

Scintigraphic Diagnosis of Experimental Pulmonary Embolism with In-111-Labeled Platelets

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Stasis thrombi, radiolabeled with Tc-99m sulfur colloid, were produced in six dogs by the injection of human thrombin into isolated jugular vein segments. After release of the thrombi, scintigraphic images demonstrated 16 pulmonary emboli containing Tc-99m. Autologous platelets labeled by the In-111 oxine method were injected. Sequential images demonstrated platelet accumulation in 14 of the 16 emboli within 1 hr. The emboli were recovered at autopsy and their activity assayed. The mean uptake of In-111-labeled platelets was 1.1% dose/g of embolus, and the mean embolus-to-blood ratio was 16. Our results demonstrate that fresh pulmonary emboli in dogs can be detected by imaging with In-111-labeled platelets, and suggest that radiolabeled platelets may provide a simple, noninvasive method for the direct visualization of pulmonary emboli in patients.

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The diagnosis of acute pulmonary embolism remains a difficult clinical problem. The symptoms and signs of this disorder, and the findings of routine laboratory examinations and chest radiography, are nonspecific (1). Although perfusion lung imaging is widely accepted as a relatively safe, simple, and sensitive test for the detection of pulmonary emboli, abnormal results due to underlying parenchymal lung disease are frequent. This difficulty is partly overcome by evaluation with combined perfusion and ventilation imaging, which results in improved diagnostic specificity (2,3). Nevertheless, in patients with lung infiltrates, congestive heart failure, or diffuse obstructive airways disease, the diagnosis cannot always be made by conventional scintigraphic means, and pulmonary angiography—with its attendant risk and high cost—may be required.

In ventilation-perfusion lung imaging, the diagnosis of pulmonary embolism is based on the indirect evidence of diminished pulmonary blood flow to lung zones with normal ventilation. Pulmonary angiography is more specific because diagnosis is based on direct demonstration of intraluminal emboli. A noninvasive scintigraphic method that would permit direct visualization of emboli would be a

useful addition to the diagnostic armamentarium (4).

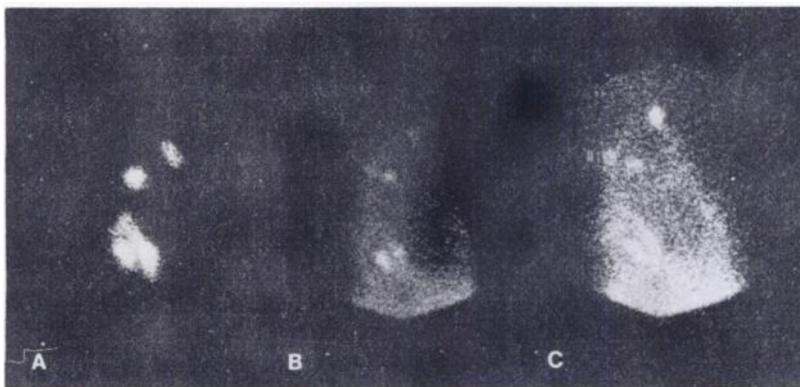
Thakur et al. (5) have recently described a new method for labeling platelets with In-111 8-hydroxyquinoline (oxine). These labeled platelets have been shown to accumulate in recent, experimentally induced venous thrombi as well as at sites of arterial endothelial injury. We have now evaluated the accumulation of In-111-labeled platelets in experimental pulmonary emboli according to the rationale that platelets would be incorporated into propagating emboli or would adhere to the surface of emboli (6).

METHODS

Six female mongrel dogs, weighing 18–23 kg, were used in these studies. Autologous platelets were labeled with In-111 oxine by the method of Thakur et al. (5). Indium-111 chloride solution (1.5 ml containing approximately 3 mCi In-111) was added to an equal volume of sterile deionized water and 200 μ l of 0.3 M acetate buffer (pH 5.3). One hundred μ l of oxine solution (1 mg oxine per milliliter

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FIG. 1. (A) Ventral image of chest showing four emboli labeled with Tc-99m sulfur colloid. The lower right embolus was in the right ventricle; the other three were in the pulmonary arterial tree. Following this, an image (not shown) was obtained using a window centered at 247 keV, which demonstrated no crossover activity from Tc-99m in the In-111 window. (B) immediately after injection of In-111-labeled platelets, uptake is seen (at 247 keV) in all four emboli. (C) Image obtained at 60 min demonstrates increasing activity in the left upper lobe embolus. The focus of increased activity lateral to the right upper lobe embolus corresponded to an area of heartworm infestation.



of ethanol) were added and the solution thoroughly mixed. The solution was extracted with three equal volumes of chloroform, and the extracts were then evaporated to dryness in a boiling-water bath. The In-111 oxine complex was resuspended in a solution of 100 μ l of 95% ethanol and 200 μ l of normal saline. Platelet-rich plasma was obtained by centrifugation (480 g for 15 min) of 30 ml of venous blood anticoagulated with 6 ml of acid citrate dextrose solution (ACD). The platelet-rich plasma was centrifuged at 1500 g for 15 min and the platelet-poor plasma removed; the platelets were then resuspended in normal saline and centrifuged again. The platelets were resuspended in 6–8 ml of normal saline and the In-111 oxine solution was added. The mixture was incubated for 15 min and then centrifuged at 1500 g for 5 min. The supernatant was discarded, and the platelets were resuspended in normal saline, centrifuged a final time, and resuspended in normal saline for injection.

The dogs were anesthetized with intravenous pentobarbital: 30 mg/kg initially and 2.4 mg/kg hourly thereafter. Radiolabeled pulmonary emboli were induced by a modification of the method of Rhodes et al. (7). Both external jugular veins were exposed and isolated for a length of 12–14 cm. All feeder veins were ligated except for one on each side, into which 20-gauge flexible catheters were introduced. A vascular clamp was placed over the caudal end of each jugular vein, which was allowed to distend. Additional clamps were then applied on the cephalic ends of the venous segments. Technetium-99m sulfur colloid (15–20 μ Ci in 0.1 ml) was injected into each venous segment through its catheter and mixed with the blood by barbotage. Next, 50 NIH units of human thrombin in 0.1 ml of 0.9% saline were injected into each segment and the neck wounds were covered with saline-soaked gauze. Thirty minutes later, the vascular clamps were removed and the thrombi released under the force of afferent venous return. None of the thrombi were adherent to the vein wall.

The dog was then placed under a scintillation camera fitted with a medium-energy parallel-hole collimator. A ventral image of the chest was obtained to demonstrate the location of the Tc-99m-labeled emboli. Subsequently, the camera spectrometer was moved to cover the 247-keV peak of In-111 and another image of the chest was obtained to verify that no Tc-99m activity was detectable in the In-111 window. The platelets labeled with In-111 (0.8–2.0 mCi) were then injected through a hindlimb vein. Ventral images were obtained immediately and every 5–10 min for 1 hr.

Within 10 min of the last image, a blood sample was taken and the thorax and abdomen were rapidly opened. The splenic pedicle, and then both pulmonary pedicles, were cross-clamped. The lungs were removed, the pulmonary arterial tree was rapidly opened, and emboli were removed to tared counting vials and weighed. Samples of normal lung, lung distal to emboli, liver, and spleen were removed to tared vials and weighed. The tissue and blood samples were assayed in a NaI(Tl) crystal well counter for both Tc-99m and In-111 activity, and an aliquot of the labeled platelet injectate was counted. Counts in the Tc-99m window were corrected for Compton crossover from In-111.

The results were calculated as percentages of the injected dose per gram (%ID/g) of wet tissue. Embolus-to-blood concentration ratios were also calculated.

RESULTS

Thrombi were successfully induced in the jugular veins of all six dogs. Sixteen emboli labeled with Tc-99m sulfur colloid were noted on the initial scintillation images in these dogs (two to four emboli per dog). Fourteen of the emboli were visualized on images obtained after injection of In-111-labeled platelets, and at least one embolus was detected in each dog. Five emboli were visualized immediately after injection of labeled platelets (Fig. 1) and the remaining nine became apparent on later images

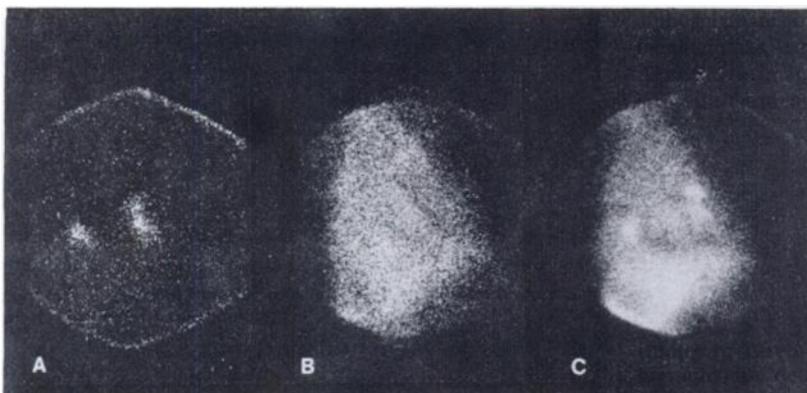


FIG. 2. (A) Ventral image of chest demonstrating three emboli labeled with Tc-99m sulfur colloid. (B) Immediately after injection of In-111-labeled platelets the emboli are not visualized. (C) At 60 min after injection of In-111-labeled platelets, uptake is detectable in all three emboli.

(Fig. 2). The studies were not continued beyond 60 min because of the relatively rapid fibrinolysis and fragmentation of canine pulmonary emboli (8) that would make their recovery at autopsy more difficult.

At autopsy, 16 emboli containing Tc-99m sulfur colloid were recovered. Four of them had lodged in the right ventricle and the remainder were in the pulmonary arteries. The mean embolus weight was 538 ± 398 (s.d.) mg (range, 92–1312 mg) and the mean uptake of In-111-labeled platelets was 1.09 ± 1.47 %ID/g (range, 0.004–6.2 %ID/g). The labeled platelet uptakes in the two emboli that could not be detected scintigraphically (one was intraventricular) were 0.004 and 0.084 %ID/g, respectively. The mean embolus-to-blood concentration ratio was 16.1 ± 12.3 (range, 4.3–42). There were no significant differences in embolus weight, embolus %ID/g, or embolus-to-blood ratio when the emboli visualized immediately were compared with those that appeared only on later images. The activity distribution in other tissues at the time of sacrifice is given in Table 1.

Seven additional clots, which did not contain Tc-99m sulfur colloid, were discovered in the pulmonary arteries of the six dogs at autopsy. Although it might be assumed that these simply represented postmortem clots, two of the "clots" were noted as discrete areas of In-111 accumulation on the pre-mortem scintigrams. The images and autopsy showed that these two foci were distal to emboli containing Tc-99m sulfur colloid. Thus, the additional uptake in these animals may reflect thrombus propagation. In another dog, In-111 platelet accumulation occurred in a pulmonary artery that was occluded by heartworms but contained no pre-mortem embolus (Fig. 1).

DISCUSSION

In a previous study of our group (5), the behavior of canine platelets labeled with In-111 has been characterized. Platelet labeling with the In-111

| Tissue | n | % dose/g \pm s.d. |
|------------------------|----|---------------------|
| Embolus | 16 | 1.09 ± 1.47 |
| Blood | 6 | 0.033 ± 0.009 |
| Normal lung | 5 | 0.037 ± 0.030 |
| Lung distal to embolus | 5 | 0.051 ± 0.029 |
| Liver | 6 | 0.054 ± 0.024 |
| Spleen | 6 | 0.124 ± 0.085 |

oxine complex is efficient: greater than 95% of added radioactivity is incorporated with the platelets, and the label is stable both in vitro and in vivo. The technique of labeling is quite simple compared with other methods; one major advantage is that 30–50 ml of whole blood provide enough platelets for labeling. The function of labeled platelets assessed by their ADP and collagen-induced aggregability is unchanged from that of unlabeled platelets. The recovery and survival of In-111-labeled platelets are similar to those of Cr-51-labeled platelets. Labeled platelets accumulate rapidly in induced venous thrombi of varying ages (up to 72 hr), permitting ready scintigraphic detection of the thrombi as early as 40–50 min after platelet injection.

In the present study, we have shown that In-111-labeled platelets accumulate in fresh pulmonary emboli and make the emboli detectable by gamma imaging. The uptake of labeled platelets by emboli is rapid. Positive images were obtained immediately after administration in five of 16 emboli, and within 1 hr in another nine emboli. These results suggest that In-111 platelets may provide a sensitive means for the direct demonstration of pulmonary embolism in patients.

The model we have chosen for the induction of venous thrombi is a variant of the serum-induction method of Wessler (9). Stasis thrombi produced by this method contain few platelets at their site of

formation, but when released and later recovered from the heart or lungs they acquire a coating of platelets, fibrin, and leukocytes (6,10). Thrombin can be recovered by washing from fresh canine pulmonary emboli (11), and platelet adherence appears to be inhibited by heparin (6). These observations have been taken to support the hypothesis that platelet adherence to pulmonary emboli is mediated at least in part by thrombin. In addition, Niewiarowski et al. (12) have reported that while platelets do not adhere to fully polymerized fibrin, they do adhere to polymerizing fibrin, the latter probably being present in fresh venous thrombi. Either of these mechanisms, which result in platelet adherence to the embolus surface, could explain the observed rapid uptake of the In-111 label by pulmonary emboli. Concurrently, labeled platelets may be actively incorporated into a propagating embolus; our finding of uptake in regions distal to emboli labeled with Tc-99m sulfur colloid suggests that this process is contributory in some cases.

The application of this method to the clinical diagnosis of pulmonary embolism will require further study to resolve several important questions. Increasing thrombus and/or embolus age may substantially reduce the uptake of In-111-labeled platelets. Our earlier results with induced venous thrombi show that scintigraphically detectable platelet accumulation occurs with thrombi as old as 72 hr (5), but the thrombus-to-blood concentration ratio appears to decrease as a function of thrombus age (13). Similarly, the finding of rapid depletion of extractable thrombin in canine pulmonary emboli (11) suggests that detection of embolism by imaging with In-111-labeled platelets may be limited to relatively new emboli.

Anticoagulant therapy may also influence the uptake of labeled platelets by pulmonary emboli. The results of Thomas et al. (6) suggest that heparin is likely to diminish the adherence of In-111-labeled platelets to the surface of pulmonary emboli. This may prove to be an important limitation, since therapy with heparin is often started before definitive diagnosis when pulmonary embolism is strongly suspected.

The specificity of labeled platelet uptake will also require further study. It is likely that pulmonary accumulation of In-111-labeled platelets will occur not only in pulmonary embolism but also in vasculitis involving the pulmonary circulation. The effects of pneumonia, atelectasis, and congestive heart failure are unknown.

Indium-111 has desirable physical properties for external imaging, but its physical half-life of 2.8 days, which is ideal for studies of platelet kinetics,

is unnecessarily long for the scintigraphic detection of thromboembolism. Our preliminary dosimetry estimates for In-111-labeled platelets (based on biodistribution data obtained in normal dogs) are: whole body 0.56 rads/mCi; spleen 30.6 rads/mCi; liver 4.2 rads/mCi; and lung 2.6 rads/mCi (S. Wagner: unpublished observations). Thus, for imaging studies the use of alternative radionuclides, including In-113m, Ga-68 (14), and Tc-99m (15), would be desirable. These generator-produced radionuclides, moreover, would be considerably less costly than In-111.

The above considerations may limit the clinical applicability of this technique. Nevertheless, our initial favorable results with this simply prepared, radio-labeled autologous blood component warrant these further investigations and a clinical trial of this diagnostic technique.

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