Pretreatment of Filtration Leukapheresis Donors With Colchicine

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Although filtration leukapheresis (FL) is a relatively simple and efficient means of harvesting neutrophils (PMN) for transfusion, the PMN obtained by this procedure have both functional and morphologic abnormalities induced when the cells adhere to nylon wool filters used in FL. Adherence to nylon wool causes a partial degranulation of PMN together with the production of superoxide at cell surfaces, and it has been suggested that this and other toxic oxygen metabolites are responsible in part for the abnormalities of FL cells. Because colchicine inhibits PMN degranulation and oxidative metabolism, we evaluated the pretreatment of donors with this drug as a means of preventing adherence-induced changes in FL PMN. The bactericidal capacity and chemotactic responsiveness of FL PMN were compared with and without pretreatment of donors with 2.4 mg colchicine (0.6 mg by mouth 12, 11, 10, and 2 hr before FL). Both the bactericidal and chemotactic functions of FL PMN were significantly impaired when donors received no pretreatment, as shown previously, but these cell functions in vitro were normal when donors received colchicine pretreatment. The improved function of FL cells with donor pretreatment was a consistent and significant finding ($p < 0.01$). Surface charge changes in PMN induced by FL were also diminished by donor pretreatment, as was lysozyme release during FL. No side effects from colchicine were observed in the donors, and FL yields were not appreciably changed by this drug. These studies indicate that colchicine pretreatment of FL donors significantly reduces adherence-induced changes in FL PMN and improves the functional quality of these cells.

When first developed, continuous-flow filtration leukapheresis (FL) promised a significant advance in the technology of collecting neutrophils from normal donors for transfusion. Not only could larger numbers of cells be collected by FL per donation than was possible by centrifugation techniques, but also FL was sufficiently simple and inexpensive to be accessible to most well-staffed medical center blood banks. It has become apparent, however, that the function of neutrophils obtained by FL in humans and in animal models is significantly changed by the interaction of these cells with nylon wool filters used in FL. FL neutrophils have been found to have markedly abnormal posttransfusion kinetics, abnormal morphology, including partial degranulation, and abnormal function in vitro. Studies of the function in vitro of FL neutrophils have shown that the abnormalities of these cells are...
related to the time and extent of cell adherence to nylon wool filters and also that the neutrophils collected by FL are functionally heterogeneous, some cells being relatively normal, some quite abnormal. Furthermore, these studies in vitro have suggested that, at most, one-fifth to one-third of neutrophils collected by standard FL techniques can be expected to perform a useful function in vivo once transfused, and this suggestion has been verified by studies in man and in animals of the posttransfusion kinetics of FL neutrophils, their ability to accumulate at inflammatory sites, and their capacity to confer protection against infection.

Various laboratories have attempted to identify FL techniques that might minimize or prevent the functional abnormalities of FL neutrophils. It is evident that shortened collection times, incomplete elution of filters, use of corticosteroid pretreatment of FL donors, and elution of cells in the presence of local anesthetic agents may augment the collection of neutrophils with relatively normal function.

In the following studies, we show that colchicine pretreatment of FL donors significantly reduces functional abnormalities of FL neutrophils. Colchicine pretreatment was studied because this drug had been shown to inhibit neutrophil degranulation as well as the burst of oxidative metabolism that accompanies degranulation, and both of these cellular events—degranulation and oxygen metabolism—are initiated by the adherence of neutrophils to nylon wool and appear to be of central importance in the induction of functional abnormalities in FL neutrophils.

MATERIALS AND METHODS

Leukapheresis donors. Healthy adult males (ages 28-37 yr) who were regular leukapheresis donors for leukocyte and platelet support of patients at the NCI were leukapheresis donors in these studies. Each donor underwent leukapheresis twice. Before the second leukapheresis, which occurred 1-2 wk after the first, each donor took a total of 2.4 mg colchicine (0.6-mg tablets taken orally at 12, 11, 10, and 2 hr before leukapheresis). In all other respects, each leukapheresis was identical. Informed consent was obtained from all donors who participated in these studies, in accordance with a protocol approved by the NIH medical review board.

Collection of neutrophils. Before each leukapheresis 50-70 ml heparinized blood (10 U heparin/ml) was obtained from the donors. Neutrophils were separated from these blood samples by centrifugation over Hypaque-Ficoll gradients followed by dextran sedimentation and hypotonic lysis of residual erythrocytes. The functions in vitro of neutrophils obtained by leukaphereses were compared in each case with the functions of these cells prepared concurrently from blood as controls.

Filtration leukapheresis procedures, using nylon wool filters (Leukopak, Fenwall, Morton Grove, Ill.), were as reported previously. Neutrophils were eluted from filters after 2.5 hr leukapheresis (with blood flow rates through filters of 40-50 ml/min) using 300 ml ACD-plasma solution per filter with gentle tapping of the filters as described previously. Like the cells obtained from blood by Hypaque-Ficoll/dextran sedimentation techniques, FL neutrophils underwent hypotonic lysis of residual erythrocytes before further study. Yield of neutrophils per filter per 2.5-hr leukapheresis was computed for all donations.

Studies of neutrophil function. Responsiveness to chemotactic stimuli, bactericidal capacity, and cell surface charge were evaluated with neutrophils purified from peripheral blood and with neutrophils collected by FL.

Neutrophil chemotaxis was measured with a radioassay that employed ³¹Cr-radiolabeling of leukocytes and double micropore filter chemotactic chambers, as described previously. For these assays, serum activated with Escherichia coli endotoxin (lipopolysaccharide B 0127:B8; Difco, Detroit, Mich.) was used as the chemotactic stimulus. The chemotactic responses of
test neutrophils to this stimulus was expressed as corrected counts per minute in the lower filter (cor cpm LF), reflecting the numbers of cells that migrated in response to the stimulus.4,19

Bactericidal assays measured the clearance of viable *Staphylococcus aureus* in neutrophil-bacteria incubation mixtures.4,20 Neutrophils (5 x 10⁶) were incubated with bacteria, prepared from washed overnight cultures, at a ratio of four bacteria to one neutrophil. Aliquots of the incubation mixtures were removed at designated intervals with a calibrated wire loop, diluted with sterile water to lyse the leukocytes, and transferred to pour plates that were counted for bacteria colonies 24 hr later. Killing of bacteria was expressed as percent of inoculated bacteria surviving after incubation with neutrophils.

Neutrophil surface charge was measured by an electrophoretic mobility assay reported previously.21 The electrophoretic mobility of neutrophils washed and suspended in phosphate-sorbitol buffer (pH 7.2) was determined with a cytopherometer (Zeiss, New York, N.Y.). Surface charge was calculated from the electrophoretic mobility of test cells as described previously and expressed as micrometers per second per volt per centimeter (μm/sec/V/cm).21

Measurement of plasma lysozyme during leukapheresis. During all leukaphereses, aliquots of blood entering and leaving the filters were obtained at designated intervals. Lysozyme activity in plasma separated from these blood samples was determined by a turbidometric assay that measured the rate of lysis of *M. lysodeikticus* (Worthington Biochemical, Freehold, N.J.) at pH 6.2.22 Enzyme activity is expressed in μg/ml egg white lysozyme standard (Worthington).

Preparation of neutrophils for morphology. Small aliquots of neutrophil suspensions prepared under the various conditions described were fixed onto glass slides by a cytocentrifuge (Cytospin; Shandon, Sewickly, Pa.) and then stained with Wright-Giemsa stain for inspection by light microscopy.

RESULTS

Release of lysozyme from leukocytes during FL. Human neutrophils purified from blood release lysozyme extracellularly when incubated with nylon wool in vitro.23,24 Degranulation and extracellular release of lysozyme also appears to occur during FL, since plasma lysozyme in blood leaving filters during FL has been found to be increased compared with that of blood entering the filters.24 Plasma lysozyme was measured in blood entering and leaving filters during FL procedures, both when donors were pretreated with colchicine and when they received no pretreatment. In every paired study there was a diminished secretion of lysozyme from leukocytes passing through the filters during FL procedures when donors were pretreated with colchicine. This diminished lysozyme secretion was evident at every time point studied between 15 and 150 min of leukapheresis and was comparable to that shown in Fig. 1 for 120 min. It could also be shown that total cellular lysozyme per number of FL neutrophils was greater when donors were pretreated.

Improved function in vitro of FL neutrophils with donor pretreatment. Both the chemotactic responses and bactericidal capacity of FL neutrophils were deficient when no donor pretreatment was used, as has been shown previously.4 However, both of these functions in vitro were normalized when donors were pretreated with colchicine.

As shown in Fig. 2A, when there was no donor pretreatment (open bars), the mean chemotactic response of neutrophils collected by FL was significantly less than that of control neutrophils obtained from the same donors at the time of leukapheresis but purified directly from blood. When donors were leukapheresed after colchicine pretreatment (shaded bars), the chemotactic responsiveness of control cells did not change, but chemotaxis of the FL neutrophils was normalized. These same results are also shown in Fig. 2B but are broken
Fig. 1. Diminished release of lysozyme during FL with colchicine pretreatment of leukapheresis donors. Plasma lysozyme activity in blood leaving filter at 120 min of leukapheresis expressed as percentage of that in blood entering filter. Results from ten leukaphereses with five donors, each of whom was leukapheresed with and without colchicine pretreatment. Plasma lysozyme activity in blood entering the filters when donors were pretreated was not significantly different from that measured when donors were not pretreated.

down for each paired study. As shown, chemotaxis of leukapheresed cells, expressed as percentage of that of control cells obtained concomitantly from each donor, was consistently improved when donors were pretreated with colchicine.

As shown in Fig. 3, the abnormal bactericidal capacity of FL neutrophils also improved with donor pretreatment. In Fig. 3A bactericidal activity of FL and control neutrophils is expressed as percent of bacteria surviving after 45 min incubation with the cells. When there was no pretreatment (open bars), survival of bacteria incubated with FL neutrophils was greater than that of bacteria incubated with control neutrophils obtained from the same donors, indicating diminished bactericidal activity of the FL cells. This reduced bactericidal activity was normalized when donors were pretreated with colchicine. As shown in Fig. 3B improved bactericidal activity of neutrophils collected by FL was a consistent finding in each paired study when donors were pretreated.

Neutrophils collected by FL show various morphologic abnormalities apparent by light microscopy. These abnormalities include ragged boundaries, vacuolization, and increased spreading and are present to varying degrees in FL neutrophils, particularly in those cells that have been most adherent to the filters. Neutrophils collected by FL without donor pretreatment showed these characteristic abnormalities, as illustrated in Fig. 1. FL neutrophils obtained after pretreatment of donors with colchicine, on the other hand, were more
Fig. 2. Improved chemotaxis of FL neutrophils with colchicine pretreatment of donors. Pretreatment, shaded bars; no pretreatment, open bars. (A) Chemotaxis of FL neutrophils compared with that of control neutrophils purified from blood of donors with and without donor pretreatment. Means ± SEM from five leukaphereses without and five leukaphereses with pretreatment. (B) Chemotaxis of FL neutrophils expressed as percentage of that of control neutrophils studied concurrently. Results for each donor with and without colchicine pretreatment.

normal in appearance. Although these cells showed some increased spreading, vacuolization of the cells was much less in evidence (Fig. 1).

Ultrastructural studies of FL neutrophils have shown that the membrane surfaces of these cells are ruffled and involuted, unlike those of normal neutrophils from peripheral blood. These morphologic changes at the cell surfaces are also accompanied by electrophysical changes. FL neutrophils could be shown to have altered cell surface charge when compared with neutrophils purified directly from blood. This abnormality of FL neutrophils was also studied with and without colchicine pretreatment of donors; as shown in Table 1, colchicine pretreatment was accompanied by a statistically significant reduction in surface charge changes of FL neutrophils.

Donor tolerance and neutrophil yields. Although the dose of colchicine given to donors was relatively small compared to those used clinically to treat acute gout, we expected that some donors might experience diarrhea or nausea after
Table 1. Surface Charge of FL Neutrophils With and Without Colchicine Pretreatment of Donors (Mean ± SEM)

<table>
<thead>
<tr>
<th>Cell Preparation</th>
<th>Surface Charge* (μm/sec/V/cm)</th>
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<tbody>
<tr>
<td>No pretreatment</td>
<td></td>
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<tr>
<td>Control neutrophils†</td>
<td>1.81 ± 0.03 (24)</td>
</tr>
<tr>
<td>FL neutrophils</td>
<td>1.61 ± 0.03 (24)†</td>
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<tr>
<td>Colchicine pretreatment:</td>
<td></td>
</tr>
<tr>
<td>Control neutrophils</td>
<td>1.85 ± 0.03 (24)</td>
</tr>
<tr>
<td>FL neutrophils</td>
<td>1.78 ± 0.04 (24)‡</td>
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*Number of replicate measurements in parentheses.
†Neutrophils separated from blood by gradient centrifugation and sedimentation techniques; blood obtained from donors at the time of leukapheresis.
‡Significance of difference (student’s t test) between these values; p < 0.01.

taking the drug. The donors, however, reported no gastrointestinal or other symptoms suggestive of a drug side effect.

Colchicine has been reported to diminish neutrophil adhesiveness in vitro at concentrations of drug that may be attained in vivo;25 thus it was expected that yields of neutrophils obtained by FL might go down after colchicine pretreat-

Fig. 3. Improved bactericidal function of FL neutrophils with colchicine pretreatment of donors. Pretreatment, shaded bars; no pretreatment, open bars. (A) Bactericidal activity of FL neutrophils compared with that of control neutrophils purified from blood of donors with and without donor pretreatment. Bactericidal activity expressed as percentage of bacteria surviving after 45 min incubation with cells. Means ± SEM from five leukaphereses without pretreatment and five with pretreatment. (B) Bactericidal activity of FL neutrophils (bacteria survival at 45 min) expressed as percentage of that for control neutrophils studied concurrently. Results for each donor with and without colchicine pretreatment.
ment of donors. After five paired studies, it appeared that there was a trend towards slightly lower yields after donor pretreatment, but this apparent difference did not attain statistical significance even after two additional paired studies (Table 2).

**DISCUSSION**

Colchicine has been reported to affect a variety of human neutrophil functions in vitro, although there has been some debate about which of these effects may be relevant to the clinical usefulness of this drug, since most effects in vitro of colchicine have been observed at suprapharmacologic concentrations of drug. Recently we showed that neutrophils from patients treated with

| Table 2. Neutrophil Yields From a Single Filter After 2.5-hr Filtration Leukapheresis With and Without Colchicine Pretreatment of Donors |
|-----------------------------|-----------------|-----------------|
| Donor Pretreatment          | Yield (Mean ± SEM)* | p Value†        |
| None                        | 1.72 ± 0.32 × 10^10 (7) | > 0.2           |
| Colchicine (2.4 mg)          | 1.34 ± 0.18 × 10^10 (7) | p > 0.2         |

*Number of leukaphereses in parentheses.
†Significance of difference between colchicine pretreatment and no pretreatment; Student's t test.
colchicine, like neutrophils from normal volunteers who have taken this drug, are protected against the effects of nonphagocytic degranulating stimuli, particularly those that can induce a selective degranulation of the specific granules (phorbol myristate acetate, ionophore A23187). At the same time, neutrophils obtained from patients taking a comparable dose of colchicine have not been found to be altered with respect to other functions (chemotaxis, phagocytosis, bacteria killing, production of leukocytic pyrogen). These observations suggested to us that colchicine pretreatment of FL donors might prevent the neutrophil degranulation that occurs during FL secondary to nylon wool adherence without adversely affecting other aspects of neutrophil function. Indeed, it was anticipated that protection of neutrophils from adherence-induced degranulation might also reduce or prevent the functional abnormalities of FL neutrophils, since there is compelling evidence to suggest that neutrophil degranulation, with its associated burst of oxidative metabolism, is of central importance to the induction of these functional abnormalities.

As indicated by these studies, pretreatment of FL donors with a moderate, well-tolerated course of colchicine during the 12 hr before leukapheresis inhibited neutrophil degranulation during FL, resulting in diminished extracorporeal secretion of lysozyme from leukocytes within nylon wool filters. At the same time colchicine pretreatment of donors significantly reduced the abnormalities of chemotaxis, bactericidal capacity, and surface charge that characterize FL neutrophils. These studies support the concept that the pharmacologic manipulation of donors may be a very useful tactic for improving FL, which remains the simplest and most accessible leukapheresis technique. Evidence has been presented previously that pretreatment of donors with corticosteroids, as has been done to induce a leukocytosis in donors, may also protect circulating neutrophils against the damaging effects of nylon wool adherence. Corticosteroids, like colchicine, also inhibit neutrophil degranulation and extracellular release of granule contents in vitro and in vivo, although likely by a different mechanism, and the benefits of FL donor pretreatment with colchicine and with corticosteroids may be additive.

Changes in the conditions of nylon wool adherence (i.e., adherence in the presence of mannitol), or changes in the conditions of filter elution (i.e., use of local anesthetic agents in eluting solutions), have been reported to improve the functional quality of FL neutrophils. The pharmacologic manipulation of donors with colchicine is another means of achieving this end and is a particularly attractive approach theoretically, since it results in functional improvements of FL cells by inhibiting the cellular events that are damaging to the cells rather than by compensating for these cellular events.

REFERENCES

COLCHICINE AND LEUKAPHERESIS DONORS


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