Effect of Platelets Stored at 22°C for 24 Hours in Patients With Acute Leukemia

By C. S. Vallejos, E. J. Freireich, G. M. Brittin, and D. S. de Jongh

We have compared the ability of transfused platelets that were fresh or stored for 24 hr at room temperature (22°C) to increase the platelet counts of thrombocytopenic patients with acute leukemia. Increments in the recipients' platelet counts after transfusion of stored platelets were substantially smaller and shorter-lived than those given by fresh platelets. This was the case even in patients who responded consistently to fresh platelets.

Clinical studies have defined the important role of platelet transfusions in the treatment and prevention of hemorrhage, especially in the supportive care of patients with neoplastic diseases. However, rapid loss of viability during storage has been a major obstacle to the wider use of platelets in therapy. Although transfusion of platelets stored at 4°C for 48 hr can increase the platelet counts of thrombocytopenic recipients 1 hr after transfusion to two-thirds of the level obtainable by the use of fresh platelets, the stored platelets have a greatly shortened life span. Attempts to store platelets by freezing them in the presence of cryoprotective agents have been modeled after studies of red cell freezing. This technique has not been applied widely, because cryopreservatives are unpleasant and potentially toxic, and because the yields of transfused frozen platelets and the increments they produce in the recipient's platelet counts have not compared favorably with those obtained with fresh platelets. Murphy et al. have reported that 22°C is the optimal temperature for storage of platelets, and these authors have provided metabolic, morphologic, and functional evidence that platelets stored in this way remain remarkably intact. However, there have been no published reports to date which document that platelets stored at room temperature are effective in increasing the platelet counts of thrombocytopenic recipients. We have attempted to provide data on this point by comparing the abilities of room-temperature-stored and fresh platelets obtained from the same normal donors.
to increase the platelet counts of thrombocytopenic patients with acute leukemia.

MATERIALS AND METHODS

Platelet concentrates were obtained from normal adults by platelet-pheresis using a closed system of bags containing ACD (U.S.P. Formula A) as the anticoagulant. The polyvinyl chloride bags were made of the investigational plastic P1-146. Red blood cells were returned to the donor before consecutive units were withdrawn. Platelet concentrates were prepared according to the Technical Methods and Procedures of the American Association of Blood Banks. Platelet-rich plasma was prepared by a light centrifugation (4000 rpm for 2 mm) in a Sorvall RC-3 centrifuge equipped with horizontal head HG-4. The temperature inside the centrifuge was maintained at 20°C. The platelet-rich plasma was then subjected to a heavy spin (4300 rpm for 5 mm at 20°C) in the same Sorvall centrifuge. One unit consisted of the platelets separated from 500 ml of whole blood. The time required to collect 4 U of platelets from a given donor was approximately 2 hr. The 4 U of platelets were pooled and suspended in a total volume of approximately 100 ml of single donor plasma. “Fresh” platelets were transfused within 4 hr and usually within 2 hr after their collection. “Stored” platelets were allowed to remain without agitation for 24 hr in a room in which air conditioning maintained the temperature at 21-22°C. Individual units of platelet concentrates were pooled immediately prior to their transfusion. The recipients were all adults who had acute leukemia with thrombocytopenia that was expected to last for at least 2 wk.

The effect of each platelet transfusion was determined by measuring the change in the platelet count of the recipient (platelet increment). Platelet counts were performed on finger-puncture blood by an automated optical technique. This method has been shown to give the same platelet counts on venous and capillary blood and to be more reliable than other methods for performing platelet counts on thrombocytopenic and finger-puncture blood samples. Venous blood platelet counts of the donors were also determined by this technique. The increment in the platelet count produced by transfusion varies directly with the number of platelets infused and inversely with the size of the recipient. These variables were taken into account in calculating the response to transfusion. The size of the recipient was expressed as his surface area in square meters calculated from height and weight. The response to a platelet transfusion was expressed as the increment in circulating platelets per cu mm of whole blood per unit of platelets transfused per sq m of body surface area of the recipient (INC/U/m²).

Eighteen patients with acute leukemia who had platelet counts of less than 50,000 per cu mm were studied. Each patient received two pairs of platelet transfusions over approximately 1 wk. Each pair consisted of fresh (F) and stored (S) platelets from a single donor. Each patient therefore served as his own control. The two different types of platelets were administered in alternating order in the following combinations: F-S F-S (seven patients); S-F S-F (six patients); F-S F-S (five patients). The number of platelets in each transfusion was not determined directly. However, the numbers of fresh and stored platelets in each pair of transfusions were believed to be approximately the same, because (1) both types of platelets were obtained with identical technique from the same donor, and (2) the mean venous blood platelet count of the donors when they gave “fresh” platelets was 233,200/cu mm and when they gave “stored” platelets was 245,000/cu mm (p > 0.50).

In the nine patients of Group I, platelet counts were performed each morning, immediately before, and immediately, 1 hr, 4 hr, and 24 hr after each transfusion of 4 U of platelets. In the nine patients of Group II, posttransfusion platelet counts were performed at 1, 24, and 48 hr. Groups I and II did not differ in any way other than the intervals at which platelet counts were performed.

Patients with active bleeding were excluded from the study, because we could not insure that a transfusion of 4 U of platelets would control hemorrhage. One patient had positive stool guaiac tests and another had petechiae during the period in which he received both fresh and stored platelets. One patient had petechiae that disappeared after one of the transfusions of stored platelets. Interestingly, this patient was one of the few who had a satisfactory increment in the platelet count 24 hr after transfusion with stored platelets.

*Quadruple plasmapheresis double Blood-Pack. FT-133, Fenwal Laboratories, Division of Travenol Laboratories, Morton Grove, Ill. 60053.
The data were analyzed statistically by pairing the platelet increments associated with transfusion of fresh and stored platelets for each patient (paired data). The significance of the differences in platelet increments was determined by a two-tailed student's \( t \) test.

**RESULTS**

The results are shown in Table 1. In the nine patients of Group I, the increments in the platelet count were determined at 0 (immediate posttransfusion), 1, 4, and 24 hr after transfusion of 4 U of platelets that were fresh or stored for 24 hr at room temperature (22°C). The increments obtained by transfusion of stored platelets were only about one-third of those given by fresh platelets, and they were short-lived. The platelet counts of the nine patients of Group II increased only slightly with transfusion of stored platelets. By 24 hr after transfusion with stored platelets their average platelet count was slightly lower than it was before transfusion.

In seven of the 18 patients in Groups I and II, both of the transfusions of fresh platelets produced positive increments at 1 and 24 hr. These patients, who responded consistently to transfusion of fresh platelets, were presumed not to have been so immunized by prior platelet transfusions. Even in these patients transfusion of stored platelets gave small increments which were short-lived. Differences between the increments given by transfusion of fresh and stored platelets were highly significant (Table 1).

A one-way analysis of variance was performed with the increments obtained by transfusion of fresh and stored platelets in order to determine if the mean values obtained were influenced by the order in which fresh and stored platelets were administered. This analysis revealed no evidence that the increments produced by the stored platelets could not be samples from a population with a common mean and similarly for the increments obtained with the fresh platelets.

<table>
<thead>
<tr>
<th>Group</th>
<th>No of Patients</th>
<th>Hr After Transfusion</th>
<th>Fresh Platelets Increment ( \times 10^3 ) Mean SE Median</th>
<th>Stored Platelets Increment ( \times 10^3 ) Mean SE Median</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9</td>
<td>0</td>
<td>10.1 1.7 8.7 2.9 0.6 2.8 (&lt;.001)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>1</td>
<td>7.1 1.8 4.4 3.4 1.4 1.9 (&lt;.01)</td>
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<tr>
<td></td>
<td>4</td>
<td>5.6 1.4 5.9 2.0 0.8 1.1 (&lt;.02)</td>
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<tr>
<td></td>
<td>24</td>
<td>5.2 1.8 5.8 0.7 0.7 -0.8 (&lt;.02)</td>
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<tr>
<td>II</td>
<td>9</td>
<td>1</td>
<td>8.0 1.7 6.4 0.5 1.1 0.5 (&lt;.001)</td>
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<tr>
<td></td>
<td>24</td>
<td>5.1 1.7 3.3 -0.5 1.0 -1.2 (&lt;.005)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients from Groups</td>
<td>7</td>
<td>1</td>
<td>12.1 1.8 9.7 2.8 2.1 1.7 (&lt;.001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II with positive increments at one and 24 hr after both transfusions of fresh platelets</td>
<td>24</td>
<td>9.8 1.8 10.0 0.5 1.1 -0.9 (&lt;.001)</td>
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*Increment change in platelet count per unit of transfused platelets per square meter of body surface area.
DISCUSSION

This study was undertaken to compare the effectiveness of transfused platelets that were fresh or stored for 24 hr at room temperature to increase the platelet counts of thrombocytopenic patients with acute leukemia. This measure of effectiveness is of practical importance for a transfusion service dealing with thrombocytopenic patients receiving chemotherapy, since the platelet count is used as the primary index for prophylactic platelet transfusion. Transfusion of stored platelets produced increments in the recipients’ platelet counts that were substantially smaller and shorter lived than those obtained with fresh platelets. These findings are supported by a review of the platelet increments obtained in adult patients with acute leukemia during the past 2 yr in our routine platelet transfusion service. Our results differ but are not necessarily at variance with those of Murphy et al., who obtained good yields of transfused platelets that had been stored at room temperature for 48 hr in some patients with aplastic anemia, but less satisfactory results in two patients with acute leukemia. Our technique of collection and concentration of platelets was that recommended by the American Association of Blood Banks and the platelet concentrates were stored at room temperature for 24 hr without agitation. Murphy et al. stored their platelet concentrates for 48 hr with continuous agitation. Continuous agitation might influence the ability of stored platelets to increase the platelet counts of thrombocytopenic recipients, and this possibility needs to be explored, for it may account for the difference between our experience and that of Murphy et al.

All of our patients had received numerous platelet transfusions prior to the study and could have developed platelet isoantibodies. It is possible that storage affects platelets so as to render them more susceptible to destruction in the immunized recipient. However, seven of our patients responded consistently to fresh platelets and can therefore be presumed not to have been sensitized. Even in these patients transfusion of stored platelets produced increments that were small and short-lived.

At the present time storage of platelets at 22°C presents two drawbacks: (1) Although we have not encountered difficulties with bacterial contamination of stored platelets in our own transfusion service, such storage has been associated with growth of contaminating bacteria. (2) Platelets stored at 22°C are less effective than fresh platelets in producing and maintaining increments in the platelet counts of thrombocytopenic patients with acute leukemia. If the biological effectiveness of a platelet transfusion is proportional to the number of circulating transfused platelets, then approximately 12–16 U of stored platelets would be required to produce the same effect obtainable by administration of 4 U of fresh platelets. Use of such large numbers of units of platelets would appear to be undesirable, because it would represent inefficient use of a limited resource and because it would increase the risks associated with transfusion of blood products, e.g., hepatitis and isoimmunization.

Our results indicate that platelets stored at room temperature (22°C) for 24 hr are not superior to platelets stored at refrigerator temperature (4°C) in their ability to increase the platelet counts of thrombocytopenic patients with acute leukemia. Other workers have found that even under the best conditions of
room-temperature storage, platelets suffer biochemical, functional, and morphological damage and that storage at cold temperatures gives better results.\(^\text{25,26}\) These findings suggest that \(22^\circ\text{C}\) may not be the optimal temperature for storage of platelets that are to be transfused to patients with acute leukemia. In this case it will be necessary to direct attention at methods to increase the supply of fresh platelets and/or to devise better methods of short-term platelet preservation in order to meet the demand for platelets used in the management of patients receiving chemotherapy.

ACKNOWLEDGMENT

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REFERENCES

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