Neutrophil Transfusion: Effect of Storage and of Collection Method on Neutrophil Blood Kinetics

By Thomas H. Price and David C. Dale

The kinetics in blood of autologous neutrophils collected by phlebotomy, filtration leukapheresis (FL), or intermittent-flow centrifugation (IFC), labeled with $^{32}$P-diisopropylfluorophosphate, and stored at $4^\circ$C for up to 2 days were measured in 41 normal subjects. Mean initial recovery for unstored IFC cells was 34.0%, compared to 7.9% for unstored FL cells. Blood half-lives were 4.1 and 2.7 hr for unstored IFC and FL cells, respectively. With neutrophils collected by phlebotomy and stored in whole blood for 1–2 days, posttransfusion recoveries and blood half-times were significantly decreased. Storage of both IFC and FL preparations resulted in only moderate kinetic abnormalities in comparison to the unstored cells. These studies indicate that the ability of unstored IFC cells to circulate is basically normal, whereas that of unstored FL cells is significantly impaired. The data further suggest that these neutrophil concentrates might be stored for 1–2 days prior to transfusion.

OPTIMAL SUPPORT of neutropenic patients with neutrophil transfusions depends on the capacity of the transfused neutrophils to function normally in vivo. Critical factors affecting neutrophil function in vivo include the technique of cell collection from the donor and the time and conditions of neutrophil storage prior to transfusion.

Currently, neutrophils are collected by continuous-flow centrifugation (CFC), filtration leukapheresis (FL), and intermittent-flow centrifugation (IFC). Kinetics in blood and function in vitro of neutrophils obtained by CFC have been found to be normal or near normal. Most investigators have found significant abnormalities in the function in vitro of FL cells, but these abnormalities can apparently be minimized or perhaps prevented by premedication of the donor with corticosteroids and by shortened collection times. Kinetic studies have shown that radioisotopically labeled FL cells have diminished recoveries and shortened blood half-times.

While the function in vitro of IFC cells may be normal, no studies have been reported on the kinetic behavior in vivo of these cells. Animal studies have shown that CFC cells are better able to accumulate in vivo in inflammatory sites than FL cells. Despite these per cell differences, it is not clear that CFC is a technique superior to FL, since much greater numbers of neutrophils can be collected by FL.

Animal studies indicate that neutrophils stored in acid citrate dextrose...
(ACD)-anticoagulated whole blood for up to 3 days retain near-normal circulatory kinetics and rates of accumulation at sites of inflammation. Recent studies have shown that human neutrophils in whole blood can be stored for 1–2 days with only minimal changes in functional capabilities in vitro. If the function in vivo of human cells can be maintained with short-term storage, it would be of practical importance to neutrophil transfusion programs.

In an attempt to study one aspect of the function in vivo of stored human neutrophils, we measured the blood kinetics of neutrophils collected by standard methods and stored for up to 2 days. Normal subjects and autologous cells were used to eliminate any effect of disease, neutropenia, infection, or isosensitization on the results.

**MATERIALS AND METHODS**

Neutrophils were collected from normal volunteers by simple phlebotomy, filtration leukapheresis, or intermittent-flow centrifugation (Latham bowl). The cell preparations were stored for up to 48 hr at 4°C, labeled with $^{32}$P-diisopropylfluorophosphate (DF$^{32}$P), and reinfused into the donor. Serial blood samples were obtained over the next 24 hr for determination of neutrophil recovery and survival.

**Subjects**

Subjects were 25 male and 16 female normal volunteers. All gave informed consent for the study, which was approved by the University of Washington Human Subjects Review Committee. All participants had neutrophil counts >1800 and a hematocrit ≥35%, prior to the study and had no history of hematologic disease or recent infection.

**Cell Collection**

**Phlebotomy.** Five hundred milliliters of blood (or estimated 15% of the blood volume in small subjects) was drawn into plastic bags (Fenwal PL130, Travenol, Deerfield, Ill.) containing ACD-A. In a few studies the blood was transferred to a different plastic bag (Fenwal PL146) for storage.

**Filtration leukapheresis (FL).** The standard Fenwal leukapheresis apparatus using two nylon fiber filters was operated at flow rates of 40–80 ml/min for 3 hr (2 hr in one subject). Subjects were given 5000 units heparin (Panheparin, Abbott, North Chicago, Ill.) intravenously at the beginning of the procedure, and 1 unit heparin/ml blood flow was added throughout the collection. During the procedure 500 ml blood was removed from the system, and the plasma from this blood was subsequently used to elute the cells from the filters. The red blood cells were returned to the subject. At the end of the collection the apparatus was flushed with 400-450 ml normal saline. Neutrophils were eluted from the filters at 50 ml/min/filter using 1000 ml of a mixture of 20%, autologous plasma, 15%, ACD, and saline while tapping the filters. Cell suspensions were concentrated to a final volume of about 300 ml by centrifugation (400 g, 15 min), and the cells were stored in the above plasma-ACD-saline mixture.

**Intermittent-flow centrifugation (IFC).** All collections were made with the Haemonetics Model 30 (Haemonetics, Natick, Mass.) using the method of Huestis et al. Six per cent hydroxyethyl starch (Volex, McGaw, American Hospital Supply, Irvine, Calif.) was used as a red cell sedimenting agent. Ten grams sodium citrate was added to the hydroxyethyl starch for anticoagulation. If the bottle of starch ran out during the collection, it was replaced by 500 ml normal saline to which 10 g sodium citrate had been added or by ACD. Large centrifuge bowls (375 ml) were used for most subjects weighing over 150 lb. The procedure was continued for six cycles (five cycles in one subject) at a maximum attainable flow rate (usually 60-100 ml/min). The flow rate was slowed to 20 ml/min when the platelet band was 1–2 cm from the exit port. Cell collection was begun at the time the platelets entered the exit port and was continued for 5 min at 20 ml/min. After the last collection cycle the red cells were allowed to sediment in the collection bag, which was not agitated during the procedure, and the majority of the red cells was drained from the bottom of the bag and returned to the donor. The final volume of the resultant leukocyte suspension was generally 300–350 ml.
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Storage

Cell preparations were stored in plastic blood bags (Fenwal PL146 or PL130) without agitation at 4°C for up to 48 hr. Samples were obtained before and after storage for determination of pH and glucose concentration.

Blood Kinetic Measurements

Neutrophils were labeled in standard fashion with 50-100 μCi DF32P. The isotope was obtained from the distributor (Amersham Searle, Arlington Heights, Ill.) as a radiochemical and was placed in propylene glycol and sterilized by the Nuclear Pharmacy at the University of Washington Hospital. After removing an aliquot for determination of neutrophil count and specific activity, these cells were reinfused into the donor over a 5–15-min period. Blood samples (20 ml) were obtained at 10 min and at 1, 2, 3, 4, 6, 8, 11, and 24 hr after infusion of the cells. Neutrophils were isolated from the phlebotomy and IFC cell preparations and from all postinfusion blood samples by Ficoll-Hypaque sedimentation and NH4Cl lysis. Contaminating red cells were removed by dextran sedimentation and NH4Cl lysis. Neutrophils were usually isolated from FL cell concentrates by NH4Cl lysis alone because these preparations contained >90%, neutrophils and few contaminating red cells. Neutrophil-specific activity was determined by measuring the radioactivity present in a known number of cells as previously described. All cell counts were performed with a Coulter counter (Coulter, Hialeah, Fla.).

Calculations

Neutrophil radioactivity per ml blood was determined at each time point. Average survival curves for each collection and storage condition were obtained by averaging the recoveries (percentage of injected neutrophil label present in the circulation at a given time) at each time point for all subjects in each category. The curves from five IFC subjects were considered unevaluable because of recovery values greater than 100%, and were excluded from the results. Most curves were reasonably linear over the first 6-10 hr when plotted on semilogarithmic paper but tended to plateau thereafter. Blood half-times (t½) were calculated for each individual curve on the basis of data from the most linear portion of the curve. Initial and 1-hr recovery were defined as the recovery at the 10-min and 1-hr samples, respectively.

RESULTS

Neutrophil yields were 1.5 ± 0.2 × 10⁹ with phlebotomy, 21.0 ± 2.4 × 10⁹ for FL, 6.2 ± 1.1 × 10⁹ for small bowl IFC, and 10.1 ± 1.6 × 10⁹ for large bowl IFC, results comparable to those reported previously. There was no appreciable change in either the white blood cell or neutrophil concentrations of the preparations with up to 2-day storage. Average pH and glucose concentration of the cell preparations and the change in these parameters with storage are shown in Table 1. There were significant differences in the red cell, white cell, and neutrophil concentrations of the three preparations. While the

| Table 1. Effect of Storage on the pH and Glucose Concentrations of the Neutrophil Preparations (Mean ± SEM) |
|-------------------------------------------------|------------|---------|------------|---------|
| WBC (× 10³/μl) | PMN (× 10³/μl) | Hct (%) | Initial | 1 day | 2 day | Initial | 1 day | 2 day |
| Phlebotomy | 4.7 | 2.6 | — | 7.00 | 6.84 | 6.74 | 420 | 390 | 422 |
| n = 10 | ± 0.4 | ± 0.4 | ± 0.06 | ± 0.03 | ± 0.03 | ± 16 | ± 14 | ± 23 |
| FL | 73.6 | 64.7 | 5.7 | 5.75 | 5.48 | 5.49 | 260 | 279 | 266 |
| n = 12 | ± 6.9 | ± 6.3 | ± 1.0 | ± 0.04 | ± 0.11 | ± 0.10 | ± 8 | ± 15 | ± 10 |
| IFC | 43.5 | 22.2 | 3.8 | 7.35 | 7.11 | 6.95 | 75 | 66 | 43 |
| n = 19 | ± 4.0 | ± 3.4 | ± 0.7 | ± 0.07 | ± 0.13 | ± 0.06 | ± 5 | ± 10 | ± 11 |

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pH of the IFC and the phlebotomy preparations were close to physiologic, the mean pH of FL concentrates was 5.75. Mean glucose concentrations were 420, 284, and 75 mg/dl for the phlebotomy, FL, and IFC preparations, respectively. With storage for 2 days there was generally a slight drop in pH. Glucose concentration did not change appreciably with storage of phlebotomy or FL cells but dropped about 35%, with IFC cell storage.

Side effects experienced by the subjects during cell donation were minimal and similar to those previously reported.9,11 These included arm discomfort due to immobility, mild vasovagal reactions, mild chilliness, and transient circumoral tingling attributable to citrate effect in the IFC collections. Rapid reinfusion of the phlebotomy and IFC cells was associated with no symptoms, and posttransfusion neutrophil counts were relatively stable (Fig. 1). With FL cells, six of the twelve subjects experienced symptoms after the infusion of autologous cells. Facial flushing occurred in four subjects at high rates of infusion, the symptoms disappearing if the infusion was slowed. Two subjects felt warm an hour or so after the infusion. Temperatures were taken only in four subjects, two of whom had temperatures of 37°–38°C 2 hr after the cells were received. One subject had mild chilling sensations after the infusion, before developing a mild fever.

Although FL resulted in a moderate increase in neutrophil count in most donors, absolute neutrophilia (>7500 cells/μl) was not observed. With FL experiments in which the cells were immediately labeled and returned, postinfusion neutrophil counts showed little change from the baseline values (Fig. 1), similar to phlebotomy or IFC cells. However, with FL cells stored for 1-2 days there was a consistent neutrophilia after reinfusion, rising to a maximum at 3-6 hr after infusion.
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Fig. 2. Disappearance of neutrophil radioactivity after intravenous injection of unstored DF32P-labeled autologous neutrophils. Vertical line, 1 SEM. a, cells collected by phlebotomy (18 subjects); b, cells collected by intermittent-flow centrifugation (4 subjects); c, cells collected by filtration leukapheresis (4 subjects).

Blood Kinetics of Unstored Cells (Fig. 2)

The blood kinetic data for unstored normal autologous neutrophils collected by phlebotomy were derived from measurements previously made in this laboratory\textsuperscript{17} and indicated an immediate postinfusion recovery of $37.6 \pm 2.1\%$ and a $\tau_{1/2}$ of $5.8 \pm 0.3$ hr. With cells collected by FL, initial recovery was $7.9\% \pm 2.3\%$. Subsequent disappearance of the cells from the circulation was reasonably exponential for the first 6–10 hr, then plateaued; the mean $\tau_{1/2}$ for the initial portion of the curve was $2.7 \pm 0.5$ hr. Initial recovery of neutrophils collected by IFC was $34.0\% \pm 0.4\%$, not significantly different from normal. The disappearance curve was not quite linear over the entire 24-hr span; the mean initial $\tau_{1/2}$ was $4.1 \pm 0.2$ hr.

Effect of Neutrophil Storage on Blood Kinetics

Phlebotomy-collected cells apparently suffered significant damage with storage (Fig. 3). With 1-day storage initial recovery dropped to $15.9\% \pm 3.4\%$ with a $\tau_{1/2}$ of $1.5 \pm 0.2$ hr. In three of these studies the blood was stored in one type of plastic (Fenwal PL130), and in the remaining three it was stored in another (Fenwal PL146). There was no apparent difference in the blood kinetics of neutrophils stored in these two plastics. Storage for an additional 24 hr did not further affect the kinetics, giving an initial recovery of $11.6\% \pm 4.4\%$ and a $\tau_{1/2}$ of $3.1 \pm 0.8$ hr.

Fig. 3. Disappearance of neutrophil radioactivity after injection of DF32P-labeled autologous neutrophils collected by phlebotomy. Vertical lines, 1 SEM. ---, no storage (18 subjects); ----, 24-hr storage (6 subjects); ·····, 48-hr storage (4 subjects).
The FL neutrophils stored for 1 day gave an initial recovery of $1.9\% \pm 0.3\%$ (Fig. 4). The number of circulating labeled neutrophils subsequently increased, reaching a maximum $(6.0\% \pm 1.4\%)$ at 1 hr. The curve thereafter was not different from that of the unstored cells. Excluding the initial point, the $\tau_1$ was $2.7 \pm 0.5$ hr. With 48-hr storage, initial recovery was $1.1\% \pm 0.3\%$; again recovery rose to a maximum at 1 hr $(4.3\% \pm 1.8\%)$, but it remained below that of the unstored or 1-day-stored cells for the remainder of the curve. The $\tau_1$ was slightly shortened $(1.6 \pm 0.2$ hr).

Storage of IFC collected cells for 1 day resulted in an initial recovery of $49.5\% \pm 13.8\%$ (Fig. 5). The $\tau_1$ was shortened to $1.8 \pm 0.4$ hr, so that the recovery was appreciably lower than that of the unstored cells by about 6 hr after the infusion. With 2-day storage initial recovery was $8.9\% \pm 1.4\%$, but circulating activity subsequently increased and the remainder of the curve was the same as that of cells stored for 1 day. The effect of storage on the recoveries and blood half-times of all the neutrophil preparations is shown in Table 2.

DISCUSSION

Neutrophil transfusions are being used with increasing frequency in the management of infected neutropenic patients. The cells are generally collected from normal donors by techniques involving centrifugation (continuous or intermittent flow) or the adherence and subsequent elution of neutrophils from nylon filters. Although most centers are currently administering neutrophils as soon
Table 2. Effect of Storage Time on Recoveries (%) and $r_2$ (hr) (Mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>No Storage</th>
<th></th>
<th>1-day Storage</th>
<th></th>
<th>2-day Storage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observations</td>
<td>Recovery</td>
<td>Initial 1 hr $r_2$</td>
<td>Observations</td>
<td>Recovery</td>
<td>Initial 1 hr $r_2$</td>
</tr>
<tr>
<td>Phlebotomy</td>
<td>18</td>
<td>37.6 ± 3.1</td>
<td>5.8 ± 2.1</td>
<td>18</td>
<td>33.1 ± 3.4</td>
<td>9.3 ± 0.7</td>
</tr>
<tr>
<td>FL</td>
<td>4</td>
<td>7.9 ± 1.4</td>
<td>2.7 ± 2.3</td>
<td>4</td>
<td>6.7 ± 2.4</td>
<td>6.0 ± 1.4</td>
</tr>
<tr>
<td>IFC</td>
<td>5</td>
<td>34.0 ± 4.1</td>
<td>7.6 ± 2.3</td>
<td>5</td>
<td>27.6 ± 2.8</td>
<td>4.1 ± 0.3</td>
</tr>
</tbody>
</table>

*Based on data of Dancey et al.17
1 Values significantly different from phlebotomy unstored, $p < 0.05$ (t test)
2 Values significantly different from FL unstored, $p < 0.05$ (t test)
3 Values significantly different from IFC unstored, $p < 0.05$ (t test).

As possible after collection, it would be advantageous if short-term storage were feasible.

In the evaluation of neutrophils prepared for transfusion, the critical consideration is their ability to function in vivo. Although function tests are simplest in vitro, they may not be reliable as an estimate of function in vivo. They may not be sensitive enough to detect subtle changes in the cells. On the other hand, they may overestimate cell damage by detecting defects that are reversible in vivo.7,13,22 The function in vivo of neutrophils comprises their ability to circulate, accumulate at inflammatory sites, and perform bactericidal functions at these sites. In these studies the blood kinetics of the neutrophils have been used as a measure of viability.

The effect of the collection technique on neutrophil viability has been the subject of numerous studies. The chemotactic and bactericidal ability in vitro of cells collected by CFC has been found to be normal by several observers.1, 3 Similar measurements on FL cells have shown modest abnormalities that appear to be quantitatively dependent on the length of time the cells are exposed to the filters and to the strength of the filter-cell bond.1,2,4,7 The phagocytic ability of IFC cells has been shown to be normal.11,12

The ability of the neutrophils in these preparations to circulate was investigated systematically by McCullough et al.,1 who studied the blood kinetics of autologous human cells collected by CFC and FL. CFC cells showed slightly but not significantly decreased recovery and normal blood half-times. FL cells had significantly decreased recovery with shortened blood half-times. These studies confirmed previous impressions based on postinfusion increments in infected neutropenic recipients,9 occasional isotopic studies,23,24 and animal studies.3,4,9 No information has been available on the blood kinetics of cells collected by IFC.

The ability of the neutrophils in these preparations to arrive at sites of inflammation has been anecdotal in humans.9,21 Applebaum et al.10 conducted studies in dogs comparing the abilities of CFC and FL cells to accumulate in skin chambers. They demonstrated that CFC cells accumulated in these chambers to a much greater degree than FL cells but suggested that the difference might be overcome by increasing the number of FL cells administered.

It would be of practical importance to know the effect of short-term storage in vitro on the viability of neutrophils. McCullough et al.14,15 studied the func-
tion in vitro of human neutrophils stored in whole blood and showed that bactericidal function and the ability to reduce nitroblue tetrazolium (NBT) remained normal in cells stored for 24 hr at 4°C. Chemotaxis was slightly impaired at 24 hr. With storage times longer than 24 hr, the results depended on the function tested. Bactericidal activity was retained through 72-hr storage, but chemotactic and NBT-reducing activities were progressively impaired. Steigbigel et al.25 showed that FL concentrates could be stored for 72 hr without significant depression in bactericidal activity. Chemotactic activity of these cells was slightly depressed at 24-hr storage. Similar results were obtained by Glasser26 in investigating the storage of IFC cells. Studies in vivo in the rabbit have shown that neutrophils may be stored up to 3 days at 4°C in whole blood without severely impairing their ability either to circulate or to accumulate at sites of inflammation.13

In these studies neutrophil viability was assessed by measuring the blood kinetics of DF32P-labeled autologous cells. Although DF32P is not an ideal neutrophil label because of elution,17 it remains the best label available for autologous studies and is considered adequate for these comparative measurements.

An attempt was made to collect neutrophils by standard methods and to store the preparations as conventionally obtained. The blood kinetics of unstored IFC cells were only slightly different from normal and indicated that the cells behaved similarly to those collected by CFC. Unstored FL cells displayed decidedly abnormal blood kinetics, with a mean initial recovery of 7.9% and shortened blood half-times. The data indicate that while neutrophils rapidly lost their ability to circulate normally when stored in whole blood, they could be stored for up to 2 days in conventionally obtained IFC or FL concentrates with only moderate additional impairment in their ability to circulate, reflected primarily by shortened blood disappearance times. With storage of FL cells and of IFC cells for more than 24 hr, a portion of the injected labeled cells was temporarily sequestered from the circulation, only to reappear within 1 hr. This phenomenon, apparently representing a storage-induced cell defect reversible in vivo, was also observed with stored neutrophils in animals.13 Since the reversal was complete by 1 hr, it seems reasonable to consider variation from normal 1-hr recovery as a measure of irreversible neutrophil damage. The initial recovery, in addition, would reflect the irreversible cell damage. Based on these considerations, neutrophils suffered significant damage when stored in whole blood for 24 hr. FL cells suffered little more damage than the unstored cells at 1 day and perhaps moderate damage by 2-day storage. IFC cells appeared at most moderately damaged after 1- or 2-day storage. While it is recognized that only one aspect of function in vivo was measured and that further studies are indicated to evaluate the ability of these cells to arrive and function at inflammatory sites, these results suggest that neutrophil concentrates may be stored for 1-2 days prior to transfusion.

The administration of FL cells is associated with a higher incidence of transfusion reactions than that of cells collected by centrifugation, and it has been presumed that some of the reactions must therefore be independent of donor-recipient incompatibility and due to the collection technique.21,27,28 That this is
true is demonstrated by the $50\%$ incidence of reactions seen in these autologous studies. This rather high figure may be due to the relatively rapid rate of administration of the cells in this study. The kinetics of the administered neutrophils were no different in subjects experiencing a transfusion reaction than in those who did not.

Of interest is the observation that subjects given stored FL cells developed a marked neutrophilia several hours after administration. The degree of neutrophilia and the fact that the additional cells were not labeled indicates that they were released from the marrow. Rapid neutropenia followed by neutrophilia is a well-described phenomenon in FL donors and is apparently related to complement activation by the foreign surface. The same mechanism may be responsible for the neutrophilia in our subjects and perhaps was not seen in those receiving unstored cells because their marrows were stimulated to release cells several hours previously. The magnitude of the neutrophilia was significantly greater than that seen during the leukapheresis procedure, however, and suggests additional marrow neutrophil-releasing properties in the stored FL preparations. Whether or not the symptoms or abnormal neutrophil kinetics seen in FL recipients are attributable to the cells themselves or to factors in the suspending plasma mixture is unknown.

The neutrophil preparations studied had widely different cellular concentrations, pH, and glucose concentrations. Whether the kinetic differences seen with either fresh or stored cells were due in part to any of these differences or wholly to the more obvious differences in collection technique is not known and requires further investigation.

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REFERENCES


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