Effect on platelet properties of exposure to temperatures below 20°C for short periods during storage at 20 to 24°C

G. Morro, S. Holme, V. M. George, and W. A. Heaton

Background: When platelet concentrates (PCs) are shipped over long distances, it is not always possible to ensure that their temperature is maintained at 20 to 24°C. In addition, PCs are not agitated as during routine shusage.

Study Design and Methods: Studies have been conducted to evaluate how exposure to temperatures below 20°C in the absence of agitation influences the properties of platelets. In initial studies, exposure to 4°C for 3 or 5 hours or to 12°C for 5 or 17 hours on Day 2 of a 5- to 6-day storage period was associated with a loss of discoid shape. This was reflected by slightly lower but statistically different morphology scores after storage compared to those observed with control platelets that were stored only at 20 to 24°C. In addition, a qualitative difference in morphology was noted in controls and PCs held at 16°C for 17 hours. In more detailed studies, both the in vivo viability and in vitro properties of platelets exposed between Days 1 and 2 to either 12°C or 16°C for 17 hours were evaluated. The protocol involved a paired study design (n = 4 for each exposure temperature) with the simultaneous storage of two identical PCs, one exposed to 12 or 16°C and the other maintained at 20 to 24°C throughout the 5-day storage.

Results: Exposure to 12°C significantly reduced (p<0.05 by paired t-test) the in vivo recovery to 37.6 ± 13.8 percent (mean ± 1 SD) from 47.8 ± 11.5 percent and the survival time to 2.0 ± 0.3 days from 6.5 ± 1.4 days. On exposure to 16°C, the differences in viability from those of control units were much less but still significant. The in vivo recovery was 42.7 ± 3.8 percent compared to 49.2 ± 3.0 percent, and the survival time was 3.5 ± 1.2 days compared to 6.5 ± 0.3 days. The loss of in vivo viability of the test platelets was associated with a loss of discoid shape, as reflected by morphology scores, extent of shape change, and mean platelet volume. In addition, platelet metabolism also appeared to be affected, as suggested by increased lactate production. All of the in vitro properties except for total ATP and residual glucose that were statistically different from those of controls on exposure to 12°C were also significantly different on exposure to 16°C.

Conclusion: These findings demonstrate that platelets undergo substantial changes in vivo viability and in vitro properties when they are exposed to temperatures below 20°C for short periods. TRANSFUSION 1994;34:517–521.

Abbreviations: PC(s) = platelet concentrate(s); PRP = platelet-rich plasma.

Platelet suspensions have been stored at 20 to 24°C since the late 1960s, when Murphy and Gardner1 showed that the posttransfusion viability of stored platelets was markedly reduced at temperatures above or below this range. During local shipment or transregional transport of platelet concentrates (PCs), they can be exposed to other conditions, in some cases for a number of hours. They cannot be agitated, which is important for maintaining in vivo viability during extended storage,2,3 and they may be subjected to temperatures below 20°C or above 24°C.

Limited data are available pertaining to the retention of platelet properties under conditions encountered during the shipping of platelets. Simon et al.4 determined that the posttransfusion viability of platelets was not influenced by short air trips but was influenced by overnight transport. Specifically, overnight courier shipment reduced survival time by 1 to 2 days relative to that of paired controls, probably as a result of prolonged exposure to temperatures outside the range of 20 to 24°C. Minimum and maximum temperatures determined during the actual shipments were 11°C and 29°C, respectively. However, the amount of time at various temperatures was not reported. Morro, and George,5 in simulated shipping studies involving the lack of agitation but retention of temperature at 20 to 24°C, found that an array of in vitro platelet properties were comparable to those of paired controls. In these studies, test platelet suspensions were held in a styrofoam shipping carton without
agitation for a period of 24 hours between Day 2 and Day 3 of a 7-day storage period. In addition, in vitro properties were maintained if the shipping carton was kept at 37°C for the first 6 hours of the 24-hour period with subsequent holding in a 20 to 24°C atmosphere.

In this report, we present the results of studies that evaluated how short-term exposure to temperatures below 20°C, which can occur during cold weather, affects in vitro and in vivo platelet viability properties. Currently, there are no data that indicate how well platelet properties are maintained during such conditions.

Materials and Methods

For the initial studies, units of platelet-rich plasma (PRP) were prepared by centrifugation of units of whole blood collected into CPDA-1 (PL-146 plastic, Fenwal Laboratories, Deerfield, IL). Pools of ABO-identical PRP were used to prepare identical PCs in another plastic (PL-132, Fenwal) with volumes of 50 ± 5 mL. For each experiment, one PC served as a control and the second was exposed to a temperature below 20°C. The control unit was stored at 20 to 24°C in an environmental chamber (Forma, Marietta, OH) with continuous agitation (6 rpm). The second unit was stored at 4°C or 5°C in a refrigerator (Thermo Scientific, Waltham, MA) throughout the 5- to 6-day storage period. The experimental unit was stored under the same conditions except for the time of exposure to a temperature below 20°C without agitation. The low-temperature exposure was conducted in an environmental chamber (Model CIOSOVR, Lomar Environmental Company, Williamsport, PA). The specific exposure conditions on Day 2 of storage were as follows: 4°C for 3 or 5 hours; 12°C for 2, 3, or 5 hours; and 16°C for 3, 5, or 7 hours. Platelet count, pH, morphology (phase microscopy), lactate dehydrogenase discharge, B-2-microglobulin release, and aggregation in response to a combination of ADP and collagen were determined at the conclusion of the storage period as previously described.54 Morphology was also examined at the conclusion of the exposure period. In addition to the quantitative scoring method routinely used for evaluation of platelet morphology, a qualitative morphologic comparison of experimental and control samples was performed. This involved microscopic scanning of the samples in a blinded fashion and judgment of whether a sample was from a control or experimental unit. The judgment was made by an observer who had substantial experience observing changes in morphology in platelets exposed to temperatures below 20°C. These prior studies indicated that the morphology was characterized altered even when the morphology score remained essentially unchanged. Results of this exercise are reported as the percentage of experimental and control pairings that were correctly differentiated.

Both in vivo and in vitro studies were conducted after the initial in vitro studies. We used a paired study design with concurrent in vivo viability and in vitro testing of test and control units obtained by double manual apheresis. For the in vivo studies, the test units were labeled with one isotope (67Ga or 111In) and the control units were labeled with the other. This study design has been shown to substantially improve the accuracy of detecting differences in the viability of stored PCs.8 Four paired studies were conducted for both temperatures investigated.

For these studies, we collected units of CPD whole blood by a double (sequential) manual apheresis procedure. PRP was prepared 1 to 4 hours after collection of the first unit of whole blood, with subsequent return of the red cells to the donor. After the red cells had been transfused, a second unit of whole blood was collected, and the PRP was prepared after a 1-hour hold. We mixed the 2 units of PRP and converted them to two PCs by centrifugation; 1 unit served as a control and the other was exposed to either 12 or 16°C for 17 hours over Days 1 and 2 of storage. Low-temperature exposure was performed by using temperature-controlled refrigerated chambers. The control and experimental units were stored as described previously in this report. At the conclusion of the storage period, we obtained samples with which to evaluate the in vivo viability of the autologous platelets and to perform in vitro testing. The platelets for in vivo transfusion and testing were prepared according to procedures described previously.9 Platelets from 1 unit were labeled with 111In, and 67Ga was used to label the second unit. Labeling of control or experimental platelets with either 111In or 67Ga was determined by random selection. Radioisotopically labeled platelets were combined and transfused. Samples for determining the circulating levels of the isotopes were obtained over a 10-day period and counted as described previously.10 We determined autologous platelet recovery and survival time by using the multiple-hit (gamma function) approach.

In addition, the mean residual lifespan of the transfused platelets was calculated as previously described.11 This variable was the average lifespan in circulation of the transfused radioisotopically labeled platelets. It may be determined by calculation of the percentage of platelets with lifespans of 0 days, 0 to 1 day, 1 to 2 days, and so on, and then by taking the arithmetic mean. Alternatively, as was done in this study, it can be calculated by estimation of the area below the survival curve (determined by the integral of the fitted curve) divided by the percentage of platelets normally in circulation (considered to be 55%). Concurrent in vivo testing, using methods that were described previously,26 measured the following variables: platelet count, pH, morphology score (phase microscopy), extent of shape change (on the addition of ADP), lactate dehydrogenase formation, residual glucose levels, response to hypotonic stress, total ATP levels, and oxygen consumption. Swirling of the PCs was determined by visual inspection; the degree of swirling was graded from 1 (poor) to 3 (good).

The data are presented as mean ± 1 SD values unless otherwise stated. We used a paired t test for statistical analysis.

Results

Initial studies were conducted to assess the extent to which a range of low-temperature exposure conditions influenced selected in vitro platelet properties. The exposure occurred over Days 2 and 3 of a 3- or 6-day storage period. Control platelets were maintained at 20 to 24°C throughout a comparable storage period. The most notable change in platelet properties was a modest but significant reduction in platelet morphology scores compared to those for control platelets at the conclusion of the storage period, after exposure to 4°C for 5 and 15 hours and to 12°C for 5 and 17 hours (Table 1). Table 1 also summarizes the extent to which paired test and control samples were correctly differentiated in qualitative microscopic examination of blinded samples. Nearly 100 percent accuracy was obtained with both 4°C and 12°C test conditions, with many of the platelets in the test units appearing plumper and denser (darker) than the control platelets. While many still retained an overall discoid shape, others were categorized as spherical. In these studies, exposure to 16°C for 3, 5, or 17 hours did not result in significant differences in morphology score. However, platelets in units exposed to 16°C for up to 17 hours also had a plumper and denser appearance, and there was good differ-
Table 1. Morphology scores after exposure to 4, 12, and 16°C.

<table>
<thead>
<tr>
<th>Exposure temperature and duration</th>
<th>Quantitative morphology score (Day 5)</th>
<th>Qualitative examination: correct differentiation (%) (test vs. control)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C, 3 hrs</td>
<td>256 ± 33</td>
<td>274 ± 34†</td>
</tr>
<tr>
<td>4°C, 5 hrs</td>
<td>256 ± 33</td>
<td>274 ± 34†</td>
</tr>
<tr>
<td>12°C, 3 hrs</td>
<td>275 ± 25</td>
<td>276 ± 40†</td>
</tr>
<tr>
<td>12°C, 5 hrs</td>
<td>252 ± 28</td>
<td>282 ± 50†</td>
</tr>
<tr>
<td>12°C, 17 hrs</td>
<td>224 ± 29</td>
<td>287 ± 40†</td>
</tr>
<tr>
<td>18°C, 17 hrs</td>
<td>205 ± 25</td>
<td>308 ± 26†</td>
</tr>
</tbody>
</table>

* Mean ± SD; n = 4 for each time and temperature condition.
† This measurement assessed the ability to differentiate between test and control samples upon qualitative microscopic examination. (See Materials and Methods for additional explanation).
‡ Difference in quantitative morphology scores between test and control platelets, p < 0.05.
§ Difference in qualitative morphology scores between test and control platelets, p < 0.01.

The influence of exposure to 12 and 16°C on in vitro platelet properties determined after 5 days of storage is shown in Table 3. Overall, exposure to 12°C had a greater influence than that to 16°C. Significant changes relative to control platelets were observed with both 12 and 16°C exposure for morphology score, extent of shape change, mean platelet volume, and swirling index, variables that reflect the retention of the discoid shape. In addition, significant differences in recovery from hypotonic stress, lactate production, and PC pH were observed with exposure to either temperature. Significant differences between test and control platelets for residual glucose and total ATP levels were observed only after 12°C exposure. Oxygen consumption and total platelet count were not altered on exposure to either temperature.

In vitro and in vivo correlations suggested that variables that reflect changes in platelet shape, morphology score, extent of shape change, swirling, and mean platelet size correlated in a significant way with in vitro viability variables of percentage recovery, survival time, and mean residual lifespan. The correlation between extent of shape change and mean residual lifespan is expressed in Fig. 2; the correlation value of r = 0.67 is significant. Significant correlations with mean residual lifespan were also determined for lactate production (r = -0.67).

Discussion

Previous studies have shown that exposure to temperatures below 20°C has a deleterious influence on the retention of in vivo and in vitro platelet viability properties during storage. Storage at 1 to 6°C was used until it was documented that platelets held for only 24 hours at this temperature underwent a marked reduction in in vivo viability, especially in relation to survival time. More recently, it has been shown that storage at 18.5°C for 3 days was associated with decreased survival in comparison to storage at 21.5°C.

In our studies, PCs were exposed to a temperature below 20°C for a limited time during a 5-day room-
Table 3. In vitro platelet properties at the conclusion of 5 days of storage after exposure to 12°C and 16°C for 17 hours

<table>
<thead>
<tr>
<th>In vitro variables</th>
<th>Exposure to 12°C (n = 4)</th>
<th>Exposure to 16°C (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test platelets</td>
<td>Control platelets</td>
</tr>
<tr>
<td>Total platelet count (×10^9)</td>
<td>62.6 ± 10.9</td>
<td>65.2 ± 18.2</td>
</tr>
<tr>
<td>pH (pH)</td>
<td>7.00 ± 0.12</td>
<td>7.14 ± 0.13</td>
</tr>
<tr>
<td>Mean platelet volume (μm³)</td>
<td>7.93 ± 0.74</td>
<td>6.93 ± 0.40</td>
</tr>
<tr>
<td>Morphology score (maximum 400)</td>
<td>249 ± 10</td>
<td>340 ± 27</td>
</tr>
<tr>
<td>Extent of shape change (% increase in optical density)</td>
<td>12.1 ± 2.2</td>
<td>260.2 ± 2.5</td>
</tr>
<tr>
<td>Swirling index</td>
<td>2 ± 1</td>
<td>3 ± 0.7</td>
</tr>
<tr>
<td>Residual glucose (mM)</td>
<td>16.0 ± 0.3</td>
<td>17.6 ± 0.8</td>
</tr>
<tr>
<td>Lactate production (mmol/10⁶ platelets)</td>
<td>13.7 ± 2.6</td>
<td>10.7 ± 2.2</td>
</tr>
<tr>
<td>Total ATP (μmol/10⁶ platelets)</td>
<td>6.38 ± 3.37</td>
<td>6.88 ± 0.54</td>
</tr>
<tr>
<td>Oxygen consumption (nmol/min)</td>
<td>1.75 ± 0.45</td>
<td>1.60 ± 0.28</td>
</tr>
<tr>
<td>Hypotonic stress response (% recovery)</td>
<td>63.4 ± 12.0</td>
<td>68.3 ± 12.8</td>
</tr>
</tbody>
</table>

* Mean ± 1 SD.
† Difference between test and control platelets, p < 0.01, by paired t-test
‡ Difference between test and control platelets, p < 0.05, by paired t-test

Temperature storage period. The intent was to simulate temperatures that may be encountered during shipping of PCs. An additional consideration in study design was the fact that, after shipment, PCs may be transfused immediately or may be stored at 20 to 24°C with agitation in a hospital blood bank or blood center for use any time prior to their 3-day outdated. The data show that even limited exposures to 16 or 12°C for 17 hours resulted in reduced platelet recovery and survival time. The marked reduction in survival time with platelet exposure to 12 and 16°C for 17 hours is thus consistent with previous studies showing that storage at 1 to 6°C has a deleterious effect on the platelets.

The loss of viability with exposure to temperatures below 20°C was associated with changes in morphology, as has been noted previously by others. The reduction in morphology score, in extent of shape change with ADP, and in swirling index and the increase in mean platelet volume are all indices of conversion of platelet shape toward the spherical. The use of an increase in apparent volume as an indicator of platelet shape change is based on previous studies in which it was demonstrated that platelets that become spherical following exposure to 4°C showed an apparent volume increase of 20 percent. Alterations in the discoid shape have been associated with a reduction in viability under a variety of conditions, including the use of EDTA as an anticoagulant, storage at 1 to 6°C, unfavorable agitation, and reduction in pH levels with storage in first-generation containers.

A significant difference in morphology score after exposure to 16°C for 17 hours relative to the score in control platelets was observed by one of the two laboratories conducting the studies. This may reflect the subjective nature of the scoring system, in which a wide array of cell shapes are separated into a small number of categories. It is probable that the interlaboratory difference was related to the scoring of those platelets that developed a plumper appearance after exposure to 16°C; one laboratory scored these platelets as discoid and the other scored them as spherical. It should be noted that the laboratory that did not obtain a statistical difference in morphology score did in most cases correctly differentiate the coded, paired test and control platelets by qualitative microscopic examination.

Metabolic platelet activity is also influenced by exposure to temperatures below 20°C. This is reflected in the small but significant difference in pH levels and, more important, in the differences in lactate production in test and control units. Increases in lactate production indicate an enhanced glycolytic rate, which has previously been demonstrated in platelets exposed to 4°C. Furthermore, increased lactate formation has been shown by Hofnc et al.,10,11,12,13 to correlate with a reduction in in vivo platelet viability in a number of situations. Although glycolysis was increased by exposure to 12 and 16°C, it should be noted that the respiration rate was not similarly affected. Lactate production exhibited a continuous rela-
tionship with in vivo variables, as a group, which suggests that the lesion and its effect on in vivo viability may be mediated by a metabolic effect secondary to the morphologic abnormality. After 12°C exposure, there was a significant breakdown of ATP, which suggested damage to some other aspects of energy metabolism, perhaps the uncoupling of oxidative phosphorylation, as suggested previously.¹⁷

The data from these studies, considered together with the conclusion by Simon et al.¹⁵ that the posttransfusion viability of platelets was reduced after overnight transit, indicate that procedures used during extended shipment of platelets should be designed with consideration of the environmental conditions that may be encountered. Use of shipping containers with enhanced insulation and cold-absorbing packages should help to minimize reduction in PC temperature. In addition, the swirling test,²⁰,²² which indirectly measures the retention of platelet discord morphology properties, can be used to evaluate the overall quality of platelet suspensions that are shipped over extended periods.

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