Development of a Practical Oral Dexamethasone Premedication Schedule Leading to Improved Granulocyte Yields With the Continuous-Flow Centrifugal Blood Cell Separator

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Studies were conducted to improve the yield of granulocytes collected for transfusion from normal donors by means of the continuous-flow centrifugal blood cell separator. Nonleukapheresed donors were medicated with varying schedules of corticosteroids to learn the magnitude and duration of granulocytosis. Normal donors were medicated with varying schedules of corticosteroids prior to a 4-hr leukapheresis and the granulocyte yields determined. It was found that maximum yields (32.2 x 10^9 granulocytes) were obtained by use of dexamethasone given orally 12 and 3 hr prior to leukapheresis. There was a good correlation between the yields and the circulating granulocyte count at the start and during the procedure.

The efficacy of allogeneic granulocytes transfused into infected granulocytopenic patients depends, among other things, on the number of the cells given and the preservation of normal function of the transfused cells. When the continuous-flow centrifugal blood cell separator (CFC-BCS) was first introduced, the granulocyte yields from single donors were small, in the range 5–10 x 10^9 granulocytes, representing less than 10% of a normal noninfected adult's daily production. Over the past 5 yr two adjunctive measures have been employed to improve yields with the CFC-BCS, (1) the production of granulocytosis using etiocholanolone or various corticosteroid preparations and (2) the use of the rouleux-forming agents hydroxyethyl starch (HES) and dextran to improve the granulocyte collection efficiency.

In 1974 we began using HES and noted the marked improvement in collection efficiency reported by others. We subsequently explored variations in corticosteroid premedication of donors in an attempt to improve granulocytosis while developing a practical oral premedication schedule for outpatient use. The following report summarizes our experience, which has led to an oral dexamethasone premedication schedule that is well tolerated and produces average yields of over 30 x 10^9 granulocytes per 4-hr 9.6-liter leukapheresis using the CFC-BCS.

MATERIALS AND METHODS

Donors were healthy volunteers who participated after giving informed consent. The following three variations in corticosteroid premedication were employed: hydrocortisone (Solu-Cortef; UpJohn, Kalamazoo, Mich.), 120 mg/m² intravenously (i.v.) 2 hr prior to leukapheresis; dexa-
methasone (Hexadrol; Organon, W. Orange, N.J.), 4.5 mg/m² by mouth 2 hr prior to leukapheresis; two oral doses of dexamethasone, 3 mg/m² each, 12 and 3 hr prior to leukapheresis. Nonleukapheresed control subjects were also medicated with varying oral dexamethasone schedules and their peripheral blood granulocyte and lymphocyte counts subsequently monitored.

A 9-10-liter leukapheresis was accomplished in a 4-hr period at an average flow rate of 40-41 ml/min using the CFC-BCS (Aminco Celltrifuge, American Instruments, Silver Spring, Md.). No systemic anticoagulation was employed, and extracorporeal clotting was prevented by the addition of either ACD (USP-NIH formula A, Travenol Laboratories, Silver Spring, Md.) or sodium citrate (30-mg vial of 56.5° trisodium citrate; McGaw Laboratories, Glendale, Calif. in 500 ml of 6° HES) to the donor line at a ratio of 1 part anticoagulant to 14 parts blood. HES (6°, hydroxyethyl starch, Volex; McGaw) was added to the donor line at a rate of 2 ml/min. Centrifugation speed was 650 rpm (30 g). Buffy coat was removed at a rate of 1 ml/min.

Complete peripheral blood counts were obtained from the donor at the start of leukapheresis, midway through, and at the termination of the 4-hr procedure. Collection efficiency was calculated by dividing the total number of cells in the collection by the number estimated to have entered the CFC-BCS [average flow rate (ml/min) x minutes of leukapheresis x average cell count/ml]. Student’s t test was used to determine the significance of the differences between samples on the hypothesis that no difference existed; p ≤ 0.05 was considered significant.

RESULTS

As summarized in Table 1, prior to the use of HES our granulocyte yields were poor even when donors were premedicated with hydrocortisone (120 mg/m² i.v. 2 hr prior to leukapheresis). With HES a marked improvement in collection efficiency resulted in a sixfold increase in granulocyte yields in the hydrocortisone-premedicated donors.

Because the intravenous hydrocortisone premedication schedule was inconvenient for outpatient use, we explored substituting a pharmacologically equivalent dose of oral dexamethasone for hydrocortisone. This resulted in poor yields compared to those obtained from donors premedicated with hydrocortisone (see Table 1). When we determined the pattern of granulocytosis following a single oral dose of dexamethasone in normal nonleukapheresed controls, the reason for the decrease in granulocyte yield became apparent (see Fig. 1). In agreement with recent observations by Mischler and Emerson, granulocytosis following oral dexamethasone was first seen 4 hr after oral administration of the steroid, a time corresponding to midway through the leukapheresis. The granulocytosis rapidly peaked and began to fall off by 6-8 hr.

Reasoning that a sustained or increasing granulocytosis during the leukapheresis would produce optimal yields, we explored combining the late sustained granulocytosis produced by oral dexamethasone7 with the early 4-hr

| Table 1. Effect of Various Steroid Premedication and HES on Granulocyte Yields |
|------------------|------------------|------------------|------------------|------------------|
|                  | No Hydroxethyl Starch | Hydrocortisone i.v. 2 hr Prior | Hydrocortisone i.v. 2 hr Prior | Hydrocortisone orally, 2 hr Prior |
|                  | n = 18               | n = 30            | n = 35            | n = 55           |
|                  | WBC (x 10⁹)         | 7.5 ± 4.0*        | 5.7 ± 2.9         | 31.2 ± 2.3       |
|                  | 24.5 ± 1.3          | 35.2 ± 1.3        |                   |                   |
|                  | Granulocytes (x 10⁹) | 2.9 ± 2.1         | 4.4 ± 2.8         | 26.8 ± 2.2       |
|                  | 21.5 ± 1.2          | 32.2 ± 1.3        |                   |                   |
|                  | Lymphocytes (x 10⁹) | 3.4 ± 2.0         | 1.1 ± 0.5         | 3.6 ± 0.3        |
|                  | 2.7 ± 0.2           | 2.6 ± 0.2         |                   |                   |
|                  | Efficiency (%)      | 7.8 ± 4.8         | 8.4 ± 4.3         | 46.3 ± 2.1       |
|                  | 46.3 ± 1.4          | 44.1 ± 1.0        |                   |                   |
|                  | Liters processed   | 9.6 ± 4.8         | 9.4 ± 0.2         | 9.4 ± 0.2        |
|                  | 9.7 ± 0.1           |                   |                   |                   |

*Mean ± SE.
peak. As shown in Fig. 2, dexamethasone 3 mg/m² administered orally to normal nonleukapheresed controls yielded a sustained granulocytosis after 12 hr. When a second dose of 3 mg/m² was added 9 hr after the first, the granulocytosis appeared to be enhanced (Fig. 2).

Based on these observations, granulocyte donors were premedicated with dexamethasone 3 mg/m² 12 and 3 hr prior to the leukapheresis procedure. Using this schedule we obtained average yields of $32.2 \times 10^9$ granulocytes, significantly better than the yields from the donors premedicated with intravenous hydrocortisone ($p < 0.025$) (Table 1). Other factors that could affect the granulocyte yield, such as collection efficiency and liters of blood processed, were constant in the different premedication groups.

As expected, the peripheral blood granulocyte count at the start of the leukapheresis and during the procedure was an important variable affecting yield.
In Fig. 3 we compare the peripheral blood granulocyte count at the start of, midway through, and at the end of leukapheresis of donors premedicated with a single i.v. dose of hydrocortisone or a single or double oral dose of dexamethasone. The granulocyte counts at the start and end of leukapheresis were significantly higher in the double-dose dexamethasone donors as compared to those donors premedicated with a single i.v. dose of hydrocortisone ($p < 0.025$). We also found a strong correlation between the granulocyte yields and the peripheral blood granulocyte count at the beginning and the average count during leukapheresis when data from the donors premedicated with a double dose of dexamethasone were analyzed ($r = 0.783$ and $0.883$, respectively).

**DISCUSSION**

Both etiocholanolone$^4$ and corticosteroids$^5-7$ are effective in improving granulocyte collections. Etiocholanolone has the disadvantages of producing pain at the injection site, temperature elevation in 60% of the donors, and flu-like symptoms in 75% of the donors.$^4$ By comparison, in our experience and that of others$^5-7$ short-term high-dose corticosteroids are well tolerated.

Because of the well-known suppressive effects that corticosteroids have on neutrophil function, there has been concern that granulocytes collected from steroid-premedicated donors might be functionally impaired.$^6,10$ When granulocyte function following corticosteroid premedication has been studied, however, no altered function has been consistently shown. Shoji and Vogler$^6$ found both yeast phagocytosis and bactericidal activity normal following intravenous hydrocortisone (120 mg/m$^2$). In a recently reported study, Glasser et al.$^{10}$ noted no impairment in neutrophil viability, phagocytosis, fungicidal activity, bactericidal activity, or chemotaxis following a single i.v. dose of dexamethasone (4 mg/m$^2$).

Oral dexamethasone appears to offer a few practical advantages over other corticosteroid preparations. Dexamethasone is a highly potent steroid available in convenient high-dosage tablets. Also, because of the $16\alpha$ methylation in dexamethasone the salt-retaining properties are minimal,$^{11}$ a possibly significant factor when the steroid is used along with HES, a blood volume expander. Finally, the initial peak of granulocytosis occurs earlier than with other oral
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steroid preparations such as prednisone, a practical consideration for early morning leukapheresis.

New methods continue to be developed to improve the efficiency of granulocyte collection. The goal is to obtain the largest number of functionally normal granulocytes in the shortest period of time. Sustaining a significant granulocytosis in the donor for the required time of collection by methods presented above clearly assists in reaching this goal.

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