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Survival of two patients with severe δ-aminolaevulinic acid dehydratase deficiency porphyria

U. Gross[1], S. Sassa[2], T. Arndt[3] and M. O. Doss[1]*
[1] *Division of Clinical Biochemistry, Philipps University Hospital, Marburg, Germany;* [2] *Rockefeller University Hospital, New York, USA;* [3] *Bioscientia, Institute for Laboratory Investigations, Mainz, Germany*

* *Correspondence: Division of Clinical Biochemistry, Philipps University Hospital, Deutschhausstr. 17½, 35037 Marburg, Germany. E-mail: Ulrich.Gross@aranea.de*

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Summary: The course of δ-aminolaevulinic acid dehydratase activity was studied over the 23 years in erythrocytes of two male patients. The enzyme activity was originally 1–2%, which then increased to ~8% of normal levels several years after clinical manifestation of the acute hepatic porphyria syndrome. Urinary excretions of δ-aminolaevulinic acid and coproporphyrin III were excessively increased in the two patients with compound-heterozygous δ-aminolaevulinic acid dehydratase deficiency porphyria.

Two male patients, not related to each other, suffered from repeated abdominal-neurological crises with cardiovascular symptoms, persistent paresis and transient respiratory paralysis from the age of 15 years. A severe acute hepatic prophyria syndrome was diagnosed on the basis of excessive excretion of the porphyrin precursor δ-aminolaevulinic acid (ALA) and porphyrins in urine. A residual activity of δ-aminolaevulinic acid dehydratase (ALAD; synonym porphobilinogen synthase; EC 4.2.1.24), was found to be the underlying cause and was first described in these two patients (Doss et al 1979). The disease was termed ALAD deficiency porphyria (ADP; McKusick 125270) or Doss porphyria.

A similar pattern with an excessive increase of ALA and porphyrin excretion with coproporphyrin III dominance was observed after oral ALA loading. Thus, ALA ingestion can be used as an *in vivo* model to study porphyrin metabolism after expansion of the ALA pool to be comparable to that of ADP (Jacob et al 1999).

ALAD is modulated by toxic and inherited conditions. Its activity is present in great excess in bone marrow and liver. Thus, a partial deficiency in heterozygotes is not accompanied by any clinical consequence (Bird et al 1979; Doss et al 1979).

Acute crises in both patients were successfully treated by high doses of glucose and intravenous haem arginate infusions, resulting in a considerable decline of urinary porphyrin precursors and porphyrins and improvement of the clinical syndrome (Groß et al 1998).

Family studies over three generations have shown that ADP is inherited as an autosomal recessive trait (Doss et al 1979, 1986). Unresponsive ALAD activity below 10% of controls indicated a homozygous variant of an acute hepatic porphyria. Immunoreactive ALAD concentration was lowered to 20% and 33% of the control level in healthy persons. Therefore, the molecular basis that accounts for the enzyme deficiency is a structurally modified enzyme (de Verneuil et al 1985). Molecular analysis revealed that both patients were compound-heterozygous with different mutations on each ALAD allele inherited from the mother and father (Ishida et al 1992; Sassa 1998). The molecular lesions of the two surviving patients reported here are different from each other and from those of the two other patients who died (Sassa 1998; Akagi et al 2000).

METHODS

ALAD activity was measured in 100 μl of packed erythrocytes, which were haemolysed in 1.4 ml water. Sodium phosphate buffer (1 ml of 100 mmol/L, pH 6.4) containing 10 mmol/L ALA was added. This mixture was incubated for 60 min at 37°C. The reaction was stopped by the addition of 20 mmol/L mercury(II) chloride in 10% (w/v) trichloroacetic acid. A centrifugation step followed for 5 min at $2000g_{max}$. The supernatant was mixed 1 : 2 with modified Ehrlich's reagent and analysed after 15 min spectrophotometrically at 553 nm against a blank. The reactivation rates were determined by addition of 0.1 mmol/L zinc chloride and 0.1 mmol/L dithiothreitol. ALA and PBG were determined spectrophotometrically after isolation by ion exchange chromatography. Porphyrins were analysed spectrophotometrically as methyl esters after separation by high-performance thin-layer chromatography (Doss et al 1979).

RESULTS

ALAD activity in two patients B. and H. with ADP was investigated over 23 years. For patient H., it was decreased to 1–2% of controls from 1977 to 1980 and in 1983. In 1982 and since 1993, ALAD activity amounted to ~8% in patient H. Patient B. had an ALAD activity below 2% of healthy controls from 1978 to 1983, which increased continuously from 1983 to 1987 to 8%. From 1987, ALAD activity in erythrocytes from patient B. was 8% (see Figure 1).

Table 1 gives the urinary excretion of haem precursors from both patients in 1999 ALA excretion in patient H. was enhanced 12-fold. In patient B. it was increased 6-fold compared to the upper level of the normal range. Urinary PBG excretion was within the normal range in patient H. In patient B. it rose to 1.6-fold above the upper limit of the normal range. Urinary total porphyrins were enhanced 24-fold in patient H. and 12-fold in patient B., with 90% coproporphyrin and dominance of

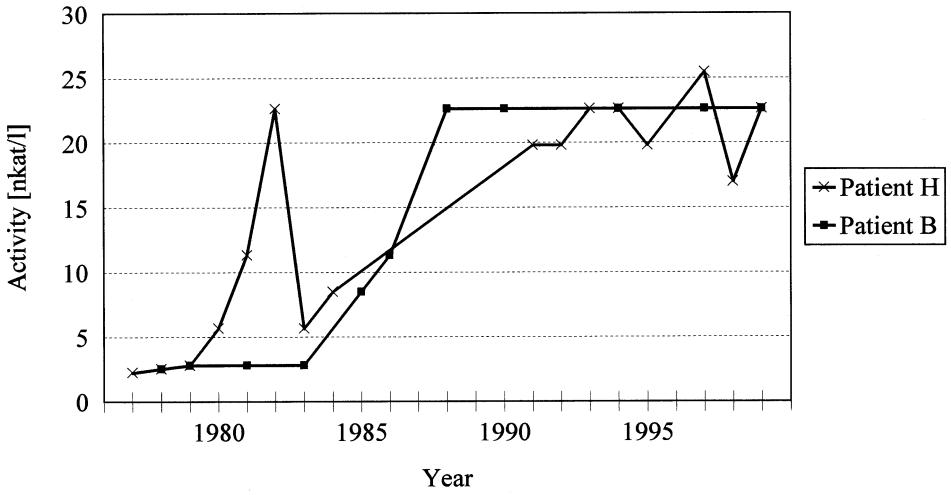

Figure 1 Course of ALAD activity in erythrocytes of patients B. and H. (normal 283±41 nkat/L; values are mean±SD, n=50)

Table 1 Urinary excretion of δ-aminolaevulinc acid, porphobilinogen and total porphyrins of the two patients with ADP in the year 1999

Excretion	*Patient H.*	*Patient B.*	*Normal*
δ-Aminolaevulinic acid (μmol/24 h)	601	305	<49
Porphobilinogen (μmol/24 h)	4	13	<8
Total porphyrins (nmol/24 h)	3894	1908	<165

coproporphyrin isomer III (95–98%) as well as 5% pentacarboxyporphyrin. Zinc protoporphyrin was 3-fold increased above the normal value (<64 nmol/dl) in erythrocytes of both patients.

DISCUSSION

The diagnosis of ADP in these patients is based on almost complete lack of ALAD activity in their erythrocytes and non-erythroid cells. This enzymatic deficiency resulted in greatly increased excretion of urinary ALA and porphyrins, with dominance of coproporphyrin III. This metabolite constellation, the excessive excretion of urinary ALA and coproporphyrin III as well as the zinc protoporphyrinaemia, imitates acute lead poisoning, which is considered an acute toxic or toxogenetic hepatoerythropoietic porphyria (Doss et al 1984). In contrast to acute lead poisoning, however, decreased ALAD activity in ADP could not be reactivated by zinc or thiols (Doss et al 1979).

The increase of zinc protoporphyrin is a characteristic finding in homozygous or compound-heterozygous porphyrias. This applies not only to the homozygous acute hepatic porphyrias but also to congenital erythropoietic porphyria and hepatoerythropoietic porphyria.

During the course of 23 years, ALAD activity in the erythrocytes in both patients H. and B. rose from 1–2% to ~8% of ALAD activity in healthy volunteers (100%). There may be two reasons for the apparent increase in ALAD activity in patients. First, it might be within the range of variation of assay results that is inherent to any assay. Second, the observed increased in ALAD activity may reflect an alteration in regulatory mechanism for haem biosynthesis over the course of 23 years. With respect to the first possibility, our ALAD assay shows 3.4% and 14.5% variation for intra- and inter-assay variance, respectively. On this basis, ALAD activity of 5.7 nkat/L and 22 nkat/L should correspond to a range of variation of ALAD activity between 4.9 and 6.5 nkat/L, and 18.8 and 25.2 nkat/L, respectively. The increases in ALAD activity of patients H. and B. were, however, distinctly greater than the ranges expected from analytical variation. While it is unclear whether the increase in ALAD activity in these patients was due to an increased transcription, a common adaption to increased needs of different gene products (Knippers et al 1990), or to a posttranscriptional event, including a direct effect on enzyme activity, it appears that the increased ALAD activity has contributed to a long-term survival of these patients, who are considered to suffer from the most debilitating acute hepatic porphyria. Since ALAD activity in normal individuals is about 100-fold higher than the activity of ALA synthase in the liver, the rate-limiting enzyme in haem biosynthesis in the liver, an increase in ALAD activity by a few per cent may have been sufficient to bring about remission in these patients. Brownlie and colleagues (1998) also observed a developmental alleviation of the genetic defect in a zebrafish model of congenital sideroblastic anaemia.

For the treatment of acute crises, glucose infusion and haem therapy were effective in some but not in other cases of ADP patients. Avoidance of drugs that are harmful in other acute porphyrias should be recommended (Sassa and Kappas, 2000).

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