

Biological Effects of Repeated Leukapheresis of Patients With Chronic Myelogenous Leukemia

By C. S. Vallejos, K. B. McCredie, G. M. Brittin, and E. J. Freireich

Fifteen patients with chronic myelogenous leukemia were managed only with repeated leukapheresis for up to 26 mo. At each procedure approximately 10 liters of blood were processed with a continuous-flow blood cell separator over a 3-hr period. Five patients had intermittent leukapheresis (1–2 procedures/wk), and ten of them had one or more series of intensive leukapheresis (4–5 procedures/wk). Addition of hydroxyethyl starch to the extracorporeal circuit was found to increase the removal of leukocytes fourfold. With intensive leukapheresis the leukocyte count decreased 80%, and the platelet count de-

creased 54% (mean values). With intermittent leukapheresis the corresponding figures were 70% and 35%. Thrombocytopenia was never clinically significant. All 15 patients experienced symptomatic improvement, and those with organomegaly had decrease in the size of the spleen and liver. Leukapheresis was not associated with morbidity, except that anemia became more severe and required periodic transfusions of packed red blood cells. Patients managed with leukapheresis did not enter bone marrow remission, and transformation of CML into blastic crisis was not prevented or delayed.

DEVELOPMENT OF THE continuous-flow blood cell separator has facilitated the harvesting of leukocytes from normal donors and from donors with chronic myelogenous leukemia (CML) for use in granulocytopenic recipients.^{1–5} Although the extraction efficiency for granulocytes of this instrument is less than 20%, it can be increased substantially by using agents which cause red blood cells to sediment, such as dextran, fibrinogen, gamma globulin, and more recently, hydroxyethyl starch (HES).¹ This latter compound is of particular interest, since it appears to be nonantigenic and is not chronically retained.^{6,7} When HES is added to the input line of the IBM-NCI blood cell separator, 0.22×10^{11} leukocytes can be obtained by processing 10 liters of blood from normal donors who have been stimulated by administration of etiocholanolone,⁸ and quantities of 10^{11} or more leukocytes may be collected from CML donors in 2–4 hr without morbidity.⁹ Previous studies of the biological effects upon CML donors of the removal of large quantities of leuko-

From the University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77025.

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C. S. Vallejos, M.D.: Project Investigator, Department of Developmental Therapeutics, the University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77025. K. B. McCredie, M.D.: Assistant Professor of Medicine, Department of Developmental Therapeutics, the University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77025. G. M. Brittin, M.D.: Professor of Pathology, Head, Department of Clinical Chemistry and Laboratory Medicine, the University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77025. E. J. Freireich, M.D.: Acting Head, Department of Developmental Therapeutics, the University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77025.

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cytes have employed conventional plasmapheresis with plastic bags. Use of this technique with removal of up to 3×10^{11} cells/day did not change the clinical condition of CML donors and resulted in little consistent decrease in their white blood cell count or spleen size.¹⁰

The present study was performed in order to investigate two aspects of the use of the continuous-flow blood cell separator in patients with CML: First, the relative efficiency of removal of granulocytes from patients with CML with and without the use of hydroxyethyl starch (HES); second, the biological effects in patients with CML of the removal of large quantities of leukocytes by repeated leukapheresis.

MATERIALS AND METHODS

Experimental Design

The effect of adding HES on the removal of leukocytes with the continuous-flow blood cell separator was studied in six patients with CML who each had two or three leukapheresis procedures in which HES-anticoagulant was compared with acid-citrate-dextrose (ACD) solution, NIH formula A. In eight procedures HES was used for processing the first 5 liters of blood and ACD solution for processing the second 5 liters. In seven procedures the order of these agents was reversed. Each patient served as his own control. The data were analyzed statistically by pairing the results obtained with HES and ACD for each patient (paired data). The significance of the differences was determined by a two-tailed student's t test.

The biological effects of repeated leukapheresis of patients with CML were studied in 15 individuals who were managed with no other therapy except periodic transfusions of packed red blood cells. Five patients received intermittent leukapheresis consisting of 4-5 procedures/mo. The frequency of their procedures was adjusted to maintain a stable clinical and hematologic status. Ten patients received one or more series of intensive leukapheresis consisting of 4-5 procedures/wk for 1-22 wk (Tables 2 and 3). Written consent for leukapheresis was obtained from all patients.

Selection of Patients

The Philadelphia chromosome was demonstrated by standard cytogenetic techniques in cultured bone marrow cells of the 15 patients admitted to the study. All patients had active disease as determined by bone marrow examination, leukocytosis, anemia, thrombocytopenia and/or organomegaly. Ten patients were male and five were female, and their median age was 53 yr (8-63). The median duration of disease from the time of diagnosis to the beginning of leukapheresis was 10 mo (3-22). One patient (C.H.) was a child 8 yr old. One patient (D.K.) had been treated previously with hydroxyurea and splenectomy. The other patients were all untreated. One patient (V.G.) probably had early blastic transformation when she entered the study (24% myeloblasts in the bone marrow; karyotype 46, XX, Ph¹). Two months later the percentage of myeloblasts increased to 64% and the karyotype of bone marrow cells changed to 51, XX, 2C+, D+, G+, 2 Ph¹.

Techniques

Leukapheresis was performed as described previously, except that heparin was not used.^{9,11} Two different machines were used, the IBM blood cell separator, Model 2990 (International Business Machines, Inc., Eddincott, N.Y.), and the Aminco centrifugal cell separator (American Instrument Company, Division of Travenol Laboratories, Inc., Silver Spring, Md.). For both machines the flow of blood through the centrifuge was maintained at a rate of 40-50 ml/min. The centrifuge was operated at 750 rpm (50 g).

In order to improve the collection of granulocytes, hydroxyethyl starch (HES), a plasma substitute with no known antigenic activity, was introduced into the extracorporeal circuit.^{7,8,12} HES was used to sediment the red blood cells in the centrifugal field of the blood cell separator.

Five hundred milliliters of 6% HES in 0.9% saline were added to 30 ml of sodium citrate anticoagulant concentrate (McGaw Laboratories, Glendale, Calif.). This HES-anticoagulant solution replaced the acid-citrate-dextrose solution, NIH formula A, used in previous leukapheresis studies and was added to the input line of the blood cell separator in a ratio of 1 ml to 14 ml of whole blood processed.^{8,12} In adults 10 liters of blood were processed during each procedure. In the child approximately one-half of this volume of blood was processed (range 3300-7500 ml per procedure).

Leukocyte and platelet counts of peripheral blood were measured before and immediately after each procedure. These counts were multiplied by the blood volume (estimated to be 2500 ml/sq m of body surface area) in order to determine the numbers of circulating leukocytes and platelets.¹³ Leukocyte counts were determined with the Coulter Counter Model F. Platelets were counted electronically with a Coulter Counter Model B.¹⁴ This method was used in preference to automated optical platelet counting because of the high ratio of leukocytes to platelets.¹⁵ An aliquot of the leukapheresis material was diluted 1:5000 with a specially designed Unopette (Unopette No. 2700 × F 188, Becton Dickinson Co., Rutherford, N.J.) for determination of the white blood cell count. The hemoglobin concentration or red blood cell count of the leukapheresis material could not be determined reliably. All patients had bone marrow examinations before starting leukapheresis and at 4-6-mo intervals thereafter.

RESULTS

The effect of added HES on the removal of leukocytes by the continuous-flow blood cell separator is shown in Table 1. Myeloblasts and eosinophils were omitted from consideration in this table, because of the large statistical errors in dealing with cell types that were enumerated only a few times in a 100-cell differential count.

The addition of HES after ACD (seven procedures) produced approximately a fourfold increase in the numbers of total leukocytes and polys removed and even greater increases in the percentages of circulating leukocytes and polys removed. Addition of HES also increased the efficiency of extraction of immature granulocytes and platelets. The differences observed between HES and ACD in the extraction of bands, lymphocytes, monocytes, and basophils were not significant.

Addition of ACD after HES (eight procedures) was associated with decreased extraction efficiency of leukocytes and platelets. However, in these eight procedures, the differences were not so large as when HES followed ACD (seven procedures), probably because HES remained in the closed circuit during the addition of ACD.

The effectiveness of HES in increasing the removal of leukocytes is also shown by a comparison of the changes in the peripheral blood leukocyte count during the first half of the procedures: leukapheresis with HES caused a large decrease in the leukocyte count compared to the small change observed with ACD.

The clinical and laboratory data for the five patients who received intermittent leukapheresis are shown in Table 2. In these patients the leukocyte counts decreased approximately 70%, and the platelet counts decreased approximately 35% during the time of the study, which ranged from 138 to 801 days in different patients. On the average, 33% of the circulating leukocytes and 7% of the circulating platelets were removed at each procedure. No platelet counts less than 50,000/cu mm were observed. In patient H.W., who had the fewest procedures, the platelet count increased moderately even though the

Table 1. Comparison of HES and ACD-A in Collection of WBC From Patients With CML

Number of Procedures	Sequence of Additives	Peripheral Blood WBC $\times 10^3/\text{cu mm}$ When Additive Started	Number of WBC Removed $\times 10^{11}$			Percentage of Circulating WBC Removed					Platelets Removed No. $\times 10^{11}$ %				
			Total	Poly	Lymph	Total	Poly	Band	Meta	Myelo		Mono	Baso	Lymph	
7	ACD	60.6*	0.21	0.06	0.02	6.0	3.0	11.6	7.9	11.7	47.6	29.9	33.4	0.30	1.8
	HES	57.0	0.82	0.20	0.12	27.4	14.0	23.7	48.6	33.3	118.8	57.6	69.1	0.52	7.3
	P	N.S.†	< 0.001	N.S.	N.S.	< 0.001	< 0.005	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	< 0.001
8	HES	66.9	0.82	0.34	0.15	24.5	15.8	48.2	62.9	43.6	64.6	74.4	42.0	0.59	3.7
	ACD	50.2	0.43	0.15	0.07	18.1	12.2	98.0	47.3	69.1	74.5	46.2	76.2	0.52	4.1
	P	< .005	N.S.	< 0.005	N.S.	< 0.005	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*All values are means.

†N.S.: $p > 0.05$.

Table 2. Clinical and Laboratory Data of Patients With CML Subjected to Intermittent Leukapheresis

Patient	Days of Study	Number of Procedures	Leukocytes × 10 ¹¹		Peripheral Blood Leukocytes (K/cu mm)		Platelets × 10 ¹¹ Collected per Procedure	Peripheral Blood Platelets (K/cu mm)		Spleen (cm)		Liver (cm)	
			Total	Polys	Start	End		Start	End	Start	End	Start	End
D.K.	801	103	0.684* (0.529)†	0.315 (0.276)	128	56	0.99 (0.65)	300	272	-	-	0	0
E.D.	748	121	0.702 (0.801)	0.336 (0.559)	170	55	0.80 (1.65)	385	75	3	0	0	0
J.M.	472	101	1.37 (1.03)	0.469 (0.386)	240	70	1.04 (0.76)	520	178	10	6	5	0
L.H.	503	60	1.11 (0.594)	0.531 (0.279)	65	63	1.02 (0.76)	300	260	0	0	0	0
H.W.	138	28	1.19 (1.07)	0.393 (0.398)	210	19	0.65 (0.33)	360	430	10	7	2	0
*Mean	532	83	1.01	0.57	162.6	52.6	0.90	373	243				
†S.D.	(264)	(17)	(0.30)	(0.38)	(68.9)	(19.7)	(0.17)	(90.2)	(130.9)				

leukocyte count decreased by 90%. In the three patients who had organomegaly the size of liver and spleen decreased. All patients experienced improvement in malaise, weakness, fatigability, and anorexia. Four of them required transfusions of 2 U of packed red blood cells every 8–12 wk. Only one of these five patients died (metastatic adenocarcinoma), and none developed blastic crisis. Leukapheresis was discontinued in two patients because of severe splenic pain and in one patient because of two episodes of pulmonary embolism. One patient (E.D.) is still being managed with leukapheresis alone.

Ten patients received a total of 20 series of intensive leukapheresis (Table 3). The second series of procedures for patient E.S. was performed without HES, and only small numbers of leukocytes and platelets were removed. This series is therefore excluded from the statistical analysis. The three series of procedures for the child (C.H.) are considered separately, because the volume of blood processed averaged only 5.6 liters per procedure. In the remaining 16 intensive series, the leukocyte counts decreased 80%, and platelet counts decreased 54% (mean values). On the average, 42% of the circulating leukocytes and 13% of the circulating platelets were removed at each procedure. During five of the series (three patients) the platelet count fell to less than 50,000/cu mm (range 21,000–46,000/cu mm), but thrombocytopenia was never associated with bleeding. All patients had symptomatic improvement and those with organomegaly had a decrease in size of spleen and liver. Anemia became more severe during each series of leukapheresis procedures and all patients required transfusions of packed red blood cells. Leukapheresis was not associated with morbidity, and patients were usually treated as outpatients.

The child (C.H.) responded well to leukapheresis. She had an exceptionally high platelet count, which was reduced to normal levels by leukapheresis alone. Following the third series of procedures she had a splenectomy, and 6 mo later she responded well to a fourth series of procedures (not included in the present study).

Of the ten patients who had intensive leukapheresis, three died of blastic crisis, one of bronchogenic carcinoma, one of uncontrolled CML, and one

Table 3. Intensive Leukapheresis of Patients With CML

Patient	Series	Days of Study	Number of Procedures	Leukocytes × 10 ¹¹ Collected per Procedure		Peripheral Blood Leukocytes (K/cu mm)		Platelets × 10 ¹¹ Collected per Procedure		Peripheral Blood Platelets (K/cu mm)		Spleen (cm)		Liver (cm)	
				Total	Polys	Start	End	Start	End	Start	End	Start	End	Start	End
D.J.	1	30	25	2.71* (2.47)†	0.460 (0.472)	283	20	5.10 (4.66)	759	182	8	0	6	0	
	2	22	14	3.95 (4.75)	1.510 (1.070)	164	36	2.50 (3.39)	250	146	6	0	5	0	
	3	37	19	2.18 (1.57)	0.809 (0.63)	142	30	1.14 (0.74)	373	46	6	0	4	0	
	4	34	26	2.32 (1.34)	0.762 (0.470)	402	27	1.08 (0.75)	230	80	9	3	6	3	
A.W.	1	29	20	1.67 (1.03)	0.703 (0.426)	310	34	1.17 (0.67)	114	66	4	0	0	0	
	2	24	19	1.10 (0.068)	0.392 (0.229)	142	25	0.56 (0.36)	260	140	10	5	0	0	
	3	23	17	1.80 (1.20)	0.385 (0.309)	136	41	0.43 (0.27)	186	64	15	8	10	7	
J.L.	1	154	78	1.86 (1.55)	0.394 (0.518)	299	35	1.40 (1.01)	710	142	18	14	7	3	
R.S.	1	36	25	1.62 (1.31)	0.605 (0.634)	345	31	0.77 (0.81)	290	131	15	6	0	0	
R.S.	2	37	25	1.40 (0.681)	0.626 (0.501)	130	28	0.34 (0.18)	136	88	10	3	0	0	
V.G.	1	16	12	2.79 (1.90)	0.325 (0.522)	284	88	0.52 (0.52)	78	21	8	3	0	0	
E.S.	1	17	11	1.29 (0.431)	0.376 (0.167)	82	53	3.90 (2.53)	974	943	8	6	10	8	
	2	11	8	0.119 (0.065)	0.009 (0.007)	97	112	0.14 (0.03)	134	124	10	10	10	9	
R.R.	1	29	25	2.42 (3.30)	0.708 (0.501)	192	56	1.08 (1.31)	770	92	18	8	0	0	
E.J.	1	9	7	0.798 (0.447)	0.253 (0.257)	72	51	0.638 (0.372)	196	430	15	5	8	5	
	2	9	7	0.482 (0.255)	0.0745 (0.0760)	43	31	0.607 (0.374)	325	340	12	8	10	6	
O.T.	1	15	13	1.27 (0.309)	0.680 (0.255)	51	30	3.71 (2.34)	1800	530	0	0	0	0	
C.H.‡	1	10	8	0.282 (0.126)	1.480 (0.680)	419	98	1.86 (1.29)	1977	220	12	9	8	6	
	2	7	6	0.274 (0.139)	0.637 (0.440)	150	40	1.89 (2.53)	435	124	9	7	6	3	
	3	11	8	0.133 (0.090)	0.639 (0.376)	219	51	1.06 (0.516)	1200	482	8	5	6	5	
*Mean		32.6	21.4	1.85	0.57	192.3	38.5	1.56	465.7	215.1					
†S.D.		(33.7)	(16.4)	(0.86)	(0.32)	(112.8)	(16.7)	(1.45)	(434.0)	(241.6)					

‡This series of procedures was performed without the use of HES.

§Child.

unexpectedly (no autopsy). One patient is being managed with chemotherapy, and three are being managed with leukapheresis alone.

The average numbers of leukocytes and platelets collected per procedure were greater for intensive than for intermittent leukapheresis (Tables 2 and 3). These differences can be attributed to the larger numbers of circulating leukocytes and platelets in the patients who had intensive leukapheresis, and also to the fact that intensive leukapheresis removed leukocytes and platelets more efficiently, as measured by the percent of circulating leukocytes and platelets removed per procedure.

DISCUSSION

Leukapheresis has been used to obtain leukocytes from patients with chronic myelogenous leukemia (CML) for transfusion to leukopenic recipients.^{16, 19} Using the original technique which employed a closed system of plastic bags, a maximum of 4 U could be processed in 4½ hr. The major limiting factor was the chronic removal of small amounts of red blood cells with each unit, which led to a decrease in the hemoglobin level with the intensive and prolonged periods of plasmapheresis.¹⁰ Use of this technique with removal of up to 3×10^{11} cells per day did not change the clinical condition of CML donors and resulted in little consistent decrease in their white blood cell count and spleen size.¹⁰

Development of the continuous-flow blood cell separator has made it possible to process 0.4–3.2 donor blood volumes during each procedure without hemolysis or excessive loss of platelets.⁶

We have found that addition of hydroxyethyl starch (HES) to the extracorporeal circuit of the continuous-flow blood cell separator increases the yield of leukocytes. This substance is a highly branched polymer of glucose which is stable in solution and has no known toxic or antigenic activity.^{6, 7} Addition of HES increased the extraction of polymorphonuclear leukocytes more than fourfold in patients with CML, with smaller increases in the extraction of other types of leukocytes and platelets. Processing of 10 liters of blood over approximately 3 hr removed an average of 1.01×10^{11} leukocytes from patients with CML who had intermittent leukapheresis and an average of 1.85×10^{11} leukocytes from patients who had intensive leukapheresis.

The 786 procedures in our 15 patients were not associated with morbidity, except that anemia became more severe during leukapheresis and required periodic transfusions of packed red blood cells. The contribution of the leukapheresis procedures to the production of anemia was not measured in our studies, because of the difficulties of obtaining reliable measurements of hemoglobin, hematocrit, and red blood cell count of the leukapheresis material. Other workers have reported that the average total red blood loss per procedure was equivalent to 75–150 ml of whole blood in the leukapheresis of CML patients with the IBM-NCI cell separator.⁹ They also reported that hemolysis was not a problem, and that autologous ⁵¹Cr red blood cell survival was normal in studies of leukapheresis of dogs with this same instrument.⁴ In our CML patients, repeated leukapheresis did not produce bone marrow remission, and it is possible that in the patients with marked granulocytic hyperplasia of the bone marrow the chronic removal of small amounts of red blood cells at each procedure contributed importantly to the development of anemia, especially in patients who had 4–5 leukapheresis procedures per wk. One patient (L.H.) had two episodes of pulmonary embolism. Although they could not be attributed definitely to leukapheresis, they were considered to be adequate reason to remove him from the study. Individual patients tolerated as many as 121 procedures, which suggests that their discomfort was small and their cooperation good.

This study has demonstrated that removal of peripheral blood leukocytes alone can result in both symptomatic improvement and objective regression of the hematologic manifestations of CML. Reduction of the white blood cell

count was associated with symptomatic improvement in all our patients, and those who had organomegaly experienced substantial decrease in size of spleen and liver. Although platelets were removed in addition to white blood cells, thrombocytopenia was never of clinical significance. Leukapheresis therefore represents a conservative but effective measure which can be employed intermittently to maintain patients with CML in stable condition for many months without the risks of drug resistance, toxicity, or bone marrow damage.

Our experience indicates that removal of large quantities of leukocytes with the continuous-flow blood cell separator can provide symptomatic control of the manifestations of CML in the benign phase, and this technique should allow prospective investigation of the natural history of this disease, without the complications of drug toxicity. Although leukapheresis is expensive and time consuming, its effectiveness in controlling the manifestations of CML would appear to compare favorably with other forms of management, i.e., chemotherapy or radiation therapy. Studies of the Philadelphia chromosome abnormality have shown that none of these forms alter the basic pathophysiologic process of CML. Three of our patients managed with leukapheresis alone have died of blastic crisis. This finding suggests that repetitive leukapheresis does not prevent or delay blastic transformation of CML. However, to determine if the frequencies of blastic crisis or late bone marrow fibrosis and failure will be altered by leukapheresis will require study of a larger series of patients with longer periods of follow-up.

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