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EXTENDED STORAGE OF PLATELET-RICH PLASMA PREPARED PLATELET CONCENTRATES IN PLASMA OR PLASMALYTE

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Abstract

Background—Using bacterial detection or pathogen reduction, extended platelet storage may be licensed if platelet viability is maintained. FDA's post-storage platelet acceptance guidelines are that autologous stored platelet recoveries and survivals should be $\geq 66\%$ and $\geq 58\%$, respectively, of each donor's fresh platelet data.

Study Design And Methods—Non-leukoreduced platelet concentrates were prepared from whole blood donations. Autologous platelet concentrates from 62 subjects were stored in 100% plasma (n=44) or 20% plasma/80% Plasmalyte (n=18), an acetate based, non-glucose containing crystalloid solution previously used for platelet storage.(1-3) Fresh platelets were obtained on the day the donor's stored platelets were to be transfused. The fresh and stored platelets were alternately radiolabeled with either ⁵¹Chromium or ¹¹¹Indium, and *in vitro* measurements were performed on the stored platelets.

Results—FDA's platelet recovery criterion was met for 7 days of plasma storage, but platelet survivals maintained viability for only 6 days. Plasmalyte stored platelets did not meet either acceptance criteria after 6 days of storage. After 7 days of storage, platelet recoveries averaged $43 \pm 4\%$ and $30 \pm 4\%$ and survivals 4.1 ± 0.4 days and 2.0 ± 0.2 days for plasma and Plasmalyte-stored platelets, respectively (p=0.03 for recoveries and p<0.001 for survivals). Post-storage platelet recoveries correlated with the commonly-used *in vitro* platelet quality measurements of HSR and Annexin V binding, while survivals correlated with ESC, morphology score, and pH.

Conclusion—There is a progressive decrease in recoveries and survivals of plasma stored platelets over time. Platelet viability is better maintained in plasma than Plasmalyte.

Keywords

Platelets; Platelet Storage; Plasmalyte

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INTRODUCTION

In 1984, the Food and Drug Administration (FDA) extended the storage time of platelets from 5 days to 7 days. However, because of an increasing number of reports of bacterial transmission following extended storage, the FDA returned the storage time to 5 days in 1986. Incorporation of a variety of assays to detect viruses prior to transfusion has substantially reduced viral transmission by transfusion. For transfused platelets, bacterial transmission remains the greatest infectious risk.(4) Room temperature storage of platelets to maintain viability facilitates the growth of contaminating bacteria that may have entered the collection bag, usually because of inadequate cleansing of the venipuncture site.

Two different methods of preventing bacterial transmission by transfusion have been introduced. The first is pathogen reduction using the addition of either S-59 or riboflavin to the platelets followed by UV-A irradiation.(5,6) In addition, three different tests for detecting bacteria prior to transfusion have been licensed.(7-9) With pathogen reduction or bacterial detection, the FDA may allow extended platelet storage as long as platelet quality is maintained. The FDA recently permitted the extension of apheresis platelet storage to 7 days with the use of bacterial monitoring.(10-11) However, post-marketing surveillance demonstrated 2 out of 2,571 units that were negative for bacteria on initial culture were positive on repeat culture taken at 8 days.(12) Therefore, although licensed, 7-day stored platelets are currently not being transfused.

The FDA has suggested a hierarchical system of monitoring post-storage platelet quality starting with *in vitro* measures of platelet metabolism, biochemistry, and function.(13) If the changes in the storage conditions from those currently licensed are relatively minor, only *in vitro* measurements may be required. However, for extended storage of platelets in plasma, the FDA is requesting autologous radiolabeled platelet recovery and survival measurements in normal volunteers. If the storage conditions are changed even further, as is necessary for pathogen reduction of platelets, then transfusion experiments in thrombocytopenic patients are needed to document both platelet hemostasis and viability.(14,15)

Previously, the FDA suggested that using radiolabeled stored autologous platelets, the recovery and survival of the stored platelets should be within 20% of that achieved for the previously licensed platelets. Because this promotes a downward “creep” in platelet quality, Dr. Scott Murphy suggested establishing a standard comparing the recovery and survival of each volunteer's stored platelets to their fresh platelets.(16) He proposed that stored platelet recovery should be $\geq 66\%$ of each donor's fresh platelets, and survival should be $\geq 50\%$ of fresh. The different criterion for recovery *versus* survival are based on prior observations demonstrating the recovery of “fresh” allogeneic radiolabeled platelets given to thrombocytopenic patients is very similar to “fresh” radiolabeled autologous platelet recoveries in normal subjects ($60 \pm 15\%$ *versus* $66 \pm 8\%$, respectively), while platelet survivals in thrombocytopenic patients are markedly reduced compared to the survival of autologous platelets (3.4 ± 1.1 days *versus* 9.6 ± 0.6 days, respectively).(17) The FDA has indicated they will accept the 66% of fresh platelet recovery criterion for licensing, but stored platelet survivals should be 58% of each donor's fresh survival data.(18)

A Supplement to *Transfusion*(19-22) includes several papers on methods of radiolabeling platelets, how and when the comparative fresh platelets should be prepared and transfused, and the criteria for evaluating platelet viability at the end of storage. Using the methods proposed in the Supplement, we report data on the extended storage of platelet concentrates prepared from healthy subjects and stored in either plasma or Plasmalyte for 5 to 8 days. Plasmalyte is the only solution licensed in the U.S. for intravenous use that has been previously used as a platelet storage solution.(1-3) It is very similar in composition to other

platelet storage solutions that are being used in Europe.(23) We had several study objectives: 1) determine the longest acceptable storage time of platelets in plasma and Plasmalyte; 2) assess the relative efficacy of the two solutions for platelet storage; 3) evaluate whether each subject's stored platelet recovery and survival data correlated with their fresh platelet data; and 4) determine whether “*in vitro*” platelet quality measurements correlate with “*in vivo*” stored platelet recovery and/or survival results.

MATERIALS AND METHODS

Study Population

Healthy subjects who met allogeneic blood donor requirements were recruited to participate in these studies, and each signed a study consent. The study protocol and consent forms were approved by the Institutional Review Board of the University of Washington School of Medicine. Between 3 and 12 normal subjects participated in each of the storage studies. Fewer subjects participated in studies in which the initial data suggested the stored platelets would not meet acceptance criteria.

Preparation Of Stored Platelets

Each subject donated 450 mls of blood into Terumo Teruflex® blood bags with Diversion Sampling Arm (BB* AGQ456A2) (Terumo Corporation, Tokyo, Japan) using routine whole blood donation procedures. This bag is used routinely at our blood center and is made of PVC with citrate plasticizer. A subject participated in each study only once. All stored platelets met the manufacturers' guidelines for platelet concentration, total platelet count, and storage volume. Platelet concentrates were prepared from the donated whole blood within 1 hour of collection to allow return of each donor's red cells within 3 hours of donation so they could participate within 56 days in more than one type of storage study.

The whole blood was centrifuged at 2300g for 4½ minutes (start to finish), with 5 minutes of brake time to prepare platelet-rich plasma (PRP) which was then transferred to a satellite bag. The PRP was centrifuged at 3000g for 6 minutes to prepare a platelet concentrate. The platelet concentrates were not leukoreduced. Most of the plasma was transferred to another satellite bag. For the storage of platelet concentrates in plasma, an average of 67 ± 6 of plasma was left with the platelet concentrate. For storage of platelets in Plasmalyte, between 10 to 20 mls of plasma were left with the platelet concentrate, and 8 mls of Plasmalyte were added for every 2 mls of platelet concentrate to give an expected final concentration of 80% Plasmalyte and 20% plasma. The volume of the Plasmalyte stored platelets averaged 67 ± 8 mls, except for the 7-day stored Plasmalyte platelets where the volume averaged $110 \text{ mls} \pm 11$ due to one outlier with a volume of 222 mls. However, this large volume unit had similar data for both the *in vivo* and *in vitro* measurements as the other 7-day Plasmalyte stored platelets. Plasmalyte contains 90 mM of NaCl, 5 mM KCl, 27 mM Na-Acetate, 23 mM Na-Gluconate, and 3 mM magnesium chloride. The platelets were allowed to rest undisturbed at room temperature for 1½ hours before placing them in a Helmer incubator Model PF96 (Helmer Corporation, Fort Wayne, Indiana) for re-suspension and continuous agitation at 70 cycles/minute during storage at $22 \pm 2^\circ\text{C}$.

For platelets stored in Plasmalyte, albumin concentrations were determined in the donor's plasma and in their platelet concentrate after the addition of Plasmalyte. Based on the albumin dilution, the percentage of Plasmalyte in the platelet concentrate was calculated.

Preparation Of Fresh Platelets

Fresh platelets were prepared from a 43 ml sample of whole blood drawn from the subject into 9 mls of Acid-Citrate-Dextrose (ACD-A) anticoagulant. The whole blood was

transferred into two 50 ml Becton Dickinson conical screw-top tubes (Franklin Lakes, NJ) and rested for 30 minutes. The tubes were centrifuged at 200g for 15 minutes to prepare PRP, and the PRP from each tube was transferred to another conical tube. ACD-A equal to 15% of the PRP volume was added, the PRP was centrifuged at 2000g for 15 minutes, and the ACD added was calculated to give a pH of 6.5 to allow immediate platelet re-suspension.

***In Vitro* Platelet Assays**

Platelet counts were performed on the day following collection and after storage using an ABX Hematology Analyzer (ABX Diagnostics, Irvine, CA). After storage, several *in vitro* measurements of platelet quality were determined; i.e., glucose concentration, pH at 37°C, extent of shape change (ESC), and hypotonic shock response (HSR),(24) Annexin V binding, morphology score,(25) and mean platelet volume (MPV). HSR evaluates a platelets ability to recover normal volume after exposure to a hypotonic solution of distilled water. ESC measures the ability of platelets to change from discoid to spherical shapes after addition of ADP. Both of these assays use a chrono-log whole blood aggregometer (Chrono-Log Model 500-Ca, Havertown, PA) to measure the response photometrically. Annexin V binds to phosphatidyl serine which becomes expressed on the platelet surface with aging. Annexin V is fluorescently labeled to allow its detection using a FACSCAN (Becton Dickinson, San Jose, CA). For the Plasmalyte stored platelets, residual donor plasma that had been stored at 4°C was warmed to 22°C and added to the platelets to adjust the platelet count to 300,000/μl before the ESC and HSR measurements were performed.(26)

Platelet Radiolabeling

A 43 ml aliquot of the stored platelet concentrate was radiolabeled with either ^{111}In or ^{51}Cr , and the opposite label was used to radiolabel the donor's fresh platelets. Radiolabeling was performed by established techniques, and platelet recoveries and survivals were corrected for elution of the label and for residual radioactivity at day 10 post-transfusion that was considered due to contaminating radiolabeled red cells.(22) The radiolabeled platelet recoveries and survivals were calculated using the multiple hit COST program.(27) Blood samples were drawn from the donor before, at 2 hours, and on days 1, 2, 3, 5, 7, and 10 days post-infusion. The radiolabels used for the fresh and stored platelets were rotated with each sequential subject enrolled in a study so that, at the end of each storage experiment, equal numbers of the subjects' fresh and stored platelets were labeled with each isotope.

Statistical Methods

Summary statistics for *in vitro* and *in vivo* measurements of the quality of stored platelet concentrates were derived. Observations from individual units have been grouped by duration of storage (days) and the storage solution (plasma or Plasmalyte). Differences between group means and other linear contrasts have been evaluated for statistical significance. For planned comparisons, significance levels have been assessed using the t-distribution. The planned comparisons were: 5-day plasma stored platelets *versus* 6-, 7-, or 8-day plasma stored platelets; 5-day plasma stored platelets *versus* 6-, 7-, or 8-day Plasmalyte stored platelets; 6, 7, and 8-day plasma stored platelets *versus* Plasmalyte platelets stored for the same times; and all plasma stored platelets *versus* all Plasmalyte stored platelets. All other comparisons are considered unplanned. For the unplanned, post-hoc, comparisons, significance levels have been assessed using Scheffe's method.(28) Scatter plots are presented to show the degree of association between stored and fresh *in vivo* platelet recoveries and survivals. Similarly, scatter plots of *in vivo versus in vitro* measurements are also presented. Stepwise linear regressions of stored platelet recovery and survival measurements on post-storage *in vitro* measurements have been fit to determine which *in vitro* measures may predict post-storage platelet viability.

RESULTS

In Vivo Platelet Recoveries and Survivals

The radiolabeled recoveries and survivals of autologous platelets in plasma and Plasmalyte are given in Table 1. Fresh platelet recoveries averaged $62\% \pm 12\%$, and survivals 7.9 ± 1.6 days (± 1 S.D.) for the 62 subjects who participated in these studies. Recoveries for plasma stored platelets decreased progressively over the storage times of 5 to 8 days (Table 1, Figure 1A) ($p < 0.001$). After 8 days of plasma storage, platelet recoveries were significantly less than 5-day stored platelet recoveries ($p < 0.001$). However, 5-, 6-, and 7-day platelet recoveries were not significantly different. Differences in platelet recoveries may have been observed between 5, 6, and 7 days of storage if the sample sizes were larger, but such differences, if observed, may not be clinically relevant.

For the Plasmalyte stored platelets, average platelet recoveries were markedly reduced regardless of the storage time ranging from only $21 \pm 5\%$ to $30 \pm 4\%$ (Table 1, Figure 1A). Comparing plasma and Plasmalyte stored platelets for similar storage times, the Plasmalyte stored platelet recoveries were less at each storage interval but only the 7 day difference achieved statistical significance ($p = 0.03$) probably due to the small number of Plasmalyte studies at 6 and 8 days of storage (3 each).

Plasma stored platelet survivals also decreased progressively with increasing storage times of 5 to 8 days (Table 1, Figure 1B) ($p < 0.001$). Platelet survivals in plasma were significantly reduced compared to 5-day stored platelets after 7 and 8 days of storage ($p < 0.001$), but not after 6 days. Again, average platelet survivals for the Plasmalyte stored platelets were markedly reduced at any storage time and varied from only 0.7 ± 0.1 days to 2.0 ± 0.2 days (Table 1, Figure 1B). Plasmalyte stored platelet survivals were significantly less than plasma stored platelet survivals at each storage interval ($p < 0.001$).

Plasma and Plasmalyte stored platelet recoveries and survivals as a percentage of the same subjects' corresponding fresh data are shown in Figures 2A and 2B, respectively. These data clearly demonstrate two things: 1) at any storage interval, the percentage of stored *versus* fresh platelet recoveries and survivals of the plasma stored platelets are significantly better than the Plasmalyte stored platelets; and 2) there is a linear decrease in both plasma stored platelet recoveries and survivals as a percentage of fresh with increasing storage time ($p < 0.001$ for both). In contrast, there is no such linear relationship for the Plasmalyte stored platelets.

Comparing each subject's fresh *versus* their stored platelet recovery and survival data for each storage interval and each storage solution are shown in Figures 3 and 4, respectively. There is a statistically significant relationship between each subject's fresh and stored recovery for both plasma and Plasmalyte stored platelets (Figures 3A and B), but the association is not as strong for the Plasmalyte stored platelets ($p < 0.001$ and 0.03 , respectively). In contrast, only plasma stored platelets show a relationship between each subject's fresh and stored survivals, but the relationship is not very strong ($p = 0.03$) (Figures 4A and 4B).

In Vitro Measurements

The results of the *in vitro* measurements for the plasma and Plasmalyte stored platelet concentrates are shown in Table 2. The baseline platelet counts for the 62 subjects averaged $240,000 \pm 56,000/\pm 1$ (± 1 S.D.). Platelet counts did not decrease over storage times of 5 to 7 days in plasma or for 6 days in Plasmalyte. However, for platelets stored in plasma for 8 days or in Plasmalyte for 7 days, post-storage platelet counts decreased by 14% and 8%, respectively ($p = 0.05$ for both compared to pre-storage platelet counts).

Average post-storage pH values ranged between 6.9 ± 0.2 and 7.5 ± 0.1 . No platelet concentrate had a pH value of less than 6.4. The average mean platelet volume (MPV) for all plasma stored platelet concentrates was 6.7 ± 0.7 , and this compares to 7.7 ± 1.0 for all Plasmalyte stored platelets ($p=0.001$).

The morphology score tended to decrease over storage time for both the plasma and Plasmalyte stored platelets. Only after 7 days of storage did plasma *versus* Plasmalyte platelets differ with respect to morphology score ($p=0.02$). The extent of shape change (ESC) for both plasma and Plasmalyte stored platelets tended to decrease over storage time, with statistically significant reductions compared to 5-day plasma stored platelets for both storage solutions ($p \leq 0.02$) and all storage intervals except 6-day plasma stored platelets. In the comparison of storage solutions with respect to ESC, Plasmalyte stored platelets showed greater reductions in ESC than plasma stored platelets ($p \leq 0.01$ for 6, 7 and 8 days). Hypotonic shock response was well maintained for platelets stored in plasma for 6 and 7 days but was significantly reduced after 8 days of storage compared to platelets stored for 5 days in plasma ($p < 0.001$). For platelets stored in Plasmalyte, HSR was comparable to HSR of platelets stored in plasma for the same times ($p=0.37$). There were no significant differences in Annexin V binding for the 5- *versus* 6-day stored platelets in plasma. Annexin V binding increased for 7- and 8-day plasma stored platelets compared to the 5-day plasma stored platelets (both $p < 0.02$). For each comparable time period, the Annexin V binding was higher for the Plasmalyte *versus* plasma stored platelets, but these differences did not achieve statistical significance. As expected, the glucose concentration was well maintained in plasma stored platelets but was markedly reduced for the Plasmalyte stored platelets ($p < 0.001$).

***In Vitro* Data As Predictors Of *In Vivo* Post-Storage Platelet Recoveries and Survivals**

Of the 62 subjects who participated in these studies, *in vitro* data were available to correlate with their *in vivo* plasma and Plasmalyte stored platelet recoveries for 52 subjects and for stored platelet survivals for 55 subjects. Backwards stepwise linear regression models of platelet recovery and survival data using the *in vitro* assays as predictors were constructed. *In vitro* measures had to have a p-value of ≤ 0.05 to be retained in a model. The best multivariable model for predicting post-storage platelet recovery results had the equation $\text{platelet recovery} = 31\% + 0.25 \times \text{HSR} - 0.50 \times \text{Annexin V binding}$. Test day glucose also showed some association ($p=0.06$) due primarily to the lower values observed for the Plasmalyte stored units. This two-variable model accounted for 48% of the variability observed in stored platelet recoveries. Figure 5A shows scatter plots of stored platelet recoveries plotted individually against ESC, HSR, and Annexin V binding. ESC, while predictive of recovery, was highly correlated with HSR results, and so its inclusion did not improve the regression model.

The best multivariable model for predicting post-storage platelet survival results had the equation $\text{platelet survival} = -16 + 0.21 \times \text{ESC} + 2.16 \times \text{pH} + 0.007 \times \text{MorphScr}$. However, the relationship between pH and platelet survival is due primarily to some of the Plasmalyte stored units that had low pH's and correspondingly shortened platelet survivals. These three *in vitro* measurements accounted for 68% of the variability in the platelet survival measurements. Figure 5B presents scatter plots of stored platelet survivals *versus* each of these three variables individually. The regression coefficients (slopes of the lines) and summary statistics for individual variables with recovery or survival are given with the legends for Figure 5.

DISCUSSION

We have assessed the storage of autologous platelet-rich plasma prepared platelet concentrates at and beyond the currently-licensed 5 days. We have also evaluated whether Plasmalyte might be a better storage solution than plasma. This study has been performed using the suggested guidelines for radiolabeling platelets(22) and for using fresh platelets as a control to assess the quality of the stored platelets.(16)

A total of 62 healthy subjects participated in these studies, and, for each storage solution and storage time, between 3 and 12 subjects participated. Fresh platelet recoveries average $62 \pm 12\%$ and survivals 7.9 ± 1.6 days. These values are well within the expected range for fresh autologous platelet recoveries and survivals.(29) As expected, both autologous plasma stored platelet recoveries and survivals decreased progressively over the storage times of 5 to 8 days (Table 1 and Figures 1A and 1B, respectively). There was an inverse relationship between both platelet recoveries and survivals with respect to storage time ($p < 0.001$). In spite of the trend for lower recoveries with increasing storage times, plasma stored platelet recoveries did not differ over 5, 6, and 7 days of storage. Failure to demonstrate a statistical differences between these storage times may be related to the number of observations. However, after 8 days of storage, platelet recoveries were significantly less than platelet recoveries at 5 days ($p < 0.001$). Platelet survivals did not differ after 5 and 6 days of storage. However, after 7 and 8 days of storage, average platelet survivals were significantly less than 5-day stored platelet survivals ($p < 0.001$). Our data are comparable to previously published studies.(30-33)

The relationship between plasma stored and fresh platelet recoveries and survivals from the same subjects over storage time are given in Table 1 and Figures 2A and 2B, respectively. The data after 5 to 7 days of storage are not significantly different, while the 8-day data is significantly less than the 5-day data ($p < 0.001$) and does not meet the guidelines of either the FDA or Dr. Murphy for stored recoveries being 66% of fresh. Stored platelet survivals as a percentage of fresh survivals met the FDA guideline (stored survivals should be 58% of fresh) only for 6 days of storage, while Dr. Murphy's guideline was met for up to 7 days of storage (stored survivals should be 50% of fresh).

In contrast to the data for plasma stored platelets, Plasmalyte stored platelets had markedly reduced viability at all storage intervals tested of 6 to 8 days (Table 1 and Figures 1A and 1B). The 80% Plasmalyte concentration used in these studies was based on studies of Haemonetics apheresis platelets stored for 7 days in varying Plasmalyte concentrations of 50% to 82%. The best autologous post-storage platelet recovery and survival results were achieved with the highest Plasmalyte concentrations (unpublished data). Whether these data are applicable to other types of platelet products is unknown. However, Rock, *et al.* stored PRP platelet concentrates for 5 days in 85% Plasmalyte in Fenwal polyolefin bags (PL-732) with excellent platelet recoveries and survivals.(3) Plasmalyte platelets were not stored for 5 days as we wanted to determine if Plasmalyte could extend the storage time beyond the currently-licensed 5 days for plasma stored platelets. Only after 7 days of Plasmalyte storage were enough studies done to show a statistically significant decrease in platelet recoveries ($p = 0.03$) and survivals ($p < 0.001$) compared to 7-day plasma stored platelets. In addition, there was no relationship between either Plasmalyte platelet recoveries or survivals over storage time as there was for the plasma stored platelets (Figures 1A and 1B). Furthermore, Plasmalyte stored platelet recoveries and survivals did not meet either the FDA's or Dr. Murphy's guidelines at any storage interval (Table 1 and Figures 2A and 2B).

If fresh platelet recovery and survival data are to be used as a control for each subject's stored platelet data, there should be a relationship between each subject's fresh and stored

data. The reason each subject's fresh data were proposed as a control is because there is known to be substantial heterogeneity in platelet recovery and survival data among subjects. (29) Indeed, there was a relationship between each subject's fresh and stored platelet recovery data whether the platelets were stored in plasma or Plasmalyte ($p < 0.001$ and $p = 0.03$, respectively) (Figures 3A and 3B, respectively). However, there was only a weak relationship between each subject's fresh and stored plasma platelet survival data ($p = 0.03$) (Figure 4A), and there was no relationship between Plasmalyte fresh and stored platelet survival data (Figure 4B).

Buffy coat platelets are well-maintained in platelet storage solutions that are similar to Plasmalyte.(23) There may be several variables that may effect the ability of platelets to be stored in platelet additive solutions: 1) the method of platelet preparation; 2) the composition of the platelet additive solution; 3) the amount of residual plasma; 4) the composition of the storage bag; and 5) the storage duration. It may be that the PRP method of preparing platelets which hard spins the platelets against the bottom of the bag requiring resting and then re-suspension *versus* the buffy coat method in which the platelets are hard-spun against the red cells is more injurious to the platelets.(34) It has been previously suggested that the optimum concentration of PAS should be 65% with 35% residual plasma to have enough glucose left at the end of storage to meet the energy needs of platelets during storage.(35) However, we have been able to store Haemonetics-collected apheresis platelets for 13 days in Plasmalyte with post-storage platelet viability that meets FDA guidelines, and these platelets have no residual glucose.(36) There is a substantial amount of controversy regarding whether residual glucose must be present to maintain platelet viability during storage.(37,38) Our recent extensive storage studies evaluating apheresis platelets stored in Plasmalyte have also indicated that the storage bag is critically important for maintaining platelet viability during storage in Plasmalyte.(36) For example, Rock, *et al.*(3) obtained excellent post-storage results with platelets stored in a polyolefin bag. At similar Plasmalyte concentrations, we obtained very poor results when platelets were stored in a PVC container with citrate plasticizer. More studies are needed storing various platelet preparations in different amounts and types of storage solutions and bags to determine the optimal parameters that will permit extended platelet storage in additive solutions.

In general, the *in vitro* assays showed greater changes for the Plasmalyte compared to the plasma stored platelets. In prior studies, a correlation between any *in vitro* measurement and either post-storage platelet recovery or survival data has often not been detected.(39,40) However, because of the range of *in vivo* results observed in these plasma and Plasmalyte extended platelet storage studies, some correlations between *in vitro* and *in vivo* measurements were observed. The best predictors of *in vivo* platelet recoveries were HSR ($p = 0.005$) and Annexin V binding ($p = 0.04$) (Figure 5A). These measurements accounted for 48% of the variability in stored platelet recoveries. ESC was highly correlated with HSR and did not add anything to the regression model. The best predictors of platelet survivals were ESC ($p = 0.000$), pH (0.004), and morphology score ($p = 0.05$) (Figure 5B). However, the pH relationship with survival was mainly related to some Plasmalyte units with low pH.

In summary, there are progressive decreases in both the recoveries and survivals of plasma stored platelets over storage times of 5 to 8 days. There is a strong relationship between each subject's fresh and stored platelet recoveries and a much weaker relationship between fresh and stored platelet survivals. When stored recoveries were expressed as a percentage of each subject's fresh recoveries, stored platelet recoveries achieved the proposed acceptance guideline of being $\geq 66\%$ of fresh recoveries after 7 days of storage (i.e., $72 \pm 11\%$), but they failed the criteria after 8 days of storage ($55 \pm 10\%$). Platelet survivals as a percentage of fresh platelet survivals met Dr. Murphy's guideline of $\geq 50\%$ of fresh after 7 days of storage (i.e., $51 \pm 16\%$) but only met the FDA's guideline of 58% after 6 days of storage (i.e., $64 \pm$

22%). However, the average survival of 7-day plasma stored platelets is 4.1 ± 0.4 days, which clearly meets the needs of thrombocytopenic patients where interval-to-next transfusion in 533 patients undergoing induction therapy for AML who received 5,848 transfusions averaged only 1.8 ± 1.3 days.⁽⁴¹⁾ In addition, the FDA's guideline for platelet survivals was established without any supportive data. Since 7-day platelets were licensed for 2 years with no reported problems with hemostasis or post-transfusion increments or survivals, it seems reasonable to, again, allow them to be licensed as long as sterility can be assured either with pathogen reduction or bacterial testing. Furthermore, when platelets are made in the time frame used by most blood centers (i.e., 2 to 6 hours from collection rather than the short time frame we used ≤ 1 hour), the viability of platelets may be better maintained.^(32,42) Finally, the data indicates that storing platelets in Plasmalyte not only does not improve post-infusion platelet viability but clearly produces an inferior product at all the storage times tested compared to plasma stored platelets; i.e., between 6 to 8 days. However, the Plasmalyte storage conditions that were used in our studies may not have been optimal.

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1A

1B

Figure 1. Autologous Radiolabeled Platelet Recoveries And Survivals Over Storage Time

1A. Platelet Recoveries *Versus* Storage Time.

1B. Platelet Survivals *Versus* Storage Time.

The relationship between platelet recoveries and survivals and storage time is shown for plasma stored platelets in circles (●) and for Plasmalyte stored platelets in triangles (▲). Data are given as the average $\pm$ 1 S.E. The regression line for the plasma stored platelet recoveries has a slope of -6.5 ($r^2=0.29$, and $p<0.001$) and for platelet survivals has a slope of -1.0 ($r^2=0.43$, and $p<0.001$). In contrast, there is not a statistically significant relationship between storage time for either platelet recoveries or survivals for the Plasmalyte stored platelets.

2A

2B

Figure 2. Stored Platelet Recoveries And Survivals As A Percentage Of The Same Donors' Fresh Platelet Recoveries And Survivals

2A. Stored Platelet Recoveries As A Percentage Of Fresh Over Storage Time.

2B. Stored Platelet Survivals As A Percentage Of Fresh Over Storage Time.

Plasma data are shown as circles (●) and Plasmalyte data as triangles (▲). The data are given as the average ± 1 S.E. of the donors' stored platelet recoveries or survivals as a percentage of the same donors' fresh platelet recoveries or survivals, respectively. In Figure 2A, the horizontal dashed line is stored recoveries at 66% of fresh, and, in Figure 2B, the horizontal lines are stored survivals of either 58% or 50% of fresh (the FDA and Dr. Murphy's acceptance criteria, respectively). The regression line for the plasma stored platelet recoveries as a percentage of fresh has a slope of -9.8, ($r^2=0.44$ and $p<0.001$), and the regression line for the plasma stored platelet survivals as a percentage of fresh has a slope of -12.8, ($r^2=0.44$ and $p < 0.001$). For the Plasmalyte stored platelets, there is no relationship between storage time and the ratios of stored to fresh platelet recoveries or survivals.

3A

3B

Figure 3. Comparison of Each Donor's Fresh Versus Their Stored Autologous Platelet Recovery
 3A. Plasma Stored Platelet Recovery Compared To The Same Donor's Fresh Platelet Recovery.

3B. Plasmalyte Stored Platelet Recovery Compared To The Same Donor's Fresh Platelet Recovery.

Five-day storage data are plotted as ($\triangleright$), 6 day data as ($\bullet$), 7 day data as ($\square$), and 8 day data as ($\blacktriangle$). The regressions for the plasma and Plasmalyte stored data are summarized by slopes of 0.75 and 0.54 ($r^2=0.39$ and 0.27 , and $p<0.001$ and 0.03 respectively).

4A

4B

Figure 4. Comparison of Each Donor's Fresh *Versus* Their Stored Autologous Platelet Survival

4A. Plasma Stored Platelet Survival Compared To The Same Donor's Fresh Platelet Survival.

4B. Plasmalyte Stored Platelet Survival Compared To The Same Donor's Fresh Platelet Survival.

Five-day storage data are plotted as ($\triangleright$), 6 day data as ($\bullet$), 7 day data as ($\square$), and 8 day data as ($\blacktriangle$). The regression line for the plasma stored data has a slope of 0.36 ($r^2=0.11$ and $p=0.03$). In contrast, there is no relationship between each donor's Plasmalyte stored platelet survival data and their corresponding fresh platelet survival data.

Figure 5. Associations Between Post-Storage *In Vitro* And *In Vivo* Platelet Measurements

5A. Post-Storage *In Vitro* Measurements Versus Stored Platelet Recoveries.

Plots of post storage platelet recovery *versus* individual *in vitro* measurements. Statistics for the regression lines: ESC (slope = 1.3, $r^2=0.25$ and $p = 0.001$); HSR (slope = 0.37, $r^2=0.28$ and $p=0.001$); and Annexin V binding (slope = -0.60, $r^2=0.26$ and $p=0.001$). Plasma data are shown by closed circles (•), and Plasmalyte data as closed triangles (▲).

5B. Post-Storage *In Vitro* Measurements Versus Stored Platelet Survivals.

Plots of post storage platelet survival *versus* individual *in vitro* measurements. Statistics for the regression lines: ESC (slope = 0.26, $r^2= 0.60$ and $p < 0.001$); pH 37°C (slope = 3.6, $r^2= 0.22$ and $p = 0.001$); and morphology score (slope = 0.02, $r^2= 0.30$ and $p = 0.001$). Plasma data are shown by closed circles (•) and Plasmalyte data as closed triangles (▲).

IN VIVO RADIOLABELED AUTOLOGOUS PLATELET RECOVERIES AND SURVIVALS

TABLE 1

Storage Conditions		N	Platelet Recoveries (%)		Stored Recoveries (% of Fresh)	Platelet Survivals (Days)		Stored Survivals (% of Fresh)
Solution	Time (Days)		Fresh	Stored		Fresh	Stored	
Plasma	5	10	62 ± 4	55 ± 4	88 ± 11	7.7 ± 0.5	6.1 ± 0.3	76 ± 17
Plasma	6	10	61 ± 2	46 ± 4	78 ± 11	8.1 ± 0.5	5.1 ± 0.5	64 ± 22
Plasmalyte	6	3	59 ± 9	29 ± 9	47 ± 15	7.2 ± 1.0	1.8 ± 0.1	26 ± 6
Plasma	7	12	60 ± 3	43 ± 4	72 ± 11	8.7 ± 0.5	4.1 ± 0.4	51 ± 16
Plasmalyte	7	12	66 ± 4	30 ± 4	46 ± 20	8.3 ± 0.3	2.0 ± 0.2	24 ± 10
Plasma	8	12	60 ± 4	34 ± 3	55 ± 10	7.2 ± 0.6	2.9 ± 0.4	41 ± 16
Plasmalyte	8	3	70 ± 2	21 ± 5	27 ± 8	8.2 ± 0.5	0.7 ± 0.1	12 ± 7

Data is reported as the average ±1 S.E.

PRO PLATELET MEASUREMENTS

TABLE 2

PLATELET COUNTS			TEST DAY						
Donor/μl	Day 1 (x 10 ¹⁰)*	Test Day (x 10 ¹⁰)*	pH**	MPV	Morphology Score	ESC	HSR	Annexin V Binding	Glucose (mgm/dl)
250,000 ± 80,000	9.2 ± 1.0	8.6 ± 0.7	7.54 ± 0.04	6.8 ± 0.2	336 ± 7	17 ± 1	62 ± 5	11 ± 2	318 ± 6
244,000 ± 44,000	6.9 ± 0.6	7.2 ± 0.7	7.47 ± 0.03	6.5 ± 0.2	278 ± 18	16 ± 2	50 ± 5	13 ± 1	306 ± 9
164,000 ± 23,000	7.1 ± 1.4	8.6 ± 2.1	6.89 ± 0.15	7.5 ± 0.6	262 ± 33	4 ± 1	39 ± 11	26 ± 7	28 ± 14
244,000 ± 46,000	6.0 ± 1.0	6.0 ± 1.1	7.44 ± 0.11	6.6 ± 0.3	288 ± 10	14 ± 1	53 ± 8	16 ± 1	269 ± 32
245,000 ± 45,000	7.1 ± 0.5	6.5 ± 0.6	7.20 ± 0.04	7.5 ± 0.3	242 ± 16	9 ± 2	36 ± 5	19 ± 2	22 ± 6
236,000 ± 62,000	7.1 ± 0.7	6.1 ± 0.5	7.51 ± 0.13	7.0 ± 0.2	245 ± 15	10 ± 1	20 ± 3	23 ± 3	224 ± 31
275,000 ± 13,000	6.3 ± 1.9	4.1 ± 1.1	6.97 ± 0.28	8.4 ± 0.8	232 ± 32	3 ± 0	30 ± 11	57 ± 18	11 ± 7

orted as average ±1 S.D.
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etelet count.
asured at 37°C.