Localization of Infection Using Streptavidin and Biotin: An Alternative to Nonspecific Polyclonal Immunoglobulin

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Since favorable images of infection are obtained with radiolabeled nonspecific IgG, streptavidin has been considered as an alternative protein in this investigation. The advantage of streptavidin is that once localized it may be targeted with radiolabeled biotin. Studies were conducted in a mouse model with an *Escherichia coli* infection in one thigh. Indium-111-labeled streptavidin showed equivalent localization to the infection as that obtained with ^{111}In-labeled polyclonal nonspecific IgG, however blood levels with streptavidin were lower at all time points; consequently, target-to-blood ratios were improved. Pretargeting with unlabeled streptavidin followed 3 hr later with ^{111}In-labeled biotin showed equivalent localization in the target and reduced activity in all organs sampled. As such, infected thigh-to-normal thigh ratios were improved 3-fold for pretargeting versus either labeled IgG or streptavidin. Improvements in infected thigh-to-liver and blood ratios were greater than 8-fold. Only in the case of kidneys was the ratio unimproved. In conclusion, we have shown that by preadministration of unlabeled streptavidin followed by labeled biotin, infectious lesions in a mouse model may be imaged earlier with lower background levels relative to the administration of labeled nonspecific IgG.

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The use of radiolabeled nonspecific polyclonal immunoglobulin (IgG) to image sites of infection has been shown to be due to nonspecific accumulation of the protein resulting from increased vascular permeability (*1*). As such, other proteins may be considered for this application and, in fact, radiolabeled albumin has been employed as an alternate agent to image infection (*2–4*). However, a general difficulty in the use of radiolabeled proteins is the excessive accumulation in normal organs such as the liver and the slow clearance of the label from circulation, both of which restrict early imaging. This situation has not been improved through the use of ^{99m}Tc-labeled proteins (*2,3, 5–8*).

A potential solution is to deliver the radiolabel on a compound which clears rapidly. The "pretargeting" concept, with the preadministration of unlabeled bifunctional antibody followed some time later with the administration of a radiolabeled hapten has been applied to tumor imaging (*9*). The use of an antibody conjugated to streptavidin to pretarget tumors in animals (*10*) and patients (*11*) prior to the administration of radiolabeled biotin has provided encouraging results because of the high affinity of biotin for streptavidin and the rapid whole body clearance of biotin.

In this study, pretargeting was applied to infection imaging. Unlabeled streptavidin administered before the administration of radiolabeled biotin was compared to the administration of ^{111}In-labeled nonspecific IgG and ^{111}In-labeled streptavidin alone in mice with an *Escherichia coli* infection in one thigh. A further objective of this investigation was to compare two ^{111}In-labeled biotin derivatives for this application: DTPA-bis-biocytinamide and EDTA-hydrazino-biotin.

MATERIALS AND METHODS

Radiolabeling

Nonspecific polyclonal IgG (Baxter Healthcare Corp., Glendale, CA), extensively dialyzed against saline to remove stabilizing agents, was coupled with the cyclic anhydride of DTPA (*12*) to an average of 1.8 groups per molecule. The coupled antibody was then radiolabeled with ^{111}In (Dupont-NEN, N. Billerica, MA) to a specific activity of 1 μCi/μg and purified before administration by passage through a 1 × 8 cm column of Sephadex G-50 with saline as eluant.

For simplicity ^{111}In-labeled streptavidin (Bio Division Societa Prodotti Antibiotica, SpA, Milan, Italy) was prepared by the addition of ^{111}In-labeled DTPA-bis-biocytinamide (DB_2) (Sigma Chemical Corp., St. Louis, MO) at a molar ratio of 1:1 as previously described (*13*). The preparation was purified from free labeled biotin by passage through a 1 × 8 cm Sephadex G-50 column. The specific activity of the labeled streptavidin was approximately 1 μCi/μg.

In addition to DB_2, EDTA-hydrazino-biotin (EB_1) was used in this study; the synthesis of EB_1 was described by Virzi et al (*13*).

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Both derivatives were radiolabeled with ^{111}In as the acetate to a specific activity of approximately 20 μCi/μg. The radiochemical purity of the labeled biotin was routinely determined by adding a 10-fold molar excess of avidin (Calbiochem, La Jolla, CA) to an aliquot of each labeled biotin followed by analysis on a 1 × 8 cm Sephadex G-50 column. In this system, radioactivity bound to biotin will associate with avidin and therefore appears earlier, i.e., higher molecular weight than free label. In all cases, greater than 90% of the radioactivity was associated with avidin, whereas controls using d-biotin saturated avidin showed less than 5% binding to avidin.

Stability of Labeled Biotins on Streptavidin

The stability of the EB_1 and DB_2-streptavidin complex in the presence of d-biotin was evaluated in vitro. Streptavidin, saturated with a 10-fold molar excess of either labeled derivative, was purified by G-50 chromatography and 1 μg was added to saline solutions of d-biotin (Sigma) ranging in concentration from 0 to 0.24 μM in a total volume of 0.1 ml. After incubation for 3 hr at 37°C, the percentage of label bound to streptavidin was determined by paper chromatography using Whatman No. 1 paper with 0.1 M sodium acetate, pH 6 as the solvent. In this system, labeled biotin migrates with the solvent, while labeled streptavidin remains at or near the origin. In one experiment, ^{111}In-DB_2 streptavidin was challenged with 24 μM d-biotin under the same conditions and the binding was determined as above. In addition, streptavidin covalently bound to polystyrene beads (Sigma) was saturated with either labeled DB_2 or EB_1 and, after washing unbound activity, the beads were incubated with agitation in normal human serum at 37°C for 3 hr. The percentage of activity remaining on the washed beads was determined.

The affinity of both labeled biotin derivatives for streptavidin was determined by equilibrium dialysis (*14*). Briefly, streptavidin was saturated with either labeled DB_2 or EB_1 and purified from excess biotin by Sephadex G-50 chromatography. The labeled streptavidins were mixed with unlabeled streptavidin to maintain a total streptavidin concentration of 0.9 μM. With EB_1, concentration varied between 0.1 to 3.6 μM and DB_2 between 0.9 to 2.3 pM. Human serum albumin was added to a final concentration of 10 mg/ml to minimize nonspecific adsorption. Each solution in duplicate was then dialyzed against 0.05 M phosphate-buffered saline for 3 days at 4°C. Aliquots of the protein solution and dialysate were removed and counted in a well type NaI (Tl) counter. The results were plotted as the concentration of biotin bound per mole of streptavidin divided by the concentration of free biotin versus the concentration of biotin bound per mole of streptavidin. The dissociation constant (K_d) is equal to the negative inverse of the slope.

Biodistribution Studies

A clinical isolate of *Escherichia coli* was grown in peptone yeast extract glucose broth (Becton Dickinson, St. Louis, MO) to a density of 10^8 organisms/ml. The isolate was stored at 4°C. Male CD-1 mice (Charles River Laboratories, Kingston, NY) weighing approximately 25 g, were anesthetized by inhalation of metofane (Pitman-Moore, Inc., Washington Crossing, NJ) and inoculated subcutaneously in the posterior left thigh with 0.1 ml of the bacterial suspension. Swelling was apparent after 24 hr. At this time, 10 μg of either ^{111}In-labeled streptavidin or labeled IgG were administered through a tail vein. For some experiments, additional animals received from 30 to 150 μg of unlabeled streptavidin by the same route and, 2–3 hr later, 1 μg of either ^{111}In-DB_2 or ^{111}In-EB_1 was administered intraperitoneally. Animals were killed by cervical dislocation at 2, 6 or 24 hr postadministration of protein and were dissected to provide samples of tissue for counting in a well type NaI(Tl) counter against a standard of the injectate. Results were expressed as percent of injected dose per gram of tissue (%ID/g). The entire infected left leg and, for comparison, the whole right leg, was removed for counting.

Imaging

The animals were anesthetized with nembutal (Abbott Laboratories, North Chicago, IL) using 1.0 mg per 25 g mouse, administered intraperitoneally in 0.1 ml of saline and then positioned directly on the collimator. Typically, three animals were imaged simultaneously. Scintigrams were acquired using a portable large field of view scintillation camera (Elscint, Hackensack, NJ) equipped with a parallel-hole, medium-energy collimator and an Elscint APEX F1 computer. Images were acquired for a preset number of counts using a 256 × 256 matrix with a 20% energy window set at 171 and 245 keV.

Statistical Analysis

All values were expressed as (±) one standard deviation. Differences between measures were made with an analysis of variance of repeat measures with organs the repeated factor. The presence of significance and/or interaction effects of pairwise comparisons were done using Fischer's LSD procedure with a Bonferonni adjustment to compensate for multiple comparisons (*15*).

RESULTS

Stability of Labeled Biotins on Streptavidin

The two labeled biotin chelates used in this study displayed significant differences in stability towards displacement from streptavidin by d-biotin. As shown in Figure 1, ^{111}In-DB_2 binding to streptavidin was not influenced by the addition of d-biotin. Not shown is negligible displacement even at a 100-fold molar excess of d-biotin (24 μM). However, the corresponding study with ^{111}In-EB_1 saturated streptavidin showed increasing displacement of label from streptavidin with increasing d-biotin concentrations over the same range. The lower affinity of labeled EB_1 for

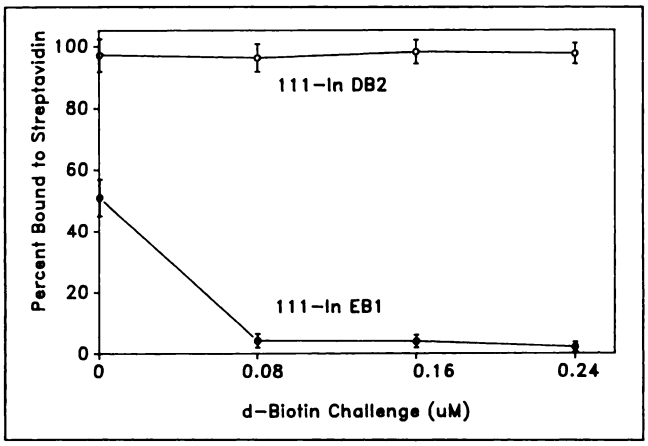

FIGURE 1. Stability of ^{111}In-labeled DB_2 and ^{111}In-labeled EB_1 on streptavidin to challenge by increasing concentrations of d-biotin.

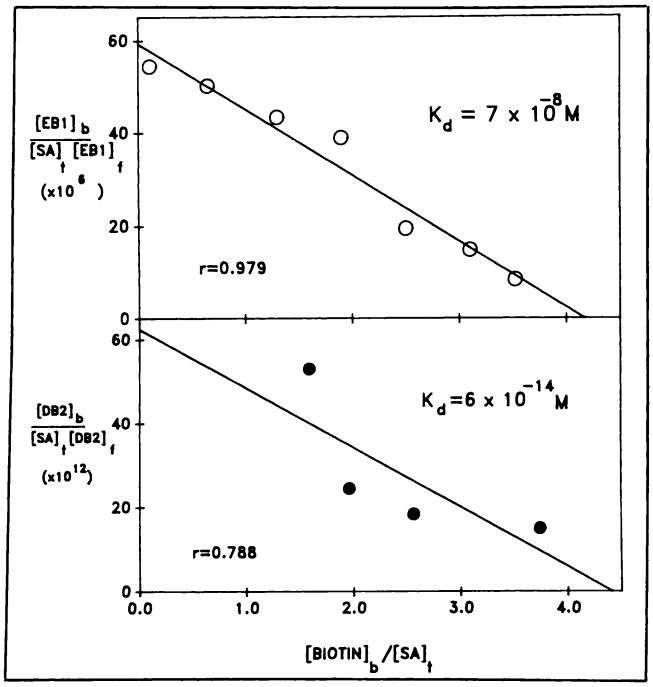

FIGURE 2. Scatchard plot for determination of the dissociation constant of EB_1 (top) and DB_2 (bottom). Each point represents an average of duplicate measurements. The dissociation constant is given by the slope of the line.

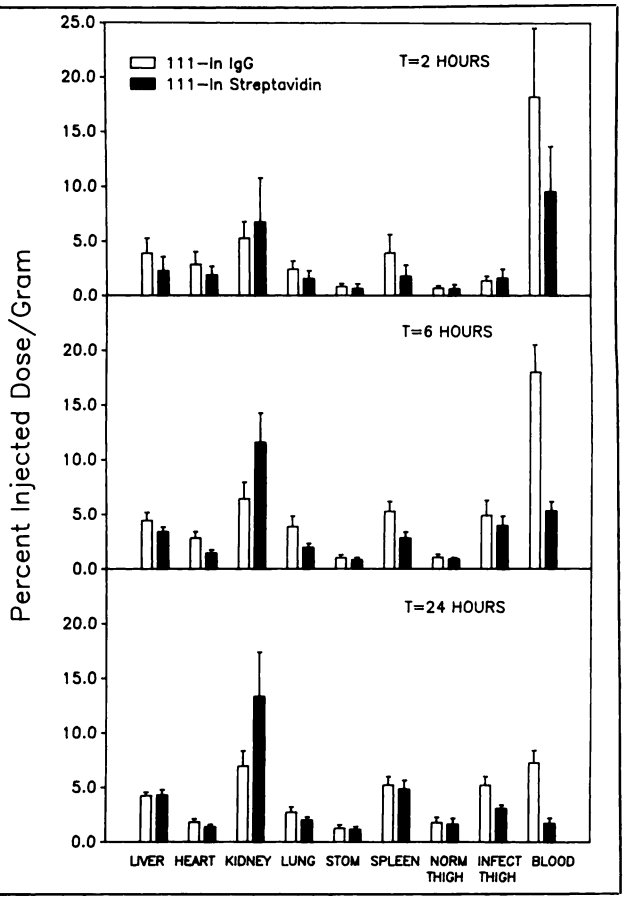

FIGURE 3. Histograms showing the accumulation (in %ID/g) of ^{111}In in infected thigh, normal thigh, normal organs and blood following administration of labeled IgG antibody and labeled streptavidin. Results are presented at 2, 6 and 24 hr following administration of the labeled protein. n = 4–6, standard deviation shown.

streptavidin was verified by the measurement of the dissociation constant (K_d) (Fig. 2). Whereas the K_d for labeled DB_2 was found to be 10^{-15} M, in agreement with measurements for d-biotin (*16*), the value for labeled EB_1 was found to be much lower at 10^{-8} M. Nevertheless, both of these labeled biotins when bound to unsaturated streptavidin in human serum, in the presence of physiological concentrations of endogenous biotin (500 pg/ml, 0.002 μM) (*17*), showed greater than 90% of the activity remaining on streptavidin.

Biodistribution Studies

To determine whether streptavidin would localize at focal sites of infection as well as nonspecific IgG, a comparison was made in the mouse model with an infection in the area of the left thigh. The biodistribution results obtained at 2, 6 and 24 hr postadministration of radiolabeled streptavidin and IgG antibody (n = 4–6 per time point) are shown in Figure 3. Following administration of labeled streptavidin, blood levels were consistently 2- to 3-fold lower ($p < 0.05$) relative to that of labeled IgG. Localization of labeled streptavidin to the infection was equivalent to that of IgG throughout 6 hr and significantly lower at 24 hr ($p < 0.05$). However, because of the reduced blood levels with streptavidin at all time points, improved infection-to-blood ratios were apparent at all times. Only in the case of kidneys were higher levels ($p < 0.05$) found for labeled streptavidin at 6 and 24 hr. High kidney levels following administration of labeled streptavidin have been previously shown (*18*). Although differences were seen for heart and lung at 6 hr ($p < 0.05$) and spleen at 2 and 6 hr ($p < 0.05$), no significant differences were observed for other tissues ($p > 0.05$).

Figure 4A presents results obtained with the intravenous administration of 30 μg of unlabeled streptavidin followed 3 hr later with the intraperitoneal administration of 1 μg of either ^{111}In labeled DB_2 or EB_1 (n = 5). Animals were killed 2 hr later. Control animals received the labeled biotin derivatives without the prior administration of streptavidin (Fig. 4B, note change in scale) As is apparent, less label localized in the tissues and target following administration of labeled EB_1 relative to DB_2, which is in agreement with that observed by Virzi et al. (*13*). However, as seen in Figure 4B, regardless of the biotin chelate administered, tissue levels were increased ($p < 0.0001$) following the preadministration of streptavidin.

Although the accumulation in the target was lower following administration of labeled EB_1 compared to DB_2 in animals receiving streptavidin (0.25% versus 0.79% ID/g, respectively), the greatly reduced blood levels (0.1% versus 2.4% ID/g, respectively) resulted in an infected

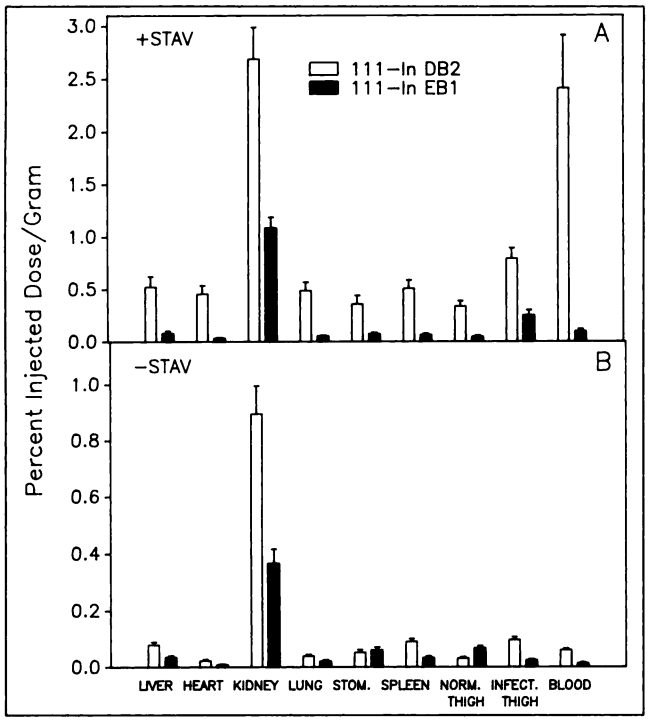

FIGURE 4. Histograms showing the accumulation (% ID/g) of ^{111}In 2 hr after intraperitoneal administration of labeled EB_1 and DB_2 in mice who did not receive a prior injection of unlabeled streptavidin (bottom panel) or who had received unlabeled streptavidin 3 hr prior to the administration of labeled biotin (top panel). n = 5, standard deviation shown.

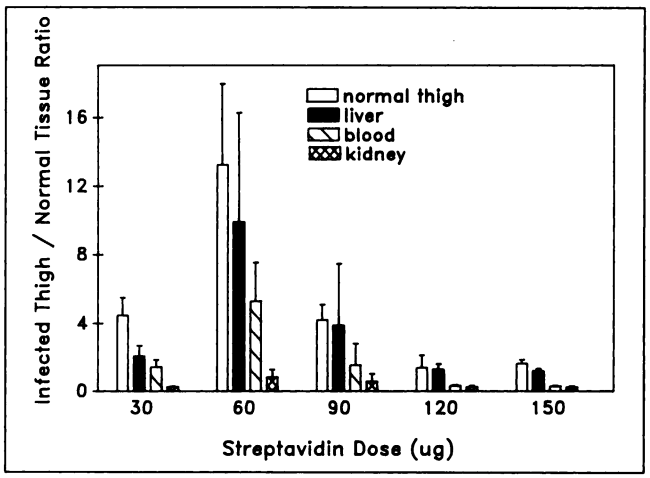

FIGURE 5. Histogram showing the infected thigh-to-normal tissue ratios of ^{111}In following administration of 30–150 μg of unlabeled streptavidin prior to the administration of labeled EB_1. n = 3–5.

thigh-to-blood ratio of 2.5 for EB_1 compared to 0.33 for DB_2. Despite the lower affinity of labeled EB_1 on streptavidin in the presence of d-biotin (see above), labeled EB_1 was selected for subsequent studies because of its more favorable biodistribution properties.

To optimize infection localization with this pretargeting approach, the dose of streptavidin was varied from 30 to 150 μg 3 hr prior to an intraperitoneal administration of 1 μg of labeled EB_1 with killing 3 hr later (n = 3–5). As shown in Figure 5, infected thigh-to-normal tissue radioactivity ratios in all tissues sampled reached a maximum at 60 μg. While the radioactivity level in the infected thigh remained constant at 2.4%–2.9% ID/g from 60 to 150 μg, the level in normal tissues increased continually with dose.

The streptavidin-biotin pretargeting approach was compared to the administration of labeled IgG and labeled streptavidin alone in the mouse infection model. For the former, 60 μg of streptavidin was administered intravenously, and 3 hr later 1 μg of labeled EB_1 was administered intraperitoneally (n = 4). The animals were killed 3 hr later (i.e., 6 hr post-streptavidin). For comparison, another group of animals received either 10 μg of labeled streptavidin or labeled IgG and were killed at 6 hr (n = 6). Control animals received only labeled biotin. The results are presented in Table 1 as %ID/g of tissue and in Table 2 as infected thigh-to-normal tissue ratios for normal thigh, blood, liver and kidneys.

As is shown in Table 1, the pretargeting approach demonstrates high incorporation of label into the target and at least a 6-fold lower accumulation in all normal tissues, other than kidneys. Blood levels were also dramatically lowered from 18% ID/ml with labeled IgG to 0.42% ID/ml for streptavidin-EB_1. The control animals, receiving labeled EB_1 without preadministration of streptavidin, showed minimal accumulations in all tissues.

A comparison of infected thigh-to-normal tissue ratios for the three methods further demonstrates the improvements with the pretargeting approach (Table 2). For example, the infected thigh-to-normal thigh ratio was 3-fold higher for pretargeting versus administration of either labeled IgG or streptavidin, while the infected thigh-to-blood ratio was 20- and 8-fold higher than labeled IgG and streptavidin, respectively, and infected thigh-to-liver ratio showed an improvement of 9-fold for pretargeting. Only

TABLE 1
Percent Injected Dose per Gram of Tissue in Mice with an Infection in One Thigh*

Tissue	^{111}In-IgG	^{111}In-Streptavidin	Streptavidin, ^{111}In-EB_1	^{111}In-EB_1
Liver	4.4 ± 0.7	3.4 ± 0.4	0.28 ± 0.1	0.04 ± 0.01
Heart	2.9 ± 0.6	1.4 ± 0.3	0.16 ± 0.05	0.01 ± 0.002
Kidney	6.4 ± 1.5	12.0 ± 2.7	3.20 ± 1.4	0.37 ± 0.09
Lung	3.9 ± 1.0	2.0 ± 0.4	0.43 ± 0.1	0.02 ± 0.01
Stomach	1.0 ± 0.3	0.9 ± 0.2	0.17 ± 0.03	0.06 ± 0.02
Spleen	5.3 ± 0.9	2.8 ± 0.5	0.23 ± 0.04	0.03 ± 0.01
Normal thigh	1.1 ± 0.3	0.9 ± 0.1	0.20 ± 0.1	0.03 ± 0.02
Infected thigh	4.9 ± 1.4	4.0 ± 0.9	2.60 ± 1.4	0.07 ± 0.03
Blood	18.0 ± 2.5	5.3 ± 0.9	0.42 ± 0.16	0.01 ± 0.004

* Mice received either ^{111}In-IgG or ^{111}In-streptavidin 6 hr earlier (1st and 2nd columns) unlabeled streptavidin 6 hr earlier with ^{111}In EB_1 administered at 3 hr (3rd column) or ^{111}In EB_1 administered at 3 hr without preadministration of unlabeled streptavidin (4th column). Values are the mean (n = 4–6) with one standard deviation.

TABLE 2
Infected Thigh-to-Normal Tissue Ratios Calculated from Table 1

Tissue	^{111}In-IgG	^{111}In-Streptavidin	Streptavidin, ^{111}In-EB$_1$
Normal thigh	4.5	4.4	13.0
Blood	0.3	0.8	6.2
Liver	1.1	1.2	9.3
Kidney	0.8	0.3	0.8

in the case of kidneys was the ratio not improved with the pretargeting approach.

The image shown in Figure 6 was obtained with all three animals in the same field of view and confirms the results reported in Tables 1 and 2. The image was obtained at 6 hr and shows the distribution of ^{111}In following administration of labeled IgG (left) and labeled streptavidin (center) compared to the pretargeting approach with unlabeled streptavidin and labeled EB$_1$ (right). The arrows point to the infection visible to the right of the bladder in all animals. It is clear that the background activity in the liver, spleen and blood is much higher in the case of the labeled proteins relative to the pretargeting approach. Although bladder activity is high in the animal receiving labeled EB$_1$ due to clearance of the label via the kidneys, it is apparent from the image that accumulation in the target is comparable to that of the labeled proteins.

Pretargeting with streptavidin was examined to see how early an infection could be visualized. Shown in Figure 7 are images of the same animal taken at 10- and 20-min intervals postadministration of labeled EB$_1$. In the top row, streptavidin was administered 3 hr prior to ^{111}In-EB$_1$. The infection is apparent by 20 min post-EB$_1$ administration and becomes more obvious at 40 and 60 min as background levels decrease. When streptavidin was administered 1 hr prior to ^{111}In EB$_1$ (center panel), the infection is apparent, although poorly, at 40 and 60 min. Most obvious is the high background activity due to circulating streptavidin at this early time. The control (bottom panel) received the labeled EB$_1$ without prior administration of streptavidin. No target localization was apparent by 60 min.

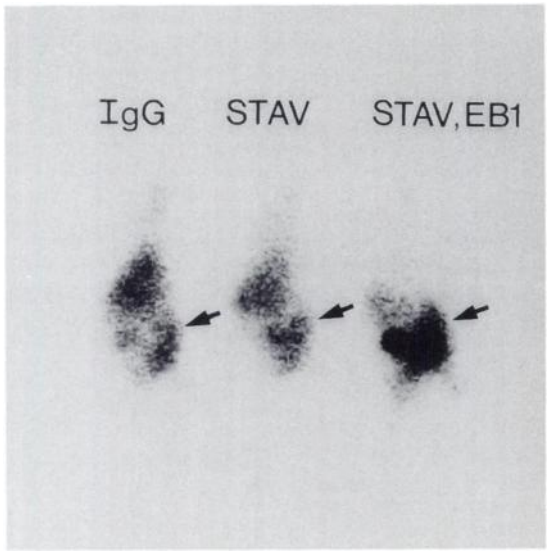

FIGURE 6. Gamma camera whole-body images obtained simultaneously at 6 hr postadministration of ^{111}In-IgG (left), ^{111}In-streptavidin (middle), and unlabeled streptavidin administered 3 hr prior to the administration of ^{111}In-EB$_1$ (right).

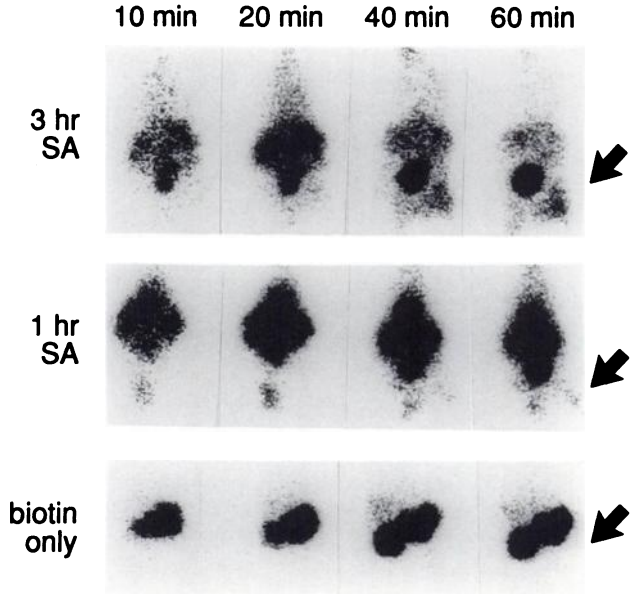

FIGURE 7. Continuous gamma camera whole-body image obtained at 3 hr (top) and 1 hr (middle) postadministration of unlabeled streptavidin (SA) and imaged at 10–20-min intervals post-labeled biotin (EB$_1$) administration. Bottom panel shows control with labeled biotin (EB$_1$) only.

DISCUSSION

When first reported, favorable images of infection obtained with the administration of radiolabeled nonspecific polyclonal antibodies were attributed to Fc-mediated antibody binding on leukocytes at the target (*19*). Subsequently, it was shown that the accumulation could be attributed simply to nonspecific diffusion of the labeled protein into infected lesions due to increased vascular permeability (*1*). Because of this nonspecific mechanism of uptake, other proteins may exhibit similar or improved infection localization properties.

The results of this investigation demonstrated that streptavidin is a suitable alternative to nonspecific IgG as an agent for imaging sites of infection in a mouse model. Both labeled IgG and streptavidin were similar in that levels of activity in most tissues, such as blood, liver, spleen and kidneys, were high with respect to the target as expected for labeled proteins. These drawbacks have been recognized for several years in connection with radiolabeled protein, e.g., anti-tumor antibodies for tumor imaging and therapy (*20,21*). Among the approaches considered to resolve these difficulties with tumor imaging was

the use of unlabeled streptavidin-conjugated antibodies administered prior to the administration of radiolabeled biotin (*22*). This approach is among those that have been referred to as "pretargeting" (*9,11,23*). In the present investigation, pretargeting was considered for infection imaging; however, in view of the nonspecific nature of the process, streptavidin alone was considered in preference to antibody-mediated targeting with streptavidin-conjugated antibody.

A related issue that was addressed in this investigation is the selection of a biotin derivative for this approach. This laboratory has synthesized and tested a series of polyaminopolycarboxylic acid biotin derivatives for labeling with ^{111}In and had identified EB_1 as an attractive alternative to the commercially available DB_2 in a mouse model (*13*). Therefore, the ^{111}In-labeled EB_1 was compared to labeled DB_2 in this investigation. Although EB_1 displayed a lower affinity for streptavidin relative to DB_2, the biodistribution results obtained with both derivatives showed that labeled EB_1 gave significantly higher target-to-nontarget ratios and was therefore used as the labeled biotin chelate for further studies.

The intraperitoneal route of administration for radiolabeled biotin was viewed as appropriate for these studies. One consideration was that most animals also received an intravenous administration of streptavidin often within a few hours. In addition, with the small molecular weight of the biotin chelates studied, 500–1200 D, the intraperitoneal route may have provided an additional advantage of prolonging clearance from circulation.

One of the interesting observations from this investigation is the existence of an optimal dose of unlabeled streptavidin (i.e., 60 μg/mouse) below and above which the target-to-nontarget ratios decrease as shown in Figure 5. This effect is the result of an increased accumulation of label with dosage of streptavidin in all tissues except the infection. The percent of label at the infection remained constant from 60 μg to 150 μg of streptavidin. The lower ratio at a 30-μg dose of streptavidin may reflect the influence of endogenous biotin occupying binding sites on streptavidin.

As is apparent from Table 1 and Figure 6, results obtained in this model with labeled streptavidin were comparable to those obtained with labeled nonspecific IgG. This observation strengthens the suggestion that IgG accumulation in sites of infection may be due to nonspecific diffusion (*1*). It is also apparent that the streptavidin-EB_1 pretargeting approach showed large improvements in localization ratios even at early times. In comparison to nonspecific IgG, pretargeting with unlabeled streptavidin followed by radiolabeled biotin may potentially improve imaging infection at earlier times in humans.

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REFERENCES

1. Morrel EM, Tompkins RG, Fischman AJ, et al. Autoradiographic method for quantitation of radiolabeled proteins in tissues using indium-111. *J Nucl Med* 1989;30:1538–1545.
2. Thakur ML, DeFulvio J, Park CH, Jungkind D, Epstein A, McAfee J. Evaluation of Tc-99m labeled proteins for localization of inflammation [Abstract]. *J Nucl Med* 1990;31:810.
3. Claessens RAMJ, Oyen WJG, Van der Neer JWM, Corstens FHM. Scintigraphic detection of inflammation foci with radiolabeled proteins [Abstract]. *J Nucl Med* 1990;31:810.
4. Rubin RH, Fischman AJ, Needleman M, et al. Radiolabeled, nonspecific, polyclonal human immunoglobulin in the detection of focal inflammation by scintigraphy: comparison with gallium-67 citrate and technetium-99m-labeled albumin. *J Nucl Med* 1989;30:385–389.
5. Abrams MJ, Juweid M, tenKate CI, et al. Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. *J Nucl Med* 1990;31:2022–2028.
6. Goh ASW, Aw SE, Sundram FX, Ang ES, Goh SK, Leong KH. Imaging of focal inflammation with ^{99m}Tc-labeled human polyclonal immunoglobulin G. *Nucl Med Commun* 1990;11:843–856.
7. Carrio I, Duncker C, Berna L, et al. Delineation of infection sites by means of Tc-99m-polyclonal immunoglobulin and Tc-99m antigranulocyte monoclonal antibody studies [Abstract]. *Eur J Nucl Med* 1990;16:426.
8. Becker W, Saptogino A, Wolf F. Tc-99m-labeled human polyclonal immunoglobulins for localization of infectious lesions in humans: uptake mechanism in vitro [Abstract]. *Eur J Nucl Med* 1990;16:426.
9. Goodwin DA, Meares CF, McCall MJ, McTigue M, Chaovapong W. Pretargeted immunoscintigraphy of murine tumors with indium-111-labeled bifunctional haptens. *J Nucl Med* 1988;29:226–234.
10. Rowlinson-Busza G, Hnatowich DJ, Rusckowski M, Snook D, Epenetos AA. Xenograft localisation using pretargeted streptavidin-conjugated monoclonal antibody and ^{111}In-labeled biotin. *J Biol Nucl Med* (submitted).
11. Kalofonos HP, Rusckowski M, Siebecker DA, et al. Imaging of tumor in patients with indium-111-labeled biotin and streptavidin-conjugated antibodies: preliminary communication. *J Nucl Med* 1990;31:1791–1796.
12. Hnatowich DJ, Layne WW, Childs RL, et al. Radioactive labeling of antibody: a simple and efficient method. *Science* 1983;220:613–615.
13. Virzi F, Fritz B, Rusckowski M, Gionet M, Misra H, Hnatowich DJ. New In-111-labeled biotin derivatives for improved immunotargeting. *Nucl Med Biol* 1991;18:719–726.
14. Scatchard G. The attractions of proteins for small molecules and ions. *Ann NY Acad Sci* 1949;51:660–672.
15. Snedecor GW, Cochran WG. *Statistical methods,* 8th edition. Ames, Iowa: Iowa State University Press; 1989:235.
16. Green NM. Avidin-1. The use of [^{14}C]biotin for kinetic studies and for assay. *Biochem J* 1963;89:585–591.
17. Mock DM, DuBois DB. A sequential, solid-phase assay for biotin in physiological fluids that correlates with expected biotin status. *Anal Biochem* 1986;153:272–278.
18. Pimm MV, Fells HF, Perkins AC, Baldwin RW. Iodine-131 and indium-111-labeled avidin and streptavidin for pre-targeted immunoscintigraphy with biotinylated anti-tumor monoclonal antibody. *Nucl Med Commun* 1988;9:931–941.
19. Fischman AJ, Rubin RH, Khaw BA, et al. Detection of acute inflammation with ^{111}In-labeled nonspecific polyclonal IgG. *Semin Nucl Med* 1988;335–344.
20. Bradwell AR, Fiarweather DS, Dykes PW, et al. Limiting factors in the localization of tumors with radiolabeled antibodies. *Immunol Today* 1985;6:163–170.
21. Hnatowich DJ, Sands H. On the accumulation in liver of ^{111}In following administration of the B72.3 antibody [Letter]. *J Nucl Med* 1989;30:1575–1576.
22. Hnatowich DJ, Virzi F, Rusckowski M. Investigations of avidin and biotin for imaging applications. *J Nucl Med* 1987;28:1294–1302.
23. Paganelli G, Riva P, Deleide G, et al. In vivo labeling of biotinylated monoclonal antibodies by radioactive avidin: a strategy to increase tumor radiolocalization. *Int J Can* 1988;2(suppl):121–125.