mature blood-brain barrier. This phenomenon has been observed with other substances (*11,12*).

CONCLUSION

Technetium-99m *d*, l-HMPAO binds to both human and guinea pig placentas. It is transfered across the placental barrier into fetal circulation mostly in the liver with increasing gestational age.

ACKNOWLEDGMENTS

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Biological Properties of Biotin–Chelate Conjugates for Pretargeted Diagnosis and Therapy with the Avidin/Biotin System

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Three-step pretargeting increases target-to-background ratios in radioimmunodetection and can potentially decrease harmful radiation to normal tissues in radioimmunotherapy. We studied four biotin–chelate conjugates (BCCs) for use in the avidin/biotin pretargeting system. **Methods:** Pharmacokinetics and biodistribution were studied in normal BALB/c (IA^k-negative), normal C_3H (IA^k-positive) and LS174T tumor-bearing BALB/c severe combined immunodeficient mice. Streptavidin alone and antibody–streptavidin conjugates [monoclonal antibody (MAb) 10-3.6 anti-IA^k IgG2a] were used. Indium-111- or ^{88}Y-BCCs were given alone intravenously; they were mixed with streptavidin or MAb–streptavidin conjugate and given intravenously; or streptavidin and MAb–streptavidin conjugate were pretargeted, and 2–3, 5 and 21 hr later, BCCs were injected intravenously. Samples were taken 2–3 hr after intravenous injection of labeled BCCs. **Results:** Three of the four BCCs were rapidly excreted by the kidneys, with <2.5%/g in any organ or tumor at 2–3 hr. Gut excretion eliminated biotinyl-(S)-1-*p*-aminobenzyl-ethylenediaminetetraacetic acid (EDTA) for use in pretargeting. Ninety percent of BCCs were bound to circulating pretargeted streptavidin at 1–6 hr, and ~15% were bound to pretargeted streptavidin at 24 hr. Kidney uptakes were: preformed streptavidin–BCC given intravenously, ~80%/g (24 hr); streptavidin pretargeted for 2–3 hr, ~60%/g; and streptavidin pretargeted for 5–21 hr, ~10%–20%/g. Kidney uptake was dose-dependent: 0.2, 0.67 and 1.0 nmol of streptavidin pretargeted for 21 hr showed increasing concentrations (24 hr). Uptake of monoclonal anti-IA^k–streptavidin–BCC complex into spleen (70% ± 10%/g; $p < 0.05$) and lymph nodes (10% ± 3.5%/g; $p < 0.01$) was higher in IA^k-positive C_3H mice than it was in IA^k-negative control BALB/c mice, and it was much higher than that in streptavidin controls. No significant target uptake was seen with anti-IA^k MAb–streptavidin pretargeted for 3 or 20 hr. Kidney uptake ~20%/g, which was lower than that of streptavidin alone. **Conclusion:** Three biotinyl chelates bind the diagnostic and therapeutic radiometals ^{111}In and ^{88}Y (and, by analogy, ^{90}Y) with the required in vivo stability and physiological properties for pretargeted diagnosis and therapy. Kidney uptake of streptavidin was decreased by conjugation to MAb. Failure of anti IA^k MAb–streptavidin conjugate to bind BCC after pretargeting may be due to rapid internalization of MAb–streptavidin-IA^k complex by the lymphocyte or to endogenous biotin. Either or both of these would make streptavidin unavailable to subsequent BCCs.

Key Words: radioimmunoimaging; radioimmunotherapy; yttrium-90; indium-111; pretargeting

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Radiolabeled monoclonal antibodies (MAbs) have shown promising clinical results in the diagnosis and therapy of cancer (*1–5*). Radionuclides such as ^{111}In and ^{90}Y have been of particular interest in radioimmunoscintigraphy and radioimmunotherapy (RAIT) due to their nuclear properties (*6–9*). Among the radionuclides for therapy, ^{90}Y is of particular interest due to its superior properties, including pure beta emission and the high-dose yield per nanomole (*10*). Bifunctional chelating agents (*11,12*) complex these metal ions and attach the chelated radionuclide to a protein or MAb (*13*). These conjugates act as carriers of radiometals for tumor targeting and radiotherapy. Chelates that hold radiometals with high stability under physiological conditions are essential to avoid excessive radiation damage to nontarget cells (*14*).

Renn and Meares (*15*) reported the large-scale synthesis of

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the macrocyclic bifunctional chelating agent, 2-p-nitrobenzyl-1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (nitrobenzyl-DOTA), which holds yttrium with extraordinary stability under physiological conditions (*16*). Nitrobenzyl-DOTA binds both yttrium and indium with superior stability, so it should be possible to use DOTA chelates of the gamma-emitting ^{111}In as a tracer for DOTA chelates of the beta-emitting ^{90}Y to gain accurate measurements of radiation dosimetry.

However, the use of radiolabeled antibodies for radiotherapy and radioimmunotargeting is not without disadvantages. The accumulation of an antibody in target tissue requires several days, so the distribution kinetics of directly labeled antibodies are not optimal for accurate imaging or delivery of toxic substances to the tumor tissue for radiotherapy. A relatively low tumor-to-background ratio and high nontarget activity are the major problems in immunoscintigraphy (*17*), due to to the slow pharmacokinetics of radiolabeled antibodies (*18*). In addition, because the high levels of circulating radioactive antibody conjugates limit the total amount of radioactivity that can be given safely, low absolute tumor uptake and low radiation dose to the tumor are a problem in RAIT. Several attempts have been made to improve biodistribution of the radiolabel, such as the use of a second antibody (*19*), local delivery (*20*), the use of metabolizable linkers between antibody and the chelate (*21*) or the use of MAb fragments that display a faster blood clearance than do whole antibodies (*22*).

Further improvements are desirable. The diagnostic or therapeutic agent should be delivered at a time when the tumor tissue is already labeled by the tumor-specific antibody. This can be accomplished if the label or the therapeutic agent, which should show a fast clearance, is captured by an antibody that is already targeted to a tumor cell. These considerations led to the concept of multistep tumor targeting, based on separate administrations of antibody and label (*18,23–26*). Several molecular pretargeting systems have been proposed, the hapten/antibody and the avidin/biotin being the two most thoroughly studied to date (*27*). A mathematical model has also been developed to determine an optimal drug delivery protocol for multistep pretargeting systems (*28*).

The avidin/biotin system is widely used for in vitro and in vivo applications, in immunohistochemistry, enzyme-linked immunosorbent assay and molecular biology (*29,30*). Avidins are proteins that bind biotin with high affinity and specificity (*31*). Theoretically, they can bind up to four molecules of biotin, and the dissociation constant of the avidin/biotin complex is on the order of 10^{-15} *M*, so binding can be considered an irreversible process (*32*).

One practical adaptation of the biotin/avidin pretargeting system has now been in Phase I/II clinical imaging trials for several years (*33*). Biotinylated antitumor MAbs are given intravenously, followed by avidin chase and then a biotin–chelate conjugate (BCC) in a three-step protocol. Improved ^{111}In human tumor images with low blood and liver background and high contrast have been obtained (*26*). Encouraging results have now been reported by these authors using a similar protocol in a Phase I ^{90}Y therapy trial (*34*).

In this article, we describe the pharmacokinetics and biodistribution of four biotinylated chelates: biotinyl-ABE, biotin-P-CITC, biotin-P-DOTA and biotinyl-LC-DOTA (Fig. 1). Detailed synthesis and chemical characterization of these conjugates have been reported elsewhere (*35*). Among these conjugates, biotinyl-LC-DOTA, biotin-P-DOTA and biotin-P-CITC bind the diagnostic and therapeutic radiometals ^{111}In and ^{88}Y with the required in vivo stability and have excellent physiological properties for pretargeted diagnosis and therapy. Some abbreviations, the pretargeting terminology and the time sequence of the pretargeting steps are given in Table 1.

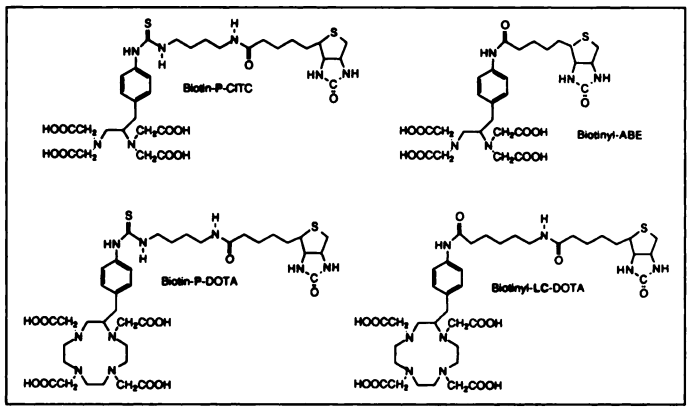

FIGURE 1. Schematic structures of the synthesized biotin–chelate conjugates.

MATERIALS AND METHODS

Synthesis of the Biotin–Chelate and Monoclonal Antibody–Streptavidin Conjugates

As BCCs are prepared for medical use, it is important to confirm that they bind to avidin. The BCCs were labeled with excess ^{57}Co/Co of known molar concentration, and then streptavidin was added. The complex was purified from excess ^{57}Co/Co and unbound BCC as described (*35*). In a separate experiment, biotin-LC-DOTA was bound to hen egg avidin, and the complex was mixed with excess ^{57}Co/Co.

Streptavidin was obtained from Boehringer Mannheim or Calbiochem and was used without further purification. The MAb 10-3.6 (IgG2a), specific for IA^k, was conjugated to streptavidin.

TABLE 1
Two- and Three-Step Pretargeting Terminology

Term	Definition
Targeting molecule	Long circulating macromolecule specific for target (MAb–streptavidin conjugate)
Effector molecule	Small, rapidly diffusing and excreted ^{111}In- or ^{88}Y-BCC
Pretargeting time (e.g., streptavidin pretargeted for 21 hr, streptavidin was given intravenously (pretargeted) 21 hr before the next step)	Time from injection of targeting molecule (bs MAb, MAb–streptavidin conjungate) until chase or effector (^{111}In- or ^{88}Y-BCC in these experiments)
Chase*	Polyvalent macromolecule that crosslinks and removes targeting molecules from the circulation but not the target
Sampling or imaging time	Time from injection of effector until kill or imaging (2–3 hr in these experiments)
Total time	Pretargeting time + chase time (if chase is used) Pretargeting + effector time [e.g., pretargeting (21 hr) + chase (1 hr) + sample (3 hr) after intravenous ^{111}In- or ^{88}Y = 24 hr total]

*bs MAb = bispecific MAb.
Note: Not used in these experiments. Given just before (0.5–1 hr) radioactive effector molecule.

IA^k is a major histocompatibility complex alloantigen (Class II) present on C_3H mouse B lymphocytes but not on BALB/c B lymphocytes. We have shown in other experiments with directly labeled ^{111}In-10-3.6 that the spleen and lymph nodes in C_3H mice are the target organs (*17*). BALB/c mice served as the control.

The synthetic pathways and detailed chemical characterization of the four BCCs studied here have been reported previously (*35*). The MAb–streptavidin conjugates were synthesized by first forming the 2-iminothiolane (Traut's reagent) derivative of anti-IA^k (IgG1) and of streptavidin. Then the streptavidin conjugate was activated with 5,5′-dithio-*bis*(2-nitrobenzoic acid) and added to 2-IT-anti-IA^k in a 3:4 molar ratio. The resulting disulfide S—S linkage coupled the two molecules together in a molar ratio of approximately 1:1.

Radiolabeling Biotin–Chelate Conjugates with Yttrium-88 or Indium-111

Indium-111 was chosen as an imaging radionuclide, and ^{88}Y was chosen as an analog of the therapeutic radionuclide ^{90}Y. Yttrium-88 or ^{111}In was dried in 1 ml of metal-free polypropylene snap-top tubes in an Eppendorf heat block at set at 95°C. Fifty microliters of 0.1 *M* NH_4Ac (pH 5) were added and vortexed, and after 5 min, 1 μl was spotted onto a thin-layer chromatography (TLC) strip (first lane). Ten microliters of 1 m*M* BCC (or a volume = 10 nmol) were added, and the solution was mixed and heated at 95° for 30 min. The solution was cooled, and 1 μl was spotted on 10-cm TLC strip in the second lane. Then, 10 nmol of nonradioactive metal were added to fill up the chelator. The solution was heated again at 95° for 30 min, and 1 μl was spotted on the TLC strip in lane 3. The TLC strip was dried and run in MeOH-NH_4Ac [10% NH_4Ac:absolute MeOH (vol/vol)].

An autoradiograph was made on DuPont film, the TLC strip was cut in 1-cm sections and counted and the percentage of labeled BCC was calculated. All activity in the first spot should be at the origin (free metal), and that in the second spot should run 100% to R_f ~2–4 (metal-DOTA complex). For injection, 1 μl of 0.1 *M* Ca/Na_2-EDTA was added during dilution, to bind any free metal and promote rapid renal excretion.

Biological Half-Life

Twenty-five to 100 μCi ^{111}In-BCC were injected intravenously for half-life measurements. For whole-body counting, the mice were placed in a small stoppered plastic beaker (with air holes) equidistant between the two 180° opposed arms of a Picker Dual Probe scintillation counter. The empty mouse holder was counted for background, and the first mouse count was obtained immediately after intravenous injection, before any renal excretion could occur. This count was used as the 100% standard zero time value for subsequent $T_{1/2b}$ calculations. Two 1-min mouse counts from each detector were performed and averaged. For later measurements, a minimum of 1000 counts were obtained by increasing the counting time. The background counts of the empty mouse holder were subtracted, and the mean net counts per minute were calculated. The time elapsed from zero time was noted, the counts per minute were decay-corrected back to zero time and the percentage of the injected dose remaining in the mouse was calculated.

Biodistribution

In control experiments, the biodistributions of four ^{111}In-labeled BCCs injected intravenously alone were measured in normal (IA^k-negative) BALB/c, LS174T tumor-bearing BALB/c severe combined immunodeficient (SCID) and normal (IA^k-positive) C_3H mice. Streptavidin and anti-IA^k (IgG2a) MAb–streptavidin conjugates were also studied. Indium-111- or ^{88}Y-labeled BCCs were mixed with streptavidin or streptavidin–MAb conjugate, the complex given intravenously and the organ concentrations were measured at 3 hr. In some experiments, the mice were also pretargeted with 0.2–1.0 nmol of streptavidin intravenously; ^{111}In-labeled BCC was injected intravenously 1, 2, 3, 4, 6 and 21 hr later; and organs were sampled at 3 hr. In other experiments, the mice were pretargeted with MAb–streptavidin conjugate, and labeled BCC was injected at 3 and 20 hr afterward. Organ and tumor samples were usually taken 2–3 hr after injection of radiolabeled BCCs.

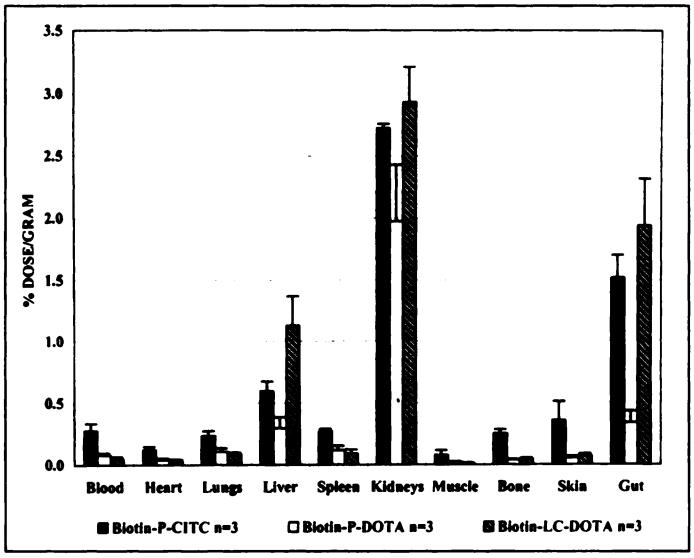

FIGURE 2. Distribution of three ^{111}In-biotin–chelate conjugates 2 hr after intravenous injection into normal BALB/c female mice. Error bars = ± 1 s.d. Kidneys are the major route of excretion, with a small amount excreted through the gut.

RESULTS

The in vitro assay of streptavidin binding gave a lower than theoretical 4:1 ratio, based on the known streptavidin valence of 4. The measured BCC-to-streptavidin ratio was approximately 1.87:1 ± 0.3 (*35*). In the hen avidin experiment, only 0.41 ± 0.08 cobalt ions (instead of 4) became bound to the complex.

The organ distributions of ^{111}In-labeled biotin-P-CITC, biotin-P-DOTA and biotin-LC-DOTA 2 hr after intravenous injection into normal BALB/c female mice are shown in Figure 2 (error bars = ± 1 s.d.). The kidneys were the major route of excretion, with a small amount excreted through the gut. Very low concentrations (<1%/g) were seen in all organs except kidney and gut, even at the early 2-hr time point.

The organ distributions of ^{111}In-labeled biotin-P-CITC, biotin-P-DOTA and biotin-ABE 2 hr after intravenous injection into LS147T tumor bearing BALB/c SCID mice at 2–3 hr are seen in Figure 3. With the exception of biotin-P-ABE, similar patterns with very low concentrations (<1%/g) in all organs except the kidney were demonstrated. In addition, the tumor showed no accumulation above the background seen in the other organs. Of interest was the very high level of biotin ABE in the gut due to the large amount excreted through the biliary system. Nothing in the structure predicted the unique metabolic route of this derivative.

The whole-body retention of ^{111}In-labeled biotin-P-CITC in BALB/c female mice injected after pretargeting with streptavidin for 1, 2, 3, 4, 6 and 24 hr is shown in Figure 4. The curves were plotted from immediately after injection of ^{111}In-labeled biotin-P-CITC up to 48 hr after injection. Almost quantitative binding of BCC to circulating streptavidin was seen up to 6 hr of pretargeting, with much less binding after 24 hr of pretargeting.

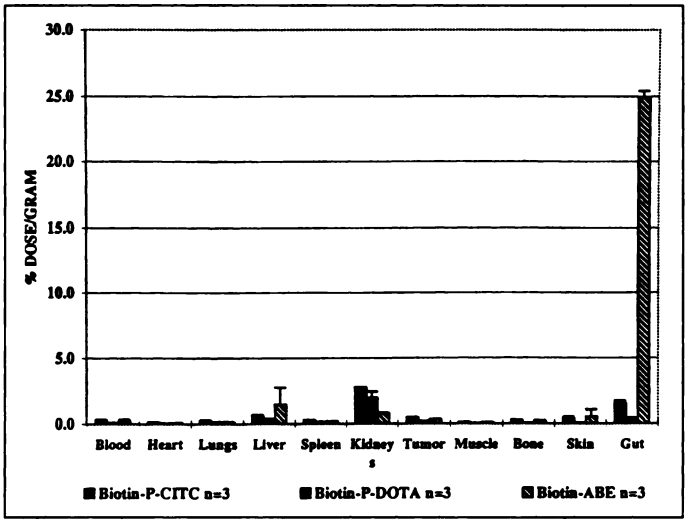

FIGURE 3. Organ distribution of three ^{111}In-biotin–chelate conjugates at 2–3 hr in BALB/c SCID mice with LS174T tumor in the left flank. Very low concentrations are seen in all organs and the tumor at the early time points, with the exception of biotin-ABE, a large fraction of which is excreted through the liver and gut.

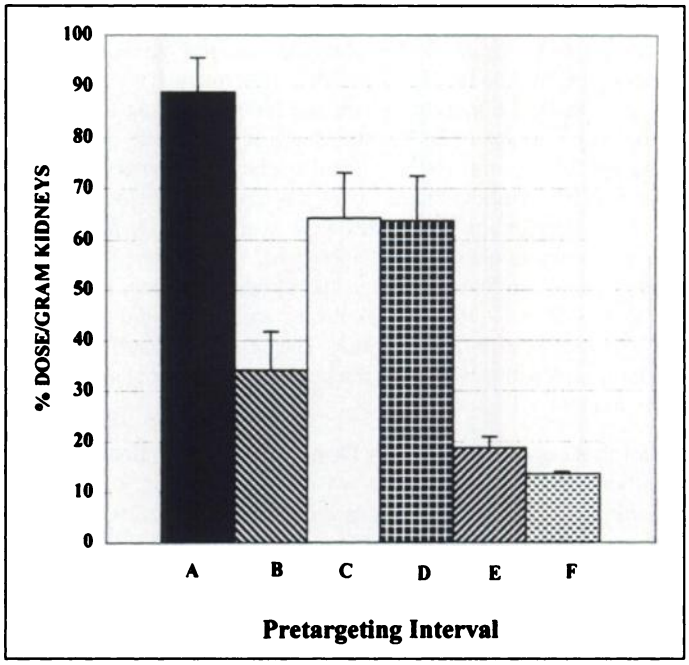

FIGURE 5. Kidney uptake in normal BALB/c female mice of streptavidin mixed with ^{111}In biotin-P-CITC and injected (Column A) or pretargeted for 1 (Column B), 2 (Column C), 3 (Column D), 4 (Column E) and 21 (Column F) hr and sampled 3 hr after intravenous injection of ^{111}In-biotin-P-CITC.

Kidney uptake of streptavidin in normal BALB/c mice is shown in Figure 5. A molar excess of streptavidin was mixed with ^{111}In-biotin-P-CITC; the complex was injected and kidneys were assayed at 24 hr (Column A); or streptavidin was pretargeted for 1 (Column B), 2 (Column C), 3 (Column D), 4 (Column E) and 21 (Column F) hr, and the kidneys were assayed 3 hr after intravenous injection of an equimolar amount of ^{111}In-biotin-P-CITC. A fairly high kidney uptake was seen at all times but especially at the earlier times, up to 3 hr. However, a significant amount (>10%/g) was also seen at 4 and 21 hr pretargeting.

The kidney uptake of increasing amounts of streptavidin pretargeted for 21 hr, 3 hr after ^{111}In-biotin-P-CITC (24 hr total), is shown in Figure 6. The kidney uptake of streptavidin is dose-dependent, with the variation among group means being significantly greater than that expected by chance ($p < 0.0001$) when compared by analysis of variance.

The organ, spleen and peripheral and mediastinal lymph node distributions of a bispecific conjugate capable of binding both the target lymphocytes and BCC are shown in Figure 7. Indium-111-biotin-P-CITC was mixed with MAb anti-IA^k–streptavidin conjugate and injected intravenously, and the 24-hr organ distribution was obtained in normal C_3H (IA^k-positive) and control normal BALB/c (IA^k-negative) mice. Figure 7 shows significantly higher uptake in the spleen and lymph nodes of C_3H mice than control BALB/c mice ($^*p < 0.05$; $^+p < 0.01$; $^\#p < 0.001$). However, there was no significant

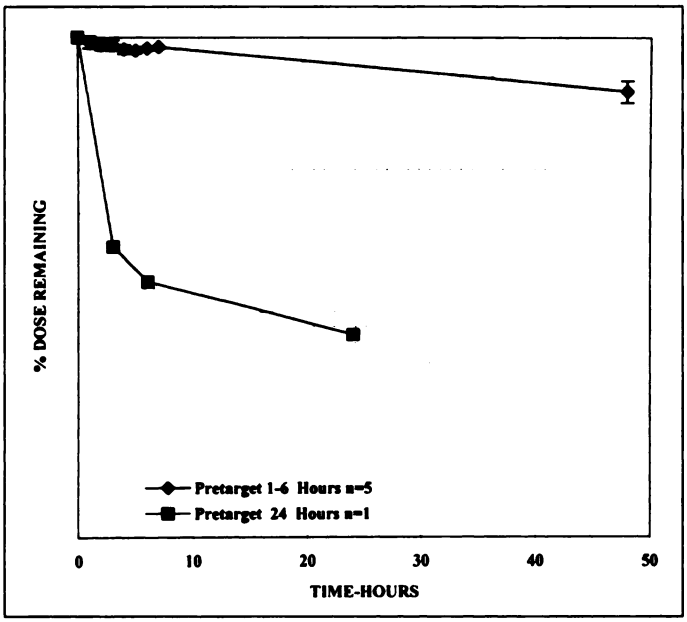

FIGURE 4. Whole-body retention of ^{111}In-labeled biotin-P-CITC in normal BALB/c female mice injected after pretargeting with streptavidin for 1, 2, 3, 4, 6 and 24 hr. Curves are plotted from immediately after injection to 48 hr after injection.

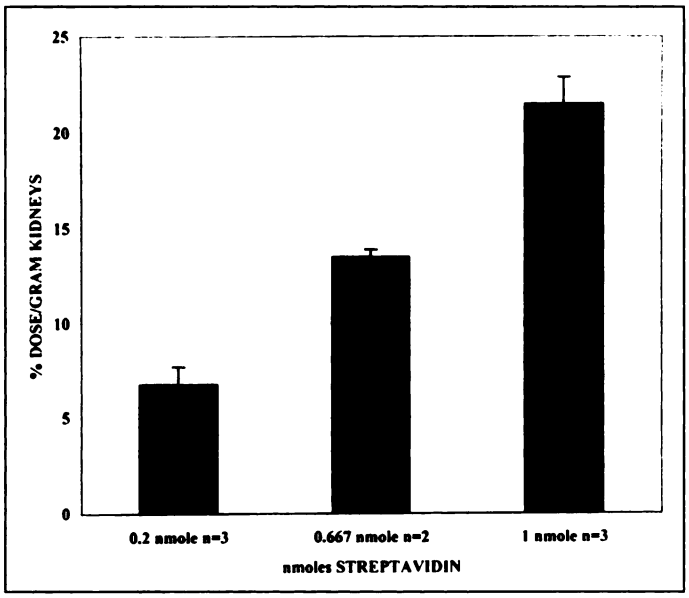

FIGURE 6. Three hour kidney uptake of ^{111}In-biotin-P-CITC in normal BALB/c female mice after streptavidin pretargeted for 21 hr. Kidney uptake is dose dependent, with the variation among group means being significantly greater than that expected by chance when compared by analysis of variance.

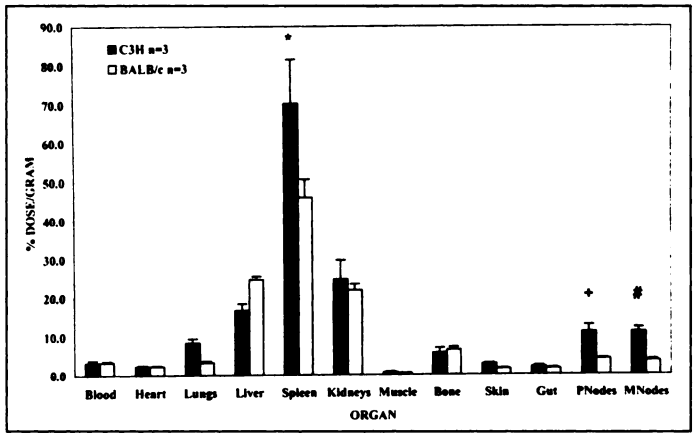

FIGURE 7. Twenty-four-hour organ distribution of ^{111}In-biotin-P-CITC mixed with MAb anti-IA^k–streptavidin conjugate in normal C_3H (IA^k-positive) and BALB/c (IA^k-negative) mice. There is significantly higher uptake in C_3H spleen and lymph nodes than in control BALB/c mice. *$p < 0.05$; $^+p < 0.01$; #$p < 0.001$.

uptake in the target spleen (0.43% ± 0.15%/g) or lymph nodes (0.35% ± 0.13%/g) when the MAb–streptavidin conjugate was pretargeted for 3 and 21 hr.

DISCUSSION

Pretargeting combines the pharmacokinetics of long-circulating targeting molecules needed for high blood and tumor concentrations and rapidly diffusing and excreted effector molecules necessary for low concentration in normal tissue and low toxicity. This is accomplished by temporal separation of the targeting and effector steps (*27*). The most popular pretargeting techniques use either the biotin/avidin system discussed here or the hapten/antibody system. However, DNA/DNA (*36*) and prodrug/enzyme systems (*37*) have also been used, and many other ligand/receptor systems are theoretically possible. All the methods depend on a long-circulating conjugate to get high target uptake with a diffusible, rapidly excreted effector molecule.

In the past, several bifunctional chelating agents were routinely synthesized and used for conjugation to antibodies in the laboratory of Claude F. Meares have shown promising results in terms of physiological stability. Among these chelates are both EDTA- and diethylenetriaminepentaacetic acid (DTPA)-based ligands. Biotinylated chelating agents have now been prepared from these precursors, thus exploring the complete set of bifunctional chelating agents, including the newer DOTA derivatives. In the biotinylated chelates studied here, the chelating site is separated by a spacer from the biotin group and, thus, from the avidin-binding site (Fig. 1).

It is interesting that only about two molecules of the chelate were able to bind to the tetravalent protein. In addition, the hen egg avidin result suggested that the avidin-bound ε-aminocaproic linker does not make chelating groups accessible for postcomplex radiolabeling. The fact that the binding sites for biotin occur in pairs that are fairly close to each other can cause steric hindrance to the binding of more than two biotinylated molecules (*26*). Thus, avidins are expected to have fewer than four biotinylated molecules bound. Too short a distance between avidin and the surface of the biotinylated chelate might also result in unsatisfactory binding of the biotinylated chelate to avidin. For sufficient binding capability to avidin, the biotin site and the chelating site of the biotinylated chelate conjugate must be separated by a spacer. The observed values we obtained are in agreement with this hypothesis and suggested that a varying degree of steric hindrance occurs between the sites (*35*).

TABLE 2
Properties of Biotin–Chelate Conjugates

Chemical properties	Physiological properties
Low molecular weight (small <10 kDa)	Renal excretion exclusively
Hydrophilic	≥GFR (100cc/min)*
Rapidly diffusible	Extracellular fluid distribution
Net negative charge	No protein binding in blood
High specific activity (≥1000 Ci/mmol)	No intracellular uptake
Choice of radionuclide; bifunctional chelate	Nonimmunogenic

*Not measured, but whole-body clearance similar to the known GFR agents ^{111}In-DTPA and ^{111}In-EDTA.
GFR = glomerular filtration rate.

In other experiments using a hapten/antibody pretargeting system, we have achieved fast in, slow out tumor kinetics in a mouse tumor model that are ideal for therapy (*38*). The tumor biological half-life of a bivalent ^{88}Y JANUS-DOTA hapten, measured over 5 days, was ~24 hr. The therapeutic ratio, obtained from the integrated tumor and blood concentrations over 5 days, was ~20:1, compared to 2–3:1 with directly labeled MAb. The total injected dose remaining in the mouse at 24 hr was 5.5%, of which 23% was in the tumor. These result suggested that it may be possible to deliver tumoricidal radiation doses with ^{90}Y using pretargeting techniques, without severe normal marrow irradiation.

The avidin/biotin system has several advantages over the hapten/antibody system, including the high affinity ($K_d = 10^{-15}$) that exists between avidin and biotin. Avidin and streptavidin are commercially available, well-defined structures that can also be made by genetic engineering techniques. The system is flexible allowing the use of several protocols; for example, the use of biotinylated MAb followed by streptavidin, chase and ^{90}Y-BCC (*39*). Despite its immunogenicity, streptavidin is preferred over avidin for the MAb conjugate due to its slower blood clearance (*40*).

The chemical and physiological properties of three of the four BCCs tested are listed in Table 2. The most important are: rapid diffusion into the extracellular fluid, no intracellular uptake in any organ and rapid excretion through the kidneys. High specific activity (>1000 Ci/mmol) of effector molecules is also necessary for receptor (antigen) targeting at nanomolar concentrations. The speed and ease of radiolabeling highly purified low molecular weight BCCs at high concentration and/or high temperature makes high specific activity readily obtainable with no-carrier-added radionuclides such as ^{99m}Tc, ^{111}In, ^{90}Y and ^{68}Ga (*35*). The half-life of the biotin-LC-DOTA in the blood was sufficient for targeting, suggesting that it was not cleaved by biotinidase in vivo as rapidly as the deferoxamine derivative of LC-biotin described by Rosebrough (*41*) was cleaved in vitro.

Failure in these initial mouse experiments of anti-IA^k MAb–streptavidin conjugate to bind BCC after pretargeting may have been due to rapid internalization of MAb–streptavidin–IA^k complex by the lymphocyte or to endogenous biotin. Either or both of these factors would make streptavidin unavailable to subsequently administered BCC. The former explanation is more likely because pretargeted streptavidin was still able to bind BCCs almost quantitatively up to 6 hr (Fig. 4) and, to a lesser extent, up to 24 hr after injection. Also, pretargeted streptavidin binding sites were still available in the kidney up to 21 hr after injection, indicating that they had not been blocked by endogenous biotin. Furthermore, using pretargeted carcinoembryonic antigen markers known not to internalize, excellent

localization of ^{111}In-BCC has been obtained with the avidin/biotin system in humans (*42*).

Recent preliminary clinical results of pretargeted ^{90}Y radioimmunotherapy using the avidin/biotin system have been very promising. Paganelli et al. (*34*) have reported a Phase I–II clinical trial in 11 patients using ^{90}Y-labeled biotinyl-LC-DOTA in a three-step protocol. Each patient received 30–50 mg of biotinylated MAb FO23C5 (anti-carcinoembryonic antigen), followed 36 hr later by an avidin chase and streptavidin, and 18–24 hr later, a second chase of biotinylated human serum albumin, followed immediately (10 min) by 5 mg of ^{90}Y-labeled biotinyl-LC-DOTA. Doses ranging from 1.85 to 5.55 GBq (50–150 mCi) of ^{90}Y bound to 5 mg of biotinyl-LC-DOTA were administered without any acute toxicity. One patient with brain tumor achieved >50% regression, whereas another showed total regression of one of his metastases. Overall, 4 of 11 patients were still in partial remission 1–5 mo after therapy.

A Phase I/II clinical pretargeted ^{90}Y RAIT trial is currently being performed by NeoRx Corporation (Seattle, WA) in human colon, lung and ovarian cancer. The pretargeted pancarcinoma MAb NR-LU-10-streptavidin conjugate is being used with ^{111}In-labeled (for dosimetry) and ^{90}Y-labeled (for therapy) biotin-DOTA. The goal is to demonstrate the pretargeting principle and lack of toxicity in humans and then to see if the the excellent preclinical therapy results in tumor mice (*43*) can be reproduced on scaling up to patients with cancer.

CONCLUSION

Biotinyl-LC-DOTA, biotin-P-DOTA and biotin-P-CITC bind the diagnostic and therapeutic radiometals ^{111}In and ^{88}Y with excellent in vivo stability and have the required physiological properties for pretargeted diagnosis and therapy. Although kidney uptake of streptavidin may be a potential problem in RAIT using the biotin/streptavidin system, renal uptake was much lower when streptavidin was conjugated to MAb.

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