Clinical Experience With Transfusion of Leukocyte-Poor Platelet Concentrates Prepared by Filtration With Prostacyclin

By Hendrik C. van Prooijen, Tineke I. Riemens, and Jan Willem N. Akkerman

Repeated transfusions with platelets from randomly selected donors lead to HLA alloimmunization in about 50% of patients due to lymphocyte contamination of platelet concentrates. Attempts to remove the leukocytes from the platelet concentrates by additional centrifugation steps led to substantial loss of platelets. We report a new procedure for removal of almost all leukocytes with excellent platelet recoveries. Single donor concentrates are treated with 50 ng/ml prostacyclin to inactivate the platelets transiently. The concentrates are then passed through a cellulose-acetate filter to remove the leukocytes. In 30 concentrates this treatment reduced the contamination by leukocytes to less than 0.1 million per concentrate with a platelet recovery of 89% ± 1% (mean ± SEM). Thirty filtered platelet concentrates transfused to ten thrombocytopenic patients within one hour after filtration were well tolerated and led to corrected count increments of (22.0 ± 1.1) x 10^3/ml blood after one hour and normal survival thereafter. In four of five patients these concentrates reduced the bleeding time. We conclude that transient inactivation of platelets by prostacyclin enables optimal removal of leukocytes and may help to reduce alloimmunization during frequent transfusions with platelet concentrates.

I N THE LAST few years chemotherapy for patients with acute leukemia has been intensified. This has rapidly increased the need for platelet concentrates as part of the supportive therapy. One of the major problems associated with repeated platelet transfusions is the development of refractoriness, primarily due to HLA alloimmunization.2-4 Only in a minority of the cases are platelet-specific antibodies triggered, which seldom form a problem for platelet transfusions.5,6 There is increasing evidence that HLA alloimmunization is induced by the contaminating lymphocytes, and clinical studies have shown that transfusion of leukocyte-poor blood components reduces the incidence of HLA alloimmunization.7,8 At present pooled or single donor platelet concentrates are depleted from contaminating leukocytes by additional centrifugation, which reduces the contamination with leukocytes to 0.8 to 2.2 x 10^6 cells per concentrate. Unfortunately this method leads to a 30% loss of platelets. More leukocytes can be removed by increasing the centrifugation speed, but this treatment further reduces the platelet yield. An alternative method is filtration which has been successfully applied to remove leukocytes from whole blood, but most of the platelets stick to the filters and the loss of platelets is about 80%.9

We here report a new procedure to prevent platelet adherence to the filter by inactivating the platelets with prostacyclin. This treatment does not interfere with the removal of leukocytes and results in platelet recoveries of about 90%. Preliminary data show that these platelets recover their hemostatic properties after transfusion.

MATERIALS AND METHODS

Preparation of platelet suspensions for in vitro studies with prostacyclin. The transient inactivation of platelets by prostacyclin was investigated in platelet-rich plasma (PRP) and in single donor platelet concentrates. For the preparation of PRP, venous blood was collected in citrate (0.1 vol of 130 mmol/L sodium citrate) from healthy donors who claimed not to have taken any medication for at least ten days. The blood was centrifuged (120 g, 10 minutes, at room temperature), and the supernatant, PRP, was collected and standardized at 2.5 x 10^6 platelets/ml by dilution in platelet-poor plasma (PPP).

Single donor platelet concentrates were prepared by cryopreservation with a Haemovet SYN using ACD-A as anticoagulant. In 30 preparations with a volume of 288 ± 7 ml (mean ± SEM) the platelet yield was (4.1 ± 0.2) x 10^8 with a contamination of (3.6 ± 0.5) x 10^6 leukocytes. For in vitro studies samples were taken from the concentrates and standardized at 2.5 x 10^6 platelets/ml by dilution with autologous plasma. The platelet suspensions were treated with different concentrations of prostacyclin, as indicated in the "Results" section. Prostacyclin (PGI1, Epoprostenol, The Wellcome Foundation Ltd, London) was approved for clinical use. The content of one vial (0.5 mg) was dissolved in 10 ml 0.01 N NaOH, distributed over 20 ampules and stored at -80°C.

Preparation of filtered platelet concentrates. Filtration was performed with cellulose acetate filters (Erypur or Cellselect, Organon Teknika, Amsterdam, The Netherlands). Just before filtration the filters were rinsed with citrate buffer (sodium citrate, 29.9 g/L, anhydrous citric acid, 563 g/L, 2 g/L human albumin, pH 6.5). The prostacyclin solution was thawed and immediately added to the platelet concentrates to obtain a final concentration of 50 ng/ml (0.1 mL of stock solution per 100 mL of platelet concentrate). No adjustments were made for the minor differences in platelet concentration. The concentrates were then passed through the filter at a flow rate of 10 mL/min, and residual platelets in the filter were collected with 200 mL of additional citrate buffer. The recovery after the filtration procedure was measured by counting the leukocytes visually in a Burker chamber and the platelets in a Baker 810 Trombocounter (Baker, Allentown, PA). The number of leukocytes after filtration was usually extremely low and was therefore measured after concentrating a 20-mL suspension by centrifugation (1000 g, 20 minutes, at room temperature) and resuspension in 0.5 mL of plasma. The filtered concentrates were used for transfusion without prior removal of the prostacyclin.

Aggregation studies. Platelet aggregation was measured in a dual channel aggregometer (Payton Ass, Scarborough, Canada) at a stirring speed of 900 rpm at 37°C following stimulation with 5 μg/mL of collagen (Hormon Chemie, Munchen, FRG).

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**Transfusion of Filtered Platelet Concentrates.** The filtered platelet concentrates were administered to ten patients on a prophylactic basis. Nine patients were treated for acute myeloid leukemia, and one patient was treated for a blast crisis of chronic myeloid leukemia. All patients were profoundly thrombocytopenic (platelet counts <15 x 10⁶/mL) but otherwise clinically stable. Platelet counts were obtained before and one hour after transfusion and every day thereafter. Post-transfusion results were expressed as corrected count increments (CCI) according to the formula:

\[
\text{CCI} = \frac{\text{absolute increment} \times \text{body surface area (m²)}}{\text{number of transfused platelets} \times 10^{11}}
\]

In five patients who showed post-transfusion counts of more than 50 x 10⁶/mL bleeding time measurements were performed by means of the Simplate II method (General Diagnostics, Division of Warner-Lambert, Morris Plains, NJ).

**RESULTS**

**Inhibition of platelet function in vitro by prostacyclin.** In an initial series of experiments with PRP the optimal concentration of prostacyclin was sought which induced complete inhibition of platelet function for at least one hour but which also permitted the platelets to recover thereafter. Platelet function was measured as the maximum aggregation in response to collagen and was expressed as a percentage of control (without prostacyclin). Figure 1 illustrates that between 1 and 20 ng/mL prostacyclin completely prevented aggregation. The inhibition was transient, and platelet function rapidly recovered upon addition of 1 ng/mL, whereas much more time was required for spontaneous recovery with higher doses of prostacyclin. Thus a dose of 5 ng/mL of prostacyclin was considered optimal for inhibition of platelet function for at least one hour. Single donor platelet concentrates contained about five times more platelets per volume, and initially the dose of prostacyclin was increased to 25 ng/mL, but we found that twice as much was required for the desired degree of inhibition (Fig 1).

The time required to recover from the prostacyclin inhibition could be greatly shortened by centrifugation and resuspension of the platelets in prostacyclin-free medium. In PRP this treatment abolished the effect of up to 20 ng/mL prostacyclin within ten minutes (Fig 2A), whereas in platelet concentrates inactivated with 50 ng/mL prostacyclin the ability to aggregate was restored after 45 minutes (Fig 2B).

**Filtration of platelet concentrates.** Single donor platelet concentrates were treated with 50 ng/mL prostacyclin within two hours after preparation and were immediately passed through the filter. The number of platelets per concentrate fell from (4.0 ± 0.2) x 10¹¹ before filtration to (3.6 ± 0.2) x 10¹¹ thereafter, resulting in a yield of 89% ± 1% (mean ± SEM, n = 30). The contamination of leukocytes fell from (3.6 ± 0.5) x 10⁶ before filtration to less than 0.1 million cells per concentrate thereafter, which equals a depletion of more than 99.9%.

**Transfusion of filtered platelet concentrates.** A total of 30 filtered concentrates was administered at a flow rate of 10 mL/min to ten patients with leukemia. The transfusions were carried out within one hour after filtration when most of the platelets were still inactive. Pulse rate and BP were measured once every ten minutes and remained normal. No other side effects were observed. Table 1 lists the post-transfusion count increments (CCI). Transfusion of filtered

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**Fig 1.** Transient inhibition of collagen-induced aggregation by different doses of prostacyclin (ng/mL, final concentrations). Aggregation induced by 5 µg/mL of collagen was measured as maximal change in light transmission and expressed as a percentage of a simultaneously run control (no prostacyclin added). The response of PRP (broken lines, 2.5 x 10⁶ cells/mL) was compared with that of platelets collected from single donor concentrates (solid line, containing 50 ng/mL prostacyclin) after dilution in autologous PPP to a final concentration of 2.5 x 10⁹ cells/mL (mean ± SEM, n = 6).

**Fig 2.** Rapid recovery of aggregability of prostacyclin-inactivated platelets by centrifugation/resuspension in plasma. PRP was treated with different doses of prostacyclin (5 minutes, 22°C) and subsequently pelletted and resuspended in prostacyclin-free, autologous plasma. The recovery of collagen (5 µg/mL)-induced aggregation was expressed as a percentage of control (Fig 2A, solid lines) and compared with the spontaneous recovery without prostacyclin removal (Fig 2A, broken lines). Figure 2B illustrates similar experiments using platelets obtained from single donor concentrates (mean ± SEM, n = 4).
FILTRATION OF PLATELET CONCENTRATES

Table 1. Post-transfusion Platelet Counts

<table>
<thead>
<tr>
<th>Time After Transfusion</th>
<th>Increment (x 10^6/mL)</th>
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<tbody>
<tr>
<td></td>
<td>Absolute</td>
</tr>
<tr>
<td>1 h</td>
<td>40.1 ± 2.5</td>
</tr>
<tr>
<td>18-24 h</td>
<td>28.5 ± 2.0</td>
</tr>
<tr>
<td>42-48 h</td>
<td>13.0 ± 1.3</td>
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</table>

NOTE: Mean ± SEM; n = 30.

concentrates resulted in CCIs of 22.0 and 14.8 x 10^6/mL blood at one hour and between 18 and 24 hours after transfusion respectively. For comparison, 26 transfusions of single donor HLA-matched platelets, administered to a comparable group of patients, produced a mean corrected count increment at one hour of 22.1 ± 2.8 x 10^6/mL and a mean corrected count increment at 24 hours of 10.7 ± 1.2 x 10^6/mL. Five patients were transfused with filtered platelet concentrates during the thrombocytopenic phase of their remission induction therapy, and none of the patients showed signs of hemorrhage. Bleeding times were performed in these five patients when post-transfusion counts were more than 50 x 10^6 platelets/mL blood. In four patients a significant reduction of the bleeding time was observed (Table 2).

DISCUSSION

For many years blood filters have been used to remove leukocytes from whole blood. The best results were obtained with cellulose-acetate filters and cotton-wool filters, both leading to a leukocyte depletion of about 98%. Unfortunately these filters also removed most of the platelets and were therefore unsuitable for removing leukocytes from platelet concentrates. The present study shows that the loss of platelets during filtration can be reduced to a minimum by transiently inactivating these cells with prostacyclin. Currently platelet concentrates are best depleted from leukocytes by additional centrifugation. Such a procedure takes about 15 minutes per concentrate and is therefore faster than our filtration technique, which takes about twice as long. The great advantage of our filtration procedure lies in the much better platelet recovery and the almost complete removal of leukocytes, resulting in concentrates that may be optimally effective for preventing HLA alloimmunization after repeated platelet transfusions. One may expect that these concentrates will rescue the need for HLA-matched platelets, which will make up for the cost of the filters and will even be more economical. Although the prostacyclin treatment appears successful in in vitro studies, two questions must be addressed before the method can be applied clinically: (1) How safe is the use of prostacyclin, which is known in certain conditions to induce hypotension? and (2) Are the transfused platelets hemostatically effective? Prostacyclin has been successfully applied in patients with peripheral vascular disease, chronic renal disease, and thrombotic thrombocytopenic purpura. In a recent study in normal volunteers, clinical tolerance, inhibition of platelet aggregation, and intracellular levels of cyclic AMP were compared during infusions of prostacyclin at rates up to 15 ng/kg/min. For short-term infusions (up to 60 minutes) the maximum tolerated rate was 10 ng/kg/min. The increase of cyclic adenosine monophosphate levels and inhibition of the circulating platelets were transient and returned to pretreatment values within 30 minutes after the end of infusion. In our study filtered platelet concentrates were transfused at a rate of 10 mL/min. Assuming that all prostacyclin added to the platelets prior to filtration was still effective at the time of transfusion, this would equal an infusion rate of about 6 ng/kg/min, which is well below the maximum tolerated rate. Indeed, no signs of hypotension or other side effects were observed. The second question is more difficult to answer. Our in vitro studies show that the prostacyclin-treated platelets regained the ability to aggregate in response to collagen after a few hours incubation at room temperature. This recovery period could be greatly shortened by pelleting the cells and resuspending them in prostacyclin-free medium. Although this provides a means to reanimate the platelets prior to infusion, we chose to transfuse the inactive platelets. Apart from bypassing a time-consuming washing step, this procedure protected the platelets optimally against in vitro activation and enabled them to recover in the blood stream. Probably the dilution in vivo in the circulating blood mimics the situation in vitro when the cells are resuspended in fresh plasma, enabling the platelets to become hemostatically active. Our in vivo data appear to support this concept and show a reduction in bleeding time in four of five patients following transfusion of prostacyclin-treated platelets. Obviously the question of whether transient inactivation in vitro improves the hemostatic functions in vivo requires a more detailed study.

The ultimate goal for the preparation of leukocyte-depleted platelet concentrates is to prevent HLA alloimmunization in patients who are frequently transfused with platelets. Recent studies by Murphy et al. have already revealed a reduction from 48% to 16% in alloimmunization when the leukocyte contamination was kept below about 2 x 10^6 per concentrate. With our technique the contamination is less than 1 x 10^5 leukocytes per concentrate, and a further reduction in alloimmunization may be expected. Thus the use of prostacyclin-treated, filtered platelet concentrates may reduce the incidence of the troublesome febrile transfusion episodes that are inherent to current transfusion regimes, prolong the time when random donor concentrates are effective, and reduce the need for HLA-matched donor platelets.

Table 2. Simplate Bleeding Times Before and After Transfusion of Filtered Platelet Concentrates

<table>
<thead>
<tr>
<th>Patient</th>
<th>Platelet Count (x 10^6/mL)</th>
<th>Bleeding Time</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>5</td>
<td>9</td>
<td>59</td>
</tr>
</tbody>
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