INVESTIGATIVE NUCLEAR MEDICINE

Imaging Endocarditis with Tc-99m-Labeled Antibody—An Experimental Study: Concise Communication

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The sensitivity and specificity of Tc-99m-labeled antibacterial antibody (Tc-99m Ab) for detecting bacterial endocarditis were evaluated in an experimental model. Rabbit-produced antistaphylococcal antibody was extracted using Rivanol and chemically labeled with Tc-99m. This Tc-99m Ab was injected intravenously in New Zealand rabbits 24 hr after producing *Staphylococcus aureus* endocarditis of the aortic valve. Imaging and tissue analyses were performed on the following day. All 11 animals developed *S. aureus* aortic-valve vegetations and showed increased uptake of Tc-99m Ab at the aortic valve, 118 times higher than at the uninfected tricuspid valve. Although high hepatic radioactivity and anatomic uncertainties interfered with in vivo delineation of these lesions, images of the excised hearts showed all affected valves. Two rabbits inoculated with *Escherichia coli* did not develop endocarditis and had little uptake of Tc-99m Ab, while six rabbits with enterococcal endocarditis had no uptake of the Tc-99m Ab in their vegetations. The findings suggest potential value of Tc-99m Ab in the rapid diagnosis of endocarditis.

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Diagnosis of endocarditis by current methods is circumstantial and at best presumptive. By the time vegetations become demonstrable by echocardiography, surgery is required for cure (*1*). Early diagnosis of endocarditis, and appropriate therapy, offer a favorable prognosis (*2*). Circulating immune complexes known to be present in the early course of the disease in most patients have been used for the diagnosis of staphylococcal endocarditis in drug addicts (*3*). The circulating antibodies can be labeled with radionuclides such as Tc-99m without loss of biologic activity of the protein (*4,5*). Radionuclide antibody imaging, a noninvasive method, is a novel—and, unlike the use of pyrophosphate (*6*), possibly specific—approach to the location of certain infections as well as neoplasms and hormone receptor sites, and its potential has not yet been fully evaluated. *Staphylococcus aureus* is a common cause of endocarditis in drug addicts; it is also a common cause of several suppurative infections. This experimental study is designed to assess the efficacy of Tc-99m-labeled antibacterial antibody for early detection of bacterial endocarditis by scintigraphic techniques.

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METHODS AND MATERIALS

Production of antibacterial antibody. The *Staphylococcus aureus* isolate used throughout this study was obtained from a patient with *S. aureus* endocarditis. The bacteria were suspended in normal saline in a concentration of 100 million CFU/ml, heat-killed at 70°C for 1 hr, and mixed with an equal volume of Freund's complete adjuvant. One milliliter of this mixture was injected subcutaneously into the inguinal area of a New Zealand white rabbit daily for 3 days, followed by 1.5 ml daily for 4 days, then 2 ml every other day for an additional 7–10 days. The antistaphylococcal antibody titer was deter-

mined by agglutination technique, and if the titer was low or absent, the process was repeated until a titer of at least 1:800 was obtained. The antiserum was then collected and stored at −70 °C.

The anti-*S. aureus* antibody was extracted from the antiserum using a modified Rivanol (2-ethoxy 6,9-diaminacridine lactate)* fractionation method of Horejsi and Smetana (*7*). In brief, the antibody was separated from other serum-protein fractions with 0.4% Rivanol solution using a ratio of one part of antiserum to four parts Rivanol. Rivanol was removed from the supernatant by the addition of 300 mg sterile activated charcoal. The antibody was then salted out from the supernatant by adding a saturated solution of ammonium sulfate (4 *M*) to the filtrate in a 1:1 ratio. The antibody precipitate was separated by centrifugation, washed with 7 m*M* Sorenson's phosphate buffer at pH 7.4, and reconstituted with the same buffer to a final concentration of 3–4 mg/ml as determined by uv spectrophotometry at 280 nm.

Labeling of the antibody with Tc-99m. One milliliter of the concentrated antibody solution was labeled with 60 mCi of pertechnetate (Tc-99m) according to a previously published chemical method (*4*). The pertechnetate was first reduced by the addition of 0.5 ml of a solution of 0.1 mg $SnCl_2$ in 0.05 *N* HCl solution. The mixture (pH 1.8) was incubated at room temperature for 10 min, then readjusted to pH 7.4 with 0.75 ml trisodium citrate/NaOH solution (pH 12.4). To the neutralized mixture, 1 ml of the antibody solution was added by very slow injection with gentle swirling into the reaction vial. After an additional 30-min incubation period at room temperature, the labeled antibody was ready for use.

Quantitative and qualitative assay of the labeled antibody was carried out by protein electrophoresis, ascending paper radiochromatography with Whatman No. 1 paper, and ITLC silica-gel plate with 85% methanol as the solvent. In addition, trichloroacetic acid (TCAA) protein precipitation was performed, with unlabeled human immune gamma globulin as carrier protein.

Production of bacterial endocarditis. Bacterial endocarditis was produced in New Zealand white rabbits of either sex (2–2.5 kg) by slight modification of a method described earlier (*8*). The anterior cervical area was shaved and cleaned with Povidone-iodine† and under anesthesia the right internal carotid artery was exposed and opened between two ligatures placed on either end of the exposed segment. The upper ligature was tightly secured while the lower was loosened to pass a No. 17 polyethylene catheter toward the heart until resistance was encountered at the aortic valve, which was then traumatized by the sharp tip of the catheter five or six times. Approximately 100 million CFU/ml of *Staphylococcus aureus* from two different strains were injected through the catheter. The catheter was then withdrawn and the lower ligature securely tied to prevent bleeding. The skin was sutured, and after recovery from anesthesia the animals were returned to their cages. With this technique all animals developed aortic-valve vegetations within 48 hr and died within 2–3 days. In another group of six animals, endocarditis was induced with enterococci using a similar technique. These animals were used to assess the specificity of Tc-99m-labeled anti-*S. aureus* antibody. Blood samples were obtained from three rabbits with enterococcal endocarditis at 15 min and at 24 hr after administration of Tc-99m-labeled anti-*S. aureus* antibody. In vivo stability of Tc-99m Ab was determined by assaying these blood samples by protein electrophoresis, TCAA precipitation, and antibody titer determination by tube agglutination assay. Two more rabbits were traumatized using similar technique but were inoculated with *Escherichia coli*. These animals did not develop endocarditis but served as controls.

Scintillation imaging. Twenty-four hours after surgery, each rabbit was injected intravenously with 20 mCi of Tc-99m-labeled anti-*S. aureus* antibody. Scintigrams images were obtained 24 hr after tracer administration to allow for reduction of background radioactivity. Previous imaging experiments showed that optimal uptake of Tc-99m Ab was seen 18–24 hr after injection. An anterior image of the heart was obtained with an Anger camera and pinhole collimator. The animal was then killed and a sample of whole blood was taken directly from the heart. The heart was then removed and washed with normal saline to restrict contamination. The left ventricle was incised to expose the infected aortic valve and carefully washed with normal saline. The heart was placed in a clean petri dish, photographed, and then scintigraphed for 45 min to collect 50,000 counts. Immediately after imaging, the aortic and tricuspid valves were removed, as well as samples of the myocardium. Tissue samples from other organs were collected, washed with normal saline, and the net wet weight of each sample determined. Urine samples were collected from the bladder. Percent uptake of the radiolabeled antibody in the tissue samples was determined by assaying the radioactivity in a well scintillation counter with the spectrometer set to count Tc-99m against a 1:1000 dilution of the injected dose. The tricuspid valves were not infected in these experiments, and were used as controls for the uptake of radioactivity in the aortic valves.

RESULTS

The labeling efficiency of 20 batches of Tc-99m antibody, as assayed by paper radiochromatography using Whatman No. 1 paper was 98.09% ± 1.81 (range 92.39–99.61%) and was 88.49% ± 6.83 (range 84.0–94.79%) with ITLC silica-gel plates. The average

binding efficiency as determined by TCAA protein precipitation was 93.45% ± 3.65 (range 87.43–98.04%). When the protein fractions in the electrophoresis plate were assayed for Tc-99m radioactivity, 98.88% (range 97.30–99.79%) of the radionuclide was found in the gamma globulin fraction, with 0.69% (range 0.01–2.07%) in the beta globulin. Neither the extraction nor the chemical labeling process affected the immunological activity of the antibacterial antibody as measured by agglutination titers.

Data from assays by antibody titer and TCAA protein precipitation demonstrated that Tc-99m Ab was stable in vivo. There was no change in the antibody activity or in the total radiolabeled protein content between the 15-min and the 24-hr serum samples obtained from the rabbits with enterococcal endocarditis. Protein electrophoresis of the 15-min serum samples indicated that Tc-99m radioactivity, hence Tc-99m Ab, remained fixed at the gamma-globulin regions, with no evidence that the tracer was transferred to other protein fractions. Because of low radioactivity level, protein electrophoresis was not done with the 24-hr serum samples.

Anterior scintigrams of the intact animal were equivocal and failed to delineate the lesion within the heart. Highest uptake of Tc-99m Ab in the intact rabbits with staphylococcal endocarditis was seen in the liver, spleen, bone marrow, and bladder. When the excised heart was imaged with the left ventricle opened and exposed to the Anger camera, all visually identifiable lesions were clearly delineated. In ten out of 11 excised hearts studied, the vegetations were large enough to show

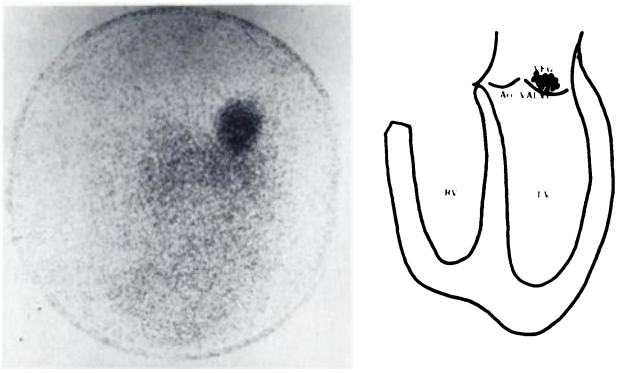

FIG. 1. Tc-99m antibody image of excised rabbit heart No. 4. (Left): vegetation shows an area of increased activity just above aortic valve. (Right): tracing from photograph of excised heart for purposes of orientation. RV = right ventricle; LV = left ventricle; Ao = aortic valve; VEG = mulberrylike cluster of vegetations.

TABLE 1. DISTRIBUTION OF Tc-99m-LABELED ANTI-*Staphylococcus aureus* ANTIBODY IN CARDIAC VALVES, MYOCARDIUM, AND BLOOD AT 24 HR AFTER INJECTION OF RADIOPHARMACEUTICAL

Case No.	Tissue sample	Radioactivity cpm/g‡ tissue	Percent inj'd dose/g tissue	Ratio† of infected valve to normal valve	myocardium	blood
1.	Blood	14770	0.022			
	Myocardium	6284	0.009			
	Aortic valve	259388	0.385	51:1	41:1	18:1
	Tricuspid valve*	5100	0.008			
2.	Blood	14179	0.023			
	Myocardium	3126	0.005			
	Aortic valve	859068	1.374	491:1	280:1	61:1
	Tricuspid valve	1778	0.003			
3.	Blood	11390	0.017			
	Myocardium	8722	0.013			
	Aortic valve	181596	0.269	34:1	21:1	16:1
	Tricuspid valve	5422	0.008			
4.	Blood	13446	0.020			
	Myocardium	3265	0.005			
	Aortic valve	80127	0.118	12:1	25:1	6:1
	Tricuspid valve	6667	0.010			
5.	Blood	39048	0.058			
	Myocardium	8519	0.013			
	Aortic valve	25033	0.030	4:1	2:1	0.5:1
	Tricuspid valve	5854	0.009			
			Average Ratio:	118:1	74:1	20:1

* Tricuspid valve was not infected.
† Based on percent injected dose/g tissue.
‡ cpm = counts per minute.

intense uptake of radioactivity (Fig. 1). The vegetations ranged from 2 to 3 mm in average size. On histologic examination they showed bacterial clumps among the fibrin threads, and *S. aureus* was recovered by culture in all of them. The tricuspid valve site, as it was not infected in this experiment and was free of vegetations, showed no increased uptake of radioactivity. In one rabbit (Table 1, Case 5), in which a single lesion under 2 mm in greatest dimension was produced, the scintigram showed only faint localization.

Two rabbits inoculated with *E. coli* did not develop endocarditis. There were no lesions or vegetations on histologic examination. The scintigrams from these excised hearts failed to show any area of increased radioactivity.

All six New Zealand rabbits infected with enterococci developed endocarditis. Microscopy showed small lesions containing the vegetations at the aortic valve site. The excised hearts gave normal scintigrams.

A tissue distribution study of Tc-99m Ab was done in five rabbits with staphylococcal endocarditis. The radioactivity in the aortic valves ranged from 0.03–1.37% injected dose/g tissue. In contrast, the tricuspid valves accumulated 0.003–0.01% injected dose/g. Based on these percentages, the uptake of Tc-99m Ab by the infected aortic valve averaged one hundred times (range 4–490) that of the normal tricuspid valves (Table 1).

Table 2 summarizes the tissue distribution data of six rabbits infected with enterococci. The radioactivity found in these aortic valves ranged from 0.015–0.05% injected dose/g tissue. The average ratios of the aortic valve to tricuspid valve, myocardium, and blood were 1.2:1, 1.2:1, and 1.5:1, respectively.

Uptake of Tc-99m Ab by other organs is summarized

TABLE 2. DISTRIBUTION OF Tc-99m-LABELED ANTI-*Staphylococcus aureus* ANTIBODY IN CARDIAC VALVES, MYOCARDIUM, AND BLOOD OF SIX RABBITS AT 24 HR AFTER INJECTION OF THE RADIOPHARMACEUTICAL. THESE ANIMALS WERE DIVIDED INTO TWO GROUPS AND WERE INFECTED WITH ENTEROCOCCI AT DIFFERENT TIMES. ALL SCINTIGRAPHIC IMAGES WERE NORMAL

Case No.	Tissue sample	Radioactivity cpm/g tissue	Percent injected dose/g tissue	Ratio* of infected valve to normal valve	Ratio* of infected valve to myocardium	Ratio* of infected valve to blood
GROUP A						
1.	Blood	5939	0.010			
	Myocardium	8951	0.016			
	Aortic valve	8683	0.015	1.5:1	1:1	1.5:1
	Tricuspid valve†	5808	0.010			
2.	Blood	4310	0.009			
	Myocardium	10181	0.022			
	Aortic valve	8966	0.019	1:1	1:1	2:1
	Tricuspid valve	9162	0.020			
3.	Blood	4521	0.010			
	Myocardium	7608	0.018			
	Aortic valve	14000	0.032	1.3:1	1.8:1	3.2:1
	Tricuspid valve	10585	0.025			
GROUP B						
4.	Blood	18053	0.005			
	Myocardium	14679	0.004			
	Aortic valve	15533	0.004	1.3:1	1:1	0.8:1
	Tricuspid valve	9555	0.003			
5.	Blood	23760	0.007			
	Myocardium	32113	0.009			
	Aortic valve	18262	0.005	0.5:1	0.6:1	0.7:1
	Tricuspid valve	37244	0.010			
6.	Blood	20540	0.006			
	Myocardium	12085	0.003			
	Aortic valve	17486	0.005	1.3:1	1.7:1	0.8:1
	Tricuspid valve	12831	0.004			
			Average Ratio:	1.2:1	1.2:1	1.5:1

* Based on percent injected dose/g tissue.

† Tricuspid valve was not infected with enterococcus.

in Table 3. Greatest uptake was found in the spleen and liver, followed by kidney and lung in decreasing order. With the exception of the spleen and liver, the concentration of Tc-99m Ab in the aortic valves of rabbits with staphylococcal endocarditis was much higher than in any other tissues. Similar results were obtained from the tissue distribution study of the rabbits with enterococcal endocarditis. However, the accumulation of Tc-99m anti-*S. aureus* antibody by the infected aortic valves was minimal in these animals. High urinary radioactivity indicated that the labeled antibody or its metabolites would be eliminated primarily by the kidneys.

DISCUSSION

The current study is based on the hypothesis that an organism-specific antibody will react with the bacterium at the site of infection, but not with normal tissue. The accumulation of Tc-99m Ab at the site of the infection may permit visualization of such lesions by scintigraphy or might be detected by external counting.

The method used in the present experiment differs significantly from that of an earlier report (*9*). Then, the antibacterial antibody was extracted by a very complicated fractionation process and labeled with Tc-99m by electrolysis. The labeled protein was further purified by column chromatography and dialysis with phosphate buffer.

The Rivanol extraction method is nonspecific but it is simple and highly selective for removing the gamma-globulin fraction from the antiserum. Relatively pure gamma globulin, including any antimicrobial antibodies present, can be obtained at room temperature in less than 2 hr. Although antibodies thus obtained are of low specificity, they are immunologically active, as shown by the present study and by earlier investigators (*10*). The whole gamma-globulin fraction containing these antibodies can be labeled with Tc-99m by the chemical labeling process, at pH 7.4, without alteration of their biological properties or immunological activity. The simplicity of the Rivanol antibody extraction and the chemical labeling process offers a practical means of producing sterile nonpyrogenic Tc-99m-labeled autologous antibodies for scintigraphic location of infectious lesions or neoplasms. Each patient could serve as his own source of antibody, which can be rapidly extracted, labeled with Tc-99m, and reinjected in less than 3 hr. Scintigrams could be obtained at 18–24 hr after tracer injection, permitting reduction of background activity. The use of Tc-99m-labeled autologous antibodies will eliminate the possible transmission of hepatitis.

Antibacterial and antiviral antibodies are known to develop in the host afflicted with various type of infectious diseases and neoplasms. Whether the antibody titer in the early course of these disease is high enough to permit visualization of these lesions remains to be investigated. However, specific uptake can be obtained, as shown in our studies with Tc-99m Ab at 1:800 titer.

The present studies clearly demonstrate the ability of radiolabeled antibodies to achieve a high concentration in experimental endocardial vegetations. All animals studied showed an increased uptake of Tc-99m Ab at the aortic valves infected with *S. aureus*, in contrast with the very low level of Tc-99m found in the lesions of enterococcal endocarditis in rabbits. Scintigrams of the excised heart, and the tissue distribution data, indicate a potentially useful concentration gradient, which may permit imaging in the larger lesions expected clinically. Alternatively, counting to determine the presence or absence of uptake, or timed clearance studies, might prove practical.

Our study strongly suggested that the imaging of infectious lesions with Tc-99m-99m-labeled antibody is a practical approach. Focal accumulations were observed in anterior scans of the intact animals. These results were equivocal owing to high hepatic and sternal radioactivity nearby, as well as a lack of familiarity with valve-plane location in the intact animal. In human patients the distances would be increased and, as in pyrophosphate imaging, accurate anatomic knowledge will allow precise localization in spite of extraneous tissue uptake.

The crucial criteria for successful imaging of infectious lesions or neoplasms are the size of these lesions, the availability of specific antibody from the host, and the concentration difference between the antibody in the

TABLE 3. TISSUE DISTRIBUTION OF Tc-99m-LABELED ANTI-*S. aureus* ANTIBODY (A) IN RABBITS WITH STAPHYLOCOCCAL ENDOCARDITIS AND (B) IN RABBITS WITH ENTEROCOCCAL VEGETATIONS. ANIMALS WERE KILLED 24 HR AFTER INJECTION OF RADIOLABELED ANTIBODY

Tissue samples	(A) Percent injected dose/g tissue* (n = 5)	(B) Percent injected dose/g tissue* (n = 4)
Blood	0.028 (0.017–0.058)	0.010 (0.005–0.010)
Myocardium	0.009 (0.005–0.013)	0.016 (0.003–0.022)
Aortic valve	0.435 (0.030–1.374)	0.022 (0.020–0.032)
Tricuspid valve	0.008 (0.003–0.010)	0.014 (0.003–0.025)
Lung	0.104 (0.038–0.208)	0.088 (0.033–0.123)
Liver	0.704 (0.505–0.750)	0.539 (0.470–0.687)
Spleen	2.157 (0.821–3.538)	0.720 (0.355–1.644)
Stomach	0.006 (0.003–0.010)	0.004 (0.003–0.005)
Kidney	0.255 (0.130–0.363)	0.234 (0.211–0.260)
Bladder	0.014 (0.010–0.017)	0.010 (0.006–0.013)
Urine	0.166 (0.083–0.377)	0.248 (0.088–0.529)

* = mean % and (range).

lesion and in adjacent tissue. The usefulness of the present approach can be evaluated with patients using autologous antibody isolated from the patient's own serum. Earlier imaging experience indicates that images can be greatly improved by proper shielding of the liver to reduce interference, by tomographic techniques, and by additional views from other angles (*9*).

It is far from proven that Tc-99m-labeled anti-*S. aureus* antibody is specific for the traumatic agent used. Presumably cross reactions can occur with other microorganisms. The possibility of accumulation of Tc-99m Ab by fibrin deposition near a damaged valve, although unlikely, cannot be ignored. While concentration of Tc-99m pyrophosphate in endocarditis has been reported in the literature (*6*), it is nonspecific and presumably is related to accompanying tissue necrosis. Imaging with Tc-99m-labeled antibody appears promising as a specific tool in the diagnosis of infectious diseases, and deserves further investigation.

FOOTNOTES

* Sigma Chem. Corp.
† Purdue Norwalk, CT.

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Program Coordinator, Maria DaCosta, along with the Scientific Program Committee Chairperson, Ted Rubel, announce the following plans for the Annual Spring Meeting of the Greater New York Chapter of the Society of Nuclear Medicine:

Friday	Saturday	Sunday
Clinical	Computer	Chief Technologist Session
Management Symposium	Cardiac	Patient Care
Adjunctive Equipment	Instrumentation	
	Scientific Papers	

The program is approved for VOICE credit; submit scientific papers to Maria Da Costa.

The Physician Section will once again be holding its conjoint meeting with technologists on Saturday.

For more information contact:

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