Successful Transfusion of Platelets Cryopreserved for More Than 3 Years

By Peter A. Daly, Charles A. Schiffer, Joseph Aisner, and Peter H. Wiernik

To determine the duration of storage for cryopreserved platelets, 14 transfusions of random-donor, pooled platelets, stored in the vapor phase of liquid nitrogen for a mean period of 1157 days (range 1060–1240), were analyzed. Twelve of these transfusions were compared in a paired fashion with fresh, random-donor, pooled platelets given within a few days to the same thrombocytopenic recipients. Platelets had been frozen using 5% dimethylsulfoxide as a cryoprotective agent either at a controlled rate of −1°C/min to −80°C or by simply placing them in the vapor phase (−120°C) of a liquid nitrogen freezer. The mean freeze–thaw loss for the 14 transfusions was 22%, and the mean corrected 1-hr increment in platelet count was 12,600/μl. In the 12 paired observations, the mean corrected 1-hr increment for frozen platelets was 11,800/μl and 25,900 for fresh platelets, giving a frozen/fresh recovery of 46%. Random donor platelets can be cryopreserved by these methods for greater than 3 yr with satisfactory posttransfusion increments. This suggests that a reservoir of frozen platelets, either random-donor for emergency transfusion or of known HLA-type for transfusion to alloimmunized patients, can be established and stored for at least 3 yr.

MANY STUDIES have shown that human platelets can be cryopreserved using dimethylsulfoxide (DMSO) as the cryoprotective agent, and that, after thawing, they can be effectively transfused to thrombocytopenic patients. In the majority of instances, such transfusions have been given prophylactically, but there is also now a sizeable number of reported patients who have shown control of bleeding and shortening of bleeding times following such transfusions. Autologous frozen platelet transfusions have become an important part of the supportive care of leukemic patients at this institution during maintenance and reinduction therapy when alloimmunization is frequently present. When frozen autologous platelet transfusions are given to patients with acute leukemia there is generally a short period of storage because of the nature of the disease. The concept, however, of establishing a reservoir of frozen platelets, either random-donor for emergency use or HLA-matched for transfusion to alloimmunized recipients, is now a distinct possibility, and prolonged storage may be necessary before a need to use such platelets arises. To study the duration of storage, we analyzed transfusions of platelets cryopreserved for >3 yr and compared posttransfusion recovery with that obtained following transfusion of fresh platelets to the same recipients.

MATERIALS AND METHODS

Patient Population

All patients were adults receiving intensive chemotherapy for a variety of malignant neoplasms. Informed consent was obtained prior to all frozen platelet transfusions. Nonalloimmunized patients...
were sought who were either receiving their first platelet transfusion or who had recently shown a good response to fresh random-donor pooled platelets. Only one patient was bleeding prior to frozen platelet transfusion. He received two transfusions of frozen platelets, but his upper gastrointestinal bleeding had been controlled with fresh platelets before he received the frozen transfusions.

**Platelet Freezing and Thawing**

The techniques used were similar to those previously described. Units of platelet concentrate were prepared by a standard serial centrifugation plateletpheresis technique, using acid citrate dextrose as the anticoagulant. Whole blood was centrifuged at 1600 g for 5 min at 25°C in a Sorvall RC-3 centrifuge to obtain platelet-rich plasma. This was then centrifuged at 6975 g for 10 min, the plasma was removed, and the platelet concentrate retained in 40–50 ml of plasma. Three to five units of the same ABO type were pooled for freezing. The mean number of platelets per freezing was $2.4 \times 10^{11}$ (range 1.3–4.0).

The pooled platelets were concentrated by centrifugation at 5000 g for 6 min at 25°C. The supernatant plasma was then removed and the platelets resuspended in a final volume of either 30 or 50 ml, depending on the number of units to be frozen. They were then transferred either to 100-ml or 200-ml polyolefin freezing bags (Hemoflex 1000-2 or 2030-2, Union Carbide, Chicago, Ill.), and an equal volume of 10% DMSO in ABO-matched plasma was slowly added. The final DMSO concentration was therefore 5%. The 10% DMSO-plasma mixture had been allowed to cool prior to its addition to allow for dissipation of heat generated by the addition of DMSO to plasma. Six of the 14 transfusions were then frozen to $-80°C$ at a controlled rate of $-1°C/min$ as previously described, and the remaining 8 preparations were merely put in metal containers and placed horizontally in the vapor phase (−120°C) of a liquid nitrogen freezer. All platelets were stored in the vapor phase of liquid nitrogen at approximately $-120°C$.

Thawing was accomplished by immersion in a 37°C water bath for 4–5 min. One-hundred milliliters of ABO-matched plasma and 10 ml of acid citrate dextrose were added slowly over 15 min. The platelets were then transferred to a polyvinyl chloride bag (TA-2, Fenwal Corporation, Morton Grove, Ill.) for centrifugation at 4400 g for 6 min at 25°C. The supernatant plasma containing most of the DMSO was removed and the platelets resuspended in 100 ml of ABO-matched plasma for transfusion. Samples were taken for platelet counts, performed electronically, to determine the loss during the freeze-thaw procedure and also for morphological evaluation by phase microscopy.

**Transfusions**

The equivalent of 4–8 U of platelets were given per transfusion, administered within 1 hr of the thawing procedure through standard blood filters over a period of 15–30 min. Platelet counts, using a Coulter Thrombocounter (Coulter Electronics, Hialeah, Fl.) were done prior to transfusion, 1 hr following completion of the transfusion, and in most cases at 18–24 hr. Stable afebrile patients without infection, bleeding, or splenomegaly were chosen for transfusions. The only exception to this was the patient with bleeding described earlier who was febrile (>101°F) during the time he had all 4 transfusions (2 frozen and 2 fresh). Three of the patients had been splenectomized. To standardize results for the body surface area (BSA) of recipients and the number of platelets transfused, increments were expressed as corrected count increments (C1) where:

$$C1 = \frac{(\text{Posttransfusion} - \text{pretransfusion platelet count}) \times \text{BSA (sq m)}}{\text{Platelets transfused} \times 10^{11}}$$

The paired transfusions were administered within a period of 1 wk in all but one patient. In that patient they were separated by a period of 3 mo without any intervening transfusions. All patients were markedly thrombocytopenic (<25,000/μl) at the time of transfusion.

**RESULTS**

The mean number of platelets administered in the frozen transfusions was $4.5 \times 10^{11}$ (range 2.8–7.4), which is equivalent to approximately 6–7 "units" of platelet concentrate. The mean freeze-thaw loss was 22% (range 4%–47%).

Thirteen patients received 14 frozen platelet transfusions (Table 1). The mean 1-hr C1 was 12,600/μl (range 5–25,000). Two patients received frozen transfusions
Values are expressed as means with ranges in parentheses. The percent recovery for the frozen/fresh paired observations was 46% at 1 hr and 32% at 18–24 hr.

without paired fresh transfusions. There were therefore 12 paired observations at 1 hr. The mean CI at 1 hr for the 12 frozen transfusions was 11,800/µl (range 5–19,000) and for fresh 25,900 (range 14–56,000). The mean percent recovery frozen/fresh at 1 hr was 46% (range 23%–83%). In 10 transfusions, paired observations were available at 18–24 hr. The mean CI at 18–24 hr for frozen platelets was 7200/µl (range 0–19,000) and for fresh 22,700 (range 6–60,000). This gave a frozen/fresh percent recovery at 18–24 hr of 32%, suggesting a decreased survival for the frozen platelets. In 2 of the 3 splenectomized patients, however, recovery at 18–24 hr was the same as that at 1 hr posttransfusion.

Nine preparations of thawed platelets were examined by phase microscopy. Three preparations with good preservation of normal discoid morphology (<50%) without microscopic clumping gave good posttransfusion increments. Three others gave poor increments when discoid morphology had been lost (<20% discs), and pseudopods and clumping were present. Two poor preparations, however, gave adequate increments, whereas one with good preservation of morphology was associated with a poor increment.

No hemorrhage occurred in patients receiving frozen transfusions, and gastrointestinal bleeding remained under control in the patient previously mentioned. There were no reactions to the residual DMSO, and patient acceptance was excellent. No febrile transfusion reactions occurred.

DISCUSSION

Two recent studies from this institution have shown the efficacy of autologous and allogeneic frozen platelet support in patients with leukemia. In the most recent study, the mean duration of storage for frozen autologous platelets was 118 days with a range of 13–400, but transfusions have been administered successfully since then after storage periods as long as 823 days (unpublished observation). There was no correlation between storage duration and posttransfusion recovery in these earlier reports. Most patients with acute leukemia undergoing maintenance or reinduction therapy need their own platelets within a short period, so studies of prolonged storage are impractical in this setting at this time. However, as survival and treatment improve in this disease, such studies may become feasible. In other circumstances, long-term storage may be important for use during times of platelet storage and in establishing a reservoir of platelets from donors with known HLA phenotypes.

The platelets used in these transfusions were frozen during 1975. At that time, it
was unclear whether controlled-rate freezing was necessary, and there was a suggestion that results were comparable if the platelets were merely placed in a freezer at $-80^\circ C$.\textsuperscript{7} Hence, some of the platelets were frozen at a controlled rate and some by placing them directly at $-120^\circ C$ in the vapor phase of liquid nitrogen. The platelet concentration necessary for optimal results was also unclear at that time, so that some were suspended in 60 ml and placed in 100-ml bags and some suspended in 100 ml and stored in 200-ml containers. Similarly, in the preparation of platelet concentrate, our understanding of variables such as centrifugation speeds and methods of storage has improved in recent years. It must be noted that the centrifugation speeds used in the preparation of these units of platelet concentrate were not optimal, particularly in the light of the study by Slichter and Harker,\textsuperscript{8} and that some damage may have been suffered by the platelets prior to freezing. With better handling of platelets there has been an improvement in posttransfusion recovery in successive studies with frozen platelets over the past few years.\textsuperscript{7}

In the most recent reported study of autologous frozen transfusions,\textsuperscript{1} none of the patients were splenectomized, and the mean 1-hr posttransfusion Cl was 13,700/\mu l. In another study,\textsuperscript{2} the mean 1-hr Cl was 12,800/\mu l, and here, a comparison with fresh transfusions in 16 patients gave a frozen/fresh recovery of 65%. The results obtained with this group of transfusions, while clinically satisfactory, were somewhat inferior. Hopefully, with correction of some of the variables alluded to above, improvement in recovery should be possible. It appears from this study that duration of storage, at least at $-120^\circ C$, is not critical in determining posttransfusion recovery. Whether this is true for platelets stored at $-80^\circ C$ remains to be seen.

Even though satisfactory increments were obtained in the majority of patients, only 4 had a rise in absolute count to 50,000/\mu l, which would be likely to produce significant shortening of the bleeding time.\textsuperscript{9} In the light of the low absolute increment, the concomitant granulocytopenia and the risk of infection bleeding time estimations were not done on these patients and therefore the functional capacity of the platelets was not fully assessed. No bleeding complications occurred in these patients, and control of upper gastrointestinal hemorrhage in a single patient persisted following transfusion of frozen platelets.

The poorer recovery at 18–24 hr would indicate that a higher percentage of the frozen platelets were damaged and therefore removed from the circulation during the first 18–24 hr following transfusion. This is further suggested by the fact that in 2 of 3 patients studied who had been splenectomized, the recovery at 18–24 hr was identical with that at 1-hr posttransfusion. This was true in the absence of spontaneous marrow recovery, as demonstrated by a fall in platelet count over succeeding days. The absence of a spleen in these patients probably allowed for longer circulation of abnormal platelets.

The shelf-life of frozen platelets, once thawed, has been shown to be short,\textsuperscript{10} and it is recommended that they be transfused within a few hours of thawing. It is not a realistic prospect then to thaw frozen platelets and hold them in reserve for possible random use. The thawing procedure takes approximately 45 min, and it is similarly less than optimal to rely on frozen platelets in the unusual circumstance of immediately life-threatening emergencies. A more realistic and exciting possibility is the establishment of a reservoir of transfusions from donors of known HLA
phenotypes for use in alloimmunized recipients. This has obvious advantages in terms of donation at the donor’s convenience and more immediate availability when needed, provided a sufficiently representative spectrum of transfusions could be stored. Presently, we are cryopreserving platelets from donors known to produce good increments in specific HLA-matched recipients as well as defined homozygotes for common HLA haplotypes who are more likely to serve as good “universal” donors.

REFERENCES

Successful transfusion of platelets cryopreserved for more than 3 years

PA Daly, CA Schiffer, J Aisner and PH Wiernik