A PRELIMINARY STUDY OF ^{51}Cr-LABELED PLATELETS FOR EVALUATION OF SPLENIC SEQUESTRATION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Chromium-51-labeled ABO-/Rh compatible platelets were used to evaluate splenic sequestration of platelets in six patients with chronic lymphocytic leukemia who were thrombocytopenic. In all patients a ratio of splenic radioactivity to hepatic radioactivity of 2.0 or greater was observed, and a sustained increase in platelet count was obtained following splenectomy.

Thrombocytopenia is a complication frequently observed in patients with chronic lymphocytic leukemia (CLL) which often limits the amount of antileukemic therapy that can be administered. The development of thrombocytopenia is commonly associated with progressive enlargement of the spleen and has prompted the suggestion that hypersplenism may be a factor (*1,2*). Evidence for such a relationship has been supported by the rise in platelet count following splenectomy in certain cases (*2*), and the hematologic improvement has permitted the administration of further therapy.

The correct selection of patients who will respond to splenectomy has consequently become an important clinical problem. For nonleukemic thrombocytopenic disease states (*3–5*), the sequestration patterns of ^{51}Cr-labeled platelets has been successfully used to predict response to splenectomy. In the present paper, preliminary observations are described that suggest the ratio of spleen-to-liver uptake of ^{51}Cr-labeled platelets may assist in the prediction of splenectomy effect in thrombocytopenic CLL patients.

MATERIALS AND METHODS

Six patients in this series have well documented CLL in the active phase, with increasing lymphadenopathy, fever, weight loss, fatigability, and clinical splenomegaly. One additional patient had well differentiated lymphosarcoma with bone marrow involvement. All patients had an adequate number of megakaryocytes in the preoperative bone marrow specimens despite the lymphoproliferative infiltration.

Platelet preparation and labeling. ABO-Rh matched platelets were separated using the Chappell method (*6*). Four to eight units of blood were collected in separate plasmapheresis packs with acid-citrate dextrose (ACD-A) as the anticoagulant. The blood was spun at 1500 G for 3 min, the platelet-rich plasma removed, and the suspension spun at 700 rpm for 10 min to remove the red and white blood cells. The supernatant platelet-rich plasma was then spun at 3000 rpm for 10–15 min to sediment the platelets. All but 15 ml of the platelet-poor plasma were then removed, and the platelets were suspended in a collection bag.

Approximately 250 μCi of sterile ^{51}Cr-labeled Na_2CrO_4 were added to the bag and allowed to incubate with the platelets for 20 min. Platelet-poor plasma supernate containing 100 mg of ascorbic acid was added in order to convert any unbound CrO_4^{2-} to Cr^{3+}. Since Cr^{3+} tightly binds to serum proteins, any ^{51}Cr that was not platelet bound was removed in this manner. The mixture was centrifuged at 3000 rpm for 10 min to sediment the platelets. The supernatant was discarded and the labeled platelets resuspended in about 30 ml of platelet-poor plasma.

Platelet survival studies. A platelet concentrate containing 60–80 μCi of ^{51}Cr-labeled platelets was injected into each patient in the late afternoon. A 15-min sample was drawn from the opposite arm to serve as the zero time specimen (100% activity

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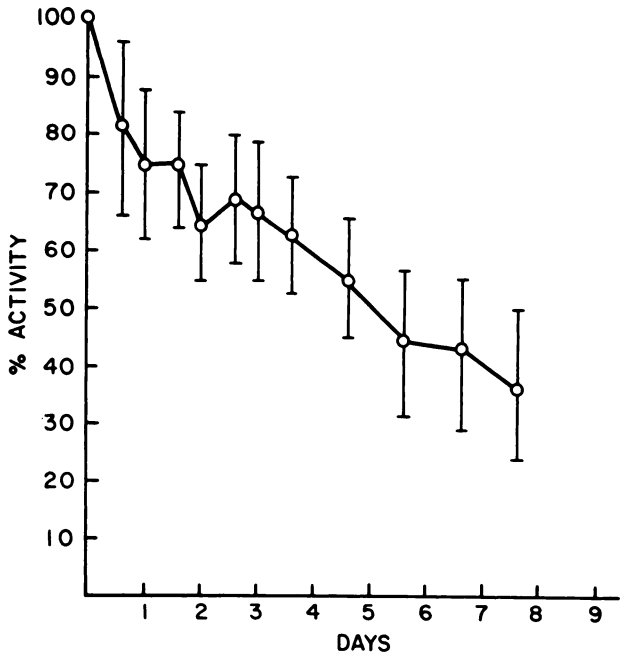

FIG. 1. Platelet survival curve. Mean platelet survival time for seven patients is shown with lymphoproliferative disorders before splenectomy.

standard). Blood was then drawn twice daily until the conclusion of the study. Two 1-ml aliquots of each sample of whole blood were lysed with saponin and counted with the 100% standard to correct for decay. The activity was plotted as a function of time on arithmetic coordinates and the best curve determined.

Splenic sequestration studies. Corrected spleen-to-liver counting rate ratios were obtained at the following time intervals: 24 hr; 48 hr; ¾ time; and ½ time after injection of the ^{51}Cr-labeled platelets. The latter two values were calculated as the times at which the plasma counting rate had fallen to ¾ or ½ of the equilibrium plasma counting rate. Counting was done as follows: Maximum counting rates over the anterior surface of the liver (L_C) and the spleen (S_C) were obtained using a 3 × 3 in. NaI(Tl) crystal with a flat-field collimator, the base of which was just touching the skin. A background count was obtained over the thigh (T_C) at a point 6 in. above the knee. The spleen-to-liver ratio (S/L) was calculated for each time period as follows:

$$S/L = \frac{Sc - Tc}{Lc - Tc}$$

The data were interpreted as follows: A S/L ratio of greater than 2.0 was considered to show definite sequestration. A S/L ratio of 1.0–2.0 was considered borderline, and a ratio of less than 1.0 was considered to show no sequestration in the spleen. This approximates the splenic, hepatosplenic, and hepatic patterns of platelet sequestration as observed by Cooper (*5*), the exception being the reservation of splenic patterns for ratios of 2.5 or greater.

RESULTS

Platelet survival. The mean platelet survival in all seven patients fell between 8 and 12 days. No survival was significantly shortened from the normal range (7–12 days).

The mean survival curve for the seven patients (Fig. 1) was a two-phase curve with an initial rapid fall over the first 2 days followed by a slower fall over the rest of the curve. The early phase of the

TABLE 1. CLINICAL DATA ON

Patient	Age/sex	^{51}Cr Plt survival (days)	^{51}Cr RBC survival (days)	S/L ratio at $T_{3/4}$	Spleen weight (gm)	Pre-op CBC* Hgb/WBC	Pre-op CBC* Diff†	Pre-op CBC* Plts × 10³	Post-op CBC* Two weeks Hgb/WBC	Post-op CBC* Two weeks Plts × 10³	Post-op CBC* Six months Hgb/WBC	Post-op CBC* Six months Plts × 10³
HC	65/M	12	22	2.0/1.0	660	12/10	60/30	75	15/15	200	14/12	150
CD	54/M	13	22	4.7/1.0	1,740	11/60	95/5	80	10/87	256	15/20	229
GH	43/M	12	—	4.5/1.0	1,295	10/2	60/30	125	12/10	498	11/4	410
HM	58/F	10	23	2.15/1.0	1,210	11/25	95/5	65	12/37	328	13/22	337
AT	64/M	8	22	4.2/1.0	535	10/20	98/2	86	12/35	181	12/25	164
PW	51/M	11	16	2.78/1.0	665	11/21	84/14	80	11/25	180	14/10	100
WC	65/M	15	—	1.14/1.0	240	11/3	35/54	50	10/9	90	9/4	80

* Hgb/WBC rounded off to nearest whole number (WBC × 10³).
† No significant change in differential counts after splenectomy (lymphocytes/granulocytes—100 cells counted).
‡ R.T. = Total-body irradiation.

curve may be secondary to platelet sequestration by the spleen. A very long tail of the curve (seen in two patients) may in part represent labeling of the red blood cell contaminants.

Platelet surface counting. As shown in Table 1, all the patients with CLL had a S/L ratio of 2.0 or greater. The one patient with lymphosarcoma (Patient 7) had a smaller ratio of 1.14. The S/L ratios in relation to time for five of the seven patients are shown in Table 2. It has been suggested by previous investigators that a rising S/L ratio is more diagnostic of sequestration than the absolute value of the ratio at any single time. Although our data were limited, no such pattern was evident in this series. The S/L ratio was maximal about the ¾ time in our patients.

Clinical correlation. Table 1 summarizes the clinical data on these patients. The impression of splenomegaly on physical examination in the CLL patients was confirmed with ^{99m}Tc liver-spleen scans (*7*). The postoperative platelet count rose in all CLL patients, and the 2½-fold rise was well maintained in five of the six patients with CLL, even in the presence of renewed chemotherapy or radiation therapy.

The hemoglobin level remained about the same, postoperatively, except in Patient 6 (Table 1) who had previously documented steroid responsive Coombs-positive anemia. The white blood cell count rose slightly postoperatively in the patients with little alteration in the differential count.

In the series as a whole, splenectomy was well tolerated and morbidity consisted of postoperative atelectasis and a low-grade fever in three of the six CLL patients and minor wound infections occurred in two cases. No serious morbidity was encountered.

The single patient with well-differentiated lymphosarcoma and a chronic clinical course not unlike CLL is also presented. The sequestration study and platelet survival values were both normal in this case, and the observed response to splenectomy was poor.

TABLE 2. VARIATION OF S/L RATIO WITH TIME

Patient	t_0	24–48 hr	$T_{3/4}$	$T_{1/2}$
HC	1.8	2.0	1.9	1.6
CD	2.65	2.6	4.7	2.2
GH	—	—	4.5	—
HM	2.01	—	2.5	1.65
AT	—	—	4.2	—
PW	2.85	2.3	2.78	2.1
WC	1.8	—	1.14	1.18

SPLENECTOMIZED PATIENTS

Comment
Chlorambucil begun 2 wks after splenectomy followed by R.T.‡ at 2 months
Chlorambucil begun 2 wks after splenectomy
Cytoxan-vincristine-prednisone started 2 wks after splenectomy
Chlorambucil begun 2 wks after splenectomy followed by R.T. at 4 months after splenectomy
R.T. begun 1 month after splenectomy
R.T. begun 1 month after splenectomy
Lymphosarcoma on cytoxan-vincristine-prednisone at one month after splenectomy

DISCUSSION

Apparent from the data presented, the platelet survival times were normal in all patients despite moderate thrombocytopenia. Aster (*8*) has suggested the existence of a large splenic pool of platelets that contains approximately one-third of the total platelet mass in normal individuals. In diseases with splenomegaly, this pool may increase so that 50–90% of an individual's platelets are in the spleen at any one time. Platelet production may be governed by the rate of platelet destruction rather than the absolute platelet count in the blood, and a large splenic pool may simply serve to remove a large portion of the platelet mass from circulation. Therefore a new steady state of platelet production and destruction may be established at a lower peripheral blood platelet count. In this situation, a thrombocytopenic patient would have a normal platelet survival time but a large splenic platelet pool detectable by scanning after infusion of ^{51}Cr-labeled platelets (*9*).

Splenic sequestration was defined in this study as a S/L ratio of 2:1 or greater at $T_{3/4}$. This value would seem consistent with the ratio used by previous investigators for the measurement of both red blood cell and platelet sequestration (*3,5,10,11*).

All of our patients with chronic lymphocytic leukemia had a S/L ratio of 2:1 or greater, and five of the six showed a sustained rise in platelet count after splenectomy. This rise was attenuated by additional therapy at 2 weeks to 1 month after splenectomy. The tolerance to further therapy was poor in the lymphosarcoma patient who did not experience a rise in platelet count after splenectomy.

In conclusion, ^{51}Cr-labeled ABO-Rh compatible platelets have been used to evaluate the splenic sequestration of platelets in six thrombocytopenic CLL

patients. In this small series, a spleen-to-liver ratio of 2.0 or greater was consistently associated with a favorable response to splenectomy, resulting in a significant increase in platelet count that was sustained over the 6-month followup period (*12*). Although the immediate effect of splenectomy (i.e., rise in platelet count) partially reflects the removal of a large platelet pool, these preliminary observations warrant the further investigation of splenic platelet sequestration as a predictor of response to splenectomy. A more critical correlation of response to splenectomy and the preoperative spleen-to-liver ratios will require the submission of splenomegalic patients with ratios below 2.0 to splenectomy. Only in this manner can the value of platelet sequestration as a response determinant be firmly established.

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