Platelet Kinetics in Patients With Bone Marrow Hypoplasia: Evidence for a Fixed Platelet Requirement

By Stephen R. Hanson and Sherrill J. Slichter

We have studied 16 normal subjects and 27 patients with stable, untreated thrombocytopenia secondary to bone marrow failure and platelet counts ranging from 12,000 to 70,000/μL. Autologous platelets were labeled with 51Cr for measurement of mean platelet life span in the normal subjects and in 20 patients. Labeled donor cells were used in the remaining subjects. Platelet survival, as determined with both autologous and homologous platelets, correlated directly with platelet count in the thrombocytopenic patients. Platelet life span was only modestly reduced in patients having counts in the range of 50,000 to 100,000/μL (7.0 ± 1.5 days vs 9.6 ± 0.6; P < .01) but was markedly reduced when the count fell below 50,000/μL (5.1 ± 1.9 days, P < .001). The recovery of donor platelets in severely thrombocytopenic recipients (60% ± 15%) was equivalent to control values (66% ± 8%; P > .2). The recovery of autologous platelets was normal when the platelet count exceeded 50,000/μL (74% ± 15%) but was reduced in patients with lower counts (50% ± 22%; P < .01). All patient and normal data were well correlated by a model predicting a maximum platelet life span of 10½ days and a fixed requirement for 7,100 platelets per microliter of blood per day, or about 18% of the normal rate of platelet turnover, which averaged 41,200 platelets per microliter per day. We conclude that although relatively few platelets are used to support vascular integrity, this requirement is reflected by a reduced platelet life span in marrow hypoplasia and may contribute to the shortening of platelet survival observed in other thrombocytopenias.

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Biased on an observed mean platelet life span of nine to ten days, it is generally accepted that approximately 10% to 15% of all platelets are removed from the circulation daily in normal individuals. However, the mechanisms of platelet removal in normal humans remain largely undefined. The concept that a finite number of platelets may be required to support vascular integrity is suggested by the observations that (1) platelets may interact directly with endothelium and subendothelial tissue components; (2) spontaneous bleeding is a frequent complication of severe thrombocytopenia; (3) platelet survival is generally reduced in thrombocytopenia due to decreased platelet production; and (4) platelet survival may be prolonged after elevation of the platelet count with transfused cells. Conversely, in normal subjects, platelets labeled with 51Cr or 111In exhibit a predominantly linear disappearance pattern, consistent with a process of senescent platelet removal. Direct evidence for platelet senescence is provided by one study in a patient recovering from drug-induced thrombocytopenia and by observations in animals with experimental thrombocytopenia, showing that newly formed platelets exhibit a prolonged disappearance pattern and finite life span in vivo. Thus, while both processes of platelet elimination may be of importance, the relative proportion of platelets lost by senescence or other mechanisms is unknown.

To help resolve this issue we have performed platelet kinetic studies in normal subjects and in patients with moderate and severe thrombocytopenia secondary to decreased platelet production. The data were subsequently analyzed to determine whether the decrease in platelet survival observed in these patients could be explained on the basis of a fixed platelet requirement.

MATERIALS AND METHODS

The control group consisted of 16 healthy volunteers, 12 males and four females, aged 21 to 60 years. The normal subjects received no medications during the study period and had no history of disorders that might alter platelet survival or function. The patient group consisted of 18 males and nine females, aged 11 to 76 years. The diagnosis of bone marrow hypoplasia was based on the presence of thrombocytopenia and reduced numbers of megakaryocytes on qualitative evaluation of bone marrow aspirates. Twenty patients with platelet counts of 17,000 to 70,000/μL were studied using autologous 51Cr-labeled platelets. Platelets were harvested from 500 mL of autologous whole blood and labeled with 51Cr as previously described. Four of these patients had undergone splenectomy and two exhibited splenomegaly on physical examination. None had received prior platelet or red cell transfusions.

In the group of seven patients studied using labeled donor cells, platelet counts averaged 24,000/μL. Donor platelets were extracted from 500 mL of whole blood and labeled according to the same method as for the autologous studies. One subject was asplenic and another exhibited moderate splenomegaly on physical examination. Two additional subjects had received prior transfusions of either platelets or red cell concentrates but showed no evidence of platelet alloimmunization. The patients were clinically stable, were taking no medications, and had no other disorders known to influence platelet survival or function. No patients had significant petechiae or purpura. Specifically excluded from these studies were patients with drug-induced or congenital thrombocytopenia or autoimmune thrombocytopenic purpura. All patients meeting the entry criteria were included in the study.

Platelet counts were performed on peripheral blood collected in EDTA as previously described and were stable during the study period. All study participants signed statements of informed consent using study protocols approved by the Human Subjects Review Committee of the University of Washington School of Medicine.

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Platelet survival was determined by measuring the disappearance of radioactivity from blood samples after injection of the \(^{51}\)Cr-labeled platelets. The initial sample was drawn one hour after the infusion of labeled cells. All blood and plasma samples were counted for radioactivity using a gamma spectrometer (Nuclear Chicago, Chicago). Platelet survival time was analyzed by computer fitting to gamma functions. The proportion of labeled platelets remaining within the systemic circulation after infusion (ie, “recovery”) was calculated from the platelet activity per milliliter, extrapolated to zero time, multiplied by the estimated blood volume (75 mL/kg body wt), and divided by the platelet \(^{51}\)Cr activity injected. Platelet turnover per microliter per day was calculated from the peripheral platelet count divided by the platelet survival time in days and corrected for recovery.

For all studies, platelet survival and platelet count were correlated according to the Mills-Dornhorst model:

\[
\frac{N(t)}{N(0)} = \frac{e^{-kt} - e^{-kT}}{1 - e^{-kT}}. \tag{Equation 1}
\]

In this formulation, the number of labeled cells surviving at time \(t\) after infusion, \(N(t)\), is determined by a fixed platelet life span (\(T\)), which may be reduced, depending on the instantaneous rate of random platelet destruction (\(k_t\)), due to extrinsic mechanisms. Because the initial slope of the platelet survival curve, \(N(t)/N(0)\), intercepts the time axis at the mean platelet life span, \(T\), it follows that:

\[
\tau = \frac{1 - e^{-kt}}{k/P}, \tag{Equation 2}
\]

where \(\tau\) is the mean platelet survival time (days), \(k/P\) is the instantaneous rate of random platelet destruction (percentage per day), \(P\) is the platelet count (platelets per microliter), and \(k\) is the fixed rate of random platelet utilization (platelets per microliter per day). Equation 2 was used to determine the dependence of platelet life span on platelet count under the assumption of a fixed platelet requirement. Platelet survival curves were also curve fitted to Equation 1 so that the disappearance patterns observed in the various patient groups could be averaged (eg, Fig 1).

All statistical analysis and curve fitting were done using the PROPHET system of the Division of Research Resources, National Institutes of Health. Statistical comparisons were made using Student’s \(t\) test (two-tailed), and the data were normally distributed. Remaining results were compared using the Wilcoxon rank sum test. All data in the Results section are given as the mean ± 1 SD.

**RESULTS**

The disappearance pattern of \(^{51}\)Cr-platelet activity was found to depend significantly on the circulating platelet concentration (Fig 1). When autologous platelets were labeled in seven patients having platelet counts ranging from 50,000 to 100,000/μL (mean; 62,000 ± 7,000/μL), a modest shortening in platelet survival was observed (Table 1). The mean platelet life span averaged 7.0 ± 1.5 days and was reduced significantly from the control values (9.6 ± 0.6 days, \(P < .01\)). When the platelet count was below 50,000 platelets/μL, the survival of both autologous platelets (13 patients; mean count; 37,000 ± 9,000/μL) and homologous platelets (six patients; mean count; 19,000 ± 6,000/μL) was further reduced (6.0 ± 1.7 days and 3.4 ± 1.1 days, respectively, \(P < .001\) vs controls). In the one additional patient receiving donor platelets, the platelet count was 52,000/μL and platelet survival averaged 6.8 days.

The recovery in the circulation of donor platelets (60% ± 15%, Table 1) was equivalent to control values (66% ± 8%; \(P > .2\)). The recovery of autologous platelets was reduced only when the platelet count was below 50,000/μL (50% ± 22%, \(P < .01\) vs controls). Excluded from the calculation of platelet recoveries were the values obtained in the five asplenic subjects (99% ± 10%) and in three patients with splenomegaly (36% ± 12%).

The relationship between platelet survival and platelet count for all patients and normal subjects is shown in Fig 2. A good linear correlation was obtained between platelet survival and platelet count in the range from 0 to 100,000 platelets per microliter. The best fit equation was \(r = 2.15 + 8.74 \times 10^{-1} \times P\), where \(P = \) platelets per microliter (\(r = .784\)). The data were also well correlated according to Equation 2 (solid line), which predicted a maximum average platelet life span of 10½ days and a finite rate of random

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Table 1. Platelet Survival in Hypoplasia

<table>
<thead>
<tr>
<th>Study</th>
<th>Platelet Count (Platelets/μL)</th>
<th>Platelet Survival (d)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous (13)</td>
<td>&lt;50,000</td>
<td>6.0 ± 1.7</td>
<td>50 ± 22</td>
</tr>
<tr>
<td>Donor (6)</td>
<td>&lt;50,000</td>
<td>3.4 ± 1.1</td>
<td>60 ± 15</td>
</tr>
<tr>
<td>Total (19)</td>
<td>&lt;50,000</td>
<td>5.1 ± 2.9</td>
<td>54 ± 19</td>
</tr>
<tr>
<td>Autologous (7)</td>
<td>50,000–100,000</td>
<td>7.0 ± 1.5</td>
<td>74 ± 15</td>
</tr>
<tr>
<td>Normals (16)</td>
<td>&gt;200,000</td>
<td>9.6 ± 0.6</td>
<td>66 ± 8</td>
</tr>
</tbody>
</table>

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platelet destruction of 4,700 platelets per microliter per day. Dividing this value by the mean recovery in normal subjects (66%) to correct for splenic pooling yielded an estimate for total random destruction of 7,100 platelets per microliter per day. Because the overall rate of platelet turnover in the normal subjects averaged 41,200 ± 4,900 platelets per microliter per day, it was concluded that less than 20% of all total random destruction of 7,100 platelets per microliter per day. The data are bounded by the region (between dashed lines) constituting all solutions to Equation 2 with $r$ in the range of from 10 to 11 days, and $k$ in the range from 1,400 to 6,800 platelets per microliter per day.

The sensitivity with respect to the data of the derived values for random platelet utilization and maximum platelet life span was also evaluated (Fig 2). Nearly all data points were consistent with a maximum platelet life span in the range from ten to 11 days, and with a rate of random platelet destruction ($k$) constituting only 5% to 25% of the overall rate of platelet removal (1,400 to 6,800 platelets per microliter per day). Also, when the curve-fitting analysis (Fig 2) was repeated excluding all patients with splenectomy, splenomegaly, or prior transfusions, the predicted values for random platelet utilization (4,700 platelets per microliter per day) and finite life span (10½ days) were unchanged. These results suggest that in at least some thrombocytopenic patients with these conditions, shortened platelet survival may be determined primarily by the reduction in platelet count.

In individuals with reduced platelet numbers, the proportion of circulating platelets removed by random vs senescent processes should be increased. This fraction ($f$) can be calculated directly from the equation: $f = 1 - \exp (-kT/P)$ given the values for the parameters $k$ (4,700 platelets per microliter per day) and $T$ (10½ days). The predicted relationship between $f$ and the circulating platelet concentration is shown by the solid line in Fig 3. Values of $f$ for all patients studied were calculated by dividing the fixed platelet requirement ($k$) by the turnover rate of circulating platelets ($P/r$). Thus, random platelet utilization was found to average 17.9% ± 2.9% of the overall platelet turnover in normal individuals. This proportion increased rapidly as the platelet count fell below 100,000/µL, consistent with the reduced platelet survival observed in our thrombocytopenic patients (Fig 3). In the severely thrombocytopenic patients studied using donor cells, random platelet utilization was nearly 100% (84% ± 26%).

**DISCUSSION**

The data demonstrate that thrombocytopenia per se may cause a significant reduction in platelet survival. An excellent correlation was found between platelet count and platelet life span over the entire range of values normally observed in humans, consistent with the concept of a fixed platelet requirement of approximately 7,100 platelets per microliter of blood per day in all individuals. It is significant that in the present study we have not attempted to interpret the platelet survival data from individual patients, ie, we have not defined kinetic parameters (eg, $k$, $T$) by curve fitting platelet survival data from individual patients or patient groups. Rather, we have attempted to correlate all patient and normal data (Fig 2) on the basis of a fixed platelet requirement and maximum platelet life span. Although not definitively proven, these assumptions would appear reasonable as discussed previously. It should be noted that the accurate determination of mean platelet life span from experimental
data requires only an appropriate curve-fitting procedure and does not require assumptions regarding the mechanisms of cell elimination.\textsuperscript{28} Therefore, platelet survival was determined according to the gamma function approach, which has been recommended\textsuperscript{16,25,26}. In previous studies, mathematical modeling of \textsuperscript{51}Cr-platelet survival curves in normal individuals has suggested that up to 70\% of all circulating platelets may be removed through random processes.\textsuperscript{1,31} However, it is now clear that such estimates may be influenced strongly by both the slight curvature of normal platelet disappearance patterns,\textsuperscript{32} and the reported variability in normal platelet life span, eg, seven to ten days.\textsuperscript{1,4,10,16,20,31,32} In addition, platelet survival may be influenced by platelet injury during collection and labeling,\textsuperscript{3,34} nonrandom sequestration of platelets by the spleen,\textsuperscript{4,32,33} the labeling of homologous v autologous cells,\textsuperscript{32} nonrandom cell labeling,\textsuperscript{33,34} and the quality of the data.\textsuperscript{30,33} We believe that these factors may result in a substantial overestimation of random platelet utilization by mathematical modeling of composite platelet disappearance patterns from normal individuals. Although measurements of platelet survival may be useful for documenting increased platelet destruction, in general, we agree with the view of earlier investigators that analysis of the shape of platelet survival curves may not provide insight into the mechanisms normally responsible for the removal of platelets from the circulation.\textsuperscript{30,31,37}

The present observations suggest that in normal individuals the requirement for platelets is modest, ie, only about 18\% of all platelets are destroyed randomly, while the remainder are presumably removed through senescent mechanisms. That few platelets are indeed required to support hemostasis and endothelial integrity is also suggested by the results reported here and by others that platelet recovery is normal in transfused thrombocytopenic recipients.\textsuperscript{17,34-40} Although the recovery of autologous platelets was modestly reduced in patients with counts less than 50,000/μL (Table 1), this result may reflect greater cell damage in vitro, since these patients provided the fewest platelets for collection and labeling. In general, these observations imply that there is no vascular platelet deficit in thrombocytopenia. In addition, Heyns et al\textsuperscript{40} were unable to demonstrate in humans by scintillation camera imaging the presence of \textsuperscript{111}In-oxine platelet activity in the vasculature of the lower limbs after the disappearance of the labeled platelets from the general circulation. These authors were, however, unable to exclude the possibility of a small platelet requirement by the vascular endothelium.

Our estimate of the number of platelets involved in maintaining normal hemostasis should be regarded as a maximum, rather than a minimum, figure. First, some cell injury may be unavoidable when platelets are harvested and labeled with \textsuperscript{51}Cr according to the acid-citrate-dextrose (ACD) method, and particularly in the patient studies with autologous platelets, in which relatively few cells were available for collection.\textsuperscript{3,34} Second, the mean age of the patient group was 42 years. Platelet survival may decrease modestly with advancing age, and it is difficult to exclude entirely the possibility of an additional, but unrecognized, component of platelet destruction, at least in some individuals. These factors could reduce platelet survival, resulting in a overestimation of random platelet destruction relative to the control group. Third, our estimate of random platelet destruction includes all possible mechanisms, of which hemostatic platelet interactions may be only one component. For example, it is conceivable that all platelets may be at some small but finite risk of removal by the reticuloendothelial system or may serve other functions unrelated to hemostasis. Finally, because spontaneous bleeding was not observed in these patients and usually occurs only when the platelet count falls below approximately 5,000/μL, increased platelet removal due to bleeding was probably not a factor affecting platelet survival in these studies.\textsuperscript{2}

Although the literature contains relatively few reports of platelet survival studies in patients with marrow hypoplasia and none, to our knowledge, involving a comparable analytic approach, the data of Slichter and Harker,\textsuperscript{2} Branehog et al,\textsuperscript{17} and Mueller-Eckhardt et al\textsuperscript{18} substantiate the correlation observed in the present studies (Fig 2). The occasional reports of normal or only slightly reduced platelet survival in patients with marrow failure may simply reflect the variability of platelet survival in these subjects (Table 1) or the methods used for platelet preparation and data analysis.\textsuperscript{4,8,30,41} Substantially greater shortening of platelet survival has been reported for patients with idiopathic thrombocytopenic purpura (ITP), suggesting an additional component of platelet destruction in this disorder, which in fact could be estimated quantitatively according to the present methods.\textsuperscript{2,4,10,16,34,41} Conversely, some of the shortening of platelet survival observed in ITP patients, as well as in other thrombocytopenic disorders, may be due to this fixed platelet requirement that becomes more apparent at lower platelet counts. Thus, in thrombocytopenic patients, accelerated rates of platelet removal due to extrinsic mechanisms can be formulated only if their platelet survival is significantly shorter than expected based on their platelet count. In addition, the present study provides some explanation for the observation that anti-thrombotic drugs generally do not prolong platelet survival beyond normal values.\textsuperscript{1,5,19,22} In our subjects, the difference between the normal and the maximum platelet survival was only 0.9 days, and it is unlikely that this difference would be significant in clinical studies. The same considerations may apply to studies involving primates having predominantly linear platelet survival patterns\textsuperscript{42} but may not apply to other species, such as the rabbit, in which the rate of random platelet destruction may be proportionally greater.\textsuperscript{30,31,43}

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REFERENCES


Platelet kinetics in patients with bone marrow hypoplasia: evidence for a fixed platelet requirement

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