DIAGNOSTIC NUCLEAR MEDICINE

Modified Technetium-99m Heparin for the Imaging of Acute Experimental Myocardial Infarcts

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We have reported previously that technetium-99m heparin (TcH) accumulates in, and allows scintigraphic identification of, damaged canine myocardium occurring with temporary occlusion and reperfusion of the left anterior descending (LAD) coronary artery. A recent modification consists of using heparin from sheep lung, with stannous phosphate as the reducing agent. In 12 dogs with permanent LAD occlusion, six were injected intravenously with TcH (3–6 mCi) at 24 hr after occlusion, and six at 48 hr. Each experimental animal demonstrated relatively high TcH uptake in the left-ventricular infarct region as compared with normal myocardium. The in vivo scintigrams in all animals with gross myocardial infarcts were positive. The results suggest that this modified TcH has value for identifying experimental myocardial infarcts and that the reduced bone uptake, compared with that occurring with Tc-99m phosphates, may be an advantage for scintigraphic infarct detection.

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Our previous studies demonstrated that Tc-99m-labeled heparin concentrated in scintigraphically detectable levels in damaged myocardium of dogs with temporary ligation of the left anterior descending (LAD) coronary artery (*1*). However, there was less marked uptake of the agent with permanent LAD occlusion (*1, 2*). In the studies described in the present report, we describe a modified form of heparin and show that it allows scintigraphic identification of damaged canine myocardium developing during permanent LAD occlusion.

METHODS

Radiopharmaceutical preparation. The original procedure for labeling heparin with Tc-99m (*1,2*) was modified. We now use an "instant kit,"* whereby the labeled product is obtained by addition of pertechnetate and mixing with a lyophilized preparation. The lyophilized kit consists of 150–200 IU of sheep-lung heparin, 80 μg Sn^{++} ion, and orthphosphate (2.34 mg phosphorus per vial). The reagent is sterile, pyrogen-free, lyophilized, and kept under nitrogen to prevent oxidation of the stannous ion. A solution of 1–2 ml (10–15 mCi) $^{99m}TcO_4^-$ is injected aseptically into a vial and the lyophilized material dissolved by gentle agitation. The reaction mixture is allowed to stand at least 10 min before drawing any doses. The radiolabeling efficiencies were routinely monitored by instant thin layer chromatography (ITLC-SG) sheets using either 85% methanol or methylethylketone as the solvent. Further analysis is performed by cellulose acetate electrophoresis with 0.1 *M* acetate buffer (pH 5.5–6.0) at 300 V for 15 min. The unlabeled heparin is also spotted at different sections of the same strip on which the final product (5 μl) is placed. The migration of the labeled product is determined by counting 5-mm sections of the strips in a gamma scin-

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tillation counter. The location of the unlabeled heparin is identified by staining with 0.5% toluidine blue.

Experimental animals. Mongrel dogs of either sex, weighing approximately 20 kg, were anesthetized with sodium pentobarbital (30 mg/kg). An endotracheal tube was inserted and the animal placed on a Harvard respirator. The chest was opened through a left lateral thoracotomy and the proximal LAD visualized, gently dissected free, then occluded with a purse-string suture. The dog's chest was closed and the animal allowed to recover. Technetium-99m heparin (3-6 mCi) was injected intravenously at 24 hr (six dogs) or 48 hr (six dogs) after LAD occlusion. Serial myocardial images were obtained up to 3 hr after injection in the anterior, left anterior oblique (45°), and left lateral projections. Using a gamma camera, 300,000 counts were collected in each image (*1*).

The animals were killed by administering 1.1 g/kg of sodium pentobarbital intravenously about 3 hr after injection of the Tc-99m heparin. The animals' chests were opened, the hearts removed and washed in saline, and imaged in vitro by placing them under the scintillation camera and collecting 100,000 counts in the anterior view.

Assays of radioactivity. Each heart was divided into transverse slices, and multiple transmural blocks were obtained from grossly normal posterior myocardium and grossly infarcted anterior myocardium. Tissue from each block was divided into epicardial and endocardial portions and counted for radioactivity. Sections for histologic examination were removed from selected blocks and fixed in phosphate-buffered 10% formalin.

All samples were weighed and counted for Tc-99m activity in a sodium iodide well counter. A standard was prepared by diluting a known amount of Tc-99m activity, and 1.0 ml of the solution was counted along with the tissue and blood samples. Counts per minute per gram of the sample were calculated, along with percentage of administered dose per gram of sample. The data were tabulated in terms of the ratio of cpm/g sample to cpm/g normal myocardium.

RESULTS

Test for radiochemical purity. Radiochromatographic analysis indicated that Tc-99m-labeled preparations had less than 5% free pertechnetate. In typical preparations, most of the radioactivity was associated with the same zone as that of unlabeled heparin, identified by the stain of toluidine blue, which migrated 5-7 cm from the point of application in the cellulose acetate electrophoresis. Most of the radioactivity (99%) remained at the point of application in those preparations that did not have heparin but had equivalent amounts of stannous chloride, indicating that reduced but unbound (or hydrolyzed) technetium remained at the point of application in this test system.

TABLE 1. ORGAN DISTRIBUTION OF Tc-99m HEPARIN (FROM SHEEP LUNG, IN PHOSPHATE BUFFER) 3 HR AFTER I.V. INJECTION IN DOGS (n = 12)

Organ	% ID/g* of organ, $\times 10^3$ (mean ± s.d.)	Ratio to normal† myocardium (mean)
Blood	5.4 ± 1.78	1.91
Bone	10.44 ± 5.10	3.69
Liver	28.12 ± 12.68	9.44
Lungs	6.16 ± 1.82	2.18
Kidneys	44.59 ± 14.82	15.76
Normal myocardium	2.83 ± 1.32	1.00

* % ID/g = Percentage injected dose per gram.

† cpm/g of tissue divided by cpm/g of normal myocardial tissue.

Scintigraphic infarct localization. Scintigrams demonstrated the accumulation of radioactivity in liver and in the kidneys, and some uptake in bone (Table 1). The blood clearance with this material was rapid enough to permit scintigraphy of the damaged myocardium by 40-60 min after injection. The rate of blood clearance was similar to that of the earlier heparin product (*1*). The blood disappearance curve was biphasic, with an initial fast component ($t_{1/2}$ = 15 min) and a slower component ($t_{1/2}$ = 305 min) (*1*).

Normal myocardium had 2.8 ± 1.32 (mean ± s.d.) percentage injected dose per gram of tissue. Blood had 1.9, bone 3.7, liver 9.4, kidneys 15.8 times the activity (cpm/g) of normal myocardial tissue (Table 1). Blood clearance data and scintigrams (Fig. 1) (1-, 2-, and 3-hr images) indicated that optimum imaging occurs at approximately 3 hr after injection of Tc-99m heparin.

All six dogs with 24-hr permanent LAD occlusions had grossly obvious myocardial infarcts, and all had

TABLE 2. RATIO OF ACTIVITY OF INJURED MYOCARDIAL TISSUE (cpm/g) TO NORMAL MYOCARDIUM (cpm/g) IN DOGS WITH PERMANENT LAD CORONARY LIGATION

	24 hr (n = 6) Transmural damage	48 hr (n = 6) Transmural damage
Peripheral EPI*	7.7 ± 3.47†	11.9 ± 4.16†
Peripheral ENDO*	9.3 ± 6.03	9.3 ± 6.12
Infarct center EPI	8.3 ± 3.21	12.1 ± 2.36
Infarct center ENDO	4.7 ± 2.65	7.0 ± 6.53

* EPI = epicardium, ENDO = endocardium.

† Mean ± S.D.

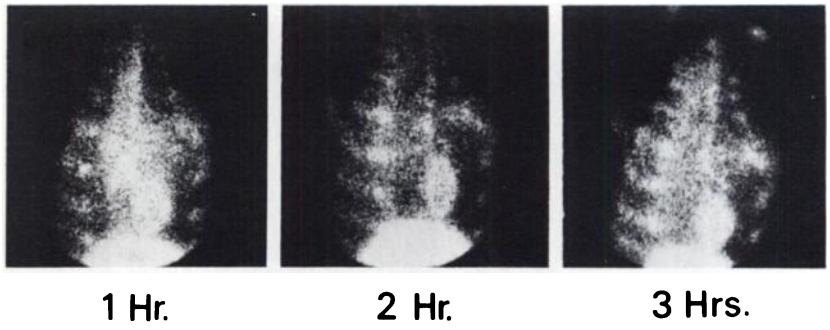

FIG. 1. Anterior scintigrams of a dog 48 hr after permanent ligation of left anterior descending coronary artery. Images obtained at 1, 2, and 3 hr after injection of Tc-99m heparin. Note improving visualization with time.

positive in vivo and in vitro scintigrams. Activity in peripheral epicardium and endocardium, and in central epicardium of the infarcts, averaged 7.0, 8.0, and 7.4 times normal myocardium, respectively, whereas the average activity ratio in central subendocardium was 4.0 (Table 2).

Six dogs with permanent LAD ligation for 48 hr had myocardial infarcts, and each of these animals had a positive scintigram both in vitro and in vivo. Activity in the peripheral epicardium and endocardium, and in the central epicardium of the infarcts, averaged 11.9, 9.3, and 12.1 times normal myocardium, whereas the average activity ratio in central subendocardium was 7.0 (Table 2). The scintigraphic visualization of the acutely infarcted myocardium was possible 40–60 min after injection of the agent, and the images remained positive throughout the study.

DISCUSSION

Our data suggest that, using a modified Tc-99m(Sn)heparin preparation, it is possible to make gamma images of experimental canine myocardial infarcts at 24–48 hr after proximal LAD occlusion. In contrast to the findings with an earlier Tc-99m heparin preparation (*1,2*), successful visualization of infarcted myocardium was achieved with the modified preparation in canine models with permanent LAD coronary artery occlusion. Thus, it appears that in this experimental model, successful imaging of myocardium acutely infarcted by permanent coronary occlusion depends on the type of heparin preparation used, and that excellent scintigraphic results may be obtained with sheep-lung heparin in combination with stannous phosphate as the reducing agent (Figs. 2 and 3).

Our previous studies with hog-mucosa heparin† had shown only a modest level of increased Tc-99m in infarcted canine myocardium with permanent LAD occlusion (*1,2*). Blood clearance rates and organ distribution of the labeled heparin agent also appear dependent on the source of heparin and the reducing agent used (*2*). However, both the original and new Tc-99m heparin preparations have much slower blood clearance rates compared with Tc-99m PPi (*3*). The Tc-99m heparins are larger-molecule compounds that are cleared primarily by soft-tissue uptake (liver) and kidneys, whereas Tc-99m PPi is cleared by bone uptake and urinary excretion, with much lower uptake in soft tissues (*3,4*). The faster blood clearance of Tc-99m PPi is one of its major advantages over other infarct-imaging agents (*4,5*), although its significant uptake in bone is a disadvantage.

A potential advantage of Tc-99m heparin compounds in the cardiac field may be the reduced uptake in bones compared with the phosphates. A potential disadvantage is that liver uptake may mask the inferior myocardium. Bone uptake of Tc-99m PPi is 12 to 19 times that of the normal myocardium at 1 hr after injection, and the factor increases to 60–87 by 6 hr (*6*). Our heparin tracer did better in the dogs: the ratio of normal myocardium

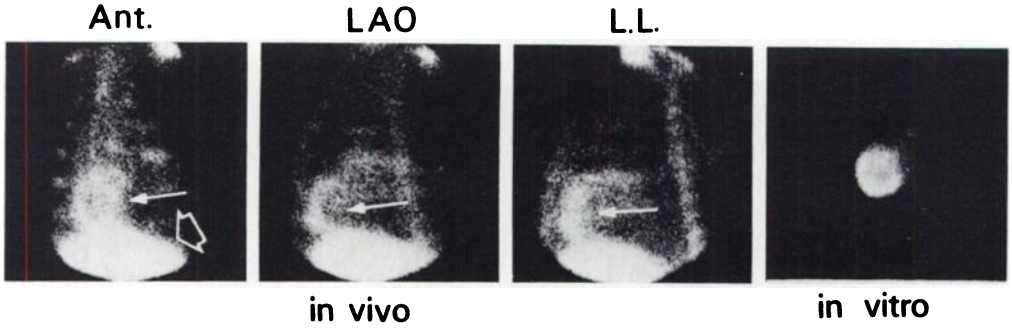

FIG. 2. Anterior, left anterior oblique, and left lateral scintigrams of a dog 24 hr after permanent ligation of LAD coronary artery. Small arrow shows Tc-99m uptake in damaged myocardium. Open arrow indicates liver uptake, and there is some uptake in the bones. Far right panel shows scintigram of excised heart in LAO position.

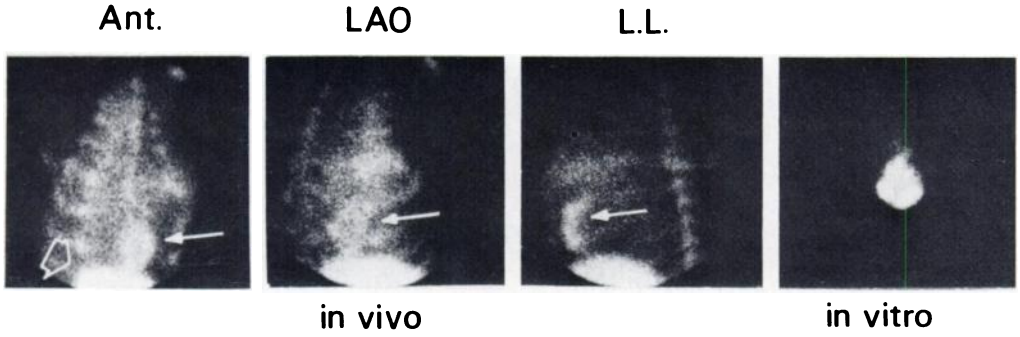

FIG. 3. Scintigrams as in Fig. 2, but at 48 hr after LAD occlusion. Small arrow shows uptake in damaged myocardium. Open arrow shows liver uptake, and there is some uptake in bones. Far right panel shows scintigram of the excised heart in the LAO position.

to bone (both cpm/g) was 2 to 5, and the maximum infarct-to-normal heart ratio reached 13.6 at 24 hr, and up to 20 at 48 hr, after permanent LAD occlusion. The average activity in the peripheral infarct at 48 hr (10.6 times normal) is lower than the 24.5 times normal seen with Tc-99m PPi (*7*), but is adequate for imaging. There was some uptake of labeled heparin in bone marrow, which may be related to the presence there of heparinase, however, the maximum cpm/g ranged from 3 to 5 times that of normal myocardium at 3–3.5 hr after injection.

Another comparison is interesting. Tc-99m PPi localizes most intensely in the peripheral region of the canine infarct, compared with the central subendocardium (*6*). A similar but less marked tendency was also noted with the Tc-99m heparin (Table 2).

Heparin is a heterogeneous polymer with components varying in molecular weight (*8*). Conformational changes in the membranes of injured cells might result in increased binding of highly reactive molecules, including such polyanions as heparin. It is also possible that there are differences in the affinity of Tc-99m heparin for damaged myocardium at various stages following infarction. The source of heparin and heparin-type compounds (polysaccharides) may also be important, but this is a matter that will need further study. It is known that heparin has affinity for thrombin and antithrombin (*9–11*). Alternatively, Tc-99m heparin may also have affinity for specific intracellular constituents of damaged myocardium, including amorphous and crystalline calcium deposits (*6*), denatured macromolecules (*12*), plasma proteins, and/or other substances that accumulate in ischemically injured cells (*13,14*). However, the exact mechanism(s) responsible for localization of labeled heparin in infarcted myocardium will require further evaluation. The optimum timing of the imaging procedure also requires additional study.

We have not been able to demonstrate increased bleeding or alteration of whole-blood clotting times in experimental animals given as much as 200–400 USP units of labeled heparin (*2*). In our studies, whole-blood clotting times at 1 hr after injection are not significantly different from the preinjection controls (*2*).

Heparin is known to induce thrombocytopenia. In one prospective study that used beef-lung heparin in 52 patients, the incidence of thrombocytopenia was 31% (*15*). In another prospective study that used hog-gut heparin in 117 patients, the incidence was only 3.4% (*16*). The incidence of thrombocytopenia appears related to either the organ or species or origin of the heparin, and is not known with sheep-lung heparin. However, the appearance of thrombocytopenia usually requires 2–10 days of administration, and it is reversible after the heparin is discontinued (*16*). Thrombocytopenia is not likely to be a complication of a single low-dose bolus.

Heparin-type (i.e., polysaccharide) molecules appear to be an untested group of compounds that can be tagged with various nuclides, and may be useful imaging agents in cardiac nuclear medicine. Further studies should explore the correlation of structure and composition of various polysaccharide molecules and their potential for scintigraphically identifying myocardial and vascular injury and thrombosis under varying experimental and clinical circumstances.

FOOTNOTES

* These kits are manufactured by Solco Nuclear Corp., Basle, Switzerland, and kindly supplied by Diagnostic Isotopes, Bloomfield, NJ.

† Abbott and Upjohn

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