Investigations of Avidin and Biotin for Imaging Applications

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The attractive properties of avidin (streptavidin) and biotin, in particular their strong affinities ($K_d = 10^{-15}M$), may be used to advantage in imaging applications. These molecules have been used in this preliminary investigation to improve the targeting of ^{111}In in animals. Antibodies have been conjugated with biotin and administered unlabeled while, at a later time, the radiolabel was administered attached to DTPA-coupled avidin or streptavidin. An alternative procedure was also considered whereby the antibodies were conjugated with avidin and administered before the administration of radiolabeled biotin. Using a model in which the target consisted of conjugated agarose beads deposited in the peritoneum of mice, it has been shown that the target/nontarget radioactivity ratios may be significantly improved with respect to the conventional procedures through the use of this approach.

J Nucl Med 28:1294–1302, 1987

A serious limitation to the in vivo use of radiolabeled antibodies for tumor localization results from the poor target/nontarget ratios which have been universally reported both in animal and patient studies (*1*). It is now clear that poor tumor localization of radioactivity is due to a combination of factors operating both in tumor (limited blood flow, limited antigen concentration) and in normal tissues (catabolism of antibody in liver, spleen) (*2*). Thus, poor localization may be expected when the radiolabel is administered in the conventional manner, i.e., attached to antibody. It is possible that improvements may result from novel procedures whereby the antibody is administered unlabeled and allowed to clear from circulation before the administration of the radioactivity. Examples of this approach have been reported in preliminary form and involve bifunctional IgG or $F(ab')_2$ antibodies in which one combining site is directed against tumor while the other is directed against a low molecular weight chelate of indium-111 (^{111}In) (*3,4*).

This laboratory is investigating the use of avidin and biotin for this and other in vivo applications. Avidin, a 66-kD protein found in egg whites, displays a strong avidity for biotin, a 244-D vitamin found in low concentrations in tissues and in blood (*5*). The association constant of the avidin-biotin bond is $10^{15}M^{-1}$ (*6*) and, as such, is 1 million-fold greater than most antigen-antibody interactions. Bond formation is rapid, and once formed, the bond is stable to extremes of pH, organic solvents and other denaturing agents (*7*). It is therefore not surprising that this interaction has been exploited in numerous in vitro applications including competitive binding assays where avidin is labeled either with radioactivity, fluors or enzymes, and used in a sandwich assay to detect biotinylated antibody (*8*). Furthermore, avidin has been conjugated with cytotoxic drugs and used to target tumor through biotinylated tumor-associated antibodies (*9*).

It is possible that avidin and biotin may be useful in vivo as well. Firstly, biotin (naturally occurring vitamin H) and avidin are likely to be nontoxic at the levels contemplated in this research (*10*). Secondly, the large affinity suggests that the avidin-biotin bond may be stable in vivo. Accordingly, the research described herein was intended to evaluate several in vivo applications in which antibodies, coupled either with biotin or avidin, are administered to animals followed by the administration of ^{111}In-labeled avidin or biotin respectively. In these studies, streptavidin as well as avidin was employed since both show similar affinities for biotin but have different biologic behaviors. Streptavidin, a 60-kD protein obtained from streptomyces avidinii (*11*) is similar to avidin in most respects; however, it is not glycosylated and is reported to show less nonspecific binding to tissues (*7,11,12*).

Received July 22, 1986; revision accepted Feb. 24, 1987.

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MATERIALS AND METHODS

Coupling of DTPA to Avidin and Streptavidin

Avidin* and streptavidin† were both conjugated with DTPA using the cyclic anhydride of DPTA‡ (*13*). The proteins were prepared at a concentration of 20 mg/ml in 0.05*M* bicarbonate buffer, pH 8.4 under conditions in which an average of 1–2 DTPA groups were attached to each protein molecule. The degree of conjugation is not critical since both proteins are tetravalent with respect to biotin and probably for this reason, it has been possible to attach 11 DTPA groups per avidin molecule without detectable effect on biotin binding (unpublished observations).

Coupling of Biotin to Antibody

The conjugation of biotin to antibody was accomplished through the use of sulfosuccinimidyl 6-(biotinamido) hexanoate (NHS-LC-biotin)‡. This biotinylating reagent couples primarily to lysine residues and contains a six-carbon spacer to reduce steric hindrance. In a typical reaction, IgG was used at a concentration of 20 mg/ml in 0.05*M* bicarbonate buffer pH 8.5. The solution was gently stirred on ice and NHS-LC-biotin in saline added at the desired molar ratio, usually between 0.25 and 5:1 with respect to IgG. The reaction was allowed to proceed for 2 hr and the product was separated from free biotin by centrifugation three times in a Centricon-30 microconcentrator§ Following each centrifugation, the sample was diluted in 0.1*M* phosphate, pH 7.0 and the final protein concentration was 12–20 mg/ml.

Determination of Biotin Groups per Antibody Molecule

The average number of biotin groups attached to each IgG molecule was determined spectrophotometrically by the method of Green et al. (*14*). The assay uses the change in absorbance at 500 nm which occurs when avidin, saturated with HABA ((2–4′-hydroxyazobenzene)-benzoic acid)*, interacts with free biotin. Briefly, the biotinylated antibody was heated to 56°C in 0.1*M* phosphate buffer for 10 min and then digested enzymatically with small volumes of 1% pronase*. The digestion was allowed to proceed at room temperature overnight. For the assay, 10 μM solution of avidin in 0.1*M* phosphate buffer was saturated with a 100-μM solution of HABA in 0.1*M* phosphate buffer, pH 7.0. The avidin-HABA solution was then titrated with increasing volumes of either the digested biotinylated IgG solution or a standard biotin solution containing 1.7 nmol of biotin, and the change in absorbance of each determined at 500 nm. From the standard curve, the concentration of biotin in the pronase-treated antibody solution could be calculated.

Avidin-Biotin Serum Stability

Avidin, conjugated with an average of 2.2 DTPA groups per molecule, was labeled with ^{111}In and added to a suspension of biotin conjugated agarose beads*. After a 45-min incubation, unbound avidin was removed and the beads were added to sterile fresh human serum to a concentration of 10 μg/ml with respect to avidin. The suspension was placed in a shaking water bath at 37°C. Initially and periodically the sample was centrifuged so that 100 μl of supernatant could be removed for counting along with a standard of the added activity. The percentage of added activity which was bound to the beads was thus determined at several time points during 24 hr.

Similarly, the stability of the avidin-biotin bond in 37°C serum was also determined by adding ^{111}In-labeled biotin to an excess of avidin conjugated agarose beads‡ and shortly thereafter adding these labeled beads to serum with sampling as above.

Coupling of Avidin to Antibodies

In this research, avidin was bound to antibodies previously conjugated to biotin. The tetravalency of avidin for biotin is here a disadvantage since dimeric and polymeric species may form which consist of several antibody molecules bound together. If high molecular weight species do form, their presence will be readily apparent in both the radioactivity and uv traces obtained by size exclusion HPLC as species with molecular weights in excess of the 220 kD expected for monomeric avidin-IgG. To avoid the formation of these unwanted species, several approaches were considered in which a threefold molar excess of biotin was added to the avidin solution just prior to or just after the addition of the biotinylated antibody. The former approach was found to be less effective in minimizing high molecular weight species. Accordingly, the procedure which was ultimately adopted involved first labeling the DTPA-conjugated avidin or streptavidin with ^{111}In in solutions containing about 1 mg/ml of protein in saline. An equal molar amount of biotinylated antibody in 0.1*M* phosphate buffer was then added with mixing. Seconds later, hydrolyzed NHS-LC-Biotin was added such that the free biotin:avidin molar ratio was 3:1.

Molecular weights of the conjugated antibodies were determined by size exclusion HPLC using a TSK 400 column¶ with 0.1*M* phosphate buffer, pH 7.0 at a flow rate of 0.2 ml/min. An in line-radioactivity detector and a uv detector operating at 280 nm were used to obtain the profile of both the radiolabeled antibody under investigation and molecular weight standards¶ The system was calibrated by determining the partition coefficient of each standard which was then plotted against the log of molecular weight (*15*). In this determination, the total bed volume was estimated by the dimensions of the HPLC column while the column void volume was measured. The retention time of antibody peaks was then applied to this calibration curve to derive an estimate of apparent molecular weight.

Whereas the above conjugation procedure offers a convenient route to the preparation of avidin-conjugated antibodies, the product is saturated with biotin and unusable for studies in which the conjugate is required to bind biotin. Consequently, an alternative procedure was also developed. A 20 mg/ml solution of IgG antibody (containing an average of 1.2 DTPA group per molecule and radiolabeled with ^{111}In) was reacted at a 0.7:1 molar ratio of NHS-LC-biotin to IgG in 0.05*M* bicarbonate buffer pH 8.0 for 5 hr. The reaction mixture was added to a 20 mg/ml solution of avidin such that the avidin:IgG molar ratio was 5:1. Soon thereafter, the product was purified by size exclusion HPLC using a TSK 250 column¶. Following purification, the biotin binding capacity of the product was established through the use of biotin-conjugated beads. For certain studies, the binding capacity of the product towards Protein-A-conjugated beads was also determined.

Labeling of Biotin

In addition to evaluating the in vivo properties of ^{111}In-labeled avidin and streptavidin, it was of interest to also

evaluate ^{111}In-labeled biotin. Therefore DTPA was covalently coupled to biotin through the use of the cyclic anhydride of DTPA and biocytin*, a lysine conjugate of biotin with an available primary amine for conjugation. Coupling was achieved initially in 0.05*M* bicarbonate buffer, pH 8.2, at a biocytin concentration of 10 mg/ml and a 1:1 anhydride:biocytin molar ratio. An aliquot of the preparation was radiolabeled with ^{111}In at tracer levels, added to an excess of avidin in the bicarbonate buffer and allowed to incubate at room temperature for 2 min. Size exclusion HPLC demonstrated that 75% of the activity was associated with labeled avidin and, therefore, that the coupling efficiency was 75%. Free DTPA was not removed from this coupled biotin preparation.

Subsequently, the conjugation was performed in nonaqueous solution. Fifty milligrams of biocytin was reacted with 10 mg of the cyclic anhydride (molar ratio 5:1) in 5 ml of dimethylsulfoxide at 117°C for 5 min. The reaction product was precipitated by the addition of 50 ml of acetonitrile and purified by repeat centrifugations between rinses in acetonitrile. After drying from traces of solvent, the product was dissolved in 2 ml of distilled water and added to a 0.9 cm × 42 cm column of P-2 gel¶ operated at 75°C to maintain solubility. The column was eluted with distilled water and the eluant monitored by uv absorbance at 220 nm. The ability of this and previous preparations of DTPA-conjugated biotin to bind to avidin was confirmed through the use of avidin-conjugated beads.

Besides DTPA-conjugated biotin, the in vivo clearance properties of other biotinylated compounds were also investigated. Six water soluble proteins ranging in molecular weight from 24 kD (trypsinogen) to 150 kD (IgG) were conjugated with both DTPA and biotin. Each protein was prepared in 0.05*M* bicarbonate buffer, pH 8.0, at a concentration of 20 mg/ml and coupled with DTPA at a 1:1 anhydride to protein molar ratio. Without purification from free DTPA, a quantity of NHS-LC-biotin as a 10 mg/ml solution in 0.05*M* bicarbonate buffer was added such that the biotin:protein molar ratio was 50:1. After labeling with ^{111}In each protein was purified by size exclusion HPLC using a I-60 column**. The avidin-binding ability of each labeled and purified protein was confirmed by a 2-min incubation in saline with avidin-conjugated agarose beads followed by filtration, rinsing and counting of the beads.

Biodistribution Studies

An important aspect of this research concerns the effect on the biodistribution of an antibody resulting from attachment of a large protein such as avidin or streptavidin. Accordingly, the biodistribution in mice was determined for ^{111}In-labeled avidin conjugated IgG antibody. These studies consistently showed large liver activity levels possibly due to nonspecific binding of avidin through its glycosylated regions to receptors within the liver (*12*). For this reason, these studies were subsequently repeated with streptavidin in place of avidin.

Streptavidin was conjugated with an average of 1.6 DTPA groups per molecule while nonspecific human IgG* was conjugated with an average of 0.25 biotin groups per molecule via NHS-LC-biotin. Streptavidin was then conjugated to IgG following the procedure described above which results in saturation of the biotin binding sites of streptavidin. The protein preparation was analyzed by size exclusion HPLC principally to establish the molecular weight of the labeled antibody. A control protein consisting of the same IgG antibody but conjugated directly with an average of 2.0 DTPA groups per molecule was also prepared. The specific activity of both preparations was ~1 μCi/μg. Both were diluted to a concentration of 50 μg/ml in 0.1*M* phosphate buffer, pH 7.0, and 0.1 ml administered by tail vein injection into male CD-1 mice. The animals were killed at 24 hr postadministration and tissues and blood removed for counting against a standard of the injectate.

Imaging Studies

Several imaging studies were performed to explore various applications of the use of avidin and biotin. In the first such study, biotin-conjugated beads were administered subcutaneously to a rat followed by the intravenous administration of [^{111}In]streptavidin. In the second study, avidin conjugated beads were administered intraperitoneally to mice to serve as targets for the i.p. administration of ^{111}In biotin. Finally, Protein-A conjugated beads were administered intraperitoneally to mice to serve as targets for the i.p. administration of avidin-conjugated antibody, the presence of which was determined by the subsequent administration of ^{111}In-labeled biotin. Protein-A binds the Fc region of antibodies.

In the first study, 100 μl of a suspension of biotin-conjugated agarose beads, 45–165 μm in diameter containing ~120,000 particles and 0.1 μmol of bound biotin were injected subcutaneously in the right arm pit of a male Sprague-Dawley rat††. One-half hour later, streptavidin, conjugated with an average of 2.2 DTPA groups per molecule and labeled with ^{111}In to a specific activity of 2 μCi/μg, was administered by i.p. injection. A total of 25 μg or 0.4 nmol of streptavidin was administered in 100 μl of saline. The animal was imaged four times in the following 7 days using a Picker camera‡‡ fitted with a pinhole collimator.

In the second study, two male CD-1 mice were injected i.p. in the upper left quadrant with avidin-conjugated beads. One animal received ~120,000 particles, 45–165 μm in diameter* containing 3 nmol of bound avidin and delivered in 0.5 ml of saline. The control animal received approximately the same number of underivatized Sepharose beads*, 45–165 μm in diameter, in the same way. Immediately following particle administration, each animal was injected i.p. in the lower right quadrant with 0.4 μg, 0.5 nmol of ^{111}In-labeled biotin with a specific activity of 140 μCi/μg and containing 24% labeled free DTPA. Both animals were counted repeatedly in an ionization chamber over 2 hr to measure whole-body activity and were then imaged.

The final imaging study was conducted to investigate further the use of avidin and biotin to improve tumor localization in the peritoneum. Two CD-1 male mice each received 2 mg of Protein-A conjugated agarose beads, 65–145 μm in diameter* containing 0.4 nmol of bound Protein-A and delivered into the peritoneum (upper left quadrant) in 0.2 ml of saline. These two animals along with an additional two who did not receive the beads immediately received in the peritoneum (lower left quadrant) 50 μg of avidin-conjugated IgG in 400 μl of 0.1*M* phosphate buffer pH 7.0. Twelve hours later, each mouse received an IP injection (lower right quadrant) of 50 ng of DTPA-biotin (40 μCi) in 100 μl of saline. These animals were killed 6 hr later.

Control animals for this study consisted of two mice who

received the Protein-A beads and two mice which did not. All four of these animals received an i.p. injection of 50 μg of avidin-conjugated IgG radiolabeled with 19 μCi of ^{111}In on the antibody. All four were killed 42 hr postinjection.

The animals were imaged prior to being killed using the Picker camera with a pinhole collimator. After the animals were killed by etherization, the intestines (where the particles become lodged), liver, spleen and kidneys were removed, and along with the carcass, activity was determined in an ionization chamber.

This latter experiment was repeated to evaluate the influence of time between the administration of avidin-IgG and [^{111}In]biotin. In this case six animals each received 3 mg of the Protein-A beads suspended in 100 μl of saline in the peritoneum (upper left quadrant). Immediately thereafter, 50 μg of avidin-IgG was delivered in 200 μl (lower right quadrant). At each time point (0, 2, or 8 hr), two mice were each administered 50 ng of [^{111}In]biotin (28 μCi) and all animals were killed at 3 hr postadministration of the activity.

RESULTS

Serum Stability

Biotin-conjugated agarose beads were employed to determine the stability of the avidin-biotin bond in 37°C serum. After 24 hr of incubation in this medium, 4% of the activity was released from the beads.

The stability of the avidin-biotin bond in 37°C serum was also investigated through the use of avidin conjugated agarose beads and ^{111}In-labeled biotin. After suspension of the beads in serum, the label was found to dissociate over a period of 45 hr at a rate of 8%/day. Dissociation at this rate may be attributed entirely to transcomplexation of label to transferrin rather than to dissociation of the avidin-biotin bond (*13*).

Biodistribution Studies

Applications in which radiolabeled biotin is administered to subjects subsequent to the administration of avidin-conjugated antibody require that the attachment of avidin or streptavidin does not seriously alter the in vivo behavior of an antibody. Therefore the biodistribution in normal mice was determined for streptavidin-conjugated IgG in which the DTPA groups and thus the ^{111}In label were placed on the streptavidin and in which the conjugation was achieved through biotin attached to the antibody. The procedure used in this study achieves monomeric conjugation through the saturation of biotin binding sites on streptavidin as described in the previous section. As a control, the same IgG antibody was radiolabeled with ^{111}In through attachment of DTPA in the usual manner. Figure 1 presents the radioactivity trace obtained by size exclusion HPLC analysis of the streptavidin-conjugated antibody. Superimposed on the figure is the uv trace obtained for several molecular weight standards. A calibration curve resulting from plotting the partition coefficient versus the log of molecular weight for these standards is linear with only modest scatter and was used to estimate the molecular weights of the observed species. Unbound streptavidin is apparent as a shoulder in the radioactivity trace and free labeled DTPA appears as a small peak at longer retention times. The major radioactivity peak has a rather broad molecular weight distribution with a mean value of about 240 kD in close agreement with the 220 kD expected for one IgG molecule bound to one streptavidin.

Table 1 presents biodistribution results obtained for the above protein and for the same IgG antibody labeled with ^{111}In in the conventional manner through direct attachment of DTPA. With the exception of heart, there are no significant differences at the $p < 0.02$ level in the two biodistributions.

Figure 2 presents the results obtained in the determination of whole-body activity following tail vein administration in mice of ^{111}In-labeled biotin and six proteins by varying molecular weight conjugated with biotin and radiolabeled with ^{111}In through attached DTPA groups. Prior to administration, each protein preparation was determined by size exclusion HPLC to be >90% radiochemically pure and by avidin-conjugated beads to display 75% or greater binding to avidin. The ^{111}In-labeled biotin clears most rapidly while in the case of the proteins, clearance is roughly proportional to molecular weight.

Imaging Studies

Figure 3 presents whole-body images obtained in a rat injected i.p. with ^{111}In-labeled streptavidin following

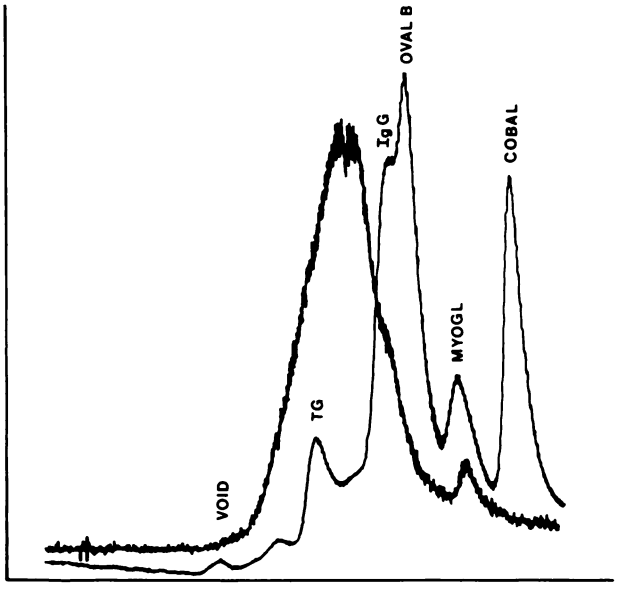

FIGURE 1
Radioactivity trace obtained by size exclusion HPLC analysis of DTPA- coupled streptavidin, labeled with ^{111}In and attached to biotinylated IgG antibody. Superimposed on the figure is the uv trace obtained for several molecular weight standards.

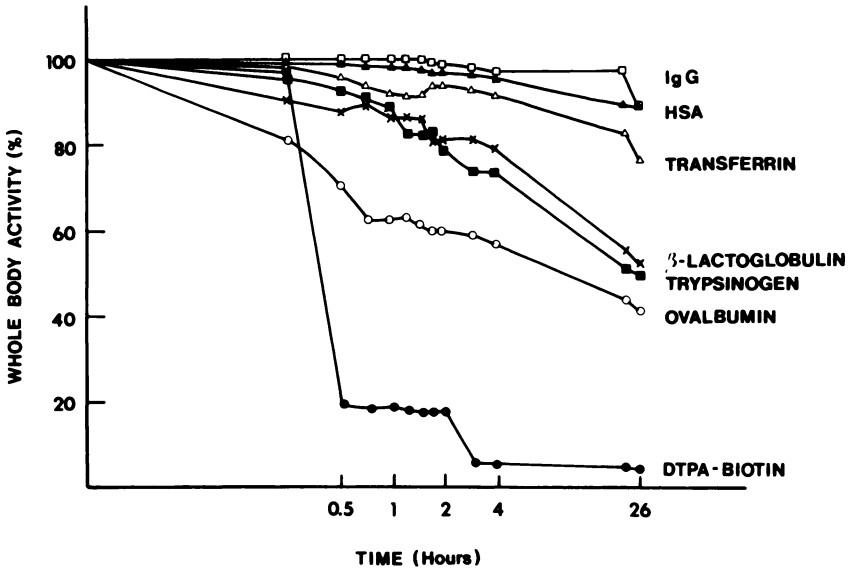

FIGURE 2
Whole-body radioactivity in mice following i.v. administration of ^{111}In-labeled biotin and six ^{111}In-labeled proteins conjugated with biotin. Semilog scale.

TABLE 1
Mouse Biodistribution at 24 hr for DTPA-IgG and DTPA-streptavidin-IgG Both Labeled with ^{111}In*

Organ	IgG	Streptavidin-IgG
Liver	5.9 (0.6)	5.0 (0.5)
Heart	1.9 (0.1)	2.2 (0.2)
Lungs	2.4 (0.9)	3.1 (0.6)
Kidneys	9.8 (0.9)	10.2 (2.1)
Stomach	0.8 (0.1)	1.0 (0.2)
Spleen	2.5 (0.2)	2.9 (0.4)
Bone	4.4 (0.5)	3.7 (0.9)
Muscle	1.2 (0.2)	1.2 (0.1)
Blood	8.9 (1.1)	7.6 (0.5)

* Percent injected dose per gram, normalized, (s.d.), N = 5.

a subcutaneous injection in the right arm pit of biotin-conjugated beads. Each image was collected for the same number of counts; the image on the left was obtained at 1 hr, the middle image at 24 hr and the right image at 48 hr postadministration. The accumulation of activity at the site of particle deposition is apparent in both of the latter images. A final image obtained at 5 days postadministration failed to show this accumulation, possibly due to count rate limitations.

Figure 4 shows the decreasing whole-body activity in two mice injected i.p. with ^{111}In-labeled biotin after the i.p. administration of avidin-conjugated beads or, in the case of the control, underivatized beads. As shown in the control study and as shown in Figure 2, the biologic half-time of labeled biotin is ~30 min. Whole body clearance in the case of the animal administered the avidin beads is also rapid, however, approximately four times greater activity is eventually retained in this animal with respect to the control. This observation is confirmed in the images shown in Figure 5. Both animals were imaged at 2 hr postadministration and for the same counting time. In the control animal (left panel), only kidneys and bladder are apparent. In the

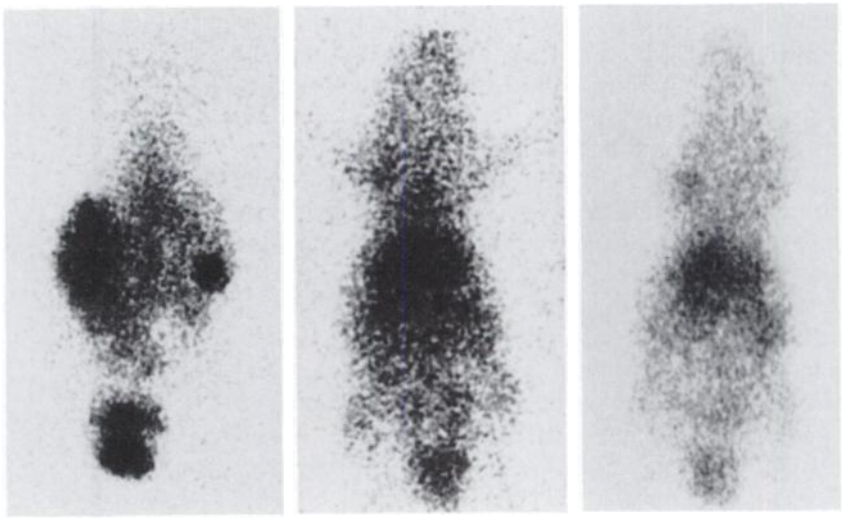

FIGURE 3
Whole-body images of a rat with biotinylated beads implanted in the right arm pit and injected intraperitoneally with ^{111}In-labeled streptavidin. Left image obtained at 1 hr, middle image at 24 hr and right image at 48 hr postadministration.

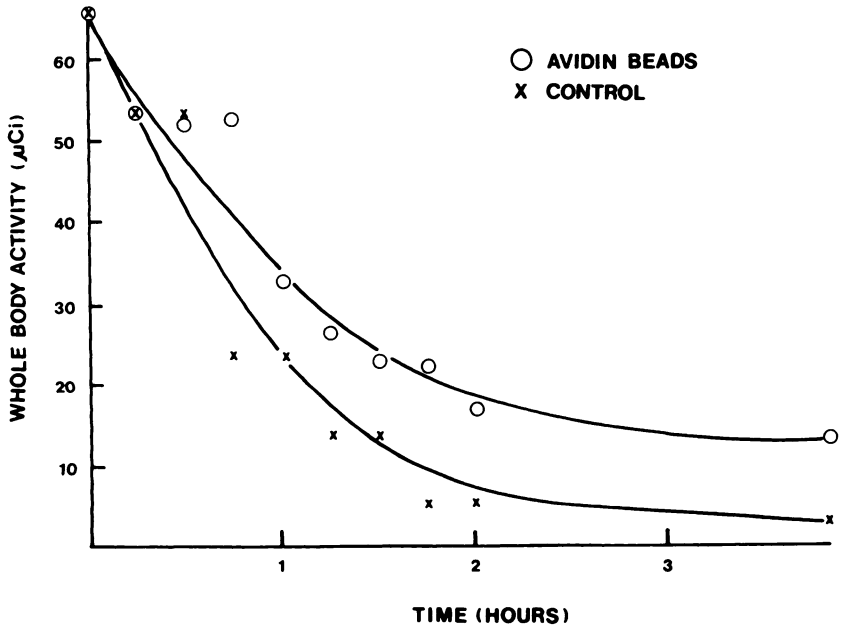

FIGURE 4
Whole-body clearance curves for two mice injected intraperitoneally with [^{111}In]biotin. One animal (control) had previously received an intraperitoneal injection of underivatized beads while the other animal received avidin-conjugated beads.

case of the animal administered avidin-conjugated beads in the upper left quadrant of the peritoneum (right panel), kidneys and bladder are again apparent along with an intense area of activity corresponding to the location of the beads. Absence of activity in the coat of this animal was confirmed by killing and dissection to eliminate the possibility that this activity was due to external contamination.

Table 2 and Figures 6–8 describe studies in which Protein-A conjugated beads were administered i.p. in mice followed by avidin-conjugated IgG antibody and then by ^{111}In-labeled biotin. Table 2 lists the activity accumulated in several organs and carcass 42 hr following i.p. administration of ^{111}In-labeled avidin-IgG as controls in mice with and without the Protein A beads. Also listed are activity levels in mice with beads who received [^{111}In]biotin i.p. 12 hr after a prior administration of unlabeled avidin-IgG, with mice killed 6 hr later. It may be seen that in the case of the controls, the presence of the beads had only a minor influence on

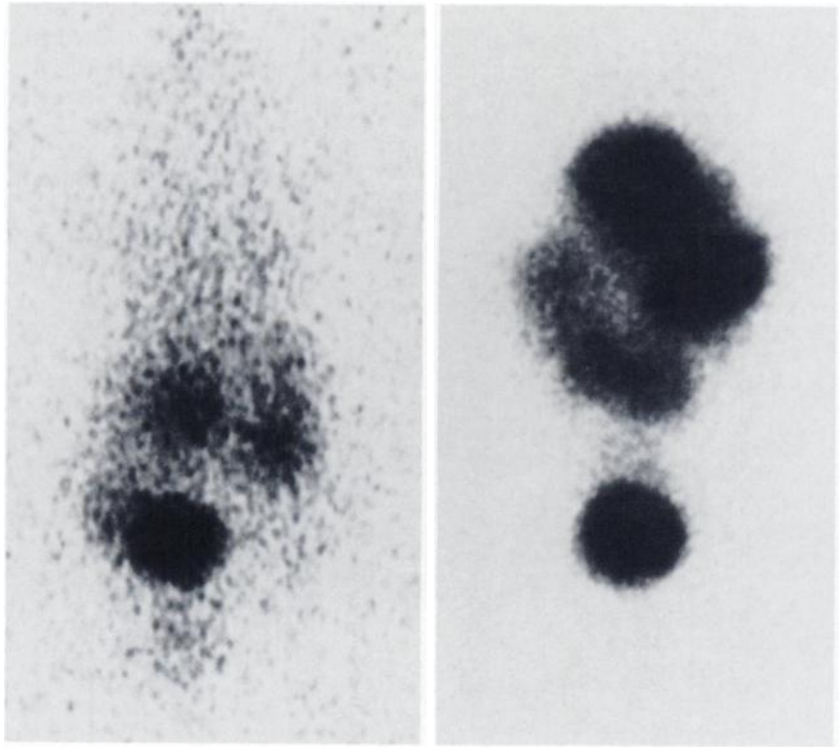

FIGURE 5
Whole-body images of two mice 2 hr following intraperitoneal administration of [^{111}In]biotin. One animal (left panel) previously received an intraperitoneal injection of underivatized beads while the other (right panel) received avidin-conjugated beads. Both animals were counted for the same length of time.

TABLE 2
Biodistribution of $^{111}In^{*}$

First injection	Second injection	Beads	Biodistribution (μCi/organ)†				
			Liver	Spleen	Kidneys	Intestines	Carcass
^{111}In-Avidin-IgG	None	yes	2.2	0.4	0.2	1.6	8.6
^{111}In-Avidin-IgG	None	no	2.9	0.1	0.3	0.8	7.4
Avidin-IgG	[^{111}In]biotin	yes	0.1	0.01	0.06	3.7	0.1

* Biodistribution of ^{111}In following i.p. administration of labeled (19 μCi) avidin-conjugated IgG antibody (controls) in mice with and without prior i.p. administration of Protein-A beads. Data compared to the biodistribution of ^{111}In following first administration of unlabeled avidin-conjugated IgG followed by second administration of radiolabeled biotin (40 μCi) in mice implanted with Protein-A beads.

† Mean values, N=2

biodistribution with activity levels in the intestines (the site of bead attachment) slightly higher in the animals receiving the beads with respect to those which did not. However, in the animals receiving the labeled biotin activity accumulation in all organs other than intestines is virtually at background levels with virtually the entire whole-body activity burden located at the site of bead deposition in the intestines. These results are displayed in Figure 6 as target (intestines) to organ ratios and show that in the case of the two controls, with the exception of spleen, this ratio is slightly superior in animals receiving the beads. However, in the case of animals receiving the labeled biotin, the ratio exceeds 10:1 in the cases and exceeds 100:1 in the case of spleen. Images obtained also show the favorable localization in the case of an animal receiving the labeled biotin. Figure 7 presents whole-body images of control animals who received labeled avidin-antibody with (left panel) and without (middle panel) beads along with an image of an animal who received the beads, unlabeled avidin-antibody, and the labeled biotin (right panel).

Figure 8 presents the results of a repeat study in mice, all of which received the Protein-A beads and the unlabeled avidin-antibody and, either 0, 2 or 8 hr later, received the labeled biotin. It may be seen that the target (intestines) to organ ratios steadily improve with time between the latter two administrations such that in the case of spleen and kidneys this ratio exceeds 1,000:1.

DISCUSSION

In this research we have demonstrated that biotin will bind rapidly to avidin and streptavidin, that this bond is stable in serum and that bond formation will occur in vivo. Consequently, a number of imaging procedures involving the use of these molecules suggest themselves. One application will involve the initial administration of biotinylated antitumor antibody to a tumor-bearing subject to be followed, after the expression of biotin has decreased in normal tissue (due to

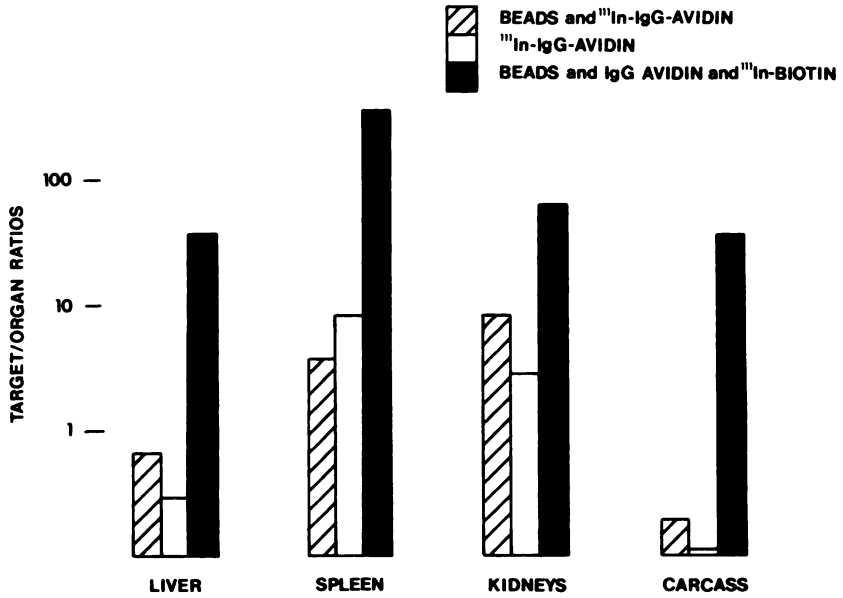

FIGURE 6
Histograms showing the ratio of radioactivity in the target (intestines) to that in three organs and in the carcass for mice receiving ^{111}In-labeled avidin-IgG IP with (cross hatched bars) and without (empty bars) implantation of protein-A beads in the peritoneum. The solid bars present results obtained for mice implanted with beads but receiving unlabeled avidin-IgG followed by ^{111}In-labeled biotin (see text). Semilog scale.

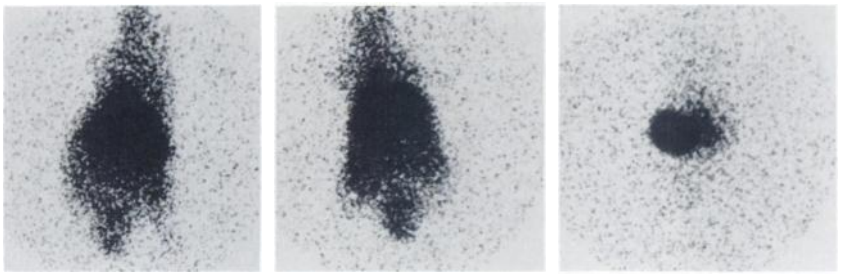

FIGURE 7
Whole-body images of mice at ~18 hr post i.p. administration of ^{111}In-avidin-IgG in mice with (left panel) and without (middle panel) prior implantation in the peritoneum of Protein-A beads. Right panel is a whole-body image obtained at ~18 hr postadministration of unlabeled avidin-IgG and 6 hr postadministration of [^{111}In]biotin in mice implanted in the peritoneum with protein-A beads (see text).

clearance and catabolism) and before expression has decreased in the target, by the administration of radiolabeled avidin or streptavidin. The study of which Figure 3 is a part illustrates this application and demonstrates a potential difficulty arising from the slow blood clearance of labeled streptavidin and the accumulation of label in normal tissues. Consequently, target to normal tissue ratios have not necessarily been improved over that now achieved by the conventional one-injection imaging procedures in routine practice. However, it is possible that localization may be improved through the intermediate i.v. administration of unlabeled avidin at a concentration which will form avidin-biotin-IgG aggregates in serum. Following clearance of these aggregates, localization may be achieved by the i.v. administration of radiolabeled biotin since biotin expression on the tumor would presumably now be replaced with avidin.

An attractive alternative to the use of biotinylated antibody is to administer the antibody conjugated to avidin or streptavidin followed by the administration of radiolabeled biotin. As has been shown, ^{111}In-labeled biotin rapidly clears the whole body of mice. Thus, activity not bound to the target may clear from the subject rapidly rather than remain in circulation or accumulate in normal tissues. However, the rate of clearance of circulating biotin may be slowed, as shown in Figure 2, by attaching biotin to a protein with the desired molecular weight. The study of which Figure 5 is a part illustrates one application in which the label localized almost exclusively on avidin conjugated beads implanted in the peritoneum following i.p. administration of labeled biotin. In this case, the rapid clearance of ^{111}In-labeled biotin was an advantage.

A similarly favorable result with regard to the localization of the label in a target within the peritoneum was achieved in a fashion more closely resembling the clinical situation. Avidin-conjugated antibody was administered i.p. and after clearance of the antibody (and therefore of avidin expression) labeled biotin was administered i.p. The absence of avidin except at the target site permitted unhindered clearance from the peritoneum and from the animal of the excess labeled biotin not bound to the target. Using Protein-A conjugated beads to simulate tumor, target to nontarget ratios were improved by more than two orders of magnitude over the conventional use of a single administration with radiolabeled antibody.

This research suggests that imaging and therapy studies may be substantially improved through the use of

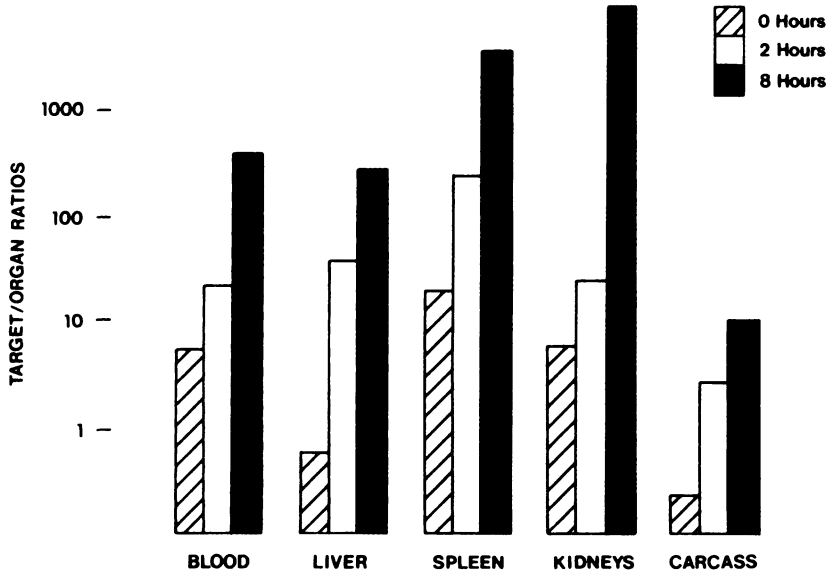

FIGURE 8
Histograms showing the ratio of radioactivity in the target (intestines) to that in blood, three organs and carcass for mice with protein-A beads in the peritoneum and receiving i.p. administration of unlabeled avidin-IgG with 0 hr (cross hatched bars), 2 hr (empty bars) and 8 hr (solid bars) between this injection and an i.p. administration of [^{111}In]biotin. Semilog scale.

avidin or streptavidin and biotin labeled with a variety of radionuclides and used in conjunction with molecules such as antibodies which target in a manner useful in nuclear medicine.

NOTES

* Sigma Chemical Co., St. Louis, MO.
† Calbiochem, Behring Diagnostics, La Jolla, CA.
‡ Pierce Chemical Co., Rockford, IL.
§ Amicon Corp., Danvers, MA.
¶ Bio-Rad Laboratories, Richmond, CA.
** Waters Associates, Medford, MA.
†† Charles River Supply, Wilmington, MA.
‡‡ Picker International, Highland Heights, OH.

ACKNOWLEDGMENTS

The authors thank Maurissa Gionet for her assistance in the imaging studies. The authors also thank Medi-Physics for supplying the ^{111}In used in this work. This work was supported in part by NIH Grant CA 33029.

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