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DEFECTIVE HUMAN ERYTHROCYTE UROPORPHYRINOGEN DECARBOXYLASE IN FAMILIAL PORPHYRIA CUTANEA TARDA: THE METABOLIC LESION OR THE RESULT OF ENDOGENOUS PORPHYRINEMIA?

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We have demonstrated that oral charcoal therapy is as effective as therapeutic phlebotomy in reducing porphyrinemia in porphyria cutanea tarda. The effects of immediate and sustained reduction of porphyrinemia on the catalytic properties of partially purified (~200-fold) preparations of red cell uroporphyrinogen decarboxylase of a patient with familial porphyria cutanea tarda were studied. All populations of the patient's red cells exhibited defective enzyme activity, and the apparent Michaelis constants (K_m) determined with penta-, hepta-, and octa-carboxylic I porphyrinogen substrates were approximately 3-4 times higher as compared to the normal controls. Mixing experiments (normal and defective enzyme), and preincubation of the normal enzyme with porphyric plasma prior to purification, yielded data supporting the concept that the catalytic defects of red cell uroporphyrinogen decarboxylase in familial porphyria cutanea tarda are independent of interactions between circulating endogenous porphyrins and the enzyme.

The metabolic defect in the heme biosynthetic pathway in porphyria cutanea tarda (PCT), the most common human porphyria, relates to diminished uroporphyrinogen decarboxylase (E.C. 4.1.1.37) activity (1). In familial PCT, an autosomal dominantly inherited disorder, the enzyme deficiency is expressed in the liver and erythrocytes. In this subgroup, we have reported that

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Abbreviations: PCT, porphyria cutanea tarda, HPLC, high-performance liquid chromatography.

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the partially purified red cell uroporphyrinogen decarboxylase has subnormal enzyme-substrate affinity (2,3), and the enzyme in the diseased state has higher susceptibility to inhibition by iron (2,4,5).

Both iron and porphyrins, present in abundance in PCT liver cytosol (1), are known to inhibit uroporphyrinogen decarboxylase activity competitively (5). Under aerobic conditions, ferrous iron might oxidize porphyrinogen substrates to corresponding porphyrins and/or cause a damaging effect on the enzyme (4), and result in hepatic porphyrin accumulation. The porphyrin-uroporphyrinogen decarboxylase interaction has been well documented in chemically induced porphyria in rodents (6) and chicken hepatocyte culture (7,8) where hepatic uroporphyrin accumulation antedates reduction of uroporphyrinogen decarboxylase activity. These data raise the question as to whether the low activity and subnormal affinity of the erythrocyte enzyme for its substrates in familial PCT is the result of enzyme interaction with circulating endogenous porphyrins.

To address this question, we first established comparable efficacy of oral charcoal therapy (9,10) to that of conventional therapeutic phlebotomy (11) on circulating porphyrin levels in familial PCT with one patient serving as his own control. The first sample of erythrocytes was procured during the active phase of the disease prior to treatment. The second population was obtained one week and a month after initiating oral charcoal therapy, at which time virtual elimination of circulating porphyrins had been achieved. These red cells, which during their life span had circulated in a porphyrin rich plasma, might still have exhibited enzyme-porphyrin binding which was not impacted by the rapid reduction of porphyrinemia. A third batch

of erythrocytes was collected three months after the last phlebotomy. These represented cells, young and old, conceived when the porphyrinemia had resolved. The kinetic abnormalities of a partially purified uroporphyrinogen decarboxylase preparation derived from erythrocytes during the three phases of the disease process were then studied. Erythrocytes from non-porphyric hemochromatosis patients undergoing phlebotomy served as control.

HUMAN SUBJECTS

Fresh whole heparinized blood (500 ml) was procured from one patient with familial PCT, and from three patients with idiopathic hemochromatosis who had normal hemolysate uroporphyrinogen decarboxylase activity and no biochemical evidence of PCT. The latter served as normal controls. The PCT patient studied was a 47 year old white male with mild alcoholic steatonecrosis and siderosis with classic photocutaneous lesions of porphyria and marked elevation of urinary uroporphyrin and coproporphyrin. Uroporphyrin (3860 μg/24 hrs) was well in excess of the normal upper limit of 50 μg/24 hr., and the diagnosis of familial PCT was confirmed by the detection of isocoproporphyrin and coproporphyrin (86.8 μg/g dry weight) in the feces, and reduced levels (60% of control) of red cell uroporphyrinogen decarboxylase activity. Blood was collected from the patient before treatment, after one week of oral administration of charcoal (ActaChar) at a dose of 30g t.i.d. and after a further four weeks course of oral charcoal at a dose of 60g t.i.d. After six weeks, phlebotomy was commenced (500ml twice a month), the endpoint being normalization of urinary uroporphyrin levels. Three months following the last phlebotomy when the patient was in sustained biochemical and clinical remission, a final sample of blood was procured for enzyme analysis.

MATERIALS AND METHODS

Materials

Sources of chemicals have been earlier described by us (2-5).

Partial purification of red cell uroporphyrinogen decarboxylase:

Blood (500 ml) from both the PCT subject and the controls was centrifuged, the red cells were removed, washed, lysed, and the uroporphyrinogen decarboxylase was partially purified (~200-fold) as previously described by us (3).

Assays:

The uroporphyrinogen decarboxylase was assayed and the Michaelis constant (K_m) determined using penta-, hepta- and octacarboxylic porphyrinogen substrates by HPLC analysis as

described by Mukerji et al (4,5). Kinetic analysis of uroporphyrinogen decarboxylase in the red cells of the PCT patient was performed before treatment, on charcoal therapy and three months following the last phlebotomy when the patient was in clinical and biochemical remission. Also, a part of the washed erythrocytes from the normal control subject was incubated with the plasma from the PCT patient, collected prior to charcoal therapy, in proportions originally present in whole blood. The mixture was left stirring for about two hours at 4°C before partially purifying the enzyme. The protein content of the preparations was measured by the method of Lowry et al (12).

Quantitative Estimation of Porphyrins:

Porphyrins were extracted from the patients' plasma, skin, urine, and feces. They were quantitated and characterized according to the elution profiles of their methyl esters by HPLC as described by Mukerji et al (9).

RESULTS

Clinical:

The patient experienced a rapid clinical response to oral charcoal therapy. This was associated with reduced formation of new bullae which previously had erupted with a frequency of 2-3 per week. These clinical improvements coincided with observations of reduced circulating plasma porphyrins and occurred long before phlebotomy was initiated.

Quantitative Analyses of Plasma and Skin Porphyrins:

Analysis of porphyrins in patients' plasma by the HPLC method showed that hepta- (40%) and hexa-carboxylic (35%) porphyrins were the major components, with total levels of 31 μg/dl (Table 1). Plasma porphyrin levels were reduced to values which were normal and near zero seven days after starting orally administered charcoal (30g t.i.d.), one month after ingestion of ActaChar at a higher dosage (60g t.i.d.), and three months following clinical and biochemical remission induced by phlebotomy (Table 1). The HPLC porphyrin elution profile of a punch skin biopsy sample of 80 mg fresh weight from the patient showed that the major component (about 90%) was pentacarboxylic porphyrin. It also contained small amounts of copro-, hexa-,

TABLE 1

KINETIC PROPERTIES OF UROPORPHYRINOGEN DECARBOXYLASE PARTIALLY PURIFIED FROM THE ERYTHROCYTES OF A PATIENT WITH FAMILIAL PORPHYRIA CUTANEA TARDA PRIOR TO AND IMMEDIATELY AFTER SUSTAINED REDUCTION OF PORPHYRINEMIA

Treatments	Total plasma porphyrin (μg/dl)	Km[a] (μM)	Vmax (nmols/hr/mg. prot.)	r^2[b]
Normal control	<0.05	0.49-0.57	3.1	0.92-0.99
Normal RBC + PCT plasma	31.1	0.50-0.75	3.2	0.98-0.99
Patient before treatment	31.1	2.11	2.0	1.00
Patient after charcoal[c]	0.06	3.08	2.3	0.99
Patient after charcoal[d]	0.04	2.86 2.00	2.0 1.9	0.99 0.98
Patient after phlebotomy	0.02	2.63 2.86	2.1 2.2	0.98 0.99

[a] K_m values, determined in triplicate as described in the text using pentacarboxylic porphyrinogen I as substrate, were measured together under identical assay conditions.

[b] r^2, coefficient of determination for the line from which the kinetic constants were derived using linear regression analysis.

[c] the patient with PCT was treated with oral administration of activated charcoal (ActaChar) as a slurry (30g every 8 hours for 7 days).

[d] the patient with PCT was treated orally with activated charcoal (60g every 8 hours for 1 month).

hepta- and octacarboxylic porphyrins. The total skin porphyrin was 0.18 μ g/g wet weight.

Kinetic Analysis of Partially Purified Red Cell Uroporphyrinogen Decarboxylase:

The data presented in Table 1 show that the apparent Michaelis constants (K_m) calculated from the Lineweaver-Burk double reciprocal plots, using pentacarboxylic porphyrinogen I as

substrate were normal (0.50-0.75 μ M) for the uroporphyrinogen decarboxylase derived from the erythrocytes of normal subjects even when pre-treated with PCT plasma prior to partial purification. On the other hand, the K_m values of the enzyme extracted and purified from the red cells of the PCT patient were 2.5 to 4.0 times higher (2-3 μ M) than similarly purified red cell enzyme from non-porphyric control subjects. These differences in K_m values also were observed after the circulating plasma porphyrins were reduced by oral charcoal therapy and after phlebotomy. The maximum velocities (V_{max}) of the enzyme from the PCT patient prior to and after the various therapeutic maneuvers were about 60-70% of normal control values. The increased K_m observed with heptacarboxylic-I (Normal = 0.24 μM; Patient = 0.60 μM), or octacarboxylic-I (Normal = 0.52μM; Patient = 1.5-2.9μM) porphyrinogen substrates, was similar to that noted with pentacarboxylic-I porphyrinogen as substrate.

Measurements of Uroporphyrinogen Decarboxylase Activity Before and After Mixing the Enzyme Preparations from the Patients and a Normal Control Subject:

Whether the observed kinetic abnormalities of red cell uroporphyrinogen decarboxylase in PCT are due to the interaction of an inhibitor such as the circulating porphyrins, or an activator, can be tested by showing absence of inhibitory or activating effects on mixing the enzyme preparations from the patient with that of a normal subject prior to assays. The observed enzyme activities on mixing the enzyme preparations in equivalent protein concentrations from normal control with that from the PCT patient prior to and after therapeutic reduction of his plasma porphyrins were more or less the same as the calculated additive activities expected from the individual assays (Table 2). These results suggest that the preparations

TABLE 2

EFFECTS OF MIXING UROPORPHYRINOGEN DECARBOXYLASE ENZYME PREPARATIONS FROM THE ERYTHROCYTES OF NON-PORPHYRIC CONTROL SUBJECT AND A PATIENT WITH PORPHYRIA CUTANEA TARDA

Enzyme Preparations	Uroporphyrinogen decarboxylase activity [(nmol/hr.)x10^2]	
	observed	calculated
1 Normal control (Ncont)	4.46	
2 PCT pre-charcoal (PCT pre-C)	2.06	
3 PCT post-charcoal (PCT post-C_1)	2.80	
4 PCT post-phlebotomy (PCT post-phle)	1.51	
5 PCT post-charcoal (PCT post-C_2)	1.50	
6 Ncont + PCT pre-C	6.45	6.52
7 Ncont + PCT post-C_1	6.68	7.26
8 Ncont + PCT post-phle	5.30	5.97
9 Ncont + PCT post-C_2	5.19	5.96

The calculated values are the additive activities expected from the individual assays. Post-C_1 = seven days after the patient was treated with oral charcoal (30g t.i.d.); Post-C_2 = 30 days on oral charcoal (60g t.i.d.); Post-phle = three months following the last therapeutic phlebotomy. The protein concentrations in these experiments were about 0.15 mg/ml. The enzymes were mixed in equivalent protein concentrations.

were free of inhibitors or activators of uroporphyrinogen decarboxylase.

DISCUSSION

We have demonstrated for the first time the equivalent clinical and biochemical efficacy of an oral sorbent, ActaChar when compared to conventional phlebotomy in the treatment of PCT. It afforded the opportunity to test whether erythrocytes conceived in a "porphyric" milieu that was rapidly restored to normal exhibited kinetic abnormalities with regard to uroporphyrinogen decarboxylase different to those cells which

were formed during a period of sustained remission. We observed no difference in the Michaelis constants (K_m) of partially purified (~200-fold) enzyme preparations from PCT erythrocytes procured at different phases of disease activity. These results strongly suggest that the inherent biochemical defect in familial PCT is low activity of human erythrocyte uroporphyrinogen decarboxylase accompanied by reduced enzyme-substrate affinity which is independent of the effects of circulating plasma porphyrins.

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