Effects of Polyhalogenated Aromatic Compounds on Porphyrin Metabolism

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Heme production is a vital metabolic process that occurs in the bone marrow and liver. Porphyrins are unused by-products of this biosynthetic process and normally occur in urine and other body fluids in low concentrations.

Various disorders can disrupt the heme biosynthetic process, causing greater quantities of porphyrins in urine. The porphyrias are a group of diseases characterized by excessive porphyrins and other precursors in urine. Porphyrias may be either hereditary or acquired through exposure to certain drugs or chemicals. Porphyrria cutanea tarda (PCT) is the disease associated with exposure to polyhalogenated aromatic compounds. The urinary porphyrin pattern is of great value in diagnosing PCT and defining the etiology of the disease. As this liver disease from chemical damage develops, the urinary pattern progressively changes. With the development of a rapid and sensitive high-performance liquid chromatography analysis, urinary porphyrin patterns can be easily monitored. All free porphyrin acids can be quantitatively analyzed in less than 15 min.

In our studies of groups exposed to porphyrinogenic chemicals, we have not observed clear differences in the urinary porphyrin patterns of cases when compared with carefully selected controls. In animal studies, however, PCT was clearly associated with polybrominated biphenyl exposure. Future evaluation of the utility of urinary porphyrin patterns as a diagnostic tool will require a cohort that has received a recent, well-documented exposure and a comparable control population. Assay of erythrocyte uroporphyrinogen decarboxylase activity will also be needed to define the form of the PCT.

Introduction

The ability to recognize disease in its early stages before overt symptoms appear is a continuing goal of medical science. Accomplishing this goal often results in treatment which arrests or prevents further development of the disease.

Monitoring various biochemical functions may provide this type of information. Our interest in this area has been in the evaluation of the heme biosynthetic pathway under the influence of environmental chemicals via urinary porphyrin analyses. This paper describes the heme biosynthetic pathway and the changes that may occur with exposure to polyhalogenated aromatic compounds. The importance of recognizing various urinary porphyrin patterns by high-performance liquid chromatography (HPLC) is discussed, and demonstrated, and suggestions are made for using these patterns as a diagnostic tool.

Heme Biosynthesis

The production of heme is a vital metabolic process, and disturbances in the biosynthesis of heme can lead to a group of diseases known as the porphyrias. Heme is produced principally in the bone marrow and the liver. Most heme (85%) is made in the bone marrow and is used to make hemoglobin for new red blood cells (1). About 15% of the body’s heme production occurs in the liver. This heme is used in making enzymes, including microsomal cytochrome P-450 and other cytochromes. The biosynthesis of heme, as depicted in Figure 1, begins in the mitochondrion of a cell with the condensation of glycine and succinate to produce 5-aminolevulinic acid (ALA). Two molecules of ALA are used to form porphobilinogen (PBG), and four molecules of PBG are condensed to form uroporphyrinogen (UPG). This molecule is successively decarboxylated and oxidized to protoporphyrin (PP), which is oxidized and converted to heme by insertion of a ferrous ion.

Porphyrins are by-products of the biosynthetic pathway to heme and are derived from oxidation of the porphyrinogens. The body produces two isomeric types of porphyrinogens and the corresponding porphyrins.
Porphyria

The porphyrias are a group of diseases caused by enzyme deficiencies along the heme biosynthetic pathway and are characterized by abnormal and excessive porphyrins and other precursors in body fluids and tissues. There are several types of porphyria, each with its own set of symptoms and biochemical signs. The porphyria that has been associated with exposure to polyhalogenated aromatic compounds (PHAs) is chronic hepatic porphyria (CHP). CHP may be a subclinical disease in which only the urinary porphyrin excretion is abnormal or it may develop into its most severe form, porphyria cutanea tarda (PCT). PCT is a skin disease manifested by increased mechanical fragility of the skin; sensitivity of exposed skin to sunlight, which produces blistering with vesicles and ulcerative lesions on the hands, neck, face, and feet; excessive growth of hair (hypertrichosis), particularly facial hair; hyperpigmentation of the skin; and excess porphyrins in the urine, which may be visibly pink and fluoresces red under ultraviolet light (2).

CHP is characterized as a membrane disease in which there is damage to the membranes of the cell walls of the hepatocytes and organelles (i.e., mitochondria, endoplasmic reticulum) within the liver cell (3, 4). The cause of damage is unknown. Recent reports provide some evidence that polyhalogenated aromatics (i.e., polybrominated biphenyls [PBBs]) produce changes in lipid metabolism, which in turn alters membrane structure (5). This change in membrane structure may cause a change in membrane permeability (damage) so that porphyrins are excreted in the bile capillaries and the intercellular substance. In addition to these changes, these porphyrinogenic chemicals or their metabolites cause the induction of ALA synthetase and inhibition of the enzyme uroporphyrinogen decarboxylase (UPD). The changes in enzyme activities produce a buildup of uroporphyrinogen and heptacarboxylporphyrinogen, and these precursors are excreted in the urine where they are easily oxidized to uroporphyrin and heptacarboxylporphyrin. A urinary porphyrin pattern in which these two porphyrins are dominant is particularly diagnostic of this enzyme deficiency and PCT (6-9).
There are two forms of PCT: familial or hereditary and acquired or chemically induced (10–12). It is possible and important to distinguish between these two forms. In the hereditary form, at least some family members exhibit overt or subclinical signs of PCT. Additionally, the activity of the enzyme UPD is substantially decreased in the liver and in the erythrocytes. In the chemically induced porphyria, there is no evidence of PCT among relatives, and UPD activity is depressed in the liver but is normal in the erythrocytes. Measuring this erythrocyte enzyme activity in subjects with abnormal porphyrin patterns is necessary to distinguish between hereditary and chemically induced porphyria.

Various chemicals have been reported to induce PCT and other subclinical states of CHP in humans. These include alcohol, estrogens, hexachlorobenzene, 2,3,7,8-tetrachlorodibenzo-p-dioxin, methyl chloride, lindane polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs) and vinyl chloride. Alcohol and estrogens are the most common chemicals which have been observed to cause PCT (1,3,13–15). In Turkey in the 1950s, several thousand persons contracted PCT and a "mixed" porphyria from exposure to hexachlorobenzene (16). Even after 25 years, many of the people still exhibit signs and symptoms of this porphyria. In Czechoslovakia, workers from a 2,4,5-T production plant became ill from exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (17). At the initial investigation, 20% had PCT and another 21% had uroporphyrinuria, but 10 years later these signs and symptoms were rare. Isolated cases of PCT and porphyrinuria from methyl chloride and lindane have also been reported (18–20). Polychlorinated biphenyls (PCBs), PBBs, and vinyl chloride have been reported to produce subclinical CHP (21–23).

**Monitoring Urinary Porphyrin Patterns**

Determining urinary porphyrin patterns is an important aid in diagnosing porphyria (6–9). In the early 1970s, Doss pointed out that thin-layer chromatography (TLC) provided quantitative and qualitative data that allowed PCT to be diagnosed from a single urine sample (6). This same author developed the theory that CHP, whether hereditary or acquired by chemical exposure, developed in stages, and these stages are recognizable by their distinct urinary porphyrin patterns (24). Strik refined these pattern classifications (Table 1) and has used these for recognizing early effects from exposure to porphyrinogenic chemicals (25).

In animals or humans exposed to porphyrinogenic chemicals, a normal liver and urinary porphyrin pattern change into early liver disease and coproporphyrinuria may be observed. As the liver disease from chemical damage develops, the urinary patterns change progressively in steps to: a type A pattern in which the uroporphyrins or heptacarboxylylporphyrins are present in amounts less than coproporphyrin; a type B pattern

| Pattern classification | U/C (%) | T/C (%) | U % | 7 % | (U + 7) % |
|------------------------|---------|---------|-----|-----|----------|
| Normal                 | 0.2–0.5 | <1      | 20  | 5   | 25       |
| Coproporphrinuria      | <0.2    | <1      | 20  | 5   | 25       |
| Type A                 | <1      | <1      | 30  | 5–15| 30–50    |
| Type B                 | >1      | <1      | 30–50| 15–20| 45–70   |
| Type C                 | >1      | >1      | 50  | 20–30| 60–80   |
| Type D (porphyria cutanea tarda, PCT) | >1 | >1 | 60 | 25–35 | 65–90 |

* *Adapted from Strik, et al. (25).*

U = uroporphyrin; 7 = heptacarboxylylporphyrin; C = coproporphyrin.
phyrin. These latter patterns (C and D) are observed in porphyria cutanea tarda. As perhaps might be expected, the uroporphyrin and heptacarboxylporphyrin are the two porphyrins found in porphyrin liver tissue (26).

With the development of HPLC, determining these urinary porphyrin patterns has become much easier, less time-consuming, and more adaptable to routine analysis.

All free porphyrin acids can be quantitatively analyzed by ion-pair chromatography with isocratic elution in less than 15 min, and the pattern can be assessed to identify abnormal hepatic conditions (9) (Fig. 3). These analyses can be used to follow the development of CHP, as in Figure 4, where the urinary outputs of groups of female rats given 1 g/kg of PBB (Firemaster) or 0 g/kg (controls) were followed. CHP becomes evident about 55 to 60 days after dosing, as indicated by the large increase in uroporphyrin and heptacarboxylporphyrin and the corresponding ratios of these porphyrins to coproporphyrin. Between 85 and 100 days, CHP develops into its most severe form (type D).

It is clear that identifying the urinary porphyrin pattern may be important in determining chronic liver disease and that applying this technique in studies of populations exposed to porphyrinogenic chemicals may be a key to early recognition of chemically induced hepatic disorders. Strik recently reported that there was a clear increase in the incidence of type A urinary porphyrin patterns among farm families exposed to PBB when compared with nonexposed farm families (22). Similarly, Doss found liver damage and an excess of type A urinary porphyrin patterns among vinyl chloride chemical workers (23).

In our studies of groups exposed to porphyrinogenic chemicals, we have not observed the clear differences in urinary porphyrin patterns that Strik (22) and Doss (23) observed: 47% and 75% abnormal patterns, respectively. Generally, 80 to 90% of our exposed groups had normal patterns, and coproporphyrinuria occurred about equally (5–8%) in cases and controls. Type A patterns (2–12%) were found in both cases and controls. These patterns occurred slightly more frequently in cases, but the differences were not significant. One type B pattern and one PCT pattern were observed. The apparent lack of ability to distinguish between cases and controls in our limited studies does not indicate that urinary porphyrins are poor indicators of chemical exposure, since our study groups were exposed to these chemicals many years ago and have been removed from that exposure, and the effects upon the liver (if ever present) were not present at the time of our studies. This may also reflect a lack of significant chemical exposure of individuals in the groups studied.

The reversibility or repair of liver disease and porphyria upon removal of chemical exposure is well supported by the Czechoslovakian study of TCDD-exposed workers (17). Porphyrinuria or PCT was initially observed in 40% of this group, but 10 years later these signs and symptoms were rare. In our animal studies, the type A porphyrin pattern proved to be only a normal variant among PBB-dosed animals and nonexposed controls. Only type B or more advanced

![Figure 4](image-url)
patterns were clearly associated only with PBB exposure. This does not mean that the type A pattern observed among the PBB-exposed animals was not produced by the PBB, but normal variations in control animals made it impossible to distinguish such effects with our small groups.

Conclusions

To evaluate the utility of urinary porphyrin patterns as a tool for recognizing exposure to PHAs, investigators must follow a population in which exposure has been recent and well documented. A comparable control population should also be selected so that any significant difference in porphyrin patterns and other biochemical values can be readily discerned.

Urine samples must be preserved properly (9) and must be analyzed by HPLC (or TLC) with proper quality control measures to provide the needed specificity. Investigators should also note exposure to alcohol and estrogenic substances, which are also porphyrinogenic agents. All cases in which PCT has been confirmed or is suspected should be assayed for erythrocyte UPD activity. A normal value, with complementary absence of PCT in family members, will likely indicate that the PCT was acquired from chemical exposure. Perhaps with the use of these and other techniques, chemically induced disease can be more clearly defined.

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