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^{111}In-8-Hydroxyquinoline Platelet Labeling in Dogs[1]

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This report validates a technique of separating and labeling canine platelets using indium-111-labeled 8-hydroxyquinoline (^{111}In-8-HQ). Platelet survival time in six normal dogs was 6.3 ± 0.7 (SD) days. Labeling efficiency using this technique was 51.8 ± 9.2% (SEM) and elution loss of ^{111}In-8-HQ label from platelets was consistently less than 4%. ^{111}In label offers no observable advantages over the more traditional ^{51}Cr label for platelet survival studies. However, the high efficiency labeling and -γ-emission characteristics of ^{111}In-8-HQ make it more suitable for noninvasive clot localization in patients with early pulmonary emboli and peripheral venous thrombi.

Radioisotopic measurement of platelet thrombokinetics has provided important information regarding thrombocytopenias and related hematologic disorders [5, 12, 13, 20, 21]. More recently, *in vivo* platelet survival studies have found wider application in many clinical areas including atherosclerotic vascular disease, the fate of arterial bypass grafts [24–26], and clinical investigation of arterial, peripheral venous, and pulmonary emboli [10, 11, 17]. The present study was undertaken to validate the efficacy of canine platelet labeling using indium-111-labeled 8-hydroxyquinoline.

Indium 111 (^{111}In) can be complexed easily to the lipophilic compound 8-hydroxyquinoline (8-HQ). When used as a platelet label for early clot localization studies, this radioisotope offers several advantages over traditional labels such as chromium 51 radiochromate (^{51}Cr)[28] and newer labels such as technetium 99m (^{99m}Tc) as pertechnetate and as the oxine complex [28, 30].

PLATELET LABELING TECHNIQUE

Six unanesthetized mongrel dogs weighing 20–35 kg were used to assess platelet survival using ^{111}In-8-HQ label. All animals were maintained on standard kennel chow and water *ad libitum*. ^{111}In-8-HQ was prepared from ^{111}InCl according to the method of Scheffel *et al.* [23]. $^{111}InCl_3$ (1 mCi) (Diagnostic Isotopes) was buffered to a pH of 5 using 0.3 *M* acetate. 8-Hydroxyquinoline (100 μg) in 100% ethyl alcohol (final concentration 1 mg/ml) was added and the mixture allowed to incubate at room temperature for 5 min. Three volumes of methylene chloride were added to extract the ^{111}In-8-HQ and the preparation was evaporated to dryness. The product was dissolved in a 1:3 mixture of ethyl alcohol and isotonic saline for addition to canine platelets.

Plastic, disposable labware was used exclusively throughout the isolation procedure. Platelets were isolated and labeled at room temperature with the following technique [23]: forty-three milliliters of blood was withdrawn into a 50-ml syringe containing 7.5 ml acid citrate dextrose (NIH solution A). After gentle mixing by inversion, the blood was transferred to a round-bottom 50-ml polycarbonate centrifuge tube and spun for 15 min at 220 *g* (VWR Scientific GS-6). The platelet-rich plasma (PRP) was aspirated and transferred to a second 50-ml centrifuge tube. The volume of PRP was measured and the pH adjusted to 6.5 to 6.7

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using more ACD-A. A sample was removed for platelet count.

The platelets were sedimented from the PRP during a second spin of 15 min at 1000 *g*. The supernatant platelet-poor plasma (PPP) was gently decanted, and the platelet button resuspended in 2 ml of the residual PPP by repeated aspiration into a 1-ml pipet. ^{111}In-8-HQ was added to the platelet concentrate such that the total amounts of hydroxyquinoline and ethyl alcohol did not exceed 12.5 μg and 12.5 μl, respectively. ^{111}In activity in the suspension was assayed using a Capintec dose calibrator. The platelet–^{111}In-8-HQ suspension was allowed to incubate at 37°C for 60 min.

At the conclusion of the incubation period, 5 ml PPP was added to the labeling suspension. The contents were mixed by swirling and the platelets resedimented by centrifuging for 15 min at 1000 *g*. The radioactive supernatant was aspirated and set aside for subsequent assay. Two additional milliliters of PPP was gently layered over the labeled platelet button, drawn off, and then added to the first radioactive supernatant. The labeled platelets were finally suspended in a known volume of PPP and the incorporated activity was reassayed with a dose calibrator. The amount of activity remaining in the supernatant washes was assayed in similar fashion. A 20-μl sample of the radio-labeled platelet suspension was used both as a standard and to observe the labeled platelet size and shape. The labeled platelets were reinfused into the animal via a peripheral vein, and rinsed in with sterile, isotonic saline. The time required for isolation and labeling was 3 to 4 hr.

Following administration of the labeled platelets, duplicate blood samples were collected in preweighed, evacuated 2-ml blood tubes containing 3 mg EDTA/tube at 0.5 and 4 hr and then daily for 7–8 days. Initially, samples of red blood cell pellet versus PRP and PPP versus platelet button were assayed separately to detect elution or reutilization of the ^{11}In label. It was found that the RBC pellet and PPP fractions contained negligible (less than 4%) radioactivity compared to the PRP or to the platelet button of the same sample (Table 1). In the last four studies only the first 24-hr samples were checked for stability of the ^{111}In-8-HQ label and subsequent measurements of platelet activity were made by assaying 2-ml specimens of whole blood. All samples were counted together with a reference standard in an auto-gamma well scintillation detector (Nuclear Chicago) to a counting error of less than 4%.

RESULTS

The mean platelet count in PRP was $228 \times 10^3/\text{mm}^3$ with a range of 195–392 $\times 10^3/\text{mm}^3$. The mean platelet count of the platelet pellet was 4.6×10^9 (range 2.9–6.6×10^9). When suspended in 10 to 15 ml of PPP, the radioactive product contained an average activity of 100 μCi ^{111}In-8-HQ. Labeling efficiency was 51.8 ± 9.2% (SEM) with a range of 21.7–79.4%. The autologous labeled platelet product was visually examined for clumping using a hemocytometer prior to reinfusion. All of the labeled products were well suspended with no observable aggregation.

The percentage recovery of labeled platelets at 30 min was calculated using the following formula:

$$\% \text{ Recovery} = [BV \times BW \times (\text{cpm/g}) \times G \times 100/(\text{cpm/ml SD}) \times D],$$

where

BV = blood volume = 70 ml/kg [3]
BW = body weight in kg
cpm/g = activity/g of first sample at 0.5 hr
G = specific gravity of blood = 1.06 g/ml [9]
cpm/ml SD = activity of diluted standard
D = volume of dose infused

In determining platelet survival raw counts per milliliter of whole blood were corrected for decay back to time of injection, and the best fit line of circulating counts versus time

TABLE 1

RELATIVE ^{111}In ACTIVITIES IN RBC VERSUS PRP AND PPP VERSUS PLATELET BUTTON IN TWO DOGS

Sample time (days)	RBC (cpm)	PRP (cpm)	Percentage	PPP (cpm)	Platelet button (cpm)	Percentage
			Dog 1			
0.02	151	3687	4.1	119	3313	3.8
0.23	127	3350	3.8	85	2948	2.9
0.63	93	2725	3.4	78	2425	3.2
2.1	56	2648	2.1	87	2357	3.7
2.6	58	1918	3.0	58	1649	3.5
3.9	40	1157	3.5	40	1018	3.9
4.9	13	474	2.8	12	403	3.1
5.6	12	337	3.7	12	297	4.0
7.2	bkgd	122	—	bkgd	106	—
			Dog 2			
0.02	253	7433	3.4	294	7344	4.0
0.29	214	7395	2.9	207	6286	3.3
0.75	268	6706	4.0	186	6657	2.8
1.7	169	5441	3.1	157	4624	3.4
2.7	134	4177	3.2	105	3634	2.9
4.0	68	2834	2.4	99	2551	3.9
4.8	71	2164	3.3	57	1839	3.1
5.8	40	1186	3.2	31	1019	3.0
6.6	16	566	2.9	16	487	3.3
8.0	12	309	3.8	10	269	3.8

was calculated using the least sum of squares linear regression. Platelet survival times ranged from 5.1 to 6.9 days with a mean of 6.3 ± 0.7 (SD) days. The coefficient of determination for the calculated platelet disappearance lines ranged from 0.93 to 0.99 (see Table 2).

Figure 1 is the composite survival curve of percentage circulating platelets versus time for all six dogs. The platelet activity seen at 30 min was designated as 100% of circulating activity and all subsequent activities were compared to that value in each dog.

DISCUSSION

Platelet survival studies using ^{51}Cr label have been reported extensively in both man and rabbit. Estimates of survival time in normal humans range from 8 to 11 days with an average of 9 to 9.6 days [1, 2, 4]. Scheffel *et al.* [23] reported platelet survival times in rabbits of 2.6 to 3.5 days using both ^{51}Cr and ^{111}In (8-HQ); values of 3.5 to 4 days were reported by earlier workers using only ^{51}Cr [8, 18]. Wistow *et al* labeled rabbit platelets with ^{111}In-8-HQ and ^{99m}Tc-oxine and observed platelet survival times of 3.7 and 2.3

TABLE 2

PLATELET SURVIVAL TIME IN SIX DOGS

Dog no.	Survival time (days)	Coefficient of determination of survival decay line (R^2)
10	5.1	0.964
11	6.6	0.965
12	6.5	0.980
13	6.9	0.993
14	6.8	0.993
15	6.0	0.926
$\bar{x}$ ± SD	6.3 ± 0.7	

Note. Survival time determined as x intercept of linear regression of circulating activity versus time.

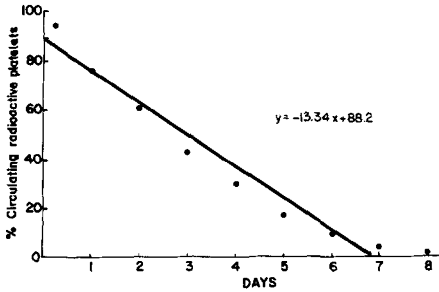

FIG. 1. Mean platelet survival curve for six dogs.

days, respectively [30]. The ^{99m}Tc-oxine complex appeared to offer no advantages over ^{111}In-8-HQ; survival time was shortened and labeling efficiency was one-third that achieved with ^{111}In-8-HQ.

Three platelet survival studies conducted specifically in dogs have been reported in the literature. Cohen and Gardner [6], using ^{51}Cr demonstrated that normal canine platelets had a survival time of 7 to 8 days. Thakur and co-workers [27], in the first reported study using ^{111}In-8-HQ for platelet labeling, observed a survival of approximately 8 days for canine platelets. Wilkinson *et al.* later established a mean platelet survival time in dogs of 7.0 days [29]. The mean 6.3-day survival time of normal canine platelets in the present study is similar to but slightly shorter than that observed by Thakur *et al.* and Wilkinson *et al.*

The ^{111}In-8-HQ platelet labeling procedures described in this report were derived from protocols established in earlier studies. Both Thakur *et al.* [27] and Scheffel *et al.* [23] demonstrated a highly stable platelet—^{111}In-oxine label. The latter investigator noted, using a rabbit model, that ^{111}In contamination in plasma and red blood cell components was minimal (less than 4%) throughout the survival study. Thakur *et al.* likewise reported in canine platelet studies that free ^{111}In in plasma and cell washings was always less than 4.5% of the injected dose. In the present study, duplicate blood samples were drawn and partitioning of the ^{111}In label to blood components other than platelets was monitored in all samples of the first two animals studied. Elution of the ^{111}In label was satisfactorily low (less than 4%) in all cases. In subsequent studies, elution and transfer of ^{111}In label was appraised only in blood samples drawn during the first 24 hr following infusion of labeled platelets. Nonplatelet-bound ^{111}In remained negligible in all these samples.

Labeling of canine platelets with ^{111}In-8-HQ was initially attempted in pH-adjusted isotonic saline, as advocated by Thakur and colleagues [23]. Plasma transferrin competes strongly for the ^{111}In-8-HQ label [16]; as a consequence, platelet labeling efficiencies are markedly reduced in plasma. In 10 studies performed prior to the 6 reported here, washed canine platelets were successfully labeled in isotonic saline with labeling yields of greater than 80%. Survival studies, however, demonstrated platelet half-lives of less than 20 hr in all cases and total life spans of less than 4 days. These data confirmed previous observations by Scheffel *et al.* [22, 23], and further suggested that despite high labeling yields, canine platelets, when labeled in isotonic saline, are either damaged in the washing process or deprived of plasma factors essential to stability and for survival [22]. Despite greater ^{111}In platelet labeling efficiency in saline, preparation in plasma is recommended to maintain viability. This is essential for thrombokinetic studies and appears to be important for clot localization studies as well [7].

Chromium-51 radiochromate (^{51}Cr) has been the traditional label for thrombokinetic studies [1, 2, 5, 21]. Radiochromate preparations containing the stable, nonradioactive carrier chromium are disadvantaged by low labeling efficiency and require withdrawal of 200 to 300 ml of blood for labeling purposes. Alternative preparations such as ^{99m}Tc-oxine require ionic tin for labeling [28, 30], and may predispose to platelet damage from toxic ions [14]. Furthermore, the low γ-emission characteristics of ^{51}Cr (less than 10%) make *in vivo* imaging all but impossible.

^{111}In-8-HQ offers several advantages for

platelet labeling. The physical half-life of ^{111}In (2.8 days) is more comparable to platelet survival time (6 to 8 days) than either ^{51}Cr ($T_{1/2}$ 28 days) or ^{99m}Tc ($T_{1/2}$ 6 hr). This factor alone makes ^{111}In-8-HQ biologically attractive for platelet kinetic studies in animals and man. In addition, ^{111}In has γ-photon energies of 173 (84%) and 245 (96%) keV with an abundance of nearly 200%, that makes it ideal for imaging purposes. Recent studies in dogs have shown that ^{111}In-labeled platelets are actively deposited or incorporated into *early* (less than 24 hr old) thrombi and emboli [7, 15, 17], and preliminary results with scintiphotoimaging using ^{111}In-radiolabeled platelets have been encouraging. This modality may provide a useful, noninvasive method of direct clot visualization in patients with *acute* pulmonary emboli and extremity venous thrombi.

SUMMARY

1. A method for *in vitro* labeling of canine platelets with ^{111}In-8-HQ is outlined.

2. In six dogs, platelet survival was found to be 6.3 ± 0.7 (SD) days.

3. The method and the advantages of the ^{111}In (8-HQ) label are discussed, including the necessity of labeling in plasma to attain adequate platelet survival *in vivo*.

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