Labeling of Platelets with Oxine Complexes of Tc-99m and In-111. Part 1. In Vitro Studies and Survival in the Rabbit

Brian W. Wistow, Zachary D. Grossman, John G. McAfee, Gopal Subramanian, Robert W. Henderson, and Marsha L. Roskopf

State University of New York, Upstate Medical Center, Syracuse, New York

We have used both Tc-99m oxine and In-111 oxine to study the effects in the rabbit of various parameters on platelet labeling and the in vivo survival of platelets harvested by four different methods. For In-111 oxine, platelet labeling in saline produces much higher efficiencies (90%) than labeling in the presence of plasma (20%), with no significant shortening of in vivo survival (1% survival at 5.9 days). For Tc-99m oxine, labeling efficiences are considerably lower (30%) and survival is shorter (1% survival at 3.8 days). For both radioagents, a 30-min labeling incubation at room temperature is suggested.

J Nucl Med 19: 483–487, 1978

Following McAfee and Thakur's introduction of 8-hydroxyquinoline (oxine) for leukocyte labeling (*1,2*), Thakur successfully visualized canine venous thrombi and mechanically damaged arterial intima by labeling platelets with In-111 oxine, and he studied their in vivo survival in dogs (*3*). Scheffel et al. then established that the survival of In-111 platelets in the rabbit compared favorably with that of Cr-51 platelets (*4*). In the rabbit, with both Tc-99m oxine and In-111 oxine, we have studied various labeling parameters and the in vivo survival of platelets following their separation by four different methods. Part 1 of this communication concerns in vitro studies and survival, and Part 2 will deal with biodistribution and imaging of experimentally induced vascular lesions.

MATERIALS AND METHODS

The Tc-99m-oxine complex was prepared by a method described previously (*5*). Indium-111 oxine was made as follows: 8-hydroxyquinoline and In-111 chloride were obtained from commercial sources. The oxine (100–300 μg) was dissolved in 0.1 ml ethanol and added to 3 mCi of In-111 chloride in 3 ml of 0.05 *M* HCl solution. The pH was adjusted to 5.4–5.5 by adding 20% sodium acetate in water. A volume of methylene chloride equal to that of the indium solution was added and mixed well in a Vortex mixer. The methylene chloride layer containing the In-111-oxine complex was separated into a conical glass test tube, evaporated to dryness in a hot-water bath under a stream of air, and the residue was dissolved in 0.1 ml of dimethyl sulfoxide (DMSO). The DMSO solution containing In-111 oxine was diluted with 0.1 ml of normal saline just before addition to the platelet suspension. This solution contained an average of 90% of the initial In-111 chloride activity.

Blood collection. New Zealand white and domestic lop-eared brown rabbits, weighing between 2 and 3 kg, were sedated with an intramuscular injection of 0.3 cc Innovar-Vet. Fifteen minutes later, the dorsal surfaces of both ears were shaved and vigorously massaged. The central artery of either ear was punctured with a 20-gauge angiocatheter, which was

Received Aug. 15, 1977; revision accepted Dec. 2, 1977.

For reprints contact: Brian W. Wistow, Div. of Nuclear Medicine, Dept. of Radiology, SUNY, Upstate Medical Ctr., 750 E. Adams St., Syracuse, NY 13210.

advanced approximately 2–3 cm. The first 2–4 ml of blood obtained were discarded. Four 8-cc aliquots of blood were then drawn into four 10-cc plastic syringes, each containing 2 cc ACD solution*. Each syringe was inverted three times immediately upon filling. A plastic stopcock filled with saline was then attached to the angiocatheter, which remained in place.

Platelet separation. Plastic test tubes and pipettes were used throughout.

1. *"Buttonless-saline" method* (*6*). Following collection, the anticoagulated blood was transferred to a 60-ml round-bottom test tube 2.7 cm in diameter and centrifuged for 40 sec at 7,700 g. The supernatant, platelet-poor plasma (PPP) was then removed with a pipette and discarded, leaving a red-cell layer covered by a thin layer of platelets. Physiologic saline equal in volume to the PPP removed was added and the tube inverted several times. Centrifugation at 7,700 g for 20 sec then resulted in a red-cell column covered by a thin platelet layer and a platelet-poor saline supernatant. This supernatant was removed and an equal volume of fresh saline was added again to the tube. If platelets were to be labeled with Tc-99m, the tube was then centrifuged at 140 g for 5 min followed by 180 g for 10 min. If platelets were to be labeled with indium-111, however, an additional saline wash and spin at 7,700 g for 20 sec was performed before the low-speed spin.

Following the low-speed spin, the platelet-rich saline supernatant, above the red cell layer, was removed and incubated with either Tc-99m oxine or In-111 oxine.

2. *"Button-saline" method.* Following collection of whole blood in ACD as above described, centrifugation at 180 g for 15 min produced a red-cell layer covered by platelet-rich plasma supernatant (PRP). The supernatant was withdrawn, transferred to a second test tube, and centrifuged at 720 g for 7 min, producing a soft platelet "button" covered by PPP. The PPP was decanted and discarded, and 5 ml of saline at room temperature were added to the button. Gentle swirling for 2–3 min resuspended the platelets.

3. *"Button-plasma" method.* Scheffel (*4*) and McIntyre (*7*) maintained a plasma-protein environment throughout both separation and labeling of rabbit platelets with In-111 oxine. We used their procedure without alteration.

4. *Addition of Prostaglandin E_1* (*8,9*). Platelets were separated by each of the three methods described above, with the addition of 50 ng prostaglandin E_1 (PGE_1) (as a 10% ethanol in Tyrode's solution) to each ml of platelet-rich plasma or platelet-rich saline following each wash.

Labeling parameters. The "buttonless-saline" technique was used for platelet collections.

1. *Incubation time.* The platelet-rich saline suspension was divided into six 2-cc aliquots, each containing 1.26×10^9 cells. Indium-111 oxine (0.25 mCi) was added to each of the six tubes, which were incubated for 5, 15, 30, 45, 60, and 120 min. After centrifugation, activity in the cells and supernatant was measured. The experiment was repeated with 4.34×10^8 cells in each sample. Similarly, aliquots containing 6.0×10^8 platelets were labeled with 0.25 mCi Tc-99m oxine.

2. *Cell concentration.* Three-cubic centimeter aliquots containing increasing numbers of platelets in saline (varying from 5.4×10^7 to 1.5×10^9 cells) were incubated with 0.25 mCi In-111 oxine for 20 min. After centrifugation, activities in the cells and supernatant were measured. Similarly, aliquots containing from 1.57×10^8 to 1.88×10^9 cells were incubated with Tc-99m oxine.

3. *Temperature.* The platelet-rich saline solution was divided into 2-cc aliquots, containing 1.2×10^9 cells, and incubated with 0.25 mCi In-111 oxine for 20 min at 4°, 25°, and 37°C. The experiment was repeated with 4.34×10^8 cells per aliquot, and also with Tc-99m oxine added to tubes containing 6.27×10^8 cells.

Platelet labeling in whole blood and platelet-rich

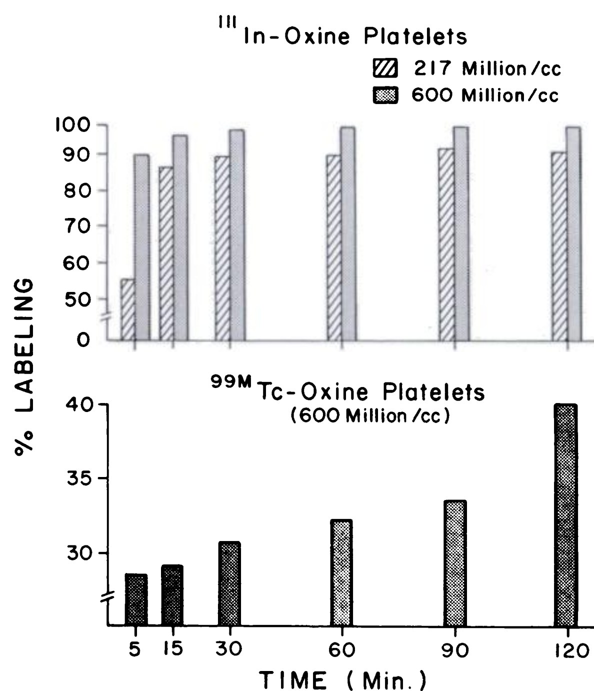

FIG. 1. Effect of incubation time on labeling efficiency with In-111 oxine and Tc-99m oxine. With In-111 oxine, there is excellent labeling at 15 min, and an incubation time of 20 min appears optimal. With Tc-99m oxine, there is gradual increase from 15 min to 2 hr.

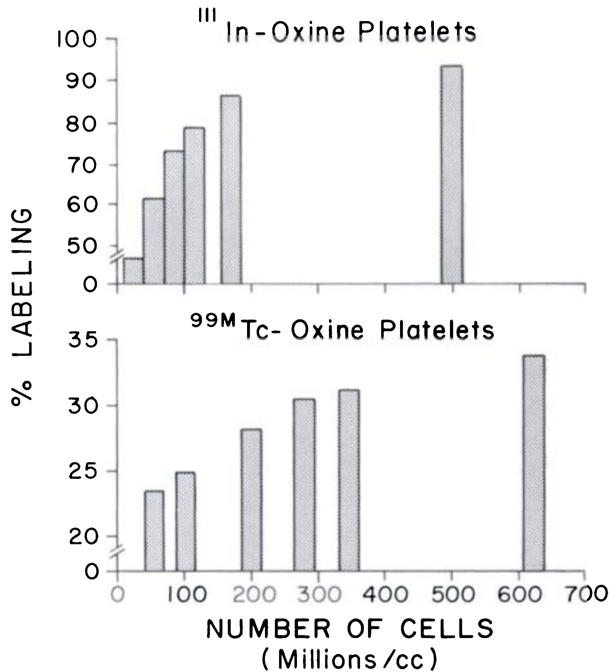

FIG. 2. Effect of cell concentration on labeling efficiency. With both In-111 oxine and Tc-99m oxine, percentage labeling is dependent on cell concentration. Platelet concentrations greater than 1.5 billion cells/cc are easily obtained.

plasma with Tc-99m oxine. Following blood collection, 1 mCi Tc-99m oxine was added to 10 cc of whole blood and incubated for 20 min at room temperature. Red blood cells and platelets were separated by the "button-saline" method, and the activity in each of these cellular fractions, as well as in the remaining cell-poor plasma, was measured. To 10 cc of platelet-rich plasma, 5 mCi Tc-99m oxine were added and incubated for 20 min; platelets were completely separated by centrifugation at 3,500 g for 15 min, and activity measured in the platelets and PPP.

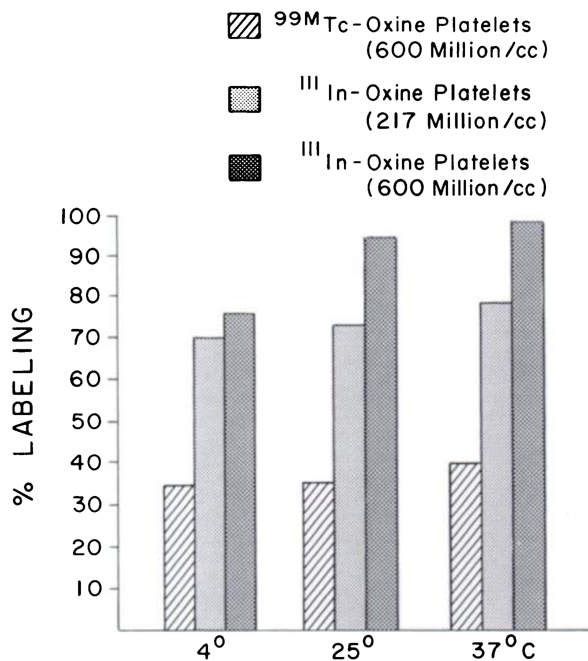

FIG. 3. Effect of temperature on labeling efficiency. Only minimal increase in percentage of labeling—both with In-111 and Tc-99m oxine—occurs at 37°C compared with 25°C. Lower labeling efficiencies were obtained at 4°C.

In vivo platelet survival. Indium-111 oxine platelet survival was determined in the rabbit following platelet preparation and labeling by four methods—"buttonless-saline," "button-plasma," and both methods with the addition of prostaglandin E_1 (three animals with each method). In addition, the survival was studied in three animals after platelet preparation by the "button-saline" method, without the addition of PGE_1. Blood was collected at 15 and 30 min, as well as at 1, 2, 3, and 24 hr. For the In-111 oxine platelet survivals, samples were collected also at 2, 3, and 4 days. Whole blood, plasma, and platelet activities were measured.

For the survival of platelets labeled with Tc-99m oxine, red cells were separated from platelet-rich plasma by centrifugation at 180 g for 15 min. Platelets were then completely collected from the PRP by centrifugation at 3,500 g for 10 min. Activities in whole blood, platelets, red cells, and platelet-poor plasma were measured. Whole-blood and PRP platelet counts were performed in order to correct apparent red-cell activity for platelets included with red cells during the separation and also to correct for this platelet loss from the platelet-rich plasma. Data were plotted on semi-log and linear paper as a percentage of the administered activity in the circulating blood volume. The blood volume—as measured in four rabbits (2.38–2.69 kg) with I-125 albumin and Cr-51-labeled red cells—averaged 6.2% of the body weight. For platelets labeled with Tc-99m oxine, the *y* coordinates corresponding to 48 and 72 hr were determined from an extrapolation of the exponential least-squares fit of measured data. Mean survival and lifespan were calculated by standard methods (7).

RESULTS

Platelet recovery. Separation by the "buttonless-saline" method collected 51% of the platelets present in whole blood. The "button-saline" method yielded 48%. Addition of PGE_1 did not affect efficiency of platelet collection.

Labeling parameters and efficiency. With both In-111 oxine and Tc-99m oxine, platelet labeling efficiency reached a practical maximum after incubation for 15–30 min (Fig. 1). Longer incubation results in only slightly increased labeling with both radiopharmaceuticals if high cell concentrations are maintained, but at much lower cell concentrations, shorter

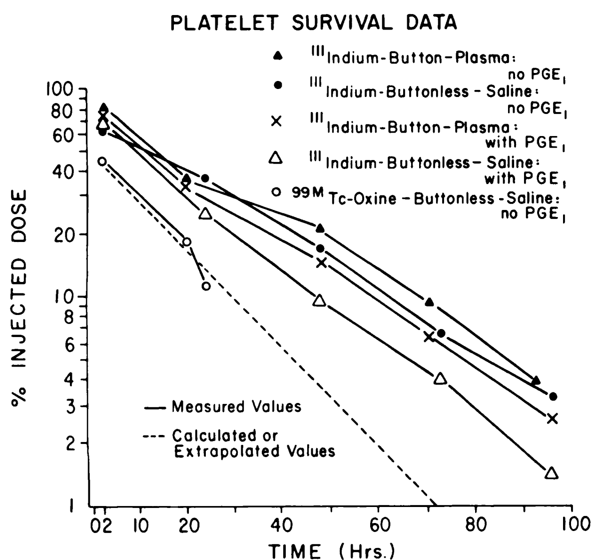

FIG. 4. Survival curves obtained with platelets prepared by five different methods. There is no significant difference between "button-plasma" and "buttonless-saline" methods. Addition of PGE_1 did not increase survival. The survival of Tc-99m-labeled platelets is shortest, but is sufficient for imaging at 24 hr.

incubations (5–10 min) result in a marked labeling decline with In-111 oxine. A direct relationship between labeling efficiency and cell concentration was observed (Fig. 2). Incubation temperature above 25°C resulted in only minimal increase in cell-labeling yield with either radiopharmaceutical (Fig. 3). On incubation of approximately half a billion rabbit platelets suspended in saline for 30 min at room temperature, the cell-labeling efficiency with In-111 oxine was approximately 90% but only about 30% for Tc-99m oxine. The labeling with In-111 oxine in the presence of plasma, by the method of Scheffel (*4*), gave efficiencies of approximately 20%.

Platelet labeling in whole blood and platelet-rich plasma. Following the addition of Tc-99m oxine to whole blood, 67% of the activity appeared in the red-cell fraction and 33% in the platelet-rich plasma. Separated platelets contained only 2% of the initial activity. Following the addition of Tc-99m oxine to platelet-rich plasma, 12.5% of the activity appeared in the separated platelet fraction.

In vivo platelet survival. Measured data for all platelet survival curves (Fig. 4) conformed best to an exponential function as indicated by correlation coefficients of 0.995 or greater for the exponential functions (Table 1). There is no significant difference in the survival parameters (life span, mean survival, or 1% exponential survival; Table 1) between platelets prepared by the "button-plasma" method and the "buttonless-saline" methods: for the former, life span, mean survival, and 1% exponential survival measured 3.6, 1.3, and 6.0 days; and for the latter 3.7, 1.3, and 5.9 days. The percentage of recovery at zero time was slightly greater for the "button-plasma" method (81%) than for the "buttonless-saline" method (77%). No improvement in survival was recorded with the addition of PGE_1. Tc-oxine platelets (using calculated and extrapolated 48 and 72 hour values) had considerably shorter survival times (mean survival 0.82 days) and a lower initial recovery (47%).

DISCUSSION

The arterial puncture technique provides a simple means of obtaining not only the initial blood sample but also the several subsequent samples required for survival studies, since the Angiocath may remain in place for several days. Unlike more invasive procedures such as cardiac puncture, the technique results in little morbidity and no mortality. Exclusion of the first 2 or 3 cc of blood, which contain tissue thromboplastin, is simple, since arterial flow rates are high.

Because plasma is removed during platelet separation by the "buttonless-saline" method, labeling efficiency with In-111 oxine is high (>90%). Thus the entire incubation mixture may be injected directly, eliminating the time-consuming steps of but-

TABLE 1

	^{111}In oxine				^{99m}Tc oxine
	Button-plasma method		Buttonless-saline method		Buttonless-saline method
	No PGE_1	PGE_1	No PGE_1	PGE_1	No PGE_1
Life span (days) (linear)	3.6	3.6	3.7	3.4	2.6
Half-life (days) (exponential)	0.90	0.83	0.88	0.71	0.57
Mean survival (days) (exponential)	1.3	1.2	1.3	1.0	0.82
1% survival (days) (exponential)	6.0	5.6	5.9	4.7	3.8
% recovery (exponential)	81	78	77	71	47
Correlation coefficient					
Linear	0.930	0.940	0.898	0.867	0.864
Exponential	0.995	0.997	0.998	0.998	0.996

ton formation and platelet resuspension after labeling. In addition, possible physical injury is avoided by preventing the formation of a platelet pellet by the cushioning effect of the red-cell layer. For Tc-99m oxine labeling, the "buttonless-saline" method requires fewer washes, since complete removal of plasma protein is not critical. Because the labeling efficiency is only about 30%, however, one button is required after incubation to separate platelet-bound activity from free Tc-99m oxine. The lower platelet recovery (47%) and shorter mean survival (20 hr) may indicate that Tc-99m oxine does not bind to cells as firmly as In-111 oxine. An equally plausible alternative explanation is that a larger percentage of the labeled cell population may not be behaving normally. The "button-saline" technique has the disadvantages of both of the other techniques when labeling is done with In-111 oxine. The two washes for plasma-protein removal and the associated button formation may produce platelet loss and possible damage. Therefore, we do not recommend this method for In-111-oxine labeling. On the other hand, for Tc-99m oxine labeling, a single wash (and single button) will suffice. This button may be resuspended in a very small volume, giving high cell concentrations in the incubation mixture. Thus the "button-saline" method may be suitable for Tc-99m oxine labeling.

PGE_1 substantially inhibits platelet aggregation and facilitates the disaggregation of platelet buttons. Images of infusion tubing through which PGE_1-treated platelets are injected show less activity than tubing used to inject platelets prepared without PGE_1, indicating a decreased adherence of the labeled cells. Since no appreciable improvement in survival is noted with PGE_1, however, we do not recommend its use.

Many factors affect platelet viability. They are maintained best at 22°C at a pH of 6.5–6.8. Oxygen and carbon dioxide tensions are also important. Without glucose or plasma proteins, platelets lose their sensitivity to ADP-induced aggregation. Washing platelets decreases their oxygen consumption and cyclic AMP levels; the former is restored by the addition of plasma albumin. Many of the biochemical changes induced by washing may be reversible after re-injection into the circulating plasma. In the present study, there was no significant difference in platelet recovery or survival between In-oxine-labeled cells prepared by the "button" method in plasma and those prepared by the "buttonless" method in a plasma-free medium; this suggests a reversal of any injury sustained during harvesting and labeling.

CONCLUSION

The "buttonless-saline" and "button-saline" methods are satisfactory for separation of rabbit platelets to be labeled with Tc-99m oxine. Although either the "buttonless-saline" or the "button-plasma" method is satisfactory for In-111 oxine, labeling in saline results in higher yields (>90%) than those obtained in plasma. Whereas these techniques are suitable for rabbit platelets, human platelet harvesting and labeling may require modified procedures.

FOOTNOTE

* 8.75 g citric acid monohydrate, 25 g sodium citrate dihydrate, 12 g dextrose anhydrous in 1000 ml distilled water.

REFERENCES

1. McAfee JG, Thakur ML: Survey of radioactive agents for in vitro labeling of phagocytic leukocytes. I. Soluble agents. *J Nucl Med* 17: 480–487, 1976

2. McAfee JG, Thakur ML: Survey of radioactive agents for in vitro labeling of phagocytic leukocytes. II. Particles. *J Nucl Med* 17: 488–492, 1976

3. Thakur ML, Welch MJ, Joist JH, et al: Indium-111 labeled platelets: Studies on preparation and evaluation of in vitro and in vivo functions. *Thrombosis Research* 9: 345–357, 1976

4. Scheffel U, McIntyre PA, Evatt B, et al: Evaluation of Indium-111 as a new high photon yield gamma-emitting "physiological" platelet label. *Johns Hopkins Med J* 140: 285–293, 1977

5. Subramanian G, McAfee JG, Gagne GM, et al: ^{99m}Tc-oxine: A new lipophilic radiopharmaceutical for labeling leukocytes & platelets. Proc. 14th International Meeting of the Society of Nuclear Medicine, Berlin, 1976

6. Bang NU, Hansen SM, Heidenreich RO: *The Influence of Plasma Proteins on Platelet Function and Metabolism*, Exerpta Medica, International Congress Series, 357/118/1975

7. *Recommended Methods for Radioisotope Platelet Survival Studies.* A Report by the Panel on Diagnostic Application of Radioisotope in Hematology of the International Committee for Standardization in Hematology, November 1976

8. Emmons PR, Hampton JR, Harrison MJG, et al.: Effect of prostaglandin E_1 on platelet behaviour in vitro and in vivo. *Br Med J* 468–472, 1967

9. Scharschmidt BF, Martin JF, Shapiro LJ, et al: The use of calcium chelating agents and prostaglandin E_1 to eliminate platelet and white blood cell losses resulting from hemoperfusion through uncoated charcoal, albumin-agarose gel, and neutral and cation exchange resins. *J Lab Clin Med* 89: 110–119, 1977