Sustained Elevation of Intracellular Cyclic 3'-5' Adenosine Monophosphate Is Necessary for Preservation of Platelet Integrity During Long-Term Storage at 22°C

By Arthur P. Bode, Stein Holme, W. Andrew Heaton, and Melvin S. Swanson

Preservation of platelet integrity and responsiveness was examined in platelet concentrates prepared in the presence of various formulations and combinations of platelet-activation inhibitors affecting intracellular levels of cyclic 3'-5' adenosine monophosphate (cAMP). Platelet concentrates were prepared and stored in an artificial medium for two weeks at 22°C. Markers of metabolic activity (pH, lactate, pO2, pCO2 in the medium), aggregation response, hypotonic shock response, and glycoprotein Ib (GPIb) expression were assessed along with direct measurements of cAMP in platelet pellets and thromboxane B2 (TXB2) in the supernate. The platelet concentrates prepared with only adenylyl-cyclase stimulators (prostaglandin E1 or forskolin) showed less maintenance of the integrity and responsiveness markers and greater loss of GPIb than concentrates prepared with phosphodiesterase inhibitors (theophylline or caffeine) or combinations with the above. These results were correlated with the ability of these compounds to sustain elevation of cAMP above basal level during the entire extended-storage period. The strong correlation (r = -0.67) between elevation of cAMP levels and suppression of TXB2 production suggests that the phosphodiesterase inhibitors provided better protection than stimulators of adenylyl cyclase alone through a reduction in platelet activation and its deleterious effects on preservation of platelets during storage. This is a US government work. There are no restrictions on its use.

MATERIALS AND METHODS

Reagents. PGE-1 (crystalline) was purchased from Sigma Chemical Co (St Louis, MO) and solubilized at 1 mg/mL in absolute ethanol before aqueous dispersion. Theophylline (anhydrous), caffeine, and IBMX also were purchased from Sigma and solubilized directly in citrated saline as needed. Forskolin was obtained from Calbiotech, Inc (Irvine, CA) and solubilized at 15 mg/mL in absolute ethanol before use. The artificial medium in which the platelets were resuspended for storage contained nonlactated Ringer's, citrate, dextrose, KCl, and MgSO4 as described elsewhere from pharmaceutical-grade components.

Preparation of platelet concentrates. Platelet concentrates were prepared by a two-step centrifugation procedure with units of citrated blood (CPDA-1) freshly collected from healthy volunteer donors by Red Cross personnel using Fenwal Blood Pack collection sets (Baxter Healthcare, Deerfield, IL) as described elsewhere. In brief, platelet-rich plasma was harvested after centrifugation on a
Sorvall RC-3C (DuPont, Wilmington, DE) at 3,100 rpm for 3 minutes, 5 seconds for a total-force integral of $1.5 \times 10^3$ rad/s. One-eighth volume citrated saline (7 parts 0.9% saline plus 1 part CPDA-1) plus or minus dissolved inhibitors was added through a sampling-site coupler to the platelet-rich plasma for a 30-minute incubation at room temperature before the second centrifugation (3,000 rpm, 10 minutes) to pellet the platelets. Artificial medium containing the same inhibitors was added after aspiration of platelet-poor plasma, and the platelets were resuspended after 30 to 45 minutes. The volume of artificial medium to be added (40 to 60 mL) was based on a target platelet count in the concentrate of $1.35 \times 10^9$/mL.

After resuspension, the platelet concentrates were treated with 2.4 mL 8.4% NaHCO$_3$ per 60 mL of concentrate and stored in room air with slow (two rotations per minute) tumbling agitation at 22°C in PL-732 plastic containers folded to give a surface-to-volume ratio of 4 cm$^2$/mL instead of the usual 7 cm$^2$/mL. The decrease in bag volume of artificial medium to be added (40 to 60 mL) was based on a target platelet count in the concentrate of $1.35 \times 10^9$/mL.

Platelet counts were determined on a Coulter Counter S Plus IV (Hialeah, FL).

Hypotonic shock response (HSR) was measured turbidimetrically as the rate of recovery after swelling of platelets in the presence of one-third volume deionized water as described elsewhere. Aggregation response was measured on a BioData PAP-4 aggregometer (Hatboro, PA) with $1 \times 10^{-3}$ mol/L adenosine diphosphate (ADP) (BioData) or 1.5 mg/mL ristocetin (Helena Labs, Beaumont, TX) (final concentrations) after pelleting and resuspension of platelet concentrate samples in inhibitor-free citrated plasma at 30°C.

Platelet concentrates were prepared with 2.4 mL 8.4% NaHCO$_3$ per 60 mL of concentrate and stored in room air with slow (two rotations per minute) tumbling agitation at 22°C in PL-732 plastic containers folded to give a surface-to-volume ratio of 4 cm$^2$/mL instead of the usual 7 cm$^2$/mL. The decrease in bag volume of artificial medium to be added (40 to 60 mL) was based on a target platelet count in the concentrate of $1.35 \times 10^9$/mL.

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Released lactic dehydrogenase (LDH) was measured on a Technicon RA-1000 discrete analyzer (Tarrytown, NY) in the supernate of platelet concentrate samples (centrifuged on an Eppendorf Microfuge (Brinkmann Instruments, Westbury, NY) at 15,600g for 2 minutes) and reported as a percentage of the total LDH appearing before freezing and thawing of unspun platelet suspension.

Flow cytometry analysis of surface glycoprotein Ibb (GPIib) was performed as described in detail elsewhere. To 100 mL of washed platelets at 1 $\times 10^8$/mL was added 100 $\mu$L (0.2 $\mu$g) of a murine monoclonal antibody (MoAb) to GPIib (clone AN-51, Dako Corp, Santa Barbara, CA) or 100 $\mu$L (5 $\mu$g) of an irrelevant murine IgG2a (Coulter Immunology). After incubation of 30 minutes at room temperature, 100 $\mu$L (6 $\mu$g) of fluorescein isothiocyanate (FITC)-labeled goat-antimouse IgG (Coulter Immunology) was added for another 30-minutes incubation without washing to minimize clumping.

The sample was further diluted 1:10 in isotonic saline before analysis on a Becton Dickinson FACS 440 flow cytometer (Mountain View, CA). Cursors were set on fluorescence histograms on the Data Consort 40 system to determine the percentage of platelets appearing to be negative for specific binding of AN-51 (GPIb-) and to determine the mean peak channel of the positive fluorescence distribution.

Intracellular cyclic AMP levels were determined from frozen platelet pellets with a commercial radioimmunoassay kit (Diagnostic Products, Los Angeles, CA) and reported in units of picomoles/10$^9$ platelets.

TxB$_2$ levels were determined in the supernate of platelet-concentrate samples (Eppendorf Microfuge, 15,600g for 2 minutes) with a commercial radioimmunoassay kit (New England Nuclear, Boston, MA) and reported in units of nanograms/10$^9$ platelets.

Inhibitor formulations. Several different CAMP-active agents were used singly or in combination in these studies; the final concentrations of these agents in the platelet concentrate were as follows: (1) no inhibitors; (2) 300 nmol/L PGE-1, (n = 8); (3) 10 $\mu$mol/L forskolin, (n = 4); (4) 200 $\mu$mol/L IBMX, (n = 4); (5) 500 $\mu$mol/L caffeine, (n = 4); (6) 2 mmol/L caffeine, (n = 4); (7) 2 mmol/L theophylline, (n = 6); (8) 300 nmol/L PGE-1 + 2 mmol/L theophylline, (n = 10). Platelet concentrates were prepared with these formulations in batches of four units per experiment. A few concentrates were excluded from analysis because of low platelet

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**Fig 1.** Metabolic activity of platelet concentrates prepared in various inhibitor formulations: 1 (C), no inhibitors; 2 (D), 300 nmol/L PGE-1 only; 6 (E), 2 mmol/L caffeine; 7 (A), 2 mmol/L theophylline; B (B), 300 nmol/L PGE-1 + 2 mmol/L theophylline. The metabolic markers were measured directly in samples of the storage medium taken at the times indicated: extracellular pH (A); extracellular lactate concentration (B); pCO$_2$ in the storage container (C); pCO$_2$ in the storage container (D).
### Table 1. Mean Ranks and Concordance Coefficients of the Performance of Metabolic Markers, Aggregations Studies, and Integrity Markers in Platelet-Inhibitor Formulations After 10, 15, and 20 Days of Storage

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Metabolic Markers</th>
<th>Aggregation Response</th>
<th>Integrity Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 d</td>
<td>15 d</td>
<td>20 d</td>
</tr>
<tr>
<td>(1) No inhibitors</td>
<td>5.5</td>
<td>5.5</td>
<td>4.3</td>
</tr>
<tr>
<td>(2) PGE-1 only</td>
<td>6.0</td>
<td>4.8</td>
<td>6.0</td>
</tr>
<tr>
<td>(4) IBMX</td>
<td>4.3</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>(5) 500 μmol/L caffeine</td>
<td>5.0</td>
<td>4.8</td>
<td>4.7</td>
</tr>
<tr>
<td>(6) 2 mmol/L caffeine</td>
<td>2.5</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>(7) 2 mmol/L theophylline</td>
<td>2.0</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>(8) PGE-1 + theophylline</td>
<td>3.3</td>
<td>2.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Concordance coefficient: 0.58, 0.89, 0.58, 0.28, 0.77, 0.52, 0.13, 0.6, 0.79

The lowest (best) ranks were accorded to the formulations with the least change in pH and lowest lactate levels, lowest pO2 in the bag, highest pCO2, highest aggregation slopes, greatest change in %T for each agonist, highest HSR, lowest released LDH, and lowest % GPIb- subpopulation.

Abbreviation: NS, not significant.

* Metabolic markers are pH, pO2, pCO2, and lactate.
† Aggregation studies are 10 μmol/L ADP and 1.5 mg/mL ristocetin; %T and slope.
‡ Integrity markers are HSR, GPIb positivity, and LDH released.

RESULTS

It would be difficult to adequately represent all the data for all the formulations of inhibitors in each of the figures. To emphasize the major effects of each type of compound, only formulations 1, 2, 6, 7, and 8 are illustrated fully. These examples show the improvements caused by addition of a phosphodiesterase inhibitor over PGE-1 alone or no inhibitors at all. Results for formulations 4 and 5 are included in the statistical summaries in text and tables below to show more subtle distinctions such as the inadequacy of IBMX or low-dose caffeine compared with other groups. The data count (less than 1 X 10^9/mL) or evidence of bacterial contamination. Assays of cAMP and TxB2 were performed on each unit, but not all of the other parameters were measured on each because of sampling-volume limitations.

**Statistical methods.** All statistical analyses were made with the SPSS/PC plus 4.0 statistical package. Because of the skewness in the numeric measures of the various cell markers used to evaluate the effectiveness of the cAMP-formulation groups, a rank transformation was used to convert the numeric measures to ranks. The lower ranks were assigned to those numeric values associated with the best marker performance, whereas the higher ranks were assigned to those numeric values associated with the poorest marker performance. Comparisons of metabolic activity, aggregation response, cell integrity, cAMP level, and TxB2 level between the various cAMP-active formulation groups was done by an analysis of variance of the ranks. Pairwise mean comparisons were analyzed with the Tukey multiple-range test. The Kendall coefficient of concordance was used to assess the extent to which the various cell markers agreed in their ranking of the CAMP-active formulation groups. The linear relationship between cAMP and TxB2 levels was determined by the Spearman rank-order correlation coefficient. Statistical significance was indicated by P values less than .05.

Fig 2. Cell-integrity markers of platelet concentrates prepared in various inhibitor formulations: symbols listed as in Fig 1 legend. Recovery from hypotonic shock is shown as percent of Day 1 response (A) and released LDH is shown as percent of total LDH in frozen-thawed platelet suspensions (B).
generated from the forskolin-treated platelet formulation 3 are mentioned only briefly in the text because of the interference we experienced in obtaining a precise platelet cAMP level in these samples.

Metabolic markers. The platelet-concentrate storage medium used in these studies contains buffering compounds and nutrients in a balanced salt solution to provide support of platelet metabolic activity for 7 to 10 days. Extension of the storage period to 20 days served to accentuate the nonlinear changes and differences in pH, lactate, pO2, and pCO2 among the treatment groups. As seen in Fig 1, the pH was maintained best in the formulations containing a phosphodiesterase inhibitor. The platelet concentrates treated only with PGE-1 showed a significant decrease in pH after the tenth day of storage similar to that in the control group. This finding was apparently caused by the increased production of lactate in the absence of a phosphodiesterase inhibitor. Continued respiratory activity was more evident in the platelet concentrates containing 2 mmol/L theophylline or caffeine as judged by lower pO2 and higher pCO2 inside the containers in the latter phases of the storage period. It seemed that PGE-1 alone did not prevent or retard the metabolic changes that occurred after 10 to 15 days in the controls.

Formulations 3 to 5 also showed severe metabolic changes. The pH decreased to less than 6.0 in each of the IBMX-treated platelet concentrates by day 15, and the lactate levels reached 40 to 50 mmol/L; the pO2 and pCO2 values at day 15 indicated no further respiratory activity. The platelet concentrates containing forskolin were in a similar state by day 15: pH less than 6.0, lactate 40 to 50 mmol/L, pO2 ≥ 180, pCO2 ≤ 12. The units prepared with 500 μmol/L caffeine showed wide variability within the group with pH between 5.67 to 6.89, lactate 30 to 57 mmol/L, pO2 = 122 to 190, pCO2 = 25 to 9 by day 15. Whereas the IBMX and forskolin formulations seemed to have failed outright, the low dose of caffeine appeared to give mixed results.

The rank transformations of the metabolic measures at days 10, 15, and 20 are given in Table 1. It is seen that formulations 6 to 8 clearly surpassed the others at all days. The common feature of these formulations was the presence of a higher concentration (2 mmol/L) of an effective phosphodiesterase inhibitor (theophylline or caffeine). The significant concordance coefficients for days 10, 15, and 20 show that all four metabolic markers were in strong agreement in ranking the formulations.

Integrity and responsiveness markers. The response of platelets to hypotonic shock has been used extensively to assess the preservation of platelets in vitro in relation to their likely recoveries in vivo in autologous infusions. In our study, the hypotonic shock test showed better responsiveness in platelet concentrates prepared with the formulations containing a phosphodiesterase inhibitor (6 to 8) after 10 to 15 days of storage (Fig 2A). Whether this finding predicts accurately an enhanced recovery or survival of these platelets after infusion is uncertain, but preliminary in vivo evidence presented elsewhere is encouraging in that platelet concentrates prepared in formulation 8 gave a much improved lifespan measurement over controls in autologous donors. Another general marker of cell integrity used in this
study was the cumulative release of LDH into the supernate during storage. Again, the formulations containing a phosphodiesterase (6 to 8) inhibitor prevented a general rise in LDH better than PGE-1 alone (Fig 2B).

Aggregation and agglutination responses were tested after the stored platelets were resuspended in inhibitor-free citrated plasma. Typical light-transmittance tracings from four types of concentrates are depicted in Fig 3. At day 10, formulation 1 without inhibitors showed little responsiveness to either 10 μmol/L ADP or 1.5 mg/mL ristocetin; at day 15, the concentrates with PGE-1 only (formulation 2) also showed significantly worse response than those formulations containing a phosphodiesterase inhibitor (formulations 7 and 8). For statistical comparisons among groups, the slope and maximum extent of aggregation for each sample tested were ranked and summarized in Table 1.

We and others have shown that GPIb expression is altered during storage of platelets; a population of platelets appears that no longer specifically binds MoAbs to surface GPIb (GPIb-). During extended storage (10 to 20 days), the specific binding of GPIb MoAbs decreases, in general, for the whole distribution of platelets, but may be moderated by the addition of PGE-1 and theophylline. In the present study, the concentrates without inhibitors (formulation 1) showed a substantial population of GPIb- platelets at day 10 and a broad shift in immunofluorescence at day 15, also evident in the concentrate with PGE-1 only (formulation 2) (see examples in Fig 4). The concentrates containing phosphodiesterase inhibitors (formulations 7 and 8) showed very little change in the broad distribution pattern and only a minor appearance of apparently GPIb- platelets. The data means for GPIb- platelets among formulations 1, 2, 6, 7, and 8 are shown in Fig 5. These data for each formulation were ranked along with HSR and supernatant LDH levels as integrity markers for statistical comparisons shown in Table 1.

As was observed in the metabolic markers, the IBMX or forskolin-treated concentrates showed variably poor results by day 15; no response in the hypotonic shock test, LDH levels between 2% to 49%, little or no aggregation with ADP or ristocetin, and 8% to 80% GPIb- platelets by flow cytometry. The units containing 500 μmol/L caffeine showed wide variability in results at day 15 and 20, but with better average values than IBMX or forskolin: 0% to 85% HSR, LDH levels between 6% to 19%, three of four units still aggregated fully with ADP or ristocetin, and only 12% to 59% platelets were GPIb-.

The rankings of these markers in Table 1 show a pattern mostly consistent with the above analysis of metabolic activity. The best rankings were achieved with formulation (6) to (8) at days 15 and 20. Concordance among the aggregation or integrity markers was less than that for the metabolic markers, except at the later time points.

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![Fig 4. Example fluorescence histograms of stored platelets stained with the MoAb AN-51 marking GPIb at day 10 (A) and day 15 (B) of storage. Same groups illustrated as in Fig 3; summary data are given in Fig 5.](image-url)
tion, CAMP levels were measured directly in platelet pellets containing 2 mmol/L caffeine or theophylline, or a combination of both. The analysis of variance clearly shows that the formulations containing a phosphodiesterase inhibitor appeared to work better than adenylate-cyclase stimulators in platelet preservation. Formulations 6 to 8, all containing 2 mmol/L theophylline, had the highest cAMP levels, had the lowest TxB2; however, this could be appreciated only on the logarithmic scale in Fig 7 and may not be relevant. Among the nonillustrated formulations, Forskolin (3) and IBMX (4) gave mean TxB2 levels at day 15 and day 20 >400 ng/10^9 platelets; formulation 5, with 500 μmol/L caffeine, gave highly variable results with mean TxB2 = 60 at day 15 and 389 at day 20.

Table 3 shows the results of transforming the numeric measures of cAMP and TxB2 to ranks. The formulations containing 2 mmol/L concentration of a phosphodiesterase inhibitor (formulations 6 to 8) had higher cAMP and lower TxB2 levels than the other formulations. The Spearman rank correlations show a strong relationship between the rank orders of cAMP and TxB2 levels at all storage times.

Overall rankings. Table 2 presents the means and standard deviations of the ranks assigned to the performance of the 11 parameters used to evaluate the cAMP-active formulations after 15 and 20 days of storage. Analysis of variance clearly shows that the formulations containing 2 mmol/L concentration of a phosphodiesterase inhibitor were superior to the other formulations in platelet preservation during storage lasting through 20 days. Formulations 1 to 5 were clearly inferior, possibly because of the lack of an effective dose of a phosphodiesterase inhibitor.

Cyclic AMP and TxB2. To illuminate the mechanism whereby phosphodiesterase inhibitors appeared to work better than adenylate-cyclase stimulators in platelet preservation, cAMP levels were measured directly in platelet pellets obtained during the storage period from each of the formulations and compared with the levels of TxB2 in the supernate. As illustrated in Fig 6, cAMP levels were highest in formulation 8 with both PGE-1 and theophylline present. In formulations 6 and 7 containing only 2 mmol/L theophylline or caffeine, cAMP reached a high of only 50 pmol/10^9 platelets as a maximum in any one platelet concentrate, but stayed above the minimal levels seen in formulations 1 and 2 without a phosphodiesterase inhibitor.

In data not shown, formulation 5, with 500 μmol/L caffeine, gave a peak cAMP value of only 32 pmol/10^9 platelets, and mean values were not significantly different from 1 and 2. Formulation 4, with IBMX, showed even lower cAMP values than 5. Assays of cAMP in formulation 3, with forskolin, were not linear with dilution, and thus were uninterpretable in our study. Because of this difficulty, forskolin-treated concentrates were not included in the figures or overall statistical analysis of cAMP effects.

TxB2 was assayed in the supernate of samples from stored platelet concentrates as an index of platelet activation because many of the changes in platelet integrity and function during storage may be described as a consequence of activation. The effect of an adequate dose of a phosphodiesterase inhibitor was plainly evident in the cAMP/TxB2 correlation plots in Fig 7. By day 15 or 20, formulation 1 and 2 were clearly higher in TxB2 levels than formulations 6, 7, and 8. Only at day 20 did it appear that formulation 8, with the highest cAMP levels, had the lowest TxB2; however, this could be appreciated only on the logarithmic scale in Fig 7 and may not be relevant. Among the nonillustrated formulations, Forskolin (3) and IBMX (4) gave mean TxB2 levels at day 15 and day 20 >400 ng/10^9 platelets; formulation 5, with 500 μmol/L caffeine, gave highly variable results with mean TxB2 = 60 at day 15 and 389 at day 20.

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Table 2. Means and SD of the Ranks Assigned to the Performance Levels of the 11 Parameters Used to Evaluate the cAMP-Active Formulations After 15 and 20 Days of Storage

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Day 15</th>
<th>Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) No inhibitors</td>
<td>6.0</td>
<td>1.19</td>
</tr>
<tr>
<td>(2) PGE-1 only</td>
<td>5.5</td>
<td>0.93</td>
</tr>
<tr>
<td>(4) IBMX</td>
<td>5.9</td>
<td>1.81</td>
</tr>
<tr>
<td>(5) 500 μmol/L caffeine</td>
<td>3.8</td>
<td>1.51</td>
</tr>
<tr>
<td>(6) 2 mmol/L caffeine</td>
<td>2.3</td>
<td>0.90</td>
</tr>
<tr>
<td>(7) 2 mmol/L theophylline</td>
<td>2.2</td>
<td>0.98</td>
</tr>
<tr>
<td>(8) PGE-1 + theophylline</td>
<td>2.4</td>
<td>1.29</td>
</tr>
</tbody>
</table>

---

**Fig 6.** Mean values of intracellular cAMP (A) or TxB2 (B) for the formulations as in Figs 1 and 2. The open circles are slightly offset from their true X-axis values to avoid overlap with the open squares.
The correlations at 15 and 20 days just failed to reach statistical significance because of the small degrees of freedom and the rank-order shifts among formulations 1 to 5.

The linear correlations between cAMP levels and each individual marker of platelet function or integrity were less robust (not shown). Rather than presume a linear correlation between cAMP level and status of platelets during storage, we formulated an hypothesis that elevation of cAMP above some threshold level was sufficient to provide long-term preservation effects, and that further increases in cAMP above that threshold had a low impact on further improvements in storage of platelets. Examination of the minimum cAMP values among the concentrates in the three best formulations (6 to 8) compared with the maximum cAMP values of the worst formulations (1 to 5) suggested that the cAMP thresholds were: greater than 22 at day 10, greater than 17 at day 15, and greater than 21 at day 20 between these groupings. In summary, it appeared that a sustained elevation of intracellular cAMP to levels greater than approximately 20 pmol/10⁹ platelets was sufficient to provide adequate protection against deterioration of platelets caused by activation during storage in concentrates.

**DISCUSSION**

During storage in the blood bank, platelet concentrates gradually lose functionality and circulatory lifespan, leading to a state referred to as the platelet “storage lesion.” The mechanisms involved in the deterioration of platelet concentrates have been debated for many years. One of the well-established hallmarks of the storage lesion has been a rapid acidification of the medium by cellular production of lactic acid; when the concentrate pH falls to 6.2 or less, platelet dysfunction is evident by most criteria. The severity of this problem was somewhat ameliorated by the advent of plastic storage containers of high gas permeability, thus reducing hypoxia and lactate production. The cause of high metabolic output by stored platelets remains unclear, although we have found that platelet-activation inhibitors reduce the metabolic rate of platelets in the new concentrate containers.

The early attempts to use prostaglandins to increase the yield of platelets during harvesting from donated blood did not appear to improve the quality of the stored platelets. The tangible benefit seemed to be in easy resuspension of platelets and less loss in clumping after centrifugation of platelet-rich plasma to make a concentrate. Addition of an acidic citrate solution on top of the pellet equally facilitated resuspension of platelets, so this effect would not seem to be specific to prostaglandins. The inhibition of platelet activation by close cell contact might explain the ease of resuspension imparted by prostaglandins or an acidic medium, but of more interest, the onset of the storage lesion was not abated by PGE-1 or PGI-2. This observation might be taken as a repudiation of the hypothesis that platelet activation during concentrate preparation contributes to the deterioration of platelet function. However, the effect of inhibitory prostaglandins such as PGE-1 or PGI-2 is known to be transient and reversible, and there are several lines of evidence that platelet-activation agonists are generated continuously in platelet concentrates; by-products of activation appear throughout the storage period. Therefore, we assumed that to adequately limit platelet activation and its sequelae during storage of platelets, a more long-lasting effect was needed.

The data presented in this report show a strong correlation between sustained cAMP levels of greater than 20 pmol/10⁹ platelets and the improved preservation of platelets in concentrates. PGE-1 alone or forskolin did not give a prolonged measurable rise in cAMP. Effective concentrations of phosphodiesterase inhibitors, especially when combined with PGE-1, gave appreciable and stable elevations in cAMP levels and significant improvements in platelet metabolism/integrity/function markers. cAMP levels also reflected the benefit of phosphodiesterase inhibitors. The added benefit of a thrombin inhibitor (Thromstop; Ameri-
can Diagnostics, New York, NY) and a general protease inhibitor (aprotinin) as shown in our other work\(^5\) suggest that elevated cAMP does not prevent all mechanisms of damage to platelets during storage. The ability of strong platelet agonists like thrombin to overwhelm the effects of prostaglandins has been shown.\(^6\) From our present findings, we conclude that the earlier work with prostaglandins in platelet concentrates failed to show an expected improvement over controls because PGE-1 (and PGI-2) cannot provide a sustained cAMP increase during a prolonged storage period.

Pharmacologic supplementation of platelet concentrates may improve quality and/or extend the shelf life of platelets in blood banking. The issues of maintaining sterility for extended periods of storage at 22°C and minimizing the potential toxicity of supplemented platelet concentrates remain to be addressed. Our results suggest that a high concentration of a relatively nontoxic phosphodiesterase inhibitor such as theophylline or caffeine alone may be the simplest effective approach to investigate. Other formulations of inhibitors, including PGE-1 and protease antagonists, may give slightly better results in certain in vitro markers, but the cost and probable toxicity risks mitigate against their potential use. In vivo platelet-survival studies may show that theophylline or caffeine provide a significant benefit equal to that which we already reported for the combination of theophylline and PGE-1.\(^2\) Successful and practical inhibition of the activation-dependent storage lesion could greatly facilitate the management of platelet-concentrate inventories in blood banking and potentially improve the quality of care in transfusion medicine.

ACKNOWLEDGMENT

We thank Lori Earls and Richard Hodges for their expert technical assistance, and Becky Boyea for preparation of the manuscript and figures. This work benefited from the generous provision of PL-732 storage containers by the Fenwal Division of Baxter Healthcare, Inc.

REFERENCES


Table 3. Ordinal Ranking of Intracellular cAMP and Supernatant TxB\(_2\) Levels, Mean Ranks, and Spearman Correlations Between the Ranks in Platelet-Inhibitor Formulations After 10, 15 and 20 Days of Storage

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Day 10 Mean Rank</th>
<th>Day 15 Mean Rank</th>
<th>Day 20 Mean Rank</th>
<th>Spearman Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inhibitors</td>
<td>cAMP</td>
<td>TxB2</td>
<td>cAMP</td>
<td>TxB2</td>
</tr>
<tr>
<td>(1) No inhibitors</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>(2) PGE-1 only</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>(4) IBMX</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>(5) 500 μmol/L caffeine</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>4</td>
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<tr>
<td>(6) 2 mmol/L caffeine</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>(7) 2 mmol/L theophylline</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>(8) PGE-1 + theophylline</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

P value = .003 NS NS

*Abbreviation: NS, not significant.*

The lowest (best) ranks were accorded to the concentrates with the highest cAMP levels and the lowest TxB\(_2\) levels.

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Sustained elevation of intracellular cyclic 3′-5′ adenosine monophosphate is necessary for preservation of platelet integrity during long-term storage at 22 degrees C

AP Bode, S Holme, WA Heaton and MS Swanson