Erythrocyte Uroporphyrinogen I Synthase Activity as an Indicator of Acute Porphyria

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ABSTRACT

The pre-clinical diagnosis of acute intermittent porphyria (AIP) is important because acute attacks can be brought about by drugs, liver toxins, hormonal changes and diet. There also may be no obvious precipitating agent. The discovery that the activity of uroporphyrinogen I synthase (URO-S) activity in the red blood cells of patients with AIP is half that found in normal persons is of great value in diagnosing this disorder and also appears useful in detecting patients with the latent disease who have normal urinary delta-aminolevulinic acid and porphobilinogen excretion. It also appears to distinguish other types of porphyria from acute intermittent porphyria. It must also be recognized that some red blood cells URO-S determinations will yield indeterminate results; therefore, repeat assays, including examination of kinship, will improve discrimination and confidence in the final diagnosis.

Introduction

Acute intermittent porphyria (AIP) is frequently familial and is inherited as an autosomal dominant trait with variable penetrance, therefore the variability of symptoms. The disease is characterized by three identified enzyme abnormalities: increased activity of delta-aminolevulinate synthase (EC 2.3.1.37), decreased activity of steroid 4-5α-reductase, and decreased activity of uroporphyrinogen I synthase (EC 4.3.1.8) (table I). There is evidence of the heterogeneity of the genetic defect as uroporphyrinogen I synthase (URO-S) forms four intermediates during synthesis of uroporphyrinogen I. Acute intermittent porphyria is the most common form of porphyria, with an incidence of one in 100,000, occurring more often in women than in men. The age of onset is usually after puberty, suggesting a steroid-linked or hormonal initiated pathobiological process. During specific phases of the menstrual cycle, acute attacks may be exacerbated, again pointing to possible endocrine mediating factors. In patients with latent AIP, attacks of this potentially fatal disease may be precipitated by drugs such as barbiturates, hydantoins, and estrogens as well as other factors such as liver toxins, hormonal changes, and diet. There also may be no obvious precipitating agent. The diagnostic assay and biochemical usefulness of URO-S as
TABLE I

Acute Intermittent Porphyria

Characterized by:

1. Erythrocyte uroporphyrinogen I synthase +
2. Delta-aminolevulinate synthase +
3. Steroid Δ 4-5α-reductase +

an indicator of AIP are discussed in the following sections along with proposed guidelines for using the assay for optimally evaluating individuals and kindred suspected of carrying the AIP trait.

Assay of Erythrocyte Uroporphyrinogen I Synthase Activity

Patients with AIP (overt or latent) may have decreased uroporphyrinogen I synthase (URO-S) activity, which accounts for the several laboratory assays that have been developed to detect this biochemical defect.5,7

The designation of the disease as acute intermittent porphyria is not an entirely appropriate nomenclature because the porphyrins are not involved but rather the porphyrin precursors, amino-levulinic acid (ALA) and porphobilinogen (PBG). The enzymatic lesion is a deficiency of URO-S in red cells and other tissues, and this synthase partially blocks the conversion of porphobilinogen into the porphyrinogens (figure 1).4,6 The deficiency of uroporphyrinogen I synthase leads to an increase in urinary PBG excretion, which is often coupled with an increased ALA excretion. The latter is the result of stimulated activity of ALA synthase secondary to diminished feedback inhibition by heme.9 During the attack, increased amounts of PBG and ALA are excreted in the urine. During the latent periods, the urinary PBG and ALA levels are still elevated, although they are much lower than acute attack concentrations and may even be normal.19,21 Since carriers of AIP have normal ALA and PBG excretion, the most appropriate test appears to be the measurement of erythrocyte uroporphyrinogen I synthase activity. The latter is reduced in latent and as well manifest forms of AIP5 thus providing enhanced specificity. Other acute forms of porphyria may have an identical clinical and chemical picture during the acute phase, but erythrocyte uroporphyrinogen I synthase activity will be normal in these porphyrin disorders. An approximately 50 percent reduction in activity of URO-S has been reported to occur in erythrocytes, leukocytes, cultured skin fibroblasts and amniotic cells. Thus, the URO-S assay also enables the intrauterine diagnosis of AIP.

Principle of Method for URO-S

Two features of this enzymatic reaction8,12 have allowed for easy adaptability of the assay to the clinical laboratory. First, the enzyme is cytoplasmic and, therefore, is retained within mature erythrocytes, which provide an ideal specimen for assay. Second, the substrate for the reaction is non-fluorescent, while the resulting product of the enzymatic reaction (uroporphyrin) is highly fluorescent. This permits its measurement in picomolar quantities. The URO-S enzyme catalyzes the formation of uroporphyrinogen from the monopyrrole precursor substrate, porphobilinogen. Under the assay conditions, the reaction forms uroporphyrinogen, which is rapidly oxidized to uroporphyrin during the acid deproteinization of the reaction mixture. Uroporphyrin fluorescence at wavelengths greater than 600 nm is then measured directly after excitation at 405 nm. The rate of uroporphyrin production is calculated and expressed in enzyme units of activity per gram of hemoglobin. The reference range in a healthy population is 1.27 to 2.01 μmol per g hemoglobin.11
Since erythrocyte URO-S activity varies with cell age and differentiation, a shift in the erythrocyte population toward younger cells (also observed in the newborn) may lead to an enzyme activity that does not accurately represent AIP. Usually an elevated reticulocyte percentage would indicate that such a condition could be present.5

Clinical Biochemistry of AIP

A partial block in hepatic heme biosynthesis at the enzymatic step of URO-S which catalyzes the conversion of PBG to uroporphyrinogen I, coupled with increased activity of ALA synthase, explains the long known increased urinary excretion of the porphyrin precursors PBG and ALA in AIP (figure 1).14 Although decreased URO-S activity has been demonstrated, the precise molecular basis for this deficiency has not been established. Nevertheless, in the absence of increased urinary excretion of PBG, the assay of URO-S activity in erythrocyte lysates has provided an improved means for detecting the genetic defect.13

There are, however, limitations of the erythrocyte URO-S assay. In some cases, enzyme activity in patients with AIP is just below the range of normal controls, and other patients with definitive AIP may have URO-S values within the reference range of normal controls.22 Others have reported that if nonaffected persons within families are used as controls, affected persons are more readily separable.10 Doss6 reported that erythrocyte URO-S increased to normal levels in a patient during the recovery phase of an acute attack, suggesting that URO-S may correlate in some manner with the clinical course and perhaps urinary findings.

Additional factors that influence erythrocyte URO-S activity are erythrocyte age and specimen storage. The URO-S activity has been found to decrease as erythrocytes age.1 It also appears that storage of erythrocytes enhances variability of measured enzyme activity. If blood is stored at 4°C, there is an inconsistent loss of activity, as much as 10 percent by day 3 and as much as 20 percent by day 5; at room temperature the losses can be 25 to 35
percent. Therefore, heparinized blood samples should be centrifuged, and, after removal of the plasma and buffy coat, the red cells washed thrice in cold isotonic saline. After packing by centrifugation and freezing in dry ice-acetone, the samples should be stored at –20°C.

Conclusion

On the basis of clinical and biochemical findings, the following guidelines are suggested in the evaluation of patients and kindred for AIP:

Patients who are ill and suspected of having this disorder because of abdominal pain, neuropathy, or other symptoms or signs, should have their urine assayed for PBG and ALA. If qualitative and quantitative studies for PBG and ALA are normal, the patients symptoms are probably not due to AIP. If the urine PBG or ALA are increased, the differential diagnosis includes AIP, variegate porphyria, hereditary coproporphyria, and lead poisoning.

A single determination of erythrocyte URO-S activity that is clearly normal is usually sufficient to rule out the diagnosis of AIP. A repeat URO-S determination should be performed when clinical suspicion is high or if urine results are abnormal and characteristic of AIP. A reticulocyte count should be obtained on the blood sample and the URO-S assay interpreted in light of the count. A reticulocyte count of five percent or more may lead to falsely elevated URO-S activity.

If urine results are characteristic and repeat erythrocyte URO-S values are low, the diagnosis of AIP is clearly suggested and the patient may be considered a carrier of the AIP trait.

Finally, it must be recognized that some erythrocyte URO-S determinations will yield indeterminate results, even when the samples are meticulously handled. Repeat assays and examination of the kinship, will improve discrimination and confidence in the final diagnosis.

References


