

## Hepatic Protoporphyrin Metabolism in Patients with Advanced Protoporphyrinic Liver Disease

Joseph R. Bloomer

*UAB Liver Center, University of Alabama at Birmingham, Birmingham, Alabama*

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Protoporphyrin is a genetic disorder in which liver damage is caused by the toxic effect of protoporphyrin accumulation in the liver. In this study protoporphyrin was measured in the resected livers of 7 patients who had liver transplantation and an additional patient from whom liver tissue was obtained post mortem. Comparison of liver, erythrocyte and serum protoporphyrin levels demonstrated a marked gradient between these compartments: erythrocyte,  $5781 \pm 655$   $\mu\text{g}/\text{dl}$ ; serum,  $384 \pm 102$   $\mu\text{g}/\text{dl}$ ; liver  $377,238 \pm 55,568$   $\mu\text{g}/100$  gm wet weight, (mean  $\pm$  SE). Protoporphyrin levels in bile of 3 patients were 55, 559, and 1,153  $\mu\text{g}/\text{dl}$ , indicating a gradient between liver and bile as well. Examination of the livers by polarization microscopy and electron microscopy demonstrated protoporphyrin pigment crystals. In one patient who had recurrent liver disease after transplantation, the protoporphyrin concentration in the graft at the time of death was similar to that in the resected liver. These data indicate that liver protoporphyrin levels in patients with advanced protoporphyrinic liver disease are much higher than levels in blood and bile, in part because protoporphyrin forms crystalline deposits in liver tissue. Thus, progressive hepatic accumulation of protoporphyrin occurs in the face of impaired biliary excretion. An intrinsic defect in hepatic excretion of protoporphyrin is probably not necessary for this condition to develop because liver disease can occur in the graft following transplantation.

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### INTRODUCTION

Protoporphyrin is a genetic disorder of porphyrin metabolism which was first described in 1961 [1]. The enzyme defect which underlies protoporphyrin is in ferrochelatase [2, 3], which catalyzes the insertion of iron into protoporphyrin to form heme. As a consequence of decreased ferrochelatase activity, there is excessive accumulation and excretion of protoporphyrin.

The major clinical feature is photosensitivity [1]. This is due to the photoactive properties of protoporphyrin on skin tissue [4, 5]. Liver disease, which is a less common but more important clinical feature [6-9], is due to the toxic effect of protoporphyrin on liver structure and function [10-12]. Protoporphyrin is excreted only in bile, and the liver is thus confronted with the excess protoporphyrin that is produced, irrespective of the tissue source. Histological examination of liver tissue from patients with advanced protoporphyrinic liver disease reveals extensive deposits of dark brown pigment which are composed of protoporphyrin [8, 13]. However, there is only limited information regarding the numerical quantity of protoporphyrin in liver tissue.

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<sup>a</sup> To whom all correspondence should be addressed: Joseph R. Bloomer, M.D., UAB Liver Center, 395 BHSB, 1918 University Blvd., Birmingham, AL 35294-0005. Tel.: 205-975-9698; Fax: 205-975-9393; E-mail: j\_bloomer@gasmac.dom.uab.edu.

In this report, information is provided regarding the concentration of protoporphyrin in the livers of 8 patients with advanced protoporphyric liver disease, comparing this to the concentrations in blood and bile. In 4 patients who had liver transplantation, protoporphyrin levels were also measured in the graft at various times after transplantation. The study was approved by the Institutional Review Boards at the University of Minnesota and The University of Alabama at Birmingham.

### MATERIALS AND METHODS

The patient population consisted of 3 males and 5 females with an age range of 13 to 51 years (Table 1). Seven patients (patients 1-7) had liver transplantation because of liver failure due to protoporphyric liver disease. Blood samples were obtained from patients just prior to transplantation, and bile was aspirated from the gallbladder of 2 patients at the time of transplantation. A portion of the resected liver specimen was obtained from each patient. In patient 8, liver tissue was obtained at postmortem exam following the patient's death from liver failure. A bile sample was collected from this patient pre-mortem through a cholecystostomy tube.

Erythrocyte and serum protoporphyrin levels were measured fluorometrically after solvent partitioning [12, 14]. The liver porphyrin concentrations were determined fluorometrically after extracting a portion of tissue with 0.6 N perchloric acid/methanol (1:1 v/v) [12, 14]. The porphyrin concentrations in bile samples were also measured fluorometrically, and high performance liquid chromatography was done on bile to determine the porphyrin profile [12, 15].

The liver specimens were all examined by polarization microscopy as previously described [16], and some were also examined by electron microscopy [8, 16].

**Table 1. Protoporphyrin levels (PP) in patients with advanced protoporphyric liver disease.**

Patient	Age/Sex	Erythrocyte PP ( $\mu\text{g}/\text{dl}$ )	Serum PP ( $\mu\text{g}/\text{dl}$ )	Liver PP ( $\mu\text{g}/100\text{g wet wt}$ )	Serum Bilirubin mg/dl
1	51/M	5276	160	395,700	10.8
2	38/F	5131	144	589,400	12.7
3	15/M	6470	236	459,900	4.7
4	18/F	6488	836	260,600	13.8
5	13/M	7679	448	522,600	8.5
6	14/F	2450	95	440,200	3.1
7	36/F	8240	362	170,800	15.0
8	33/F	4516	790	178,700	18.1
Mean $\pm$ SE	27 $\pm$ 5	5781 $\pm$ 655	384 $\pm$ 102	377,238 $\pm$ 55,568	10.8 $\pm$ 1.8
Normal/control		20-65	0	20-100	0 - 1.5

## RESULTS

The erythrocyte and serum levels of protoporphyrin in the patients (Table 1) were increased compared to patients with protoporphyria who do not have liver disease, in whom the levels usually are less than 2,000  $\mu\text{g}/\text{dl}$  and 50  $\mu\text{g}/\text{dl}$  respectively. This difference is due principally to the retention of protoporphyrin in blood because of the impaired liver function. Following liver transplantation, the erythrocyte and serum protoporphyrin levels declined to  $1,765 \pm 365$   $\text{mg}/\text{dl}$  and  $31 \pm 27$   $\text{mg}/\text{dl}$ , respectively, concomitant with a decrease in the serum bilirubin level to  $0.9 \pm 0.3$   $\text{mg}/\text{dl}$  and reflecting the improved hepatic function.

The liver of each patient was enlarged, firm in consistency and black in color. Diffuse red fluorescence was demonstrated by fluorescence microscopy in most of the hepatocytes. Histological examination demonstrated a finely nodular cirrhosis with active hepatocellular necrosis, portal inflammation and cholestasis. There were extensive deposits of brown pigment in hepatocytes, Kupffer cells and biliary structures. Pigment deposits in hepatocytes were usually in the form of small homogenous droplets or coarse granules. Several of the pigment deposits were birefringent when examined by polarization microscopy, and electron microscopy demonstrated amorphous material in which were imbedded numerous electron-dense crystals. The crystalline deposits lay free in the cytoplasm of the hepatocytes, but in Kupffer cells were exclusively in lysosomes.

The protoporphyrin levels in the livers were greatly in excess of the levels in erythrocytes and serum (Table 1). It could not be determined by the method used to extract and measure protoporphyrin in liver as to how much was in crystalline form and how much was soluble. Bile protoporphyrin levels in 3 patients were elevated compared to control subjects without protoporphyria, but were less than those in patients with protoporphyria who do not have liver disease (Table 2) [15]. This suggests that biliary excretion of protoporphyrin was impaired in the patients, as would be expected because of the structural liver damage which had occurred.

**Table 2. Bile porphyrin levels in patients with protoporphyria.**

		Total Bile Porphyrin ( $\mu\text{g}/\text{dl}$ )	Distribution of Porphyrins (percent)		
			Protoporphyrin	Coproporphyrin	Other
Patient	2	1240	93	7	0
	7	621	90	10	0
	8	58	95	5	0
Patients without liver disease (n=6)*		$2874 \pm 1185^\dagger$	$84 \pm 7$	$7 \pm 2$	$9 \pm 4$
Control (n = 17)*		$46 \pm 9$	$12 \pm 2$	$79 \pm 3$	$8 \pm 2$

$^\dagger$  Mean  $\pm$  SE

\*These data were included in a previous publication [15].

**Table 3. Protoporphyrin concentrations gradients.**

Patient	Erythrocyte/Serum*	Liver/Serum*	Liver/Bile*
1	33	2473	—
2	36	4093	511
3	27	1949	—
4	8	312	—
5	17	1167	—
6	26	4634	—
7	21	467	306
8	6	226	3249
mean ± SE	22 ± 4	1915 ± 605	1355 ± 949

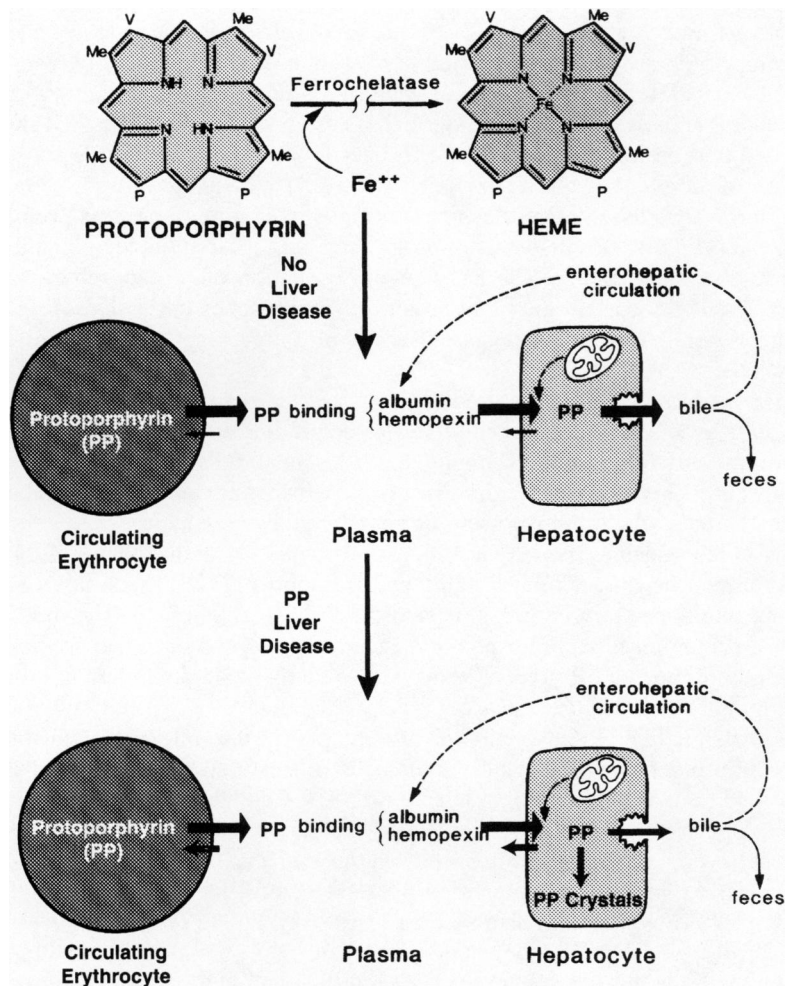
\*Concentration ratios: Erythrocyte/serum = ( $\mu\text{g}/\text{dl}$  cells)/( $\mu\text{g}/\text{dl}$  serum); liver/serum = ( $\mu\text{g}/100$  g wet wt liver)/( $\mu\text{g}/\text{dl}$  serum); liver/bile = ( $\mu\text{g}/100$  g wet wt liver)/( $\mu\text{g}/\text{dl}$  bile).

As a consequence of the marked hepatic levels of protoporphyrin, a significant concentration gradient existed between liver and blood and between liver and bile (Table 3). Thus the liver was the most significant repository of protoporphyrin accumulation.

In 4 patients, protoporphyrin levels were measured in the graft at varying periods following liver transplantation (Table 4). In each case the level was increased when compared to nonporphyric control specimens. In one patient there was a progressive increase in the protoporphyrin level, and this patient developed a recurrence of protoporphyric liver disease which led to his death 5 years after transplantation (patient 5). Post mortem examination revealed a black, cirrhotic liver with extensive protoporphyrin pigment deposits. The protoporphyrin level was 507,800 mg/100 g wet weight, compared to a level of 522,600 in his resected liver. A detailed report of this patient's post-transplant course has been previously published [17].

**Table 4. Protoporphyrin levels (PP) in the graft after liver transplantation.**

Patient	Time after Transplantation	Graft PP ( $\mu\text{g}/100$ g wet wt)
2	13 mos	1180
	3 yrs	2700
	5 yrs	350
3	13 mos	44,900
4	11 mos	11,700
5	8 mos	620
	3 yrs	134,000
	5 yrs	507,800



**Figure 1. Development of liver damage in protoporphyria.** As a consequence of deficient ferrochelatase activity in heme-forming tissues, there is excess protoporphyrin that must be excreted in bile. When liver injury develops as a result of protoporphyrin toxicity, biliary excretion does not keep pace with the excess protoporphyrin that is formed. This causes progressive protoporphyrin accumulation in liver tissue and accelerated injury. Protoporphyrin crystals form in liver tissue when a high concentration is reached

## DISCUSSION

Hepatic ferrochelatase activity is deficient in patients with protoporphyria [3], and the liver may therefore contribute to the excess production of protoporphyrin. Several different types of studies have provided evidence for this possibility [18-22]. However, the extent of the liver's contribution is uncertain, and the bone marrow is probably the major source of the excess protoporphyrin in most patients. Nevertheless, the significant decrease in hepatic ferrochelatase activity that has been observed in patients with

advanced liver disease (to a level that is 4 to 20 percent of that in normal or control liver tissue) suggests that the liver may make an important contribution in this situation [23].

The more critical role of the liver in protoporphyria is to excrete the excess protoporphyrin that is formed, irrespective of the tissue source. The excretory pathways of the porphyrin compounds are determined primarily by their aqueous solubility, and hence by their number of carboxyl groups. Protoporphyrin, which has only 2 carboxyl groups, is poorly aqueous soluble and is excreted virtually entirely in bile. Experimental studies have documented that protoporphyrin is toxic to liver function, impairing bile formation and the activity of membrane-bound enzymes [10, 11]. As patients develop liver injury from protoporphyria toxicity, the liver becomes less able to excrete the excess protoporphyrin that is formed (Figure 1). This leads to progressive protoporphyria accumulation in liver tissue and accelerated injury. The basis of the formation of protoporphyria crystals is uncertain. The high concentration that protoporphyria reaches in the liver is undoubtedly one factor. Preliminary studies suggest that complexation of protoporphyria to calcium may be another factor [24].

Studies with the isolated perfused rat liver indicate that hepatic uptake of protoporphyria occurs by simple or facilitated diffusion, continuing at a significant rate even when the excretion into bile becomes impaired [25]. The intracellular transport of protoporphyria is probably by nonvesicular carriers, which are targeted to the canalicular membrane, since intracellular transport is not inhibited by colchicine or monensin [26]. Cytosolic proteins such as the fatty acid binding protein and/or phospholipid transfer proteins may affect the intracellular binding and transport [27, 28]. Secretion of protoporphyria into bile appears to be linked to phospholipid secretion [26]. The *mdr2* P-glycoprotein is essential for biliary phospholipid excretion [29], and mice that are homozygous for disruption of the *mdr2* P-glycoprotein also have a marked impairment of biliary secretion of protoporphyria [30].

Bile acids facilitate the excretion of protoporphyria into bile by increasing the concentration of protoporphyria that can be attained. This is related to the number of hydroxyl groups and their location in the bile acid [26, 31]. Cholates increase the biliary excretion of protoporphyria more than chenodeoxycholate, which in turn has a greater effect than ursodeoxycholate. Biliary protoporphyria thrombi also form at a lower concentration of protoporphyria in bile during infusion with ursodeoxycholate than with the other two bile acids [31]. Thus, the administration of ursodeoxycholate to patients with protoporphyria liver disease must be done cautiously. Chenodeoxycholate has been administered to patients, with the unexpected result that fecal, bile and blood levels of protoporphyria decreased, suggesting that protoporphyria synthesis was reduced [32].

Since protoporphyria liver disease may occur in the graft following liver transplantation, it is unlikely that an intrinsic defect in hepatic protoporphyria excretion is a necessary factor in the development of this condition. Every patient who is transplanted for protoporphyria liver disease thus has the risk of recurrent disease in the graft. Further definition of the mechanism by which the liver excretes protoporphyria into bile is desirable. Measures to facilitate biliary excretion could be instituted in patients with early liver damage and would hopefully reverse that condition, and could be routinely used in patients who have had liver transplantation to prevent damage to the graft.

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