Effect of Metabolism on Retention of Indium-111-Labeled Monoclonal Antibody in Liver and Blood

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The effect of a chelator structure on the metabolic fate of the ^{111}In-labeled monoclonal antibody (Mab) T101 was investigated in normal Balb/c mice to assess the importance of this chemical parameter in the reduction of the background radioactivity in blood and liver. **Methods:** Mab T101 was conjugated with either 2-(p-isothiocyanatobenzyl)-6-methyl-diethylaminetriaminepentaacetic acid (DTPA) (1B4M), 2-(p-isothiocyanatobenzyl) cyclohexyl-DTPA (CHX-B) or cyclic DTPA dianhydride (cDTPA) and then radiolabeled with ^{111}In. Normal mice were injected intravenously with these ^{111}In-labeled T101 conjugates and sacrificed in groups of five up to 5 days postinjection for comparative biodistribution studies and analyses of liver, blood and urine samples for radioindium products. **Results:** The biodistribution of ^{111}In-1B4M-T101 and ^{111}In-CHX-B-T101 were similar to each other but significantly different from that of ^{111}In-cDTPA-T101, particularly in the blood and liver. Size-exclusion high-performance liquid chromatography indicated that the concentration of the intact ^{111}In-immunoglobulin (Ig)G in liver decreased with similar rates for the three conjugates. Meanwhile, the concentration of a small DTPA-like metabolite in liver increased to a different peak value (4.6% ID/g for the cDTPA conjugate and 1.6% ID/g for the 1B4M and CHX-B conjugates) approximately at 24 hr and maintained a steady-state concentration up to 5 days. **Conclusion:** The thiourea linkage between T101 and the ^{111}In-labeled chelates and a higher complex stability and higher lipophilicity of ^{111}In-1B4M and ^{111}In-CHX-B appear to be responsible for lower liver and higher blood radioactivity for the 1B4M and CHX-B conjugates.

Key Words: ^{111}In-Mab DTPA conjugates; metabolism

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The use of ^{111}In-labeled antibodies for tumor imaging has shown promising clinical results (*1–4*). Initially, ^{111}In labeling was performed with either cyclic diethylenetriaminepentaacetic acid (DTPA) dianhydride (cDTPA) (*5,6*) or DTPA carboxycarbonic mixed anhydride (*7*). Unfortunately, when applied clinically, the high degree of liver accumulation resulted in poor visualization of hepatic lesions and unfavorable dosimetry. Because of the negative impact of this accumulation in the liver, numerous studies to evaluate the metabolism and biodistribution of ^{111}In-labeled antibodies have been performed (*8–15*). Although the mechanisms of ^{111}In accumulation in the liver are not completely known, investigators have shown that, at early times after antibody infusion, the ^{111}In in the liver is associated with intact immunoglobulin (Ig)G, whereas at later times, the radioactivity is associated with smaller molecular weight metabolic products. Isolation of cell fractions from the liver of rodents that received ^{111}In-labeled antibody conjugates has indicated that the hepatocytes are the main site of metabolism and accumulation of the radioindium (*16*).

In an attempt to lower the ^{111}In accumulation in the liver, a search for DTPA derivatives with more favorable characteristics has been pursued. Brechbiel and Gansow (*17,18*) synthesized various p-isothiocyanatobenzyl DTPA derivatives that differ in the position of the benzyl and methyl substituent on the diethylenetriamine backbone. These derivatives were conjugated to monoclonal antibody (Mab) B72.3 (*19,20*) or Mab B3 (*21,22*) and radiolabeled with ^{111}In. Biodistribution studies in mouse xenograft tumor models indicated that the conjugated antibodies with a methyl substitutent on the diethylenetriamine backbone or a cyclohexyl group, which replaced an ethylene backbone, had less liver accumulation. Studies by Carney et al. (*23*) with a similar chelate with a methyl group on the acetic acid backbone showed low liver accumulation that did not differ significantly from that of the iodinated antibody.

The aim of this study was to define important chemical parameters further, such as the stability of ^{111}In-labeled complexes, lipophilicity and chemical bonding of bifunctional chelators, to reduce the accumulation of the radioactivity in nontarget organs.

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MATERIALS AND METHODS

Monoclonal Antibody

T101, an IgG_{2a} murine monoclonal antibody that recognizes CD5 (a human pan-T-cell antigen), was used. The production, purification and characterization of this antibody have been previously described (*24*). No cross-reactivity with murine T cells has been described (*25*).

Chelating Agents and Indium-111 Radiolabeling

DTPA was conjugated to T101 with the use of the cyclic DTPA dianhydride (cDTPA) (*6*). Briefly, 400 μl of T101 (800 μg in 0.1 *M* $NaHCO_3$, pH 8.3) was mixed with 40 μl of freshly made cDTPA (*26,27*) (11.4 μg in dimethyl sulfoxide). The mixture was incubated at room temperature for 1 hr and then radiolabeled with 20 mCi of ^{111}In-Cl_3, as described previously (*6*). The cDTPA-T101 conjugate was labeled with ^{111}In in the presence of excess free DTPA. The number of DTPA molecules conjugated per T101 was calculated based on the percent ^{111}In distribution between DTPA T101 conjugate and free DTPA (*28*). The radiolabeled T101 was purified by size-exclusion high-performance liquid chromatography (HPLC) (TSK-3000, Thompson Instruments, Springfield, VA, 30 cm), with 0.02 *M* sodium phosphate and 0.1 *M* sodium sulfate as the elution buffer (pH 6.7, 1 ml/min).

T101 was also conjugated with 2-(p-isothiocyanatobenzyl)-6-methyl-DTPA (1B4M) and 2-(p-isothiocyanatobenzyl)-cyclohexyl-DTPA (CHX-B) (*17,18*). Both chelating agents were prepared with ^{14}C. The 1B4M and CHX-B conjugates were purified by the HPLC method described earlier. The number of chelators conjugated per T101 was determined based on scintillation counting of ^{14}C activity, as described previously (*29*).

Stability Test

The labeled T101 conjugate (25 μl) was mixed with 250 μl of normal mouse serum that contained 0.1% sodium azide. The final concentration of the T101 conjugates was 15 to 20 μg/ml. The mixture was incubated at 37°C for 5 days in a humidified incubator maintained with 5% CO_2/95% air. The pH of the serum remained constant, and there was no evidence of bacterial growth during the 5-day period. An aliquot (20 μl) was taken daily, and the stability of labeled antibodies was determined by size-exclusion HPLC with a TSK-3000 (30 cm) and a TSK-2000 (30 cm) column connected in series. The columns were eluted with 0.02 *M* sodium phosphate and 0.1 *M* sodium sulfate at pH 6.7 (1 ml/min).

Immunoreactivity

The immunoreactivities of the radiolabeled antibodies were determined with CCRF-CEM cells, a CD5-positive human leukemic cell line. Briefly, 5 ng of labeled T101 conjugates was incubated with 0.5×10^6 to 8×10^6 cells in a total incubation volume of 200 μl at 4°C for 1 hr. Nonspecific binding was determined by the addition of 25 μg of unlabeled T101 (5000-fold excess) in the assay solution. The maximum cell-bound counts (corrected for nonspecific binding), expressed as a percentage of the total counts added, were taken as the immunoreactivity.

Biodistribution Studies

Balb/c mice (Harlan Sprague-Dawley, Indianapolis, IN, female, 5–8 wk) were injected intravenously through a tail vein with HPLC-purified monomeric T101 conjugates (4–5 μCi/0.7 μg). The animals (n = 3–5) were killed by exsanguinization at 10 min and 2, 7, 19, 43 and 115 hr after injection. The organs were excised, blotted with gauze and weighed. The radioactivity was measured with a gamma counter (Packard Auto-Gamma 5650, Meriden, CT). Organ uptakes were expressed as the percent of the injected dose per gram of tissue (% ID/g) and normalized to a 20-g body weight. Urine and feces were collected separately and counted for radioactivity. Whole-body retention was obtained by a count of the whole carcass, including all organs. Biodistribution, urinary and fecal excretion and whole-body retention were obtained from the same mice. Statistical analysis was performed with one-way analysis of variance (ANOVA) with a two-tailed F test to compare some of the biodistribution data from the three conjugates. The blood clearance half-life of the distribution and elimination phase was obtained with a nonlinear regression analysis by the use of the Graphpad InPlot program (Intuitive Software for Science, San Diego, CA) for a two-compartment exponential clearance model, [Blood concentration] = $Ae^{-k\alpha t} + Be^{-k\beta t}$, where $k\alpha$ and $k\beta$ are the distribution and clearance rate constant, respectively, and t is the time. The nonlinear regression method was used to fit a curve to the experimental data.

Metabolism Studies

For the analysis of the radioactive products in vivo, 113 to 135 μCi per 19 to 24 μg of ^{111}In-labeled T101 was injected intravenously into normal Balb/c mice, and the serum, urine, bile and liver supernatants were obtained at 10 min and 2, 4.5, 19, 43 and 115 hr after the injection.

To prepare the liver supernatant, liver samples were soaked in 50 ml of ice-cold saline for 30 min to remove blood and then treated with three cycles of freezing and thawing. These samples were homogenized with 0.02 *M* cold phosphate-buffered saline at a 1:1 v/w ratio with the use of a motor-driven glass homogenizer. The homogenates were spun at 20,000 rpm for 1 hr at 4°C (Beckman J2-21, JA20, Palo Alto, CA). To extract as much radioactivity into the supernatant as possible, the pellet was homogenized and spun again, and then the supernatants from the two centrifugations were combined for analysis. The supernatant and pellet were counted in a dose calibrator to assess the percentage of radioactivity extracted into the supernatant. The supernatant was filtered through a 0.2-μm filter (Millex-GV, Bedford, MA) before analysis. The radioactivity of the filtrate and filter was measured by a dose calibrator (Squibb CRC-6A, Ramsey, NJ).

For a control experiment to prove that the fragmentation of the ^{111}In-labeled antibody does not take place during the homogenization process, each of the three ^{111}In-labeled T101 conjugates was mixed with whole liver. This mixture was homogenized, and the supernatant was prepared as described before for the sample preparation. Bile was obtained by puncturing the gallbladder with a 30-gauge needle and 1-ml syringe. It was diluted with 100 μl of chilled saline.

These samples were analyzed by size-exclusion HPLC with a TSK-3000 and a TSK-2000 column connected in series (0.02 *M* sodium phosphate and 0.1 *M* sodium sulfate, pH 6.7, 1 ml/min), paper chromatography (Whatman no. 1, Maidstone, England) with normal saline as a solvent and thin-layer chromatography (silica gel, Macherey-Nagel) with a solvent mixture of 10% ammonium acetate-methanol-0.5 *M* citric acid (2:2:1). The urine and liver supernatants were also analyzed by reverse-phase HPLC (C18 radial pack cartridge connected to Z-module, 5% ethanol in 0.01 *M* phosphate buffer at pH 6.7, 1 ml/min). The liver supernatant was treated with ultrafiltration (10-kD cutoff), and the filtrate was injected into the reverse-phase HPLC column.

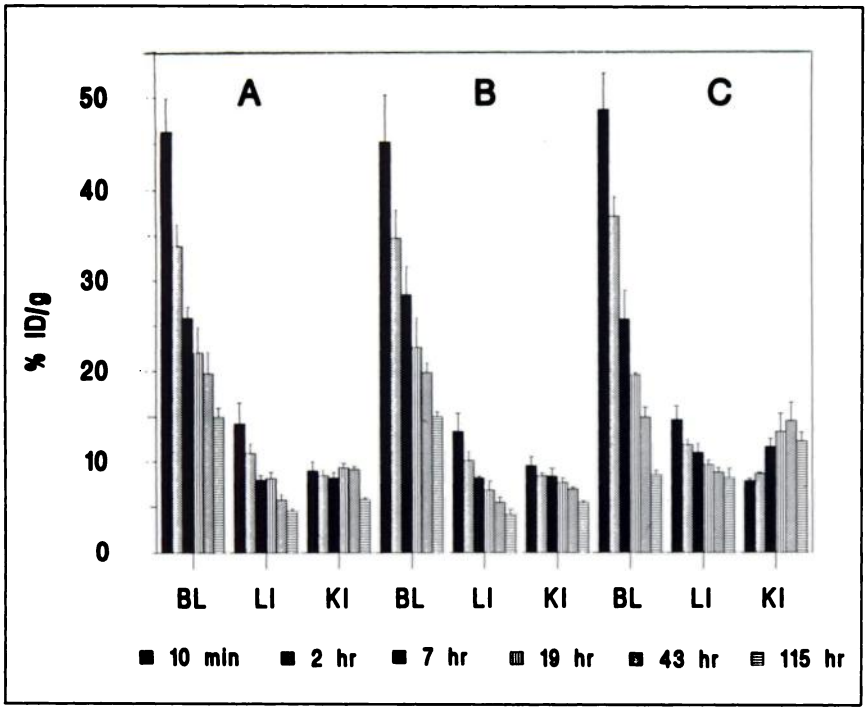

FIGURE 1. Comparative biodistribution of ^{111}In-T101 chelate conjugates in normal Balb/c mice. (A) ^{111}In-1B4M-T101. (B) ^{111}In-CHX-B-T101. (C) ^{111}In-cDTPA-T101. BL = blood; LI = liver; KI = kidney. Mean values of three to five mice.

RESULTS

Radiolabeling and Immunoreactivity

All T101 preparations were determined to contain an average of 0.6 to 1.6 chelators conjugated per antibody and labeled with ^{111}In at a specific activity of 4.7 to 7.1 mCi/mg. The immunoreactivity of ^{111}In-labeled T101 conjugates ranged from 60% to 80% when assessed with a cell binding assay at an antigen excess condition.

In Vitro Stability in Mouse Serum

The ^{111}In-1B4M-T101 and ^{111}In-CHX-B-T101 were stable in mouse serum, with no dissociation detected up to 5 days of incubation. In contrast, by day 5, 2.0% of ^{111}In activity transchelated from the ^{111}In-DTPA-T101 to a substance with the molecular weight of transferrin.

Biodistribution, Urinary and Fecal Excretion and Whole-Body Retention

The biodistributions of ^{111}In-1B4M-T101 and ^{111}In-CHX-B-T101 were similar to each other but different from that of ^{111}In-cDTPA-T101. The difference was particularly prominent in liver and blood samples.

The radioactivity of ^{111}In-1B4M-T101 and ^{111}In-CHX-B-T101 was rapidly cleared from the liver. The corresponding activity at 115 hr was 4.5% and 4.1% ID/g, respectively (Fig. 1). In contrast, the liver uptake (8.2% ID/g) of ^{111}In-cDTPA-T101 at 115 hr was twice those of the 1B4M and CHX-B conjugates ($p < 0.001$, analyzed by one-way ANOVA with a two-tailed F-test). The blood clearances of ^{111}In-1B4M and ^{111}In-CHX-B conjugates were similar to each other but slower than ^{111}In-cDTPA conjugate. This resulted in a higher liver-to-blood ratio for the cDTPA conjugate at late time points (Table 1). The nonlinear-regression analysis of the blood clearance data by the In-Plot program displayed a biexponential clearance curve, fitting well to the experimental data from each of the three conjugates ($R^2 > 0.990$). Although this curve fitting was achieved with a limited number of data points (six), the R^2 value close to 1 indicates a high degree of curve matching. The $T_{1/2\alpha}$ obtained by the nonlinear-regression analysis was similar for these three conjugates (2.4, 2.4 and 2.2 hr for cDTPA, 1B4M and CHX-B, respectively). However, the $T_{1/2\beta}$s of the 1B4M (154 hr) and the CHX-B (145 hr) conjugates were twice that of the cDTPA conjugate (76 hr). Interestingly, whole-body clearance of ^{111}In-cDTPA-T101 ($T_{1/2}$ = 193 hr) was similar to that of the 1B4M ($T_{1/2}$ = 186 hr) and the CHX-B-T101 ($T_{1/2}$ = 185 hr) conjugates. The excretion rates into urine and feces were also similar for the three conjugates with 18%–22% of the ID excreted cumulatively in the urine and 17%–19% in the feces at day 5 (Fig. 2).

Metabolism Study

An average of 77.4% of the initial total radioactivity in the liver was recovered in the liver supernatant preparations. When the supernatant was filtered, less than 10% of the radioactivity remained on the 0.2-μm filter. Most of the remaining activity on the filter was due to the contamination with some of the pellet and fat-like substances from the supernatant. Paper chromatography of serum, urine or

TABLE 1
Tissue-to-Blood Ratio* for ^{111}In-1B4M-T101, ^{111}In-CHX-B-T101 and ^{111}In-cDTPA-T101 in Normal Balb/c Mice

	10 min	19 hr	43 hr	115 hr
1B4M (n = 3)				
Liver	0.30 (0.02)	0.37 (0.06)	0.29 (0.01)	0.29 (0.02)
Kidney	0.19 (0.01)	0.43 (0.01)	0.47 (0.08)	0.38 (0.02)
Bone	0.08 (0.00)	0.20 (0.05)	0.23 (0.02)	0.21 (0.03)
Muscle	0.04 (0.01)	0.09 (0.01)	0.13 (0.02)	0.10 (0.00)
Intestine†	0.05 (0.00)	0.17 (0.03)	0.14 (0.00)	0.14 (0.00)
CHX-B (n = 3)				
Liver	0.29 (0.02)	0.29 (0.02)	0.28 (0.05)	0.29 (0.03)
Kidney	0.20 (0.03)	0.33 (0.02)	0.35 (0.03)	0.37 (0.01)
Bone	0.11 (0.02)	0.16 (0.01)	0.21 (0.04)	0.20 (0.02)
Muscle	0.05 (0.02)	0.09 (0.01)	0.09 (0.01)	0.10 (0.00)
Intestine†	0.05 (0.01)	0.14 (0.01)	0.13 (0.02)	0.15 (0.01)
cDTPA (n = 5)				
Liver	0.30 (0.02)	0.49 (0.02)	0.45 (0.03)	0.97 (0.14)
Kidney	0.16 (0.02)	0.68 (0.04)	0.74 (0.06)	1.44 (0.11)
Bone	0.07 (0.01)	0.19 (0.03)	0.19 (0.02)	0.50 (0.10)
Muscle	0.02 (0.00)	0.07 (0.01)	0.07 (0.01)	0.12 (0.01)
Intestine†	0.04 (0.01)	0.16 (0.02)	0.15 (0.01)	0.30 (0.01)

For simplification, data at 2 and 7 hr were omitted.
*Percentage of the injected dose per gram (%ID/g) in each organ was divided by the %ID/g in the blood at the given time interval. Data are reported as mean (1 s.d.).
†Small and large bowel with its content.

liver supernatant samples showed no evidence of the radioactivity in colloids.

To estimate the molecular weight of the metabolic products, a standard curve from the plots of HPLC retention times versus molecular weights was constructed; IgG (150,000 D), ovalbumin (44,000 D), myoglobin (17,000 D) and vitamin B_{12} (1350 D) were used as standards.

Size-exclusion HPLC of serum samples obtained from the in vitro incubation or from the mice indicated that the radioactivity of the 1B4M and CHX-B conjugates was

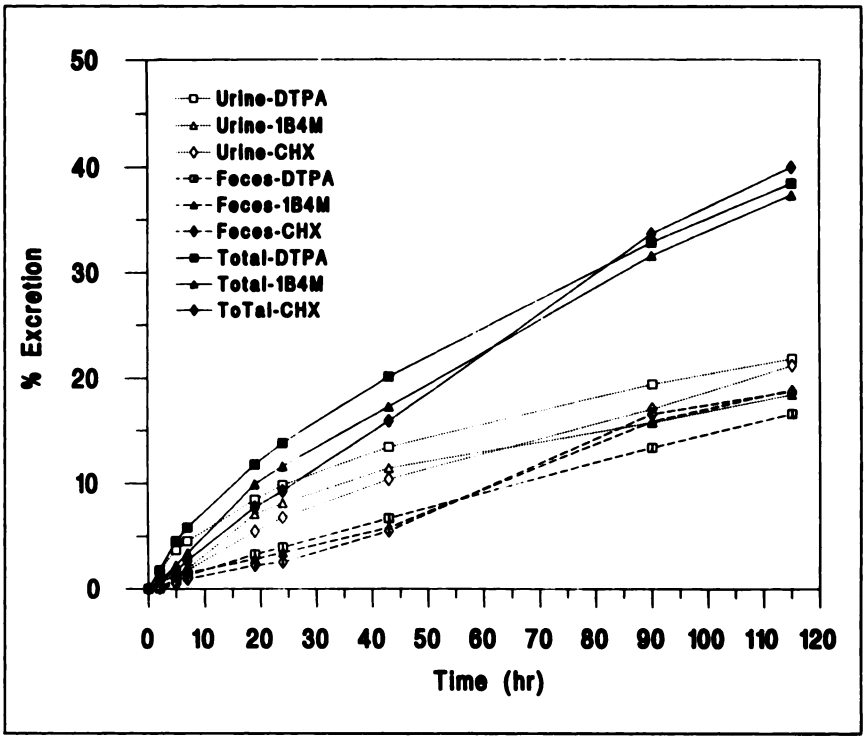

FIGURE 2. Cumulative radioactivity excreted into urine and feces.

solely associated with the intact IgG peak (R_t = 15.5 min) up to 5 days. In contrast, ^{111}In-cDTPA-T101 showed transchelation of approximately 1.5% of ^{111}In to serum transferrin (R_t = 17.5–18.0 min) in the in vivo experiment and 2.0% in the in vitro experiment at day 5.

The radioactivity in the processed liver was analyzed by size-exclusion HPLC. The control sample from the homogenization of liver mixed with each of the three conjugates showed only one peak, which corresponded to IgG. In contrast, the liver supernatant for the 1B4M and CHX-B conjugates showed two radioactive substances with an estimated molecular weight of 150,000 D and 1500 D (R_t = 25.5–26 min). In addition to these two species, the cDTPA conjugate showed a third radioactive substance with a molecular weight identical to transferrin. The liver concentration of the radioactive substance with the 150,000-D molecular weight was similar for the three T101 conjugates. In all cases, the concentration of this high molecular weight species decreased with time, whereas the concentration of the small molecular weight radiometabolite increased approximately up to 1 day and became constant thereafter up to 5 days (Fig. 3). The steady-state metabolite concentration from the cDTPA conjugate was about three times higher than those of 1B4M and CHX-B conjugates (4.8% ID/g for cDTPA versus 1.7% ID/g for 1B4M and CHX-B). Only the cDTPA conjugate showed a transferrin peak in the liver; however, this was small and never exceeded 1.1% ID/g.

The radioactivity in the urine for all preparations was identical to the 1500-D metabolite, as determined by size-exclusion HPLC and thin-layer chromatography. Bile radioactivity for the cDTPA conjugate was mainly in the form of intact IgG, although approximately 10%–20% of the total bile activity was in the small metabolite form for up to 19 hr. For the 1B4M and the CHX-B conjugate, 28% and 34%, respectively, of the total bile activity was in the small metabolite form at 2 hr. The bile samples obtained at later times were also analyzed by HPLC, but the radioactivity was too low for the determination of the peak intensities.

With reverse-phase HPLC, a polar radiometabolite peak was observed at a retention time of 5 min for the urine or liver samples for the cDTPA conjugate. For the 1B4M conjugate and the CHX-B conjugate, the reverse-phase HPLC of the urine and liver samples showed one major radiometabolite peak at 6 and 7.5 min, respectively. In addition, one minor peak was observed for the 1B4M conjugate and three minor peaks were detected at 5, 13 and 16 min for the CHX-B conjugate. The intensities of these minor peaks were negligible compared with the intensity of the major peaks.

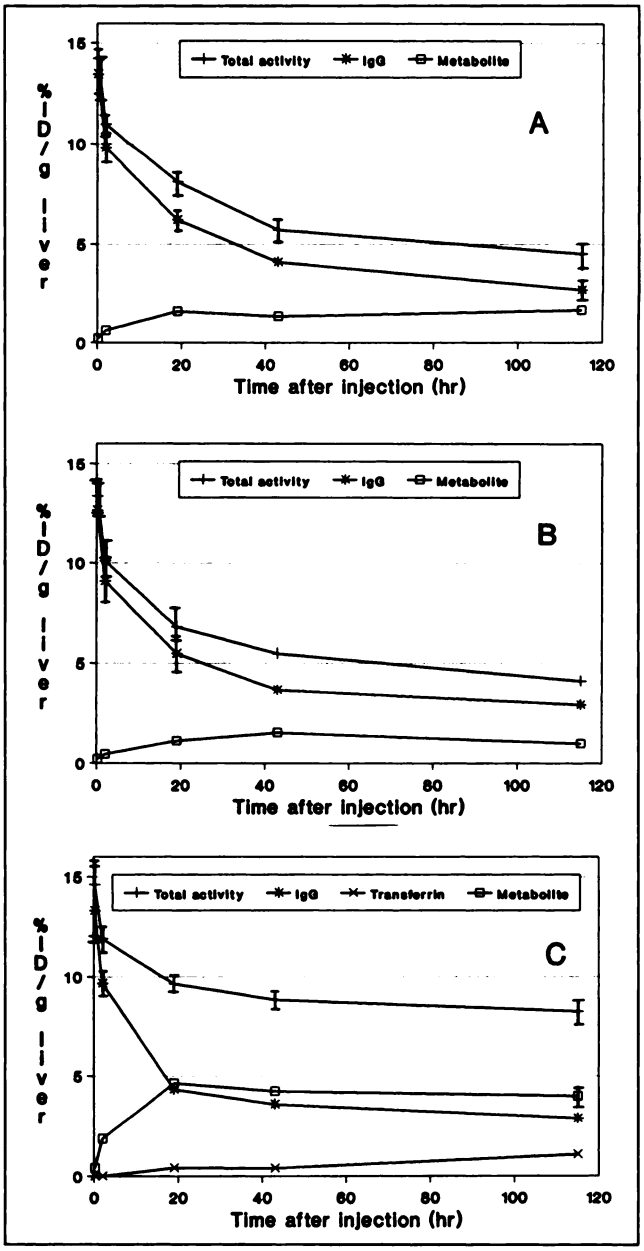

FIGURE 3. Indium-111 products in liver. Values are expressed as %ID/g of liver tissue, based on results of biodistribution studies and size-exclusion HPLC analysis of liver supernatant. Bars indicate the s.d., which are omitted when the range is within the symbol. (A) ^{111}In-1B4M-T101. (B) ^{111}In-CHX-B-T101. (C) ^{111}In-cDTPA-T101.

DISCUSSION

The development of bifunctional chelating agents for ^{111}In-labeling of Mab that will result in a lower accumulation in nontarget tissue is an important endeavor. The authors were encouraged to find that both ^{111}In-1B4M-T101 and ^{111}In-CHX-B-T101 produced lower liver accumulation of radioindium than did ^{111}In-cDTPA-T101 (Fig. 1). This lower liver accumulation of ^{111}In-1B4M-T101 and ^{111}In-CHX-B-T101 was similar to that of ^{111}In-1B4M-B3 and ^{111}In-CHX-B-B3 (*22,23*) (B3 is another murine Mab IgG directed against a carbohydrate antigen expressed on epithelial tumors). The liver accumulation of the control conjugate, ^{111}In-cDTPA-T101, was similar to that reported by Hnatowich et al. (*5*) who used antiprostatic acid phosphatase Mab conjugated with cDTPA. In the current

radiolabeling experiments, the cDTPA conjugate was labeled in the presence of a molar excess of free DTPA. It was proposed that labeling with excess free DTPA minimizes the binding of ^{111}In to weaker chelators, such as DTPA diamide, possibly produced by the reaction of cDTPA dianhydride with two intra- or intermolecular amino groups of the Mab.

For the comparative studies, the 1B4M, CHX-B and cDTPA conjugates cleared from the whole body with similar rates. However, the two new conjugates, the 1B4M and the CHX-B, cleared faster from the liver but slower from the blood than the cDTPA conjugate at later time points. The cDTPA conjugate produced increasing liver-to-blood ratios that were consistent with accumulation in this organ (Table 1). In contrast, the liver-to-blood ratios for the two new conjugates did not vary with time, which suggests that the clearance from the liver is proportional to the clearance from the blood (Table 1). These differences in biodistribution, that became pronounced at later time intervals, suggest that the differences were caused by the differences in metabolic rates for the radiolabeled conjugates and subsequent elimination of metabolites.

The 1B4M, CHX-B and cDTPA conjugates looked similar in the serum with respect to metabolism and ^{111}In transchelation to transferrin for a 5-day period. The analysis of serum samples indicated that the radioindium in serum was solely associated with IgG for the two new conjugates. In addition, a negligible amount of ^{111}In-transferrin (less than 2% of the total serum activity at day 5) was detected for the cDTPA conjugate.

However, substantial differences were found between the two new conjugates and the cDTPA conjugate, primarily in the formation of the small radiometabolite in the liver and, to a lesser extent, the formation of ^{111}In-transferrin. For the cDTPA conjugate, the small radiometabolite was formed more rapidly than that for the two new conjugates (Fig. 2). However, this small radiometabolite was eliminated from the liver more slowly than the small radiometabolites from the 1B4M and CHX-B conjugates.

The precise structure of the small radiometabolites was not identified in this study. The molecular weight of the ^{111}In-labeled metabolites in the liver was less than 1500 D for the three conjugates. The HPLC study did not show any intermediate molecular weight metabolite with a molecular weight in the range from 50 to 30 kD. This result is similar to the findings of Motta-Hennessy et al. (*10*), Schuhmacher et al. (*11*) and Beatty et al. (*13*) but different from those of Paik et al. (*14*) and Jones et al. (*16*). This difference might be caused by differences in the antibody subclasses and animal species used for the experiments.

The metabolite from the cDTPA appeared to be most polar and that from the CHX-B conjugate, the least polar, based on reverse-phase HPLC. The analysis of urine samples showed results similar to the radiometabolites extracted from the liver samples. This suggests that the small metabolites diffuse out of the liver cells, enter into the circulation and are excreted through the kidney. The extent of the biliary excretion of the metabolites was not well defined because the samples from the gallbladder, especially for the two new conjugates, did not contain enough activity for HPLC analysis at later time intervals. Nevertheless, the analysis of gallbladder samples for the cDTPA conjugate indicated that 80%–90% of the total radioindium was bound to the intact antibody for up to 19 hr. This suggests that the small polar radiometabolite from the cDTPA conjugate is not excreted readily by the biliary system. For the two new conjugates, the contribution of the small radiometabolites to the total gallbladder activity was somewhat higher (28% for the 1B4M and 34% for the CHX-B at 2 hr) than that shown for the cDTPA conjugate, even though this was determined at an early time point. This appears to indicate that the small radiometabolites of the 1B4M and the CHX-B conjugates are excreted better through the biliary system than that of the cDTPA conjugate. This result is consistent with the findings of Arano et al. (*30*) and Duncan and Welch (*31*) that the small radiometabolite from ^{111}In-labeled cDTPA-conjugated galactosylated human serum albumin (HSA) was not excreted through the biliary system as readily as the radiometabolite of ^{111}In-labeled p-isothiocyanatobenzyl EDTA-conjugated galactosylated HSA. It appears that the addition of a benzyl group to the bifunctional chelate increases the lipophilicity of the metabolite and thereby enhances the biliary excretion. They reported that the metabolites were the lysine adducts of the corresponding chelates. However, in the current study, the authors were not able to separate unequivocally the lysine adducts from the ^{111}In-labeled chelates by reverse-phase HPLC.

The faster blood clearance but the slower liver clearance of the radioindium from the cDTPA conjugate could also be explained if the cDTPA conjugate was recognized by the immune system as foreign and removed by the reticuloendothelial system (RES) organs. However, this possibility was ruled out because the radioindium concentration in the liver was similar for the three conjugates at 10 min and the radioindium concentrations in the other RES organs, such as the spleen and bone marrow (bone), were similar for the three conjugates during a 5-day period.

A reasonable assumption is that murine antibody IgG_{2a} is not rapidly metabolized in mouse liver and that a substantial amount of the internalized but not metabolized murine antibody diffuses out of the liver and into the circulation. The percentage of the radioactivity found in the circulation might depend on the stability or the nature of the chemical bond that links the chelating agent to the antibody. The higher blood concentrations for the 1B4M and CHX-B conjugates appear to indicate that the thiourea linkage for these two conjugates delays the metabolism of the 1B4M and CHX-B conjugates to the small metabolites and results in the higher concentration of the radioactivity diffusing out of liver cells into the circulation.

In conclusion, the lower accumulation of the radioactivity in the liver for both the 1B4M and the CHX-B conjugates is more favorable than that of the cDTPA conjugate.

This study suggests that the improved stability of the ^{111}In chelate complexes of the 1B4M and CHX-B and their increased lipophilicity are important chemical parameters that are responsible for the reduction of the liver's radioactivity in mice injected with ^{111}In-1B4M-T101 and ^{111}In-CHX-B-T101. The thiourea linkage of the two new conjugates appears to be responsible for the reduced radiometabolite concentration in the liver and the increased total radioindium concentration in blood compared with the cDTPA conjugate.

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