Kinetics, Distribution, and Sites of Destruction of Canine Blood Platelets with In-111 Oxine

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In five normal dogs we have studied the survival, tissue distribution, and fate of autologous platelets labeled with indium-111 oxine. The methods include blood sampling, computer-assisted scintigraphy, and whole-body profile scanning. Mean In-111-platelet recovery in the circulation was 45 ± 22.5 (s.d.) and survival 124.6 ± 10.5 hr. Platelet survival curves fitted a linear function best. Initially platelets pooled rapidly in the spleen with a single exponential function, and at zero-time equilibrium (35 ± 4)% of the injected In-111 was located in this organ. Early hepatic uptake was also significant, and constituted (20 ± 4)% of total-body radioactivity. As labeled platelets disappeared from the circulation, In-111 activity in the spleen increased progressively and linearly to reach (59 ± 9)% of the body activity at 120 hr. Hepatic radioactivity decreased with time but to a lesser extent than that of the heart. The results indicate that in the dog the major site of destruction of platelets is the spleen, with the liver playing a less important role.

J Nucl Med 21: 36–40, 1980

Indium-111 is a cyclotron-produced nuclide ($T_{1/2}$ = 2.8 days), with 183% gamma emissions at 173 and 247 keV. These physical characteristics permit quantitative gamma imaging in the in vivo distribution of labeled cells. In-111 8-hydroxyquinoline (In-111 oxine), a lipophylic agent, is an efficient and "physiological" platelet label (*1,2*). Good external imaging of experimentally induced thrombi has been obtained with this agent (*1,3,4*).

We report on an in vivo study of canine platelet kinetics. The life span, early distribution in spleen and liver, and sites of destruction of In-111-labeled platelets were investigated with serial blood sampling, a scintillation camera interfaced with computer-assisted imaging system, and whole-body profile scanning.

Received May 7, 1979; revision accepted July 20, 1979.

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MATERIALS AND METHODS

Platelet labeling (*5*). Platelets were separated and labeled by a technique modified after Thakur (*1*) and the recommendations of the International Committee for Standardization in Hematology (ICSH) (*6*). All procedures were carried out aseptically at 22°C in plastic or siliconized equipment. Mongrel dogs weighing 23–33 kg were venesected, and after discarding the first 3 ml blood, collected in a separate syringe, 42 ml blood was collected with an 18-gauge needle into a 50-ml polystyrene syringe containing 8 ml ACD-A (ACD in NIH formula A) as anticoagulant. The blood was transferred to 50-ml polystyrene conical tubes* and platelet-rich plasma (PRP) was prepared by differential centrifugation at 180 *g* for 10 min. Contaminating erythrocytes were removed by centrifuging PRP at 180 *g* for 5 min. The pH was adjusted to 6.2–6.5 by addition of 5 ml ACD-A per 100 ml PRP. Platelets were sedimented by centrifuging at 800 *g* for 15 min and resuspended in 2–3 ml physiological saline. The platelet concentrate was

incubated for 30 min with In-111 oxine† at a constant concentration of 6.25 μg oxine/ml platelet suspension. At this concentration the oxine and ethanol in the In-111 oxine do not affect platelet function adversely, and In-111 labeling efficiency is high (*5*). The labeled platelets were washed once with two volumes of platelet-poor autologous plasma (PPP) and finally resuspended in 2–3 ml PPP.

Aggregation of labeled platelets was assessed in vitro by phase microscopy. Two drops of PRP were placed on a glass slide and ~1 μl collagen fibril suspension‡ was added with a micropipette. Formation of aggregates consisting of more than 10 platelets was considered as the normal in vitro platelet aggregation response (*5*). Only platelets reacting this way were used for in vivo studies.

Platelet survival. Preparation of standards, determination of volume of platelets injected, blood sampling, and counting of radioactivity for platelet survival studies were done as recommended by ICSH (*6*). Mean autologous platelet survival was calculated by a least-squares analysis of the linear and logarithmic estimates. The standard deviation of an estimate measures the discrepancies between the fitted line and the data (Ref. *6*, Method 1). The recovery of injected platelets in the circulation after equilibrium was derived by extrapolation of the platelet survival curve back to zero time from Days 1–5.

Platelet distribution and destruction. A scintillation camera was interfaced with a computer-assisted imaging system with a 64 × 64 element acquisition matrix and a 128 × 128 interpolated display (*7*). The pulse-height analyzer was set to include the 173 keV photopeak. The dogs were positioned under a high-energy divergent collimator to visualize the heart, spleen, and liver. Geometry in day-to-day imaging was stabilized by immobilizing the unanesthetized dogs in a fixed frame on a rigid table.

In-111-labeled platelets were injected as a bolus, and images were obtained by integrating for 30-sec intervals for 15 min. Areas of interest were selected and time-activity curves of early organ radioactivity generated.

Subsequent redistribution of In-111 activity was studied by the following method. Whole-body profile scanning, with a shielded slit collimator placed as close as possible above the subject, was done daily in a low-background steel room. Counts from both energy peaks were integrated at 10-mm intervals along the length of the dog to generate a radioactivity profile. Activity in the spleen, liver, and heart was measured for 9 min daily by computer-assisted scintillation-camera imaging and selection of areas of interest. Radioactivity of a selected thoracic area was integrated by both the scintillation camera and the whole-body counter. These activities were related and a correction factor derived. Radioactivity as measured by the scintillation camera was in all cases normalized to that of the whole-body activity by applying the correction factor. Time-radioactivity distributions for the whole body and the specific organ on Days 1 to 5 were subjected to linear least-squares analysis. Zero-time equilibrium was derived by back-extrapolation of regression activity.

Evaluation of accuracy of external quantitation of radioactivity. In a separate experiment, the radioactivity distribution in four dogs was quantitated by whole-body profile scanning and gamma imaging at 120 hr after injection of In-111-labeled platelets. The dogs were then killed and the radioactivity of the excised liver and spleen counted again with the scintillation camera and the whole-body scanner.

RESULTS

Platelet survival. The relevant subject data, radioactivity administered, recovery, and linear and logarithmic functions of platelet disappearance from the circulation are given in Table 1. The radioactivity recovered in the circulation at equilibrium varied considerably from dog to dog, with a mean of (45.7 ± 22.5)% (±1 s.d.). Disappearance curves of labeled platelets in the six dogs are illustrated in Fig. 1. These curves fitted a linear function best, with a mean platelet survival of 124.6 ± 10.5 hr (Table 1).

Early splenic pooling of platelets. This was assessed by analysis of the early (0–15 min) camera images. Time-activity curves of the spleen were fitted by a least-squares Newton iteration method to a closed two-compartment model (*8*). Data were normalized relative to the maximum value for each curve. The mean accumulation of platelets (±1 s.e.m.) observed in six dogs is depicted in Fig. 2, and camera images in Fig. 3. Platelets accumulate in the spleen as a single exponential function

TABLE 1. SUBJECT AND PLATELET SURVIVAL DATA

Dog	Radioactivity administered MBq (μCi)	Recovery in circulation (%)	Mean survival time (linear) ±1 s.d.* (hr)	Mean survival time (log.) ±1 s.d.* (hr)
A	12.9 (350)	82	113 ± 2.1	64.4 ± 2.7
B	11.8 (320)	23	123.7 ± 3.3	61.7 ± 4.2
C	18.1 (490)	45	116.9 ± 5.1	52.4 ± 5.6
D	10.5 (284)	62	138.9 ± 4.0	72.8 ± 5.8
E	5.4 (145)	33	136.0 ± 3.7	66.1 ± 5.5
F	11.1 (300)	29	119.1 ± 6.8	46.3 ± 7.9
Mean ± s.d.		45.7 ± 22.5	124.6 ± 10.5	60.6 ± 9.7

* This standard deviation measures the discrepancy between the fitted line and the data.

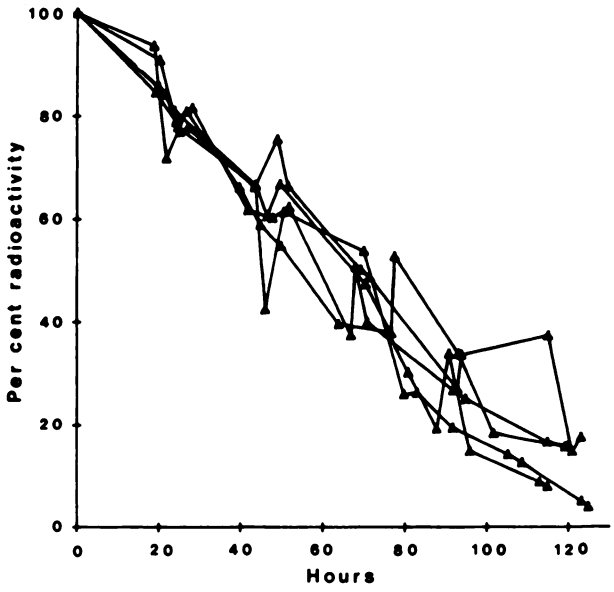

FIG. 1. Platelet survival curves in normal dogs. Experimental values are reported as normalized curves (equilibrium value at 100%) for easier evaluation of mode and rate of platelet disappearance. Recovery values of In-111-labeled platelets in circulation are given in Table 1.

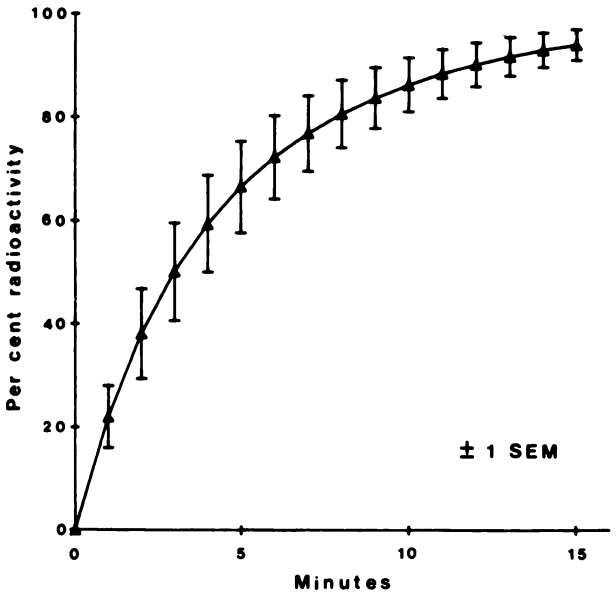

FIG. 2. Early accumulation of platelets in spleen. Splenic radioactivity is expressed as percentage of maximum value. Platelet accumulation follows as single exponential function, reaching 94% of maximum activity at 15 min after injection.

with a mean rate constant of 0.26 ± 0.17 min^{-1}, reaching 94% of maximal activity at 15 min after injection.

The percentage of In-111 activity pooled in the spleen at equilibrium, as determined by whole-body counting and the scintillation camera, was (36 ± 4)% of the total-body activity (Table 2).

It is noteworthy that the spleen size in any given dog varied considerably from day to day (Fig. 3).

Early platelet activity in the liver. Changes in radioactivity in the liver and heart were determined during the initial 15 min after injection at In-111 platelets. The curves were normalized to 100% at 1 min after injection. Early-phase hepatic activity is considerable and does not decrease in parallel with that of the heart (Fig. 4).

Initial equilibrium radioactivity in the liver, expressed as a percentage of total-body activity, was also determined. The mean derived hepatic activity at zero-time equilibrium was (21 ± 4)% of total-body radioactivity (Table 2, Fig. 5).

Determination of sites of platelet destruction. Organs were visualized, areas of interest selected, radioactivity

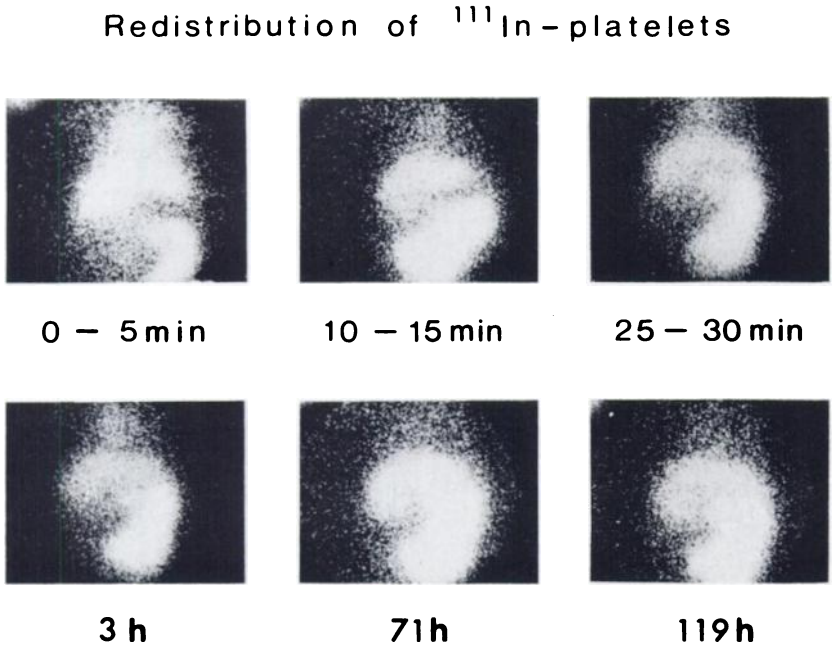

FIG. 3. Scintillation-camera images of organs of interest after injection of labeled platelets. Accumulations in heart, spleen, and liver are prominent in first 30 min after injection. Later, In-111 activity is redistributed: spleen, and to a lesser extent liver, are the major sites of platelet sequestration. Note variation in size of spleen at different times.

TABLE 2. EARLY AND LATE IN VIVO ORGAN In-111 DISTRIBUTION

Subject	Equilibrium percentage activity			120-hr percentage activity		
	Heart	Spleen	Liver	Heart	Spleen	Liver
A	5.6	36.9	23.1	1.0	72.3	10.8
B	6.2	32.8	23.7	1.4	52.4	21.3
C	1.5	37.6	16.3	0.3	60.9	12.2
D	2.2	41.1	15.8	0.6	62.0	13.1
E	4.5	29.7	24.8	1.4	48.8	15.2
Mean ± 1 s.d.	4.0 ± 2	35 ± 4	20 ± 4	0.9 ± 0.5	59 ± 9	14 ± 4

quantitated serially, and time-activity curves generated (Fig. 5, Table 2). Liver activity (as a fraction of the whole body) decreased but to a lesser extent than that of the heart. In-111 activity in the spleen increased progressively and linearly to reach (59 ± 9)% of total-body activity at 120 hr, and remained constant thereafter. Comparing the changes in initial and final percentages for organ radioactivity with the t-test for paired data, we found a significant increase in activity in the spleen ($p < 0.001$) and a significant decrease in activity in the liver ($p < 0.05$) and heart ($p < 0.01$).

Accuracy of external quantitation of organ radioactivity. This was assessed by comparing quantitation of hepatic, splenic, and whole-body radioactivity in vivo and after removal of organs from sacrificed animals. The ratios between in vivo and ex vivo hepatic, splenic, and whole-body radioactivity were 0.84 ± 0.14, 1.18 ± 0.13, and 1.00 ± 0.10, respectively.

DISCUSSION

The use of In-111 oxine as a platelet label, in conjunction with a computerized scintillation-camera system with dynamic imaging capability, allows a new approach to the study of platelet kinetics.

The technique of labeling platelets in a saline medium has the disadvantage of damaging rabbit and human platelets (*2,6,9*), but permits more efficient platelet uptake of In-111 oxine (*1*). The dog platelet survival curves fit a linear function best and show no evidence of an early rapid clearance, which would indicate platelet damage (Fig. 1). In vitro platelet function was normal. We are thus satisfied that we are labeling a physiologically normal platelet population.

The results are typical of our experimental conditions. Several factors that are difficult to control—such as preparation of platelets, age of animals, and reaction of animals to stress—may influence the results. We acknowledge that because of geometrical complexities, surface counting as an estimate of total radioactivity is subject to error. Interpretation of results of quantitative estimates of In-111 activity redistribution must take this into account. The experiments in which quantitations of radioactivity of the organs in vivo and in removed post

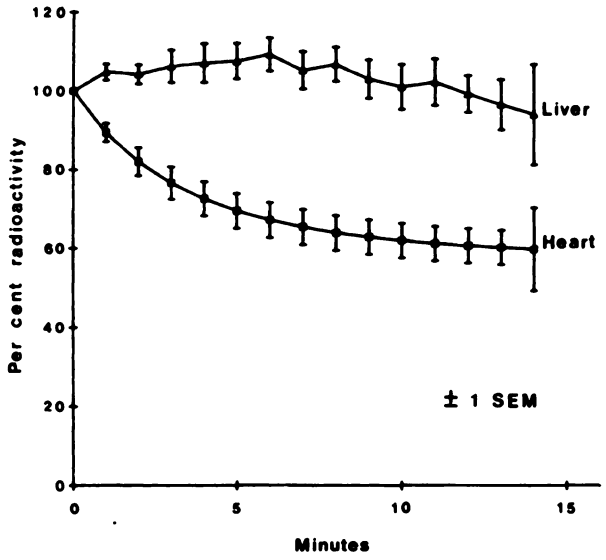

FIG. 4. Time-activity curves showing early disappearance of In-111-labeled platelets from heart, and accumulation in liver. Hepatic activity is prominent and does not decrease parallel with that in heart. Curves were normalized to 100% at 1 min after injection of In-111 platelets.

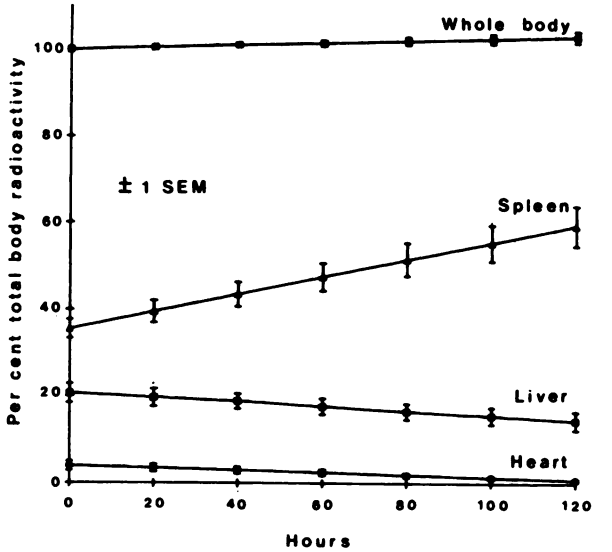

FIG. 5. Organ distribution of In-111 activity, expressed as percentage of total-body radioactivity, following injection of In-111 platelets. There is marked increase in activity in spleen. Total-body radioactivity remains virtually constant.

mortem specimens showed that the radioactivity of the spleen is overestimated and that of the liver underestimated by in vivo external counting. This may be due to the shapes and anatomical situations of these two organs. Geometrical and attenuation errors may be minimized in future experiments by adopting anterior, posterior, and transmission imaging (*10*).

A pool of platelets, consisting of about one third of the total platelet number and exchanging freely with circulating platelets, exists in the spleen (*11,12*). Our results, measured as In-111-platelet recovery in the blood at equilibrium, are in accord (Table 1) but there is marked variation from subject to subject. This is possibly due to the great variation in splenic size observed in individual dogs from day to day (Fig. 3). Contraction and relaxation of the spleen will affect the splenic pool size. The accumulation of platelets in the spleen is rapid (Fig. 2), maximal after 15 min, and the size was estimated to be 36% of the total platelet mass.

There was excess In-111-platelet activity in the liver in the period immediately after injection, and this activity did not decrease in parallel with blood flow as reflected by activity in the heart (Fig. 4). This suggests the presence of a hepatic platelet pool. A proportion of these pooled platelets recirculated eventually (Fig. 4). The size of the remaining hepatic platelet pool was not estimated in this study, but this aspect should be studied further.

Little is known about the fate of senescent platelets. Studies with Cr-51-labeled platelets suggest that the liver and spleen are the major sites of destruction in man (*13–15*) and in rats (*15*). Our results indicate that in the dog the major site of destruction of platelets is the spleen, with the liver playing a minor role (Fig. 5, Table 2). These two organs were responsible for clearance of 73% of total radioactivity. This corresponds closely to values obtained in the rat (*15*). It is not clear where the remaining platelets go. In thrombocytopenic animals platelet radioactivity was detected in vascular endothelial cells by autoradiography (*16*), but the utilization of platelets in maintaining normal endothelium has not been assessed. The reticuloendothelial component of the bone marrow may also provide a site of platelet destruction.

These results demonstrate the advantages of In-111 over Cr-51 as a platelet label. Serial imaging and analysis of data with a comptuer-assisted imaging system allows more reliable quantitation of the in vivo distribution of the tracer. The technique may also be readily applied to the study of the kinetics and sites of destruction of platelets in diseases of abnormal platelet utilization and pooling.

FOOTNOTES

* Falcon 2070, Oxnard, CA.

† Diagnostic Isotopes, NJ.

‡ Hormon-Chemie, Müchen, FRG.

ACKNOWLEDGMENTS

This research was supported in part by the South African Medical Research Council and the Atomic Energy Board. The authors thank Mrs. E. Joubert for secretarial assistance in the preparation of the manuscript.

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