Pretargeting of Bacterial Endocarditis in Rats with Streptavidin and $^{111}$In-Labeled Biotin

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A radioimaging approach for the detection of endocarditis has been investigated using two-step pretargeting with streptavidin and radiolabeled biotin. **Methods:** Hemodynamic alterations within the rat heart were induced by placing an indwelling catheter into the left ventricle through the aortic valves. The animals were subsequently infected with *Staphylococcus aureus* through a tail vein. After an incubation period, rats were first injected with streptavidin and, 2 h later, with $^{111}$In-labeled ethylenediaminetetraacetic acid–biotin. Whole-body gamma camera images were taken 4–5 h postinjection of the radiolabeled biotin. Control animals consisted of catheterized but uninfected, infected but uncatheterized and normal untreated rats. As a further control, the labeled biotin was administered to a study animal without the preadministration of streptavidin. **Results:** Histology showed typical endocarditic changes in the hearts of study animals with massive deposition of gram-positive cocci. Catheterized but uninfected animals showed alterations corresponding to nonbacterial thrombotic endocarditis. Macroautoradiography showed accumulation of radiolabel in the endocarditic vegetations of study animals. Whole-body gamma camera images showed important cardiac uptake in 7 of 8 catheterized and infected animals and in 3 of 6 catheterized but uninfected animals. Normal rats and those infected but not catheterized showed negative results by histology, autoradiography and imaging. The percent uptake of the injected dose in the heart was 0.20 (SD = 0.13) in catheterized and infected animals, 0.12 (SD = 0.10) in catheterized but uninfected animals, 0.10 (SD = 0.04) in infected but uncatheterized animals and 0.04 (SD = 0.01) in normal control animals. **Conclusion:** The two-step pretargeting approach using streptavidin and $^{111}$In-labeled biotin was used successfully to detect *S. aureus*-induced bacterial endocarditis in rats.

**Key Words:** endocarditis; streptavidin; biotin; pretargeting


Early diagnosis of infective endocarditis is a complex clinical problem. The general signs of bacterial endocarditis include fever, skin lesions, splenomegaly, new or changing heart murmur, peripheral embolization, congestive heart failure or conductance system disturbances. However, none of these signs is diagnostic of the disease. Blood cultures are also unreliable because patients frequently are treated with antibiotics before a diagnosis is established. Therefore, emphasis is placed on methods such as transthoracic echocardiography, Doppler sonography, CT (1) and, most recently, transesophageal echocardiography. All but the last has limited sensitivity to detect small vegetations. Transesophageal echocardiography has shown improved sensitivity to small lesions, but it is a technology that is costly, is not widely available and creates considerable patient discomfort. The most difficult diagnosis is that of small, hidden vegetations, which are common in patients with a prosthetic valve or calcification of the annulus or valves. The vegetations can grow rapidly, leading to valve perforation or abscess (2), thus worsening the patient’s prognosis (3).

Radionuclide imaging has the potential to provide a sensitive diagnosis of this condition. Previous studies on the radioisotopic detection of inflammatory heart conditions used $^{67}$Ga citrate (4,5), $^{99m}$Tc-pyrophosphate (5–7), $^{111}$In-labeled thrombocytes (8) and $^{99m}$Tc-antistaphylococcal antibody (9). White blood cells labeled with $^{111}$In or $^{99m}$Tc (16,10–12) and radiolabeled anti-NCA-95 antigranulocyte antibody (11) were used successfully in a limited number of patients. Each approach has had varying degrees of success.

This article describes a pretargeting approach using streptavidin and radiolabeled biotin to detect bacterial endocarditis in a rat model. The two-step approach depends on the nonspecific accumulation in the lesion and clearance elsewhere of unlabeled streptavidin from the first injection. The location of streptavidin within the lesion is then detected by imaging the radiolabeled biotin after the second injection. We have shown previously that this approach can detect soft tissue infections in an animal model (13) and chronic infection in patients (14).

A well-characterized rabbit model with catheter-induced bacterial endocarditis (15) was adapted to the rat. Surgical insertion of a catheter into the left ventricle followed by the administration of *Staphylococcus aureus* through a tail vein can efficiently induce bacterial endocarditis, as was shown by histopathological methods. The endocarditic rats were studied with streptavidin followed by $^{111}$In-labeled biotin.

**MATERIALS AND METHODS**

**Injectates**

A solution of streptavidin for injection (Societa Prodotti Antibiotici, Milan, Italy) at 10 mg/mL in normal saline was prepared and divided into 0.2-mL aliquots. Ethylenediaminetetraacetic acid—
biotin (EB1) was synthesized in our laboratory as described previously (16) and was labeled to a specific activity of either 10 or 30 μCi/μg with 111In acetate (New England Nuclear–DuPont, North Billerica, MA). The low-specific-activity biotin was used for imaging and biodistribution, whereas the higher-specific-activity biotin was used for autoradiography. To test labeling efficiency, an aliquot of 111In-labeled biotin was mixed with an excess of streptavidin. After a 10-min incubation at room temperature, the mixture was applied to a 0.7 × 20-cm Sephadex G-50 column (Sigma Chemical Co., St. Louis, MO) eluted with saline and fractions of five to seven drops collected. The percentage of eluting with the protein defined the labeling efficiency and was determined by counting each fraction in an NaI (TI) well counter. For imaging studies, 10 μg (90–110 μCi) of 111In-labeled biotin was administered intraperitoneally in 0.1 mL saline. For autoradiographic purposes, the specific activity was 30 μCi/μg.

Animal Model

Bacterial endocarditis in male Wistar rats (weight range, 300–400 g; Charles River, Willmington, MA) was induced as described by Perlman and Freedman (15). A polyethylene tubing was surgically placed through the aortic valve as follows. The neck area of an anesthetized animal (Ketaset/Rompun, 60–7.5 mg/kg [Ketaset; Aveco, Fort Dodge, IA; Rompun; Mobay Corp., Shawnee, KS]) was shaved and cleaned with aseptic solution. The right common carotid artery was exposed above the clavicle and a polyethylene catheter (PE-10; inner diameter = 0.28 mm; outer diameter = 0.61 mm; Intramedic Clay-Adams, Parsippany, NJ) filled with diluted heparin (1000 U/mL saline; Elkins-Sinn, Inc., Cherry Hill, NJ) was inserted through an incision in the artery and gently fed toward the heart. Vigorously pulsating resistance on the catheter indicated when the left ventricular wall was reached. The catheter was then secured in place at the point of entry with the cervical end bent over and tied to the surrounding muscle using 3–0 silk (Deknatel, Queens Village, NY) and the skin was sutured with Ethicon 4–0 monofilament nylon (Ethicon, Somerville, NJ).

The day after the surgical insertion of the catheter, rats were infected with S. aureus (no. 29213; American Tissue Culture Collection, Rockville, MD) by tail vein administration of a 0.2-mL bolus containing approximately 107 colony-forming units. Bacterial cultures were maintained on blood agar plates, and cultures were grown in peptone-yeast-glucose culture broth (BBL; Becton-Dickinson, Cockeysville, MD) overnight at 37°C in preparation for animal inoculation. Thereafter, 48 h was allowed for bacteremia to become established. Mortality was near 30% (including anesthesia-related losses), similar to that reported by others (17). On the fourth day, 0.6 mg unlabeled streptavidin in 0.2 mL saline was administered through a tail vein. Two to three hours later, 10 μg 111In-labeled EB1 was administered intraperitoneally and the animals were imaged 5 h thereafter. The study group was catheterized and infected, and the three control groups were as follows: untreated, uncatheterized but infected and catheterized but uninfected with 6–8 animals in each group. A catheterized and infected animal was administered 111In-labeled biotin alone without the preadministration of streptavidin.

Imaging

To minimize blood-pool radioactivity, animals were not imaged until 5 h after the administration of 111In-labeled biotin. Before imaging, animals were anesthetized with pentobarbital (7.5 mg/kg intraperitoneally) and were placed directly on the medium-energy collimator of a portable gamma camera (Apex 409M; Elscint, Haifa, Israel). Typically, posterior whole-body views were acquired for 5 min each.

Biodistribution

After imaging, animals were killed by administration of 15–20 mg/kg pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, IL) and a sample of whole blood was obtained through the thoracic aorta. The heart, including the catheter and carotid artery (from point of entry), was removed as one piece, gently rinsed with saline so as not to disturb the pathology, weighed and then counted in an NaI(TI) well counter. The left ventricle of the heart was opened through the anterior wall with a scalpel to locate the position of the catheter and to record signs of infection, especially in the region of the aortic valves. Animals were excluded if the catheter was not in the correct position.

For the determination of radioactivity biodistribution, other tissues, including liver, kidney, lung, stomach, spleen, skin, muscle and intestine, were removed, rinsed, weighed and counted in an NaI(TI) well counter for radioactivity. Student unpaired t test was used for statistical comparison of the various control groups (Sigma Plot SPSS, Chicago, IL).

Histology

For histological examination, longitudinally cut, formalin-fixed hearts were processed through alcohols and xylene, embedded in paraffin and sectioned in preparation for histology with hematoxylin–eosin or Gram staining using standard procedures.

Autoradiography

Autoradiography was used to identify regional intracardiac distribution of the radiolabel. After excision, weighing and counting of total radioactivity, some hearts (with intact catheters and carotid arteries) were frozen at −20°C for 1 h and then embedded in Tissue Tek OCT compound (Miles, Inc., Elkhart, IN) and stored at −25°C for 2 h. Using a cryomicrotome (PMV 2250; KLB Instruments, Gaithersburg, MD), each heart was sliced along the long axis and representative 50- to 100-μm slices were immobilized onto adhesive tape (type 820; 3M, St. Paul, MN) and were left to freeze dry. Twenty-four hours later, the tapes with tissues were mounted on cardboard and placed in contact with x-ray film (Ektrascan M EM-1; Eastman Kodak Co., Rochester, NY) in a vacuum-sealed black plastic bag. The vacuum-sealed bag ensured tight contact of the slices with the film. The radioactivity content of 50- to 100-μm slices was low so that samples were left undeveloped until all radioactivity decayed (10–14 d). The film was developed in an automatic film processor (Kodak RP X-OMAT Processor, model MGB; Eastman Kodak Co.). Overlaying the autoradiographs and tissue slices allowed the correlation of anatomic structure with the corresponding radioactivity. Because the distribution of the label was not to be quantified, 111In standards were not included during the autoradiographic process.

RESULTS

Quality Control

The 111In-biotin labeling efficiency was always >90% at either 10 or 30 μCi/μg when assessed with excess streptavidin followed by Sephadex G-50 column chromatography. The streptavidin used in this investigation bound an average of three labeled biotin groups per molecule when 111In was added at a 1:4 streptavidin/biotin molar ratio.
**Biodistribution**

The distribution of radiolabel in tissues at 5 h postadministration of labeled biotin for the study and three control groups is shown in Figure 1 (n = 6–8). As expected, accumulations in the major organs for all four groups are low, with the exception of the kidneys. Of significance to the detection of endocarditis is the low radioactivity levels in blood and organs near the heart, such as lung and liver. In the heart, the untreated animals show the lowest accumulation of label among the control groups at 0.04 %ID/g. The other two control groups showed significantly (P < 0.05) decreased heart radioactivity at 0.10–0.12 %ID/g. However, the study group, i.e., catheterized and infected, showed the highest heart accumulation at 0.20 %ID/g, representing a 5-fold increase over the untreated animals (P < 0.05) and twice that of the other two control groups (P < 0.05).

An additional control animal was treated with 111In-labeled biotin without the preadministered streptavidin. In this case, the percentage of accumulated radioactivity at 5 h was 5-fold to 20-fold lower in all organs relative to the other animal groups being treated with streptavidin (data not presented). For example, radioactivity in the heart was reduced to 0.006 %ID/g.

**Gross and Microscopic Pathology**

For gross examination before sectioning, hearts were cut through the anterior wall of the left ventricle. The animals that were catheterized and infected showed the most severe tissue alterations. In most of the specimens from these animals, inflammation, including abscess formation, was visible, sometimes extending into the aortic root and the left ventricle. Pink excrescences, compatible with bacterial vegetations, were attached to the valve. The group of animals that were catheterized but uninfected showed only white and brown thrombi in the area of the aortic valve. The tissues of animals that were inoculated with *S. aureus* but were not catheterized appeared normal on gross examination.

Histological sections of hearts from untreated control animals showed an intact aortic valve and cardiac muscle with normal myocardial fibers, as expected (Fig. 2a). Similarly, infected but uncatheterized hearts also showed intact structures (Fig. 2b). Uninfected animals that were catheterized showed a thickening of the aortic valve on which a mixed fibrin-platelet cap was visible (Fig. 2c). Granulocytic infiltration of surrounding tissues, diagnostic of acute endocardial and myocardial inflammation, was also present. Gram stains were negative in all 3 controls (data not shown). The pathology of the catheterized, uninfected control can be summarized as foreign body-induced, nonbacterial thrombotic endocarditis.

On hematoxylin-eosin staining, hearts from catheterized and infected animals typically showed large deposits of fibrin on the thickened valve (Fig. 2d). Valve destruction was the most severe in this group. Large deposits of coecal bacteria were present under the layers of the fibrinous cap and can be seen as dark staining areas (Fig. 2d, arrow). Gram stains of adjacent sections showed large gram-positive coecal vegetations on the valve leaflet and base that sometimes extended distally into the aortic root.

**Imaging**

In general, the images obtained at 5 h showed radioactivity in kidneys and bladder with low uptake in the upper abdomen (liver) and chest (lungs and blood pool). The low radioactivity accumulation in the thorax is an advantage in evaluating cardiac radioactivity. In the case of animals that were catheterized, all images showed radioactivity at the incision in the anterior neck. This accumulation may be explained by the nonspecific uptake of radioactivity in edemic or traumatized tissue. However, this accumulation did not interfere with determinations in the cardiac region.

![FIGURE 1. Histograms show biodistribution at 5 h of 111In-EB1 in rats. Data from untreated normal rats, infected but not catheterized controls, catheterized but not infected controls and catheterized and infected rats are shown (n = 6 per group). Error bars represent 1SD of mean.](image-url)
addition, several animals showed accumulations of label in the interscapular region on the back, which proved to be in subcutaneous brown adipose tissue. Because this uptake overlapped with the heart on anterior images, lateral views were very useful in determining cardiac uptake in these animals.

All but 1 animal in the study group of 8 catheterized and infected animals showed marked focal uptake in the anterior chest. The one exception showed faint indeterminate anterior mediastinal radiolabel uptake. After dissection, the catheter in this case was found in the descending aorta rather than the desired location in the left ventricle. Therefore, the remaining 7 animals, properly catheterized and infected, all showed strong positive chest uptake.

Figure 3a presents images of a pair of untreated rats administered streptavidin followed by $^{111}$In-labeled biotin. No accumulation is evident in the heart, whereas radioactivity is obvious in the kidneys and bladder as expected because of the clearance of $^{111}$In-labeled biotin. Figure 3b shows images of 2 infected but uncatheterized animals. Once again, the kidneys and bladder are visible in contrast to the heart. Figure 3c presents lateral images of a pair of rats that were both catheterized; however, only the animal on the left in this panel was infected. Distal from the accumulation of radioactivity at the site of catheter insertion is accumulation of radioactivity in the anterior chest in the vicinity of the heart. Camera resolution was insufficient to allow more definitive identification of the radioactivity to the heart. The catheterized but uninfected animal on the right in Figure 3c shows no similar heart uptake. Figure 3d shows a catheterized and infected animal. Heart accumulation is evident in the image, and subsequent histology revealed severe endocarditis in this animal. The animal on the left in Figure 3d was subjected to a sham operation in which the right common carotid artery was isolated and ligated as is usual, but no catheter was inserted. The animal was infected as usual with S. aureus. Although there is accumulation of radioactivity in the area of incision, it is almost certainly because of tissue trauma. There is no indication of cardiac uptake.

Of the 6 animals that were catheterized but not infected, the whole-body images showed no heart accumulation of radioactivity in 3 animals, faint accumulation in 1 animal and positive uptake in the remaining 2 animals. Of those 2 animals with positive heart images, 1 showed more radioactivity retained in the whole body. Thus, in this case, the imaging result may have been in part caused by slow clearance of radiolabel probably because of the animal's
condition. Histological examination of the other animal with a positive chest image showed large thrombotic changes in the heart, with myocardial infiltration of inflammatory cells but negative Gram staining. Because of excessive inflammatory involvement, this case was not classified as pure nonbacterial thrombotic endocarditis. These 2 animals were therefore not included in the biodistribution analysis; 2 similarly treated animals that were not imaged made up the group for biodistribution analysis.

Figure 4 shows additional histological sections of a representative rat heart showing extensive bacterial colonization of the aortic wall in both the hematoxylin-eosin–stained (top) and Gram-stained (bottom) sections of the aortic root, extending distally into the aorta.

**Autoradiography**

Autoradiography was performed to locate more precisely the accumulation of radioactivity within the heart. A representative section of tissue from a catheterized and infected animal with two corresponding autoradiographs of adjacent slices are shown in Figure 5. The tissue section (Fig. 5A) shows the catheter in the aorta extending into the left ventricle. A large thrombus is evident adjacent to the tip of the catheter next to the region of the aortic valve. A small abscess on the aorto-ventricular border is also apparent. These changes were typically observed in the catheterized and infected animals and represent a pathology consistent with severe bacterial endocarditis. One autoradiograph (Fig. 5B) shows increased radioactivity uptake in the endocarditic thrombotic region and in the abscess. The other autoradiograph (Fig. 5C) shows accumulations in the region of the aortic root paralleling the catheter and corresponds to extended inflammatory areas. The uptake of radioactivity is greatest in regions that appear infected; however, the radioactivity is distributed among focal regions and is not uniformly distributed throughout the heart. The areas of accumulation can be clearly delineated from uninvolved areas. Endocarditic animals administered only 111In-labeled biotin without the preadministration of streptavidin had insufficient radioactivity in the heart for autoradiographic analysis.

**DISCUSSION**

*Staphylococcus aureus* is a particularly frequent pathogen in acute bacterial and drug-addict endocarditis and is an important cause of prosthetic valve endocarditis (18,19). Bacteria, especially *S. aureus*, are strong initiators of platelet aggregation (20). Hidden under growing layers of the fibrin plaque, the infection can be relatively inaccessible to white blood cells. This study used a catheter-induced animal model of acute staphylococcal endocarditis that was modified from
that of Perlman and Freedman (15). The insertion of the catheter alone resulted in sterile fibrin and platelet vegetation, whereas S. aureus administration led to the development of endocarditis. The severity of the model was characterized in this study by a mortality rate of 30%, similar to that reported elsewhere (17). Animals died of postoperative heart failure, complications of anesthesia or overwhelming bacterial sepsis.

The rat model mimics the clinical pathology and mortality of human staphylococcal disease (21). The functional importance of the catheter in the model is in preventing the valve from completely closing during diastole, thus creating aortic insufficiency. In human aortic insufficiency, the retrograde diastolic flow passes through the valve as a high-pressure and high-velocity jet and, on impact, can damage the endocardium (22).

Also, as the regurgitating flow slows down beyond the valvular orifice, it creates abnormal turbulence and low-pressure sinks that are favored by colonizing bacteria (23). In the case of aortic insufficiency, these predisposing conditions render the ventricular surface of the aortic valves and the chordae tendineae of the mitral valve a primary site of bacteremic colonization (22). Bacterial adherence to the catheter facilitates colonization or thrombus development (24). In addition, the tip of the catheter can irritate the endocardium and contribute to colonization in this way. A direct role of the catheter in the model was supported by histological evidence showing moderate nonbacterial thrombotic endocarditis in the group of animals that was catheterized but uninfected. There appeared to be no physiological effect of the catheter on the rest of the body.

Ring abscesses are found in almost one third of the clinical cases with natural valve disease (25) and in about 60% of prosthetic valve endocarditis (26). The rat endocarditis model showed similar physiological characteristics and may therefore be relevant to endocarditis conditions in patients. Catheterized but uninfected rat hearts showed milder valve thickening and destruction accompanied with sterile thrombotic inflammatory changes, whereas normal and infected rats showed intact heart pathology.

Pretargeting approaches have been applied previously to cardiac imaging. Streptavidin-conjugated antimyosin F(ab')2 antibody followed by 111In-labeled biotin was used to detect myocardial infarction (27). In addition, a two-step approach with avidin and labeled biotin unexpectedly showed cardiac uptake in a patient with septic endocarditis (28). Two-step pretargeting with unlabeled streptavidin followed by radiolabeled biotin was first used for the detection of soft tissue infection in a mouse model (13). Higher target-to-blood ratios were reported over that achievable with one-step 111In-labeled immunoglobulin G in that model. The same pretargeting approach has been used clinically in patients and provided focal accumulations of radioactivity in chronic infections (14) and vascular graft infections (29). In these investigations, the first step of the proposed mechanism was the nonspecific extravasation of streptavidin into the edemic inflamed tissues caused by the increased capillary permeability in the infected regions. The histological structure of endocarditic vegetations does not allow similar speculation here. There are no blood vessels in the normal valve and mural endothelium and, at least initially because of lack of capillarization, there is no edema formation to explain the leakage of streptavidin into tissues. However, the underlying mechanism may again be nonspecific accumulation of streptavidin into the vegetation. Furthermore, the growing fibrin mass might entrap circulating streptavidin at the site of endocarditic vegetations.

One advantage of pretargeting for cardiac imaging is that time may be allowed for the streptavidin in circulation to clear before the radioactivity is administered. Along with the rapid clearance of radiolabeled biotin, the result can be levels of radioactivity in blood and other tissues below that which interferes with heart imaging. For example, contrary to other imaging techniques (i.e., 111In-labeled white blood cells), liver radioactivity levels were not a problem in this study.

In general, the two infected groups (catheterized and uncatheterized) both showed higher retention of radioactivity than normal or catheterized but uninfected rats. This can
be caused by slower clearance of the radioactivity secondary to bacteremia, which can cause capillary dilatation with consequent slowing of the peripheral circulation. Among heart uptakes, the highest accumulation of radioactivity was in the study group (i.e., catheterized and infected).

The moderate differences observed in heart accumulation of radioactivity between the study and control groups (Fig. 1) may be, at least in part, because of sampling. It was not possible to separate areas of endocarditis from normal heart for separate counting, and as a result, regions of the heart muscle with high radioactivity content were counted along with areas of low radioactivity. The images, however, do not suffer from this drawback. Anterior and left lateral whole-body images taken 5 h postadministration of radiolabel show strong accumulation in the hearts of study animals. Differences in the images of the controls and study animals were sufficient to differentiate clearly between normal, infected but uncatheterized, catheterized but uninfected and catheterized and infected animals. However, the small rat heart combined with the limited resolution of the gamma camera prevented the identification of focal uptake to intracardiac structures.

Because of the limitations of in vitro counting and in vivo imaging, histology and autoradiography were used to determine the physiological condition of the heart and to characterize the distribution of radiolabel. Histological analysis of hearts from the study animals showed strong evidence of infection and endocarditis often with partial destruction of the aortic valve and abscess.

Autoradiography showed a homogeneous distribution of label in normal hearts, whereas endocarditic hearts showed increased uptake of radioactivity in the infected valves and accumulation in adjacent thrombi and abscesses. In the more severe cases, when endomyocardial infection extended distally into the aortic root, uptake of label was also observed in the involved aortic wall. The nonaffected regions of the left ventricle had markedly lower uptake, thus creating a heterogeneous intracardiac distribution of label (focal endocarditic high uptake versus low basal uptake). Based on this result, one might speculate that the cardiac uptake as observed on the whole-body images reflected the selective intracardiac uptake of the infected region.

CONCLUSION

Histopathological and autoradiographic results in this study show evidence that radioactivity accumulation was consistent with the pathology. The results of the biodistribution, imaging, histology and autoradiographic studies indicate that radioactivity accumulated in the infected alterations of rat hearts using the two-step streptavidin-labeled biotin pretargeting protocol. Furthermore, the pretargeting approach resulted in reduced background radioactivity in the tissues around the heart.

REFERENCES


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