Abnormal Chromatographic Patterns of Porphyrins in Urine

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Abstract. Urine is the most frequent specimen used in the initial evaluation of adult patients who present with photosensitivity. When urine porphyrins are abnormal, characterization of the chromatogram is facilitated by calculation of uroporphyrin-to-heptacarboxylate porphyrin (uro/hepta) and uroporphyrin-to-isocoproporphyrin (uro/iso) ratios. The most frequent abnormal pattern, and that most consistent with porphyria cutanea tarda (PCT), is an uro/hepta ratio \( \leq 2.0 \) and an uro/iso ratio \( \leq 18 \). When the uro/hepta or uro/iso ratios are less consistent with PCT, other less common porphyrin disorders should be considered. These include variegate porphyria, coproporphyria with manifestations of photosensitivity only, adult onset congenital porphyria, mixed porphyrias, and other less frequent porphyrin disorders. After initial evaluation, the diagnosis should ideally be confirmed by additional testing of blood and fecal specimens. Most attacks of the acute porphyrias are associated with a uro/hepta ratio >4, and can be confirmed by an elevated urine porphobilinogen concentration. (received 20 March 2001; accepted 7 May 2001)

Key words: porphyrins, porphyria cutanea tarda, photosensitivity, porphobilinogen, HPLC

Introduction

When patients develop photosensitivity, porphyrin disorders are among the diseases considered. In adult patients, urine is often the specimen selected for initial study [1,2]. Analysis of urine porphyrins by high performance liquid chromatography (HPLC) can distinguish porphyrin disorders from other causes of photosensitivity, such as drug reactions, with a high degree of confidence [2-4]. Porphyria cutanea tarda (PCT) is the most common porphyrin disorder in adults. Although it is easy to distinguish PCT from non-porphyrin diseases, other less frequent porphyrin disorders also occur in adults, and distinguishing these from PCT is more difficult. These disorders include variegate porphyria and coproporphyria (which manifest only photosensitivity in about one-half of cases), adult onset congenital porphyria, mixed porphyrias, and other rare conditions.

There are many published examples of the chromatographic patterns that are associated with porphyrin disorders [1-4]. However, these are almost always single examples, and they provide little discussion of the variability that occurs among patients with the same disorder. The general portrait of PCT has 2 parts, elevated uroporphyrin and heptacarboxylate porphyrin concentrations of approximately equal magnitudes, and the appearance of an unusual intermediate, isocoproporphyrin [2,3]. Therefore, 2 ratios were selected to quantitate the patterns of abnormal results: (1) the uroporphyrin-to-heptacarboxylate (uro/hepta) ratio, and (2) the uroporphyrin-to-isocoproporphyrin (uro/iso) ratio. The goals of this study were to characterize the chromatographic pattern that is commonly seen in PCT and to identify the chromatographic patterns that suggest less frequent porphyrin disorders. This report can be described as a reference interval study of an abnormal population.

Methods and Materials

The study population was selected from patient specimens received for porphyrin analysis at ARUP
Laboratories, Salt Lake City, UT, USA. ARUP is a clinical reference laboratory associated with the University of Utah. It receives specimens from a wide geographic area. After routine urine porphyrin analysis was complete, abnormal results were collected and analyzed in a Microsoft Excel spreadsheet. If not already performed in response to the physician's request, or to facilitate laboratory interpretation, a urine PBG analysis was performed. Specimens and specimen identifiers were not retained, and patient consent was not required. This study complied with the ethical guidelines of the University of Utah.

As previously recommended [2], prompt refrigeration was the only specimen preservation precaution that was taken for analysis of urine porphyrins and porphobilinogen (PBG). The traditional use of sodium carbonate for urine pH adjustment [5] was not employed because it caused minor foaming problems in the porphyrin analysis. The following reference intervals were used for both random and timed collections: uroporphyrin >4 mmol/mol creatinine, heptacarboxylate porphyrin >2 mmol/mol creatinine, and PBG >8.8 mM [2]. Isolated elevations of coproporphyrin were not studied, since this pattern is typically associated with non-porphyrin disorders, such as liver disease. Isolated elevations of hexacarboxylate and pentacarboxylate porphyrins and isocoproporphyrin were also not included, since these are typically due to interferences.

Urine porphyrins were analyzed by HPLC as described elsewhere [2]. Briefly, specimen aliquots were filtered and loaded onto an automated HPLC system (Alliance 2690 Millennium System, Waters, Milford, MA). Portions (200 µl) were injected onto a C18 reverse-phase column (Waters Symmetry, 5 mm, 4 x 150 mm), eluted with a phosphate/methanol gradient (0.10 mol/L phosphate, pH 3.5, methanol 10-90%), and monitored by fluorescence (Waters 474 detector, excitation 404 nm, emission 618 nm). Concentrations (nM) were determined from peak areas. Porphyrins and standards were purchased from Porphyrin Products (Logan, UT). Because isocoproporphyrin was not commercially available, the isocoproporphyrin peak was standardized using coproporphyrin.

Specimen aliquots were submitted for creatinine analysis, and porphyrin-to-creatinine ratios were calculated to facilitate interpretation of porphyrin reference intervals. Creatinine was determined on a model 717 analyzer (Hitachi Instruments, San Jose, CA) using an automated kinetic Jaffé method. To convert porphyrins to units of mmol/mol creatinine, nM were multiplied by 11.3 and divided by mg/dl creatinine. The uro/hepta and uro/iso ratios were calculated from nM.

Urine PBG was analyzed by anion-exchange chromatography as described elsewhere [2]. Briefly, specimen aliquots (1.0 ml) were passed through an anion-exchange column (Bio-Rad Laboratories, Hercules, CA), washed with 10 ml of deionized water, and eluted with 4.0 ml of 1 M acetic acid. Eluates were combined with Ehrlich's reagent, and color reactions quantitated at 555 nm. All elevated results were examined in a spectrophotometer to verify peak absorbances at 525 and 555 nm and a peak height ratio (525-to-555) of about 0.8.

Results

Typical normal and abnormal chromatograms are illustrated in Fig. 1. Over a period of 6 months, 156 abnormal porphyrin results were collected. These were placed in the following 3 categories: 14 acute porphyria, 127 elevated porphyrins, and 13 slightly elevated porphyrins. The slightly elevated group was placed in a separate category to reduce possible effects of relatively marginal elevations. All 13 cases showed normal PBG, and this group was not examined further.

The category of acute porphyria was defined by the presence of elevated urine PBG (reference interval >8.8 mM). Fourteen cases were identified in which the PBG range was 80-243 mM (mean 168, SD 68, skew 0.04). In 8 of the 14 cases, PBG analyses was specifically requested by the ordering physician, whereas 6 were added by the laboratory. In addition to elevated PBG, all 14 cases demonstrated a consistent pattern of elevated porphyrins, with a uro/hepta range of 4.0-21 (mean 12, SD 4.5, skew 0.17), and uro/iso range of 3.1-280 (mean 69, SD 75, skew 2.0).

The category of elevated porphyrins (n = 127) was defined by urine porphyrins ≥ twice the reference intervals for uroporphyrin or heptacarboxylate porphyrin. These cases showed a uro/hepta range of 0.53-5.7. In Fig. 2, the uro/hepta ratios are arranged in rank-order by increasing value, and plotted against
Abnormal chromatographic patterns of urine porphyrins

Fig. 1. Urine porphyrin chromatograms. Lower tracing is a normal pattern. Upper tracing is typical for porphyria cutanea tarda (PCT). Early peaks are due to normally occurring fluorescent compounds in urine.

the uro/hepta ratio. The strong inflection point in Fig. 2 occurs at a uro/hepta ratio of 2.0, with 11 results above the inflection point. Below the inflection point, there were 116 results with an uro/hepta range of 0.53-1.99 (mean 1.3, SD 0.34, skew 0.11).

The uro/iso ratio was also examined. For the elevated porphyrins category, the uro/iso range was 0.34-178. In Fig. 3, the uro/iso ratios are arranged in rank-order by increasing value, and plotted against the uro/iso ratio. Although not as distinct as in Fig. 2, an inflection point is present in Fig. 3 at a uro/iso ratio of 18. Eleven values were again found above the inflection point. Below the inflection point were 116 results with an uro/iso range of 0.34-17.3 (mean 7.1, SD 4.3, skew 0.64). Only 6 of the 11 values above the inflection point in Fig. 3 correspond to those in Fig. 2. Results that fall above at least one of the inflection points have the following distribution: 6 cases above both inflection points, 5 cases above uro/hepta and below uro/iso, and 5 cases below uro/hepta and above uro/iso.

In the elevated porphyrins category (n = 127), the average age was 49 yr (SD 12), with no age given for 12 patients. Specimens consisted of 23 random and 104 24-hr collections. For the 24-hr collections, the average volume was 2000 ml (SD 950, range 550-6000). For all specimens, the average creatinine concentration was 77 mg/dl (SD 42, skew 1.0, range 16-220). The average uroporphyrin concentration was 1200 nM (SD 2200, range 62-24,000), and exhibited a strongly positive skew of 8.5. The average uroporphyrin-to-creatinine ratio was 220 mmol/mol creatinine (SD 560, skew 9.7, range 11-6,200). Heptacarboxylate porphyrin showed characteristics of similar magnitude.
Among the elevated porphyrins in Figs. 2 and 3, 91 specimens were from males and 36 from females. Using chi-square analysis for goodness of fit, this is significantly different from a random 50-50 distribution (n = 127, 1 degree of freedom, p < 0.01, critical chi-square = 6.63, observed chi-square = 22.8). However, the 16 cases above the uro/hepta and uro/iso inflection points consisted of 6 males and 10 females. Although these numbers are small, chi-square analysis revealed this was significantly different than the study sample (n = 16, 1 degree of freedom, p < 0.01, critical chi-square = 6.63, observed chi-square = 9.3). However, 6 males and 10 females is not significantly different from a random distribution (observed chi-square = 1.0). In contrast, the cases of acute porphyria were from a preponderance of female patients (2 males and 12 females).

Repeat analysis of several specimens showed no significant loss (< 15%) of porphyrins or PBG after 10 days of refrigeration (data not shown). Repeat specimens from the same affected individuals (n = 3) showed essentially the same pattern of elevated porphyrin intermediates (data not shown).

Discussion

Specimen preservation for urine porphyrins and PBG can be a contentious issue. Refrigeration is the most important aspect of preservation [2], and is adequate when refrigeration, transport, and analysis are prompt. Adjustment of pH can decrease analyte loss after several days of storage, although it is not practical for some collection sites in this era of managed care. It should be emphasized that the specimens in the present study were collected from a variety of distant sites under conditions unknown to the laboratory, and that some loss of analytes probably occurred. Analyte loss does not appear to have been a significant factor for PBG, since the results were either normal or markedly elevated. However, that was not the case for porphyrins. In part to compensate for the probable porphyrin loss, the present study excluded those specimens which showed only slight porphyrin elevations (reference intervals < twice normal).

Using a simple rank order approach (Figs. 2 and 3), most of the study specimens were found to have a uro/hepta ratio ≤ 2.0 and a uro/iso ratio ≤ 18. In the present study, 111 of 127 results (87%) demonstrated these characteristics. This pattern conforms to what is expected for PCT [2-4], namely: (1) the uroporphyrin and heptacarboxylate porphyrin concentrations are close to the same magnitude, and (2) an isocopro-porphyrin peak is prominent. This is by far the most frequent abnormal pattern in the study sample. While these chromatographic characteristics are consistent with PCT, this does not mean that all the cases in this

Fig. 2. The uro/hepta data for 127 urine samples are arranged in rank-order and plotted as a function of uro/hepta ratio. The sharp inflection point occurs at a uro/hepta ratio of 2.0. Cases of acute porphyria (n = 14) were not included, but all showed a uro/hepta ratio > 4.

Fig. 3. The uro/iso data for 127 urine samples are arranged in rank-order and plotted as a function of uro/iso ratio. An inflection point occurs at a uro/iso ratio of 18.
category are PCT. Likewise, while different results do not exclude PCT, they are more suggestive of less common porphyrin disorders.

The acute porphyrias represent a special category, since attacks can be identified unambiguously by an elevated urine PBG [2,4]. In the present study, 14 cases of acute porphyria were found, and all showed elevated porphyrins in a pattern distinct from PCT. Where PCT is commonly associated with a uro/hepta ratio ≤2.0, the acute porphyrias are associated with a uro/hepta ratio significantly larger. Specifically, in the present study the uro/hepta range was 4-21, meaning that uroporphyrin concentration was always 4 or more times the heptacarboxylate porphyrin concentration. While this does not indicate that all cases of acute porphyria will demonstrate a larger uro/hepta ratio, the vast majority would be expected to show this characteristic.

While a uro/hepta ratio >2.0 is less suggestive of PCT and more characteristic of an attack of an acute porphyria, there are other porphyrin disorders that should also be considered. In the present study there were 11 of 127 cases where uro/hepta was >2.0 and PBG was normal. Since a normal PBG excludes an attack of an acute porphyria with a high degree of confidence [2,4], other conditions that should be considered include variegate porphyria and coproporphyria exhibiting only manifestations of photosensitivity, adult onset congenital porphoria, mixed porphrias, and a variety of other rare porphyrin disorders [2,4]. While the uro/hepta ratio can help identify those urine porphyrin results that are more likely to be associated with disorders other than PCT, it should be emphasized this is intended as an initial evaluation. Final diagnosis should be based on additional analyses of blood and fecal specimens [4].

The uro/iso ratio does not appear to furnish as much information as the uro/hepta ratio for 2 reasons: rank ordering of the uro/iso ratio (Fig. 3) did not show as clear an inflection point, and the uro/iso inflection point did not differentiate as clearly between PCT and acute porphyria (2 of 14 cases were below the inflection point). However, the uro/iso ratio still contained useful information. The present study identified 5 cases where the uro/hepta ratio was <2.0 (suggestive of PCT) but where the uro/iso ratio was >18 (not consistent with most cases of PCT). In other words, the isocoproporphyrin peak was markedly lower than what was seen with the majority of PCT cases. Thus, the uro/iso ratio can identify a few additional cases in which the less common porphyrin disorders should be considered as more probable.

While PCT is described as showing uroporphyrin and heptacarboxylate porphyrin concentrations of approximately equal magnitude [2-4], a more quantitative estimate of the variability can be deduced from Fig. 2. The 116 results below the inflection point are those most consistent with PCT, and show a uro/hepta range of 0.53-1.99. Uroporphyrin is anywhere from one-half to twice the concentration of heptacarboxylate porphyrin. In Fig. 1, for example, the uro/hepta ratio is 1.8. Likewise, PCT is described as demonstrating a significant isocoproporphyrin peak [2-4]. The isocoproporphyrin variability can be estimated from Fig. 3, where the 116 results below the inflection point show a uro/iso range of 0.34-17.3 and an average ratio of 7.1. The uroporphyrin concentration averaged about 7 times larger than the isocoproporphyrin concentration. In Fig. 1, for example, the uro/iso ratio is 8.9.

Patients who present with attacks of acute porphyria are more likely to be female, and in the present study, 12 of 14 cases of acute porphyria were from women [4]. Among the 111 results identified as most consistent with PCT, 77% were from men. PCT is well known to occur more frequently in men [1], in contrast to the acute porphyrrias. Above the inflection points in Fig. 2 and 3, there were 16 patient results that showed characteristics different from both the acute porphyrias and PCT. The distribution in this group was more random (6 males and 10 females). Although this is an interesting finding, 16 patients comprise a small number, and the sex of the patient is not useful for diagnosis. It suggests, however, that the study sample is consistent with at least one characteristic expected in a population of patients with porphyrin disorders.

In conclusion, urine is the most frequent specimen used in the initial evaluation of adult patients who present with photosensitivity. In the characterization of urine porphyrin chromatograms, calculation of uro/hepta and uro/iso ratios can provide useful information when the porphyrins are elevated. The most frequent abnormal pattern, and that most consistent with PCT,
is an uro/hepta ratio ≤2.0 and an uro/iso ratio ≤18. When either ratio is larger, additional testing for urine PBG is an essential aid for accurate laboratory interpretation. When the uro/hepta or uro/iso ratios are not consistent with the commonly occurring PCT pattern, less common porphyrin disorders should be considered. This approach is intended for initial evaluation, and the final diagnosis of porphyrin disorders manifesting photosensitivity should be made only after additional tests of blood and fecal specimens.

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References