5-Aminolevulinic acid dehydratase deficiency porphyria: a twenty-year clinical and biochemical follow-up

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5-Aminolevulinic acid dehydratase (ALAD) activity in two patients with compound heterozygous 5-aminolevulinic acid dehydratase deficiency porphyria was studied over the last 20 years. The patients’ enzyme activity was <10% from 1977 to 1997. An acute crisis in each patient was successfully treated by infusion of glucose and heme arginate. After this therapy both urinary 5-aminolevulinic acid (ALA) and total porphyrins were diminished to 65% in patient B. In patient H, ALA was decreased to 80%, and total porphyrins were reduced to 15% after treatment with heme arginate and glucose. The patients remained free of symptoms after this therapy. Family studies of patient B showed cross-reactive immunological material (CRIM), in which the maternal mutation is CRIM(1), whereas the paternal mutation is CRIM(2). Incubation of erythrocyte lysates with ALA decreased porphyrin formation, whereas incubation with porphobilinogen produced porphyrin concentrations within reference values in both patients, confirming that ALAD activity is rate-limiting in these cells.

5-Aminolevulinic acid dehydratase (ALAD)⁵ is the second enzyme in the heme biosynthetic pathway, which is cytosolic and nonlimiting in heme synthesis in healthy cells. The enzyme catalyzes the condensation of two molecules of 5-aminolevulinic acid (ALA) to form one molecule of the monopyrrole porphobilinogen (PBG). Activity of this enzyme is markedly inhibited by environmental toxins such as lead (1) or markedly decreased in an inherited enzyme deficiency. ALAD activity is present in great excess in the healthy liver, and a partial enzyme deficiency such as in heterozygous ALAD deficiency is not accompanied by any clinical consequence (2).

This study deals with the two currently surviving patients (patients H and B) with ALAD deficiency porphyria (ADP). ADP was first reported in these two young men, not related to each other, who have an intermittent severe acute hepatic porphyria syndrome and ALAD activity ~1% of controls (3). Since 15 years of age, both patients suffered from repeated abdominal-neurologic crises with cardiovascular symptoms, persistent paresis, and transient respiratory paralysis. Despite marked ALAD deficiency, there was no anemia in either patient. Family studies demonstrated that ALAD deficiency was inherited as an autosomal recessive trait (4). Cloning and expression of the defective genes of one patient demonstrated that the patient was heteroallelic for ALAD deficiency with two separate point mutations, one producing an inactive enzyme and the other producing an unstable enzyme (5). Another point mutation has been detected in the second patient. Because he has a wild-type residue at this site in the other allele, this patient is also compound heterozygous for ALAD deficiency, although the second mutation has not yet been defined (6).

Because there have been few studies that followed enzymatic and biochemical courses of acute hepatic porphyrias, we thought it important to report complete immunological and enzymatic data, as well as biochemical changes over a period of 20 years.

Materials and Methods

Determination of ALAD activity was performed with 100 μL of packed erythrocytes in 80 mmol/L sodium phos-
phate buffer, pH 6.4, containing 8 mmol/L ALA according to the European standard method. PBG formed in the assay was determined spectrophotometrically with Ehrlich’s reagent (7). ALA and PBG were determined spectrophotometrically after isolation by ion-exchange chromatography. Porphyrins were analyzed spectrophotometrically as methyl esters after separation by high-performance thin-layer chromatography (8). Total porphyrins were calculated from the sum of individual porphyrins. Urinary coproporphyrin isomers I-IV were quantitated by isocratic ion-pair HPLC (9). Zinc protoporphyrin and protoporphyrin in erythrocytes were analyzed using reversed-phase ion-pair HPLC (10), with simultaneous fluorometric detection of both substances. Porphobilinogen deaminase (PBGD) and uroporphyrinogen decarboxylase activities in erythrocytes were determined as described previously (11). Uroporphyrinogen-III synthase activity was measured using erythrocyte lysates by a coupled enzymatic assay (12). Coproporphyrinogen oxidase, protoporphyrinogen oxidase, and ferrochelatase activities were assayed using lymphocytes, according to the methods reported previously (13–15). Incubation of erythrocyte lysates with exogenous ALA and PBG was carried out in the dark at 37 °C for 2 h, as described previously (16). ALAD and PBGD activities in lymphocytes were determined, and lymphocytes of patient B and his family members were isolated and transformed by infection with Epstein-Barr virus according to Sassa et al. (17). ALAD concentrations in erythrocytes were determined by rocket immunoelectrophoresis with an antibody against purified human ALAD. ALAD from erythrocytes from subjects of family B was partially purified using anion-exchange chromatography. Three microliters of enzyme fractions was applied to each well. A calibration curve of homogeneously purified ALAD from nondiseased erythrocytes ranged from 10 to 50 mg/L (17). Total soluble protein was measured according to the method of Bradford (18). These investigations were in accordance with the current revision of the Helsinki Declaration of 1975.

**Results**

**CLINICAL AND LABORATORY FINDINGS**

ALAD activity and urinary excretion of heme precursors in both patients with ADP were determined over a period of 20 years. The erythrocyte ALAD activity of both patients was <10% of that in controls [reference range, 283 ± 41 nkat/L, (x ± SD, n = 50), CV = 5.2%] during a period of 20 years.

In patient B, erythrocyte PBGD, uroporphyrinogen-III synthase, and uroporphyrinogen decarboxylase as well as lymphocyte coproporphyrinogen oxidase, protoporphyrinogen oxidase, and ferrochelatase activities were additionally examined; all were within reference values.

Urinary ALA excretion was 44-fold and 50-fold in 1979 and 1983, respectively, in patient H. Urinary ALA excretion was increased 44-fold in 1979 and 80-fold in 1985 in patient B (Fig. 1). Urinary PBG excretion was increased fourfold in 1983 in patient H. It increased fourfold in 1979 and fivefold in 1986 in patient B (Fig. 2). Urinary total porphyrins of both patients (Fig. 3) followed the course of ALA and PBG, with 90% coproporphyrin and 5% pentacarboxyporphyrin. The ratio of the urinary coproporphyrin isomers I:II:III:IV was 3.1% ± 0.5%; 4.2% ± 1.7%; 84.0% ± 1.7%; 8.7% ± 2.5% (x ± SD). Fecal porphyrins of...
both patients were within reference values (data not shown).

In 1983 patient H suffered from an acute porphyric crisis that was associated with an excessive intake of alcohol (300 g in one day). Urinary excretion of ALA, PBG, and total porphyrins were 2.6 mmol, and 29 and 10.7 μmol per day, respectively.

After intensive treatment with glucose, heme arginate, diet, and physiotherapy, the clinical status of both patients improved, despite persistently high levels of ALA (between 0.45 and 2.1 mmol), PBG (between 4 and 17 μmol), and total porphyrin (between 2 and 9 μmol) excretion. In 1994, ALA excretion of ~1.6 mmol was found; however, it was not associated with an acute attack.

In the first half of 1997, patient H was in good health.

TREATMENT OF ACUTE CRISIS
In the summer of 1997, patient H worked strenuously for ~9 h without eating. The next morning, he suffered from abdominal colic, weakness in the legs and arms, paresthesia and partial paresis, hypertension, and tachycardia, and he lost appetite. His urinary ALA was 2.2 mmol/24 h. Heme arginate infusion was initiated immediately, which produced good clinical and biochemical responses, and the symptoms disappeared after 3 days. After he was treated with heme arginate, his coproporphyrin excretion was found increased, probably because of improved metabolism of ALA into porphyrins. His ALA excretion increased after the cessation of heme arginate treatment but remained at lower levels than during the acute period (Fig. 4).

The response to heme arginate in patient B is shown in Fig. 5. This patient was more resistant to heme arginate treatment than the first patient. He had no response to the first heme arginate treatment. Probably his condition was compromised because of vomiting and refusal of food at that time. A much better response, however, was achieved (days 15–20) when heme arginate treatment was combined with glucose infusion. In the spring of 1997, he suffered again from a severe acute abdominal neurologic manifestation and responded well to heme arginate.

PORPHYRINS IN ERYTHROCYTES
In the erythrocytes of patients H and B, protoporphyrin was increased 12- and 3-fold, respectively, compared with healthy controls. Zinc protoporphyrin was enhanced 15- and 9-fold in patients H and B, respectively, compared with healthy controls. In the erythrocytes of the parents of patient H, these porphyrins were within reference values (Table 1).

IN VITRO STUDIES
Porphyrin formation from ALA and PBG was evaluated in erythrocyte lysates in both patients (Table 2). With $10^{-5}$, $10^{-4}$, and $10^{-3}$ mol/L ALA, the amount of total porphyrins formed by erythrocyte lysates of patients B and H was 69%, 41%, and 19% compared with the amount formed by healthy controls. With $0.5 \times 10^{-3}$ mol/L PBG, total porphyrins formed were not different from healthy controls in both patients.

In Epstein-Barr virus-transformed lymphoblastoid cells, ALAD and PBGD activities were examined in patient B and in his family members. ALAD activity was 2.5%, 33%, 46%, and 30% from the patient, the mother, the sister, and the brother, respectively (Table 3). PBGD activity was within the reference range for all these subjects (Table 3).

![Fig. 4. Time course of ALA and total porphyrins of patient H under therapeutic treatment of heme arginate and glucose.](image)

![Fig. 5. Time course of ALA and total porphyrins of patient B under therapeutic treatment of heme arginate and glucose.](image)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Zinc protoporphyrin, nmol/L</th>
<th>Protoporphyrin, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father of patient H</td>
<td>370</td>
<td>70</td>
</tr>
<tr>
<td>Mother of patient H</td>
<td>440</td>
<td>50</td>
</tr>
<tr>
<td>Patient H</td>
<td>4300</td>
<td>830</td>
</tr>
<tr>
<td>Patient B</td>
<td>2400</td>
<td>210</td>
</tr>
<tr>
<td>Reference range</td>
<td>280 ± 110</td>
<td>70 ± 30</td>
</tr>
</tbody>
</table>
**Table 2. Total porphyrins in nmol · g⁻¹ total soluble protein · h⁻¹ from lysates of erythrocytes from patients B and H after incubation with various concentrations of ALA and PBG.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>ALA 10⁻⁶ mol/L</th>
<th>ALA 10⁻⁴ mol/L</th>
<th>ALA 10⁻² mol/L</th>
<th>PBG 0.5 x 10⁻³ mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient B</td>
<td>3.8</td>
<td>8.6</td>
<td>12.8</td>
<td>68.8</td>
</tr>
<tr>
<td>Patient H</td>
<td>3.1</td>
<td>7.4</td>
<td>13.2</td>
<td>62.1</td>
</tr>
<tr>
<td>Reference range (n = 4)</td>
<td>5.0</td>
<td>19.6</td>
<td>66.7</td>
<td>69.3</td>
</tr>
</tbody>
</table>

**Table 3. ALAD and PBGD activities in Epstein-Barr virus-transformed lymphocytes from family B.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>ALAD, fkat/g</th>
<th>PBGD, pkat/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient B</td>
<td>2.0</td>
<td>3.7</td>
</tr>
<tr>
<td>Mother</td>
<td>26</td>
<td>3.4</td>
</tr>
<tr>
<td>Sister</td>
<td>36</td>
<td>3.5</td>
</tr>
<tr>
<td>Brother</td>
<td>24</td>
<td>3.3</td>
</tr>
<tr>
<td>Reference range (x ± SD, n = 5)</td>
<td>79 ± 19</td>
<td>4.4 ± 1</td>
</tr>
</tbody>
</table>

**IMMUNOLOGIC STUDIES**

The ALAD concentrations of patient B, his father, and his mother were 27%, 35%, and 77%, respectively, when compared with the concentrations in healthy controls. The erythrocyte concentrations of ALAD were 62% and 45% in his sister and brother compared with reference values, respectively. Erythrocyte ALAD activities were 1%, 43%, and 25% in the patient, his father, and his mother, respectively. The sister and the brother showed 39% and 37% erythrocyte ALAD activity. Thus in this patient, his mother, his sister, and his brother, ALAD activity was lower than its concentration, indicating that the ALAD mutation in these subjects is cross-reactive immunological material-positive [CRIM(+)]. In contrast, the patient’s father had CRIM(−) mutation.

**Discussion**

**ALAD ACTIVITY**

ADP was characterized by deficiency of erythrocyte ALAD activity in two patients. They are both alive and generally in good health at the age of 40, indicating that markedly diminished ALAD activity is still sufficient for heme synthesis. An acute attack was associated with excessive alcohol ingestion in one of our patients. Alcohol was shown experimentally to increase 5-aminolevulinic acid synthase activity in the liver (19).

**REGULATORY ASPECTS**

Our results demonstrated that acute crises in both patients can be managed by therapy with heme arginate in combination with glucose.

Protoporphyrin and zinc protoporphyrin concentrations in the erythrocytes of these patients were markedly increased. Despite the decreased ALAD activity in these patients, an overproduction of PBG and porphyrins occurs. The excessive urinary coproporphyrin excretion cannot be caused by an enzymatic deficiency, because coproporphyrinogen oxidase activity was within reference values. Oral ALA loading tests in healthy persons show an excretion of ~3.3 μmol/L urinary total porphyrins with a portion of 62% coproporphyrin during the first 24 h (20). The production of coproporphyrin from ALA in patients with ADP may be the consequence of an alteration in the regulation of heme biosynthesis, which is mimicked by the dominance of urinary coproporphyrin in healthy persons after loading with ALA.

**PORPHYRIN FORMATION FROM ALA AND PBG**

Urinary ALA excretion was increased ~50-fold, whereas urinary PBG excretion was ~5-fold in both patients. Although this finding is compatible with the relative rate-limiting nature of ALAD deficiency in these patients (21), it is necessary to show that in fact ALAD in cells functions as a rate-limiting enzyme in porphyrin formation. Our findings in Table 2 demonstrate that porphyrin formation from ALA is diminished, whereas its formation from PBG is within reference values. This clearly indicates that ALAD functions as a rate-limiting enzyme for porphyrin synthesis. This finding is consistent with our finding on deficient ALAD activity, decreased ALAD protein, and the aberrant phenotype of the mutant ALAD expressed by the patient’s cDNA (5, 6).

**IMMUNOLOGICAL FINDINGS**

Our results indicate that ADP in patient B is caused by two separate point mutations (5). These data agree with the observation of Table 3, where ALAD activity in lymphocytes of the compound heterozygous patient B (5) is <10% and is 32% in his heterozygous mother, sister, and brother, all of whom have the maternal mutation (5). The latter molecular genetic analysis is also confirmed by the data from erythrocytes, which show that all these persons have a CRIM(+) mutation. The father’s mutation is CRIM(−). The different CRIM types of the patient’s mother and father show that one must deal with two different mutations in the patient and thus with compound heterozygosity. The maternal CRIM(+) and the paternal CRIM(−) mutations produce a CRIM(+) mutation in the compound heterozygous patient.

**ADP PATIENTS**

ADP is an extremely rare disease. Only four patients have been reported thus far, which include the two young men from Germany (3), one child from Sweden (22), and one elderly patient from Belgium (23). The Swedish child was
also a compound heterozygous subject for ALAD deficiency (24). The Swedish child underwent liver transplantation (25); however, he died 2 years and 9 months after the transplantation at the age of 9 years (S. Thunell, personal communication). The Belgian patient also died at the age of 63, 2 years after the onset of the disease. Thus the two subjects studied in this report are the only ones who are alive.

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References


