
Comparison of Oxine and Tropolone Methods for Labeling Human Platelets with Indium-111

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The effect of the chelates oxine and tropolone, used to label platelets, on the kinetics of indium-111- (^{111}In) labeled platelets was studied in twelve normal human subjects. Autologous platelets were labeled either in saline with ^{111}In -oxine or in plasma with ^{111}In -tropolone. Mean platelet lifespan was estimated by fitting the disappearance curve of platelets from the circulation to the multiple hit and other mathematical models. The in vivo distribution of platelets was quantitatively imaged with a scintillation camera. The in vivo recovery of ^{111}In -oxine and ^{111}In -tropolone did not differ, and the mean platelet lifespan was also similar (^{111}In -oxine: 230 ± 29 hr; ^{111}In -tropolone: 226 ± 13 hr). At equilibrium (90 min after reinjection of labeled platelets) and at the end of platelet lifespan, ^{111}In -oxine and ^{111}In -tropolone radioactivities in the spleen and liver were similar. These results demonstrate that the results of kinetics measured with ^{111}In -oxine or ^{111}In -tropolone do not differ significantly.

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Indium-111 (^{111}In) is the radionuclide recommended by the International Committee for Standardization in Hematology as the platelet label of choice (1). Although other ^{111}In -chelates have been advocated (2,3), this Committee recommends oxine and tropolone as the most suitable for platelet studies. It is, however, not clear from the results of several studies whether one of these is superior (2-12).

Theoretically, oxine is not an ideal chelate. First, some of the commercially available ^{111}In -oxine complexes contain alcohol, which may affect platelet function (5). Second, the labeling efficiency of ^{111}In -oxine is considerably higher in a medium with no plasma, than in plasma (5-8); this is unfavorable because labeling and handling platelets in an artificial medium may induce in vitro damage to platelets (9,10). These negative aspects may be overcome with ^{111}In -tropolone, a

chelate which permits labeling of platelets with relatively high efficiency in a plasma medium (4).

There is a prevailing view (2-4) that tropolone is superior to oxine because with the former, platelets are labeled in plasma and not in saline. This is considered the reason why the kinetics of ^{111}In -oxine and ^{111}In -tropolone labeled platelets have been reported to differ (2, 3,10). Thus, it has been stated that ^{111}In -tropolone labeled platelets do not accumulate transiently in the liver immediately after their reinjection (2,3). Also, the recovery fraction of ^{111}In -oxine labeled platelets in the circulation was reported to be higher than those labeled with ^{111}In -tropolone (10).

However, this view has not been substantiated by convincing scientific data. Indeed, the results of the few well-documented studies have not clarified the issue. For instance, Vallabhajosula et al. (11) conclude that platelets labeled with ^{111}In -tropolone in plasma are preserved better in the circulation and have an equal or longer lifespan than platelets labeled with ^{111}In -oxine in ACD-saline. Furthermore, there was evidence that the ^{111}In -oxine labeled platelets were temporarily sequestered in the liver to a greater extent than those labeled in tropolone. Hill-Zobel et al. (12) expressed a similar view on the basis of results in a rabbit model but were not sure that these results can be extrapolated to human conditions. In contrast, in a preliminary study, we found no evidence that the results of our method of labeling platelets with ^{111}In -oxine differs from those with tropolone (13).

It is important to establish whether there is a difference between these two chelates. Indium-111-oxine is widely used and marketed commercially as an approved platelet label. If results with oxine do indeed differ substantially from those of tropolone, it may, therefore, have important economic implications. Furthermore, the recommendation that either tropolone or oxine may be used (1) will also need to be revised if the commendable goals of standardization are to be attained.

We addressed this question by comparing, in normal human subjects, the labeling efficiency and kinetics of platelets labeled with ^{111}In -tropolone in plasma with those of platelets labeled with ^{111}In -oxine in saline. The

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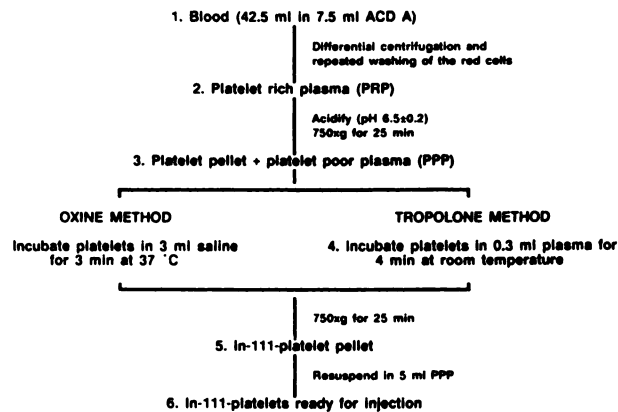


FIGURE 1
Methodology for labeling platelets with ^{111}In -oxine and ^{111}In -tropolone.

techniques that we used are appropriate for such a comparative study: they have been validated and provide quantitative data on the in vivo distribution of the labeled platelets.

MATERIALS AND METHODS

Subjects

Twelve normal human subjects (6 males) ranging in age from 18 to 35 yr, volunteered for a study approved by the Ethical Committee of the Provincial Administration and University of the Orange Free State. The blood platelet counts of the subjects were normal (range 200/l to $304 \times 10^9/\text{l}$). The subjects were instructed not to take any drugs, known to affect platelets, for at least 2 wk before and during the 7-day study period.

The volunteers were randomly divided into two groups of six. Autologous platelets were labeled either with ^{111}In -oxine (Amersham International plc, UK) or ^{111}In -tropolone (^{111}In -chloride supplied by Amersham International plc, UK, and tropolone by Sigma Chemical Corp., St. Louis, MO).

Platelet Labeling with ^{111}In

After discarding the first 2 ml, 42.5 ml blood were drawn from the antecubital vein with a 19-g Abbot "butterfly" needle directly into a polystyrene syringe containing 7.5 ml ACD A (Baxter Travenol, Baxter Laboratories). The blood was transferred to 2×50 ml polystyrene tubes (Falcon 2070, Falcon Labware, Oxnard, CA) and platelets were harvested from the blood by differential centrifugation and repeated washing of the red cells (7,8,14). The mean recovery of platelets from the blood was $95\% \pm 3\%$.

The platelets were labeled as outlined in Figure 1. In the

oxine method (8), the platelet pellet was gently resuspended in 3 ml saline and incubated with the radionuclide. In the tropolone method (15), the labeling medium was 0.3 ml plasma. Tropolone (40 nM final concentration), followed by ~ 17 MBq ^{111}In -chloride, were added. In both methods, unbound radionuclide was removed by withdrawing the supernatant from the centrifuged labeled platelets. The latter were resuspended in platelet poor plasma for reinjection.

Labeled platelets were considered viable if aggregates consisting of 20 or more platelets were induced by ADP (7). Contamination of the labeled platelets with red cells and plasma proteins, the labeling efficiency, and the radioactivity of the injected labeled platelets, were measured (16).

In Vivo Platelet Kinetic Studies

Mean Platelet Lifespan (MPLS). MPLS was estimated from the rate of disappearance of the labeled platelets from the circulation. The disappearance curves were constructed from the radioactivity in blood samples collected at 5 and 90 min after reinjection of the labeled platelets, and then daily for 6 days. The count rate of the blood samples was measured in a well-type scintillation counter. MPLS was estimated by fitting several mathematical models to the blood clearance curves (17).

The recovery of labeled platelets in the circulation at equilibrium was calculated from the radioactivity/ml injected, an estimate of the blood volume (18), and extrapolation of the survival curve back to zero time (8,14).

Blood platelets were counted with an electronic particle counter (19). Mean platelet turnover was calculated from the MPLS and the platelet count was corrected for the splenic platelet pool (20). The size of the latter was determined with quantitative imaging.

In Vivo Quantification of the Distribution of Labeled Platelets. The methods have been described in detail elsewhere (8, 21,22). Briefly, the distribution of platelets was imaged with a large field of view scintillation camera and the scintigrams were analyzed on an A² MDS data processing system. Whole-body and region of interest (ROI) ^{111}In radioactivity was corrected for geometry with the geometric mean method. The ROI activity was expressed as a percentage of whole-body activity. The radioactivity not in the liver or the spleen, is referred to as the "remainder." At the end of platelet lifespan, most of this radioactivity is located in the bone marrow (8, 21). The initial (equilibrium) radioactivity was derived by back extrapolation of linear least-square regression analysis of data, and the final organ radioactivity was determined at the end of the MPLS.

Statistical Methods

Data were assessed for normal distribution with the Shapiro-Wilks test. Unpaired data were evaluated with the t-test

TABLE 1
Platelet Labeling Data

	Oxine	Tropolone	t value	p value	95% confidence interval
Labeling efficiency (%)	92 ± 2	79 ± 10	3.1225	0.03	4-22
% Contamination with:					
red cells	1.2 ± 0.4	1.8 ± 2.5	-0.5805	0.59	-1.7-2.9
plasma proteins	2.2 ± 0.5	1.7 ± 1.1	0.0136	0.99	-1.4-2.4

TABLE 2
Survival Data of Platelets Labeled with ^{111}In -oxine and ^{111}In -tropolone

	Oxine	Tropolone	t value	p value	95% confidence interval
Recovery (%)	70 ± 10	58 ± 11	1.9772	0.11	-1-25
Platelet turnover ($\times 10^9/\text{hr}$)	1.41 ± 0.32	2.00 ± 0.49	2.4694	0.06	-0.026-1.20
"Hits"	44 ± 30	20 ± 20	1.6305	0.16	-8-56
Mean Platelet Lifespan (hr):					
Linear	242 ± 23	228 ± 13	1.2980	0.25	-14-42
Weighted mean	225 ± 33	221 ± 11	0.2817	0.79	-33-41
Multiple hit	230 ± 29	226 ± 13	0.3083	0.77	-24-32
Dornhorst	219 ± 25	222 ± 10	0.2729	0.80	-31-25
Alpha order	224 ± 37	221 ± 10	0.1917	0.94	-37-43

for different means, or the non-parametric Wilcoxon rank sum test where appropriate. The difference between sample means was compared by determining the 95% confidence intervals (23).

RESULTS

Platelet Labeling

The mean blood platelet count of the two groups did not differ significantly (oxine $249 \pm 39 \times 10^9/\text{l}$ versus tropolone $282 \pm 45 \times 10^9/\text{l}$; $p = 0.23$). The results are summarized in Table 1. Labeling efficiency with oxine was significantly ($p = 0.03$) higher than with tropolone. Contamination of the labeled platelet product with red cells and plasma proteins was slight and similar with both methods. There was less than 1 leukocyte per 1,000 platelets present in the ^{111}In -labeled platelet suspensions that were reinjected.

In Vivo Platelet Kinetics

Recovery of ^{111}In -oxine ($70\% \pm 10\%$) and ^{111}In -tropolone ($58\% \pm 11\%$) labeled platelets in the circulation did not differ significantly (Table 2).

Mean Platelet Lifespan. The mean curves describing the disappearance of the labeled platelets are illustrated

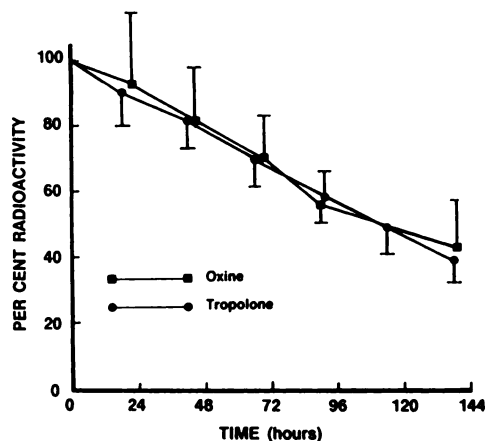


FIGURE 2

The curves demonstrate the disappearance of ^{111}In -oxine and ^{111}In -tropolone labeled platelets from the circulation. The values are expressed as a mean (± 1 s.e.m.) percent of the count rate of the blood sample collected at 5 min.

in Figure 2. The MPLS, calculated with the different mathematical models and number of "hits" of the multiple hit model, did not differ significantly for oxine- and tropolone-labeled platelets (Table 2). The large number of "hits" of the multiple hit model indicate a near linear platelet survival curve with both labels.

In Vivo Distribution and Sites of Sequestration at Equilibrium. The results are summarized in Table 3, and the initial postinjection phase is illustrated in Figure 3.

The time-radioactivity curves, illustrating the redistribution of oxine- and tropolone-labeled platelets in the spleen and the liver for the first 90 min after reinjection of the labeled platelets, are presented in Figure 3. The radioactivity in the spleen increased monoexponentially. The rate constant of the increase of oxine-labeled platelets in the spleen was 0.177 ± 0.041 per minute and that of tropolone-labeled platelets was 0.155 ± 0.041 per minute. This difference was not significant ($t = 0.7675$, $p = 0.46$; 95% confidence interval $-0.052-0.096$).

A transient accumulation of radioactivity in the liver was evident with both the labeling methods (Fig. 3); this represents the "collection injury" (24). The extent of the injury observed with the two labeling methods was compared by measuring the area under the respective time-activity curves. This did not differ significantly ($t = 0.797$, degrees of freedom = 10, $p = 0.44$).

At equilibrium, 90 min after reinjection of the ^{111}In -platelets, the size of the splenic platelet pool and the radioactivity in the liver were similar for the two methods (Table 3).

In Vivo Distribution and Sites of Sequestration at the End of Platelet Lifespan. The results are summarized in Table 3. The contributions of the various components of the reticuloendothelial system to the sequestration of senescent ^{111}In -oxine- and ^{111}In -tropolone-labeled platelets did not differ significantly.

DISCUSSION

The methods to label platelets either with ^{111}In -oxine or ^{111}In -tropolone differ in one important aspect, namely the labeling medium which was used. A high

TABLE 3
In Vivo Distribution and Sites of Sequestration of Platelets Labeled with ^{111}In -oxine and ^{111}In -tropolone*

	Oxine	Tropolone	t value	p value	95% confidence interval
Equilibrium distribution					
Spleen	30.0 ± 6.3	30.1 ± 6.3	0.1442	0.89	-7.1-8.1
Liver	9.6 ± 1.2	9.5 ± 1.4	0.1328	0.90	-1.5-1.7
Distribution at MPLS					
Spleen	37.2 ± 8.8	45.3 ± 6.9	1.7743	0.14	-12.7-3.5
Liver	29.2 ± 9.6	31.3 ± 6.4	-0.4458	0.67	-12.4-8.2

* Values are given as a mean (± 1 s.d.) percent of whole-body radioactivity.

labeling efficiency is achieved with ^{111}In -oxine only if the platelets are suspended in an artificial medium with no plasma (3,8). In contrast, platelets may efficiently be labeled in plasma with ^{111}In -tropolone (4,15). This difference may be important because platelets are apparently better preserved in plasma than in artificial media (3,4,10). It is, therefore, conceivable that the tropolone method may be superior to the oxine method. Indeed, this has been reported (2,3,10), although the

advantage of tropolone is not evident when the mean platelet lifespan is measured (10).

It should, however, be noted that it is difficult to standardize the methods of isolating and labeling platelets. Differences observed with the two principal labeling methods may therefore be related to some aspect of one of the methods used in a particular laboratory. We have in this study compared our method of labeling of platelets with ^{111}In -oxine (8) with the recommended ^{111}In -tropolone method (1,15). We studied the influence of the two labeling methods on some aspects of platelet labeling and on the results of platelet kinetics.

The labeling efficiency was acceptable with both methods and the administered activity was sufficient to estimate the MPLS and perform quantitative imaging. Contamination with other blood cells was low with both methods. The labeled platelets aggregated well in response to ADP ex vivo. The result of our simple method to assess the in vitro aggregation response, was similar to that reported by others (10). The recovery of oxine- and tropolone-labeled platelets in the circulation did not differ. In essence, these results show that with either of the two methods, a viable platelet population can be labeled efficiently.

The measurement of the MPLS is an important clinical and research tool. In normal subjects, platelets disappear from the circulation in an age-dependent manner (20,24). Thus, labeling a random sample of the circulating platelet population, would result in a near-linear platelet survival curve (24). It is evident (Fig. 2, Table 2) that the estimate of the MPLS with ^{111}In -oxine and ^{111}In -tropolone labeling methods, correspond closely. The number of "hits" in the multiple hit model is substantial and the difference between the MPLS calculated by fitting the data to a linear function and the multiple hit model, was not significant. This and the fact that our estimate of the MPLS is at the upper limit of the accepted normal value (2,10,24) indicates that with both methods there is minimal random destruction of the labeled platelets. This shows that the platelets are not adversely affected by either of the labeling procedures.

Vallabhajosula et al. (11) found a slightly longer

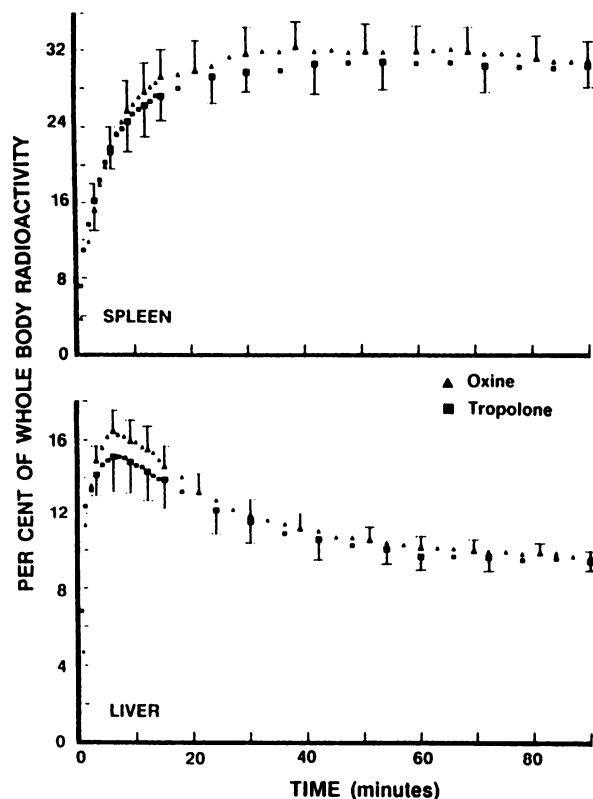


FIGURE 3
The redistribution of the labeled platelets in the spleen and liver in the first 90 min after their reinjection. Values are expressed as a mean ± 1 s.e.m. The accumulation of oxine- and tropolone-labeled platelets in the spleen was monoexponential and was not different. There was a measure of "collection injury" in the liver for both oxine- and tropolone-labeled platelets. The collection injury was not significantly different.

MPLS with the tropolone method than with oxine; however this was only evident with linear regression analysis. Using five mathematical models, we did not find such a difference. This discrepancy may be explained by a difference in methodology. Our method of labeling platelets with ^{111}In -oxine differs in one aspect from that of Vallabhajosula et al. (11). We do not include the extra washing step, which involves sedimenting of the platelets for 10 min at $2000 \times g$. Our method is, therefore, potentially less harmful to the platelets since additional mechanical stress to the platelets is avoided.

The influence of the "collection injury" on platelet kinetics may be assessed by quantifying the extent of the transient accumulation of labeled platelets in the liver immediately after their reinjection (8,14,25,26). It is evident from Figure 3 that both labeling methods slightly activate platelets. The accumulation of the platelets was transient in both instances, and the duration and the extent of the accumulation corresponded. Peters et al. (2,3) have suggested that ^{111}In -tropolone labeled platelets do not accumulate in the liver. However, they did not quantitate the hepatic radioactivity. It is, therefore, difficult to directly compare our and their results. It has been shown that the "collection injury" has no influence on the MPLS or the in vivo redistribution of platelets during their lifespan (25). The finding that the "collection injury" induced by ^{111}In -oxine labeling is reversible is supported by the results of the present study.

Our data demonstrate that the in vitro and in vivo function, MPLS, and the in vivo distribution of platelets labeled with ^{111}In -oxine in saline and ^{111}In -tropolone in plasma do not differ significantly. Our results, therefore, do not support the contention that platelets labeled with ^{111}In -tropolone are better preserved. Evidently, the methods used for the labeling, is critical. Our results indicate that it is acceptable to compare results obtained with the two methods. It would, therefore, seem unwarranted to discard the established and widely accepted method of labeling platelets with ^{111}In -oxine for the tropolone method.

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