Indium-111 Platelet Kinetics in Normal Human Subjects: Tropolone Versus Oxine Methods

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The effect of labeling media on the kinetics of [^{111}In]platelets was evaluated by performing a paired crossover study in eight normal human subjects using tropolone and oxine methods. Platelets were labeled in autologous plasma with [^{111}In]tropolone (In-tr) and in ACD-saline with [^{111}In]oxine (In-ox) and reinjected. Starting at 1 hr, ten blood samples were obtained over an 8-day period. The in vivo platelet recovery was higher at 1 hr and throughout the 8 days of study with In-tr and the gamma camera images showed less uptake in liver and spleen than with In-ox. When platelet life-span (PLS) was estimated using all ten samples, only linear regression showed that the platelet life-span was longer with In-tr (10.7 ± 1.5) than with In-ox (9.5 ± 0.8). When the PLS was estimated excluding the 1-hr sample point, the life-span of platelets was significantly longer with In-tr than with In-ox based on three out of four models of curve fitting. These results demonstrate that platelets labeled with In-tr in plasma are preserved better in circulation and have equal or longer life-span than platelets labeled with In-ox in ACD-saline.

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Platelets, preformed in bone marrow megakaryocytes, are released into circulation with a finite "life span" or "survival time" (*1,2*). Measurement of platelet life-span is a sensitive and specific procedure for the understanding of platelet activation and consumption in thromboembolic and atherosclerotic disease and for the assessment of therapeutic effects of platelet-inhibitor drugs (*3*).

Platelet life-span (PLS) can be estimated by measuring the rate of disappearance of radiolabeled platelets from the circulation. Most of the literature on platelet life-span in normal and patient populations is based on ^{51}Cr platelets (*1,4*), a technique first introduced in 1955 by Morgan et al. (*5*). Chromium-51 sodium chromate labels platelets in plasma, but poor labeling efficiency leads to a requirement for 425 ml blood to provide sufficient platelets. Chromium-51 also has disadvantageous physical properties of a long half-life (27.7 days) and poor photon abundance (10% of 320 keV), precluding external imaging. These disadvantages were overcome with the introduction of indium-[^{111}In]oxine in 1976 by Thakur et al. (*6*). Indium-111 has a relatively short half-life (2.8 days) and emits two gamma photons (172 and 247 keV) which permit quantitative imaging of in vivo distribution of labeled platelets. Indium-111 In-oxine, however, is not platelet specific. Isolated platelets, therefore, are suspended in nonplasma media for specific labeling. In order to achieve high labeling efficiency and to preserve in vitro function of labeled platelets a number of investigators have tried different buffer systems (*7*). Although the life-span of [^{111}In] oxine-labeled platelets was comparable to ^{51}Cr-platelets in both animals (*6,8*) and humans (*9–11*), Mathias and Welch have concluded (*7*) that labeling platelets in plasma is necessary if platelet viability is of primary importance.

In 1981, Dewanjee et al. (*12,13*) introduced [^{111}In] tropolone, a water soluble radiochemical which labels platelets in plasma with higher affinity than [^{111}In] oxine. Since platelets are better preserved in plasma

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than in artificial media (*14*), we performed a paired, crossover study in normal human volunteers and compared the kinetics of ^{111}In platelets labeled with tropolone.in plasma to those labeled with oxine in ACD-saline.

METHODS AND PROCEDURES

Subjects

Informed consent was obtained from eight normal human volunteers, six men and two women, ranging in age from 23 to 39 yr. The subjects were instructed not to take aspirin or any other drug containing aspirin 2 wk before and during the 8-day period of platelet study. The platelet life-span was measured in the same subject using both tropolone and oxine methods. The interval between the two studies was 79 ± 25 days. Six out of eight subjects had the tropolone study first. At the time of both studies, subjects had a normal platelet count.

Radiochemicals

1. Indium-111 tropolone (250 μCi/50 μg tropolone) in 0.5 ml was prepared according to the method of Dewanjee et al. (*12*) using [^{111}In]chloride* and tropolone.†

2. Indium-111 oxine (1 mCi/50 μg oxine) in 0.5 ml was obtained and decayed for 5 days to achieve the same specific activity as [^{111}In]tropolone. At the time of platelet labeling, 250 μCi ^{111}In activity remained in the vial.

Preparation of ^{111}In-Platelets

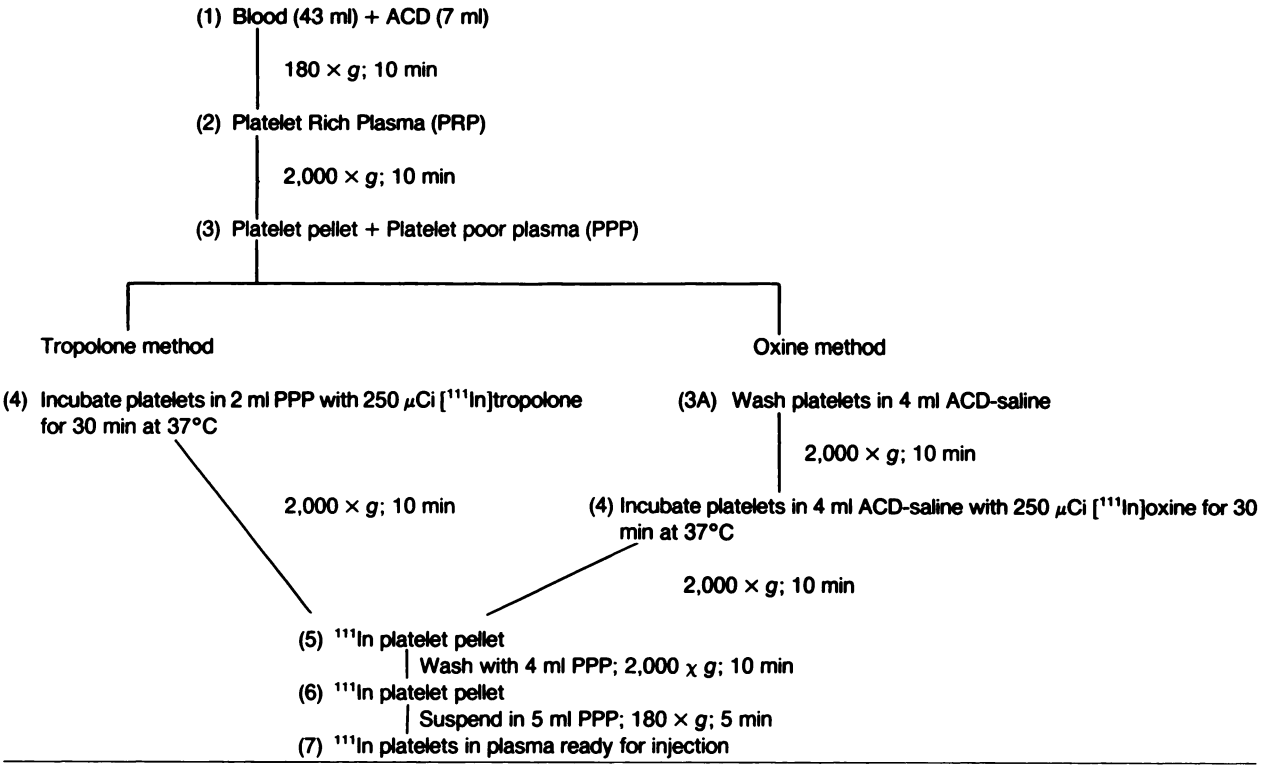
Whole blood was collected from normal human volunteers using a two syringe technique (*10*). Eighteen milliliters of blood were drawn into a first syringe containing 2 ml of 3.8% sodium citrate solution. The platelet rich plasma from this blood sample was used for in vitro platelet aggregation studies. Forty-three milliliters of blood were drawn into a second syringe containing 7 ml modified ACD solution. Platelets were separated from the blood using differential centrifugation and labeled with ^{111}In as shown in Table 1.

In the tropolone method, platelets were suspended in 2 ml autologous PPP and incubated with 250 μCi [In]tropolone (50 μg) at 37°C for 30 min. In the oxine method, platelets were washed first with ACD-saline buffer at a pH of 6.5 and incubated with 250 μCi [^{111}In] oxine in the same buffer (*10*). At the end of the incubation period, the unbound ^{111}In was removed by centrifugation. The ^{111}In-platelets were resuspended in PPP after washing once with PPP. The [^{111}In]PRP was gently spun to remove platelet aggregates before injecting into the donor.

Platelet Aggregation Studies in Vitro

Platelet aggregation studies were performed in the standard manner (*15*) using a Payton model 800B aggregometer. We studied the effects of two aggregating

TABLE 1
Procedure for Labeling Platelets



agents, ADP (10 μg/ml) and collagen (170 μg/ml) upon unlabeled and labeled PRP from the same individual. The percent changes in light transmission were determined and a qualitative assessment of aggregation tracings was also made in order to compare the in vitro function of tropolone and oxine labeled platelets.

Platelet Kinetic Studies in Vivo

One hundred to one hundred fifty microcuries of ^{111}In-platelets ($2–3 \times 10^9$) in 3–4 ml autologous plasma were injected intravenously into the donor. The syringe containing the labeled platelets was weighed before and after the injection to determine the net weight of the injected dose. An aliquot (50 μl) of [^{111}In]PRP was weighed separately into a tube (standard). Following injection of ^{111}In platelets, ten blood samples were obtained over an 8-day period—at 1 hr, twice daily for next 2 days and once a day for the remaining 5 days. EDTA was used as an anticoagulant. Two milliliters aliquots of the whole blood were pipetted into tubes. The ^{111}In activity in all samples and the standard were counted in a gamma counter. The in vivo kinetics of ^{111}In-platelets (tropolone and oxine) were compared by evaluating the results of the following four measurements.

1. Imaging studies. Anterior and posterior images of the lower chest and upper abdomen were obtained 30 min postinjection of ^{111}In-platelets, using a gamma camera (GE-400) fitted with a medium-energy collimator. Both 173 and 247 keV energy peaks of ^{111}In were imaged with a 15% window. The images were recorded by an imaging computer in a 128 × 128 matrix. Regions of interest were drawn manually around the spleen, liver, and heart and total counts in each organ were obtained. The geometric means of ^{111}In activity in the posterior and anterior views were calculated for the spleen, liver, and heart. The results were expressed as organ ratios.

2. In vivo recovery. The percent of injected dose (^{111}In-platelets) remaining in the circulation at 1 hr and at all times of blood sampling was calculated using the donor's estimated blood volume based upon height and weight (*16*).

$$\% \text{ Recovery} = \frac{^{111}\text{In (cpm/ml blood)} \times \text{blood volume (ml)} \times 100}{^{111}\text{In-PRP standard (cpm)} \times \text{dilution factor}}.$$

The recovery of labeled platelets at time zero postinjection was also calculated. The amount of ^{111}In activity in circulation at time zero was estimated by extrapolating the time-activity curves.

3. Platelet life-span. The plot of ^{111}In activity in the blood samples as a function of time was fitted with a linear regression, exponential function, weighted mean, and multihit model analysis (*17*) as recommended by the International Committee for Standardization in Hematology (ICSH) (*18*). Using these methods, the mean platelet life-span was estimated by calculating the rate of platelet disappearance from circulation using (a) all ten samples obtained over an 8-day period and (b) nine samples obtained over an 8-day period excluding the first sample at 1 hr.

4. Statistical analysis. Platelet survival times were compared between the tropolone and oxine methods, for each of the four methods of estimation of survival time using two-way analysis of variance (ANOVA) without replication (*19*). The number of hits calculated in the multihit model and organ count ratios were compared using Wilcoxon's signed pair nonparametric test because these variables do not distribute normally (*19*). The labeling efficiency, and in vivo recovery for the tropolone and oxine methods were also compared using two-way analysis of variance without replication.

TABLE 2
Labeling Platelets with ^{111}In: Tropolone Versus Oxine

Item	Tropolone Mean ± s.d.	Oxine Mean ± s.d.
Labeling efficiency (%)	77 ± 5	73 ± 8
Aggregation*: ADP	56 ± 42	48 ± 30
Collagen	64 ± 29	68 ± 30

* % Change in light transmission compared with control.

RESULTS

Labeling Efficiency and In Vitro Function

The labeling data and the results of in vitro aggregation studies are summarized in Table 2. The labeling efficiency of [^{111}In]tropolone was 76.6 ± 5.3%; [^{111}In]oxine was 72.6 ± 8.1%. These results are not significantly different.

There was no significant difference in the aggregation of tropolone- and oxine-labeled platelets as determined by percent changes in light transmission (Table 2). Furthermore, the aggregation tracings of both groups of labeled platelets were qualitatively indistinguishable.

In Vivo Recovery

The recovery of ^{111}In platelets is shown in Table 3. In general, mean platelet recovery is higher with trop-

TABLE 3
In Vivo Recovery of ^{111}In Platelets

Model	[^{111}In]tropolone Mean ± s.d.	[^{111}In]oxine Mean ± s.d.	p
Linear	66 ± 17	57 ± 11	N.S.
Exponential	69 ± 17	61 ± 11	N.S.
Multihit	67 ± 17	56 ± 10	N.S.
Based on 1 hr blood sample	65 ± 16	51 ± 10	<0.05

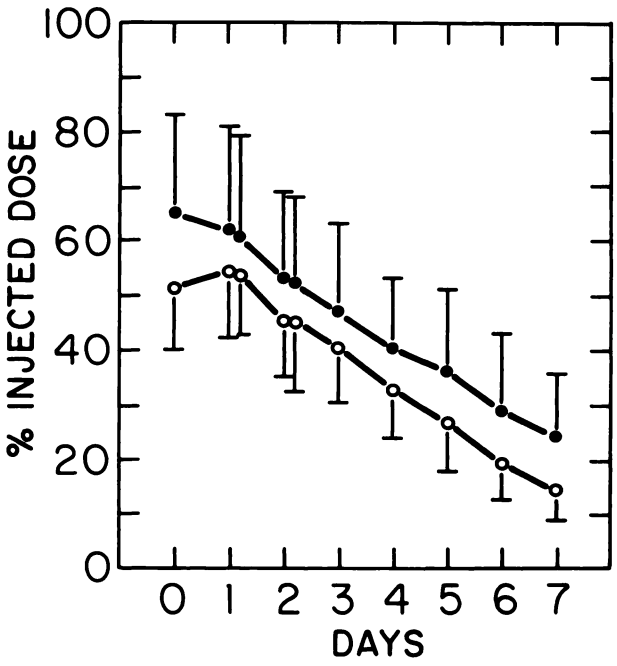


FIGURE 1
Percent injected dose (in vivo recovery) of ^{111}In-platelets as function of time. (●) Tropolone; (○) Oxine

olone than with oxine by all methods of analysis. At 1 hr postinjection, the fraction of [^{111}In]tropolone platelets (65 ± 16%) remaining in the circulation is significantly higher ($p < 0.05$) than [^{111}In]oxine platelets (51 ± 10%).In both tropolone and oxine methods, the amount of free ^{111}In in the 1-hr blood sample is <3% of the injected dose. The mean recoveries estimated based on the amount of ^{111}In activity at zero time were also higher with the tropolone than with the oxine method but not significantly different by statistical analysis.

The recovery of platelets (% injected dose) at each sampling time is shown in Fig. 1. The fraction of ^{111}In-platelets remaining in the circulation is higher during the entire 8-day period with tropolone than with oxine. The recovery of [^{111}In]oxine platelets is slightly higher

TABLE 4
Relative Distribution of ^{111}In-Platelets in Heart, Liver, and Spleen

	[^{111}In]tropolone Mean ± s.d.	[^{111}In]oxine Mean ± s.d.	p
Injected dose (μCi)	128 ± 35	108 ± 27	
cpm/(μCi) ^{111}In			
Heart	15.4 ± 4.3	15.0 ± 5.5	
Liver	20.4 ± 8.6	34.0 ± 12.4	
Spleen	73.4 ± 35.4	76.4 ± 24.2	
Organ ratios			
Liver/heart	1.3 ± 0.3	2.6 ± 1.3	<0.05
Spleen/heart	4.8 ± 1.5	5.5 ± 1.8	N.S.
Spleen/Liver	3.8 ± 0.9	2.6 ± 1.4	<0.05

TABLE 5
Indium-111-Platelet Mean Life-Span* in Normal Human Subjects: Tropolone Versus Oxine

Model	[^{111}In]tropolone Mean ± s.d. (days)	[^{111}In]oxine Mean ± s.d. (days)	p
Linear	10.7 ± 1.5	9.5 ± 0.8	<0.05
Exponential	7.3 ± 1.5	6.3 ± 0.9	<0.10
Weighted mean	9.2 ± 1.4	8.4 ± 0.7	<0.10
Multihit	9.3 ± 1.4	9.3 ± 1.2	N.S.

*Based on ten-point analysis including 1-hr sample.

at 24-hr postinjection than at 1 hr while this difference was not observed with tropolone method.

Organ Uptake

The relative uptake of ^{111}In-platelets in the liver, heart and spleen 30 min postinjection was expressed as organ ratios (Table 4). The mean liver/heart ratio was significantly higher with oxine than with tropolone. By contrast, the mean spleen/liver ratio was significantly higher with tropolone than with oxine. These results indicate that there was greater liver uptake of ^{111}In-platelets with oxine than with tropolone.

Mean Platelet Life-Span

The PLS estimated using various mathematic models is shown in Tables 5 and 6. When all ten samples (Table 5) were used to fit the curves a linear regression analysis showed that the life-span of ^{111}In-platelets with tropolone (10.7 ± 1.5 days) is significantly higher ($p < 0.05$) than with oxine (9.5 ± 0.8 days). The life-span estimated by the exponential and weighted mean methods also tended to be longer with tropolone. The multihit analysis showed no difference in PLS between tropolone and oxine methods. Paired analysis, however, showed that the number of hits (Table 7) is greater with oxine (35 ± 23) than with tropolone (15 ± 22).

The analysis of platelet recovery data (Fig. 1) showed a lower recovery of ^{111}In platelets labeled with oxine at 1 hr postinjection than at 24 hr. In order to determine the effect of this systematic error on platelet survival kinetics, the 1-hr sampling point was excluded from the curve fitting. The PLS using nine samples (Table 6) showed significant differences between the tropolone

TABLE 6
Indium-111-Platelet Mean Life-Span* in Normal Human Subjects: Tropolone Versus Oxine

Model	[^{111}In]tropolone Mean ± s.d. (days)	[^{111}In]oxine Mean ± s.d. (days)	p
Linear	10.7 ± 1.5	9.1 ± 0.7	<0.05
Exponential	6.9 ± 1.5	5.3 ± 0.7	<0.02
Weighted mean	9.1 ± 1.0	7.6 ± 0.8	<0.02
Multihit	8.8 ± 1.2	7.4 ± 1.4	<0.10

*Based on nine-point analysis excluding 1-hr sample.

TABLE 7
Indium-111-Platelet Life-Span: Evaluation of Mathematic Models

	Best fit (n = 8)			Multihit no. of hits
	Points	Linear	Exponential	
[^{111}In]tropolone	10	5	3	15 ± 22
	9	5	3	8 ± 14
[^{111}In]oxine	10	8	0	35 ± 23
	9	4	4	10 ± 14

and oxine methods based on the first three models of curve fitting. Multihit analysis showed nearly significant difference in the PLS between the tropolone and oxine methods.

When comparing the goodness of fit between the linear and exponential models (Table 7) as shown by the sum of squares, linear regression was a better fit in all eight subjects studied with oxine compared with five out of eight in case of tropolone. By excluding the 1-hr sample point, linear regression was the better fit only in four out of eight studies with oxine, compared with five out of eight with tropolone. These findings are compatible with the number of hits in multihit analysis.

DISCUSSION

There are two important aspects to platelet labeling with ^{111}In; one is to achieve high labeling efficiency and the other is to preserve both in vitro and in vivo platelet function. The extent to which labeling media (plasma versus ACD saline) affect the in vivo kinetics of ^{111}In-platelets has not been investigated. Therefore, the in vivo kinetics of ^{111}In-platelets labeled in autologous plasma with tropolone and in ACD saline with oxine were compared in the same human subjects (n = 8) allowing paired analysis.

Our results clearly show that platelets were labeled efficiently (>70%) in 2 ml plasma with [^{111}In]tropolone. Similar labeling efficiencies were obtained with [^{111}In] oxine in ACD saline buffer. The labeling efficiency with 5 days old [^{111}In]oxine in our studies is comparable to the published data (*7*). There were no significant differences noted in the aggregation response of the platelets labeled by tropolone or oxine methods. Because of the great diversity of approaches in quantitating aggregation (*20*), we also examined the two groups of tracings qualitatively and found them to be indistinguishable.

The percent of injected dose (labeled platelets) remaining in the circulation (recovery) is an indirect measure of platelet activation during the in vitro radiolabeling procedure. Indium-111-platelets labeled with tropolone in plasma tended to have higher recovery than platelets labeled with oxine suggesting that platelets were better preserved in plasma during radiolabeling procedure. There are no significant differences in the free ^{111}In activity in plasma between tropolone and oxine methods. We have unpublished data which shows similar fractions of free ^{111}In activity in the plasma regardless of labeling method. However, the amount of free ^{111}In activity in plasma depends upon duration of centrifugation, rpm, and the interval between sampling time and centrifugation.

An important finding in our results (Fig. 1) is that the recovery of [^{111}In]oxine platelets was slightly greater at 24 hr than at 1 hr. This suggests that oxine labeled platelets were temporarily sequestered by the spleen and liver to a greater extent than tropolone labeled platelets upon injection. This observation is also supported by the gamma camera imaging studies performed 30 min postinjection which suggest that the liver uptake of ^{111}In-platelets was higher with the oxine than with the tropolone method (Table 4).

Another important aspect of platelet kinetics is the life-span and some kind of empirical survival model is therefore needed to estimate PLS. Platelet disappearance patterns for a total population label are neither strictly linear nor exponential and frequently vary from person to person. The multihit model encompasses both linear and exponential models in the sense that if the number of hits is only one, the curve is a simple exponential; as the number of hits increases, the curve becomes linear (*16,4*).

When the platelet life-span was estimated using all ten sample points (Table 5), only linear regression clearly showed that platelets labeled in plasma with tropolone have a significantly longer life-span than oxine labeled platelets. Multihit analysis, however, showed no difference in PLS (9.3 ± 1.3 days) between tropolone and oxine methods. Evaluation of the different mathematic models suggests that with oxine, a linear regression provided a better fit (8/8) as opposed to an exponential model and the number of hits in a multihit model was also larger (and hence more linear) than with tropolone.

Since the blood samples obtained within the first few hours may not represent plasma platelet steady state, we also estimated the PLS based on the nine samples obtained 24 hr after injection. The exponential and multihit models turned out to be particularly more sensitive to the first point. Omitting the 1 hr sample significantly reduced the estimated life-span of [^{111}In] oxine labeled platelets. The difference in the mean PLS (multihit model) with and without the first point was 1.9 days with oxine compared to only 0.4 days with tropolone. The results (Table 6) clearly show that the life-span of ^{111}In-platelets is significantly longer when labeled with tropolone than with oxine based on linear, exponential and weighted mean models and nearly significant with multihit analysis. On the other hand, the average number of hits derived from the nine-point multihit analysis (Table 7) became similar for both tropolone and oxine methods. None of the standard

methods of estimating PLS are designed to handle systematic deviation of the samples as was observed with the first sample point. We, therefore, feel that the results based on the samples obtained 24 hr after administration of labeled platelets are more accurate.

The data on recovery and imaging studies suggest that platelet function is better preserved when labeled with [^{111}In]tropolone in plasma. In addition, the PLS estimated based on the blood samples obtained 24 hr after injection also suggests that labeling platelets in plasma tend to prolong the surival time of ^{111}In-platelets. In conclusion, platelets can be labeled efficiently in plasma with [^{111}In]tropolone and the technique is less time consuming and does not require nonplasma buffer systems. The ^{111}In-platelets labeled with tropolone method appear to be preserved better in circulation and have an equal or longer life-span compared with ^{111}In-platelets labeled with oxine in nonplasma media.

FOOTNOTES

* Medi-Physics, Inc., Richmond, CA.
† Sigma Chemical Corporation, St. Louis, MO.

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