Erythrocyte Porphobilinogen Deaminase Activity in Porphyria Cutanea Tarda

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Porphyria cutanea tarda (PCT) results from a metabolic block in hemep synthesis at the level of uroporphyrinogen decarboxylase. We measured the activity of one of the enzymes preceding it in the heme biosynthetic pathway, porphobilinogen deaminase (PBGD; EC 4.3.1.8), in erythrocytes of 47 patients with symptomatic or asymptomatic familial or sporadic PCT. PBGD activity was significantly increased in all four PCT groups, compared with controls. To study the mechanism of this increased PBGD activity, we determined, using polyclonal antibodies, the amount of immuno-detectable PBGD per 100 units of PBGD activity (Ig PBGD/100 U) and the total amount of immuno-detectable PBGD (Ig PBGD) in erythrocytes from all 47 patients and from controls. In both familial and sporadic PCT, Ig PBGD/100 U was decreased compared with that in controls (P < 0.05). Especially in asymptomatic patients of the familial PCT group there was an inverse correlation between increasing PBGD activity and Ig PBGD/100 U (r = 0.90). In familial PCT, and to a minor degree in sporadic PCT, an increase in PBGD activity was accompanied by an increased Ig PBGD, compared with controls (familial PCT: P < 0.001, sporadic PCT: P < 0.05). In familial and sporadic PCT an increase in erythrocyte PBGD activity can, at least partly, be explained by a diminished degradation of PBGD. In familial PCT, in the symptomatic group more than in the asymptomatic group, and to a minor degree in sporadic PCT, there is in addition an increase in the absolute amount of PBGD.

Porphyria cutanea tarda (PCT) is a disorder of porphyrin metabolism that usually presents in adult life with photosensitivity.2 The disease is characterized biochemically by the excretion of large amounts of uroporphyrin and heptacarboxylporphyrin in the urine and by a decreased activity of the enzyme catalyzing the conversion of uroporphyrinogen (URO) to coproporphyrinogen, uroporphyrinogen decarboxylase (UROD, EC 4.1.1.37), in the liver (1).

Sporadic and familial forms of PCT have been described. In the sporadic form, UROD activity is within normal limits in extrhepatic tissue; however, a 50% reduction of UROD activity is found in the liver in the symptomatic phase (2, 3). In familial PCT, liver and extrhepatic UROD activities are about 50% of normal (4, 5). The enzymatic defect is inherited as an autosomal dominant trait. Clinical disease is produced when hepatic siderosis and (or) another disease-precipitating factor such as high alcohol intake or estrogen use is present (1, 6).

Normally, the sequence of enzymatic reactions in the heme biosynthetic pathway (Figure 1) proceeds with little or no accumulation of enzyme substrates. However, reduced activity at any enzyme step can lead to accumulation of that enzyme's substrate, because decreased production and concentration of the end-product, heme, will lead to induction of the first enzyme, delta-aminolevulinic acid synthase (ALAS; EC 2.3.1.37) and increased production of porphyrin precursors (6).

Two enzymes in the heme pathway, ALAS and porphobilinogen deaminase (PBGD; EC 4.3.1.8), have low activities as compared with the remaining enzymes (Figure 1) (6). In all porphyrias, including PCT, the rate-limiting enzyme ALAS is increased (6, 7). In patients with forms of porphyria associated with an acute attack (acute intermittent porphyria, coproporphyria, and variegate porphyria) induction of ALAS is associated with increased excretion of aminolevulinic acid and porphobilinogen (PBG); i.e., PBGD forms a partial metabolic block (6). In PCT, however, aminolevulinic acid and PBG concentrations in plasma are slightly increased (6, 8) and PCT is not associated with acute attacks of neurological dysfunction.

Some authors have reported an increased PBGD activity in erythrocytes (9-12) and livers (8, 12) of patients with PCT. However, in most of these studies they did not discriminate between patients with familial or sporadic PCT or between symptomatic and asymptomatic porphyria.

For this reason we assessed the activity of PBGD in sporadic and familial PCT, both in the symptomatic and asymptomatic phase. When we found an increased erythrocyte PBGD activity in both familial and sporadic PCT, we

![Fig. 1. The heme biosynthetic pathway, with special reference to porphyria cutanea tarda](image-url)

C7, C5, C5: hepta-, hexa-, and pentacarboxylporphyrinogen; Corpo, coproporphyrinogen; other abbreviations spelled out in text footnote.2

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2 Nonstandard abbreviations: PBG, porphobilinogen; PCT, porphyria cutanea tarda; URO, uroporphyrinogen; UROD, uroporphyrinogen decarboxylase; ALAS, aminolevulinic acid synthase; PBGD, porphobilinogen deaminase; FePy, FePy, familial PCT patients with and without dermatological symptoms after phlebotomy; SPaym, SPaym, sporadic PCT patients with and without dermatological symptoms after phlebotomy; and Ig PBGD, immuno-detectable PBGD.

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extended the study to examine the mechanism of this increase in enzyme activity.

In erythrocytes, PBGD is still active even after long storage at −20 °C. Under normal conditions, during the lifetime of an erythrocyte a part of this PBGD is degraded and no longer is catalytically active, although it still is immuno-detectable. In this study this has been called "Ig PBGD/100 U," which is the amount of immuno-detectable PBGD per 100 units of PBGD activity; in other publications (13, 14) this has been called "cross-reacting immune material." Conditions that result in a relatively diminished PBGD degradation result in higher concentrations of catalytically active PBGD, and therefore a decrease in Ig PBGD/100 U in erythrocytes.

To study whether a change in erythrocyte PBGD activity is somehow related to changes in PBGD degradation, Ig PBGD/100 U was determined in both groups of PCT patients and in controls. Apart from this we also determined the total amount of immuno-detectable PBGD (Ig PBGD) in erythrocytes in all four categories of patients and in control subjects.

Materials and Methods

Patients

We made observations in 23 patients with familial PCT, 12 with characteristic skin symptoms (Fsym), and 11 without symptoms at the moment of blood sampling (Fasym). The 11 patients without symptoms had been treated in the past by phlebotomy. The diagnosis of familial PCT was based on a positive family history for PCT, skin symptoms, and a characteristic pattern of porphyrins produced from PBG by hemolysates (indirect UROD activity) (11). We also studied 24 patients with sporadic PCT, 12 with characteristic skin symptoms (SPsym), and 12 who were symptom-free after phlebotomy (SPasym). The diagnosis of sporadic PCT was based on a negative family history for PCT, skin symptoms, and a pattern of porphyrins produced from PBG by hemolysates comparable with normals (indirect UROD activity) (11).

Patients with symptoms had increased urinary excretion of uro- and heptacarboxylporphyrin, whereas such excretion in patients without symptoms was within normal limits at the time of testing.

We also determined PBGD activity, Ig PBGD/100 U, and Ig PBGD in 60 individuals without liver disease. Male/female ratios and mean (± SD) ages in the different patient groups and in the controls were as follows: Fsym: 7/5, 49 ± 16; Fasym: 8/3, 52 ± 17; SPsym: 8/4, 60 ± 10; SPasym: 8/4, 64 ± 14; and controls: 49/21, 41 ± 21.

Methods

Hemolysates. Erythrocytes in heparinized blood samples were washed three times in isotonic saline (NaCl, 154 mmol/L) at 4 °C and then hemolysed by freeze-thawing four times in solid CO₂/facetone.

Indirect UROD activity. UROD activity was measured indirectly by analyzing the pattern of porphyrins formed during incubation of fresh hemolysate in the presence of PBG under conditions identical to those in the PBGD assay. The reaction was stopped by freezing the mixture, which subsequently was stored at −20 °C. The pattern of formed porphyrins after incubation was analyzed as described by Alleman et al. (11). Porphyrins were methylated and the resulting methyl esters were applied to a silica gel column (Merckosorb SI 100, 20-μm particle size; Merck) for high-pressure liquid chromatography. The porphyrins were measured in a Perkin-Elmer spectrofluorometer. Using the results by this method, we calculated the uro- + heptacarboxylporphyrin/coproporphyrin ratio.

Porphobilinogen deaminase assay. Hemolysates were diluted 20-fold with Tris·HCl buffer (50 μmol/L, pH 8.0 at room temperature), and 200 μL of the diluted preparation was added to 400 μL of a mixture containing PBG (10 μmol/L) and Tris·HCl buffer. This was incubated at 37 °C for 60 min. The reaction was stopped by addition of 600 μL of a 250 g/L solution of trichloroacetic acid. After centrifugation the porphyrins formed were measured in the supernatant fluid with a Perkin-Elmer spectrofluorometer (excitation 399 nm, emission 640 nm, bandwidth 20 nm). Under the conditions of our assay the optimum fluorescence of coproporphyrin is measured at 635 nm and of uroporphyrin at 645 nm. Results were expressed as total porphyrins (in picomoles) per milligram of protein per hour at 37 °C, with coproporphyrin I as the standard (14). Protein was measured according to the method of Lowry et al. (15).

Urinary porphyrins. Urine was lyophilized before the methylation of the porphyrins. The methylated porphyrins were determined fluorometrically as described previously (11, 16).

Immuno-detectable PBGD/100 units PBGD activity. We determined the amount of immuno-detectable PBGD per 100 units of PBGD activity (Ig PBGD/100 U) in erythrocytes by using rabbit IgG anti-human PBGD, which was raised by use of human PBGD purified according to the method described by De Rooij et al. (17). Hemolysates from patients were diluted to a standard PBGD catalytic activity, and Ig PBGD/100 U was detected as follows: 50 μL of PBGD/100 U was added to wells, and the mixture was incubated for 1 h at room temperature. After 50 μL of the hemolysate was added, the mixture was incubated for 1 h at room temperature. The plates were centrifuged and the residual (unbound) PBGD activity was determined in the supernatant according to de Rooij et al. (14, 17). Ig PBGD/100 U is defined as the amount of antibodies needed to bind 100 units of PBGD (1 unit of PBGD activity is that producing 1 pmol of URO per hour at 37 °C) and was calculated by using linear-regression analysis of the duplicate PBGD-binding values found in five different IgG dilutions (14, 17).

Total immuno-detectable PBGD in erythrocytes. The total amount of Ig PBGD in erythrocytes, expressed in microliters of IgG per milligram of erythrocyte protein per hour, was calculated as the product of Ig PBGD/100 U (μL IgG/100 U) and the PBGD activity (units/mg of erythrocyte protein per hour).

Statistics. We tested PBGD activities, Ig PBGD/100 U, and Ig PBGD of the various groups for significant differences using the Wilcoxon test.

Results

The analysis of urine from both familial and sporadic PCT patients with symptoms showed the characteristic pattern of PCT with a predominant increase in urinary uro- and heptacarboxylporphyrin. The treated, symptom-free patients showed a normal rate of urinary excretion of both uro- and heptacarboxylporphyrin (Table 1). The indirect UROD activity of patients with familial PCT showed a
increased erythrocyte uro- + heptacarboxylporphyrin/coproporphyrin ratio of \(>2.80\), whereas in sporadic PCT this ratio was \(<2.80\) (Table 1) (11).

The results for mean (± SD) erythrocyte PBGD activity (in pmol per mg of protein per hour) in familial and sporadic PCT patients and in the controls were as follows: Fasym: 136 ± 12, Fasy: 111 ± 16, SPsym: 99 ± 17, SPasym: 97 ± 18, and controls: 80 ± 14. PBGD activity was significantly greater in all PCT patients than in the controls (Fasym, Fasy, and SPsym: \(P < 0.001\), SPasym: \(P < 0.005\)). In the Fasym group the activity of PBGD was significantly higher than that in both groups with sporadic PCT (\(P < 0.005\)).

Figure 2 illustrates PBGD activity, Ig PBGD/100 U, and Ig PBGD in the groups of familial and sporadic PCT patients and controls. In both groups of familial and sporadic PCT patients, Ig PBGD/100 U was less than in the controls (\(P < 0.05\)).

Ig PBGD was found to be significantly (\(P<0.001\)) increased in familial PCT compared with controls. In sporadic PCT, Ig PBGD was only slightly increased (\(P < 0.05\)). In the Fasym group, Ig PBGD was significantly (\(P<0.01\)) more increased than that in the Fasy group (Figure 2).

Figure 3 illustrates the individual relation between PBGD activity and Ig PBGD/100 U in all four groups of PCT and controls. Especially in the Fasym group there was a strong inverse correlation (\(r = -0.90\)) between individual increasing PBGD activity and decreasing Ig PBGD/100 U.

Discussion

After the initial enzyme of the heme biosynthetic pathway, ALAS, PBGD has the next lowest endogenous activity of all of the eight enzymes in the heme pathway (6). This makes PBGD a possible second control point in the heme pathway (10).

An increased PBGD activity in PCT has been described previously (8–12). Here, we demonstrate an increased erythrocyte PBGD activity in familial and sporadic PCT, both symptomatic and asymptomatic, compared with controls (Figure 2). Apart from an increased erythrocyte PBGD activity, an increased PBGD activity in liver cells has been described in PCT (8, 12). An increased PBGD activity could provide an explanation for URO accumulation, but also for the absence of acute attacks in PCT (10).

Increased erythrocyte PBGD activity has been found in patients with liver cirrhosis (18), in patients with lymphoreticular malignancies (19), and also in reticulocytosis (20). Results of liver-function tests (bilirubin, alkaline phosphatase, and aminotransferases) were, however, within normal limits in our asymptomatic patients (results not shown). Also, reticulocytosis has not been described in PCT, and reticulocyte counts were normal in our patients. To our surprise we found an increased erythrocyte PBGD activity in both the symptomatic and asymptomatic form of PCT and also in sporadic PCT. Sporadic PCT has been described to
be confined to the liver, and a 50% reduction of UROD in the liver has been found only in the symptomatic phase of the disorder (2, 3). An increased activity of PBGD in erythrocytes can be due either to decreased rates of inactivation of PBGD or to increased PBGD enzyme protein.

Ig PBGD/100 U was found to be slightly decreased in both groups of sporadic and familial PCT (P < 0.05) (Figure 2); especially in the Fasym group there was a strong inverse correlation between increasing PBGD activity and decreasing Ig PBGD/100 U in individual patients (r = -0.90) (Figure 3). These findings suggest that in both sporadic and familial PCT, increased PBGD activity can be explained, at least partly, by diminished degradation of PBGD enzyme.

The mechanism of this proposed diminished degradation is not clear; however, we speculate that in erythroid cells of PCT patients there is a small but increased production of the substrate of PBGD—i.e., PBG. This seems likely because the rate-limiting enzyme ALAS is increased in erythroid (5) and liver (7) cells of patients with PCT. Also, plasma aminolevulinic acid and PBG have been found to be slightly increased in PCT (6, 8). These observations suggest an increased supply of PBG in erythroid cells initiated by diffusion of aminolevulinic acid from plasma. PBGD reportedly is protected from degradation when the enzyme is bound to its substrate, PBG (21). Therefore it is possible that in PCT an increased amount of PBG is bound to PBGD, protecting the enzyme from degradation, leading to a diminished Ig PBGD/100 U. Apart from a diminished degradation of PBGD enzyme in PCT, there is in addition in familial PCT—and to a minor degree in sporadic PCT—an increased total amount of immuno-detectable PBGD (Ig PBGD) in erythrocytes present (Figure 2). The mechanism of this increase in enzyme protein is not clear and needs further elucidation.

References