New Approaches to the Transfusion Management of Thalassemia

By Richard D. Propper, Lawrence N. Button, and David G. Nathan

Recent advances in the treatment of patients with thalassemia major have centered around the removal of iron from individuals already overloaded due to repeated transfusions. In this report we present therapeutic maneuvers designed to decrease the rate of iron accumulation. We demonstrate that the persistent maintenance of hematocrits above 35% ("supertransfusion") is not associated with an increased transfusion requirement because it produces a decrease in whole blood volume (21% ± 2%). Supertransfusion is also associated with normalization or even prolongation of plasma iron turnover. In addition, we describe a method for obtaining units of blood from normal donors that contain primarily young red cells ("neocytes"). These cells have prolonged in vivo survival as measured by the interval between transfusions (30 ± 2 days to 43 ± 4.5 days) and 51Cr red cell survival (43.8 days versus 27.8 days). Supertransfusion with neocytes is effective in decreasing the rate of iron accumulation in thalassemia.

Since the introduction of chronic transfusion regimens for the treatment of thalassemia major,1,2 therapeutic emphasis has been placed primarily on the removal of iron from these overloaded patients by means of various chronic chelation programs.3-10 Although many of these trials appear promising with respect to maintenance of net negative iron balance in severely overloaded patients,9,10 none have attempted to prevent irreversible iron-induced toxicity by minimizing the rate of iron accumulation. To determine the effect of maintaining hematocrits continually in the "normal" range on iron accumulation, we have compared the usual hypertransfusion regimen (maintenance of hematocrit >27%) with a "supertransfusion" regimen in which the hematocrit is never allowed to fall below 35%. We examined transfusion requirement, blood volume, and the rate of plasma iron clearance in both regimens. The latter primarily measured suppression of erythropoiesis and may also offer an indirect index of iron absorption.

Since the fall in hematocrit in aplasia or in patients with totally ineffective erythropoiesis is approximately 1% per day, more transfused blood should be required to maintain hematocrits above 35% than above 27%. In this article we present evidence that such supertransfusion is not associated with an increased transfusion requirement in patients with thalassemia major and conclude that this discrepancy is probably secondary to a transfusion-induced decrease in whole blood volume. We also demonstrate that supertransfusion produces a marked prolongation of the plasma iron clearance (T½) and a decrease in the plasma iron turnover (PIT) to levels seen in the normal population.

Finally, we present an approach to the collection of young red cell populations from normal donors by a process we call "neocytophoresis." The transfusion of such cells lengthens the interval between transfusions in thalassemic patients. A program of supertransfusion of neocytes produces a significant decrease in the rate of iron accumulation in patients with thalassemia.

Materials and Methods

Twenty splenectomized thalassemic patients, ages 5-25 yr, were eligible for this study. Transfusion histories varied greatly between patients prior to the initiation of the study. All patients over age 18 gave informed consent for these studies, and parental consent was obtained in all cases where minors were involved. In addition, all patients included were undergoing chronic subcutaneous deferoxamine therapy, previously described.3

Red cell volumes were determined by 51Cr dilution studies and simultaneous plasma volumes by 125I RISA dilution studies, as described by Button et al.11 51Cr red cell survivals were performed on the patient's circulating red cells according to Button.11 Predicted whole blood volumes were calculated according to the method of Linderkamp,12 in which the volumes are related to surface area. All blood volume data are reported as ml/kg for ease of comparison.

Plasma iron clearance was performed after injection of 15 ml of AB normal plasma labeled with 3 μCi of 51Fe. Samples were obtained at 3, 15, 30, 45, 60, and 120 min and the T½ calculated from the slope of the semilogarithm plot of cpm/ml of plasma as a function of time, using the method of Huff et al.13

Red blood cell enzyme levels were adapted from Beutler14 after removal of contaminating white cells by passage of the sample through microcrystalline cellulose columns. Red cell ages were estimated according to Corash and coworkers.15

Red cells obtained by neocytophoresis were collected on an Amino Celltrifuge continuous flow cell separator after the appropriate informed consents were obtained from the donors. The collecting port was located 1 mm lateral to the edge of the buffy coat. All units were obtained at a flow speed of 60 cc/min, and the collection port removed cells at 4 cc/min. After collection of 500 cc of blood by this method (mean hematocrit, 38%), the cells were glycerolized and frozen according to the method of Meryman.16 On

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the day of transfusion, the cells were washed and thawed and each unit was given over 1.5 hr. Samples of these cells were used for 51Cr labeling studies of in vivo survival and for red cell volume distribution curves. The red cell volume determinations were performed with a Coulter channelizer integrated with a model B Coulter counter (Coulter Electronics, Hialeah, Fla.).

All statistical data were presented as mean ± standard error of the mean. p Values were obtained from partial sampling and evaluated via the Student’s t test where applicable.

**RESULTS**

**Effect of Supertransfusion on Whole Blood Volume**

On the standard transfusion regimen, all patients received washed, frozen packed cells every 4–5 wk to maintain hematocrits greater than 27%. Occasionally, over a 1-yr period, a pretransfusion hematocrit was obtained that was recorded to fall below this level (see Table 1), but in no case did this occur on more than one occasion. The introduction of supertransfusion was accompanied by an initial increase in red cell requirement as expected. However, after a period of time, which varied from 1 to 4 mo, all patients maintained hematocrits greater than 35% on a transfusion schedule identical to that of their previous standard transfusion regimen. A composite example utilizing the first six patients studied is shown in Fig. 1. We therefore concluded that whole blood volumes in these patients must have decreased considerably.

To test this hypothesis, 51Cr red cell and simultaneous 125I plasma volumes, which had been measured in 21 patients while they were on the standard transfusion schedule, were reviewed and compared to predicted values. The results are listed in Table 1. Actual whole blood volumes greatly exceeded predicted levels in all patients studied by a mean of 33.8% ± 3.3%. Whole blood volume determinations were repeated in the 12 patients who had completed at
least 9 mo on a supertransfusion regimen (mean interval 12.6 mo). These values were compared to the previously obtained whole blood volumes. The results are shown in Table 2. In these patients, a reduction in whole blood volume was demonstrated over the interval amounting to a mean decrease of 21% ± 2%. This observation suggested that supertransfusion might effectively decrease erythropoiesis. To test this hypothesis, the following studies were performed.

Effect of Supertransfusion on Plasma Iron Clearance

To demonstrate that a supertransfusion schedule that persistently maintains hematocrits above 35% is associated with a significant decrease in erythropoiesis, we compared plasma iron clearances performed 4 wk after the last transfusion in 10 of these patients before and at least 3 mo after the initiation of the supertransfusion regimen. The results, presented in Table 3, demonstrate the half-life of transferrin-bound $^{59}$Fe was lengthened from a mean of 23.5 ± 4.4 min to a mean of 108.1 ± 15.5 min when the mean hematocrit was raised from 29.1 ± 0.6% to 41.8 ± 0.5%. Coincident measurements of plasma iron turnover (PIT) demonstrated a decrease from 10.0 ± 1.4 mg/100 ml/24 hr to 1.7 ± 0.3 mg/100 ml/24 hr. This represents a greater than fivefold decrease in the mean plasma iron turnover to a level in the normal range for our laboratory. The plasma iron clearance curves of a representative patient before and after supertransfusion are compared in Fig. 2.

Neocytopheresis

The primary source of iron for deposition in thalassemic patients is the transfused red blood cell. Since iron release occurs only after cell death, prolongation of the lifespan of the transfused red cells must result in decreased iron deposition per unit time. To accomplish such prolongation, we developed a technique for obtaining young red cells ("neocytes") from normal blood donors. Using an Aminco continuous flow cell separator, we consistently obtained units of neocytes from standard blood donors. The separator uses the technique of differential centrifugation to separate cells. The younger, less dense cells eventually position themselves in the uppermost portion of the red cell layer, permitting their aspiration and collection. Red cell volume distribution was determined in each unit with a Coulter sizer, and red cell age was estimated by the method of Corash. The cells were then washed and frozen according to standard practice and deglycerolized and thawed before administration to two patients over a period of 8 mo. The results of such neocyte transfusions were compared to those obtained when the patients were transfused with ordinary...
frozen cells. Table 4 and Fig. 3 show that the red cells in the neocyte units had a considerably younger mean age and larger volume distribution than did ordinary units. The interval between transfusions (which were administered when the hematocrit dropped to 36%) lengthened from 30 ± 2.5 days to 43 ± 4.5 days in the two patients treated with these cells. The neocyte and ordinary units were also compared with respect to 51Cr red cell kinetics. A composite curve using four separate donors is shown in Fig. 4. The daily in vivo loss of frozen and thawed neocytes was considerably less than the loss of ordinary frozen and thawed cells from the same donor.

DISCUSSION

In the treatment of thalassemia, we and others have focused our attention on the removal of iron from individuals who are already overloaded.3-10 Until recently, little attention has been focused on methods by which we may normalize hemoglobin concentration, thereby reducing the impact of ineffective erythropoiesis on bone development and simultaneously decreasing the rate of iron loading. Since most of the pathologic changes in thalassemia are thought to be secondary both to chronic anemia and iron overload, we addressed these areas directly.

Here we present evidence that persistent maintenance of normal hematocrits in transfusion-dependent patients by supertransfusion is not permanently associated with an increased transfusion requirement because such treatment is associated with a decrease in blood volume. Modell and coworkers have performed a retrospective analysis of transfusion

<table>
<thead>
<tr>
<th>Table 4.</th>
<th>Reticulocyte Count (%)</th>
<th>Enzyme Activity (IU/min/g Hb)</th>
<th>Calculated Red Cell Age (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predonation sample</td>
<td>0.8 ± 0.1</td>
<td>13.6 ± 1.2</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>Neocyte unit</td>
<td>4.1 ± 0.3</td>
<td>21.5 ± 3.7</td>
<td>9.8 ± 0.7</td>
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Comparison of neocytes and routinely obtained red blood cells from donors; n = 11 for both sets of determinations.
requirements, blood volumes, and mean hemoglobin concentrations in a thalassemic population in Britain. In this study, none of the patients were maintained at a hematocrit continuously above 35%. In fact, the highest mean hemoglobin concentration in these patients was slightly over 13 g/dl. The hemoglobin nadirs were, therefore, in the 10.5–11.0/dl range. Nonetheless, two patients whose mean hemoglobin values were 13 g/dl or higher had mean blood transfusion requirements not significantly higher than those patients whose mean hemoglobin values were 11.5 g/dl. This, we conclude, was probably due to the decrease in blood volume associated with the chronic maintenance of normal hemoglobin values.

In addition to maintenance of normal hemoglobin concentration by supertransfusion, erythropoiesis was also inhibited by this transfusion technique, as evidenced by a marked reduction of plasma iron turnover. This reduction in the rate of erythropoiesis has the established benefit of reducing the impact of ineffective erythropoiesis on bone development and a further theoretical benefit in that it is likely that the decrease in plasma iron turnover was associated with a decrease in iron absorption from the gastrointestinal tract. Cavill and coworkers have pointed out that iron absorption is a function of the product of the plasma iron turnover times the fraction of the total exchangeable iron pool that is found in the gut. Therefore, an acute reduction in plasma iron turnover must reduce iron absorption. Whether this effect of supertransfusion on iron absorption would be significant in the face of continuous Desferal administration or would be additive if the patients consumed adequate amounts of tea is not known, but at least the effect of supertransfusion on iron absorption is apt to be a salutory one.

Since the largest component of iron deposition in patients with thalassemia is derived from transfusion, we decided to attempt to decrease the amount of iron delivered by improving the quality of the red blood cell transfusions, as originally suggested by Piomelli and coworkers. Their data show that younger red cells have a prolonged in vivo survival when compared to older cells. Therefore, we used an Aminoceltrifuge to obtain neocytes from normal donors at the rate of 1 U every 2 hr. Neocytes obtained from this procedure demonstrate markedly increased in vivo survival even after glycerolization, freezing, and thawing. This prototype system is presently both time-consuming and expensive. The technology itself is similar to that presently employed by many institutions to obtain white cells and platelets for transfusions and includes heparinization of the donor during the procedure. In fact, the cost of the unit of such red cells is presently comparable to that of a white cell collection.

We are hopeful that a significant decrease in the rate of iron accumulation, coupled with continuous chelation therapy, will markedly alter the prognosis for patients with thalassemia major. This decrease in the rate of iron accumulation may permit us to use less aggressive and expensive chelation regimens in the future. In addition, it may now be appropriate to reconsider the approach to the treatment of some of the other debilitating hyperplastic anemias (specifically, the sickle syndromes). A therapeutic regimen consisting of supertransfusion with neocytes and moderate chelation might decrease overall morbidity in severely affected individuals.

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REFERENCES

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