A Controlled Comparison of the Efficacy of Hetastarch and Pentastarch in Granulocyte Collections by Centrifugal Leukapheresis

By J.-H. Lee, S.F. Leitman, and H.G. Klein

Compared with hetastarch (HS), the low molecular weight analog pentastarch (PS) has been reported to be equally effective for granulocyte collection by centrifugal leukapheresis, to result in fewer adverse donor reactions (ADR), and to have a more rapid elimination profile. We prospectively compared the granulocyte collection efficiency (GCE), granulocyte yield, and ADR in 72 randomly paired granulocyteapheresis procedures from 36 volunteer donors using the model CS-3000 Plus Blood Cell Separator (CS) and either PS or HS as the sedimenting agent. Paired collections from each donor allowed us to compare the two agents directly while controlling for intrinsic donor differences. In 33 of 36 (92%) donors, HS procedures were significantly more efficient than PS procedures ($P < .001$). As an average, HS collections yielded $2.3 \pm 0.67 \times 10^{10}$ granulocytes at 58% ± 8.8% GCE, whereas PS procedures resulted in $1.4 \pm 0.76 \times 10^{10}$ granulocytes at 33% ± 15% GCE. No starch-induced ADR were seen with either agent. For granulocyte harvests using the CS, (1) in most donors, using HS as the red blood cell sedimenting agent during centrifugal leukapheresis results in significantly higher (nearly twofold) GCE and larger granulocyte yields in comparison with using PS, (2) ADR were not observed with either agent, and (3) the potential benefit of more rapid PS elimination should be balanced against significantly lower granulocyte yields. This is a US government work. There are no restrictions on its use.

ACROMOLECULES have been routinely administered to volunteer blood donors during granulocyte collection to promote red blood cell (RBC) sedimentation and increase granulocyte yield. During the past decade, 6% hydroxyethyl starch (hetastarch [HS]) has been the preferred sedimenting agent because it greatly enhances granulocyte yield, the transient plasma expansion is generally well tolerated, and it has few clinically significant side effects in conventional dose and administration frequency. However, significant HS blood levels persist for weeks and trace amounts can be detected years after its administration. Delayed HS clearance and consequent long-term donor safety concerns resulted in a search for alternative agents. Ten percent pentastarch (PS), a more recently developed low molecular weight HS analog, is cleared from the circulation relatively rapidly and has been reported to be equally effective in enhancing granulocyte collection. The ability of PS to separate blood cells and induce RBC sedimentation, as assessed in vitro by the recovery of blood cells in supernatant plasma, has been reported to be similar to that of HS. The results of a subsequent multicenter clinical trial involving 75 volunteer donors who underwent 179 centrifugation leukapheresis procedures strongly suggested that using PS as the sedimenting agent results in an adequate granulocyte yield comparable to that from using HS, and also documented almost no clinically significant adverse donor effects. Initial studies could not detect PS in the blood within a few days of its administration, and the adverse donor reactions (ADR) profile has been inferred to be even more favorable than that of HS. These initial studies have encouraged most collectors to substitute PS for HS in procuring granulocytes. However, equivalent efficacy for PS and HS has not been shown in a controlled trial. The present controlled study examines the granulocyte collection efficiency (GCE), granulocyte yield, and the ADR in randomly paired PS-HS collection procedures from the same donor and directly compares the performance characteristics of the two agents for use in centrifugal granulocapheresis.

MATERIALS AND METHODS

We prospectively studied 72 consecutive granulocyte collections from 36 volunteer blood donors using the model CS3000 Plus Blood Cell Separator (CS; Fenwal Laboratories, Deerfield, IL). Each donor underwent paired collections separated by approximately 2 months (range, 2 weeks to 7 months) and randomly received a standardized 500 mL dose of either 10% PS (McGaw, Inc, Irvine, CA) or 6% HS (NPBI, Emmer-Compascuum, The Netherlands) during the first procedure and the alternate agent during the subsequent collection. The HS preparation used was chemically and pharmacologically identical to the preparation more widely used in the United States (McGaw, Irvine, CA). Thirty milliliters of 46.7% trisodium citrate added to 500 mL of either PS or HS was evenly distributed over approximately 7 L of processed whole blood (1:13 ratio with blood). After obtaining informed consent for the procedure for procuring granulocytes, all donors were premedicated with 8 mg of oral diazepam 12 hours before each procedure. The two RBC sedimenting agents are compared in Table 1. The specific instrument settings used are summarized in Table 2.

GCE. The GCE of each procedure was calculated by dividing the granulocyte yield by the total number of granulocytes processed. The yield was calculated as the product of the component cell count and volume. The number of granulocytes processed (GP) was calculated as follows: GP = (WBC)(%G)(L), where WBC represents the donor peripheral white blood cell count per liter, %G represents the granulocyte fraction (neutrophils including bands), and L represents the total volume of processed whole blood in liters. We used the average of the cell counts immediately before and after each procedure, for both WBC and %G. The accuracy of averaging the two cell counts has been previously verified. All cell counts were performed on the Coulter Counter Model S-PLUS V (Coulter Electronics, Inc, Hialeah, FL).

PS versus HS. For each donor, we plotted the GCE (Fig 1) and the granulocyte yield (Fig 2) from the procedure using HS as a function of the donor’s corresponding values using PS. We also

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HYDROXYETHYL STARCH IN GRANULOCYTOPHERESIS

Table 1. A Comparison of the Biochemical and Pharmacokinetic Characteristics of 10% PS and 6% HS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PS</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average molecular weight (MW)</td>
<td>264,000</td>
<td>480,000</td>
</tr>
<tr>
<td>No. average molecular weight (MN)*</td>
<td>63,000</td>
<td>71,000</td>
</tr>
<tr>
<td>Hydroxyethyl starch concentration (g/dL)</td>
<td>10.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Hydroxyethyl groups per glucose residue</td>
<td>0.45</td>
<td>0.70</td>
</tr>
<tr>
<td>Time to reach 50% peak blood level (h)*</td>
<td>2.5</td>
<td>25.5</td>
</tr>
<tr>
<td>24-h plasma distribution (% dose)*</td>
<td>7</td>
<td>38</td>
</tr>
<tr>
<td>24-h urinary excretion (% dose)</td>
<td>70</td>
<td>33</td>
</tr>
<tr>
<td>24-h extravascular distribution (% dose)*</td>
<td>33</td>
<td>23</td>
</tr>
<tr>
<td>Overall survival in blood*</td>
<td>96 h</td>
<td>17.26 wk</td>
</tr>
</tbody>
</table>

* Mishler et al. Characteristics not taken from Mishler et al. have been listed directly from the package insert of each agent.

...compared the mean and the standard deviation of the GCE and the granulocyte yield for all procedures using PS versus all procedures using HS. The GCE distribution of the 36 procedures on each sedimenting agent is compared in Fig 3.

The donors were verbally questioned to detect any ADR from either starch preparation at completion of each procedure and were instructed to report by phone any problems arising subsequent to each procedure. All granulocyte donors in this study were also regular platelet donors at our collection center, and subsequent donor visits allowed the opportunity to confirm absence of ADR.

*Statistical methods.* The significance of the results on GCE and granulocyte yield was analyzed using the sign test. In brief, the data points above the line of identity were labeled plus in determining the $\chi^2$ (with one degree of freedom) and $P$ values. The adequacy of the sample size was inferred from the resulting degree of significance. In Figs 1 and 2, we used linear regression analysis (sum of least squares method) to generate trend lines.

RESULTS

Figure 1 compares the expected relationship between HS and PS if the two agents are equal (dashed line) with the observed relationship (solid line). The increased $y$-intercept of the observed line describes minimization, by using HS instead of PS, of unacceptably poor GCE or collection failures. Thirty-three of the 36 donors (92%) showed a higher GCE with HS than with PS ($P < .001$). The actual granulocyte yields are plotted in Fig 2, in which comparable predonation leukocyte counts for the two collections from each donor resulted in a line comparable to that in Fig 1. The overall mean predonation leukocyte count of approximately $8 \times 10^7$L with 80% granulocytes showed substantial interdonor but relatively little intradonor variability. In comparison with PS, using HS resulted in a component with substantially more granulocytes in 32 of the 36 donors (89%, $P < .001$). The wide interdonor variability in the GCE and the granulocyte yield using PS were minimized by using HS, as indicated by the relatively horizontal slope values less than 1 (0.24 and 0.52 for Figs 1 and 2, respectively). For all 72 procedures, using HS resulted in a significantly higher mean GCE (58% ± 8.8% vs 33% ± 15%, $P < .001$) and a larger mean granulocyte yield (2.3 ± 0.67 vs 1.44 ± 0.76 × 10^9 granulocytes, $P < .001$) than using PS.

The greater variability in cell yield with PS than with HS may be also appreciated from Fig 3, which shows a distribution plot of the granulocyte yield for the 36 procedures with each agent. The less efficient and more variable PS collections included 9 procedures (25%) with unacceptably low cell numbers (<1 × 10^9 granulocytes) and were more likely (12 procedures [33%]) to result in a yield between 1.0 and 1.5 × 10^9 granulocytes. In contrast, only one HS collection (3%) generated less than 1 × 10^9 granulocytes. HS collection commonly (13 procedures [36%]) generated components with a cell dose between 2.0 and 2.5 × 10^9 granulocytes. Using HS assured a minimum GCE of 40%, whereas using PS resulted in a collection failure with a GCE less than 10% in 3 of the 36 procedures (8.3%). Infrequent ADR

Table 2. Apheresis Parameters for Granulocyte Collection on the Model CS3000 Plus Blood Cell Separator

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood flow rate</td>
<td>50 mL/min</td>
</tr>
<tr>
<td>Centrifuge RPM</td>
<td>1,000</td>
</tr>
<tr>
<td>Blood volume processed</td>
<td>6.5-7.0 L</td>
</tr>
<tr>
<td>Interface detector setting</td>
<td>33</td>
</tr>
<tr>
<td>Program</td>
<td>2</td>
</tr>
<tr>
<td>Chamber type</td>
<td>Granulo</td>
</tr>
</tbody>
</table>

Fig 1. A comparison of the GCE for PS and HS. The GCE using HS was roughly linearly correlated with the GCE using PS, and a given donor with a relatively high GCE on PS was also more likely to show a high GCE on HS. In 33 of 36 donors (92%), HS resulted in a superior GCE in comparison to PS. The 3 remaining donors showed a comparable GCE with either PS or HS. The dashed line indicates the hypothetical plot assuming that the two agents are equally effective.
from dexamethasone premedication (sleep disturbance) and
the apheresis procedure (mild vasovagal reactions and citrate
toxicity) did not favor collections using either agent. None
of the 36 donors reported ADR directly attributable to either
agent.

**DISCUSSION**

Advances in blood component therapy and the therapeutic
efficacy of RBC, platelet, and plasma components allow
effective transfusion support of patients with complicated
hematologic disorders. As a result, infectious complications
unresponsive to optimal antibiotic therapy have become an
increasing cause of morbidity and mortality in patients with
prolonged cytopenias. In contrast to RBCs and platelets,
granulocytes have developed slowly as a therapeutic blood
component, owing in part to the difficulty in collecting suf-
ficient numbers of cells that permit well-controlled studies
to assess granulocyte transfusion efficacy. Thus, despite nu-
merous studies that have attempted to assess the therapeutic
role of granulocyte transfusions (GTs) in neutropenic pa-
tients, definitive information is not available and GTs have
fallen out of favor. Seven controlled studies comparing anti-
biotic therapy alone versus GT in addition to antibiotic ther-
apy in infected neutropenic patients have generated conflict-
ing conclusions. Five of the seven studies report at least
partial success of GT; no benefit was seen in the remaining
two studies involving 47 and 22 subjects. However, the latter
two studies used extremely low cell doses, approximately
only 20% of currently achievable dose.\(^{11}\) Recent reports us-
ing granulocyte colony-stimulating factor to stimulate nor-
mal blood donors again raise the possibility of routinely
collecting greater numbers of granulocytes in the future.\(^{11}\)
Advances that increase cell dose may significantly improve
the efficacy of GT, especially in the pediatric setting, in
which a higher dose of granulocytes per kilogram of body
weight is possible. It thus seems appropriate to reanalyze the
technique for collecting granulocytes in optimal numbers.

The granulocyte yield depends primarily on the efficiency
at which the cells are extracted during an apheresis collection
procedure. The similarity of RBC and granulocyte sedimenta-
tion rates during centrifugation\(^{12,13}\) has necessitated the use of
macromolecules to enhance RBC sedimentation and to achieve
optimal separation of these cells for a successful granulocyte
collection.\(^{14,15}\) The macromolecules most widely used include
hydroxyethyl starch preparations, dextrans, and modified fluid
gelatins; of these, HS gained initial favor because it effectively
enhanced granulocyte yield with few ADR in conventional

**Fig 2.** A comparison of the granulocyte yield for PS and HS. A
plot of the actual granulocyte yield rather than the GCE generated a
line analogous to the line shown in Fig 1. In 32 of 36 donors (89%),
HS resulted in a superior granulocyte yield in comparison to PS. The
4 remaining donors showed a comparable yield with either agent.
The dashed line indicates the hypothetical plot assuming that the
two agents are equally effective.

**Fig 3.** The granulocyte yield distributions for the two sedimenting
agents. The number of procedures generating a particular granulo-
cyte yield was plotted as a function of that yield (x 10\(^{10}\) granulocytes,
grouped in increments of 0.5) for the 36 procedures with each agent.
Using HS resulted in more uniform components with a higher granu-
locyte yield than using PS.
dose and administration frequency.1 However, significant HS blood levels persist for weeks, and trace amounts may be detected years after its administration.2,3 Incidence estimates of clinically apparent HS-induced adverse reactions range from 0.09% to 0.7%.1,6,17 In doses of less than 1,500 mL, HS induces only transient, mild, clinically insignificant laboratory alterations, including prolonged partial thromboplastin and prothrombin times, decreased platelet counts and fibrinogen levels, von Willebrand-like syndrome, and hyperamlasemia.1,6,19 However, serious reactions, including severe pruritus, disseminated intravascular coagulopathy, and shock, have been reported.20,22 Although they are exceedingly rare, these serious reactions and concern about long-term toxicity as a result of delayed HS clearance have prompted a search for a sedimenting agent with a more favorable elimination profile. The initial studies which suggested that PS is as effective as HS while being eliminated much more rapidly encouraged many collectors to choose PS for use in granulocyte harvests by centrifugal leukapheresis.6,8

The separation of granulocytes from erythrocytes, which dictate GCE, depends on the relative sedimentation rates of the two cell types and the granulocyte sedimentation rate relative to the upward plasma flow resulting from downward cell displacement. Macromolecules that promote RBC rouleaux formation increase the effective cell mass to cell surface area ratio and thereby increase both the cell sedimentation rate and the consequent upward plasma flow. RBCs, with a highly negatively charged outer cell surface, are much more susceptible to polar macromolecules than are neutral granulocytes, and RBC sedimentation is selectively increased over granulocyte sedimentation through both direct cell and indirect plasma effects.23 Although the precise molecular mechanism of RBC rouleaux formation is unknown, kinetic studies using nuclear magnetic resonance relaxation methods have shown that HS rapidly induces over 20 seconds the formation of aggregates containing 4 RBCs on average at equilibrium, which effectively doubles the mass to surface area ratio in comparison to a single RBC.24

Two properties of macromolecules independently influence RBC sedimentation. There appears to be a critical molecular weight (CMW) as well as a critical concentration (CC) below which RBC sedimentation is not enhanced. Although rigorous numbers are difficult to establish, the CMW and the CC for hydroxyethyl starch compounds have been determined to be 300,000 and 0.3 g/dL, respectively.1 HS, with a weight-average molecular weight (MW) of 480,000 and relatively slow elimination over several days, readily meets these criteria under virtually all collection conditions used today. In contrast, the substantially lower PS MW of 264,000 (below the CMW) may explain in part the decreased efficacy relative to HS. Because the number-average molecular weights of the two agents are fairly comparable, the relatively few but large molecules present to a greater extent in HS than in PS may be an important element that contributes to the more uniform, increased HS efficacy. Furthermore, the rapid PS plasma clearance within hours (and within the time frame of a single leukapheresis procedure) decreases the potential cumulative effect of infusing the sedimenting agent into recirculated donor blood already containing the agent in significant amounts.

The initial studies on PS, which included one multicenter clinical trial, concluded that PS is as effective as HS in centrifugal granulocytapheresis.7,8 In view of the current literature on the effects of macromolecules on the physics of blood cell separation23 and the biochemical differences between PS and HS,1 it would be surprising if in fact PS were as effective as HS. One intrinsic donor variable that predictably influences granulocyte harvest by centrifugal leukapheresis is the donor erythrocyte sedimentation rate (ESR).9 Results of studies from our laboratory have suggested that PS may be less effective than HS, based on an indirect comparison of the respective formulas that predict GCE from the donor ESR. The slope and the y-intercept of the linear relationship GCE (%) = 1.3 ESR (mm/h) + 45 when one uses HS as the cell sedimenting agent decreases to 0.8 and 20, respectively, when PS is substituted for HS in otherwise identical collection procedures.25 In this study, we directly compare the two agents in a controlled clinical trial; our results confirm the indirect comparison study. The more consistent HS collections assured a 40% minimum GCE, whereas a substantial fraction (8.3%) of the less predictable PS procedures resulted in collection failures at less than 10% GCE. Routine PS use will more likely result in fewer granulocytes than the criteria established by the American Association of Blood Banks Standards Committee, or $1.0 \times 10^{10}$ granulocytes per unit in 75% of tested units.26 Finally, the lack of ADR from either agent in our series of only 36 collections in each of the two arms from 72 donors is not unexpected in view of the previously reported incidence of less than 1%.1,16

We conclude the following for centrifugal granulocytapheresis using the model CS3000 Plus blood cell separator and either PS or HS as the RBC sedimenting agent. (1) In most donors, HS results in a significantly higher (nearly twofold) GCE and a larger granulocyte yield in comparison to PS. (2) Sedimenting agent-induced ADR were not observed with either HS or PS. (3) The potential benefit of more rapid PS elimination should be balanced against the significantly lower granulocyte yield. The relative efficacy of PS and HS using other blood cell separators and under other collection conditions requires further study.

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