Bone Marrow Delta-Aminolaevulinate Synthase Deficiency in a Female With Congenital Sideroblastic Anemia

By George R. Buchanan, Sylvia S. Bottomley, and Ruprecht Nitschke

Heme biosynthesis was examined in erythroid tissue of a 4-yr-old girl with severe sideroblastic anemia since infancy, as documented by the presence of intramitochondrial deposits of iron in erythroblasts. Free red cell protoporphyrin, urinary porphyrins, and activities of erythrocyte porphobilinogen synthase, uroporphyrinogen I synthase, aspartate aminotransferase, and pyridoxine kinase were normal or increased. Bone marrow ferrochelatase activity was normal. Activity of bone marrow delta-aminolaevulinate (ALA) synthase was markedly reduced to 7 pmole ALA/10³ erythroblasts/30 min (normal 127 ± 29) but was enhanced fivefold by pyridoxal phosphate (normal 0%–25% increase). Therapy with oral pyridoxine and parenteral pyridoxal-5'-phosphate did not increase effective red cell production. The sideroblastic anemia in this patient appears to be related to a congenital defect in the initial step of heme biosynthesis.

CONGENITAL forms of sideroblastic anemia are a rare and heterogeneous group of disorders. Most affected patients are males who develop hypochromic microcytic anemia in childhood or early adult years. An X-linked recessive inheritance pattern has been demonstrated in some kindreds. In only a few instances have females with congenital sideroblastic anemia (CSA) been identified. Some mildly affected females have been heterozygous carriers of a defective X-linked gene, but the precise inheritance pattern has not been ascertained in the few reported patients with severe anemia.

Defects in erythroid heme synthesis have been observed in both acquired and congenital sideroblastic anemia but have not been fully defined. Diminished activity of delta-aminolaevulinate synthase (ALA-S), the enzyme that catalyzes the first and rate-limiting reaction in heme biosynthesis, has been described in five instances of CSA and in a limited number of patients with acquired forms of the disease. In several reports, activity of ferrochelatase, the most distal enzyme in the heme synthetic pathway, was found to be reduced; in others, a defect of coproporphyrinogen oxidase was implicated. The anemia in some children and adults has been partially responsive to administration of pyridoxine, a necessary cofactor for optimal activity of ALA-S.

In this article we describe a unique patient—a girl with severe, transfusion-dependent sideroblastic anemia present since early infancy. Examination of the erythroid heme biosynthetic pathway showed a single enzymatic defect, namely, diminished activity of ALA-S, which was partially augmented by pyridoxal-phosphate (PLP).

CASE REPORT

A 4½-yr-old black girl was first noted to be anemic at 3 mo of age when the hemoglobin was 9 g/dl. She was treated with oral and then parenteral iron but failed to demonstrate a rise in hemoglobin. On initial evaluation at our institution at 9 mo of age, the physical examination was normal except for pallor. The hemoglobin was 6.8 g/dl, hematocrit 21%, reticulocytes 0.5%, mean cell volume (MCV) 52 fl, mean cell hemoglobin (MCH) 13 pg. Red blood cell morphology (Fig. 1) was characterized by marked hypochromia, microcytosis, fragmented red blood cells, teardrop forms, basophilic stippling, and occasional nucleated red blood cells. Total and differential white blood cell counts were normal, and platelets were present on the blood smear in adequate numbers. Bone marrow examination revealed micronormoblastic erythroid hyperplasia. Stainable iron was abundant, and a few ring sideroblasts were noted. Serum iron was 231 µg/dl, total iron binding capacity 276 µg/dl, and hemoglobin electrophoresis on cellulose acetate at pH 8.6 was normal (98.7% hemoglobin-A, 1.0% hemoglobin-A₂, and 0.3% hemoglobin-F).

Following the administration of folic acid (1 mg/day) and oral pyridoxine (25 mg/day), the hemoglobin remained in the range of 6–7 g/dl. The patient remained well except for periodic episodes of decreased activity. At 2 yr of age, the hemoglobin was 5.1 g/dl and hematocrit 19%, and she required the first of many red blood cell transfusions. The hemoglobin and hematocrit remained in the range of 3–7 g/dl and 12%–20%, respectively. Reticulocytes were never greater than 4.8% and usually were less than 0.5%. At 3½-yr of age she was reevaluated. Frontal bossing and mild splenomegaly were noted. The general hematologic studies are indicated in Table 1. The peripheral blood smear (Fig. 1) was unchanged from prior examinations. The results of the following studies were normal or negative: serum copper, blood lead, serum bilirubin, Ham test, isopropanol and heat stability tests for unstable hemoglobin, and citrate agar gel hemoglobin electrophoresis at pH 6.3. No hemoglobin-H inclusions or Heinz bodies were observed after supravital staining of the peripheral blood with crystal violet or brilliant cresyl blue, and no inclusions were noted in the bone marrow after incubation with methyl violet. Measurement of globin chain synthesis rates revealed a slightly decreased α/β ratio of 0.83 (normal 0.90–1.10). Family history indicated no known anemia or
consanguinity. Both parents and a 13-mo-old female sibling had normal values for most routine hematologic studies as well as hemoglobin electrophoresis (Table I). The slightly decreased MCV in the father is thought to represent alpha-thalassemia trait, and the sister appears to have mild iron deficiency. A number of special investigations were performed at this time and are detailed below.

At age 4 yr, the patient failed to demonstrate a reticulocytosis or rise in hemoglobin and hematocrit following an empirical 2-wk trial of prednisone (2 mg/kg/day). At present, she receives folic acid (1 mg/day) and oral pyridoxine (50 mg/day) but continues to require red blood cell transfusions every 2–3 mo. She is developing normally and her general health remains good. The serum ferritin is presently 1300 ng/ml. Plans are underway to initiate subcutaneous dexferrioxamine chelation treatment in the near future.

Table 1. Hematologic Data in the Patient and Her Family

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Value</th>
<th>Patient*</th>
<th>Father</th>
<th>Mother</th>
<th>Sister</th>
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<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>19</td>
<td>44</td>
<td>36</td>
<td>34.38</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>5.9</td>
<td>14.5</td>
<td>12.9</td>
<td>11.0, 12.8</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>0.7</td>
<td>-</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>50</td>
<td>76.80</td>
<td>82</td>
<td>67.65</td>
<td></td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>15</td>
<td>27</td>
<td>30</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>RBC morphology</td>
<td>See text</td>
<td>Normal</td>
<td>Normal</td>
<td>Slightly hypochromic and microcytic</td>
<td></td>
</tr>
<tr>
<td>Leukocytes ($\times 10^9$/liter)</td>
<td>14.2</td>
<td>5.5</td>
<td>5.9</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>Platelets ($\times 10^9$/liter)</td>
<td>660</td>
<td>172</td>
<td>214</td>
<td>Adequate</td>
<td></td>
</tr>
<tr>
<td>Serum iron (µg/dl)</td>
<td>58–179</td>
<td>278</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum iron binding capacity (µg/dl)</td>
<td>249–409</td>
<td>289</td>
<td>403</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum ferritin (ng/ml)</td>
<td>10–273</td>
<td>407</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin electrophoresis †</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent A</td>
<td>&gt;95</td>
<td>97.9</td>
<td>97.0</td>
<td>96.5</td>
<td>97.0</td>
</tr>
<tr>
<td>Percent A₂</td>
<td>1.5–3.5</td>
<td>1.6</td>
<td>2.5</td>
<td>3.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Percent F</td>
<td>&lt;2.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*Studied at 3½ yr of age. 3½ mo after transfusion.
†On cellulose acetate, pH 8.6.
CONGENITAL SIDEROBLASTIC ANEMIA

MATERIALS AND METHODS

Bone Marrow Morphological and Cytogenetic Studies

Bone marrow aspirates and needle biopsy were obtained from the posterior iliac crest. Smears were stained with Wright-Giemsa and Prussian blue reagents for examination by light microscopy. Additional bone marrow was fixed in 2.5% buffered glutaraldehyde and processed for transmission electron microscopy by standard techniques. Bone marrow chromosomal analysis was performed using standard methods, including Giemsa banding.

Investigation of Heme Biosynthesis

The following enzymes were measured in peripheral blood erythrocytes: porphobilinogen synthase in the presence and absence of dithiotreitol,22 uroporphyrinogen I synthase,23 pyridoxine kinase,24 and aspartate aminotransferase (EGOT).25 Aspirates of heparinized marrow were assayed for ferrochelatase and for ALA-S according to previously described methods.5,26 Erythrocyte-free protoporphyrin and urinary levels of ALA, porphobilinogen, uroporphyrin, and coproporphyrin were measured by standard techniques.27,28

RESULTS

Bone Marrow Morphology

The bone marrow was hypercellular and showed erythroid hyperplasia (M:E ratio 1:2.5). There was markedly reduced hemoglobinization of most late basophilic and orthochromic normoblasts. Prussian blue stains revealed markedly increased reticuloendothelial iron for a child of this age. Many of the erythroblasts were ring sideroblasts (Fig. 2).

On electron microscopy, early normoblasts contained some dispersed ferritin particles and small quantities of intramitochondrial iron. Numerous polychromatophilic and orthochromic normoblasts contained large intramitochondrial deposits of osmophilic material, resembling iron micelles (Fig. 3). In many mitochondria, the iron deposits were noted between the cristae. These findings thus confirmed the observations by light microscopy.

Chromosomal Studies

Karyotypic analysis, including G-banding of peripheral blood lymphocytes as well as bone marrow cells, showed a normal 46 XX chromosomal complement. Eight percent of spreads were aneuploid but no consistent abnormalities were found.

Enzymes and Intermediates of the Heme Biosynthetic Pathway (Table 2)

The patient’s bone marrow contained markedly diminished ALA-S activity—only 6 and 7 pmole ALA/10⁶ erythroblasts/30 min—when compared

Fig. 2. Bone marrow smear (Prussian blue stain), showing ring sideroblasts (arrows).
with control values. The enzyme activity was significantly (fivefold) enhanced when PLP was added to the assay mixture, although it remained subnormal. All other heme synthesis enzymes and intermediates that were measured in bone marrow, peripheral blood, and urine were normal or increased (Table 2).

Results of Therapeutic Trials With Oral Pyridoxine and Parenteral Pyridoxal-Phosphate

For 6 mo the patient continually received oral pyridoxine, 50 mg twice daily, without an increase in reticulocytes, hematocrit, hemoglobin, or MCV, or

Table 2. Studies of Heme Biosynthetic Enzymes and Intermediates

<table>
<thead>
<tr>
<th></th>
<th>Patient's Data</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ-ALA synthase - PLP*</td>
<td>7.6 pmole ALA/10⁶ erythroblasts/30 min†</td>
<td>127 ± 39 (SD)‡</td>
</tr>
<tr>
<td>Δ-ALA synthase + PLP</td>
<td>35.25 pmole ALA/10⁶ erythroblasts/30 min†</td>
<td>0%-25% increase‡</td>
</tr>
<tr>
<td>Ferrochelatase</td>
<td>108 pmole heme/10⁶ erythroblasts/30 min</td>
<td>84-284‡</td>
</tr>
<tr>
<td>Red blood cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBG synthase - DTT</td>
<td>841 nmole PGB/ml RBC/30 min</td>
<td>363 ± 83 (SD)‡</td>
</tr>
<tr>
<td>PBG synthase + DTT</td>
<td>2.115 nmole PGB/ml RBC/30 min</td>
<td>932 ± 296 (SD)‡</td>
</tr>
<tr>
<td>Uroporphyrinogen I synthase</td>
<td>99 pmole URO/ml protein/hr</td>
<td>67.7 ± 7.4 (SD)‡</td>
</tr>
<tr>
<td>Protoporphyrin</td>
<td>27 μg/dl</td>
<td>15-60</td>
</tr>
<tr>
<td>Pyridoxine kinase</td>
<td>1.84 nmole/min/g Hb</td>
<td>1.38 ± 0.05 (SD)§</td>
</tr>
<tr>
<td>EGOT - PLP</td>
<td>27.6 IU/g Hb</td>
<td>3.02 ± 0.67 (SD)†</td>
</tr>
<tr>
<td>EGOT + PLP</td>
<td>47.5 IU/g Hb</td>
<td>5.04 ± 0.90 (SD)†</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ-Aminolaevulinic acid</td>
<td>4.9 mg/g creatinine</td>
<td>0-4/24 hr†</td>
</tr>
<tr>
<td>Porphobilinogen</td>
<td>2.6 mg/g creatinine</td>
<td>0-4/24 hr†</td>
</tr>
<tr>
<td>Uroporphyrin</td>
<td>7 μg/g creatinine</td>
<td>0-40/24 hr‡</td>
</tr>
<tr>
<td>Coproporphyrin</td>
<td>147 μg/g creatinine</td>
<td>50-300/24 hr‡</td>
</tr>
</tbody>
</table>

PLP, pyridoxal-phosphate; PGB, porphobilinogen; ALA, Δ-amino-laevulinic acid; DTT, dithiothreitol.
* - PLP, without added PLP (0.2 mM); + PLP with added PLP (0.2 mM).
† Values of 2 separate bone marrow aspirates.
‡ Values in adult healthy controls. In one normal child comparable values were obtained.
§ Normal values from data of Chern and Beutler.24
‖ Normal values from data of Beutler.29
decrease in transfusion requirements. A therapeutic trial with intramuscular pyridoxal-5'-phosphate (PLP) (kindly provided by Dr. John Hines, Cleveland Metropolitan General Hospital, and its use approved by the Human Research Review Committee of the University of Texas Health Science Center), 25 mg every 12 hr for 14 days, produced no change in hemoglobin, hematocrit, and reticulocyte count during treatment and for 4 wk thereafter.

**DISCUSSION**

Sideroblastic anemia is a disorder characterized by variable anemia, increased body iron stores, ineffective erythropoiesis, and the presence of ring sideroblasts in the bone marrow. Unlike the normal marrow sideroblast, the pathologic sideroblast in subjects with sideroblastic anemia contains prominent accumulations of iron within mitochondria. Sideroblastic anemia is usually an acquired defect occurring in middle-aged or older patients. It may occur with ethanol abuse, with intake of drugs such as isoniazid, and infrequently with inflammatory disorders and premalignant states. Patients in whom no such associated problem can be identified are designated as having idiopathic sideroblastic anemia.

On rare occasions, sideroblastic anemia appears to be congenital and/or familial. Nearly 100 such cases have been reported since the initial descriptions by Cooley and by Rundles and Falls. These patients nearly always have a hypocromic microcytic anemia, which is of variable severity and usually is detected during the first several decades of life. Nearly all anemic subjects have been males, and in several kindreds, an X-linked recessive inheritance pattern has been demonstrated. In two kindreds, the defect of sideroblastic anemia appeared to be linked either to that of glucose-6-phosphate dehydrogenase deficiency or the blood group antigen Xg. Light microscopic examination as well as ultrastructural study of marrow erythroblasts show abnormal iron accumulation primarily in late normoblasts (as in our case), in contrast to patients with acquired sideroblastic anemia in whom mitochondrial iron overload is more prominent in the earlier proliferating erythroid cells.

Only a small minority of patients with CSA have been females. They appear to fall into two groups, the larger of which includes subjects who are presumed or proven heterozygous carriers for a defective X-linked gene. Although most of these individuals have minimal or no anemia, abnormal red blood cell morphology, particularly a dimorphic peripheral blood picture, has usually been observed, and in several ring sideroblasts were seen in the bone marrow. A few female patients have been described in whom the precise inheritance pattern was not clear, and anemia in these was more severe. They may represent sporadic cases or homozygotes for an abnormal autosomal or X-linked gene. The patient reported here appears to belong to this category. In only one such previously described female patient was the anemia severe enough to require blood transfusion.

The underlying pathophysiology in sideroblastic anemia has been considered by most authors to be impairment of heme biosynthesis. The evidence supporting this concept includes the observation that most patients have hypocromic microcytic anemia with increased iron stores and minimal or no impairment in globin biosynthesis. White and Ali observed that heme enhanced globin synthesis in congenital and acquired sideroblastic anemia in contrast to controls. The abnormal accumulation of iron in the mitochondria (the site of the first and last steps of heme biosynthesis) and the occasional responsiveness of these disorders to pyridoxine (a cofactor necessary for ALA synthesis) also render support to this notion. Early attempts to define specific abnormalities of heme synthesis in patients with congenital and acquired sideroblastic anemia with indirect methods, such as quantitation of intermediates in the heme synthetic pathway and relative precursor incorporation rates into heme, suggested decreased activities of ALA synthase in some and coproporphyrinogen oxidase or ferrochelatase in others. More recently, by direct assay of the heme biosynthetic enzymes, a number of adult patients with acquired sideroblastic anemia have been shown to have diminished activity of bone marrow ALA-S. Studies in patients with CSA are more limited. Aoki and coworkers studied a single adult male patient with CSA and found marrow ALA-S to be markedly reduced. Konopka and Höfbrand have recently reported that four of their five patients with CSA, including one female with moderate anemia, had low bone marrow ALA-S activity, which in one case was partially restored by PLP. To our knowledge, the patient described in this report is the first child, and only the second female, with CSA to have decreased ALA-S activity in erythroblasts. The normal erythrocyte-free protoporphyrin and the normal marrow ferrochelatase activity by direct assay indicate that the last step in heme biosynthesis is not disturbed in this patient. The raised activities of the cytosol enzymes (porphobilinogen synthase, uroporphyrinogen I synthase, pyridoxine kinase, and EGOT) probably represent a relatively young population of circulating erythrocytes, since these enzymes are known to decrease in activity with red cell age. The markedly increased activities of pyridoxine kinase and EGOT may also be a consequence of the prior pyridoxine therapy.

Some patients with X-linked or with acquired sid-
Sideroblastic anemia show improvement after therapy with oral pyridoxine. Such responses have provided support for impaired ALA synthesis being important in the pathogenesis of these anemias and imply that diminished ALA-S activity can be in part enhanced by its cofactor PLP. Although our patient was not responsive to oral pyridoxine therapy, it is of interest that in vitro PLP significantly enhanced the ALA-S activity (Table 2). A similar finding was observed in one of Konopka and Hoffbrand’s patients with CSA. Several patients with acquired sideroblastic anemia have been described who failed to respond to oral pyridoxine but in whom an increase in reticulocytes and/or hemoglobin occurred after parenteral PLP therapy. Although it has been suggested that in such individuals the mechanism(s) for conversion of pyridoxine to PLP is defective due to diminished activity of pyridoxine kinase, evidence has also been produced that this enzyme, as well as the in vivo cellular content of PLP, is normal. At present, it appears that the clinical and in vitro effects of pyridoxine or PLP represent secondary effects on a defective ALA synthesis step that vary from case to case.

In view of current knowledge, it is concluded that the markedly reduced activity in ALA-S in our patient was at least in part, if not entirely, responsible for her refractory hypochromic anemia. There was no evidence for other causes of severe congenital hypochromic anemia. The slightly decreased \( \alpha/\beta \) globin chain synthetic ratio in the reticulocytes is consistent either with clinically insignificant alpha-thalassemia trait or with sideroblastic anemia itself, as White and coworkers have demonstrated slight diminution in alpha-chain synthesis in a number of patients with sideroblastic anemia. The thrombocytosis in this patient is most likely a secondary effect of a stimulated marrow state, and it is noteworthy that high platelet counts have been observed in many patients with acquired sideroblastic anemia. Since the data in the family members are inconclusive, an acquired nonfamilial defect cannot be fully excluded. However, it seems more likely that she is a homozygote for a faulty autosomal recessive gene, as acquired sideroblastic anemia in young children has not been well described. This patient also differs in many respects from a recently described child with acquired sideroblastic anemia who had an antibody directed against erythroid colony forming cells.

Whether the defects in heme biosynthesis, including the observation in this case, are a result rather than a cause of the iron-laden mitochondria remains unsettled. This question might be resolved when an opportunity arises to examine the enzymes in the heme synthetic pathway in an iron-depleted individual with the sideroblastic defect or following effective chelation therapy.

The prognosis for this patient remains guarded, for many severely affected, transfusion-dependent patients with the X-linked recessive form of CSA have eventually died of complications of iron overload. It is our hope that this can be prevented by iron chelation therapy.

ACKNOWLEDGMENT

We are grateful to Dr. M. Dean Jacoby for referral of this patient, to Dr. Michael Waterman and LaVerne McElroy who kindly performed the globin chain synthetic studies, and to Nan Collins and Sharon Johnson for technical assistance. We thank Dr. Jacqueline Coalson, Department of Pathology, University of Oklahoma College of Medicine, for the preparation and interpretation of the electron micrographs and Dawn Miller for secretarial assistance.
CONGENITAL SIDEROBLASTIC ANEMIA

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