Imaging Experimental Coronary Artery Thrombosis with Indium-111 Platelets

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SUMMARY The ability of cardiac scintigraphy with indium-111 (111In)-labeled platelets to detect coronary artery thrombosis (CAT) was assessed in a canine model. Cardiac imaging and tissue distribution studies were performed shortly after administering 111In-labeled platelets to 12 dogs (group 1) with acute CAT. Four dogs (group 2) with acute CAT were studied 2 and 22 hours after administering 111In platelets. In addition, four dogs (group 3) with 24-hour-old CAT were similarly evaluated. In all group 1 animals, in vivo imaging 1–2 hours after 111In platelet administration revealed intense uptake in the region of thrombus-containing left anterior descending arteries that was readily discernible from background blood pool activity. Sequential imaging of the four group 2 animals over a 22-hour period revealed no change in the scintigraphic pattern of the thrombosed arteries. In contrast, 111In platelet imaging in the four group 3 animals with 24-hour-old CAT failed to reveal enhanced activity within the region of the thrombus-containing coronary artery. In the 12 group 1 animals, the CAT accumulated 69 ± 10 (mean ± SEM) times greater activity than present in blood and 651 ± 135 times greater activity than normal left ventricular myocardium. There was 24 ± 7 times greater 111In activity in the damaged left anterior descending arteries compared with normal circumflex arteries. Similar uptake ratios were seen in group 2 animals. The 24-hour-old thrombi from group 3 animals showed no enhanced 111In uptake.

This study demonstrates that experimental acute CAT can be detected readily with 111In platelet cardiac scintigraphy. Acutely formed thrombi accumulate labeled platelets, but 24-hour thrombi do not. Serial imaging of acutely formed thrombi over 22 hours after the administration of 111In platelets shows no significant change in the scintigraphic appearance. This technique may provide a better understanding of the role of thrombosis in myocardial infarction.

THE ROLE of coronary artery thrombosis in the pathogenesis of myocardial infarction and sudden death is controversial.1-7 Platelet function and survival are variable in patients with coronary artery disease,8-12 and the value of antiplatelet agents is unclear.13-19 Assessing the role of thrombosis and platelet deposition in the pathogenesis of acute and chronic coronary artery disease has been limited by the lack of suitable in vivo methods of detection. Since platelets play a primary role in arterial thrombosis20,21 and may be important in the development and progression of established atherosclerotic lesions,22-24 we investigated whether cardiac imaging with indium-111 (111In)-labeled platelets could identify acutely formed experimental coronary artery thrombosis. Previous studies have confirmed the efficacy of this approach in a number of cardiovascular processes that involve platelet deposition.25-32

Methods

Induction of Experimental Acute Coronary Artery Thrombosis

Acute coronary artery thrombosis was established in adult mongrel dogs using a modification of the catheter electrode method of Salazar.28 After administering 30 mg/kg of intravenous sodium pentobarbital, a cervical cutdown was performed and the left carotid artery was isolated. A #7F Sones catheter was passed down the carotid artery and advanced into the left coronary artery ostium and proximal portion of the left anterior descending artery under fluoroscopic guidance. The use of a Y-shaped adaptor at the end of the catheter permitted injection of contrast material and passage of a guidewire through the catheter. A teflon-coated guidewire with its stainless steel tip exposed was placed in the catheter and advanced 2–3 cm beyond the catheter tip into the proximal portion of the left anterior descending artery. The catheter then was withdrawn slightly, leaving only the guidewire in the coronary artery (fig. 1). The free end of the guidewire was connected to the positive side of a DC 9-V battery connected to a potentiometer. The negative pole of the electrical source was grounded by alligator clips to the animal's tongue to complete the circuit. While a precordial surface electrocardiographic lead was monitored, 500–900 μA of current were passed continuously along the guidewire. The current flow was maintained until ST-segment elevation or frequent ventricular ectopy appeared. Then (usually after about 30 minutes), the current was discontinued and the catheter and guidewire were
removed. The animal was treated with lidocaine (50–100-mg bolus) to suppress ventricular ectopy.

Preparation of $^{111}$In-Platelets

Indium-111 is a radionuclide well-suited for labeling platelets. It emits two photons per disintegration with energies of 173 and 247 keV, both of which can be detected by conventional scintigraphic cameras. The 67-hour half-life of the radionuclide permits imaging for several days after the initial administration. Indium-111 bound with 8-hydroxyquinoline (supplied commercially as $^{111}$In-oxine) is a lipid-soluble compound that effectively labels blood cellular components without altering their functional properties.26–27 The survival of $^{111}$In platelets is comparable to that of chromium-51 platelets. Both have in vivo half-lives of 4.5 days.28 Labeled platelets are cleared by the reticuloendothelial system of the liver and spleen.

Autologous platelets were isolated and labeled by a modification of the Thakur method.28 From each dog, 43 ml of venous blood were drawn into a 50-ml disposable plastic syringe that contained 7 ml acid citrate dextrose (ACD) solution (Squibb). The blood was transferred into two 50-ml conical (Falcon) plastic test tubes and centrifuged at 180 g at room temperature for 15 minutes using a calibrated horizontal swing rotor tabletop centrifuge (IEN). The platelet-rich plasma was transferred with a glass Pasteur pipette, pooled into another plastic 50-ml tube and centrifuged again at 1500 g for 10 minutes. The platelet-poor plasma was removed by pipette and the platelet button was suspended in 5 ml 0.9% sodium chloride and centrifuged at 100 g for 15 minutes. This step was repeated and the platelets were finally suspended in 5 ml normal saline. Platelet suspension was accomplished by gentle admixture with a Pasteur pipette. The platelet suspension was transferred into a 15-ml Falcon round bottom polyethylene test tube. Indium-111 oxine (Diagnostic Isotopes, calibrated as 1 mg in 50 μg oxine and 50 μl ethanol) was used for labeling. Approximately 450 μCi $^{111}$In were diluted fourfold with normal saline and added dropwise to the platelet suspension with a Pasteur pipette. The mixture then was incubated at room temperature for 15 minutes. The labeling efficiency then was assessed by sampling an aliquot of the platelets and assessing its radioactivity. With this labeling technique, greater than 90% of the radioactivity is usually found in association with the platelets.29 The labeled autologous platelets were administered intravenously to the dogs. The number of platelets administered was approximately $8–9 \times 10^9$.

Experimental Groups

Coronary artery thrombosis was produced in 20 dogs. The animals were divided into three experimen-

**Figure 1.** Radiographs showing the technique for creating left anterior descending coronary artery thrombosis. Radiographs were obtained with the dog in the left lateral position. The panel on the left demonstrates the positioning of the coronary artery catheter selectively within the left anterior descending coronary artery; contrast material has been injected to delineate its anatomy. The panel on the right shows the guidewire that has been passed through the coronary catheter into the left anterior descending coronary artery. The catheter has been withdrawn from the coronary artery and is in the ascending aorta. The electric current then is passed through the guidewire, resulting in endothelial damage and subsequent coronary thrombosis.
tial groups. In group 1 (12 animals), $^{111}$In platelets were administered $\frac{1}{2}$ to 1 hour after coronary artery thrombosis was induced. Cardiac imaging then was performed 1–2 hours later, after which the animals were sacrificed. Group 2 included four dogs also given $^{111}$In platelets $\frac{1}{2}$ to 1 hour after coronary artery thrombosis was induced. These dogs were imaged sequentially 2 and 22 hours thereafter. After the initial imaging study, the dogs were allowed to recover from anesthesia. They then were reanesthetized before the second imaging. After the latter imaging study, they were sacrificed. Group 3 consisted of four dogs that received $^{111}$In platelets 22 hours after induction of thrombosis and were imaged 1–2 hours later. This last group was studied to assess whether “older” coronary artery thrombi accumulate enough radiolabeled platelets to allow external detection by in vivo imaging.

Radionuclide Imaging

After platelet administration, the dogs were placed in the left anterior oblique position and cardiac imaging was performed with either a Searle Radiographics Pho Gamma HP scintillation camera fitted with either a 4-mm pinhole collimator or a medium-energy, parallel-hole collimator, or a Picker Dynamo portable gamma camera fitted with a medium-energy, parallel-hole collimator. All images were recorded on Polaroid film. The collimator was placed directly over the precordial impulse with a field of view encompassing the cardiac region as defined by the blood pool radioactivity and the superior border of the spleen. The half-life of both $^{111}$In (67 hours) and of transfused autologous platelets (4.5 days) accounts for the visualization of blood pool activity during the imaging times chosen for this study. Twenty-five thousand-count images (imaging time of 10–15 minutes) were obtained using a 20% window centered on the 247 keV photo peak of $^{111}$In. The left anterior oblique position was the best for imaging, since it placed the left anterior descending artery tangential to the left border of the cardiac silhouette as defined by the radioactive blood pool image. Blood pool and hepatic-splenic activity of $^{111}$In provided comparative anatomic reference points for localization of any abnormal focal cardiac activity. Abnormal $^{111}$In uptake was considered present in the region of the left anterior descending artery if the activity had an intensity greater than the blood pool activity or equal to or greater than the hepatic-splenic activity and was appropriately located for that coronary artery. Cardiac imaging was performed using constant imaging geometry in the four animals studied serially. All cardiac scintigraphs were interpreted independently as either positive or negative by at least two of the authors.

Excised Heart Imaging, Tissue $^{111}$In Distribution

Upon completion of in vivo imaging, each dog was sacrificed with a lethal dose of intravenous sodium pentobarbital. The beating heart was excised rapidly and washed completely to remove blood and non-adherent postmortem clot. Weighed samples of blood were taken when the heart was removed. The intact excised heart then was placed under the gamma camera in the same relative anatomic orientation as obtained in vivo, and 10,000-count images were obtained. The excised heart was examined grossly for the presence of occlusive thrombosis within the left anterior descending artery. The site of thrombosis was directly compared with that noted on the in vivo cardiac and excised heart scintigraphs. The left anterior descending artery then was dissected carefully from surrounding tissue. The artery was opened lengthwise and any thrombus was removed and weighed. Samples of the remaining damaged left anterior descending artery, left ventricular myocardium adjacent to the site of thrombosis, normal circumflex artery, apical and anterolateral left ventricular myocardium, and myocardium from the inferior and posterolateral walls were likewise weighed in plastic test tubes. The weighed samples then were counted in a Beckman Gamma 8000 automated well-type scintillation counter. Radioactivity concentrations of each sample were expressed as counts/min per gram of tissue and multiple samples from each tissue were averaged. In each animal, radioactivity ratios were determined for coronary artery thrombus-to-blood, coronary artery thrombus-to-normal left ventricular myocardium (from posterolateral and inferior portions of the left ventricle) and damaged left anterior descending-to-normal circumflex arteries. In selected cases, samples of thrombus and damaged left anterior descending artery were placed in formalin and submitted for standard histopathological examination.

Results

Coronary artery thrombosis was produced in each of the 20 dogs. The thrombi appeared as firm, thick, bluish-red, chord-like structures within the left anterior descending artery (fig. 2). The occlusive thrombus usually occupied the entire proximal left anterior descending artery, with some animals demonstrating thrombus within the first and second diagonal branches. After dissecting and opening the artery, the thrombus was found partially adherent to the left anterior descending artery. At points of previous contact with the guidewire, there were raised brownish gold or hemorrhagic lesions of the left anterior descending artery. The entire thrombus detached readily from the artery. Spotty raised hemorrhagic lesions on the intimal surfaces of the left anterior descending artery could not be removed. In animals sacrificed 24 hours after the thrombus was induced, pale areas of necrosis were seen in the zones of the left ventricular myocardium supplied by the occluded coronary arteries. There was no gross evidence of infarction in animals sacrificed within 2–3 hours of clot induction. The circumflex arteries did not contain any thrombi. Microscopic examination of the left anterior descending artery showed adherent premortem thrombus composed mostly of fibrin and
platelets. There was disruption of the intimal layer, but retention of the internal elastic membrane.

**Indium-111 Platelet Cardiac Imaging**

In each animal in group 1, enhanced radioactivity uptake was seen in the region of the left anterior descending artery (figs. 3 and 4). The linear uptake was tangential to the left border of the cardiac silhouette and originated proximal to the aortic outflow tract as demarcated by the blood pool radioactivity within the ascending aorta. Excised heart imaging in the 12 dogs demonstrated intense tracer activity within the occluded left anterior descending artery. Acceptable images were obtained with both parallel-hole and pinhole collimators (figs. 3 and 4). The best in vivo scintigraphic definition, however, of the occluded artery was obtained with the parallel-hole collimator because it provided a more exact geometric definition of coronary artery accumulation of indium-111 corresponding to the region of coronary thrombosis in the left anterior descending coronary artery. In the left interior oblique position, this uptake in the coronary artery is tangential to the upper left border of the cardiac radioactive blood pool. Definition of the cardiac blood pool is most evident in the image obtained with the parallel-hole collimator, in which the liver is also apparent at the bottom of the figure. Note the hot spot in the excised heart image on the right corresponding to the left anterior descending artery.
relationship of the blood pool, ascending aorta and coronary arteries. With the pinhole collimator, the enhanced activity was magnified, which allowed detection of relatively small lesions. The intracoronary radioactivity uptake seen on the excised heart image conformed to the extent of coronary artery thrombosis seen on gross pathology. It also confirmed that the enhanced in vivo cardiac uptake was localized to the left anterior descending artery.

Similar scintigraphic delineation of the coronary artery thrombus was seen in group 2 dogs during both early and late imaging. The presence of the increased activity within the coronary artery persisted during serial imaging (figs. 5 and 6). Again, excised heart imaging confirmed that all of the enhanced $^{111}$In uptake was confined to thrombus within the left anterior descending artery (fig. 6).

In contrast to the results in dogs with acute coronary artery thrombosis, four dogs with 22-hour-old coronary artery thrombi imaged 2 hours after $^{111}$In platelet administration did not reveal any enhanced localization of radioactivity in the region of the left anterior descending artery. Cardiac imaging revealed only blood pool activity (fig. 7). Imaging of the excised heart showed only minimal left ventricular myocardial uptake. The heart from each dog contained occlusive clot within the left anterior descending artery and a pale area of necrosis in the distribution of the myocardium perfused by the occluded artery. No enhanced $^{111}$In platelet uptake concentrated in either the occlusive clot or infarcted myocardium.

### Tissue $^{111}$In Distribution

The average radioactivity uptake ratios in the three groups of animals studied are summarized in table 1. In the 12 group 1 animals, the thrombi accumulated $69 \pm 10$ (mean $\pm$ SEM) times greater activity than present in blood and $651 \pm 135$ times greater activity than normal left ventricular myocardium. There was $24 \pm 7$ times greater $^{111}$In activity in the damaged left anterior descending arteries compared with normal

![Figure 5](image)

**Figure 5.** Comparison of images obtained in a group 2 study animal evaluated serially 2 and 24 hours after induction of thrombosis. The image on the left was obtained with a parallel-hole collimator 2 hours after thrombus was induced, the image in the middle panel was obtained at the same time with a pinhole collimator; the image on the right was obtained 24 hours after thrombus was induced with a pinhole collimator. Note the region of increased indium-111 uptake corresponding to the thrombus-containing coronary artery in all images.

![Figure 6](image)

**Figure 6.** Images obtained in a group 2 animal 4 and 22 hours after thrombus induction. The image on the left was obtained shortly after thrombus induction, that in the middle panel 18 hours later, while that on the right was obtained from imaging the excised heart at the conclusion of the study. Note the region of increased indium-111 uptake at 4 and 22 hours corresponding to the region of coronary thrombosis, and the abnormal region of indium-111 uptake in the excised heart. All images were obtained with a pinhole collimator.
circumflex arteries. Similar uptake ratios were seen in group 2 animals, with a mean thrombus-to-blood ratio of 52 ± 12. Enhanced uptake of 111In activity was confined solely to the thrombus and damaged left anterior descending artery since 111In activity in left ventricular myocardium adjacent to the thrombus was not appreciably different from that of normal left ventricular myocardium. Furthermore, infarcted left ventricular myocardium did not concentrate 111In platelets to any substantial extent compared with normal left ventricular myocardium.

The 1-day-old thrombi from group 3 dogs showed no enhanced uptake of 111In activity compared with blood, thus explaining the negative in vivo images (thrombus-to-blood ratio 1.4 ± 0.4). In these four dogs, 111In activity in the infarcted myocardium compared with normal left ventricular myocardium was 1.7 ± 0.3.

Discussion

This study demonstrates that 111In-labeled platelets readily incorporate into freshly formed experimental coronary artery thrombi, enabling detection by in vivo cardiac imaging. Acutely formed thrombi were visualized as intense activity within the course of damaged left anterior descending arteries of canine hearts shortly after administration of the radioactive platelets. The significant thrombus uptake of 111In platelets as early as 1–2 hours after platelet administration accounted for the sensitive detection by scintigraphy. Excised heart imaging confirmed that the thrombus was the major site of enhanced 111In platelet uptake, and in each case this was confirmed further by gross pathology. Since it has been shown that unbound 111In-oxine is not concentrated to any significant extent in the thrombotic process,21, 22 the marked 111In activity in the coronary artery thrombus represented intense platelet deposition. Once 111In platelets incorporated into freshly formed thrombi, serial imaging demonstrated a stable scintigraphic appearance over the subsequent 22 hours. Thus, the coronary artery thrombus was not significantly modified in vivo during this time. In contrast to the results obtained with acutely formed coronary artery thrombi, 24-hour-old thrombi failed to accumulate significant amounts of 111In platelets when administered at that time.

Injury to the intima of a blood vessel results in endothelial disruption and exposure of collagen. At the site of injury, a fresh thrombus, composed initially almost entirely of platelet aggregates, forms rapidly. The platelet thrombus rapidly becomes surrounded by fibrin, and the platelet mass degenerates as more fibrin is laid down. By 24 hours, intact platelets are no longer recognizable, as the thrombus consists primarily of fibrin. This sequence of events, based on pathological analyses,21 is compatible with our imaging results.

The experimental model chosen for this study results in dramatic thrombus formation. Histologically, the lesion is similar to that formed spon-

<table>
<thead>
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<th>Experimental group</th>
<th>Positive image</th>
<th>Thrombus/blood</th>
<th>Thrombus/LV myocardium</th>
<th>Damaged LAD/normal CX</th>
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<td></td>
<td>n</td>
<td>111In radioactivity ratio (mean ± SEM)</td>
<td>111In radioactivity ratio (mean ± SEM)</td>
<td>111In radioactivity ratio (mean ± SEM)</td>
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<td>Acute coronary artery thrombosis (2 hrs)</td>
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<td>390 ± 78</td>
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<td>24-hour-old thrombosis</td>
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<td>1.4 ± 0.4</td>
<td>32 ± 10</td>
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Abbreviations: LV = left ventricular; LAD = left anterior descending coronary artery; CX = circumflex coronary artery.
taneously in humans. Study of such thrombi over time shows slow resolution and canalization, such that experimentally induced thrombi remain 100% occlusive for at least 1 week after induction. Thus, it is not surprising that the scintigraphic appearance of acutely formed thrombi did not change appreciably over 24 hours. Indium-111 platelet scintigraphy, therefore, may be useful in studying both the evolution of acute thrombus formation and whether the size of such thrombi can be modified once formed. Since fibrin adherence to the surface of the thrombus is an ongoing process, it is expected that iodine-131-labeled fibrinogen continues to deposit in experimentally induced coronary artery thrombi as long as 72 hours after thrombus induction. The kinetics of labeled platelet inclusion into forming coronary artery thrombi suggest that its usefulness in detecting thrombotic coronary occlusion may be limited to its early acute-onset phase.

The role of occlusive thrombosis in precipitating clinical coronary syndromes, notably myocardial infarction, ischemia and sudden death, is controversial. Fatal transmural myocardial infarction is associated with a high incidence of occlusive coronary artery thrombi in the major epicardial arteries, with a reported frequency of 31-96%. However, study of patients with subendocardial myocardial infarction or those with sudden cardiac death reveals thrombosis in only a small percentage of cases. Although patients with sudden cardiac death rarely have occlusive thrombosis, there is an increased frequency of platelet aggregates in their microcirculation, presumably arising from microembolization of coronary artery mural thrombi. In addition, recent data in man suggest that occlusive coronary thrombosis may occur secondary to spasm. In a group of patients given iodine-125-labeled fibrinogen 15-47 hours after the onset of symptoms compatible with myocardial infarction, postmortem analysis revealed radioactivity in the entire coronary artery thrombus in six of the seven patients. The findings suggested that thrombus formation was a secondary effect of infarction, but the results were equally compatible with radioactive labeling of a preexisting primary thrombus. Unfortunately, labeled fibrinogen incorporation cannot be discriminated by in vivo cardiac imaging because of the high background activity of the circulating blood pool. Indium-111 platelet cardiac imaging, therefore, may be better for studying patients with myocardial infarction.

Platelet imaging may also provide a method for detecting and studying the development of atherosclerosis. The relationship between platelets and thrombosis in the development and progression of atherosclerotic lesions is not completely defined. The presence of fibrin and platelets in atherosclerotic plaques suggests that atherosclerosis derives at least in part from thrombosis. Immunofluorescent techniques have identified fibrin and platelets in plaques. Experimentally induced thrombi can be transformed into atherosclerotic plaques. Platelet release factors may contribute to vessel injury and provide a nidus for atherosclerosis.
thrombus-to-blood radioactivity ratio was 3:1, cardiac imaging could not readily discriminate the in vivo thrombus from the background activity of the circulating blood. They obtained better scintigraphic definition after direct intracoronary administration of the tracer. Both Salimi et al. and Moschos et al. showed that iodine-131 fibrinogen continues to incorporate in coronary artery thrombi as late as 72 hours after clot induction. The fibrinogen technique, therefore, would be more useful to detect older thrombi, which do not incorporate 111In.

In platelets. Chromium-51 platelets also have been infused into dogs with experimentally induced coronary artery thrombi. One hour after thrombus formation, the thrombus-to-blood radioactivity ratio was 150:1, results comparable to those in the present study. The long half-life of chromium-51 and its low gamma photon emission make it unsuitable for external detection by scintigraphy.

The present study demonstrates the feasibility of imaging coronary artery thrombosis. Our model provides a method for studying the pathogenetic role of platelets in ischemic heart disease and the temporal relationship of thrombosis to the clinical onset of myocardial infarction. For the technique to be clinically useful, the 111In platelets would have to be administered at the onset of clinical symptoms during the time of presumed rapid platelet deposition. Despite the relatively long half-life of 111In, the radioactive burden to the patient is acceptable. Although caution is advised in applying any animal study to clinical investigation, the acutely formed experimental coronary artery thrombus is histologically similar to that formed spontaneously in humans. The results of this study are encouraging enough to justify further platelet imaging studies in man to evaluate the role of platelet thrombosis in myocardial infarction. Furthermore, 111In in platelet cardiac scintigraphy may have wider application in the detection of a variety of intracardiac processes involving platelet deposition.

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