Platelets Labeled with Oxine Complexes of Tc-99m and In-111. Part 2. Localization of Experimentally Induced Vascular Lesions

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Using rabbit platelets harvested and labeled with either Tc-99m or In-111 oxine as described in Part 1, we have successfully imaged experimentally induced fresh venous thrombi and newly injured arterial intima. Visualization of lesions up to 6 hr old is striking. Thrombi and arterial damage 24 hr old, however, were usually not imaged successfully; nor were preformed platelet-poor arterial emboli. The varying rates of platelet deposition in vascular lesions of different ages and types account for these observations. Should human cells prove as effective, widespread clinical application is anticipated.

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Using rabbit platelets labeled with both Tc-99m and In-111, we have imaged and examined the dynamics of platelets aggregation in venous thrombi, endarterial damage, and preformed arterial emboli.

MATERIALS AND METHODS

Platelets were separated and labeled by both the "buttonless-saline" and "button-saline" methods described in Part 1.

Production of venous thrombosis. Venous thrombi were produced by injection of suspended iron particles (0.5–1 g of 1.3-μ particles in 3 cc Renografin-76) into an ear vein while a magnet was positioned anteriorly over the rabbit's neck (*1–3*). After 30 min the magnet was removed and radiographs demonstrated clumps of i.v. iron under the former position of the magnet, far proximal to the i.v. ear injection site (Fig. 1A and B). Thrombi were produced 1, 3, 6, or 24 hr before the injection of labeled platelets, as well as immediately, 15 min, or 3 hr following platelet injection—i.e., with labeled platelets already circulating. A minimum of three animals was studied for each time interval. Platelets were always injected slowly into the contralateral ear. Initially the platelets were injected intra-arterially, in order that any clumped cells could be filtered out by the ear's capillary bed. Subsequently, however, the i.v. route was found to be equally satisfactory, since clumping was not significant. Both autologous and heterologous cells were studied.

Endarterial damage. Following blood collection, the "floppy" tip of a pediatric angiographic wire

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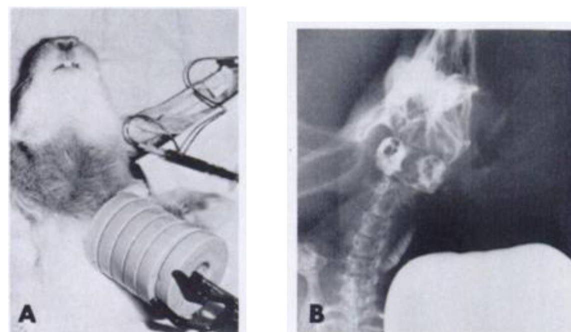

FIG. 1. (A) Sedated rabbit with magnets positioned over antero-superior thorax. Iron particles suspended in Renografin-76 are injected into a left ear vein. (B) Radiograph of same animal demonstrates iron particles in a neck vein. Note "feathery" appearance of particles, due to alignment along magnetic lines of force.

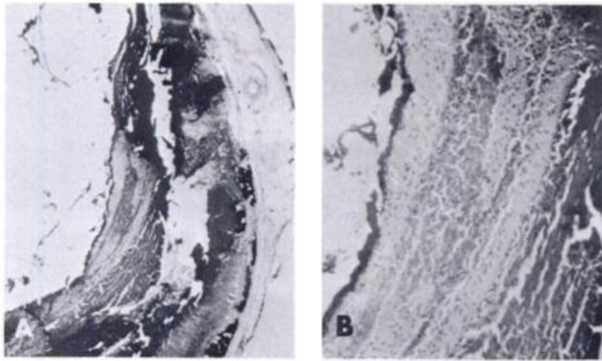

FIG. 2. (A) Low-power H&E section reveals clumps of i.v. iron (black), as well as both platelet-rich and fibrin-rich-cell thrombus. Vessel wall remains intact. This thrombus was successfully imaged at 3 hr. (B) Higher power photomicrograph demonstrates classical alternating layers of platelet-rich and fibrin-rich-cell thrombus ("lines of Zahn"). This pattern closely parallels that observed in spontaneous human venous thrombi.

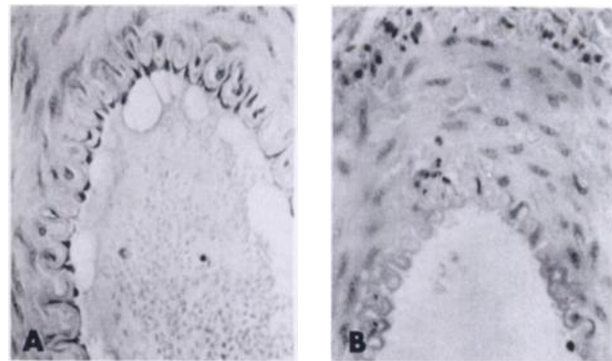

FIG. 3. (A) High-power photomicrograph of normal undamaged central ear artery of rabbit. Note sub-intimal internal elastic lamella and single layer of intimal cells with deeply stained nuclei adjacent to lumen. (B) High-power photomicrograph of a rabbit's injured central ear artery. Muscularis and internal elastic lamella remain intact. The single layer of intimal cells, however, has been sloughed off.

guide (0.018 French) was inserted through the indwelling angiocath (Part 1) and advanced proximally into the central ear artery until resistance at the junction of the ear and the head was encountered. Alligator clips connected to the positive and negative terminals of a dc power supply were attached to the wire guide and the lateral edge of the rabbit's ear, and a constant 4-mA current was passed between the clips for 40–50 sec. The clips were then removed and the wire guide and angiocath withdrawn. Bleeding from the arterial puncture site was easily controlled by 2–3 min of mild pressure. Arteries were injured 1, 3, 6, or 24 hr before labeled platelet injection, as well as immediately, 1, and 3 hr after labeled platelet injection. Again, a minimum of three animals was studied for each time interval. All labeled platelets were injected intravenously into the contralateral ear. Both autologous and heterologous cells were studied.

Production of preformed systemic arterial emboli. Following puncture of the central ear artery, 4 ml of blood were withdrawn into a plastic syringe containing 1.5 mCi Tc-99m MAA in 0.2 cc saline, mixed, and permitted to clot, thus producing a Tc-99m-MAA-labeled "red" thrombus. This platelet-poor thrombus was then forcibly injected, retrograde, into the central ear artery, and was observed on the gamma camera to enter the systemic arterial circulation in fragmented form. Platelets labeled with In-111 oxine, which had been prepared from previously drawn blood, were immediately injected intravenously. The animal was imaged using the Tc-99m and In-111 "windows" alternately, to establish whether indium-labeled platelets would localize in preformed "red" systemic-arterial emboli.

Histopathology. One and one-half hours after production of venous thrombi and endarterial damage, the involved vein and artery, as well as contralateral "control" vessels, were isolated by dissection, removed, and fixed. Tissue sections were prepared and stained with hematoxylin and eosin.

Control studies. Control studies of venous thrombi and endarterial damage were performed with [^{99m}Tc] pertechnetate, Tc-99m oxine incubated with whole blood, Tc-99m oxine incubated with platelet-poor plasma and Tc-99m sulfur colloid. The vascular lesions were produced 15 min after the i.v. radiopharmaceutical injection. Because of rapid sulfur colloid clearance by the reticuloendothelial system, thrombosis was also initiated immediately after a control i.v. injection of the Tc-99m sulfur colloid. In an additional experiment, sulfur colloid was injected 2 hr after thrombus formation.

RESULTS

Histopathology. Tissue sections of the induced venous thrombi showed the vessel wall to be com-

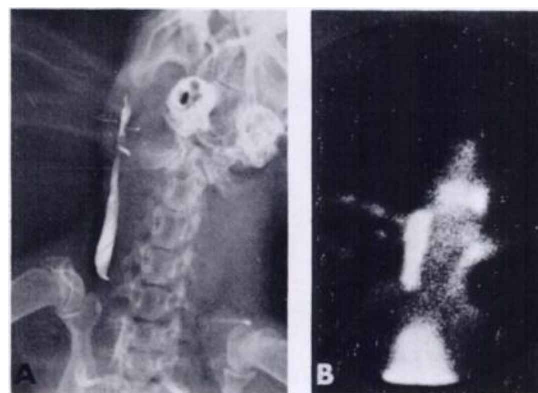

FIG. 4. (A) AP radiograph demonstrates i.v. iron thrombus in neck on right. Thrombus formation was initiated 2 hr after i.v. injection of autologous platelets labeled with 4 mCi Tc-99m oxine. (B) Anterior scintiphoto obtained 1 hr after thrombus formation reveals intense activity along the course of the iron thrombus in (A).

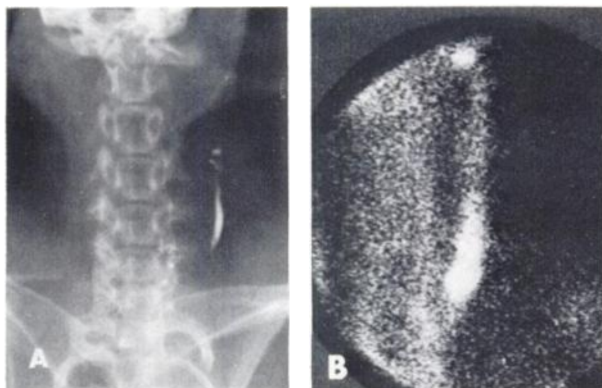

FIG. 5. (A) AP radiograph demonstrates i.v. iron thrombus in neck on left. Thrombus formation was initiated 1½ hr before labeled-platelet injection. (B) Anterior scintiphoto, obtained 1 hr after i.v. injection of autologous platelets with 3 mCi In-111 oxine, reveals accumulation along course of iron thrombus in (A).

pletely intact. The thrombi contained clumps of iron and alternating layers of "white" platelet thrombus and "red" fibrin-erythrocyte thrombus. Classical "lines of Zahn" were clearly demonstrated (Fig. 2)—closely simulating spontaneous human thrombi. Tissue sections of the electrically-induced arterial damage revealed loss of the intimal cell layer with integrity of the internal elastic lamella, no disruption of the muscularis, and no thrombus formation (Fig. 3).

Venous thrombus detection. All thrombi produced 15 min after injection of 0.5–2.0 mCi of platelets labeled with Tc-99m oxine or In-111 oxine were well visualized within 1 hr of their formation (Fig. 4). Preformed thrombi were also demonstrated by subsequent labeled-platelet injection (Fig. 5), with either autologous or heterologous cells. As the interval between thrombus formation and platelet injection increased, the time between platelet injection and thrombus visualization also increased. For example, whereas the 1.5-hour-old thrombus visualized within 1 hr of platelet injection, the 3-hour-old thrombus required 3.5 hr to visualize.

FIG. 6. Posterior scintiphoto reveals intense residual activity in a polyethylene infusion tube, as well as in medial and lateral i.v. injection sites in right ear. Note absence of accumulation along course of undamaged right-sided central ear artery. The injured left central ear artery is clearly observed. Injury preceded i.v. injection of 3 mCi Tc-99m-oxine-labeled platelets by 2 hr.

Thrombi produced 6 hr before labeled platelet injection visualized poorly, and thrombi produced 24 hr before the injection usually failed to visualize at all. This remained true when imaging was continued for 6 hr after injection of platelets labeled with Tc-99m oxine and for 24 hr after those labeled with In-111 oxine.

In five control animals, whose thrombi were initiated 15 min following i.v. injection, respectively, of [^{99m}Tc] pertechnetate, Tc-99m oxine incubated with whole blood, Tc-99m oxine incubated with platelet-poor plasma, and Tc-99m sulfur colloid, no lesions were visualized. Moreover, when Tc-99m sulfur colloid was injected 2 hr after thrombus formation, the thrombus was not detected. However, the thrombus created immediately following injection of Tc-99m sulfur colloid visualized faintly.

Endarterial damage. Platelets labeled with either Tc-99m or In-111 clearly accumulated along injured vessels (Fig. 6) when damage was produced up to 6 hr before platelet injection. As in the case of venous thrombi, however, 24-hour-old damage failed to accumulate enough platelets for successful gamma-imaging. Autologous and heterologous cells were equally effective.

Systemic arterial embolization with preformed thrombi. Alternate imaging with the technetium and indium windows of the camera's spectrometer demonstrated widely scattered technetium-labeled, platelet-poor, "red" thrombi throughout the arterial tree. However, these failed to accumulate In-111-labeled platelets significantly (Fig. 7) in images obtained 1, 2, and 3 hr after the platelet injection.

DISCUSSION

Many methods for the induction of thrombi and arterial injury have been developed. Most require some surgical or chemical manipulation of blood vessels, through a skin incision or puncture site over the area of interest. Such procedures usually superimpose soft-tissue trauma upon the clot formation or damaged artery, complicating image interpretation. The iron-thrombus method avoids such difficulties, since the sole venapuncture is far removed and the magnets produce no soft-tissue damage. In addition, the iron particles serve as their own contrast material, so that radiographs may be directly compared with scintigrams, obviating the need to locate each thrombus by dissection. Similarly, the endarterial damage produced by passage of a mild electrical current between the intraluminal guide wire and a distant soft-tissue electrode produces minimal injury, proximal to the arterial puncture.

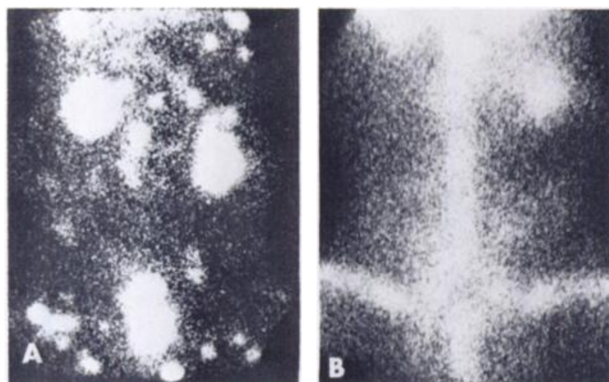

FIG. 7. (A) Anterior gamma image of a rabbit 10 min after forcible retrograde injection of Tc-99m-MAA-labeled blood clots into central ear artery. Note multiple scattered "hot" emboli throughout arterial tree. (B) Gamma image using the In-111 window reveals no In-111-oxine-labeled platelet accumulation at sites of Tc-99m-MAA-labeled systemic arterial emboli.

Striking visualization of fresh venous thrombi and newly damaged arterial intima is consistent with the theory that platelets accumulate at the "head" of a venous thrombus and on any abnormal intraluminal surface. As venous thrombi age, however, they become more "red" (i.e., predominantly fibrin with entrapped erythrocytes) and their rate of platelet acquisition declines. This sequence agrees with our observation that the older thrombi require a longer interval between platelet injection and gamma-camera visualization than younger thrombi, and it is also consistent with our inability to visualize thrombi a day or more old. Our results with end-arterial damage are similar. Thus, failure of In-111-labeled platelets to localizing in platelet-poor, preformed, systemic-arterial "red" emboli is not surprising.

Gastrointestinal and urinary-tract radioactivity observed after i.v. injection of Tc-99m oxine platelets suggests some instability of the cell label. A similar biodistribution is observed after i.v. injection of Tc-99m oxine alone. Platelet labeling with In-111 oxine is much more stable, and little gastrointestinal or urinary-tract excretion is observed.

The relative clinical values of Tc-99m-labeled and In-111-labeled platelets will depend on various factors, including the location and age of the lesion in question. For the examination of intra-abdominal organs and the pelvic and ileo-femoral veins—the usual sites of potentially fatal thrombi—In-111 oxine platelets will probably prove superior. However, with bladder emptying, shielding, and oblique views, Tc-99m-labeled platelets may also be effective. Indium-111, with its longer half-life, may prove superior for thrombi one or more days old, which may require at least 24 hr between platelet injection and imaging. Moreover, extremely high-risk post-surgical patients could be imaged for several days after platelet injection, in an attempt to demonstrate new thrombi as they form. Fresh vascular lesions in the arms, mid-to-lower legs, and neck, however, may be studied best with the Tc-99m, in view of its favorable characteristics for gamma-camera imaging.

In conclusion, both venous and arterial lesions have been successfully imaged with heterologous and autologous platelets labeled with Tc-99m oxine and In-111 oxine. Should similarly labeled human cells prove as effective, widespread clinical application is anticipated.

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