

**Comparison of In-111-Labeled Platelets and
Iodinated Fibrinogen for the Detection
of Deep Vein Thrombosis**

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Indium-111-labeled platelets and I-125 fibrinogen were administered to dogs with experimentally induced deep-vein thrombosis. The thrombus uptake of both labeled compounds was studied as a function of thrombus age. In thrombi less than 24 hr old, thrombus-to-blood ratios for In-111 platelets were about twice as great as those obtained for I-125 fibrinogen. Excellent scintiphotos were obtained with In-111 platelets. In thrombi older than 24 hr, uptake of both agents was low, but good images were still obtained with In-111 platelets. The images in this case are not of thrombi, but rather of damaged vessel wall. Indium-111-labeled platelets are superior to iodinated fibrinogen for imaging fresh thrombi, but offer no advantage for thrombi over 24 hr old.

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The fibrinogen uptake test using I-125 fibrinogen is widely accepted as a useful, accurate method for the detection of deep-vein thrombosis (1-3). If either I-131 or I-123 is used to label fibrinogen, thrombi may be imaged with a scintillation camera (4,5). Unfortunately, the thrombus uptake of radioiodinated fibrinogen is poor when the thrombus is several days old (6), resulting in low thrombus-to-blood ratios; this makes imaging difficult, especially in regions of large blood pool.

Thakur et al. (7) have recently reported a method for labeling platelets with indium-111. Preliminary experiments in dogs indicated uptake of labeled platelets in venous thrombi, permitting scintigraphic visualization of thrombi as old as 72 hr at the time of labeled platelet administration.

In the present study, we have further investigated the uptake of In-111-labeled platelets in deep-vein thrombi as a function of thrombus age, using I-125 fibrinogen as a standard of comparison.

METHODS

Experimental deep-vein thrombosis was induced in dogs as previously described (6). The dogs were anesthetized with sodium pentobarbital (30 mg/kg).

A vinyl catheter containing a stainless steel wire was inserted into the jugular vein and advanced under fluoroscopic guidance into the femoral vein. The stainless steel wire was connected to the anode of a variable power supply. The cathode was connected to a skin clamp on the leg bearing the catheter. A direct current (5 mA, 2 V) was applied for 1 hr. The wire and catheter were then withdrawn. The thrombi were allowed to age for 1, 2, 4, 6, 12, 19, 24, 48, or 72 hr before i.v. injection of labeled platelets and labeled fibrinogen into a forelimb vein. Indium-111-labeled autologous platelets were prepared by the method of Thakur et al. (7). Platelets were obtained from 43 ml of blood drawn into a syringe containing 7 ml of acid citrate dextrose (ACD). The blood was carefully transferred to two 50-ml polypropylene test tubes and spun at 180 g for 15 min. The platelet-rich plasma (PRP) was removed with siliconized Pasteur pipets, placed in polypropylene test tubes, and further centrifuged at 1700 g for 15 min. The platelet-poor plasma

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(PPP) was removed, and the platelets were gently resuspended in 10 ml of 6:1 saline:ACD. They were centrifuged and suspended twice more in the same manner. This final suspension of platelets was used for the labeling.

Indium-111-oxine complex was formed as previously described (7). Briefly, 1–1.5 ml (approximately 3 mCi) of In-111 chloride solution (pH 1–3) were added to an equal volume of deionized sterile water and 200 μ l of 0.3 M acetate buffer, pH 5.3. Fifty μ l of oxine in ethanol (1 mg/ml) were added and the solution mixed thoroughly. The resulting complex was extracted into two equal volumes of chloroform. The chloroform was evaporated to dryness in a boiling-water bath and the residue was dissolved in 50 μ l of ethanol and diluted with 150 μ l of normal saline.

The ethanol-saline mixture containing the In-111-oxine complex was added to 10-ml of platelet suspension and incubated at room temperature for 15 min. A small aliquot of the resulting suspension was then removed and centrifuged at 1700 g to check labeling efficiency. If the incorporation of label into the platelets was greater than 90%, the suspension of labeled platelets was used directly for injection; otherwise they were centrifuged at 1700 g and resuspended in 3–5 ml of PPP. An average of 700 μ Ci was injected into each dog. It has been shown that >70% of the platelets are recovered in the normal dog and that their survival is similar to that of chromium-51 labeled platelets (7).

Radioiodinated fibrinogen was prepared by a modification of the iodine monochloride (ICl) technique of McFarlane (8,9). A small amount of ICl was allowed to exchange with 2 mCi Na¹²⁵I in 200 μ l of 2 M NaCl. To this was added 200 μ l borate buffer, pH 7.9 (0.4 M, 0.11 M NaCl). The mixture was immediately jetted into 400 μ l of the same borate buffer containing 3 mg of fibrinogen, Blomback fraction I-4 (10), isolated from fresh canine plasma by the method of Mosesson and Sherry (11). After 15 min, the labeled fibrinogen was purified by ammonium sulfate precipitation at 30% saturation followed by dissolution in 0.02 M Tris buffer (pH 7.4, 0.135 M NaCl). Labeled fibrinogen was stored at –20°C for up to 2 wk before use. Typically, 200–400 μ Ci were used per dog in these experiments. This method of preparation has been shown to give labeled fibrinogen that behaves as fibrinogen both in vitro (12) and in vivo (13).

Each dog received both In-111 platelets and I-125 fibrinogen, injected at the same time. At various intervals during the 24 hr immediately following administration of labeled platelets, images of In-111 activity were made of the dogs' hind limbs with a

TABLE 1. THROMBUS ACCUMULATION OF LABELED PLATELETS AND FIBRINOGEN

Thrombus age (hr)* at time of injection	Thrombus accumulation	
	In-111 platelets	I-125 fibrinogen
1	32	7.7
2	26	14
	37	20
4	18	15
	21	17
6	32	16
12	25	†
19	15	8.4
20	2.0	3.4
24	2.5	2.5
	1.9	2.4
	1.1	1.6
48	3.0	2.3
	5.2	3.6

* Thrombus weights ranged from 53 to 400 mg. Mean weight of 24-hour-old thrombi was 78 ± 27 mg.

† I-125 counts in this animal were too low to permit accurate calculation.

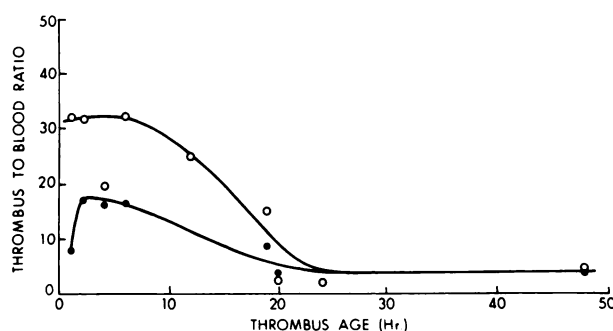


FIG. 1. Thrombus-to-blood ratios of tracers in induced deep vein thrombus as a function of thrombus age at time of injection. ○ = In-111 platelets. ● = I-125 fibrinogen.

scintillation camera. Twenty-four hours after injection of the tracers the thrombus was removed. A blood sample and samples of vessel wall at the thrombus site, and from the same site in the contralateral leg, were also collected. The samples were weighed and counted for both In-111 and I-125 in a NaI(Tl) well counter. Thrombus-to-blood ratios were calculated as (cpm/g thrombus) \div (cpm/g blood).

RESULTS

Thrombus-to-blood ratios obtained in these experiments are shown in Table 1, and are plotted in Fig. 1 against age of thrombus at time of injection of the labeled blood components. For thrombi less than 24 hr old, the uptake of In-111 platelets was significantly greater than that of labeled fibrinogen;

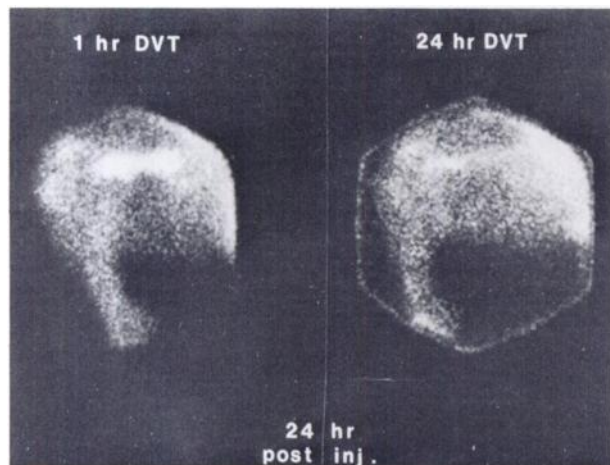


FIG. 2. Scintiphotos of hind limbs of experimental dogs showing sites of thrombosis. Left: 1-hour-old thrombus at time of In-111 platelet injection; Right: 24-hour-old thrombus at time of In-111 platelet injection. Images made 24 hr after In-111 platelet injection. Thrombus-to-blood ratios for 1-hour-old thrombus (24 hr post injection) was 32, and for 24-hour-old thrombus (24 hr post injection) 2.5.

the thrombus-to-blood ratios were about twice as great. However, for thrombi over 24 hr old at the time of injection, uptake of both agents was low and the thrombus-to-blood ratios were similar.

Scintiphotos of the hind limbs demonstrated In-111 platelet accumulation at the site of thrombosis in all cases (Figs. 2-4). The best images were observed 12-15 hr postinjection (Fig. 3), although all thrombi were visualized as early as 1-2 hr after injection. Thrombi older than 24 hr at the time of tracer injection were quite small at the time of their removal (>48 hr after thrombus induction). A 72-hour-old thrombus could not be identified 24 hr after

tracer injection (96 hr after thrombus induction), although In-111 platelet uptake in the femoral vein was observed scintigraphically (Fig. 4). This finding suggests that the images detected platelet deposition on damaged endothelium. Further evidence for uptake of platelets by injured vessel wall was obtained from activity determination in the samples of vein wall. After removal of a thrombus that was 24 hr old at the time of tracer injection, a section of vessel from the thrombus site contained more than four times as much In-111 radioactivity by weight as did a section of vessel from the corresponding site in the opposite hind leg. In another dog with a 48-hr deep vein thrombus, vessel ratios were 2:1 (damaged side:undamaged side) after thrombus removal.

DISCUSSION

Our results show that In-111 platelets produce higher thrombus-to-blood ratios than radioiodinated fibrinogen in thrombi less than 24 hr old. The highest uptake of labeled platelets is by thrombi that are 6 hr old or less. The effects of thrombus age on thrombus uptake of radioiodinated fibrinogen noted in this study was similar to those previously reported (6,14). It is clear that neither agent is taken up well by thrombi greater than 24 hr old.

This model for induction of deep-vein thrombosis apparently produces at least some degree of endothelial injury, since the labeled platelets adhere to the damaged area long after the thrombus has lysed. Preliminary encouraging reports of the imaging of older thrombi (7) probably involved images of injured vessel walls, not thrombi, since the same model was used. Because thrombolysis in dogs is more rapid

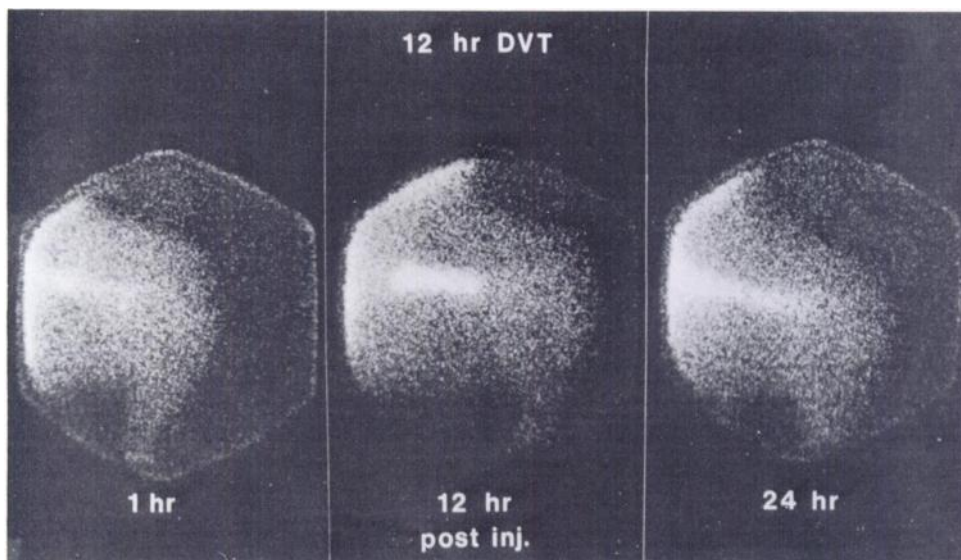


FIG. 3. Scintiphotos of site of thrombosis in hind limb of a single dog. Thrombus was 12 hr old at time of injection of In-111 platelets. Imaging times (L to R): 1, 13, and 24 hr postinjection. Thrombus/blood ratio 24 hr postinjection was 25:1.

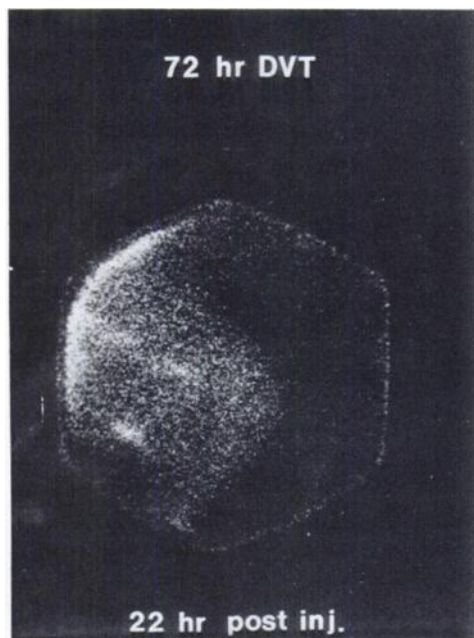


FIG. 4. Scintiphotos of hind limb of dog. Indium-111 platelets administered 72 hr after thrombus induction. Image was made 22 hr after tracer injection. Although a thrombus was apparently visualized, no gross clot was visible in vein.

than in humans, it may be possible to obtain good images of older deep-vein thrombi in humans.

There has been a common impression that venous thrombi differ from arterial thrombi in that the former are composed primarily of red cells and fibrin rather than masses of platelet aggregates (15,16). This implies that there should be no significant accumulation of platelets by venous thrombi. Paterson (17), however, claims that platelet deposition is always present in some part of a venous thrombus, particularly at the thrombus head. Harker et al., and Steele et al. both report that platelet survival times are frequently shortened in patients with venous or arterial thrombosis (18,19). Our results agree with these latter workers in that platelets do accumulate on venous thrombi.

We conclude that In-111 platelets are superior to radioiodinated fibrinogen for the imaging of deep-vein thrombosis.

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