Noninvasive Detection of Coronary Thrombi with In-111 Platelets: Concise Communication

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The need for rapid, definitive identification of coronary thrombosis has been intensified by the advent of thrombolytic therapy and by interest in the role of thrombosis in the etiology of coronary artery disease. To determine whether platelet thrombi can be detected noninvasively with In-111 platelets, a method was developed in which Tc-99m-tagged red blood cells were used to correct for activity within the blood attributable to platelets circulating but not associated with thrombus. In 18 dogs coronary thrombi were induced closed-chest with a copper coil introduced into the coronary artery. Indium-111 platelets and Tc-99m RBCs were administered either before or 1 hr after induction of thrombus, and serial scintigrams obtained. Coronary thrombus was identified readily in the processed scintigrams. In six dogs, thrombolysis was achieved with intracoronary streptokinase. In each case serial scintigraphy demonstrated resolution of the clot. The dual radiotracer technique should permit serial noninvasive delineation of the temporal relationship between platelet deposition and coronary heart disease in patients, and should facilitate the evaluation of interventions designed to prevent platelet aggregation or to lyse existing thrombi.

J Nucl Med 24: 130–135, 1983

Since Herricks' description (*1*) of the association between coronary thrombosis and myocardial infarction in the early 1900s, the role of thrombosis as a primary or a secondary phenomenon has been controversial (*2–5*). More than 85% of patients presenting with acute myocardial infarction exhibit angiographically demonstrable coronary thrombi (*6*), and platelet aggregation has been implicated as responsible for the cyclical diminution of blood flow after experimental coronary stenosis in dogs (*7–8*). Such considerations, coupled with the promise of nonsurgical methods of revascularization—such as administration of intracoronary streptokinase—have intensified the need for early characterization of the presence and extent of thrombus in patients with ischemic heart disease, and for clarification of the role of platelet aggregates and thrombus in other cardiac disorders such as unstable angina.

Using a technique developed in this institution (*9,10*), platelets labeled with indium-111 have been used to detect myocarditis (*11*), venous thrombosis (*12–13*), atherosclerosis (*14–17*), and the platelet component of transplant rejection (*18*). The labeled platelets retain physiological activity (*19–23*) and exhibit normal survival (*9,10,24*).

Noninvasive detection of thrombus within the heart has been demonstrated with In-111 platelets (*25,26*), iodinated fibrinogen (*27–28*), and other coagulation factors (*29*). Although conventional scintigraphy using In-111 platelets alone has been shown to permit detection of coronary thrombi, scintigraphy using In-111 platelets alone has been insensitive in demonstrating smaller amount of incorporation of platelets into thrombi (*30–31*). Limitations are encountered due primarily to persistence of high levels of radioactivity associated with the labeled components in the vascular space. The dif-

Received Apr. 12, 1982; revision accepted Sept. 13, 1982.

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ficulties of differentiating labeled platelets incorporated into the thrombus from those still circulating are considerable in studies of the heart because of the large volume of the cardiac blood pool (*32*).

Investigation of the role of platelets in the pathogenesis of ischemic cardiac disease has been impeded by the lack of a sensitive and specific technique for measuring platelet deposition in vivo. This study was performed to determine whether platelet thrombi could be detected reliably in the coronary arterial bed through the use of a method developed to correct for radioactivity in circulating platelets. Red blood cells labeled with Tc-99m were used to serve as a blood-pool tracer so that digital subtraction of activity attributable to circulating platelets could be used to delineate activity associated with platelets incorporated in thrombi (*14,33,34*). The computer-processed scintigrams provided an index of radioactivity attributable to deposition of platelets in thrombi. The results demonstrate detection of experimentally induced coronary thrombosis in dogs and its resolution after administration of intracoronary streptokinase, events not readily evident by scintigraphy using In-111 platelets alone.

METHODS

Animal preparation. Eighteen conditioned mongrel dogs, average weight 18 kg, were anesthetized with thiopental (12.5 mg/kg) and alpha-chloralose (60 mg/kg). The left femoral artery and vein were cannulated for measurement of arterial pressure and for administration of drugs. Lidocaine was administered as necessary for dysrhythmia resulting from coronary thrombosis or reperfusion.

For induction of thrombus, a copper coil (5–7.5 mm long) was inserted into either the left anterior descending or the left circumflex coronary artery as previously described (*35,36*). With the animal in the right anterior oblique position, a modified USCI #1 Amplatz type left coronary catheter was inserted selectively with fluoroscopic guidance into the coronary artery. After the control angiogram had been obtained, a 0.021-in. guide wire was inserted into the coronary artery and the angiographic catheter removed. A copper coil (0.5 mm diameter copper wire wrapped in a helix around a 19-g needle) was slid over the guide wire and advanced into the artery with a 0.025-in. i.d. × 0.038-in. o.d. Formacath catheter. The guide wire was then removed. The coil usually lodged at the mid level of the LAD or at the level of the marginal branch of the circumflex. Formation of occlusive thrombus was heralded by electrocardiographic signs of ischemia, including ST elevation and ventricular dysrhythmia, and was confirmed angiographically. Such thrombi developed consistently within 5 to 15 min.

Radiotracer preparation and analysis. In ten dogs, autologous In-111 platelets (~500 μCi) and Tc-99m red blood cells (~2 mCi), prepared as previously described (*9,14*), were injected intravenously 15 min before induction of thrombus. To simulate more closely the potential clinical applications of the technique, labeled cells (~500 μCi In-111 platelets and 2 mCi Tc-99m RBCs) were administered to eight dogs 1 hr after induction of thrombus.

The radioactivity in any region of interest (ROI) attributable to In-111 platelets will consist of activity carried in labeled platelets circulating in the blood pool (In_{BP}) plus radioactivity due to labeled platelets incorporated into a plaque or thrombus (In_{TH}). The activity due to In_{bp} can be calculated with the use of a different radiotracer (such as Tc-99m red blood cells) that is confined to the vascular space. If a reference region is identified in which all In-111 activity is confined to the vascular space, then the ratio In_{ref}/Tc_{ref} will describe the relative activity of the two radiotracers in the circulating blood pool. Then for any ROI, In-111 in the blood pool can be calculated as

$$In_{bp} = \left(\frac{In_{ref}}{Tc_{ref}}\right) Tc_{roi}$$

Activity due to In-111 incorporated into thrombus in the ROI is equal to In_{tot}-In_{bp}. This assumes that the amount of labeled red cells incorporated into the thrombus is small compared with those incorporated into the thrombus. The assumption is valid in the current model of thrombus, based on histological examination of the thrombus (*36*).

Before scintigraphy, dogs were placed in the supine position. A total of 250,000 counts were collected for each tracer from the thoracic region with the use of a large-field gamma camera fitted with a medium-energy, parallel-hole collimator. Since radioactivity emanating from the spleen and liver was minimized by placement of the camera, special shielding was not used. The camera's energy discriminators were set at 247 keV for the indium-111 photo peak, and at 140 keV for the Tc-99m. Since the Tc-99m energy is close to the lower energy emission of In-111 (173 keV), the interference from In-111 in the measurement of Tc-99m was minimized by administering at least four times the activity of Tc-99m as In-111. Indium contamination of the Tc channel was determined in separate studies in which only In-111 platelets were administered, and the crosstalk never exceeded 14%. Because of this and the 1:4 dose ratio, we estimated that In-111 contribution to the Tc-99m scintigrams is less than 5%; therefore, correction for crosstalk was not used. Other camera systems, of course, might not have fared as well.

Scintigrams were stored single-channel, first with the In-111 window, then with the Tc-99m window. Images were obtained in the 30° RAO, straight AP, and 30° LAO projections. The camera was interfaced to a digital computer, and scintigrams were digitized in a 64 × 64

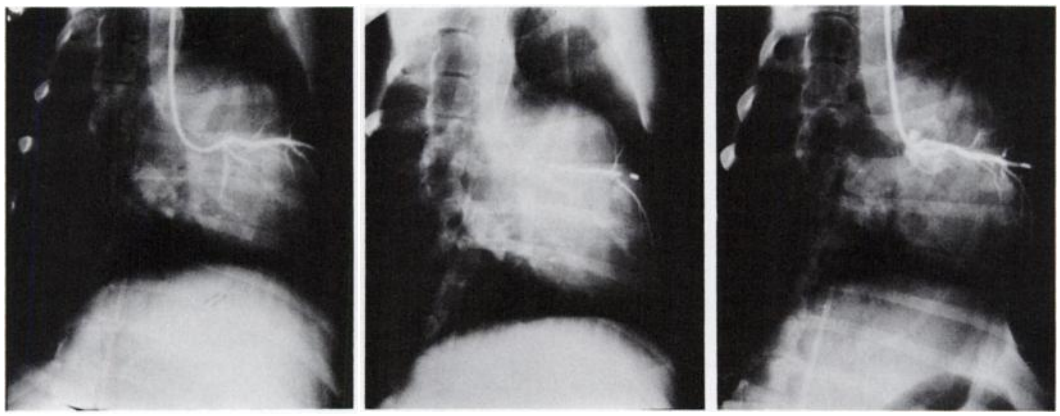

FIG. 1. Left: Control angiogram obtained from dog in LAO projection. Center: Angiogram obtained 5 min after placement of copper coil in left anterior descending coronary artery. Right: Angiogram obtained after thrombolysis has been induced with intracoronary streptokinase.

matrix. Images were processed by subtracting the technetium images (representing activity in the blood pool) from the indium images, pixel by pixel, after normalizing counts for each tracer in the circulating blood (obtained from assessment of vascular radioactivity attributable to each in the undamaged carotid artery at its origin). Operationally, scintigrams were processed by multiplying the Tc-99m matrix in the ROI (placed over the whole heart outline) by the value of In_{ref}/Tc_{ref} obtained from the region of the carotid artery. The resulting Tc matrix was subtracted pixel by pixel from the matrix obtained from In-111 in the ROI. Serial scintigraphy was performed generally from 15 min to 4 hr after administration of tracer, and, in some dogs, for as long as 24 hr after administration.

The percentage of indium excess (% IE)—i.e., the excess radioactivity attributable to platelet accumulation in the thrombus relative to platelet activity in the blood pool—was calculated from the digitized images as previously described (*14*). Briefly, a region of interest over the thrombus was identified after the scintigrams were processed, as noted above. Total radioactivity from In-111 (In_{tot}) is equal to radioactivity from platelets in the blood pool (In_{bp}) plus the activity of platelets incorporated into the thrombus (In_{th}). Indium activity in excess of that in the blood of the ROI (% IE) can be calculated as:

$$\% \text{ IE} = In_{tot} - In_{bp} \times 100 = In_{tot} - \frac{In_{ref}}{Tc_{ref}} (Tc_{roi}) \times 100$$

The % IE represents a measure of the amount of deposition of labeled platelets.

Dogs were killed at selected intervals for analysis of tissue radioactivity by gamma well counting of samples in vitro.

To assess the efficacy of the technique developed to detect dissolution of clot, three dogs given tracer before thrombus induction, and three dogs to whom tracer was administered after induction of thrombus were given an intracoronary infusion of streptokinase (4,000 units/min = 2 ml/min) 3 hr after the induction of coronary thrombus. Scintigraphy was performed throughout the streptokinase administration and for 1 hr after the onset of reperfusion dysrhythmia. Tissues were then analyzed for radioactivity in vitro.

RESULTS

In all dogs coronary thrombus occurred within 15 min after placement of the coil in the coronary artery. Occlusion was manifest by typical electrocardiographic changes of ischemia and confirmed by coronary arteriography (Fig. 1). In dogs with persistent thrombus (those not given streptokinase), clot mass averaged 40 ± 26 (s.d.) mg (range 11 to 88 mg) (Table 1). Histologically, the clot was a "white" thrombus consisting primarily of platelets and fibrin with some trapped red blood cells. The clot usually extended both proximal to and distal from the coil and completely occluded the artery in each case.

Figure 2 shows a series of digitized scintigrams obtained from three dogs with permanent thrombus. The accumulation of platelets in the thrombus is evident clearly in the subtracted image, in a position corresponding to the middle of the left anterior descending coronary artery. Independently the indium-platelet and technetium-RBC images alone do not demonstrate clearly the presence of coronary thrombus. In the subtracted image, platelet accumulation in the thrombus is well defined. Analogous scintigrams were obtained from two dogs with occlusion of the circumflex coronary artery. In these dogs, thrombus was seen most clearly in the 30° LAO projection because of tissue attenuation in the AP projection.

As noted above, a total of 250,000 counts were obtained from each tracer. Of these, 26.6 ± 2.0% emanated from the region of the heart itself (with the majority originating from the spleen and liver), and 3.1 ± 0.3%

TABLE 1. COMPARISON OF SELECTED MEASUREMENTS IN THE FOUR GROUPS OF DOGS STUDIED*

Group (n)	Clot weight (mg)	% IE	Clot/blood ratio
Prelabeled, permanent thrombus (7)	46.8 (11.4 – 87.6)	35.4 (7.4 – 71.8)	38.1 (19.9 – 51.5)
Postlabeled, permanent thrombus (5)	32.9 (12.8 – 69.4)	20.7 (8.9 – 40.6)	27.5 (5.6 – 61.1)
Prelabeled, thrombolysis (3)	11.0 (1.5 – 17.5)	16.8 (0.8 – 28.1)	22.1 (2.3 – 61.1)
Postlabeled, thrombolysis (3)	5.1 (1.1 – 9.5)	8.1 (0.9 – 9.4)	9.1 (7.7 – 10.4)

* All values indicate the mean (with range). % IE indicates the percentage indium excess (see text) determined from the scintigrams, and the clot-to-blood ratio was obtained by postmortem well counting, and is normalized per gam of tissue and blood.

of collected counts originated from the region of the thrombus (as identified on the processed scintigram).

In dogs in which label was administered before induction of thrombus, the mean % IE, calculated from the processed scintigrams, was 35.4, and the clot-to-blood indium ratio based on measurements in vitro was 38.1 (Table 1). In dogs with permanent occlusion but in whom labeled cells were administered after induction of thrombus, these mean values were 20.7 and 27.5 respectively (Table 1). The between-group values were not significantly different. They indicate the rapidity of incorporation of platelets into thrombus early after clot formation.

In dogs subjected to coronary thrombolysis with streptokinase, residual clot mass averaged 6 ± 6 mg. In these dogs all scintigrams were positive for coronary clot before thrombolytic therapy, and all scans became negative after thrombolysis. Figure 3 shows a series of scintigrams from one dog in this group. In dogs subjected to thrombolysis, the IE averaged 16.8 and 8.1% in dogs in which label was administered before or after, thrombolysis (Table 1). The mean indium clot-to-blood ratios were 22.1 and 9.1 respectively (Table 1).

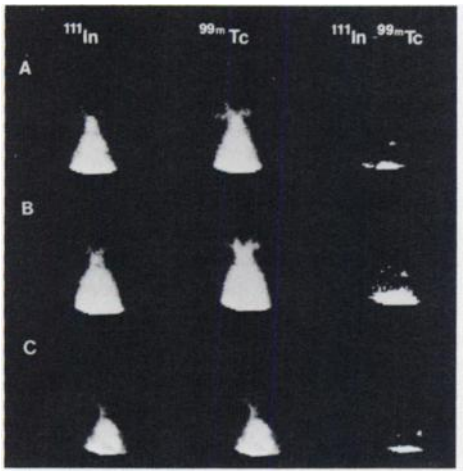

FIG. 2. Digitized scintigrams obtained from three different dogs with thrombus in LAD coronary artery; dogs supine, A-P projection. Scintigrams shown are those obtained with either In-111 photopeak alone, Tc-99m photopeak alone, or subtraction technique described in text. Thrombus is clearly seen only in subtraction scintigrams.

DISCUSSION

The results of this study indicate that coronary thrombi can be visualized noninvasively with the use of In-111-labeled platelets when appropriate correction is made for platelet activity in the circulating blood pool.

Without digital subtraction, images are dominated by radioactivity in cavitary, circulating, labeled platelets. A previous study of experimental coronary thrombosis indicated that visualization was adequate without the use of the subtraction technique (*26*). However, correction for radioactivity emanating from the vascular pool should improve the specificity and sensitivity of detection of platelet aggregation. Of particular interest, dogs with persistent occlusion had clot-to-blood ratios that were not significantly different, regardless of whether labeling was implemented before or after induction of thrombus. Thus, turnover of labeled platelets was rapid for at least 1 hr after formation of clot (Table 1). This observation may explain the findings of Erhardt

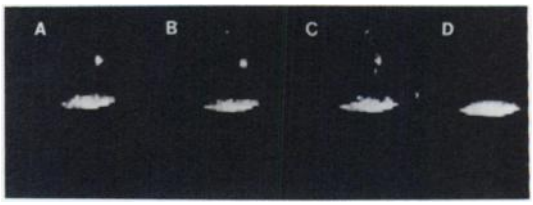

FIG. 3. Processed scintigrams obtained from dog with thrombus in left anterior descending coronary artery, before and after intracoronary administration of streptokinase. Scintigrams were obtained 1 and 3 hr (panels A and B) after induction of thrombus, and 30 min and 1 hr after thrombolysis (panels C and D). Thrombus is seen clearly before thrombolysis, but subtracted scintigram becomes negative after clot dissolution.

et al., who injected labeled fibrinogen in patients and found that formation of clot follows rather than antecedes myocardial infarction in some cases (*28*). Their conclusion may, in fact, reflect persistently rapid turnover of elements within thrombi formed relatively recently.

In the present study, all dogs treated with streptokinase demonstrated diminished radioactivity in the thrombus during administration of the thrombolytic agent, and all exhibited negative scans 1 hr after the onset of reperfusion dysrhythmia. Thus, the technique developed appears to be promising as a means for determining the efficacy of thrombolytic therapy.

Autopsy studies have demonstrated that myocardial infarction is associated frequently with coronary thrombosis (*2–5*). However, such reports may be biased by inclusion of only the fatal cases. Results of angiography early after the onset of infarction have strongly implicated thrombosis as an early event (*6*), but widespread use of this approach is limited in the acute setting because of its invasive nature. Clinical application of the technique developed in the present study should permit serial, noninvasive delineation of temporal relations between platelet deposition and the onset of myocardial infarction in patients with ischemic heart disease, and also characterization of the contribution of platelet aggregation or incorporation into thrombi to other cardiac disorders such as bypass graft occlusion, cerebral vascular disease, unstable angina, and peripheral vascular disease. In addition, it should permit objective, noninvasive evaluation of the efficacy of interventions designed to prevent platelet aggregation or induce lysis of existing thrombi.

ACKNOWLEDGMENTS

The authors thank Alice Rand, Lennis Lich, and Mark Albertina for their technical assistance and Barbara Donnelly for preparation of the typescript.

This work is supported in part by NHLBI SCOR in Ischemic Heart Disease, Grant HL 17646, and NHLBI SCOR in Thrombosis, Grant HL 14147. Published in abstract form, *Circulation* 64 (Suppl IV): IV33, 1981.

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