Inappropriately Low Red Cell 2,3-Diphosphoglycerate and p50 in Transfused β-Thalassemia

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REPEATED BLOOD TRANSFUSIONS are the basis of therapy for thalassemia major. Maintenance of the hemoglobin (Hb) concentration above 10 g/dl suppresses bone marrow hyperactivity and thereby alleviates most clinical symptoms of the primary disease.1 Historically, the development of secondary hemachromatosis has led to the ultimate demise of such patients, typically from cardiac failure, presumably due to cardiac iron overload.2 The achievement of iron balance through chelation therapy is the primary goal of modern management of this disease,3 but the long-term prognosis associated with effective chelation therapy remains to be established.

In various anemic states, as the Hb concentration declines, there is a corresponding increase in 2,3-DPG and decrease in Hb oxygen affinity, thereby ensuring adequate tissue oxygen delivery.4 Two previous reports1,5 have suggested that this compensatory response to hypoxia may be inadequate in patients with thalassemia major. Both of these studies were performed in patients transfused with a regimen incapable of completely suppressing endogenous red cell production. The red cells produced in thalassemia major contain fetal hemoglobin (HbF) nearly exclusively, which has different oxygen-dissociation characteristics. We have therefore studied 2,3-DPG levels and Hb–oxygen dissociation in 20 patients with thalassemia major whose own red cell production has been nearly completely suppressed by a hypertransfusion regimen.

MATERIALS AND METHODS

Twenty patients with homozygous β-thalassemia, ranging from 3 to 22 yr of age, were studied. They were in excellent clinical status, except for two of the oldest ones, who were suffering from heart failure but were well compensated by digoxin therapy. The oldest ten patients had undergone splenectomy. None of them were cigarette smokers. Each patient received approximately 15 ml/kg of either packed red cells (2 patients), leukocyte-poor (7 patients), or frozen-thawed red cells. The hypothesis that the time of blood storage was a factor was excluded by repeatedly transfusing one patient with packed red cells administered within 4 hr of collection in CPDA-1. A second hypothesis, that red cell function might be impaired by the iron-overloaded thalassemic environment, was excluded by studying a newly diagnosed, newly transfused patient with aplastic anemia. In both cases, the same inability to appropriately increase 2,3-DPG and p50 as the Hb fell during the intertransfusion interval was noticed. These data suggest that red cells of chronically transfused patients are unable to adapt to the decline in Hb that occurs during the intertransfusion interval.

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20 normal individuals. In addition, we have studied 12 patients with thalassemia major had inappropriately low 2,3-DPG concentrations and p50s. These findings occurred in all patients, regardless of whether they had received packed, leukocyte-poor, or frozen-thawed red cells. The hypothesis that the time of blood storage was a factor was excluded by repeatedly transfusing one patient with packed red cells administered within 4 hr of collection in CPDA-1. A second hypothesis, that red cell function might be impaired by the iron-overloaded thalassemic environment, was excluded by studying a newly diagnosed, newly transfused patient with aplastic anemia. In both cases, the same inability to appropriately increase 2,3-DPG and p50 as the Hb fell during the intertransfusion interval was noticed. These data suggest that red cells of chronically transfused patients are unable to adapt to the decline in Hb that occurs during the intertransfusion interval.

METHODS

Hb-oxygen dissociation in 20 patients with thalassemia major had inappropriately low 2,3-DPG concentrations and p50s. As is well established. In contrast, however, prior to transfusion, at the nadir of Hb, patients with thalassemia major had inappropriately low 2,3-DPG concentrations and p50s. These findings occurred in all patients, regardless of whether they had received packed, leukocyte-poor, or frozen-thawed red cells. The hypothesis that the time of

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acquired aplastic anemia during the first 2 mo of transfusion. His diagnosis was confirmed by bone marrow aspirate and biopsy; reticulocyte counts were between 0.0% and 0.1%. This patient differed from the thalassemics in that he was not iron overloaded. He was studied on 13 occasions, while his Hb concentration ranged from 7.3 to 12.8 g/dl. He received approximately 15 ml/kg of frozen-thawed red cells once every 3 wk.

RESULTS

In the 24 normal individuals, the mean hemoglobin was 14.3 ± 0.8 g/dl, with a mean 2,3-DPG concentration of 13.8 ± 1.4 μmole/g Hb, and a mean p50 of 27.2 ± 0.8 mm Hg. The 13 anemic, nontransfused patients (see Materials and Methods) had a mean Hb of 8.5 ± 1.4 g/dl, a mean 2,3-DPG concentration of 20.1 ± 1.3 μmole/g Hb, and a mean p50 of 33.8 ± 2.0 mm Hg.

In this nontransfused control group, regression analysis confirmed the well-known phenomenon that the 2,3-DPG concentration is an inverse function of the Hb (p < 0.001; Fig. 1). The slope of the regression line (-0.93) is in good agreement with data previously reported by others. In contrast, however, no correlation between Hb concentration (pretransfusion) and 2,3-DPG concentration could be identified in the 20 transfused thalassemia major patients (r = 0.18, p = 0.45). This group of patients had inappropriately low 2,3-DPG concentrations for their Hb levels, with a mean Hb of 10.6 ± 0.5 g/dl (expected p50 = 32.7 mm Hg). Red cell 2,3-DPG was equally low regardless of whether the patient received packed, leukocyte-poor, or frozen-thawed red cells.

Similarly, whereas in the controls the p50 was inversely correlated to the Hb concentration (r = -0.90, p < 0.001; Fig. 2), in chronically transfused patients with thalassemia major, the p50 was always inappropriately low for their Hb level (r = -0.25, p = 0.14), with a mean p50 of 27.1 ± 0.9 mm Hg for a mean Hb of 10.6 ± 0.5 g/dl (expected p50 = 32.7 mm Hg). It is important to realize that these absolute values of 2,3-DPG and p50 would be within “normal limits” for a normal Hb level, but are low for the observed Hb.

In the three patients who have been studied during the interval between transfusions, the p50 and 2,3-DPG concentration showed little movement from week to week. In this group, in whom the pretransfusion p50 averaged 25.3 mm Hg, with a mean 2,3-DPG concentration of 9.5 μmole/g Hb, the p50 reached a maximum mean level of 27.0 mm Hg 1 wk after transfusion, with a corresponding maximum mean 2,3-DPG concentration of 12.7 μmole/g Hb.

To evaluate the hypothesis that the inability of transfused red cells to synthesize adequate quantities of 2,3-DPG might be due to the “thalassemic environment,” we studied a newly diagnosed, newly transfused, non-iron-overloaded patient with aplastic anemia. This patient demonstrated a similar inability to appropriately increase 2,3-DPG and p50 as the Hb fell during the intertransfusion interval (Fig. 3).

To explore the possibility that a functional defect might be related to the duration of storage of transfused red cells, a 3-yr-old thalassemic patient was transfused consecutively 3 times with packed red cells that had been collected in CPDA within 4 hr of transfusion. At the 3 subsequent transfusion visits, the
mean hemoglobin concentration was 10.6 g/dl, in association with a mean 2,3-DPG level of 13.1 μmole/g Hb, and mean p50 of 26.0 mm Hg. These values for 2,3-DPG and p50 are, once again, inappropriately low for the Hb level.

DISCUSSION

Following the collection and storage of blood, the oxygen affinity of Hb increases due to the depletion of 2,3-DPG, a compound that regulates oxygen affinity by binding to the β-chains of the Hb molecule. After infusion, transfused red cells are able to synthesize 2,3-DPG up to “normal” values, thereby decreasing Hb oxygen affinity. It is well documented that, in various nontransfused anemic states, as the Hb concentration declines, there is a compensatory increase in 2,3-DPG and a decrease in Hb oxygen affinity, thereby ensuring adequate tissue oxygen delivery. But in 1974, deFuria and coworkers reported that, in chronically transfused patients with thalassemia major, Hb oxygen affinity was inappropriately high for the degree of anemia, suggesting an impairment of the ability of red cells to adapt to the decline in Hb during the intertransfusion interval. The patients studied by deFuria had been typically maintained at pretransfusion Hb concentrations of 8 g/dl, a regimen that was incapable of suppressing endogenous HbF production. Their findings were therefore somewhat inconclusive, as HbF does not readily bind 2,3-DPG and has an intrinsically higher oxygen affinity.

Current more intense transfusion regimens are aimed at completely suppressing endogenous red cell production. One would expect that, in such chronically hypertransfused patients, if the biochemical response of the transfused cells to hypoxia were intact, the circulating red cell 2,3-DPG concentration would be elevated above normal immediately prior to transfusion, when the Hb concentration is at its nadir. The data shown in Figs. 1 and 2 indicate that the concentration of 2,3-DPG and the p50 remain at the “normal” level, fail to rise when the Hb falls, and therefore become inappropriately low for the corresponding level of Hb. In thalassemic patients, the observed p50s were always appropriate for the red cell 2,3-DPG concentrations, suggesting that the impaired response to the fall in Hb is due to inadequate 2,3-DPG synthesis. Additional studies of blood obtained from three patients during the weeks between transfusions support these findings and suggest that, during the intertransfusion interval, chronically transfused patients develop a “functional anemia,” in that there is inadequate modulation of Hb oxygen affinity in response to a declining Hb concentration.

It is well known that, in normal individuals, the concentration of 2,3-DPG and the p50 decrease as red cells age. Furthermore, Opalinski and Beutler have reported that the increase of 2,3-DPG synthesis in response to anemia is much greater in patients with hemolytic anemia (including sicklers) with a young red cell population, than in patients with aplastic anemia, who have an older red cell population. Thus, young red cells appear to be more capable of responding to hypoxia than older red cells. It is possible that our observations of inappropriately low 2,3-DPG and p50 in chronically transfused patients simply reflect the relatively older red cell population present immediately prior to transfusion. This hypothesis is now being tested in a clinical trial of young red cell transfusions that is ongoing in our laboratory.

Initially, the possibility that the impaired synthesis of 2,3-DPG could be related to the iron-overloaded milieu of the thalassemic patient was considered. However, our observations of a similar pattern in a newly transfused, non-iron-overloaded patient with aplastic
anemia clearly indicate that this phenomenon is due to the transfused blood itself. In this patient, in whom the Hb was allowed to decline to lower levels than in the thalassemics, the concentration of 2,3-DPG and the p50 increased above the normals, an observation previously reported by Dickerman et al.17 However, the metabolic response of the transfused red cells, as in the thalassemic patients, was never adequate, considering the observed Hb concentration. When examined carefully, the data of Dickerman et al.17 are consistent with our findings.

We also attempted to determine whether the duration of blood storage was somehow responsible for impaired 2,3-DPG synthesis by repeatedly transfusing packed red cells within hours after collection in CPDA. This limited trial in one patient failed to increase 2,3-DPG and p50 before transfusion, suggesting that the impairment of red cell function occurs immediately upon blood collection.

Is the relatively high Hb oxygen affinity of transfused red cells of clinical importance in patients with β-thalassemia major? Oxygen transport to tissues is primarily a function of three variables: blood flow, Hb concentration, and the arteriovenous oxygen difference, the latter of which is a function of Hb oxygen affinity.18 It is clear that, in order to maintain a constant supply of oxygen to the tissues, given their relatively low level of Hb and relatively high oxygen affinity, in thalassemic patients the blood flow (i.e., cardiac output) must be chronically increased. Some of the residual pathophysiology associated with thalassemia major may be due, in part, to poor tissue oxygenation and high cardiac output. Thus, even with modern adequate transfusion modalities, a relative degree of tissue hypoxia may persist as a result of “functional anemia.”

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