

Anesthesiology
83:1167-1172, 1995
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Effects of Storage Time on Quantitative and Qualitative Platelet Function after Transfusion

Brian A. Rosenfeld, M.D.,* Barbara Herfel, B.S.,† Nauder Faraday, M.D.,‡ Alice Fuller, P.A.C.,§
Hayden Braine, M.D. ||

Background: Platelet transfusions are being used increasingly in patients with thrombocytopenia to improve hemostatic function before surgery and invasive procedures. However, there are limited data on the immediate quantitative and qualitative platelet response after transfusion. Some authors have suggested that transfused platelets require time *in vivo* to regain maximal function, which is dependent on the duration of platelet storage. Therefore, the timing of surgery and invasive procedures with optimal platelet function may not be occurring.

Methods: Twenty-five patients with thrombocytopenia from ablation chemotherapy and total body irradiation (before bone marrow transplantation), were randomized to receive either 1-day (fresh) or 4-day stored platelets. No patient had infection, organ system dysfunction, or previous platelet transfusion. Single-donor platelets were transfused (1 unit/10 kg body weight) over 60 min. Whole blood from an indwelling central venous catheter was obtained before, immediately after, and 1, 2, and 24 h after transfusion. Platelet number was measured on a Coulter counter and platelet reactivity was measured using agonist-induced whole blood impedance aggregometry (ohms) and dense granule release (adenosine triphosphate [ATP]).

Results: Platelet number increased similarly ($21,000 \pm 2,000/\text{mm}^3$ to $76,000 \pm 7,000/\text{mm}^3$ and $20,000 \pm 1,000/\text{mm}^3$ to $65,000 \pm 4,000/\text{mm}^3$) after transfusion in the 1- and 4-day stored platelets, respectively. These levels were maintained for 2 h after transfusion in both groups and then decreased similarly (26% and 27%) at 24 h. Agonist-induced platelet aggregation increased immediately after transfusion to 5 $\mu\text{g}/\text{ml}$ collagen (0.7 ± 0.4 to 11.4 ± 1.0 ohms and 0.1 ± 0.1 to 5.2 ± 1.0 ohms),

10 $\mu\text{g}/\text{ml}$ collagen (1.5 ± 0.7 to 18.0 ± 1.9 ohms and 0.6 ± 0.4 to 10.0 ± 1.6 ohms) and ristocetin (0.7 ± 0.4 to 10.1 ± 1.7 and 0.1 ± 0.7 to 6.2 ± 1.0 ohms), in 1- and 4-day, stored platelets, respectively and persisted unchanged in both groups for 2 h. Fresh platelets were hyperaggregable compared to 4-day stored platelets for collagen-induced (5 $\mu\text{g}/\text{ml}$ and 10 $\mu\text{g}/\text{ml}$) aggregation. Agonist-induced platelet dense granule release (ATP) increased immediately after transfusion to 5 $\mu\text{g}/\text{ml}$ collagen (42 ± 18 to 410 ± 49 picomoles ATP and 20 ± 7 to 186 ± 22 picomoles ATP), 10 $\mu\text{g}/\text{ml}$ collagen (60 ± 22 to 449 ± 53 picomoles ATP and 44 ± 13 to 219 ± 25 picomoles ATP) in 1- and 4-day platelets, respectively. Ristocetin-induced ATP release increased immediately after transfusion of fresh platelets only (0 ± 0 to 69 ± 17) and remained unchanged for 2 h. Fresh platelets also demonstrated greater dense granule release to collagen (5 μg and 10 $\mu\text{g}/\text{ml}$) and ristocetin than 4-day stored platelets.

Conclusions: In patients with chemotherapy-induced thrombocytopenia, platelet transfusion causes an immediate increase in number and function, which is independent of storage time. This quantitative and qualitative increase persists unchanged for 2 h after transfusion, suggesting that there is no acute "warm-up time" necessary for transfused platelets to regain maximal function. Fresh platelets demonstrate increased aggregation and dense granule release compared to 4-day stored platelets and may impart improved hemostatic function *in vivo*. (Key words: Platelet function. Thrombocytopenia. Transfusion.)

DURING the last 2 decades, there has been a rapid increase in the use of platelet transfusion for the prevention of thrombocytopenic bleeding.^{1,2} Transfusions are thought to improve *in vivo* hemostatic function, thereby decreasing potential blood loss and morbidity. In this context, platelet transfusions are routinely administered to patients with severe thrombocytopenia before placement of invasive catheters and surgical procedures.³ However, it is not clear from the literature when the optimal hemostatic function is achieved after platelet transfusion for attempting invasive procedures and surgery.

Platelets obtained from donors for transfusion are processed and stored at room temperature for up to 5 days.^{4,5} During this time, platelet activation⁶⁻⁸ and agonist desensitization^{9,10} occurs, along with glycogen,¹¹

* Assistant Professor, Anesthesiology, Medicine and Surgery.

† Research Assistant.

‡ Assistant Professor, Anesthesiology.

§ Physician's Assistant.

|| Associate Professor, Oncology and Medicine.

Received from the Departments of Anesthesiology and Critical Care Medicine, Medicine, and Oncology, The Johns Hopkins Medical Institutions, Baltimore, Maryland. Submitted for publication January 12, 1995. Accepted for publication August 29, 1995.

Address reprint requests to Dr. Rosenfeld: Anesthesiology and Critical Care Medicine, Johns Hopkins Hospital, 600 North Wolfe Street, Meyer 297A, Baltimore, Maryland 21287-7294.

glutathione,^{12,13} and high-energy adenine nucleotide depletion.¹⁴ After transfusion, platelets have been shown to require recovery time *in vivo* before regaining maximal qualitative function.^{11,15,16} This warm-up time may be owing to energy depletion, acidosis, and/or other aforementioned changes that occur during storage. However, any temporal improvement in qualitative platelet function occurring *in vivo* after transfusion, may be negated by the quantitative decrease in platelet number that also occurs after transfusion.^{17,18} The time course of maximal platelet effectiveness (quantitative and qualitative function) immediately after transfusion for platelets stored for various lengths of time has not been fully studied. Therefore, strategies currently used to coordinate platelet transfusion with the timing of surgical procedures may not be effective. We therefore studied the temporal relationship between platelet number and function after transfusion of 1- and 4-day stored platelets.

Materials and Methods

After Joint Commission on Clinical Investigation (institutional review board) approval, informed consent was obtained from 25 patients. All patients were thrombocytopenic from chemotherapy (cyclophosphamide, busulfan, carboplatinum, VP-16) either alone, or in combination with total body irradiation administered before bone marrow transplantation. Patients were administered platelets according to protocol when counts approximated 20,000/mm³. All patients had indwelling central venous catheters placed before induction of chemotherapy and were receiving only prophylactic antibiotics according to protocol (acyclovir, norfloxacin, vancomycin, fluconazole, cefotaxime, and amphotericin-B). Patients were excluded from the study if they had received previous platelet transfusions. Platelets were prepared by plateletpheresis from single donors with platelet concentrates collected in adenine-citrate-dextrose-4 (Baxter Healthcare Corp., Deerfield, IL) using automated apheresis and stored at room temperature on a flat bed rotator. Platelets were transfused within 24 h of collection (fresh) or 4 days after collection. Platelet concentrates (1 and 4 day) were transfused at 1 unit/10 kg body weight (1 unit = 5.5 × 10¹⁰ platelets) for a period of 60 min.

Blood for analysis of platelet number and function was obtained from indwelling central venous catheters before, immediately after, and 1, 2, and 24 h after

transfusion. Platelet count was measured at all time points and platelet function was measured at all time points except 24 h. After disposal of the initial 3 ml blood, whole blood was immediately placed in tubes containing ethylenediaminetetraacetic acid and 3.8% sodium citrate (9:1), respectively. The ethylenediaminetetraacetic acid-containing tube was analyzed for platelet count on a Coulter counter (Coulter, Inc., Hialeah, FL) and a corrected count index (adequate response > 5,000) was calculated using the formula:

$$\text{CCI} = \frac{\text{posttransfusion count} - \text{pretransfusion count}}{\text{number of units transfused}} \times \text{body surface area.}$$

Whole blood in 3.8% sodium citrate was immediately analyzed for platelet reactivity using a Model 500 Chronolog lumi-aggregometer (Chronolog Corp., Havertown, PA). Platelet reactivity was assessed by quantifying agonist-induced platelet aggregation and dense granule secretion (release of adenosine triphosphate [ATP]). Briefly, 0.5-ml aliquots of whole blood and 0.45 ml of isotonic saline were placed in plastic cuvettes and warmed to 37°C. Luciferin-luciferase reagent (50 μl, Chronolog Corp.) was added, and the sample was stirred at 1,200 RPM using polytetrafluorethylene-coated magnetic bars. Platelet reactivity was studied using four platelet agonists: (1) collagen (5 μg/ml and 10 μg/ml); (2) adenosine diphosphate (ADP; 20 μM); (3) ristocetin (1.0 mg/ml); and (4) thrombin (0.1 unit/ml). Agonists were added to the sample during stirring, and the resultant aggregation and ATP release measured until a maximal response was attained, or for 10 min. Peak response for aggregation was reported in ohms of impedance and dense granule release in picomoles of ATP (luminescence). An internal aggregation impedance standard was measured before the addition of each agonist, and ATP release was calculated by measuring an ATP standard added to the patient's diluted blood.

Statistical Analysis

All results are mean ± standard error of the mean (SEM). Demographic data were analyzed by Chi-square and *t* test. Platelet number and reactivity data were examined by nonparametric analysis, using Friedman analysis of variance for repeated measures and Wilcoxon's test with Bonferroni corrected *P* values for pairwise comparisons. Kruskal-Wallis analysis of variance was used to elicit effects owing to group differences.

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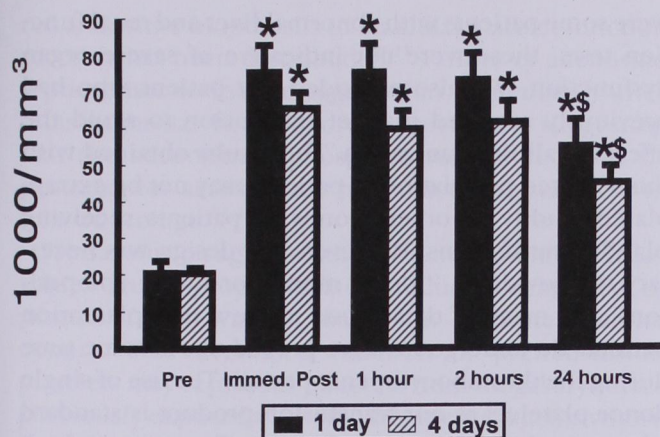


Fig. 1. Platelet counts over time for 1- and 4-day stored platelets in $1,000/\text{mm}^3$. * $P < 0.01$ versus pretransfusion. \$ $P < 0.01$ versus immediately after, and 1 and 2 h after.

Results

Twenty-five patients were examined for peritransfusion quantitative and qualitative platelet function. The group included 18 men and 7 women with a mean age of 46 ± 2 yr. Two patients received allogeneic and 23 patients received autologous bone marrow transplantation for lymphoma ($n = 16$) multiple myeloma ($n = 5$), chronic lymphocytic leukemia ($n = 1$), Hodgkins disease ($n = 1$), neuroblastoma ($n = 1$), and chronic myelogenous leukemia ($n = 1$). Two patients had abnormal renal function (creatinine level > 1.3 mg/dl) and ten had abnormal liver function tests (either one or more of the following: total bilirubin > 1.2 mg/dl, alkaline phosphatase > 120 international units per liter (IU/l), aspartate aminotransferase > 35 IU/l, alanine aminotransferase > 30 IU/l) before platelet transfusion. Twelve patients received fresh platelets and 13 received platelets stored 4 days. There were no differences in age, diagnosis, laboratory abnormalities, or antibiotic use between groups. Patients tolerated platelet transfusion without problems and there were no febrile responses. There were no differences in platelet number or function in the three patients receiving amphotericin treatment, nor in the patients with elevated renal or hepatic chemistries (data not shown).

Platelet counts for both groups are shown in figure 1. These demonstrated an immediate increase of $55,000/\text{mm}^3$ ($21,000 \pm 2,000/\text{mm}^3$ to $76,000 \pm 7,000/\text{mm}^3$) and $45,000/\text{mm}^3$ ($20,000 \pm 1,000/\text{mm}^3$ to $65,000 \pm 4,000/\text{mm}^3$) for 1- and 4-day platelets,

respectively, after transfusion. These platelet counts were maintained unchanged for 2 h and then decreased to $55,000 \pm 6,000/\text{mm}^3$ (20%) for 1 day and to $45,000 \pm 5,000/\text{mm}^3$ (27%) for 4 day at 24 h, still remaining greater than pretransfusion levels. Corrected count index values are shown in figure 2. These demonstrate similar levels in both groups after transfusion that are maintained through 24 h in the fresh platelets, but decrease in the 4-day group (41%) at 24 h.

Agonist-induced platelet aggregation data are shown in figure 3. These demonstrated an increase in collagen-induced (5 and 10 $\mu\text{g}/\text{ml}$) and ristocetin-induced aggregation immediately after transfusion in both groups. This increase in aggregation was maintained unchanged for 2 h in both groups. There was no appreciable change in adenosine-diphosphate-induced aggregation at any point after transfusion. Fresh platelets demonstrated increased collagen-induced (5 and 10 $\mu\text{g}/\text{ml}$) aggregation compared to 4-day stored platelets after transfusion.

Agonist-induced dense granule release data are shown in figure 4. These demonstrated an increase in collagen-induced (5 and 10 $\mu\text{g}/\text{ml}$) ATP release immediately after transfusion that was maintained unchanged for 2 h in both groups. Ristocetin-induced dense granule release was increased for 1-day platelets only after transfusion and was maintained for 2 h unchanged in this group. Fresh platelets demonstrated increased dense granule release compared to 4-day platelets for both collagen (5 and 10 $\mu\text{g}/\text{ml}$) and ristocetin-induced stimulation.

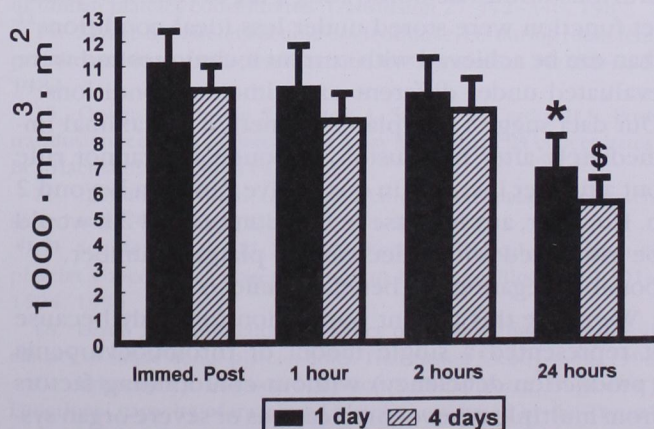


Fig. 2. Corrected count index over time for 1- and 4-day stored platelets in $1,000/\text{mm}^3/\text{m}^2$. * $P < 0.01$ versus immediately after and 1 hr after. \$ $P < 0.01$ versus all time points.

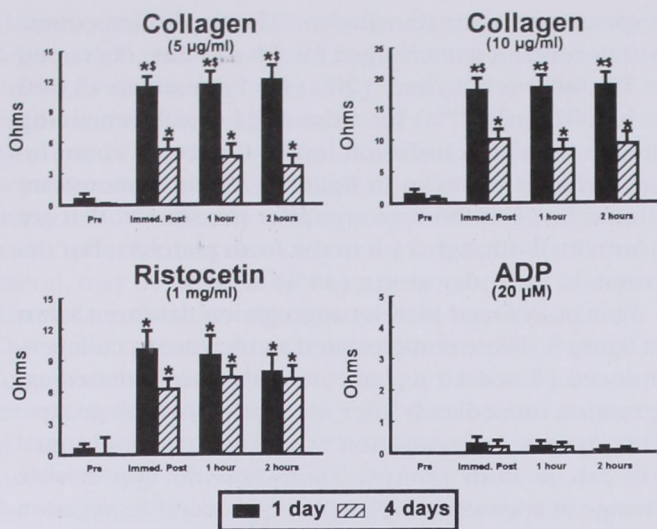


Fig. 3. Agonist-induced platelet aggregation over time for 1- and 4-day stored platelets in ohms of impedance. Agonists and their concentrations administered are denoted on each individual figure. * $P < 0.01$ versus pretransfusion values. § $P < 0.01$ versus 4-day stored platelets.

Discussion

Most platelet function studies after transfusion have been designed primarily to examine the long-term effects (days) of transfused platelets, while ignoring the immediate effects that are germane to planned invasive interventions. Some previous studies that have examined short-term platelet effectiveness after transfusion, have demonstrated that *in vivo* warm-up time is necessary to attain maximal platelet function.^{11,15,19} These previous studies demonstrating a lag in return of platelet function were stored under less ideal conditions¹¹ than can be achieved with current techniques and were evaluated under different experimental conditions.¹⁶ Our data suggest that platelet function is maximal immediately after transfusion, although we cannot rule out a further increase in qualitative function beyond 2 h. However, any increase in function beyond 2 h would be associated with a decrease in platelet number,^{17,18} possibly negating the beneficial effects.

We chose this patient population for study because it represented a single model of thrombocytopenia (production deficiency) without confounding factors from multiple types of medications or severe organ system disease (increased destruction or utilization). No patients in this trial were infected and all received prophylactic antibiotics according to protocol. While there

were some patients with abnormal liver and renal function tests, these were not indicative of severe organ dysfunction. We also excluded any patient who had previously received platelet transfusion to avoid the effects of alloimmunization. The results obtained with this selected population of patients may not be extrapolated readily to other groups of patients receiving platelet transfusions. However, this design was necessary to provide an "ideal" model for future comparisons, and many of these bone marrow transplantation patients do undergo invasive procedures at some time during the thrombocytopenic period. The use of single donor platelets as our transfusion product is standard of care in this patient population, offers potential advantages over random donor platelets,² and is frequently supplied by blood banks to other patients (personal communication, Dr. Paul Ness, Blood Bank Director, Johns Hopkins Hospital). In patients without prior platelet sensitization, random donor platelets might be equally efficacious,^{2,18} but similar experiments would be necessary to confirm this.

Whole blood aggregation and dense granule release were used for evaluating platelet function. This *ex vivo* technique does not require platelet preparation and closely recreates *in vivo* hemostasis, which involves both red and white blood cells. The contribution of

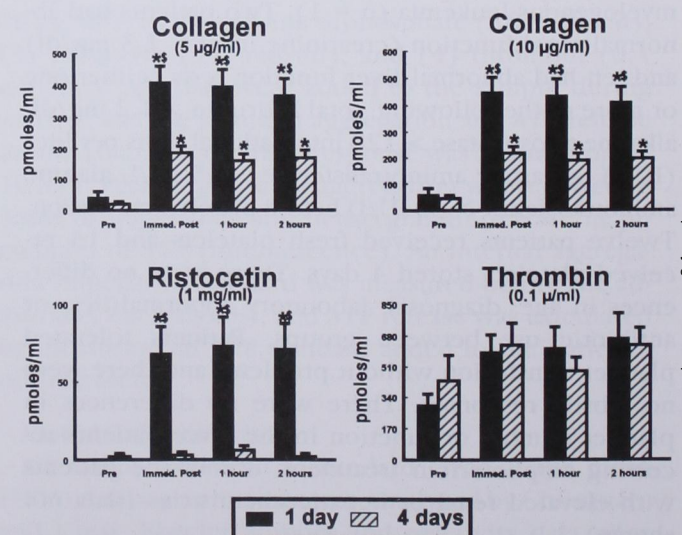


Fig. 4. Agonist-induced platelet dense granule release over time for 1- and 4-day stored platelets in picomoles of adenosine triphosphate per milliliter. Agonists and their concentrations administered are denoted on each individual figure. * $P < 0.01$ versus pretransfusion values. § $P < 0.01$ versus 4-day stored platelets.

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red and white blood cells to maximal platelet function recently has been described.^{20,21} Failure of adenosine diphosphate to induce aggregation immediately after transfusion has been reported previously, suggesting that the adenosine diphosphate surface receptor or postreceptor activity may be particularly sensitive to *ex vivo* storage.^{11,15,22}

Pretransfusion aggregation and dense granule release values are disproportionately reduced compared to posttransfusion values (based on absolute platelet count). This decreased function of pretransfusion platelets may represent platelets from megakaryocytes previously exposed to chemotherapeutic agents whose function is impaired, and/or that residual platelets were a much older cohort with reduced function.^{23,24} Posttransfusion increases in qualitative function may be coupled to increased platelet-platelet interaction (endogenous alpha and dense granule release further stimulating reactivity) from higher platelet counts, but this would not explain the difference in function between fresh and 4-day stored platelets. Functional differences between fresh and stored platelets have been shown, which demonstrated increased agonist-induced thromboxane production²² and improved *ex vivo* bleeding times^{19,25} for fresh *versus* stored platelets. These results support our findings, which show a differential effect between quantitative and qualitative function depending upon storage time. The most likely explanation for this is that stored platelets are an older cohort with decreased function.^{23,24}

Increased platelet count and reactivity up to 2 h after transfusion provide a framework to approach patients with severe thrombocytopenia for invasive procedures. Based on our data, surgery, intravascular line insertion, or epidural catheter placement could commence immediately after transfusion of platelets stored for 1–4 days and may also be performed up to 2 h without any *ex vivo* decrement in number or function. This has significance to anesthesiologists who may be contemplating procedures in thrombocytopenic patients or preoperatively advising our surgical colleagues about the timing of surgery. Additionally, fresh platelets have improved *ex vivo* function compared to platelets stored for 4 days and these may provide better *in vivo* hemostatic function.^{25,26} If this is confirmed *in vivo*, specific requests for fresh platelets could be made to the blood bank when patients are actively bleeding.²⁷ However, it should be kept in mind that the risk of bleeding depends on many additional hemostatic fac-

tors and data from this model of thrombocytopenia may not be extrapolated to other situations.

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