The Effect of Erythroid Hyperplasia on Iron Balance

By Pensri Pootrakul, Kriengkrai Kitchaoren, Pornpan Yansukon, Prawase Wasi, Suthat Fucharoen, Preecha Charoenlarp, Gary Brittenham, Martin J. Pippard, and Clement A. Finch

Measurements of erythropoiesis and iron balance were made in eight normal and 32 anemic subjects. The latter consisted of 12 individuals with ineffective erythropoiesis (β-thalassemia/hemoglobin E), 13 subjects with ineffective erythropoiesis and hemolytic anemia (hemoglobin H), and seven subjects with hemolytic anemia (hereditary spherocytosis). A consistent relationship within each existed between the degree of erythropoiesis and radioiron absorption. Although the effect of erythropoiesis on iron absorption was of similar magnitude in the two thalassemia groups, the effect in hereditary spherocytosis was much less. There was agreement between absorption and ferritin or magnetic susceptibility (SQUID) measurements of iron stores in thalassemia, but in hereditary spherocytosis a discrepancy existed between absorption and ferritin. It is concluded that, although increased erythropoiesis is associated with increased iron absorption, some additional factor associated with red cell breakdown is more directly responsible for the positive iron balance in thalassemia.

IRON OVERLOAD is known to occur in a number of anemic states in humans, most frequently in the thalassemic and sideroblastic syndromes. Overload has also been demonstrated in certain patients who have a refractory anemia with a hyperplastic erythroid marrow and in a variety of hemolytic states. In these conditions, transfusion does not seem necessary for the development of iron overload. It is not clear whether the positive iron balance is simply a reflection of the rate of erythropoiesis because thalassemia is known to have the highest rate of red cell production and also the greatest iron loading or whether other factors are involved. In this study we compare iron absorption and other measurements of iron overload with ferrokinetic measurements of erythropoiesis in three groups of patients with hyperproliferative anemia.

MATERIALS AND METHODS

Forty-two male subjects from the Hematology Clinic of Siriraj Hospital were studied according to a protocol approved by the Human Subjects Committee of the University of Washington and Mahidol University. All subjects gave their informed consent. The subjects included eight normal individuals, 12 patients with β-thalassemia/hemoglobin E and 13 subjects with hemoglobin H disease whose criteria for diagnosis were as published elsewhere, and seven subjects with hereditary spherocytosis identified by a chronic hemolytic anemia, splenomegaly, and increased osmotic fragility. All subjects had been in a steady state without infection for the preceding 3 months. Stool examination in thalassemic subjects was negative for hookworm and other parasites. In the past, six of the patients with β-thalassemia/hemoglobin E had been transfused with 4 to 15 units of red cells. Only one of the patients with hemoglobin H had been previously transfused, and he had been given 4 units of red cells. No subject had received blood in the preceding 2 months.

On the first day of the study a plasma iron turnover (PIT) procedure was performed by using FeS04. If the transferrin saturation of the subject was less than 80%, the radioiron (4 μCi, 0.1 μg) in a saline solution, pH 2, was injected intravenously over a period of five minutes. If the saturation was greater than 80%, normal plasma was labeled in vitro and saturated to the level present in the patient’s plasma. Blood volume was calculated from the dilution of radioiron in the circulation, and PIT was corrected for any increase above the assumed normal of 75 mL/kg. PIT was performed and calculated as described elsewhere, with the additional correction for blood volume change according to the following formula:

PIT (mg/dL whole blood/d) = plasma iron (μg/dL)/t1/2(min) x plasmatocrit x patient’s blood volume (mL/kg)/75.

The PIT index was calculated by relating the patient’s PIT to the normal PIT of 0.7 mg/dL whole blood/d and expressing the PIT as a multiple of normal. Iron kinetics were also calculated as erythroid iron turnover (EIT) and as erythroid transferrin uptake (ETU). Methods of calculation are described elsewhere. Because statistical analyses of the results by these two methods showed no advantage over the simpler calculation of PIT, they were not used in the calculations of relationships. Samples to determine red cell utilization were drawn at 2 weeks, expressed as the amount of radioactivity in circulation over the amount injected, and calculated as described elsewhere.

Iron absorption was measured on the second day of the study. One to 2 μCi of 59Fe in 5 mg FeS04 at pH 2 was given in 50 mL of water containing 2 mol ascorbic acid/mol iron to the subject who had been fasted overnight. Total-body activity was determined in a shadowed total-body counter at two hours to establish the initial activity, and the count was repeated at 2 weeks. This counter was constructed by Earl Palmer, Battelle Institute, and costs were partially provided through a grant from CIBA-Geigy, Horsham, West Sussex, U.K. Absorption was calculated from the difference by taking into account background and decay.

Magnetic susceptibility measurements were made with a specially designed SQUID susceptometer whose characteristics and performance have been discussed elsewhere. The measurement was made.

From the Division of Hematology, Department of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand; Cleveland Metropolitan General Hospital; the Section of Haematology, Medical Research Council Clinical Research Center and Northwick Park Hospital, Harrow, Middlesex, England; and the Division of Hematology, Department of Medicine, University of Washington, Seattle.

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Address reprint requests to Clement A. Finch, MD, Hematology Division, Department of Medicine, RM-10, University of Washington, Seattle, WA 98195.

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Table 1. Characterization of Four Groups of Subjects

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Normals (8)</th>
<th>β-Thalassemia/βE (13)</th>
<th>HbH (12)</th>
<th>Hereditary Spherocytosis (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>27</td>
<td>5</td>
<td>18-33</td>
<td>25</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>15</td>
<td>2</td>
<td>13-17</td>
<td>8</td>
</tr>
<tr>
<td>Blood volume (mL/kg)</td>
<td>78</td>
<td>5</td>
<td>70-85</td>
<td>90</td>
</tr>
<tr>
<td>Plasma iron (μg/dL)</td>
<td>122</td>
<td>30</td>
<td>96-187</td>
<td>170</td>
</tr>
<tr>
<td>Tf Sat (%)</td>
<td>37</td>
<td>10</td>
<td>29-51</td>
<td>75</td>
</tr>
<tr>
<td>Corrected PIT (mg/dL wb/d)</td>
<td>0.77</td>
<td>0.12</td>
<td>0.82-0.94</td>
<td>7.04</td>
</tr>
<tr>
<td>Corrected ETV (mg/dL wb/d)</td>
<td>0.5</td>
<td>0.16</td>
<td>0.3-0.7</td>
<td>6.2</td>
</tr>
<tr>
<td>RBC ut (%)</td>
<td>66</td>
<td>17</td>
<td>42-89</td>
<td>512</td>
</tr>
<tr>
<td>RBC ut (%)</td>
<td>89</td>
<td>2</td>
<td>86-93</td>
<td>27</td>
</tr>
<tr>
<td>Reticulocyte index</td>
<td>0.8</td>
<td>0.5-0.9</td>
<td>2.0</td>
<td>0.8-4.2</td>
</tr>
<tr>
<td>Iron absorption (%)</td>
<td>18</td>
<td>5</td>
<td>12-16</td>
<td>51</td>
</tr>
<tr>
<td>SQUID Fe (mg)</td>
<td>268</td>
<td>200</td>
<td>(50-512)</td>
<td>4,961</td>
</tr>
</tbody>
</table>

Abbreviations: Tf Sat, transferrin saturation; RBC ut, RBC utilization of radioiron; wb, whole blood.
†Geometric mean and geometric SD.
in the preceding year in normal and thalassemic subjects before the other procedures were carried out.

Other laboratory studies include the blood hemoglobin concentration performed by the cyanmethemoglobin method, the hematocrít by the microtechnique, the red cell count by Coulter Counter, plasma iron and total iron-binding capacity by standard methods, and plasma ferritin by a radioimmunometric technique. Reticulocyte counts of 1,000 red cells were performed after new methylene blue staining. Results are expressed as the reticulocyte index in which the red count and blood volume were used to derive the absolute number of circulating reticulocytes and the hemoglobin concentration to derive maturation time.

RESULTS

A characterization of the four groups of subjects studied including measurements to characterize iron balance and erythropoiesis are shown in Table 1. Subjects with \( \beta \)-thalassemia/hemoglobin E were more anemic and had a greater accumulation of body iron than subjects with hemoglobin H; they were more anemic than subjects with hereditary spherocytosis but had comparable ferritin values. The erythroid marrow activity (total erythropoiesis) as estimated by the PIT was nearly the same in patients with \( \beta \)-thalassemia/hemoglobin E and hereditary spherocytosis, whereas patients with hemoglobin H were at a much lower level. The reticulocyte index, representing effective erythropoiesis, was only slightly increased in thalassemic subjects and was significantly higher in patients with hereditary spherocytosis. Red cell utilization was lowest in patients with \( \beta \)-thalassemia/hemoglobin E, thus reflecting the high degree of ineffective erythropoiesis.

Iron stores in 25 thalassemic patients were estimated from the plasma ferritin determination and by magnetic measurements of liver iron. In view of the skewed distribution of normal ferritin values, the log ferritin value was used for comparisons. These measurements were positively correlated with each other as well as with iron absorption values (Fig 1). A further calculation was made in which the plasma ferritin and SQUID measurements of iron accumulation to be compared to absorption were adjusted for age as published elsewhere. In our study, this did not improve the correlation.

Blood volume and erythropoiesis were evaluated by radioiron measurements. It was noted that the blood volume increased as the PIT increased (Fig 2, right). There was also an inverse relationship between PIT and hemoglobin concentration, the lower the hemoglobin concentration, the greater the PIT (Fig 2, left).

There were relationships between PIT and the SQUID or serum ferritin measurements of iron balance \((r = .75 \text{ and } .55, P < .05)\). Of particular interest was the degree of increase in absorption for the increase in PIT, which in thalassemia was about four times that observed in hereditary spherocytosis (Fig 3). A further comparison was made of patients with \( \beta \)-thalassemia/hemoglobin E according to whether they had been previously transfused. The six previously transfused had a mean PIT of 8.9 ± 1.7 mg/dL whole blood/d and a mean absorption of 64% ± 20%. Corresponding values for those not transfused were 5.1% ± 2.5% and 39% ± 21%. The greater severity of the thalassemic process in the transfused patients appeared to be an adequate explanation for the difference in absorption.

There was a good correlation between absorption and the plasma iron concentration or transferrin saturation in thalassemic patients (Fig 4). No correlation was seen in hereditary spherocytosis between absorption and either the plasma iron concentration or transferrin saturation.

Table 2 summarizes the correlations between the different individual parameters measured in the total subject group as well as the individual groups.

DISCUSSION

Iron overload has been recognized as a frequent complication in thalassemia, even when transfusions or oral iron have not been administered. The increased absorption in such patients is reduced when erythropoiesis is suppressed by transfusion. A relationship between erythropoiesis and absorption has been also validated by animal studies, most dramatically by the reversal of iron loading in the pyruvate kinase-deficient dog after normal erythropoiesis was instituted by a marrow transplant. For these reasons, attention has been focused on erythroid activity as a regulator of iron absorption.

Clinical reports suggest a distinction between the frequency with which symptomatic iron overload occurs in patients with ineffective erythropoiesis (thalassemia, sideroblastic and some refractory anemias with hyperplastic mar-
Fig 2. Relationships between erythropoiesis and blood volume (right) or hemoglobin concentration (left). Least-square extrapolations for patients with hereditary spherocytosis (upper line) show a higher hemoglobin value for any given PIT as compared with thalassemic patients (lower line). Symbols: O, $\beta$-thalassemia/hemoglobin E; HbH disease; and A, hereditary spherocytosis.

Fig 3. Relationships between PIT and iron absorption in patients with thalassemia (left) and hereditary spherocytosis (right). Symbols: O, $\beta$-thalassemia/hemoglobin E; HbH disease; and A, hereditary spherocytosis.

Fig 4. Relationships between iron absorption and plasma iron (left) or transferrin saturation (right) in patients with thalassemia. Symbols: O, $\beta$-thalassemia/hemoglobin E; and A, hemoglobin H disease.

were made not only of PIT but also of erythroid iron uptake in which the purpose is to exclude nonerythroid turnover and also of erythroid transferrin uptake in which the effect of a variation in plasma iron supply is excluded so as to measure more specifically marrow transferrin receptors.10 Relationships between the PIT, EIT, and ETU in the patient groups agreed closely, and therefore the simpler PIT was used in correlations with other measurements. With all of these measurements, it was necessary to correct the usual expression of iron turnover per decaliter whole blood per day for blood volume changes. The increase in blood volume in thalassemia observed in these studies has been previously described.24 The extent of the volume increase appeared related to the rate of erythropoiesis, presumably a reflection of the increased vascularity of the marrow.

An inverse relationship was found between erythropoiesis and the degree of anemia. Assuming no impairment in the proliferative capacity of the marrow, this would be expected

Table 2. Correlation in Patient Groups

<table>
<thead>
<tr>
<th>Comparison</th>
<th>All (32)</th>
<th>Thal (25)</th>
<th>Thal/HbE (12)</th>
<th>HbH (13)</th>
<th>HS (7)</th>
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<tbody>
<tr>
<td>Correlation PIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v BV</td>
<td>.69*</td>
<td>.70*</td>
<td>.60†</td>
<td>.48</td>
<td>.95*</td>
</tr>
<tr>
<td>v Hb</td>
<td>.71*</td>
<td>.79*</td>
<td>.53</td>
<td>.44</td>
<td>.78†</td>
</tr>
<tr>
<td>v Absorption</td>
<td>.69*</td>
<td>.80*</td>
<td>.62†</td>
<td>.80*</td>
<td>.92†</td>
</tr>
<tr>
<td>v SQUID</td>
<td>.75†</td>
<td>.59†</td>
<td>.80*</td>
<td>.45</td>
<td>.73</td>
</tr>
<tr>
<td>v log ferritin</td>
<td>.55*</td>
<td>.52†</td>
<td>.53</td>
<td>.45</td>
<td>.73</td>
</tr>
<tr>
<td>v Tf Sat</td>
<td>.51†</td>
<td>.62*</td>
<td>.57†</td>
<td>.88*</td>
<td>.33</td>
</tr>
<tr>
<td>v Tf Sat</td>
<td>.56*</td>
<td>.69*</td>
<td>.53</td>
<td>.88*</td>
<td>.03</td>
</tr>
<tr>
<td>Absorption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v SQUID</td>
<td>.88*</td>
<td>.91*</td>
<td>.57†</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>v log ferritin</td>
<td>.64*</td>
<td>.75*</td>
<td>.86*</td>
<td>.48</td>
<td>.56</td>
</tr>
<tr>
<td>v Pi</td>
<td>.77*</td>
<td>.78*</td>
<td>.87*</td>
<td>.68†</td>
<td>.39</td>
</tr>
<tr>
<td>v Tf Sat</td>
<td>.80*</td>
<td>.78*</td>
<td>.77†</td>
<td>.67†</td>
<td>1.0</td>
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<tr>
<td>SQUID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v log ferritin</td>
<td>.81†</td>
<td>.81*</td>
<td>.79†</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>v Pi</td>
<td>.82*</td>
<td>.86*</td>
<td>.77†</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>v Tf Sat</td>
<td>.75*</td>
<td>.67†</td>
<td>.74†</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Log Ferritin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v Pi</td>
<td>.71*</td>
<td>.78*</td>
<td>.88*</td>
<td>.52</td>
<td>.16</td>
</tr>
<tr>
<td>v Tf Sat</td>
<td>.53*</td>
<td>.65*</td>
<td>.70*</td>
<td>.46</td>
<td>.02</td>
</tr>
</tbody>
</table>

Abbreviations: Thal, $\beta$-thalassemia; HS, hereditary spherocytosis; BV, blood volume; PI, plasma iron.

*P < .001.
†P < .05.
because the degree of erythropoietin stimulation of the erythroid marrow is a function of the decrease in circulating hemoglobin content.

Several methods were used to evaluate the iron status. Because most of the excess iron is located in the liver in patients with erythroid hyperplasia, the magnetic measurement of liver iron provided the most direct noninvasive quantitation. More widely used because of its greater availability is the measurement of plasma ferritin levels, which generally reflects iron stores throughout the body, however, plasma ferritin is not considered quantitative at levels over 1,000 µg/L, and even at lower levels, its relationship to storage iron is nonlinear. For purposes of storage measurement, the log value of plasma ferritin was considered more appropriate. Magnetic and ferritin measurements showed a reasonable correlation with each other in the 25 thalassemic patients (r = .81). Similar relationships were found between absorption and these two measurements in all 32 patients (r = .88 and .75). This suggested that there was reasonable agreement between these three indicators of iron balance, although each measurement is quite different in its implications and in factors affecting it. SQUID reflects cumulative liver iron and would be influenced by past diet, blood losses, and past transfusions. Plasma ferritin reflects all of these and, in addition, nonhepatic storage iron. Its relationship to stores is known to be disturbed by changes in tissue metabolism or tissue damage. Absorption in the normal individual is regulated so as to maintain iron balance, but in the thalassemic individual it is unaffected by the enlarged iron stores.

Although the ferritin and SQUID measurements might be expected to reflect the time of iron accumulation, allowance for age did not seem to improve their correlation with the PIT, perhaps because there was not a great variation in age among the subjects studied. Likewise, the sporadic transfusions that had been given to patients with β-thalassemia/hemoglobin E and would have increased stores to some degree, had no obvious effect on the relationship between PIT and absorption; however, transfusions had been given to those patients with greater anemia, higher marrow proliferations, and higher absorption so that transfused iron represented a small portion of the total iron stores in those individuals.

Within the separate groups, PIT and absorption showed a strong relationship. There appeared to be little difference between β-thalassemia/hemoglobin E and hemoglobin H disease in the rate of increase in absorption for any given increase in PIT. There was an even closer relationship between PIT and absorption in hereditary spherocytosis, but absorption occurred at a much lower level for any given value of PIT. In other words, no difference in absorption could be seen between thalassemic patients with predominately ineffective erythropoiesis and those with combined ineffective erythropoiesis and increased destruction of circulating red cells, but the level of absorption in relation to the PIT was much reduced in patients with hemolytic anemia.

The correlation between absorption and plasma iron or transferrin saturation found in thalassemia was not seen in hereditary spherocytosis. This suggests that the elevation of plasma iron levels was in some way related to the thalassemic state but not the rate of erythropoiesis. Hyperferremia appears to be characteristic of conditions in which increased iron is mobilized from both absorption and the RE cell, ie, in both thalassemia and idiopathic hemochromatosis. Under such conditions, the only storage area for excess iron would be the hepatocyte. Thus the increase in plasma iron and transferrin saturation in thalassemia is yet another index of increased iron absorption. To place these findings in sequence, an increase in iron absorption resulted in an increased transferrin saturation, increased liver iron, and increased plasma ferritin, all reflecting the positive iron balance.

A puzzling finding in hereditary spherocytosis was the apparent discrepancy between the elevation of ferritin levels, which suggested increased iron stores, and the essentially normal absorption. Although other measurements of plasma ferritin and hereditary spherocytosis have indicated little if any increase in most subjects, these elevated ferritin levels were validated by repeated laboratory measurement. In view of this contradiction, iron stores in these patients are in question. Unfortunately, it was not possible to carry out SQUID measurements in these individuals; however, the transferrin saturation of 44%, lower than in the other two groups, suggests that iron loading at that time was not great. Conceivably, some iron loading had occurred in the past, but absorption was being downregulated in these subjects because of the increased stores.

The greater absorption in thalassemia is consistent with the high frequency of symptomatic iron overload in patients with this disorder and its rarity in hereditary spherocytosis. Yet it has been shown that the increased absorption is reversed if red cell turnover is normalized by hypertransfusion or marrow transplantation. This would suppress not only red cell production but also the increased red cell destruction as defective cells are replaced by normal cells and raises the possibility that iron loading in the thalassemic patient may relate partly, perhaps predominately, to the nature of the red cell destruction. If destruction occurs in a manner that would make iron less available for recirculating to transferrin, there might be a greater need for an increased iron supply through absorption. As compared with hereditary spherocytosis, there is a greater degree of intravascular hemolysis in thalassemia that leads to uptake of the hemoglobin-haptoglobin complex or the heme-hemopexin complex by the liver. Were such iron less easily mobilized than iron processed by the RE cell, there might be a greater stimulus for iron absorption. Following this line of thought, one might expect iron sequestration to lead to hypoferremia in the plasma that would be compensated by increased absorption. In that case the increased absorption should cease once the plasma iron concentration becomes normal. It seems more likely, even if sequestration of catabolized red cell iron does occur, that the regulator for increasing the iron supply originates in immature erythroid cells in need of additional iron. Evidence for this has already been presented in the rat transfused with reticuloctyes where, like the thalassemic patient, additional iron is mobilized from both RE cells and the intestinal mucosa.
REFERENCES


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