Haem biosynthesis and human porphyria cutanea tarda: Effects of alcohol intake

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The review describes the structural and biochemical properties of the haem biosynthetic enzyme, uroporphyrinogen decarboxylase (UROD), which sequentially catalyzes the removal of the four carboxyl groups from the acetate side chains of octacarboxylic uroporphyrinogen to form coproporphyrinogen, and the possible biochemical mechanism of the genesis of porphyria cutanea tarda (PCT). The disease is caused when the activity of UROD is significantly reduced. PCT is a multifactorial disease where both inherent and environmental factors such as alcohol, estrogens, halogenated aromatic hydrocarbons and viral infection (mainly hepatitis C) are involved in biochemical and clinical expression. In PCT, hepatic iron plays a key role. Alcohol intake could induce mobilization of iron from protein-bound ferritin. PCT should be managed by avoidance of these toxins and removal of iron by vigorous phlebotomy. Such iron-reduction therapy would provide additional benefit for hepatitis C patients by interferon therapy.

Pathway of haem biosynthesis

In animals, haem is synthesized from the precursors of glycine, succinyl coA and iron in a series of reactions catalyzed by eight different enzymes (Fig. 1). The first and the last three steps of the synthesis take place within the mitochondria, whereas the intermediates are carried out in the cytoplasm. With the exception of the first enzyme, deficiencies of each enzyme of the pathway are associated with at least one type of porphyria.

Porphyrin, a tetrapyrrole, is an ubiquitous "pigment of life". By chelating with iron, the metalloporphyrin participates in the transfer and consumption of oxygen via haem in animals. On the other hand, chlorophyll, the magnesium-chelated porphyrin, is responsible for the generation of oxygen in plant leaves. Haem is a vital constituent of cellular hemoproteins necessary for important functions such as the transport and storage of oxygen (e.g. hemoglobin, myoglobin), electron transport (e.g. respiratory cytochromes) and oxidation-reduction reactions (e.g. cytochrome P450 enzymes). The bone marrow is the site where the largest amount of haem is synthesized for hemoglobin formation. A large amount of haem is also synthesized in the liver, mostly as cytochrome P450 enzymes. Both in the bone marrow and the liver, the pathway of haem synthesis is the same, but the regulation in these tissues is different in a number of important respects.

In this overview, the author presents the enzymatic pathway for haem biosynthesis with special emphasis on uroporphyrinogen decarboxylase, the fifth enzyme whose activity in the liver is significantly reduced in porphyria cutanea tarda, and the current understanding of the etiology and the pathogenesis of the disease.
cyclize non-enzymatically to form uroporphyrinogen I which cannot undergo metabolism beyond coproporphyrinogen I. Alternatively, it can cyclize enzymatically in the presence of uroporphyrinogen III cosynthase in the fourth step to form, more rapidly, uroporphyrinogen III.

![Diagram](image)

Fig. 1—Synthesis of haem and enzyme deficiencies in porphyria

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Classification of disease</th>
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<tr>
<td>1) ALA Synthase</td>
<td>ALA Dehydratase Deficient Porphyria</td>
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<td>2) ALA Dehydratase</td>
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<td>3) PBG Deaminase</td>
<td>Congenital Erythropoietic Porphyria</td>
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<td>4) URO III Cosynthase</td>
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<td>5) URO III Decarboxylase</td>
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<tr>
<td>6) Cepro III Oxidase</td>
<td>Hereditary Coproporphyria</td>
<td>Dominant</td>
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<td>7) Proto IX Oxidase</td>
<td>Variegate Porphyria</td>
<td>Dominant</td>
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<tr>
<td>8) Ferrochelatase</td>
<td>Erythropoietic Protoporphyria</td>
<td>Dominant</td>
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Abbreviations: ALA—Delta-aminolevulinic acid, PBG—Porphobilinogen, Uro—Uroporphyrinogen, Cepro—Coproporphyrinogen, Proto Protoporphyrinogen

The fifth step of haem biosynthesis is catalyzed by the cytosolic enzyme uroporphyrinogen decarboxylase (UROD). It catalyzes the sequential removal of the four carboxyl groups from the acetate acid side chains of octacarboxylic uroporphyrinogen III to form the tetracarboxylic coproporphyrinogen III (Fig. 2). At low substrate concentration, uroporphyrinogen III is decarboxylated via a preferred clockwise route that begins at the acetate side chains of ring D, through rings A and B and ends at ring C. On the other hand, uroporphyrinogen III (at higher concentration) and uroporphyrinogen I are decarboxylated by a random mechanism. Among the uroporphyrinogen isomers, uroporphyrinogen III is the preferred substrate for the enzyme. The full length cDNA for the human enzyme has been isolated and sequenced. The gene is about 3 kb in length, contains 10 exons, and is found on chromosome 1p34, as shown by in situ hybridization techniques. The gene for human UROD encodes a cytosolic protein of 367 residues with a predicted molecular weight (MW) of 40.8 kDa. In one study it was demonstrated that a single monomeric enzyme from human erythrocytes catalyzes all the four decarboxylation steps. On the other hand, the enzyme purified from chicken erythrocytes was shown to be a dimer with MW 79 kDa. Evidence for two distinct UROD activity peaks (present as a complex in its native state) with MW ~ 60 kDa and ~ 40 kDa from human red cells, purified to homogeneity by uroporphyrin-sepharose affinity chromatography, and absence of immunological cross-reactivity of anti-UROD 60 kDa antibodies with purified UROD 40 kDa, clearly ruled out the existence of only a single monomeric enzyme in vivo. Other studies indirectly support this observation. The recombinant enzyme has now been crystallized and its structure has been defined. The authors predicted from their equilibrium ultracentrifugation data that recombinant UROD is a dimer with MW 92-98 kDa which dissociates during gel filtration chromatography into a 66 kDa protein. Their SDS-PAGE data showed that the monomer had a MW of 44 kDa. The enzyme may contain multiple active sites or there may be a single active site for each UROD subunit. The possible modes of reactions for UROD are: (i) two uroporphyrinogen molecules may attach and then they are decarboxylated separately by the adjacent monomers or enzyme molecules. It has been observed that the two UROD activity peaks decarboxylated porphyrinogen with distinctly different substrate specificities, (ii) the partially decarboxylated products from uroporphyrinogen are shuffled between the enzyme molecules where each has different catalytic mechanism, or one is involved in specific catalysis and the other for transfer of intermediates; (iii) uroporphyrinogen III formed in vivo by the action of uroporphyrinogen III cosynthase is “handed over” in a specific orientation, which then undergoes a series of rotations on UROD before the enzyme catalyzes the reaction in an orderly clockwise pathway.

In the sixth step, coproporphyrinogen oxidase which is localized in the intermembrane space of liver mitochondria, catalyzes the oxidative decarboxylation of two of the four propionic acid groups of coproporphyrinogen III (on ring A followed by ring B) to form the two vinyl groups at positions 2 and 4 of protoporphyrinogen IX, a decarboxylic porphyrino-
gen. Protoporphyrinogen oxidase in the seventh step then catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX by the removal of six hydrogen atoms. This enzyme is localized in the inner mitochondrial membrane. In the eighth and final step, Ferrochelatase catalyzes the insertion of ferrous iron into protoporphyrin IX.

The tetrapyrrole intermediates preceding protoporphyrin IX (which is an oxidized form) are porphyrinogens (reduced form). In the human liver, ALAS and PBG deaminase are the rate-limiting enzymes, and the activity of UROD is approximately 90 times higher than the rate-limiting level\(^1\). Any uroporphyrinogen formed is therefore likely to be converted very rapidly to protoporphyrin IX.

**Human diseases with uroporphyrinogen decarboxylase defects**

The subnormal activity (50% of the normal value) of UROD in the liver is the defect observed in patients with overt porphyria cutanea tarda (PCT), the most common form of porphyria in humans\(^{13,14}\). Both inherited and acquired factors are involved in the clinical expression of the disease. PCT is often developed in later life (hence the name tarda, which in Latin means “late”). Classification of the disease distinguishes: (i) Type I sporadic PCT (S-PCT) which exhibits decreased UROD activity in the liver, but not in the erythrocytes, and there is no family history of the disease; (ii) Type II familial PCT (F-PCT) which is transmitted as an autosomal dominant genetic trait, the half-normal enzyme activity and immunoreactivity of the enzyme (i.e. CRIM negative) is apparently present in all tissues; (iii) Type III PCT manifests itself in a few patients with at least one other family member with PCT, but a normal UROD activity in the erythrocytes; (iv) another variant, which is rare, has been called hepatoerythropoietic porphyria (HEP). It occurs in individuals with two mutant UROD alleles, either homozygotes for a single mutation or as compound heterozygotes with two different mutant alleles. HEP is developed during childhood and may be quite severe. Mutational analyses in UROD show that in F-PCT the decreased UROD activity reflects only expression of normal allele\(^1\), and in more common S-PCT no mutations have been identified in the UROD gene\(^1\). In F-PCT, the UROD might exist as a normal-mutant heterodimer\(^8\), but the recombinant techniques used to study the mutation could be performed on mutant homodimers\(^8\). The heterodimers could show different catalytic properties as compared to the normal homodimers existing in non-porphyric subjects\(^9\). In the human red cell, an intrinsically abnormal UROD in F-PCT with subnormal affinity for its substrate has been observed\(^17,18\). Since human UROD is encoded by a single gene which is transcribed to produce the same mRNA in erythroid and non-erythroid tissues\(^8\), the same enzyme defect is expected to be present in the liver tissue. This should not be sufficient for clinical and biochemical expres-

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**Fig. 2—Decarboxylation of uroporphyrinogen III; the letters a, b, c, and d denote the position on which the acetic acid group on ring A, B, C, and D respectively are decarboxylated to a methyl group.**
sion of active disease, because the normal enzyme activity in the human liver is about 90 times higher than the rate-limiting level\textsuperscript{13}. Indeed, most relatives with the same enzyme defect as the propositi in FPCT do not show disease activity. In S-PCT, it is possible that mutations occur in uncharacterized promoter region or at other loci that may predispose to hepatic UROD deficiency\textsuperscript{16}. Another variant is toxic PCT which is associated with exposure to halogenated aromatic hydrocarbons such as hexachlorobenzene (HCB), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and the polychlorinated biphenyls (PCB). Between 1956 and 1961 an epidemic affecting more than 3000 people in Turkey occurred following exposure to HCB that had been used as a seed wheat fungicide. Experimental uroporphyria resembling PCT can be induced in some mouse strains (e.g. SWR, C57BL/10 and C57BL/6) by feeding HCB or TCDD with a single injection of iron-dextran or ALA in drinking water. Some form of inherited factors are present in the mouse strain prone to develop uroporphyria\textsuperscript{19}. In another strain, e.g. DBA/2, mice are resistant to developing porphyria. An absolute requirement for hepatic cytochrome P-450 isoform CYPIA2 in causing experimental uroporphyria in knockout mice following injection of iron-dextran and giving

![Diagram of metabolic pathways](image)

Fig. 3—Reviewer’s hypothetical mechanism for ethanol-induced mobilization of physiological iron (Fe\textsuperscript{2+}) from ferritin, which amplies the metabolic defect in hepatic uroporphyrinogen decarboxylase (UROD) in porphyria cutanea tarda (PCT).

Ferritin iron (Fe\textsuperscript{2+}) can be mobilized from the protein by O\textsubscript{2} due to one-electron reduction. Fe\textsuperscript{2+} can bind at the cation binding site of UROD and competitively inhibit the enzyme, and generate oxyradicals which may oxidize proximal amino acid residues and cause peptide bond cleavage. Fe\textsuperscript{2+}-catalyzed Haber-Weiss reaction may produce hydroxyl radical (OH\textsuperscript{-}) that cause lipid peroxidation and tissue damage. The oxyradicals can oxidize the porphyrinogen substrate to nonmetabolizable porphyrins and cause disease.

Abbreviations: ADH—Alcohol dehydrogenase, MEOS—Reaction catalyzed by cytochrome P4502E1, ALDH—Aldehyde dehydrogenase, UO—Uric oxidase, UROD—Uroporphyrinogen decarboxylase, XO—Xanthine oxidase, XDХ—Xanthine dehydrogenase
ALA has been reported. When haem synthesis is impaired due to the reduction of UROD activity to a certain level, the substrate and intermediates (particularly uro- and heptacarboxylic porphyrinogens) accumulate and oxidize at the site of synthesis, which is mainly the liver, and spill into the blood, from where they may be deposited in the skin and also be excreted in the urine. Porphyrins emit intense red light when excited by light of the 400 nm Soret band wavelength. The light-absorbing and fluorescent properties of porphyrins stem from the resonance of the system of conjugated double bonds. In the skin, porphyrins absorb the sun’s radiant energy, get activated and promote formation of highly reactive oxidizing forms of oxygen, which in turn damage tissue by peroxidation of lysosomal membranes. Cells are damaged due to the release of hydrolytic and proteolytic enzymes from the lysosomes. Cutaneous lesions at the light-exposed areas on the hands and face are formed which subsequently lead to the appearance of vesicles and bullae. Occasionally, the lesions can become infected. In PCT, there is an increased risk of development of hepatocellular carcinoma in the liver.

“Trigger” effects of alcohol intake for the pathogenesis of uroporphyria (Porphyria cutanea tarda)

PCT appears to be an iron-dependent disorder. This is based on the following observations: (i) hepatocellular siderosis is found in almost all patients; (ii) removal of iron by phlebotomy induces both clinical and biochemical remission; (iii) administration of iron is followed by relapse in patients in whom a remission has been induced by phlebotomy therapy. In recent studies, increased frequency of genetic mutation in hereditary hemochromatosis in HFE gene has been shown to be associated with the hepatic iron overload in patients with PCT. Inhibition of UROD activity has been observed by ferrous iron. Also, free radicals are generated when Fe²⁺ is oxidized which then convert porphyrinogen to nonmetabolizable porphyrins, and may damage UROD protein. In vivo, excess iron is stored mainly as ferritin and hemosiderin. In the patients with PCT, the ferritin level was reported to be elevated relative to healthy controls and patients without liver siderosis. In the hepatocytes of patients with S-PCT and F-PCT and in C57BL/10 porphyric mice, a morphological co-occurrence of uroporphyrin crystals and ferritin iron have been reported. The accumulation of iron precedes uroporphyrin crystal formation. Iron has to be mobilized from ferritin in order to play a key role in the pathogenesis of PCT.

Alcohol ingestion has long been shown to be a major factor in precipitating the symptoms of PCT. In the majority of reported cases, PCT is more common in patients with alcoholic liver disease. Patients with S-PCT frequently have a history of heavy alcohol consumption. Ethanol has been reported to increase uptake of iron in patients with PCT and also in normal subjects, and to increase liver iron content. The level of iron in the liver has been reported to be significantly higher in patients with alcohol-related chronic hepatitis, alcoholic hepatitis and chronic viral hepatitis patients in comparison to controls. Super oxide radicals (O²⁻) produced by xanthine oxidase is able to release iron from ferritin. The metabolism of ethanol in the liver via alcohol dehydrogenase (ADH) pathway involving xanthine oxidase generates O²⁻ radicals. Cytochrome P4502E1 is the main enzyme that oxidizes ethanol in the non-ADH pathway, but the degradation of acetaldelyde produced by the non-ADH pathway is slower than that produced by the ADH pathway. During ethanol metabolism, increased nucleotide catabolism has been observed, which produces substrates such as xanthine and hypoxanthine with greater affinities for xanthine oxidase, and the concentration of hepatic NADH is increased. A shift of xanthine metabolism from the xanthine dehydrogenase (XDH) to the oxidase pathway (due to NADH-mediated inhibition of XDH activity) may contribute to O²⁻ production (Fig. 3). Step by step, we observed in vitro that O²⁻ radicals are generated during the metabolism of ethanol via ADH pathway involving xanthine oxidase. O²⁻ reductively released Fe²⁺ from ferritin leading to the inhibition of UROD activity and induction of microsomal lipid peroxidation. We also observed in vivo that SWR mice became porphyrinic within two weeks when iron-dextran was injected and fed an ethanol liquid diet. The urinary porphyrins (mainly uro- and heptacarboxylic porphyrins) were significantly increased and the hepatic UROD activity was concomitantly decreased in alcohol-treated mice as compared to non-treated mice. No urinary porphyrin could be detected in the DBA/2 mice, when treated under similar conditions as SWR mice. This demonstrates first how alcohol intake amplifies the inherent metabolic defect of reduced enzyme-substrate affinity to cause disease.

Autooxidiation of the Fe²⁺ ion in the presence of atmospheric oxygen generates O²⁻ radicals due to the acceptance of a single electron by a ground state oxy-
gen molecule. A univalent oxidation (by abstration of hydrogen atom) of uroporphyrinogen by a superoxide anion could initiate a chain reaction in which O_2^- is a propagating species (UPH_6 + 3O_2 + Me^n → UP + 3H_2O_2 + Me^(n+1), where UPH_6 and UP are the reduced and oxidized forms of porphyrins and Me is a metal cation, e.g. Fe^{2+}). Fe^{2+} is an important catalyst in the production of the hydroxyl radical (OH) from H_2O_2 through the Fenton reaction (H_2O_2 + Fe^{2+} → OH + OH + Fe^{3+}). OH radicals can oxidize uroporphyrinogen by abstration of hydrogen atoms with extremely high rate constants. OH radicals may damage UROD and induce lipid peroxidation and liver damage. The dual effect of Fe^{2+} on UROD activity could be studied by using Zn^{2+} in parallel experiments, because Zn^{2+} exists only in one oxidation state. Therefore, Zn^{2+} would not function as a redox system that makes it unable to facilitate the formation of active oxygen species.

Other etiological factors

PCT seems also to be provoked by estrogens and viruses. It is currently known that in certain countries PCT patients have a high prevalence of hepatitis C virus (HCV) infection. A frequent association has also been reported between PCT and the hepatitis B virus, and also between PCT and human immunodeficiency virus (HIV). In southern Europe the prevalence of HCV infection in patients with active PCT is high (70-90%), low (8-18%) in northern Europe, Australia, and New Zealand, and moderate (50-60%) in North America. It is also observed that sporadic PCT patients are more significantly infected by hepatitis C than familial PCT patients. The marked variation from country to country in the incidence of positivity of HCV infection in S-PCT patients could be explained by the sensitivity of different alleles of modulatory genes of UROD to viral infection. Most patients with chronic HCV infection did not develop PCT. Among the men in the North American patient selection, about 80% used alcohol. A potential link between chronic hepatitis C and PCT is iron in the liver. Alcohol, iron, and chronic HCV infection all produce increased oxidative stress in the liver, a condition conducive for the development of PCT. Hepatic iron reduction therapy ameliorates the severity of chronic hepatitis C, and may improve its response to interferon therapy and bring remission to active PCT. Heavy alcohol use may exacerbate HCV infection. HIV infection, likewise, has other risk factors for development of PCT including heavy alcohol use, iron overload, and/or concomitant HCV infection. These agents may act by unmasking PCT in genetically susceptible individuals.

Treatment of PCT by phlebotomy or low-dose chloroquine is highly effective. Patients should cease exposure to alcohol, estrogens, iron supplements or other exogenous agents that are judged to have contributed to the disease. Although some patients improve dramatically after the cessation of alcohol, the results are generally unpredictable and slow. Therefore, it is generally advisable to begin phlebotomy as well. For patients with end-stage renal disease, recombinant erythropoetin treatment with or without phlebotomy has become the treatment of choice for PCT.

Conclusion

In the human liver, the uroporphyrinogen decarboxylase (UROD) has activity greatly in excess of the rate-limiting level. Therefore, the uroporphyrinogen substrate and the intermediates are rapidly converted to coproporphyrinogen and then to haem, a vital constituent necessary for life. Even in approximately 20% of patients from families showing autosomal dominant inheritance in familial porphyria cutanea tarda (F-PCT) with half-normal enzyme activity in all tissues, further inactivation of the enzyme in the liver appears to be necessary for clinical expression of the disease. In this group, the inherent defect in UROD apparently is the reduced affinity for its substrate. Until now, no causative mutations at the UROD locus have been detected in the more common sporadic PCT. Hepatic iron has a dual effect in that it inhibits the enzyme and oxidizes the porphyrinogen to non-metabolizable porphyrin, causing the disease. Only in certain individuals prone to develop PCT, the intake of alcohol and polyhalogenated aromatic hydrocarbons exacerbate the metabolic defect in UROD. Intake of alcohol can induce mobilization of iron (Fe^{2+}) from protein-bound ferritin. Iron not only may damage UROD and oxidize porphyrinogen, but it also induces lipid peroxidation and liver damage. Protection from these factors and removal of iron by phlebotomy are the treatments of choice for the disease.

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References


