Comparison of Human and Bovine Protoporphyria¹

DAVID A. BRENNER AND JOSEPH R. BLOOMER

Howard Hughes Medical Institute
Laboratory, Yale University School of Medicine,
New Haven, Connecticut

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Protoporphyria (PP) is an inherited disorder of porphyrin metabolism in man in which there is excessive accumulation and excretion of porphyrin. Recently, a similar disorder has been described in cattle. In this report, the clinical, biochemical, and genetic features of bovine and human PP are compared.

Human and bovine PP are characterized by photosensitivity and elevation of erythrocyte and fecal protoporphyrin levels. In both disorders, a deficiency of heme synthase activity is present in all tissues which have been examined. The diseases differ clinically in that hepatobiliary disease has been found thus far only in human PP. They also have different inheritance patterns. Human PP is an autosomal dominant disease, while initial studies strongly suggest that there is an autosomal recessive pattern of inheritance in bovine PP.

INTRODUCTION

The porphyrias are a group of inherited disorders in man in which there is increased formation and excretion of porphyrins and/or porphyrin precursors [1,2]. These compounds are intermediates in the biosynthetic pathway for heme, the prosthetic group for hemoproteins such as hemoglobin and the cytochromes.

The biochemical abnormalities characteristic of congenital erythropoietic porphyria have been found in animals, and the disease has previously been compared to that in humans [3,4]. Recently, an inherited disorder in cattle has been described in which the biochemical abnormalities are the same as in human protoporphyria (PP) [5,6]. This is the first animal model of the human disease. In this review, PP in cattle is compared to that in humans in order to identify both similarities and differences between the diseases.

Biochemical and Enzymatic Abnormalities (Table I)

The biochemical manifestations in each of the inherited porphyrias in man reflect a partial deficiency in the activity of a specific enzyme in the heme biosynthetic pathway. An accumulation of porphyrins and/or porphyrin precursors occurs because of the partial enzymatic defect.

The first reaction in the heme biosynthetic pathway, in which glycine condenses with succinyl-CoA to form 8-aminolevulinic acid (ALA), is the rate-limiting step [1,2]. The reaction is catalyzed in the mitochondria by ALA synthase. Hepatic activity of ALA synthase may increase markedly during acute attacks of porphyria,

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Address reprint requests to: Joseph R. Bloomer, M.D., University of Minnesota School of Medicine, 420 Delaware St. S.E., Minneapolis, MN 55455

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presumably as a secondary phenomenon in order to maintain adequate formation of heme [1,2].

Two molecules of ALA undergo dehydration condensation in the cytoplasm to form porphobilinogen. The pathway's next steps involve porphyrinogens, which are colorless, reduced compounds [1,2]. Uroporphyrin and coproporphyrin are fluorescent byproducts that are produced by the nonenzymatic oxidation of porphyrinogens. Protoporphyrin is formed by the enzymatic oxidation of protoporphyrinogen in mitochondria. In the final step of the pathway, the intramitochondrial enzyme heme synthase (also named ferrochelatase) catalyzes the chelation of ferrous iron to protoporphyrin to form heme.

Humans with PP have a partial deficiency of heme synthase activity in all tissues which have been examined, including bone marrow [7], peripheral blood cells [7–9], liver [10], and cultured skin fibroblasts [10–12]. The level of residual activity is 10 to 30 percent of normal. Most patients have a two-to-threefold increase of ALA synthase activity in their bone marrow and liver [7,13–16].

Cattle with PP also have a deficiency of heme synthase activity in all tissues which have been examined: liver, kidney, heart, spleen, and lung [5,6]. The level of residual activity is less than 10 percent of normal. Hepatic ALA synthase activity is increased threefold [5].

As a result of the deficiency in heme synthase activity, protoporphyrin is accumulated and excreted in excessive amounts. The biochemical hallmark of PP in humans and cattle is an elevated level of red cell and fecal protoporphyrin [1,2,5]. Since protoporphyrin is excreted only into bile, the urine does not contain increased amounts of porphyrins or porphyrin precursors in humans with the disorder. Urinary uroporphyrin and coproporphyrin are slightly increased in cattle, however [5].

When calves with PP are placed in dim light for several weeks, their red cell protoporphyrin levels remain unchanged, but fecal protoporphyrin falls from 1850 to 59 μg/g [5]. The effect of sun exposure on fecal and red cell protoporphyrin levels in humans has not been examined.
Clinical Manifestations (Table 1)

Photosensitivity that begins early in life is the most common clinical manifestation in both human and bovine PP [1,2,5]. After a variable exposure to sunlight (often within minutes), humans with PP experience itching and burning of the sun-exposed skin, followed by erythema and edema. Chronic skin changes may persist, with thickening and scarring of the exposed skin. Some patients report that the photosensitivity decreases with age [17].

Calves with PP which are exposed to sunlight develop cutaneous changes over their snouts, ears, and backs that consist of edema, erythema, fissures, alopecia, and scabbing. There is no erythrodontia. The cutaneous lesions heal when the calves are kept out of the sun. One calf lost her photosensitivity by three years of age [5].

Hepatobiliary disease also occurs in human PP. There is a greater frequency of cholelithiasis than in the normal population [17–21]. The gallstones contain a large amount of protoporphyrin [18,21].

The incidence of significant hepatic disease in patients with PP probably is less than 10 percent [21,22]. However, 15 cases have been reported in the world literature in which death has occurred from cirrhosis and hepatic failure [22]. At autopsy the livers of these patients are black due to massive protoporphyrin deposition. The deposits give a characteristic birefringence when examined by polarizing microscopy [23] and are composed of crystals [24].

Neither hepatic disease nor cholelithiasis has been reported in bovine PP [5,6]. The liver histology has not been examined.

Although a deficiency of heme synthase activity is the fundamental enzymatic abnormality in PP, anemia is not an important problem in either humans or cattle. Some patients have a slight decrease in hemoglobin and/or hematocrit levels, usually with hypochromic, microcytic indices [21,25]. Iron kinetics and red cell survival studies are usually normal [21,26,27]. Sideroblastic anemia has been reported in one patient who died in hepatic failure [28]. The bone marrow of patients with PP is normal except for fluorescence in late normoblasts and reticulocytes due to the presence of protoporphyrin [14,27].

In bovine PP, the red cell morphology and indices are normal, and there is no indication of abnormal hemoglobin metabolism or erythropoiesis [5,6]. The bone marrow has not been examined.

Inheritance (Table 1)

Human PP is inherited as an autosomal dominant disease [29]. Individuals may be asymptomatic despite biochemical abnormalities, demonstrating variable penetrance of the clinical manifestations of the disease. A study of heme synthase activity in cultured skin fibroblasts from members of three families with PP has been consistent with autosomal dominant inheritance [11].

The inheritance pattern in bovine PP appears to be autosomal recessive. The disease was originally found only in female calves [5,6]. Subsequently, male calves with PP have been identified (Schwartz, personal communication). The dams of the calves are clinically normal, but they show approximately a 50 percent reduction in hepatic heme synthase activity [5]. These data are consistent with a recessive inheritance pattern in which the heterozygous parents (each having a partial deficiency of heme synthase activity) produce homozygous offspring who have biochemical and clinical manifestations due to a severe deficiency of enzymatic
activity. All of the reported calves with PP were sired from one clinically asymptomatic bull, in whom heme synthase activity has not been measured.

DISCUSSION

The fundamental enzymatic defect in both human PP and bovine PP is a deficiency of heme synthase activity. Protoporphyrin, the substrate for heme synthase, accumulates because of this deficiency.

The liver and bone marrow, which are the major organs of heme synthesis, have both been implicated as sources of the excess protoporphyrin in human PP. The relative contributions of the liver and bone marrow may vary with each individual. Two studies support a predominantly erythropoietic origin for the excess protoporphyrin [30,31]. The relative contributions of the liver and bone marrow in bovine PP have not been determined.

The primary clinical manifestation of both human and bovine PP is photosensitivity, which begins early in life. The skin reaction is caused by exposure to light of wavelength around 400 nm [32], which is the point at which porphyrins absorb maximally (called the Soret band). The cutaneous changes are mainly confined to the upper dermis [33,34]. Circulating protoporphyrin or protoporphyrin deposited in skin absorbs light in the Soret region, leading to damage of the skin and/or blood vessels in the skin [35,36]. The reason why some calves and humans with PP apparently outgrow their photosensitivity is unknown.

Hepatobiliary disease also occurs in human PP, but is absent in bovine PP. The explanation for this may be that the small number of calves studied to date may be insufficient to detect a rare complication or that the calves may not develop hepatobiliary disease until they are older. Since hepatic disease may be a critical complication in humans with PP, its failure to occur in cattle with the disease would limit the usefulness of this as a model.

The most striking difference between human and bovine PP is the pattern of inheritance. Human PP is inherited as an autosomal dominant trait. Bovine PP, on the other hand, appears to be inherited as an autosomal recessive trait. Confirmation requires measuring heme synthase activity in tissues of the bull which sired the affected calves, as this should be intermediate between that observed in normal animals and calves with protoporphyrin. If the measurement substantiates autosomal recessive inheritance, it remains to be determined why cattle express the disease only in the homozygous condition, whereas in humans it is expressed in the heterozygous state. Direct comparison of the specific activity of heme synthase in several tissues from cattle and humans may provide an explanation, as it is possible that the specific activity is higher in bovine tissue (i.e., a higher catalytic rate/mg of enzyme protein or higher concentration of enzyme).

Despite these differences, bovine PP could provide a useful model to study aspects of the human disease. It will be of particular interest to investigate factors which may modify the production and excretion of protoporphyrin. This would include the effect of drugs [22,37], diet [38-40], and intravenous infusion of hematin [41]. Further study of this model may also provide information which is basic to our understanding of heme biosynthesis.

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