Long-Standing Changes in the Urinary Profile of Porphyrin Isomers After Clinical Remission of Porphyria Cutanea Tarda

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Abstract. Patients with overt porphyria cutanea tarda (PCT) show a distinctive and abnormal urinary profile of porphyrin excretion. It is not known, however, whether clinical remission of the disease produces complete normalization of this profile. We selected 46 patients, previously diagnosed with PCT, who after treatment presented normal levels of total porphyrins in urine (<35 nmol/mmol creatinine). We analyzed their urine specimens by hplc to identify and quantify the various porphyrins and we compared the urinary porphyrin profiles to those of 40 healthy volunteers. While healthy volunteers gave a pattern dominated by excretion of coproporphyrin III, 80% of the PCT patients in clinical remission showed the characteristic profile of PCT, with decreased coproporphyrin-to-uroporphyrin ratio and/or inversion of the normal coproporphyrin III-to-coproporphyrin I ratio. Detection of uroporphyrin III and heptacarboxyl III intermediates was significantly more common among the patients than the controls (p <0.05). This study shows that PCT patients demonstrate persistent changes in urinary porphyrin profiles during clinical remission, even when total urinary porphyrin excretion has fallen to the normal range. (received 28 March 2003; accepted 15 May 2003)

Keywords: porphyrins, porphyria cutanea tarda, high performance liquid chromatography

Introduction

The porphyrias are a group of diseases produced by deficiencies of various enzymes involved in heme biosynthesis [1]. Porphyria cutanea tarda (PCT), one of the most prevalent, is caused by a deficiency of uroporphyrinogen decarboxylase (Uro-D). This enzyme catalyzes decarboxylation of uroporphyrinogen to coproporphyrinogen; inhibition of Uro-D induces the accumulation of polycarboxylated porphyrins, resulting in the typical phototoxic skin reactions and liver disturbances [2]. The Uro-D deficiency may be inherited or acquired; in the later case, the known etiopathogenic factors include chronic viral infections, estrogens, alcohol abuse, and exposure to polyhalogenated aromatic hydrocarbons. The Uro-D inhibition in acquired PCT is a complex multifactorial process in which the pathogenic mechanisms have not been completely elucidated. Iron overload appears to be a major factor, triggering the formation of reactive oxygen radicals. The hemochromatosis gene, which controls ferritin-dependent regulation of iron absorption from the gut, plays a role in PCT; several of its mutations may cause predisposition to PCT [3].

The typical clinical manifestations of PCT in the form of cutaneous photosensitivity are due to the accumulation of fluorescent polycarboxylated porphyrins derived from oxidation of the corresponding accumulated porphyrinogens. Hereditary forms of PCT account for roughly 20% of cases. Several mutations in the Uro-D gene (chromosome 1p34) induce 50 to 60% decrease of Uro-D activity in all tissues. This reduction, per se, may not produce overt PCT in most individuals with the mutations; additional genetic or non-genetic factors are usually needed to trigger the disease [4,5].

PCT is usually diagnosed by analyzing porphyrin excretion in urine and feces. Patients with overt PCT show a very high excretion of total
porphyrins in urine. The urinary analysis usually reveals high levels of 8- and 7-carboxylated porphyrins and less pronounced elevations of 6- and 5-carboxylated intermediates. Some of the lipophilic intermediates are also increased in feces, with the presence of isocoproporphyrin being a characteristic of PCT. Porphyrin profiles in urine and feces can be assessed by several analytical tools, most notably by high performance liquid chromatography (hplc), which is generally used to diagnose PCT and to distinguish it from other types of porphyria [6-9].

Once the diagnosis of PCT has been established, therapeutic strategies are available. Phlebotomy and/or chloroquine administration are measures to control excessive porphyrin production [10,11]. (Editor’s note: chloroquine is seldom used in North America because of concerns about its hepatotoxicity in PCT.) A monitoring program that includes periodic analysis of urinary porphyrins is also normally established during the treatment and follow-up of PCT patients.

It is unclear, however, if clinical remission of PCT, and the consequent diminution of total porphyrin excretion in urine, is always attended by normalization of the porphyrin excretion profile. It seems likely that alterations of porphyrin metabolism, accumulation, and elimination may persist for a long time in the familial and sporadic forms of PCT. Specifically, it is uncertain whether the characteristic hplc profile of urinary porphyrins undergoes complete restoration to normal after clinical remission of PCT.

In this study we investigated this issue by selecting a group of patients previously diagnosed with PCT. We performed a detailed analysis of their urinary porphyrin excretion profiles during clinical remission and compared them to the corresponding profiles in healthy subjects.

Materials and Methods

We surveyed 105 PCT patients who were seen during 2002 in the Dermatology Unit of the Hospital Clinic at the University of Barcelona. We measured the total porphyrin excretion in the urine of these patients by fluorimetry. Fifty-nine patients (56%) gave a value above our upper limit of normal (35 nmol/mmol creatinine), while 46 (44%) gave normal results. The latter group was selected for this study. These patients were all previously diagnosed as PCT at the same hospital, but were currently in clinical remission and were seen at the clinic for monitoring and follow-up. The clinical histories in all cases included several previous results with elevated total porphyrin levels in urine. The subjects comprised 38 men and 8 women, age 28 to 84 yr. These patients were informed of the aims of study and they gave permission for further urine analyses.

Forty members of the hospital staff volunteered to serve as healthy subjects. They included 20 men and 20 women, age 32 to 66 yr. None were related to persons with known or suspected porphyria. The volunteers were informed about the objectives of the study and they gave consent for participation in the study. In all PCT cases and controls, early-morning urine samples were collected. Urine creatinine concentrations were measured with a Bayer ADVIA 1650 analyzer; the urine samples were frozen in the dark at -80°C until the porphyrin analyses were performed.

Quantitative assessment of urinary total porphyrins employed a spectrofluorimeter (model F-2000; Hitachi). Fifty µl of urine was diluted in 2.7 ml of HCl (2.7 mol/L) and fluorimetry was performed with excitation at 398 nm and emission at 603 nm. A calibration curve was constructed with acid dilutions of coproporphyrin III standard (Porphyrin Products, Inc., Logan, UT; 0.5 µg/ml in 1 M HCl). The concentration of the standard was established by measuring its absorption at 399.5 nm in 0.1N HCl (ε= 489 mM). Total urinary porphyrins were expressed as nmol/mmol creatinine. The threshold for positive/negative discrimination was set at 35 nmol/mmol creatinine.

Urinary porphyrins were fractionated by reverse-phase hplc according to Lim and Peters [8]. Briefly, 1 ml of urine was acidified with 50 µl of concentrated HCL and 250 µl was injected into a chromatograph (model 474, Waters Corp., Milford, MA) with a fluorescence detector (excitation and emission wavelengths 405 nm and 618 nm, respectively), 2 pumps, programmed gradient system, an automatic injector with 2000-µl sample loop, and a Milenium data management station.
The analytical column (BDS-Hypersil; Shandon HPLC, Cheshire, England) was 250 mm x 4.6 mm, 5 µm particle size. Solvents for gradient elution were 10% (v/v) acetonitrile in ammonium acetate (1 mol/L, pH 5.16; solvent A), and 10% (v/v) acetonitrile in methanol (solvent B). The column was equilibrated with 100% solvent A before the sample was injected. Porphyrins were separated with a 30-min linear gradient elution from 100% solvent A to 35% solvent A, followed by isocratic elution for 10 min (flow rate, 1 ml/min.). A calibration curve was constructed with dilutions in 0.1 M HCl of a mixture of 10 ± 1 nmol each of the 8-, 7-, 6-, 5-, and 4-carboxyl porphyrins of the I isomer series, plus uroporphyrin III and coproporphyrin III (Porphyrin Products, Inc., Logan UT).

Each porphyrin and isomer fraction was quantified independently in urine and expressed as nmol/mmol creatinine [12]. The detection limit for each of the individual porphyrins in urine was 0.1 nmol/mmol creatinine.

Results

Urine samples from the 40 controls contained total porphyrin concentrations <35 nmol/mmol creatinine, which was the level used to select the 46 PCT patients in clinical remission.

Hplc fractionations (illustrated in Figs. 1 and 2) were performed on the urine samples from the controls and PCT patients in remission. The chromatograms all showed well defined and quantifiable peaks. The control urines all contained measurable concentrations of coproporphyrin III; coproporphyrin I, and uroporphyrin I (Table 1). In 8 (20%) of controls, uroporphyrin III and heptacarboxylyporphyrin III were also detected. The individual porphyrins in urine specimens from the controls were within the normal limits (nmol/mmol creatinine) reported by Hindmarsh et al [13] (ie, uroporphyrin I: <3.9; uroporphyrin III: <2; heptacarboxyly III: <1.3; coproporphyrin I: <8.5; coproporphyrin III: <26).

Urine coproporphyrin III-to-coproporphyrin I ratios (Copro III/I ratio) were calculated for each control. The mean ratio was 2.98 (SD ± 1.67). In only one case did the coproporphyrin I level exceed the coproporphyrin III level (Table 1). The total coproporphyrin (coproporphyrin III + I)-to-total uroporphyrin (uroporphyrin III + I) ratio (Copro/Uro ratio) was >1 in all cases, with a mean of 28.11 (SD ± 35) (Table 1).

The concentrations of individual porphyrins in the urine samples from PCT patients in remission are summarized in Table 1. Although the sum of the various porphyrin concentrations yielded total porphyrin levels similar to those of controls, detailed analysis of the chromatograms of PCT patients in remission showed significant differences from the normal porphyrin profiles of the healthy controls.

Notably, 27 (58.7%) of the patients showed a decreased Copro III/I ratio with preponderance of

<table>
<thead>
<tr>
<th>Porphyrin/isomer</th>
<th>Healthy volunteer controls, n = 40</th>
<th>PCT patients in remission, n = 46</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uroporphyrin I</td>
<td>0.49 ± 0.61, (0.10-2.14)</td>
<td>3.92 ± 3.64, (0.10-18.12)</td>
</tr>
<tr>
<td>Uroporphyrin III</td>
<td>0.22 ± 0.11, (nd-0.24)</td>
<td>1.58 ± 2.12, (0.10-11.56)</td>
</tr>
<tr>
<td>(detected in 20% of urines)</td>
<td></td>
<td>(detected in 69% of urines)</td>
</tr>
<tr>
<td>Heptacarboxyl III</td>
<td>0.15 ± 0.10, (nd-0.20)</td>
<td>1.32 ± 1.67, (0.10-10.23)</td>
</tr>
<tr>
<td>(detected in 20% of urines)</td>
<td></td>
<td>(detected in 76% of urines)</td>
</tr>
<tr>
<td>Coproporphyrin I</td>
<td>1.20 ± 1.18, (0.11-5.81)</td>
<td>5.30 ± 5.84, (0.20-8.40)</td>
</tr>
<tr>
<td>Coproporphyrin III</td>
<td>3.18 ± 2.76, (0.11-14.35)</td>
<td>5.58 ± 6.22, (0.30-9.23)</td>
</tr>
<tr>
<td>* Copro III/I ratio &lt;1</td>
<td>n = 1* (2.5 %)</td>
<td>n = 27* (58.7 %)</td>
</tr>
<tr>
<td>b Copro/Uro ratio &lt;1</td>
<td>n = 0</td>
<td>n = 21* (46.5 %)</td>
</tr>
</tbody>
</table>

* Copro III/I ratio = coproporphyrin III / coproporphyrin I concentration ratio.

b Copro/Uro ratio: coproporphyrin III + coproporphyrin I / uroporphyrin III + uroporphyrin I concentration ratio.

c The number of individuals (%) with the inverted ratio.
Fig. 1. Typical chromatogram of porphyrins in urine from a healthy subject.

Fig. 2. Typical chromatogram of porphyrins in urine from a PCT patient in remission.
Urine porphyrin profiles in porphyria cutanea tarda

III and an increased excretion of heptacarboxyl III. There are also increased concentrations of hexacarboxyl- and pentacarboxyl-porphyrins, the accumulation of series III intermediates being a direct consequence of Uro-D inhibition.

Use of certain porphyrin ratios facilitates the characterization of abnormal chromatograms, notably the Copro/Uro ratio and the uroporphyrin-to-heptacarboxyl porphyrin ratio [20]. In our PCT patients in remission, some of these features clearly appear, eg, increased excretion of uroporphyrins relative to coproporphyrins and decreased Copro/Uro ratio, often combined with increased presence of the heptacarboxyl III intermediate. The profile of the patients was abnormal compared to controls in whom coproporphyrin excretion was predominant. The inverted Copro/Uro ratio and the presence in urine of series III polycarboxylated intermediates are distinctive of overt PCT when large amounts of porphyrins are excreted in urine and feces [8]. This profile is also seen in remission, in a circumstance of low total porphyrin elimination.

Although less known than the Uro/Copro inversion, a significant change in relative proportion of coproporphyrin isomers I and III was apparent in several of the patients. In normal urine, the concentration of coproporphyrin III isomer usually exceeds that of the I isomer, and the CoproIII/I ratio is usually >3. Some authors have calculated a normal ratio and reported a significant lowering of this ratio in PCT [13], possibly reflecting a pronounced reduction of the coproporphyrin III isomer after the onset of the disease. In patients with overt PCT and high porphyrin excretion studied during 2002, we observed 54% of cases with Copro III/I <1 (unpublished data). The excretions of the isomers have fundamental differences, since the III isomer, an intermediate of the heme biosynthesis, exists in equilibrium between its production, utilisation, and elimination, while the I isomer is not further metabolized and is totally eliminated. The coproporphyrin isomeric proportion in PCT is complex; it is affected by previous or concomitant alcoholic liver damage and by the relative amounts excreted by urine and feces [21]. In our PCT patients in remission, the Copro III/I isomeric ratio in urine was shifted in most cases. The urinary level of the coproporphyrin I isomer (Copro III/I <1; Table 1). The Copro/Uro ratio was shifted in 21 (46.5%) of the patients, with an abnormal excess of uroporphyrins over coproporphyrins (Copro/Uro <1). In 11 patients Copro III/I was <1 and Copro/Uro was <1. Thirty-seven patients presented at least one of the two abnormal inversions. The means ± SD of these ratios in this group of PCT patients were 1.35 ± 1.22 (CoproIII/I) and 4.30 ± 7.47 (Copro/Uro) respectively (p <0.05 versus controls).

Discussion

This study indicates that some PCT patients, after treatment, enter a period of clinical remission with lowering of the total urine porphyrins. When total urine porphyrins are assessed by screening methods [14,15], a normal result is obtained in most of these cases, with the total porphyrin fluorescence close to that of urine from healthy persons. This occurs in the acquired and the hereditary forms of PCT, consistent with substantial normalization of the heme biosynthetic pathway, relative reactivation of hepatic Uro-D activity, and diminished porphyrin accumulation in skin and tissues.

Our results show that a normal level of total urinary porphyrins is compatible with subtle abnormalities in the isomeric pattern and the relative abundance of specific porphyrins. In many of our PCT patients in clinical remission, the chromatographic profile was typical of overt PCT, but of a reduced magnitude. Reverse-phase hplc profiles of urine porphyrins in PCT patients have been described by several authors [16-19]. The features are elevation of uroporphyrin isomers I and III and an increased excretion of heptacarboxyl III. There are also increased concentrations of hexacarboxyl- and pentacarboxyl-porphyrins, the accumulation of series III intermediates being a direct consequence of Uro-D inhibition.
coproporphyrin III isomer exceeded the I isomer in all but one of the normal controls; there were many patients (58.7%) in whom the urinary coproporphyrin I isomer was dominant. This subtle isomeric shift was compatible with normal urinary total coproporphyrin excretion.

In conclusion, our results show that many patients with PCT show persistent subtle changes in their urinary porphyrin profiles despite clinical remission, even though the total urinary porphyrin excretion falls within the normal range. These abnormalities may reflect persistence of the disordered heme pathway. Other factors may contribute to the continuance of the abnormalities, since chronic hepatitis C virus infection or toxic metal exposures [22-24] can produce some of the urinary changes here described. However, since all of our patients had a previous history of overt PCT with greatly elevated excretion of porphyrins in urine and faeces, persistence of the biochemical disorder seems the most logical explanation.

The authors plan further studies of these PCT patients to follow the altered excretion profiles of urinary porphyrins during remission, in relation to genetic and exogenous etiologic factors.

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References