co-injected with ^{125}I-labeled antiTac(Fv)-PE38 at a final concentration of 150 mg/mL L-lysine, for a total of 30 mg per mouse. Another 30 mg/200 μL lysine was injected intraperitoneally 15 min later (*24*).

Internalization of AntiTac(Fv)-PE38 into the ATAC4 Cell

To perform internalization assays, 10^6 ATAC4 cells were plated per well in 6-well microtiter plates and incubated in the RPMI 1640 medium as described above for 1 d. The medium of each well was then replaced with 2 mL ice-cold medium containing 25 ng/mL ^{125}I- or ^{111}In-labeled antiTac(Fv)-PE38 and incubated at 4°C for 30 min. This procedure allowed surface binding to IL-2Rα and minimized the chance of internalization. The non–cell-bound radioactivity was removed by aspirating the medium from each well and washing twice with 1 mL cold PBS. The PBS was removed, 2 mL ice-cold medium was added, and the plates were incubated at either 4°C or 37°C for 0 (baseline), 1, 3, 6, or 24 h. At each time point the media were removed and counted to determine the non–cell-bound fraction. The cell-surface radioactivity was then determined by using an acid wash with 1 mL 0.1N acetic acid containing PBS, pH 4.0, at 37°C for 5 min and then detaching the cells from the wells using trypsin/ethylenediaminetetraacetic acid (EDTA) medium (GIBCO Laboratories) at 37°C for 5 min. The cell pellets were separated from the supernatant by centrifugation and counted to determine the internalized fraction. The supernatant and the washing solution with the acid were counted, and the cell-surface–bound radioactive fraction was determined. Each data point was obtained in triplicate, and the data were expressed as the mean percentage ± SD recovered in the non–cell-bound, the cell-surface–bound, or internalized fraction.

Biodistribution of Radiolabeled AntiTac(Fv)-PE38 and HPLC Analysis of Serum and Urine

Seven groups, each containing 5 mice bearing both A431 and ATAC4 tumor xenografts, were injected intravenously with both 0.5 μg/296 kBq ^{125}I-labeled antiTac(Fv)-PE38 and 0.25 μg/148 kBq ^{111}In-labeled antiTac(Fv)-PE38. One group was co-injected with L-lysine as described above. The mice were killed at 15 and 45 min; 2 and 6 h; and 1, 2, and 4 d after injection. Their blood was drawn and aliquoted for counting, and their serum was separated and analyzed by size-exclusion HPLC using a TSK G2000SW column (TosoHaas, Philadelphia, PA; 0.067 mol/L sodium PBS and 0.1 mol/L KCl; pH 6.8; 0.5 mL/min) equipped with an on-line NaI γ detector (γ RAM; IN/US Systems, Inc., Fairfield, NJ). The entire organs were then harvested and counted in a γ counter (Packard Auto-Gamma, Meriden, CT), with exception of the bone and muscle, where representative pieces were sampled. To determine whole-body retention, the remaining carcass was divided into 3 portions and also counted, and the sum of the activity in the carcasses, blood, and individual organs was determined. The data were expressed both as percentage injected dose per gram (%ID/g) of tissue and as receptor-positive tumor-to-normal tissue ratios. In addition, when the mice were killed, urine was collected for HPLC analysis.

Serum and urine samples from the individual mice were analyzed by size-exclusion HPLC. The fractional distribution of activity present as intact antiTac(Fv)-PE38 (retention time, 16.5 min for the antiTac(Fv)-PE38), as high-molecular-weight complexes, or as catabolites was quantified from the HPLC tracing. To ascertain the total amount of the %ID/g of blood present as intact antiTac(Fv)-PE38, complexes, or catabolites, the fractional distribution in each compartment was determined from the total radioactivity retained in the blood and multiplied by the fraction determined by HPLC.

To validate the reproducibility, a repeat labeling of antiTac(Fv)-PE38 was performed and a similar biodistribution study was performed at four time points (15 min, 2 h, 1 d, and 4 d; n = 5).

Effect of Co-injection with L-Lysine

Two groups, each containing 5 mice bearing both A431 and ATAC4 tumor xenografts, were injected intravenously with both 0.5 μg/296 kBq ^{125}I-labeled antiTac(Fv)-PE38 and 0.25 μg/148 kBq ^{111}In-labeled antiTac(Fv)-PE38. Both groups were co-injected with 30 mg L-lysine. The mice were killed 45 min after injection, and biodistribution studies and HPLC of serum and urine were performed as described above.

Cytotoxic Assay

SP2/Tac cells were plated at 40,000/well in 50-μL aliquots and diluted with 50 μL radiolabeled immunotoxins or control nonlabeled immunotoxin. After overnight incubation at 37°C, the cells were pulsed with [^{3}H]leucine (37 kBq/well), harvested, and counted. The 50% inhibitory concentration (IC_{50}) was then determined (*15*). In the SP2/Tac line as well as most other cell types, protein synthesis inhibition by LMB-2 and derivatives leads to and correlates with cell death.

Statistical Analysis

Statistical analysis was performed using the nonpaired *t* test or 1-way analysis of variance with pairwise comparison using the Bonferroni method (Sigmastat; Jandel Scientific, San Rafael, CA).

RESULTS

Quality Control Study of Radiolabeled AntiTac(Fv)-PE38

The antiTac(Fv)-PE38 was labeled with up to 2.96 GBq/mg ^{111}In (range, 0.41–2.96 GBq/mg). The maximum binding of the ^{111}In-labeled antiTac(Fv)-PE38 to SP2/Tac cells ranged from 48% to 57%. The maximum binding of the higher specific activity labeled immunotoxin was 10% lower than that of the lower specific activity ^{111}In-labeled immunotoxin and that of the ^{125}I-labeled immunotoxin, in which binding ranged from 57% to 62%.

The SDS-PAGE results of the 2 different labeled samples (n = 4) treated by furin digestion showed that 58% ± 2% of ^{125}I and 78% ± 2% of ^{111}In were located on the 28-kDa band (single-chain Fv part) of the antiTac(Fv)-PE38 rather than on the 35-kDa band (immunotoxin part).

Cytotoxicity assays were performed to determine the bioactivity of the radiolabeled preparations of immunotox-

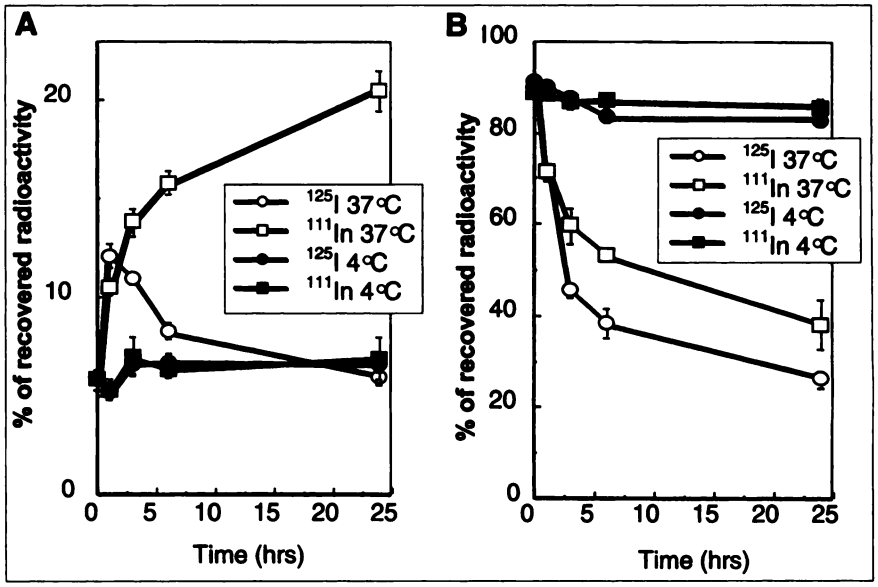

FIGURE 1. Data of internalized fraction (A) and cell-surface fraction (B) of ^{111}In-labeled antiTac(Fv)-PE38 (squares) and ^{125}I-labeled antiTac(Fv)-PE38 (circles) were plotted at either 4°C (■ or ●) or 37°C (□ or ○) for 0, 1, 3, 6, or 24 h. At each time point, media were removed and counted to determine non–cell-bound fraction. Cell-surface radioactivity was then determined by using acid wash. Cells were detached using trypsin/EDTA, and cell pellets were separated from supernatant by centrifugation and counted to determine internalized fraction. Supernatant and acid wash solution were counted to determine cell-surface–bound fraction. Each data point represents results of measurements performed in triplicate. Data are expressed as mean percentage ± SD.

ins. Two or 3 preparations of each radiolabeled immunotoxin were produced to confirm reproducibility. Based on IC_{50} values of the radiolabeled and unradiolabeled immunotoxin, ^{125}I-labeled antiTac(Fv)-PE38 retained 37% ± 15% of the activity of unlabeled antiTac(Fv)-PE38, and ^{111}In-labeled antiTac(Fv)-PE38 retained 35% ± 15%.

Internalization of AntiTac(Fv)-PE38 into the ATAC4 Cell

In the case of ^{125}I-labeled antiTac(Fv)-PE38, the peak concentration of the ^{125}I internalized fraction was 12% at 1 h. This retention decreased over time, returning to ~6% within 6 h, similar to the baseline value obtained in samples incubated at 4°C or at time 0. In contrast, the internalized fraction of ^{111}In increased over time and reached up to 20% at 24 h (Fig. 1). The cell-surface–bound fractions of ^{125}I-labeled antiTac(Fv)-PE38 and ^{111}In-labeled antiTac(Fv)-PE38 decreased over time but at 24 h were 26% and 38% of the initial cell associated activity, respectively.

Biodistribution of Radiolabeled AntiTac(Fv)-PE38 and HPLC Analysis of Serum and Urine

The uptake of ^{125}I-labeled antiTac(Fv)-PE38 in the ATAC4 tumor was slightly more than that of ^{111}In-labeled antiTac(Fv)-PE38 at 2 h or earlier, but it was significantly less than that of ^{111}In-labeled antiTac(Fv)-PE38 at 1 d and later ($P < 0.001$). The maximum ratio of ^{111}In-to-^{125}I was 13 at 96 h after injection (1.4 %ID/g ^{111}In versus 0.1 %ID/g ^{125}I) (Tables 1 and 2). The accumulation of ^{111}In-labeled antiTac(Fv)-PE38 in the liver and the kidney was significantly higher than that of ^{125}I-labeled antiTac(Fv)-PE38 at all time points ($P < 0.001$). The accumulation of ^{111}In-labeled antiTac(Fv)-PE38 in the normal tissue, excluding the blood, was significantly

TABLE 1
Biodistribution of ^{111}In-Labeled AntiTac(Fv)-PE38

Site	Time (h)					
	0.25	0.75	2	6	24	96
Blood	29.22 ± 1.11	15.84 ± 1.06	5.68 ± 0.82	1.28 ± 0.11	0.24 ± 0.05	0.04 ± 0.00
Liver	22.38 ± 1.03	23.27 ± 2.49	26.83 ± 1.11	22.84 ± 1.30	16.51 ± 0.69	9.04 ± 0.41
Kidney	59.09 ± 1.72	89.59 ± 4.22	117.31 ± 7.95	118.97 ± 10.91	102.38 ± 2.73	58.39 ± 4.75
Intestine	2.24 ± 0.12	2.18 ± 0.11	1.98 ± 0.31	1.53 ± 0.11*	1.05 ± 0.14	0.39 ± 0.01
Stomach	2.06 ± 0.29	1.86 ± 0.39	1.74 ± 0.28	0.89 ± 0.28	0.82 ± 0.25*	0.26 ± 0.04
Spleen	10.39 ± 0.60*	10.47 ± 1.59	10.34 ± 1.66	8.03 ± 0.94	5.82 ± 1.43	3.68 ± 0.15
Lung	12.90 ± 2.09	7.47 ± 0.92	3.82 ± 0.44	1.93 ± 0.22	1.16 ± 0.22	0.51 ± 0.05
Muscle	0.94 ± 0.09*	1.04 ± 0.10	0.82 ± 0.09	0.80 ± 0.11	0.63 ± 0.15	0.33 ± 0.05
Bone	4.58 ± 0.88*	4.98 ± 0.48	4.73 ± 0.33	3.92 ± 0.70	3.58 ± 0.74	2.59 ± 0.34
A431 tumor	1.88 ± 0.45	2.43 ± 0.23	2.43 ± 0.73	1.71 ± 0.29*	1.06 ± 0.09	0.42 ± 0.09
ATAC4 tumor	2.44 ± 0.26	4.70 ± 0.37	6.70 ± 1.40*	5.70 ± 0.92	3.08 ± 0.19	1.44 ± 0.19
Whole body	103.46 ± 2.40	101.90 ± 0.80	95.97 ± 1.53	91.59 ± 1.28	71.21 ± 1.14	39.54 ± 1.52

Each group contains 5 animals. Data are expressed as %ID/g (mean ± SD).
*Nonsignificant differences at $P > 0.01$ compared with ^{125}I-scFv(Tac)-PE38.

TABLE 2
Biodistribution of ^{125}I-Labeled AntiTac(Fv)-PE38

Site	Time (h)					
	0.25	0.75	2	6	24	96
Blood	35.74 ± 1.71	23.86 ± 0.64	11.11 ± 0.79	3.21 ± 0.34	0.36 ± 0.03	0.05 ± 0.01
Liver	12.42 ± 0.58	6.90 ± 0.97	4.00 ± 0.46	1.15 ± 0.14	0.17 ± 0.01	0.06 ± 0.01
Kidney	30.62 ± 1.61	22.15 ± 2.12	9.78 ± 1.31	1.79 ± 0.40	0.22 ± 0.03	0.04 ± 0.01
Intestine	2.76 ± 0.14	3.42 ± 0.14	2.93 ± 0.23	1.42 ± 0.32	0.12 ± 0.02	0.03 ± 0.01
Stomach	6.83 ± 0.56	20.14 ± 2.78	31.08 ± 10.66	14.44 ± 3.84	0.48 ± 0.19	0.06 ± 0.02
Spleen	9.91 ± 0.50	7.16 ± 0.81	4.12 ± 0.31	1.48 ± 0.19	0.25 ± 0.07	0.07 ± 0.01
Lung	17.29 ± 2.26	12.79 ± 1.13	7.12 ± 0.52	2.34 ± 0.09	0.32 ± 0.10	0.07 ± 0.01
Muscle	1.27 ± 0.12	1.90 ± 0.16	1.20 ± 0.08	0.55 ± 0.08	0.05 ± 0.01	0.01 ± 0.00
Bone	4.31 ± 0.65	3.98 ± 0.31	2.41 ± 0.16	0.86 ± 0.09	0.13 ± 0.03	0.06 ± 0.01
A431 tumor	2.60 ± 0.64	4.31 ± 0.21	3.80 ± 0.62	1.31 ± 0.06	0.17 ± 0.04	0.04 ± 0.04
ATAC4 tumor	3.21 ± 0.27	7.31 ± 0.42	8.67 ± 2.03	4.20 ± 0.30	0.64 ± 0.06	0.11 ± 0.02
Whole body	92.94 ± 1.91	88.79 ± 2.31	66.59 ± 4.80	30.20 ± 5.42	6.40 ± 0.66	3.90 ± 0.94

Each group contains 5 animals. Data are expressed as %ID/g (mean ± SD).

higher than that of ^{125}I-labeled antiTac(Fv)-PE38 at 1 d and later ($P < 0.001$). Likewise, the ATAC4 tumor-to-blood ratio of ^{111}In-labeled antiTac(Fv)-PE38 was significantly higher than that of ^{125}I-labeled antiTac(Fv)-PE38 at 120 min and later (Fig. 2; $P < 0.001$).

HPLC analysis of serum taken at 15 min showed that with both ^{125}I- and ^{111}In-labeled antiTac(Fv)-PE38, more than 80% of the radioactivity was found in the intact antiTac(Fv)-PE38 fraction. The radioactivity decreased with an ~35 min half-life. However, in both cases there was a high-molecular-weight fraction, which cleared with an ~2.5 h half-life for ^{125}I and an ~2 h half-life for ^{111}In (Figs. 3A and B). HPLC analysis of urine from the mice showed that all the radioactivity was present as catabolites.

At all time points, whole-body retention of ^{111}In-labeled antiTac(Fv)-PE38 was significantly higher than that of ^{125}I-labeled antiTac(Fv)-PE38 ($P < 0.01$).

Effect of Co-Injection with L-Lysine

The mice co-injected with L-lysine showed significantly lower renal accumulation (55 ± 6 %ID/g) of ^{111}In-labeled antiTac(Fv)-PE38 than the mice without co-injection (90 ± 4 %ID/g; $P < 0.001$). In contrast, lysine co-injection did not change the renal accumulation of ^{125}I-labeled antiTac(Fv)-PE38. In mice receiving ^{125}I-labeled antiTac(Fv)-PE38, HPLC analysis of serum showed significantly less catabolites (1.43 ± 0.05 %ID/g) in the L-lysine co-injection group than in the group not receiving lysine (2.47 ± 0.02 %ID/g; $P < 0.01$). When L-lysine was not co-injected, there was no excretion of intact radiolabeled antiTac(Fv)-PE38 (data not shown). When L-lysine was co-injected, HPLC analysis of urine showed the presence of intact ^{125}I-labeled and ^{111}In-labeled antiTac(Fv)-PE38, which was significantly greater than the no-lysine control ($P < 0.01$).

DISCUSSION

The biodistribution of ^{111}In-labeled antiTac(Fv)-PE38 was different from that of ^{125}I-labeled antiTac(Fv)-PE38. The greatest differences were manifest as markedly increased and prolonged hepatic and renal accumulation, likely related to differences in the catabolism of the 2 radionuclides. Once internalized into a cell, iodine is released quickly as a result of catabolism and most likely returned back into the circulation and excreted by the kidney (*20,37*). Because the released ^{125}I may not be attached to the toxin, it may not trace the toxin's fate and will underestimate the amount of immunotoxin molecules internalized in cells (*15*). This is likely to occur in all tissue internalizing the immunotoxin. In the case of ^{111}In, our results show that after internalization, the internalized radiometal is retained much longer and accumulates in the cells. When iodine is compared with radiometals, similar findings have been observed in vitro (*20,21*), in preclinical tumor models (*24,38*), and in humans (*39*). It is important to realize in this and other studies that when radioactivity in tissue is measured, it is difficult to tell whether it is on the intact immunotoxin or on catabolites. Nevertheless, in the case of the radiolabeled immunotoxin, ^{111}In, a tracer retained by cells in vitro, is more likely to reflect immunotoxin delivery to tissue.

With both ^{125}I- and ^{111}In-labeled antiTac(Fv)-PE38, we found significant amounts of high-molecular-weight radioactivity in the serum. This activity had a slower clearance than that of the intact antiTac(Fv)-PE38. This higher molecular weight component may be the result of interaction of the immunotoxin with a serum protein. When either ^{125}I- or ^{111}In-labeled immunotoxins were incubated in murine serum, the high-molecular-weight fractions appeared and slowly grew larger (data not shown). These findings in serum could result in inactivation and help explain the faster decrease in cytotoxic activity compared with the decrease in the amount of radiolabeled immunotoxin that can be bound to antigen (*15*).

The biodistribution of ^{111}In-labeled antiTac(Fv)-PE38 suggests that the critical organ of antiTac(Fv)-PE38 toxicity should be the kidney. On the basis of what is known about

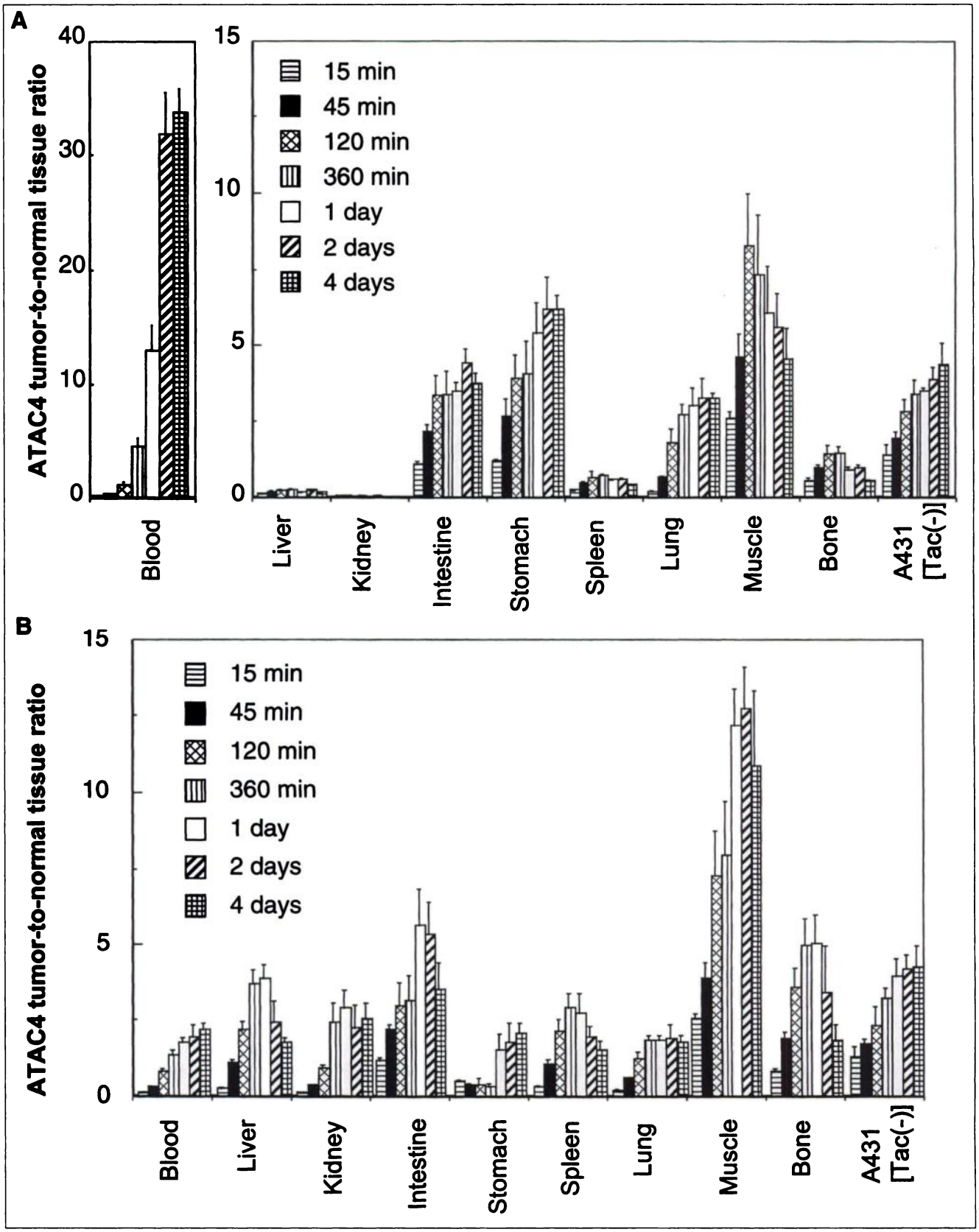

FIGURE 2. Plot of ATAC4 tumor-to-normal tissue ratios of ^{111}In-labeled antiTac(Fv)-PE38 (A) and ^{125}I-labeled antiTac(Fv)-PE38 (B) at 15, 45, 120, and 360 min and 1, 2, and 4 d after injection (n = 5; mean ± SD).

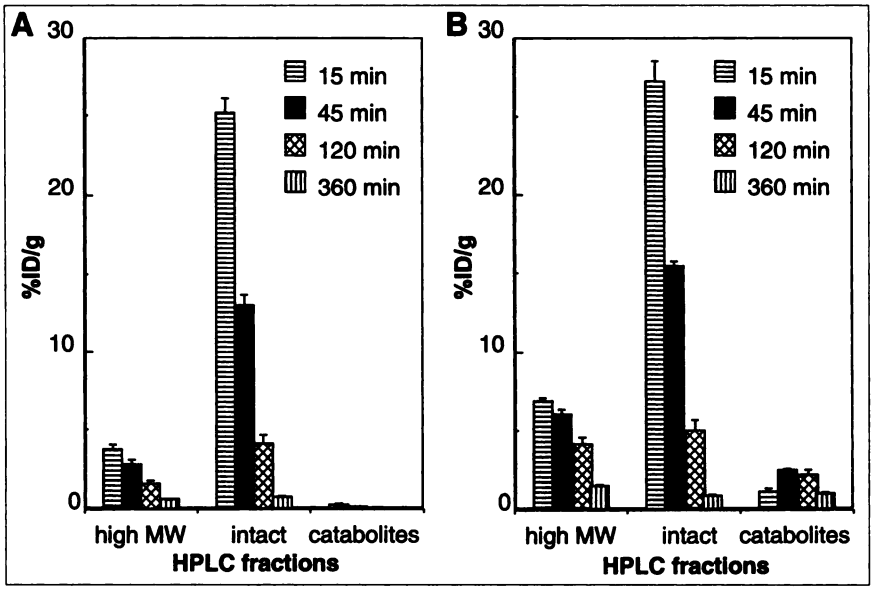

FIGURE 3. Plot of serial serum HPLC fractions of ^{111}In-labeled antiTac(Fv)-PE38 (A) and ^{125}I-labeled antiTac(Fv)-PE38 (B) at 15, 45, 120, and 360 min after injection. (Retention time, high molecular weight (high MW): 14.3 min; antiTac(Fv)-PE38: 16.7 min; low molecular weight: > 25 min). Data were normalized to %ID/g of blood (n = 5; mean ± SD).

renal handling of small-molecular-weight proteins, it is likely that the radiolabeled immunotoxin was filtered and then internalized into the proximal tubules and then catabolized, and the ^{111}In was retained. In the kidney, this catabolic process may be associated with inactivation of the toxin to a less toxic molecule, because a major percentage of toxin localizes there without dose-limiting renal toxicity. The second highest ^{111}In accumulation was in the liver and may explain the increase in transaminases encountered when this reagent has been used in the clinic (*40*).

Most (78%) of the ^{111}In was bound to the Fv portion of the immunotoxin, whereas 58% of the ^{125}I was bound to the Fv portion. These values are similar to the proportions of the lysines for ^{111}In and the tyrosines for ^{125}I in the Fv portion. The high specific activities achieved by ^{111}In-labeled anti-Tac(Fv)-PE38 resulted in a product that had only a minor decrease in immunoreactivity compared with ^{125}I-labeled antiTac(Fv)-PE38, which had lower specific activity and a 4-fold decrease in cytotoxicity compared with the nonlabeled immunotoxin. The immunoreactivity values for the ^{125}I-labeled antiTac(Fv)-PE38 are slightly lower than those reported (*15*). These slightly lower values could be related to the higher specific activity labeling in our study. Although the PE38 portion of the immunotoxin contains 11 tyrosines and 3 lysines, the cytotoxicity assay showed similar values for the ^{125}I- and ^{111}In-labeled immunotoxins, which were lower than the control. This finding suggests that we were not preferentially labeling at the key amino acids. When contemplating translation into clinical trials, these specific activities suggest that we may be able to label at 148 MBq/50 μg ^{111}In for clinical trials. Currently, that level represents <10% of the single, therapeutic dose of anti-Tac(Fv)-PE38 used in clinical trials.

CONCLUSION

We successfully labeled antiTac(Fv)-PE38 with ^{111}In and evaluated its biodistribution, pharmacokinetics, and catabolism. Our results demonstrated differences between ^{111}In- and ^{125}I-labeled antiTac(Fv)-PE38 on these parameters. These studies suggest that ^{111}In-labeled antiTac(Fv)-PE38 can be used to trace antiTac(Fv)-PE38 in humans.

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