reduced values of RBF may be found that are even lower than those in patients with RAS.

CONCLUSION

Our data demonstrates that measurements of RBF from first-pass radionuclide angiography using ^{99m}Tc-MAG3 are of little help as a diagnostic test in patients in whom a diagnosis of renovascular hypertension is suspected on clinical grounds.

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Imaging Osteomyelitis with Streptavidin and Indium-111-Labeled Biotin

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Animal studies of infection imaging by a two-step protocol have shown that important improvements in target to nontarget ratios are possible. In this protocol, unlabeled streptavidin is administered and allowed sufficient time to accumulate in the lesion, probably by nonspecific processes, and to clear elsewhere. Thereafter, ^{111}In-biotin is administered. A fraction of the labeled biotin may be retained in the lesion because of biotin's high affinity for streptavidin while most of the activity is cleared through the kidneys. **Methods:** Radioscintigraphy with unlabeled streptavidin followed with ^{111}In-labeled biotin was performed in 15 patients with chronic osteomyelitis. As controls, each patients received either ^{111}In-labeled biotin without the preadministration of streptavidin or ^{111}In-labeled nonspecific IgG. **Results:** Regions of focal uptake were identified in all patients receiving streptavidin followed by radiolabeled biotin as early as 10 min postadministration of radioactivity, and retention of label was evident through 24 hr. Coincident regions of abnormal accumulation were apparent with ^{111}In-IgG, but only in delayed images. Moreover, with ^{111}In-biotin alone, without the preadministration of streptavidin, focal accumulations were detected in areas similar to that identified with the two-step protocol. Although, these observations were only in the earliest images. **Conclusion:** The results of this preliminary clinical investigation suggest that a two-step protocol with unlabeled streptavidin and radiolabeled biotin may be an alternative for the detection of infection.

Key Words: streptavidin; radiolabeled biotin; pretargeting; infection imaging; osteomyelitis

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Recently, radiolabeled nonspecific polyclonal IgG has been shown to be useful in the diagnosis of infection and inflammation (*1*). The accumulation of this protein is thought to be due to nonspecific diffusion resulting from increased vascular permeability and edema at these sites (*1–3*). Despite the obvious success of this method, a major disadvantage to the use of ^{111}In-IgG is its slow rate of accumulation into a lesion and its slow clearance from normal tissues and circulation. As a result, diagnosis is often delayed 24 to 48 hr postadministration of radioactivity. We have previously considered in an animal model whether streptavidin may be a more useful agent to detect infection (*4*). Unlabeled streptavidin was administered and allowed to accumulate nonspecifically into the lesion, probably in a manner similar to polyclonal IgG. Some time later, the radiolabel was delivered bound to biotin, a low mole weight vitamin. Biotin has an extremely high affinity for streptavidin (*5*). Therefore the labeled biotin may diffuse rapidly into the lesion, and bind irreversibly to streptavidin therein, while the unbound biotin clears from the circulation and normal tissues. Thus, one advantage with this approach is the low accumulation of label in normal tissues, mainly the result of biotin's rapid whole-body clearance. As such, target-to-nontarget tissue ratios on the order of 10 have been reported in a mouse model within hours of postadministration of labeled biotin (*4*). We report the results of the use of this two-step approach to detect infection in patients.

MATERIALS AND METHODS

Reagents

Streptavidin was obtained as a sterile, pyrogen-free lyophilized powder in single use vials. Each vial contained 10 mg of streptavidin that was solubilized in 2 ml sterile saline before administra-

tion. The biotin derivative, biotinyl-hydrazino- ethylenediaminetetraacetic acid (EB1) (*6*), was obtained as a sterile lyophilized powder with 1 mg packaged in single-use vials. A vial chosen at random was tested for sterility and pyrogenicity (Limulus lysate). Before administration, the EB1 was solubilized in 1.0 ml sterile saline and 0.2 ml (0.2 mg) was transferred to a sterile vial. To this was added 1.4 to 3.5 mCi ^{111}In in sterile 0.25 *M* acetate buffer, pH 6. Before administration, radiochemical purity was assessed by two methods. For one test, a molar excess of avidin was added to an aliquot of the labeled biotin preparation. Radiolabeled biotin binds to avidin and remains at or near the origin on ITLC-SG thin-layer chromatography using saline as eluant, while unbound label migrates. As a control, the assay was repeated with biotin-saturated avidin that should show no retention of radiolabel at the origin. As a second test, an aliquot of the labeled biotin preparation, after addition of avidin, was analyzed by size-exclusion Sephadex G-50 chromatography. The percentage of activity eluting in the void volume, i.e., labeled avidin, was used as a determination of radiochemical purity. For administration, a minimum of 85% radiochemical purity was required by both analyses.

Nonspecific polyclonal IgG available for patient administration was coupled with diethylenetriamine pentaacetic acid anhydride (DTPA) (*7*) under sterile, apyrogenic conditions. An average of 1.6 chelate groups per molecule was attached and the product tested for both sterility and pyrogenicity (Limulus lysate). The DTPA-IgG was stored in sterile, pyrogen-free, single-use vials, each containing 1 mg of protein. For radiolabeling, 1.3 to 3.7 mCi ^{111}In were added as the acetate complex in sterile 0.25 *M* acetate buffer as previously described (*7*). Radiochemical purity was determined on an aliquot of each preparation before administration by Sephadex G-50 chromatography. In this analysis the percentage of activity eluting in the void volume with the antibody reflected the radiochemical purity. As before, 85% radiochemical purity was required for administration.

Patient Protocol

The study included 15 patients with radiological evidence of chronic infection associated with osteomyelitis, each of varying origin. Each patient was entered into the study protocol, whereby 10 mg of unlabeled streptavidin was administered through an arm vein at 24 hr (and in one patient at 6 hr because of scheduling difficulties) before the intravenous administration of ^{111}In-EB1. As a control, five of these patients on another occasion received 0.2 mg of ^{111}In-EB1 without the previous administration of streptavidin. As an additional control, 7 of the 15 patients were imaged after the administration of ^{111}In-IgG. Only one patient was included in all three protocols. An interval of 4 to 7 days was planned between studies to avoid study interference, and the sequence of protocols was randomly assigned. Twelve of the 15 patients were receiving medication that was maintained throughout the study, although the clinical status of each patient remained unchanged. Before entry into the investigation each patient gave informed consent in compliance with the guidelines provided by the Institute H. San Raffaele Ethics Committee. After administration of streptavidin and labeled EB1, all patients were closely monitored for any adverse reactions.

Serum Analysis

Human Antistreptavidin Antibodies. The presence of human antistreptavidin antibodies (HASA) was measured in serum samples collected before the first study and at 4 to 8 wk after streptavidin administration. An ELISA using streptavidin-coated microtiter plate wells was used as described previously (*8*). Briefly, dilutions of serum samples were added to wells for 1 hr at 37°C. The wells were washed and horseradish peroxidase-conjugated rabbit antihuman antibody added. After incubation, the wells were again washed and the enzymatic reaction developed with the chromogenic substrate, o-phenylenediamine blocked by addition of 1*M* H_2SO_4, and the absorbance read at 492 nm.

Blood Chemistry. A 10-ml blood sample was obtained in heparinized vacutainer tubes from each patient before entry into the protocol and at 1 wk after the last study. Standard blood chemistry analysis and characteristic blood cell profiles were obtained to determine if any changes occurred as a result of test agents. The analysis included hemoglobin, hematocrit, white blood cell, lymphocyte, monocyte, platelet, granulocyte, blood sugar, BUN, creatinine, bilirubin, alkaline phosphate, SGOT and albumin. These analyses were performed by the Blood Chemistry Laboratory at San Raffaele Hospital, Milan, Italy.

Biotin Binding Capacity of Streptavidin in Serum. In the two-step protocol, streptavidin is administered and in circulation hours before labeled biotin is administered. It is possible that during this time endogenous biotin has the opportunity to bind to streptavidin in circulation, hence reducing or eliminating the extent to which ^{111}In-labeled biotin, administered at a later time, can bind. Therefore, the biotin binding capacity of streptavidin in circulation for 24 hr was determined. Briefly, to an aliquot of serum collected at various times over 24 hr, after streptavidin administration, was added a fixed amount of ^{111}In-labeled EB1. The samples were analyzed after a 10-min incubation by size-exclusion HPLC using a Superose-12 column (Pharmacia, Piscataway, NJ). Fractions of 0.3 ml were collected for counting in a well counter against a standard and results reported in percent of added activity. The activity eluting with streptavidin at higher mole weight was presumed bound to streptavidin. As control, labeled EB1 was added to serum from a normal volunteer and run in a similar manner. The percentage bound to streptavidin, corrected for the minimal activity associated with these fractions in the control sample, was converted to milligrams of streptavidin, assuming 1 mole EB1 bound per mole streptavidin. Thus, streptavidin concentration in serum is reported as a function of time. For comparison, data on clearance of label from patients enrolled in an independent investigation, receiving ^{125}I-labeled streptavidin, is graphed in a similar manner. In this latter study, 1 mg ^{125}I-streptavidin (Iodogen method) was administered intravenously at a specific activity of 1 mCi/mg. Blood was collected at intervals for the next 48 hr and aliquots of serum counted against a standard of the injectate in a Na(Tl) well counter.

Pharmacokinetics

The clearance of activity from circulation, after labeled biotin administration, was measured in five patients who received labeled EB1, with streptavidin administered 24 hr earlier. Blood samples (1 ml) were collected in heparinized tubes at 1, 5, 10, 20, 30 and 60 min and at 2, 3 and 24 hr after administration of labeled biotin and aliquots were counted in a well counter against a standard of the injectate. The time-activity curves were analyzed using the software program PharmKit (A. Johnston and S. Jackson, Saint Bartholomews, London) and the rate of label clearance described.

The clearance of label into urine was also determined. Urine was collected at intervals of 0–4, 4–8, 8–12, 12–24 and 24–48 hr. Total urine volume was recorded for each collection, and aliquots were counted in a gamma well counter against a standard. Results are expressed as cumulative label excreted with time through 48 hr and as the percent injected dose excreted per hour.

Imaging

Planar anterior and posterior whole-body images and planar anterior spot images were obtained using a Siemens 7500 Orbiter gamma camera equipped with a large field of view, high-energy collimator set on the 170- and 247-keV photons for ^{111}In. On some occasions, a gamma camera was used fitted with an ^{111}In collima-

tor and set for the same parameters. Planar spot views (using a 64 × 64 pixel matrix) were acquired with the camera positioned over suspected areas. In all patients, a dynamic acquisition at one frame per minute for 10 min was obtained simultaneously with the administration of labeled EB1. This was followed by static images acquired for a preset number of counts at 0.5, 1.5, 4 and, in some cases, 24 hr. After administration of labeled IgG, imaging was performed in an identical fashion but the 10-min dynamic image was obmitted due to the slow clearance of labeled antibody. SPECT images were obtained in some patients using a 40-cm field rotating gamma camera.

Target-to-nontarget ratios were estimated from the images by drawing regions around the area of interest. For background accumulation a corresponding site of equal pixel size was chosen, typically on the contralateral side, or when chest lesions were involved an area in the shoulder was chosen. The images were evaluated by clinicians participating in the investigation and by two physicians unfamiliar with the patient's clinical history and assigned protocols. The images obtained were compared to plain radiographs available from the patient's clinical files.

RESULTS

Patients

All 15 patients enrolled in this investigation had documented radiological evidence of chronic infection associated with osteomyelitis. A summary of each patient's condition, treatment and the protocols performed as part of this investigation is presented in Table 1. The study group consisted of 10 men and 5 women, mean age of 61 yr (range 28–86 yr). The location of disease varied, and 12 patients were receiving antibiotics or nonsteroidal anti-inflammatory drugs at the time of study, although in each case the status of disease was unchanged. All patients were included in the streptavidin/labeled biotin protocol. On another occasion, seven were studied with ^{111}In-IgG, and five received ^{111}In-biotin without the preadministration of streptavidin. Only one patient (Patient 3) was enrolled in all three protocols. The time interval between protocols was 4 to 7 days, and the order of assigned protocols was not controlled.

Included in Table 1 are test results for human antistreptavidin antibody (HASA). Five patients were tested for HASA 4 to 8 wk postadministration of streptavidin; all patients had negative results. The test samples were measured against a panel of normal serum with the minimum sensitivity of the assay placed at 0.3 units/ml. Through the course of this investigation, no adverse reactions were observed after administration of either streptavidin or ^{111}In-labeled EB1. In addition, blood chemistries, such as bilirubin and creatine, as well as characteristic blood cell counts, for example, lymphocyte, monocyte, granulocyte and neutrophils, remained unchanged from pretest values in all patients (data not presented).

Serum and Urine Clearance

As illustrated in Table 2, serum clearance of ^{111}In-labeled EB1 24 hr after streptavidin administration, best fit two components: an early phase with a $T_{1/2}$ of 13.5 min (range, 3–23 min, n = 5) and a terminal phase with a $T_{1/2}$ of 20 hr (range, 12–28 hr, n = 5). Clearance of label was not measured in the control protocols. These results represent slower clearance than that reported in two other studies using a similar ^{111}In-biotin derivative. Paganelli (*8*), in a three-step approach using biotinylated antibody, followed with 4 to 6 mg avidin and then 0.2 to 0.3 mg of ^{111}In-labeled DTPA-biotin, reported clearance with a fast component, $T_{1/2}$ of 5 min ± 3 min, and a slow component, $T_{1/2}$ of 2.4 hr ± 1.2 hr. Similar results were described in the first report of ^{111}In-labeled DTPA-biotin in patients after administration of 1 mg of streptavidin conjugated HMFG1 antibody (*9*), i.e., an early phase of 2.4 min (1.2 min, s.d.) and a slow phase of 4.2 hr (1.8 hr, s.d.).

Clearance of label into urine postadministration of streptavidin is presented in Figure 1 for five patients. The excretion of label is illustrated as cumulative urinary radioactivity over time (panel A) and the percent injected dose excreted per unit of time (panel B). The data are varied, ranging from nearly 100% to 30% recovery of activity over 48 hr. Regardless, as shown in panel B, most of the activity is excreted in the first 8 hr.

Biotin Binding Capacity of Streptavidin in Serum

After administration of streptavidin, serum was tested for biotin binding capacity with the addition of an equal molar amount of ^{111}In-biotin (equivalent to that administered) with analysis by size-exclusion HPLC. As illustrated in Figure 2, a decrease in streptavidin concentration in serum over time was observed, as defined by a decrease in the amount of label that coeluted with streptavidin in size exclusion HPLC. The decrease in streptavidin concentration over time was similar to that observed for the clearance of label after administration of ^{125}I-labeled streptavidin from an independent investigation. If endogenous biotin was saturating streptavidin's four binding sites, then a more rapid decrease in streptavidin concentration would have been observed by this analysis. These results demonstrate that streptavidin remaining in the circulation for 24 hr retains a capacity to bind labeled biotin. These results also suggest that the level of endogenous biotin is not enough to saturate streptavidin (at the dosage delivered in this study, 10 mg) that was in circulation an extended time.

Images

In each of the 15 patients studied with the streptavidin/labeled biotin protocol, one, and often multiple sites of abnormal accumulation of radioactivity were visualized typically as early as 10 min after administration of labeled biotin. Clear delineation from background of sharp focal regions was apparent in all cases within 1 to 2 hr. In 7 of 10 patients the images showed retention of activity in a focus through 24 hr.

An example of whole-body distribution of label at 1.5 hr after ^{111}In-EB1 administration, with streptavidin administered 24 hr before, is shown in Figure 3A (Patient 9). At this early time highest activity is seen in bladder and kidneys due to the quick clearance of this agent. Blood pool activity and normal tissue accumulation is minimal. The lesions (indicated by arrows) are apparent in the region of the left hip. Figure 3B shows anterior spot views of the pelvic area in the same patient acquired at 30 min, 1.5 and 24 hr following labeled biotin administration. A focus to the right of the bladder is visible at 30 min, however by 1.5 hr the lesion is more clearly delineated. Furthermore, activity is detected in the focus through 24 hr, although the lesion intensity has decreased.

Identification of a sternal lesion after the streptavidin/labeled biotin protocol is demonstrated in a patient diagnosed with sternal osteomyelitis (the result of coronary artery by-pass surgery, Patient 14). The thorax typically is a difficult region in which to identify abnormal uptake of activity with labeled antibodies or labeled white cells due to high liver accumulation and slow clearance of label from the blood pool. The anterior 30-min image (Fig. 4A) shows activity in the chest with no apparent liver accumulation. The transverse SPECT image, 1.45 hr postadministration of labeled biotin, shows accumulation of activity at the outermost edge of the chest as might be expected with sternal involvement (Fig. 4B). Blood-pool is discernable (in rows 3 and 4) as activity towards the midline.

TABLE 1
Patient Characteristics and Studies Performed

Patient no.	Sex	Age	Location	Treatment	Studies			
					STAV, EB1	IgG	EB1 alone	HASA
1	M	65	L foot chronic OM	Antibiotics	+			Neg
2	F	65	R femur arthritis	Antibiotics	+		+	
3	M	32	R hip infection	—	+	+	+	Neg
4	M	79	R hip infected prosthesis	NSAID	+	+		Neg
5	F	72	L hip infected prosthesis	Antibiotics	+		+	
6	F	86	L foot suspected OM	Antibiotics	+	+		Neg
7	M	79	R hip infected prosthesis	Antibiotics	+			
8	F	70	R hip infected prosthesis	—	+		+	
9	F	53	L hip OM	Antibiotics	+	+		
10	M	68	Sternum chronic OM	Antibiotics	+	+		
11	M	50	Sternum acute OM	Antibiotics	+	+		Neg
12	M	64	L femur tuberculosis	—	+	+		
13	M	28	R clavicle septic	Antibiotics	+			
14	M	69	Sternum postop OM	Antibiotics	+		+	
15	M	37	R humerus septic	Antibiotics	+			

OM = osteomyelitis; NSAID = nonsteroidal anti-inflammatory drug; HASA = human antistreptavidin antibody.

Blood pool activity and liver accumulation are low with respect to the lesion.

Another example of a study in the upper body is presented in Figure 5. This patient presented with sepsis in the right clavicle (Patient 13). This image, shown after administration of streptavidin and labeled biotin, demonstrates clear discrimination of focal accumulation at 10 min. By 2 hr, activity persists in a focus while blood pool and normal tissues have cleared. Activity remains in the focus through 24 hr, although lesion intensity has decreased.

The one patient in whom labeled biotin was administered at 6 rather than 24 hr after streptavidin administration, is presented in Figure 6. Probably as a result of this short interval, high blood-pool activity and increased normal tissue accumulation is demonstrated in the 2-hr image. This activity remains through 24 hr, and the lesion is most clearly delineated at this time, unlike that observed in other examples presented in this report. As suggested in Figure 2, approximately 40% of streptavidin may be in the circulation at 6 hr. It is likely that at administration, labeled biotin binds to the circulating streptavidin. Therefore, it is probably the labeled streptavidin that is accumulating and accounts for activity in the lesion, as well as retained activity in normal tissues and blood pool. Also of note is the apparent activity in the bowel. The control study, labeled biotin alone, (bottom row, right) was obtained at 2 hr in an identical fashion. In contrast, only slight focal accumulation was seen in the left hip and background activity levels were low.

A similar control with labeled biotin administered alone is presented in Figure 7. The patient was diagnosed with septic pseudoarthrosis of the right femur (Patient 2). Shown are spot images at 10 min, 1.5 hr and 24 hr with the streptavidin/labeled-biotin protocol (top row), and with labeled biotin alone, administered at another time (bottom row). In both cases, activity is observed in the region of the lesion within 10 min. By 1.5 hr, the lesion appears more diffuse with biotin alone (bottom row), with focal areas more discernable when streptavidin is

TABLE 2
Plasma Clearance of Indium-111-EB1

Patient no.	$T_{1/2\lambda 1}$(min)	$T_{1/2}$(hr)
3	23.0	17.8
5	17.0	28.2
6	2.7	18.8
7	21.0	21.5
11	3.2	11.8
Average	13.5	20.0

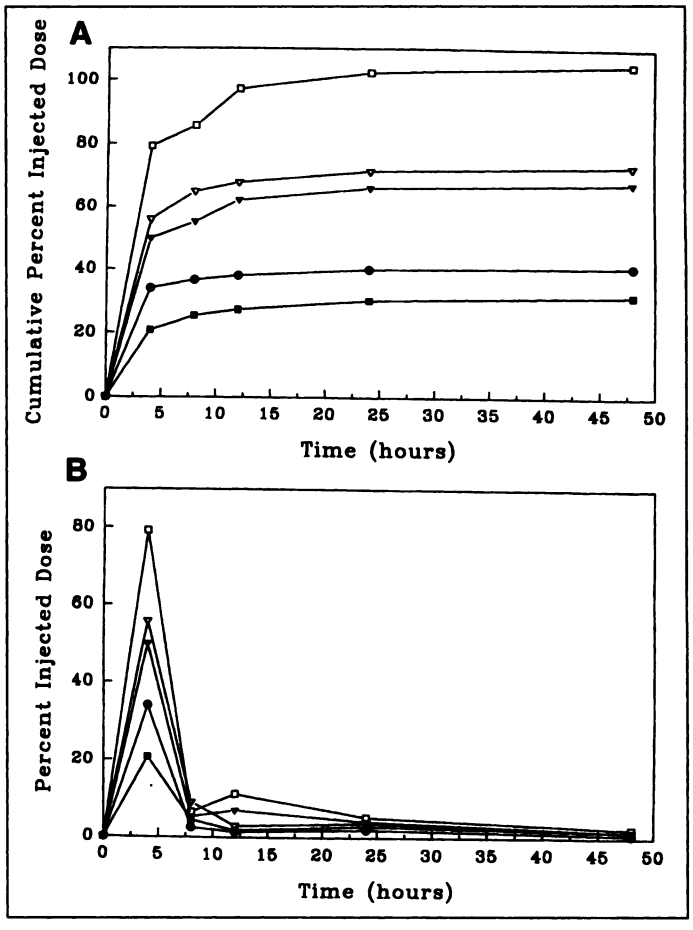

FIGURE 1. Clearance of activity into urine from five patients. (A) Cumulative percent injected dose over 48 hr. (B) Percent injected dose excreted per unit of time.

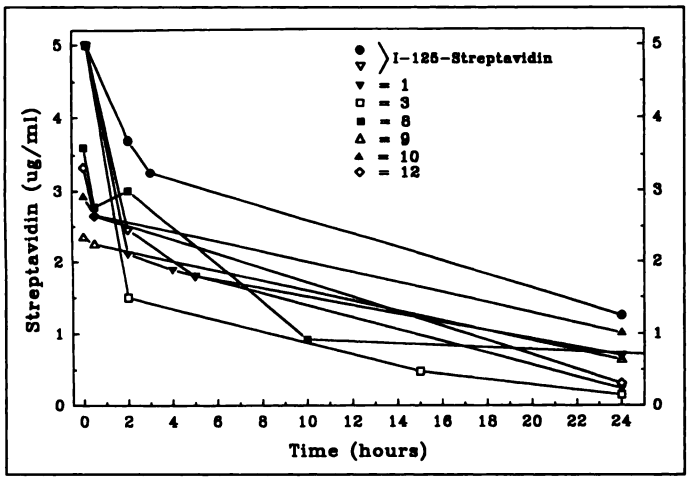

FIGURE 2. Streptavidin concentration in serum over 24 hr measured in six patients using a biotin binding assay. Included are data from two patients who received ^{125}I-labeled streptavidin.

administered beforehand (top row). Also, there is minimal retention of activity at 24 hr with the streptavidin/labeled biotin study, as a result, no image was taken at this time in the control study. The difference in observed lesion retention of label among patients may be due in part to circulation into and out of the lesion.

Figure 8 presents images obtained under two protocols. The patient presented with a pseudo ascentabularia of the right hip, with no evidence of trauma to the area, but possible signs of degenerative disease (Patient 3). The spot views along the top are with the streptavidin/labeled biotin protocol taken at 10 min, 1.5 and 24 hr. Along the bottom are images obtained with ^{111}In-labeled IgG at 2, 24 and 48 hr. Activity is high in nontarget tissues in the labeled IgG study, with blood pool remaining high through 48 hr. Three focal regions are apparent in the 24-hr image (indicated by arrows). With the streptavidin/labeled biotin protocol in the same patient, abnormal accumulation is apparent in the left thigh in the 10-min image. This activity clears rapidly so that at 1.5 hr blood-pool activity is low and three focal regions are evident (noted by arrows), with little interference from the blood pool and other tissues at this time. In contrast, in the labeled IgG study, blood-pool and normal organ activity remain high at 2 hr, although the lesions are evident at this time.

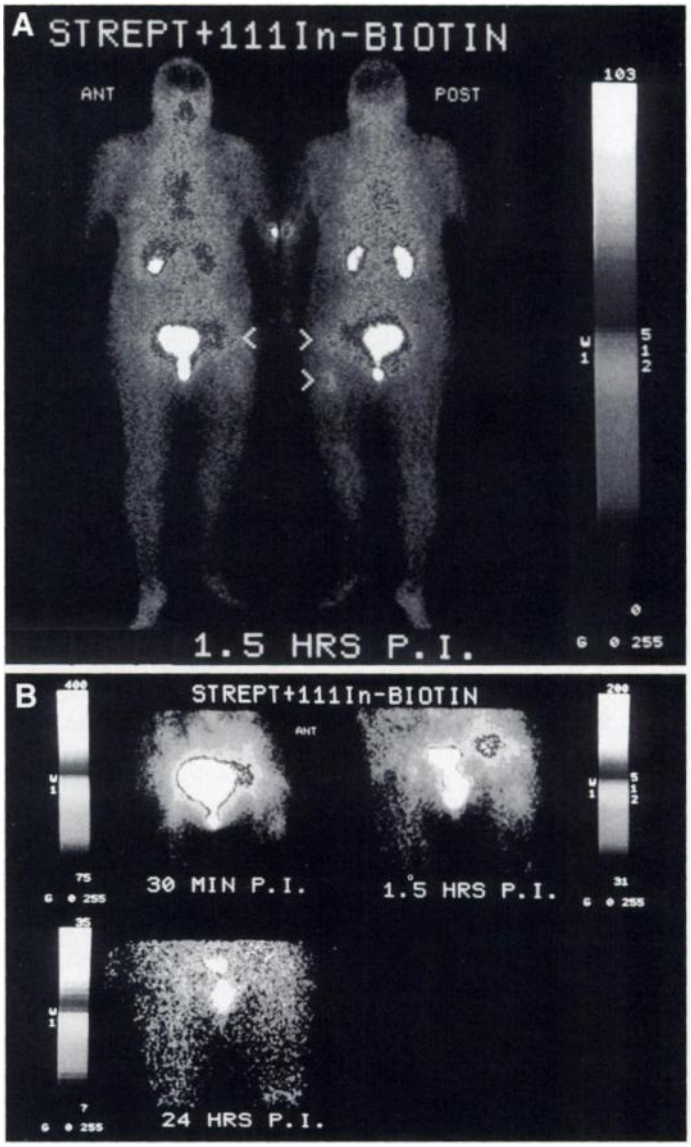

FIGURE 3. (A) Whole-body anterior and posterior image acquired 1.5 hr after administration of ^{111}In-EB1 with streptavidin, administered 24 hr earlier, demonstrates minimal normal tissue retention of activity. (B) Spot anterior views in the same patient at 30 min and 1.5 hr show clear delineation of the lesion and retention of focal activity at 24 hr postadministration of ^{111}In-biotin.

Target-to-Normal Tissue Ratios

The target-to-normal tissue ratios listed in Table 3 were determined by comparing counts in an area drawn around the lesion, to an area on the contralateral side, or in a region of similar size normalized for pixel number. Results are presented for the three protocols at 10 min, 1.5 to 4 hr, 24 hr and 48 hr. The ratios of 2.5 and 2.1 for streptavidin/labeled-biotin and IgG, respectively, at 1.5 to 4 hr were obtained, with little change at 24 hr: 2.0 and 2.5, streptavidin/labeled-biotin and IgG, respectively. As noted by the range given in parentheses, the data are varied within each protocol. As such, comparisons between protocols failed to establish any significant difference at any time.

DISCUSSION

This investigation reports on an application of pretargeting with unlabeled streptavidin and labeled biotin to detect infection in patients. An important observation in this investigation is that ^{111}In-EB1, with or without the preadministration of streptavidin, shows focal accumulation within minutes. However, persistence of a focus was only apparent when streptavidin was given previously. A report describing the use of ^{18}F-biotin for infection detection in an animal model with PET (*10*) also demonstrated that biotin alone was successful in detecting a lesion. The limitations of this preliminary investigation did not permit evaluation of the sensitivity and specificity of the streptavidin/labeled-biotin approach.

An important observation in this investigation is the absence of an antistreptavidin (HASA) response in all 5 of the patients tested at 4 to 8 wk. Previous investigations with administration of 1 mg streptavidin conjugated antitumor antibody (*9*), or a similar biotin binding protein, avidin (4–6 mg), used as a clearing agent (*8*), showed an antistreptavidin response at 2 wk in 3 of 5 patients, in the former, and an antiavidin response in 7 of 12 patients at 15 to 20 days, in the latter. The absence of a titer in this investigation at 4 wk may be due to the small patient sample. More recent experience in a large number of patients suggests that avidin and streptavidin are highly immunogenic are: a 20% response may be expected for avidin (Paganelli G, *personal communication*, 1995). In addition, no adverse reac-

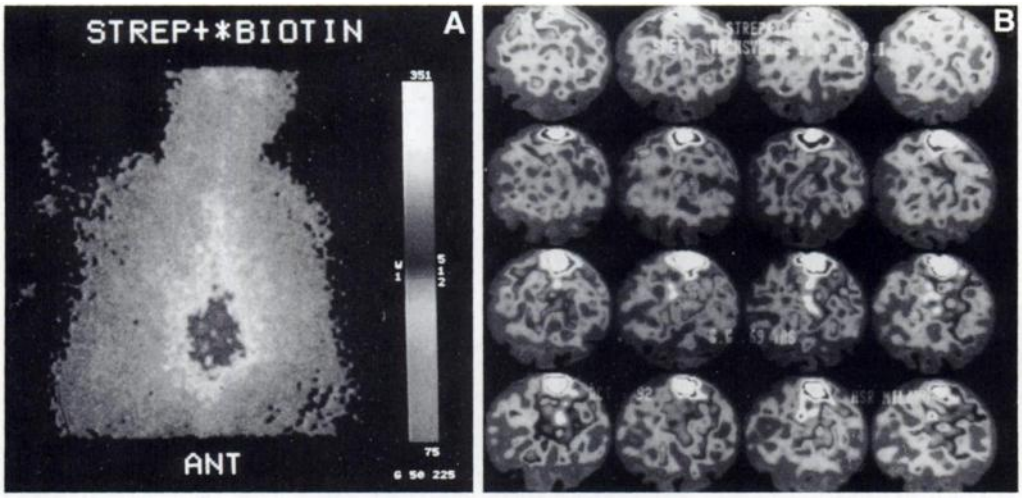

FIGURE 4. (A) Anterior spot view at 1.5 hr with the streptavidin/labeled-biotin protocol in patient with osteomyelitis of the sternum. (B) SPECT transverse images in the same patient clearly differentiate activity in the lesion (along the top edge of each view) from blood pool (rows 3 and 4 in center of view).

tions nor toxic effects from either streptavidin nor labeled biotin were observed.

This study also demonstrated that endogenous biotin is not an apparent problem with this two-step approach. First, streptavidin was detected in serum collected through 24 hr following administration using labeled biotin as a tracer. Thus, streptavidin in the circulation for 24 hr was not saturated with endogenous biotin. Second, in 7 of 10 patients, retention of activity in a lesion was maintained through 24 hr. Since labeled biotin alone was not retained beyond 2 hr, the persistence of activity in a focus may, in part, be explained by the probable binding to streptavidin located therein. Therefore, the streptavidin that is localized is also not saturated with endogenous biotin. An example of this is shown in Figure 6: with biotin alone at 2 hr (bottom right) the lesion is slightly visible above background, but with streptavidin administered previously a substantial region of abnormal accumulation is maintained even at 24 hr. The control studies with labeled biotin alone without the previous administration of streptavidin were not routinely imaged through 24 hr because of low activity remaining. Only when streptavidin was included in the protocol was activity maintained in a focus at later times. Thus, one can speculate that persistence of activity in a focus through 24 hr is likely due to binding to streptavidin.

As the data on the clearance of labeled streptavidin from the circulation demonstrate (Fig. 2), about 40% of streptavidin may remain in circulation at 6 hr. With labeled biotin administered at 6 hr after streptavidin, it is likely that the streptavidin is labeled in the circulation (Fig. 6). So, in this example, accumulation may be attributed to labeled streptavidin. In this investigation, accumulation of radiolabeled streptavidin in a lesion was not obtained. This one case may serve as indirect evidence that streptavidin accumulates in a lesion.

In all patients, focal accumulations were seen with streptavidin followed with radiolabeled biotin as early as 10 min with improved discrimination of the focus within the next 2 hr. In half of these patients, accumulation persisted through 24 hr. The

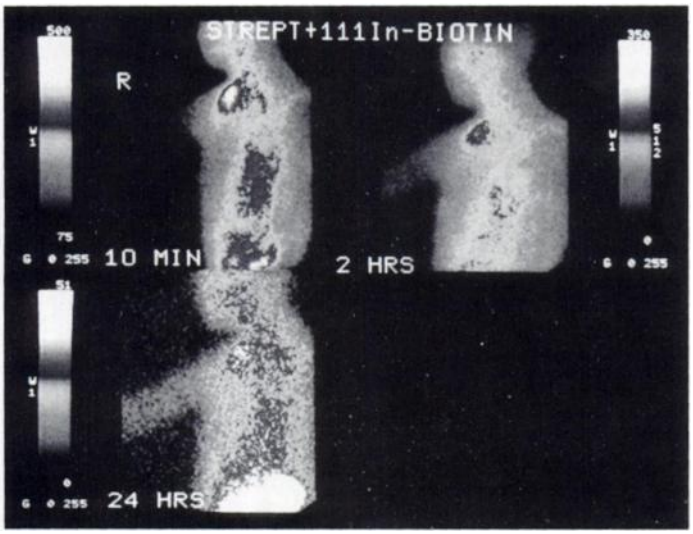

FIGURE 5. Patient with right clavicle sepsis after the streptavidin/labeled-biotin protocol. The spot view at 10 min easily depicts focal accumulation. Activity is retained through 24 hr.

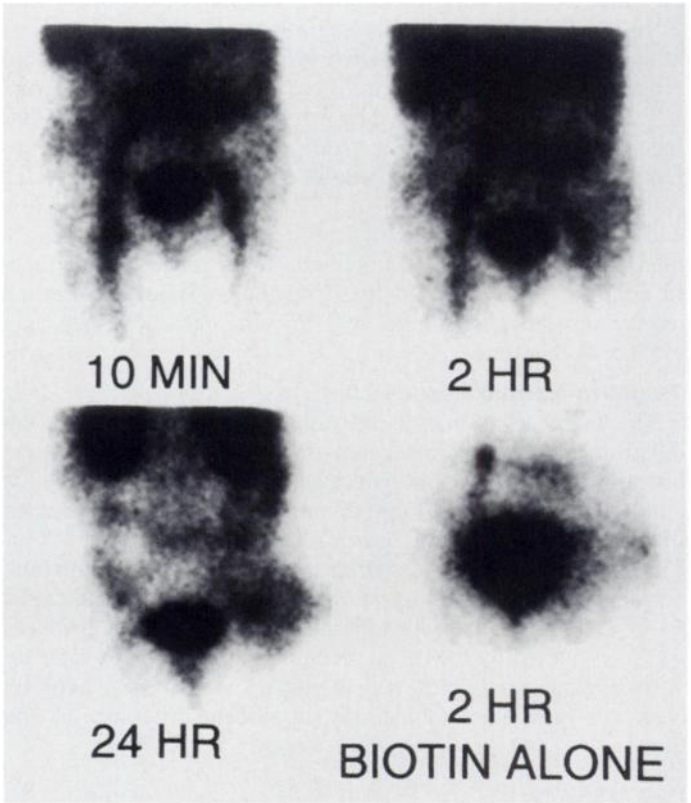

FIGURE 6. Streptavidin/labeled biotin protocol with labeled biotin administered 6 hr after streptavidin. Images were taken at 10 min, 2 hr and 24 hr, and during a second study without streptavidin administration (lower right). Blood-pool activity is higher than that observed in other patients and the lesion is most clearly discernable at 24 hr. The control study with biotin alone (2 hr image) shows minimal accumulation in normal tissues and in the lesion as well.

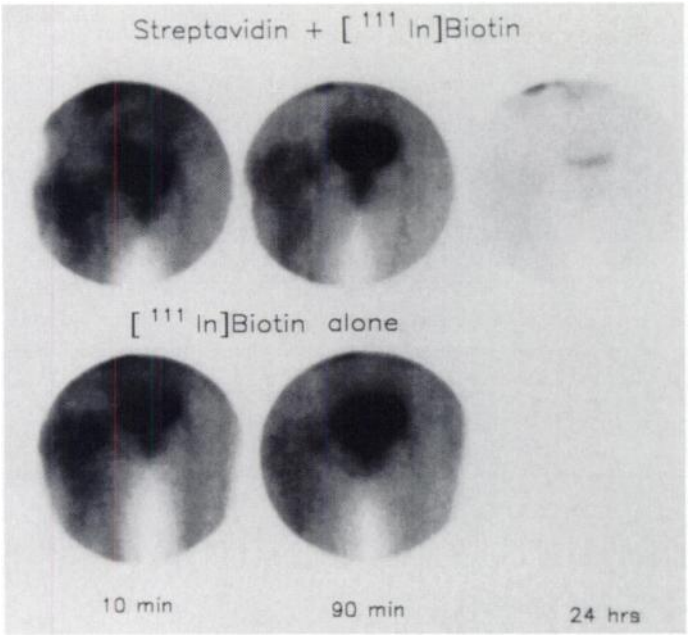

FIGURE 7. Streptavidin/labeled biotin study (top row) and control with labeled biotin alone (bottom row). Access of labeled biotin to the lesion is immediate in both cases with more focal accumulation with the streptavidin/labeled-biotin protocol at 90 min.

lesion-to-normal tissue contrast was excellent, in part due to low blood-pool activity and low normal-tissue accumulation of labeled biotin. Studies with nonspecific IgG identified abnormal regions of accumulation similar to that seen with the streptavidin/labeled-biotin protocol and produced similar target-to-nontarget ratios at 1.5 to 4 hr through 24 hr (Table 3). Comparisons cannot be made at the earliest times, since no images were performed at 10 min with labeled IgG, due to the expected slow accretion of this labeled protein into lesions.

This particular patient population had lesions predominately in the extremities. It will be interesting to see more studies of chest and abdominal lesions comparing IgG and the streptavidin/labeled-biotin protocol. Abdominal lesions are difficult to

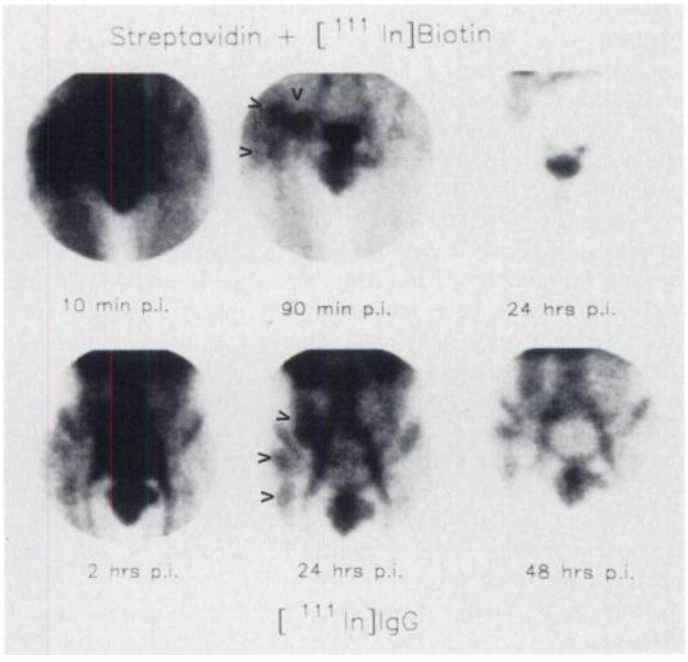

FIGURE 8. Streptavidin/labeled biotin study (top row) and control with labeled IgG (bottom row). The 90-min streptavidin/labeled-biotin study shows minimal normal tissue and blood pool relative to the 2 hr ^{111}In-IgG study.

TABLE 3
Target-to-Normal Tissue Ratios

	10 min	1.5–4 hr	24 hr	48 hr
STAV + EB1	2.7 (1.1–3.7) n = 5	2.5 (1.3–3.9) n = 12	2.0 (1.3–3.3) n = 10	ND
EB1	2.1 (1.7–2.4) n = 3	2.2 (1.6–2.7) n = 5	ND	ND
IgG	ND	2.1 (1.3–4.2) n = 6	2.5 (1.6–4.3) n = 5	2.9 (2.3–1.9) n = 5

STAV = streptavidin. ND = not done. There is no significant statistical difference between the data.

detect due to high blood-pool activity and organ accumulation of label. Thus, the small molecular weight biotin may have an advantage, as suggested in this investigation.

The similar target-to-nontarget ratios for the streptavidin/labeled-biotin and the labeled IgG protocols suggest that the former is likely to be as reliable as labeled IgG for infection imaging applications. These data also suggest that labeled biotin alone, without streptavidin, may have applications for infection imaging not previously anticipated, at least at the earliest times. The absence of data with labeled biotin alone at 24 hr for comparison to the streptavidin/labeled-biotin protocol may have indicated differently at this later time due to the quick clearance of labeled biotin and retention at 24 hr in the latter. Notwithstanding, the present study suggests that ^{111}In-biotin alone without the previous administration of streptavidin may have potential as an infection imaging agent.

This study has demonstrated lesion accumulation at early times and retention of activity through 24 hr, it remains to be determined if early lesion uptake is driven by the presence of streptavidin. The activity was administered as ^{111}In-EB1. Therefore, early focal accumulation is most likely due to ^{111}In-EB1, which may or may not be bound to streptavidin assumed to be in the lesion. Of importance is that labeled biotin has quick access to the lesion, which is discernable within minutes. Also, images as late as 24 hr show focal activity. At this late time, the activity is unlikely to be simply ^{111}In-EB1. It is likely retained bound to streptavidin, although direct evidence for this was not part of this investigation.

There are two advantages with this approach. First, activity is administered on a small molecular weight molecule that localizes and clears quickly. This study has demonstrated that after ^{111}In-EB1 administration, activity is observed in a lesion with reduced background as early as 10 min. Second, for imaging at later times, the streptavidin may participate in retaining activity in the lesion. Incorporation of streptavidin into the protocol is possibly more advantageous for late rather than early imaging.

CONCLUSION

This preliminary study describes infection detection using streptavidin and labeled biotin in a pretargeting approach. Although, the study sample was not adequate to evaluate sensitivity and specificity, the positive observations described substantiate further study in patients with a varied infectious clinical condition. Furthermore, the dosage and timing of agents are parameters to be investigated. Only then can this approach be evaluated for consideration as an alternative infection imaging agent. Moreover, since background levels decreased more rapidly with the streptavidin/labeled-biotin protocol than with labeled IgG, facilitating imaging within minutes postadministration of label, shorter lived nuclides such as ^{99m}Tc may be more advantageous and will be considered in future studies.

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Evaluation of Sequential Thallium and Gallium Scans of the Chest in AIDS Patients

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With decreasing incidence of pneumocystis carinii pneumonia (PCP) in AIDS as a result of prophylactic regimens, there is a higher incidence of tuberculosis (TB), mycobacterium avii complex (MAC), kaposi sarcoma and malignant lymphoma. There is a need for differentiating these various pathological entities. The purpose of this study was for a retrospective evaluation of sequential thallium and gallium scans in AIDS patients for differentiating intrathoracic kaposi sarcoma from malignant lymphoma and opportunistic infections. **Methods:** A total of 181 patients had both studies completed between March 1992 and May 1994. The final diagnosis was verified only in 83 patients. Results were correlated with the CD4 counts, bronchoscopic and chest radiograph findings. **Results:** In patients with pulmonary kaposi sarcoma and no opportunistic infections (19 patients), a thallium-positive, gallium-negative pattern was detected in 17 patients with a sensitivity of 89%. In the presence of kaposi sarcoma plus opportunistic infections, this pattern was only detected in 7 of 19 patients (sensitivity dropped to 37%). In 45 patients with opportunistic infections and no kaposi sarcoma, only two false-positive findings were found in patients with cytomegalic virus pneumonia for a specificity of 96%. For the whole group of 83 patients, sensitivity was 63%; specificity 95%; positive predictive value 92%; accuracy 81%; and negative predictive value 75%. **Conclusion:** A thallium-positive, gallium-negative pattern in AIDS patients has a high specificity for the diagnosis of kaposi sarcoma, however, the sensitivity dropped from 89% to 37% in the presence of opportunistic infections.

Key Words: AIDS; kaposi sarcoma; opportunistic infections; gallium-67-citrate; thallium-201-chloride

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AIDS has reached epidemic proportions in the United States with more than 800,000 Americans thought to be infected with the causative agent of HIV (*1*). Early in the epidemic, PCP occurred in 75% of patients with the syndrome (*2*). With the common use of primary and secondary prophylaxis against PCP, its incidence decreased and the life of AIDS patients is prolonged without stopping a decline in immune function. Accordingly, there has been a shift in the clinical manifestations of HIV infection from PCP to other illnesses that occur when immune function is depressed. Mycobacterium avii complex disease, waisting syndrome, cytomegalic virus disease and esophageal candidiasis occur more frequently in patients who received prophylaxis against PCP than in those who did not (*3*).

Kaposi sarcoma is the most common neoplasm observed in HIV-infected individuals, followed by non-Hodgkin's lymphoma (*4,5*). The frequency of pulmonary involvement of kaposi sarcoma varied between 21% to 44% (*6–8*). Non-Hodgkin's lymphoma was reported in homosexual men shortly after recognition of the AIDS epidemic (*9*). Subsequently, other studies confirmed the increased incidence of this tumor in patients at risk for AIDS (*10–17*) and is now recognized as the second most common HIV-associated malignancy (*18*). Although an association between kaposi sarcoma and the development of lymphoma had been reported before AIDS, patients with AIDS and kaposi sarcoma do not appear to be at greater risk of developing lymphoma than do patients with AIDS without kaposi sarcoma (*11*). Thoracic involvement does occur with non-Hodgkin's lymphoma, but it is usually not the major presenting feature of AIDS-associated lymphoma. The incidence of thoracic involvement by lymphoma varied from zero to 25% in most of the large series (*13,19*). In both kaposi sarcoma and malignant lymphoma, the behavior of the disease is more aggressive and patients die because of additional opportunistic infections.

Problems in AIDS patients occur for several reasons: (a) the existence of more than one disease at any given time, (b) the difficulty in early verification of the diagnosis, (c) nonspecificity of the clinical presentation of symptoms and (d) the abnormalities seen on morphological imaging modalities. In

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