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

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The relationships among hemoglobin concentration (Hb), red cell 2,3-diphosphoglycerate (2,3-DPG), and p50 were studied in 20 chronically hypertransfused patients with thalassemia major. In the nontransfused control group, which included normal individuals as well as patients with sickle cell disease or iron deficiency anemia, the Hb correlated inversely with both 2,3-DPG concentration and p50, 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 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.

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 reports5,6 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 packed red cells (2 patients), leukocyte-poor (7 patients), or frozen-thawed red cells (11 patients) at intervals of 3–4 wk. All blood units were collected in CPDA-1 and either administered or frozen within 5 days. Frozen units were then thawed and deglycerolized within 24 hr of transfusion. The patients' mean pretransfusion Hb concentration was 10.6 g/dl, the mean HbF concentration was 11.3%, and the mean reticulocyte count was 0.4%, indicating a low concentration of endogenous thalassemic cells in the circulation. Capillary pH, serum CO2 base excess, and electrolytes were within normal limits.

Both red cell 2,3-DPG and p50 were measured on 1–5 occasions in each patient immediately prior to transfusion. Where multiple analyses were performed, the mean value has been used in data analysis. In addition, in three patients, 2,3-DPG and p50 were measured twice per week during the interval between transfusions. Red cell 2,3-DPG was measured by the technique of Keitt.7 The Hb–oxygen dissociation curve was determined with a Hemox-Analyzer (TCS Medical Products, Philadelphia, PA), an instrument that utilizes 50 μl of blood under controlled conditions (pH 7.4, temperature 37°C).8 From this curve, the p50, i.e., the partial pressure of O2 (mm Hg) at which Hb is 50% saturated with O2, was calculated.

In an attempt to determine whether the duration of blood storage prior to transfusion influenced the 2,3-DPG and p50 findings, one 3-yr-old patient with thalassemia major was transfused 3 times with packed red cells that had been collected in CPDA-1 within 4 hr of transfusion. 2,3-DPG and p50 were studied as above.

As controls, we have measured p50 and 2,3-DPG concentration in 24 normal individuals. In addition, we have studied 12 patients with anemia due to other causes, including 4 with iron deficiency and 8 with sickle cell disease; 2 of the 2,3-DPG samples in this group were destroyed during the analysis. In this group of controls, the relationships among Hb concentration, 2,3-DPG level, and p50 were determined by regression analysis.

We have also studied one newly diagnosed patient with an
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
\( r = -0.93 \) 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 demonstrated 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, and a mean 2,3-DPG of
13.8 ± 1.7 µmole/g Hb (expected value 18.5 µmole/g
Hb). 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
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To explore the possibility that a functional defect
might be related to the duration of storage of trans-

cused 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

![Fig. 1. Red cell 2,3-diphosphoglycerate (2,3-DPG) as a function
of Hb concentration. The control population (•) consisted of
normal individuals as well as patients with anemia due to iron
deficiency or sickle cell disease. The line is the result of a
regression analysis of samples obtained from control subjects.
2,3-DPG concentration increased in controls as the Hb decreased
(\( p < 0.001;\ r = -0.93 \)). There was no correlation between
2,3-DPG and Hb concentration in thalassemics (□) (\( p =
0.45;\ r = 0.18 \)).](https://www.bloodjournal.org/content/80/4/804)

![Fig. 2. Red cell p50 as a function of Hb concentration. The
control population (•) was identical to that in Fig. 1. The line is the
result of regression analysis of samples obtained from control
subjects. In controls, p50 increased as the Hb decreased (\( p <
0.001;\ r = -0.90 \)). There was no correlation between p50 and Hb
concentration in thalassemics (□) (\( p = 0.14;\ r = -0.26 \)).](https://www.bloodjournal.org/content/80/4/804)
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,9,10 a compound that regulates oxygen affinity by binding to the β-chains of the Hb molecule.11 After infusion, transfused red cells are able to synthesize 2,3-DPG up to “normal” values, thereby decreasing Hb oxygen affinity.12,13 It is well documented⁴ 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 coworkers⁵ 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.¹⁴

Current more intense transfusion regimens are aimed at completely suppressing endogenous red cell production.¹ 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.¹⁵ Furthermore, Opalski and Beutler¹⁶ 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.\textsuperscript{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.\textsuperscript{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 \(\beta\)-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.\textsuperscript{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, 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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