Survival and Recovery of Human Platelets Stored for Five Days in a Non-Plasma Medium

By G.A. Adams, S.D. Swenson, and G. Rock

Human blood platelets were stored for five days as concentrates in 60 mL of: (a) plasma; (b) non-plasma medium with anticoagulant; and (c) non-plasma medium without anticoagulant. All preparations were equally functional when tested for platelet aggregation and release reaction in response to single agonist or synergistic pairs of agonists in vitro. Platelets stored in non-plasma medium with anticoagulant had lower kallikrein, fibrinogen/platelet A, lactate, and β-thromboglobulin than did plasma controls after five days. In vivo recovery and survival of platelets stored in non-plasma medium with anticoagulant were 51.2% ± 4.3% and 8.7 ± 0.3 days, respectively, which were not statistically different from plasma controls of 39.2% ± 4.9% and 7.2 ± 0.8 days, respectively. It is concluded that platelets can be stored for five days in a non-plasma medium and still have good in vivo recoveries and survivals.

Materials and Methods

Materials. 14C-Serotonin (5-hydroxytryptamine creatinine sulphate, Amersham Corporation, Oakville, Ontario) was diluted to 150 μmol/L in 2% ethanol in distilled water and frozen. Bovine tendon collagen (SIGMA Chemical Company, St Louis) was prepared by the method of Cazenave et al. ADP (SIGMA) was dissolved in normal saline to 10 μmol/L and frozen. The ionophore, A23187, (SIGMA) was dissolved in dimethylsulfoxide (DMSO) to 10 μmol/L and then diluted to 5 × 10–3 mol/L in normal saline. Epinephrine (Eastman-Kodak, Toronto) was prepared at 1.0 mmol/L in 0.15 mol/L NaCl and 1.0 mmol/L of tartaric acid, diluted to 5 × 10–4 mol/L with normal saline, and frozen. Lyophilized sodium arachidonate (Bio Data Corporation, Hatboro, Pa) was reconstituted according to the company's instructions. Non-plasma media were prepared by mixing concentrated sodium citrate (46.7% trisodium citrate, Haemonetics Corporation, Braintree, Mass), dextrose for injection (50%, sterile and nonpyrogenic), and Plasma-Lyte A (90 mmol/L NaCl, 23 mmol/L of sodium gluconate, 27 mmol/L of sodium acetate, 5 mmol/L of KCl, 3 mmol/L of MgCl2, 294 mosm/L, pH 7.4, Travencol Canada Inc, Mississauga, Ontario). The standard non-plasma medium was composed of 59 mL of Plasma-Lyte, 0.5 mL of concentrated citrate and 0.5 mL of 50% dextrose and designated PCD. One other non-plasma medium, PCD, was stored; it was free of citrate and designated PD. Mixtures were made with the aid of (Millex-GS, Millipore Corp, Bedford, Mass) 0.22-μm filter units and medication injection sets.

Paired studies, in vitro. Whole blood from nine normal healthy donors was collected into CPDA-1 anticoagulant. One tube was collected from each bag so that the platelets could be tested for biphasic response with epinephrine. Seven responsive units were chosen and centrifuged at 1,000 g for seven minutes in a Sorvall RC-3B at 22°C. The PRP was extracted into satellite bags and subsequently pooled and divided into six new transfer packs (Fenwal PL732, 300-mL capacity) to give 72 × 108 platelets per bag. All six units were centrifuged at 3,350 g for six minutes on the Beckman Model J-6M centrifuge, and most of the plasma was removed, leaving 7 to 9 mL. The concentrated platelets were left to sit at room temperature undisturbed for one hour, after which plasma was added back to the controls and non-plasma media was added to the others. Final volumes of platelet concentrates were 60 mL. The platelet concentrates were stored at 22°C on a vertically rotating shaker at 6 rpm (Helmer Labs Inc, St Paul). After five days, the platelets were tested for in vitro function.

Platelet function testing. Platelet concentrates were diluted to 3 × 108 platelets per milliliter with either autologous plasma, non-plasma medium, or both, to standardize the aggregation conditions to three parts plasma and one part non-plasma medium. After incubating with 1.0 μmol/L of 14C-serotonin at 22°C for one hour, imipramine to 2 μmol/L was added to prevent reuptake of serotonin, and platelet aggregation and the release reaction were measured. Platelet stimuli were prepared at ten times final concentration and added to platelet suspensions equally or in pairs. The following final concentrations of the aggregating agents were used: epinephrine 5 × 10–3 mol/L, ADP 10–3 mol/L, collagen 5.0 μg/mL, A23187 10–4 mol/L, and arachidonic acid 0.33 mg/mL. Aggregation was defined as the maximum change in transmittance after stimulation. Four minutes after stimulation, the samples were centrifuged at 15,000 g for one minute in an Eppendorf centrifuge [Brinkman Instruments (Canada), Rexdale, Ontario] to obtain supernatant samples. The percentage of release of 14C-serotonin was calculated as previously reported. Platelet and WBC counts were done on the Coulter Counter (Model ZBI, Coulter Electronics, Hialeah, Fla). Aggregations were performed on Payton aggregometers (Payton Instruments, Toronto), and radioactivity was measured using the Beckman LS7500 liquid scintillation system (LS7500, Beckman Instruments Inc, Irvine, Calif). To illustrate the results graphically, a "synograph" was constructed by plotting the level of aggregation or release response to the various stimuli on each spoke of a 16-spoke polar graph. By joining the points, an image is generated that is easily remembered and that facilitates comparison between different platelet preparations and periods of storage. The upper right quad-
rants shows the results from single stimuli; the three other quadrants indicate the responses obtained with pairs of stimuli.

**Analysis of media.** The pH, pCO₂, and pO₂ of the platelet concentrates were measured with a Corning 165/2 blood gas analyzer (Corning Medical, Corning Glass Works, Medfield, Mass) at 37 °C. Glucose concentrations were determined using Fisher Diagnostics (Fair Lawn, NJ) Glucotall reagent. Lactate measurements were performed using the Boehringer-Mannheim (F.R.G.) lactate kit. Kallikrein was measured by the Diagnostica Stago (Asnieres, France) kit. β-Thromboglobulin was measured using the radioimmunoassay kit from Amersham and fibrinogen/peptide A by the kit from Mallinckrodt (St. Louis).

**Autologous reinfusion studies.** Autologous reinfusion studies were approved by the Medical Ethics Committee of the Ottawa General Hospital. A unit of whole blood was withdrawn from volunteers into a CPDA-1 triple pack using standard blood banking procedures. The platelets were concentrated, and as much plasma was removed as was possible. PCD was added to the platelet concentrate, which had rested undisturbed for one hour, to a final volume of 60 mL. A sample was removed for blood culture. The platelet concentrate was stored at 22 °C on a vertically rotating shaker at 6 rpm (Helmer Labs, Inc). After five days, the platelets were radiolabeled with ¹¹¹chromium according to recommended procedures. The platelet concentrates were infused, and blood samples were collected for seven days. Platelet recovery was calculated using the average of the samples taken two and three hours after infusion; platelet survival was calculated using Murphy’s multiple-hit program.

**RESULTS**

Two non-plasma media, PCD and PD, were used to support human platelets during storage for five days at 22 °C. The fibrinogen/peptide A levels were greater in the PD medium with no anticoagulant than in the media, PCD, to which anticoagulant was added (Table 1). Platelet count, WBC count, pH, pCO₂, pO₂, glucose, lactate, and kallikrein were not significantly different for either non-plasma media. The plasma concentration of lactate (122 mg/dL) was twice as high as the PCD concentration of lactate (60 mg/dL). The dextrose levels were 434 mg/dL in the PCD as compared with the plasma levels of 373 mg/dL (Table 1). The kallikrein and β-thromboglobulin levels were also lower in the non-plasma medium than in the plasma after five days. Between the media and plasma, no other parameters were significantly different.

In vitro evaluation of these preparations demonstrated that platelets stored in PCD (Fig 1), PD (Fig 2), and plasma (Fig 3) had maintained their functions of aggregation and the release reaction to synergistic pairs of stimulants. Platelet responses to single stimulants were similar for PCD, PD, and plasma, but were less than the responses exhibited on the day of blood collection (data now shown). Statistical analysis of the synographs (Figs 1 through 3) were performed by calculating the paired difference for all the stimuli for both platelet responses. Platelets stored in PCD had better aggregation responses with a mean (SE) of 6% (1.3), n = 120, but had less average release of serotonin by 7.7% (1.4) compared with the plasma controls after five days of storage. Platelet concentrates stored in PD had an average 4.0% (1.0), n = 90, more aggregation and 1.8% (1.5) less release of serotonin than did plasma controls after five days of storage.

Autologous platelets stored for five days in the non-plasma medium and transfused into volunteers yielded excellent in vivo platelet recoveries of between 40% and 60% and mean platelet survivals of between 8.3 and 9.4 days (Table 2). The survival curves were all linear (Fig 4). The control values, reported in Table 2, were obtained with platelets stored in PL732 bags for five days as reported previously. Blood cultures were negative on all preparations.

**DISCUSSION**

Plasma is generally not required by patients receiving platelet therapy and would be used more efficaciously if directed to fractionation. A preliminary report of the ability

**Table 1. Analysis of Storage Media After Five Days of Platelet Storage at 22 °C**

<table>
<thead>
<tr>
<th>Medium</th>
<th>n</th>
<th>PCT (10⁹/mL)</th>
<th>WBC (10⁹/mL)</th>
<th>pH</th>
<th>pCO₂ mm Hg</th>
<th>pO₂ mm Hg</th>
<th>DEX (mg/dL)</th>
<th>LAC (mg/dL)</th>
<th>FPA (ng/mL)</th>
<th>KALL (%)</th>
<th>βTG (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-plasma medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCD</td>
<td>14</td>
<td>1.00</td>
<td>2.40</td>
<td>7.00</td>
<td>21</td>
<td>116</td>
<td>434</td>
<td>60</td>
<td>2.1</td>
<td>32</td>
<td>4.4</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01</td>
<td>0.30</td>
<td>0.03</td>
<td>1</td>
<td>4</td>
<td>10</td>
<td>2</td>
<td>0.7</td>
<td>2</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>7</td>
<td>0.95</td>
<td>1.90</td>
<td>6.80</td>
<td>20</td>
<td>129</td>
<td>373</td>
<td>54</td>
<td>3.4</td>
<td>40</td>
<td>—</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01</td>
<td>0.28</td>
<td>0.04</td>
<td>1</td>
<td>4</td>
<td>14</td>
<td>1</td>
<td>0.5</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Plasma controls</td>
<td>4</td>
<td>0.98</td>
<td>2.10</td>
<td>7.10</td>
<td>30.0</td>
<td>130</td>
<td>373</td>
<td>122</td>
<td>7.8</td>
<td>139</td>
<td>6.4</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01</td>
<td>0.50</td>
<td>0.01</td>
<td>0.8</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0.8</td>
<td>29</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

n, Number of determinations; PCT, platelet count; WBC, white blood cell count; LAC, lactate; DEX, dextrose; FPA, fibrinogen/peptide A; KALL, kallikrein concentration as percentage of normal citrated pooled plasma obtained by venipuncture and vacutainers; βTG, β-thromboglobulin.
of non-plasma media to support platelet storage was presented by us in 1982 and recently confirmed by another group. This report documents the ability of a non-plasma medium to support platelets adequately during five days of storage. There are many possible advantages to storing platelets in a non-plasma medium. The more precise control of the environment during storage should lead to a more consistent product. This, in turn, should lower the number of units required per infusion and hence reduce demand. The removal of plasma should diminish the risk of transfusion of infectious agents, toxins, allergens, or drugs. Last, the recovery of additional plasma for fractionation should help prevent the adoption of expensive alternate plasma procurement procedures.

The decreased levels of lactate in the non-plasma media may offer a clue as to how the media were able to maintain platelet function so well. It has been suggested that increased oxygen transfer through the PL732 bags promotes aerobic metabolism and recent analyses have suggested that the rate of lactate production is five to eight times less in well-oxygenated conditions relative to anaerobic conditions. It is unlikely that the non-plasma medium could have facilitated oxygen diffusion because the bag is the limiting resistance. However, the non-plasma medium was at atmospheric oxygen tension when it was added to the concentrated platelets. This is in contrast to the situation with plasma, which is at venous oxygen levels when collected and which takes hours to days to return to atmospheric tension during storage. Therefore, it may be this rapid return to high oxygen concentration on addition of the non-plasma medium that maintains aerobic metabolism and reduces lactate production during storage.

The low levels of fibrino(gen)peptide A demonstrated that anticoagulant carried over with the residual plasma in the platelet concentrates with non-plasma medium was sufficient to guard against activation of coagulation during storage. Because there was still ~15% plasma in the platelet concentrates, this could have generated a maximum of 13,000 ng/mL (3.0 mg/mL of fibrinogen in plasma x 10,000/330,000 [mass ratio of fibrino(gen)peptide A to fibrinogen] x 15%), whereas only 3.4 to 4.4 ng/mL was detected. Therefore, although the non-plasma medium for the in vivo study (PCD) contained anticoagulant as a safety precaution, it may not have been necessary.

The decline during storage of platelet aggregation and release of dense granules in response to activation by single

| Table 2. Survival and Recovery of Autologous Platelets Stored for Five Days in a Non-Plasma Medium, PCD, and Transfused Into Healthy Volunteers |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Volunteer | Linear | Weighted Mean | Multiple Hit | No. of Hits | Recovery (%) |
| Wi | 8.4 | 7.8 | 8.4 | 25 | 40.6 |
| Bl | 8.5 | 8.0 | 8.5 | 25 | 54.0 |
| He | 8.3 | 7.9 | 8.3 | 25 | 60.9 |
| Be | 9.4 | 8.6 | 9.4 | 25 | 49.1 |
| Mean (SE) | 8.7(0.3) | 8.1(0.2) | 8.7(0.3) | 25(0) | 51.2(4.3) |
| Laboratory averages | 7.2(0.8) | 6.0(1.4) | 39.2(4.9) |
PLATELETS IN NON-PLASMA MEDIUM

stimuli, but not pairs of stimuli, has been reported previous-
ly. A similar pattern of dysfunction was observed in plate-
lets after storage in the non-plasma media either with or
without anticoagulant (Figs 1 through 3), implying that the
acquired defects are not induced by plasma but are due to
other factors, which could include contact with the plastic
bag, leachables from the bags, or self-inflicted injury by the
platelets themselves.

The in vivo recoveries of platelets that were stored in a
non-plasma medium (PCD) were higher but not statistically
different from those platelets that were stored in plasma,
51.2% vs 39.2%, \( P > .05 \), respectively. The in vivo survivals
were also higher but not statistically different (8.7 days vs 6.0
days \( P > .05 \)) for platelets stored in PCD when compared
with storage in plasma. The linear nature of the survival
curves (Fig 4) is indicative of the nonconsumptive removal of
platelets by healthy individuals. The excellent in vivo recov-
ery and survival of the platelets that were stored in the
non-plasma medium encourages the clinical testing of these
preparations in thrombocytopenic patients to demonstrate
their therapeutic capacities. The similar levels of \( \beta \)-thrombo-
globulin in the plasma and non-plasma media suggest that
the platelets have undergone identical degrees of activation
during storage and should have equal hemostatic properties.
This contention remains to be proven.

ACKNOWLEDGMENT

We would like to acknowledge the assistance of Rod Butt,
McMaster University, Hamilton in analysis of the platelet survival
and recovery data and of P. Tittley in performing the in vivo
experiments.

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    retain full aggregating potential in response to pairs of aggregating
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