In-Oxine Platelet Survivals in Thrombocytopenic Infants

By Valerie Castle, Geoffrey Coates, John G. Kelton, and Maureen Andrew

Thrombocytopenia is a common occurrence (20%) in sick neonates, but the causes have not been well studied. In this report we demonstrate that thrombocytopenia in the neonate is characterized by increased platelet destruction as shown by shortened homologous In-oxine-labeled platelet life spans. Thirty-one prospectively studied thrombocytopenic neonates were investigated by measuring the In-labeled platelet life span, platelet-associated IgG (PAIgG), and coagulation screening tests. In every infant, the thrombocytopenia was shown to have a destructive component since the mean platelet life span was significantly shortened to 65 ± 6 (mean ± SEM) hours with a range of one to 128 hours compared with adult values (212 ± 8; range, 140 to 260; gamma function analysis). The platelet survival was directly related to the lowest platelet count and inversely related to both the highest mean platelet volume and duration of the thrombocytopenia. In

Thrombocytopenia occurs frequently (20%) in sick neonates admitted to a neonatal intensive care unit. The mechanism responsible for the thrombocytopenia has been the subject of controversy, with some investigators suggesting that decreased platelet production is the cause of the thrombocytopenia, whereas others have reported that increased platelet destruction is the dominant mechanism. In a previous study, we postulated that the thrombocytopenia in neonates was caused by an increased rate of platelet destruction. However, these conclusions were primarily based upon measurements of platelet volume and assessments of bone marrow megakaryocytes mass and response to platelet transfusions. Although all are consistent and compelling, they remain indirect measures. The benchmark for classifying the mechanism of thrombocytopenia into underproduction, sequestration, and increased destruction is the performance of radiolabeled platelet survival studies. Until recently, such studies could not be performed in infants primarily because of the low specific activity of the traditional platelet label, Chromium 51. The recent application of In-oxine, which has a high labeling efficiency for platelets, now allows the performance of platelet survival studies in the investigation of thrombocytopenic infants. We found that all thrombocytopenic infants studied had shortened platelet life spans when compared with adults and that in some patients platelet sequestration occurred.

MATERIALS AND METHODS

Patient Population

Thrombocytopenic infants less than 2 months of age with a platelet count less than 100 x 10^9/L admitted to the McMaster Regional Neonatal Intensive Care Unit were eligible for this study. Patients were entered into the study if informed consent was obtained and the infant was in stable condition. The study was approved by the university-approved Ethics Review Committee and Nuclear Physics Review Committee.

A data base was collected for each infant, and the information included Apgar scores, the presence of respiratory distress syndrome (RDS), evidence of infection, umbilical lines, exchange transfusions, antibiotics, and maternal drug use. The gestational age was determined by a combination of maternal dates and Dubowitz scores.

Investigations

Platelet life span studies. Random donor platelets obtained from the Canadian Red Cross were used for the performance of the platelet survival studies. Platelets were obtained from an ABO-compatible, hepatitis B surface antigen-negative, unrelated donor. After centrifugation and washing with acid-citrate-dextrose (ACD), the platelets were resuspended with 30 to 50 uCi of In-oxine and incubated at room temperature for 30 minutes. The labeled platelets were then centrifuged with ACD and subsequently resuspended in platelet-poor plasma. Each infant received 8 to 15 uCi/kg of In-labeled platelets (2 to 4 mL), the higher dose used for organ imaging. The labeled platelets were injected into a peripheral intravenous line and blood sampling performed at 1, 6, 8, 12, 24, 48, 72, and 96 hours after injection. The blood samples were obtained by microtechniques and consisted of 0.5 mL of whole blood collected into EDTA. The In radioactivity in the samples was measured in a gamma scintillation well counter (1085 Nuclear Chicago Gamma Counter, Chicago), and platelet survival curves were constructed. The survival curves were fitted to linear, exponential, and gamma functions by using the least-squares method and a digital computer.
Cytomegalovirus, and one with an enterovirus [Echo 1 or Echovirus 3]. The diagnosis of disseminated intravascular coagulation (DIC) required prolongation of the screening tests for coagulation. The gamma function was constructed from the multiple-hit program developed by Murphy and Francis, and this was used for all calculations of platelet survival.1 Laboratory investigations. The laboratory investigations performed in all infants included platelet counts, measurements of the mean platelet volume (MPV) and platelet-associated IgG (PAIgG), and coagulation assays. All blood samples were obtained from nonheparinized umbilical catheters or antecubital veins. The platelet counts and MPV were measured on EDTA-anticoagulated specimens by using a Coulter-S+ Counter (Coulter Electronics, Hialeah, FL), with confirmation by examination of a stained blood film. PAIgG was measured on 2 mL of whole blood collected into ACD. The platelets were isolated and washed and the total PAIgG (lysed platelets) measured by using an immunoradiometric assay.6 The coagulation assays were performed by microtechniques7 on blood collected into polystyrene tubes containing 3.8% sodium citrate and 0.1 mol/L e-aminocaproic acid. The prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin clotting time (TCT), and fibrinogen level were measured on all samples. Abnormal results, (greater or lower than 2 SD of the age-matched normal range) led to further investigations including measurement of factors V and VIII procoagulant (VIII:C), and fibrinogen-fibrin degradation products (FDP, Thrombo-Welco Test; General Diagnostics, Morris Plains, NJ). The diagnosis of disseminated intravascular coagulation (DIC) required prolongation of the screening tests plus the presence of elevated levels of FDP or low levels of factors V and VIII:C and fibrinogen.

Differences between groups were analyzed by Student’s unpaired t test. For multiple comparisons the Bonferroni correction was used. The platelet survival in 31 thrombocytopenic infants required assisted ventilation. Three infants had positive blood cultures, all with Propionibacterium, and four infants had viral infections documented by culture (three with cytomegalovirus and one with an enterovirus [Echo 11]).

Platelet Survival and Imaging

All 31 infants studied had evidence of a destructive thrombocytopenia as demonstrated by a shortened platelet life span compared with adults. The mean gamma function life span in the infants was 65 ± 6 hours (mean ± SEM) with a range of 1 to 128 hours compared with 212 ± 8 hours in the adult. There was a correlation between the lowest platelet count for each infant and platelet survival (r = .40; P = .02; platelet count = 16.704 + 0.338 · platelet survival; Fig 1). There was an inverse relationship as well between both the duration of the thrombocytopenia and platelet survival (r = −.63; P < .01; duration = 124.825 − 55.8600 · log platelet survival) and between the highest MPV and platelet survival (r = −.42; P < .05; MPV = 15.4976 − 1.9696 · log platelet survival).

The immediate percent recovery of the radiolabeled platelets was >50% (the lower limit for the normal adult) in nine infants; however, in 22 infants there was a percent recovery <50% (24% ± 3.4, mean ± SEM) that suggested increased platelet sequestration. Platelet turnover was calculated for each infant by using a standard approach,9 and only three infants had definite evidence of decreased production contributing to their thrombocytopenia. Organ imaging was performed in 11 infants who were sufficiently stable to be transported to the nuclear medicine department. All 11 had a low percent recovery, with 13% ± 1.7% (range, 5% to 18%) uptake in the liver and 40% ± 4.9% (28% to 54%) uptake in the spleen, for a spleen-to-liver ratio of approximately 3:1.

The clinical characteristics of the 31 infants studied are shown in Table 1. The infants are grouped as either premature or full term because of the differing spectrum of disorders present in these patients. A five-minute Apgar score <7 was present in 12 infants. Twenty-five infants

<table>
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<th>Characteristic</th>
<th>Premature</th>
<th>Full-Term</th>
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<tr>
<td>Number</td>
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<td>10</td>
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<tr>
<td>Gestational age (wk)*</td>
<td>30 ± 3.8</td>
<td>39.5 ± 1.5</td>
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<td>Birth weight (kg)*</td>
<td>1.460 ± 0.738</td>
<td>3.143 ± 0.765</td>
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<tr>
<td>5-min Apgar score (&lt;7)†</td>
<td>9</td>
<td>3</td>
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<tr>
<td>IPPV†</td>
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<td>RDS†</td>
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</tr>
<tr>
<td>Positive viral culture†</td>
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Abbreviation: IPPV, intermittent positive pressure ventilation.

*Mean ± 1 SD.

†Reported as the number of infants affected.

Fig 1. The relationship between the lowest platelet count and platelet survival in 31 thrombocytopenic infants (r = .40; P = .02; platelet count = 16.704 + 0.338 · platelet survival).
cause for the abnormal sites of accumulation was uncertain but may have been due to active bleeding in these areas.

Laboratory Investigations

Further evidence that the thrombocytopenia was due to increased platelet destruction was provided by the demonstration of an increased MPV in all infants (12.3 ± 0.4 fl, normal <10.0 fl) plus a poor (<30 x 10⁹/L) incremental response to random donor platelets in 13 of 24 infants. The cause for the increased platelet destruction was classified as immune (based upon the presence of PAIgG), DIC, or neither. Eight infants had evidence of DIC, 13 had increased PAIgG levels, and ten had neither DIC nor increased PAIgG levels (Table 2). Infants for whom either DIC or increased PAIgG levels were present had a higher MPV, a lower platelet nadir, and a longer duration of thrombocytopenia (Fig 2). In addition, on subanalysis the infants with either DIC or increased PAIgG levels had a significantly shorter platelet life span compared with infants in whom neither DIC nor increased PAIgG levels were present (Fig 3).

DISCUSSION

The systematic application of platelet survival studies to thrombocytopenic adults has increased our understanding of the pathophysiology of a variety of thrombocytopenic disorders. The classification of neonatal thrombocytopenias also would benefit by the performance of such studies. In this report we describe the use of ¹¹¹In-oxine for the performance of platelet survival studies plus organ imaging in 31 neonates with thrombocytopenia. The shortened platelet survivals observed correlated with the basic mechanisms responsible for the thrombocytopenia. Our results are compatible with a previous report of shortened platelet survival studies using the traditional label ⁵¹Cr in 15 ill newborn infants, not all of whom were thrombocytopenic.¹ The ⁵¹Cr label has a number of limitations including a low labeling efficiency and the inability to quantitate platelet localization in various organs.
The recent availability of $^{111}$In-oxine as a platelet label overcomes many of these problems. $^{111}$In has a high labeling efficiency (90%) that permits the radio labeling of small volumes of platelets, but, however, the volumes required were still too large to permit autologous platelet survival studies in the infant. The $\gamma$ emission characteristics of $^{111}$In-oxine allow this radiolabel to be used for organ imaging, which was performed for some of the infants in this report.

In a previous study, we had postulated that the mechanism of thrombocytopenia, which is seen in approximately 20% of ill neonates, was due to an increased rate of platelet destruction. This conclusion was based largely on the measurement of platelet volume, assessment of megakaryocyte mass, and response to platelet transfusions, which are all indirect measures of platelet life span. In the current study using $^{111}$In-labeled platelet survival measurements, we confirmed our previous impressions and showed that thrombocytopenia in ill neonates was primarily due to an increased rate of platelet destruction. In three infants, underproduction based on the calculated platelet turnover also contributed to the thrombocytopenia. A fixed daily turnover alone could not account for the short $^{111}$In-labeled platelet survivals; however, it may have contributed in part to the shortened survivals. The shortest platelet life spans were observed in infants with the lowest platelet count, highest MPV, and the longest duration of thrombocytopenia. In addition, a low percent recovery was observed for about two thirds of the infants, thereby suggesting that increased splenic sequestration could have contributed to the thrombocytopenia. The platelet life spans and percent recoveries in the thrombocytopenic neonates were compared with the same measurements in healthy adults. Ideally, the estimates of the platelet life span and platelet recoveries should have been compared with $^{111}$In platelet studies performed in healthy, non-thrombocytopenic neonates. However, we did not perform such studies. Nonetheless, there is no reason to believe that the platelet life span in healthy neonates is different from that in adults with the identical normal range for the platelet count and the same platelet volume. In addition, we have recently shown that platelet life spans and percent recoveries using $^{111}$In-labeled platelets are identical for neonatal rabbits compared with adult rabbits.

In an attempt to correlate the platelet life span with the underlying cause of the thrombocytopenia, we grouped our patients into three groups: immune platelet destruction (evidenced by elevated levels of PAIgG), DIC (evidenced by abnormal coagulation test results consistent with increased thrombin activity), and thrombocytopenic disorders not due to detectable immune mechanisms or DIC. As we found in our previous study, there was little overlap among these three groups, and the proportion of infants in each group was similar to what was found previously. The infants with the thrombocytopenia in whom a cause for the platelet destruction could be found (DIC or elevated PAIgG levels) had significantly more severe thrombocytopenia compared with the infant in whom no cause could be found (Fig 2). Consistent with the severity of their thrombocytopenia was the demonstration of significantly shorter platelet life spans in these two groups compared with the thrombocytopenic infants with neither elevated PAIgG levels nor DIC (Fig 3).

One of the advantages of $^{111}$In is the ability to perform quantitative in vivo imaging. In vivo distribution studies of $^{111}$In-labeled platelets in the human healthy adult have shown that the liver and spleen are the major sites of platelet deposition. Our study shows the same to be true in the infant with a predominant uptake in the spleen and liver. $^{111}$In-labeled platelets have also been used in certain pathological circumstances to image abnormal accumulation of platelets in the human adult and in animals. There has been only one report of the use of $^{111}$In-labeled platelets in an infant, and this was to image a hemangioma in an infant with Kasabach-Merritt syndrome. In our study, of the 11 infants who were imaged, two showed evidence of abnormal platelet accumulation. One very premature infant had an abnormal accumulation in the posterior occipital area, and a full-term infant with an overwhelming enteroviral infection and hepatic necrosis had abnormal accumulation in the lungs and scalp. The etiology of these abnormal accumulations is uncertain; however, bleeding into these areas is a distinct possibility. Although these represent single cases, it is possible that the systematic application of these techniques will improve our understanding of the mechanism of thrombocytopenia in these and other infants.

In this study, the use of $^{111}$In-labeled platelets has shown that thrombocytopenia in the newborn infant is primarily caused by increased platelet destruction and, in some patients, platelet sequestration.

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