【 CASE REPORT 】

Erythropoietic Protoporphyria-related Hepatopathy Successfully Treated with Phlebotomy

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Abstract:
A 27-year-old man bearing an erythropoietic protoporphyria (EPP)-associated ferrochelatase (FECH) mutation was admitted to our hospital for general malaise and marked elevation of the serum levels of hepatobiliary enzymes and bilirubin. Initial treatment with plasma exchange did not reduce the blood protoporphyrin or serum liver enzyme levels, so phlebotomy was started. Surprisingly, weekly phlebotomy normalized the serum levels of liver enzymes, accompanied by a marked reduction in the blood protoporphyrin levels. The clinical course of this case strongly suggests that phlebotomy may be a suitable treatment option for EPP-related hepatopathy.

Key words: erythrocyte protoporphyrin, plasma exchange, phlebotomy

(Intern Med 57: 2505-2509, 2018)
(DOI: 10.2169/internalmedicine.0673-17)

Introduction

Porphyrias are hereditary disorders in the heme biosynthesis enzymes and are classified into eight types according to genetic abnormalities of the enzymes and the accumulation of biochemical intermediates of the heme biosynthesis pathway (1). Erythropoietic protoporphyria (EPP) is one type of porphyria caused by a reduced activity of ferrochelatase (FECH) and the accumulation of protoporphyrin, a substrate of this enzyme (2). In most cases of EPP, loss of function mutations in FECH result in the massive accumulation of protoporphyrin in erythroid cells residing in the bone marrow due to the disturbance of erythroid heme biosynthesis, subsequently leading to high concentrations of protoporphyrin in the plasma and skin. Such high concentrations of protoporphyrin in the skin are primarily responsible for the immediate photosensitivity in EPP patients who develop erythema and edema in sun-exposed areas. Thus, EPP is a prototypical erythropoietic porphyria associated with skin lesions.

The liver is another target organ of EPP. Plasma protoporphyrin is taken up by hepatocytes, followed by biliary excretion into the feces. A significant proportion of patients with EPP develop hepatobiliary diseases secondary to the massive accumulation of protoporphyrin in the hepatocytes. EPP-associated hepatopathy can be used as a prognostic factor for this disease, since approximately 5% of EPP patients present with life-threating liver diseases, such as liver cirrhosis. It should be noted, however, that no treatment has been established thus far for EPP-related hepatopathy, although some studies have reported improvement in patients' conditions by ursodeoxycholic acid (3), cimetidine (4-6), cholestyramine (7), adsorption therapy with activated carbon (8), plasmapheresis (9, 10), and exchange blood transfusion (11). Furthermore, a study reported the efficacy of liver transplantation, but its long-term results have not been clarified (12).

We herein report a case of EPP-related hepatopathy successfully treated with phlebotomy.

Case Report

A 27-year-old man was admitted to Kindai University Hospital due to general malaise and jaundice. He had suffered from photodermatosis since childhood. He had been diagnosed with systemic lupus erythematosus (SLE) due to pancytopenia, butterfly rash, and an oral ulcer in May 2015 and was being treated with prednisolone (45 mg). A marked elevation in his hepatobiliary enzymes (aspartate transaminase, AST 130 U/L, alanine aminotransferase, ALT 39 U/L) was seen at this time point, so he was referred to our department. A microscopic examination using liver biopsy specimens revealed an interlobular bile duct and Maltese-cross-positive porphyrin deposition in hepatocytes. Hepatocyte deposition of porphyrin, the marked elevation of serum protoporphyrin level (895 μg/dL, normal range; 30-86 μg/dL), and photosensitivity prompted us to consider a diagnosis of EPP-related hepatopathy. A definitive diagnosis of EPP was made since he had a loss of function mutation (IVS3-48T > C) in FECH. The patient was therefore ultimately diagnosed with EPP-related hepatopathy and treated with ursodeoxycholic acid (600 mg/day), cimetidine (800 mg/day), and cholestyramine (27 g/day) along with a reduction in sun exposure to minimize the toxicity of protoporphyrin.

He complained of general malaise in March 2016, and his serum levels of AST (295 U/L), ALT (200 U/L), gamma-glutamyl transpeptidase (GGT, 617 U/L), and total bilirubin (T-bil, 4.0 mg/dL) were again elevated (Table). His serum levels of albumin and prothrombin time levels were normal. In addition, his serum levels of Fe and ferritin were normal and his serum was negative for hepatitis B surface antigen and hepatitis C virus antibody. Since the blood concentration of protoporphyrin remained high (8,500 μg/dL), we considered this to be a case of exacerbation of EPP-related hepato-

Table.   Laboratory Data on Admission.

Blood count	WBC (3,300-8,600)	11,400 μL
	Hb (13.7-16.8)	12.3 g/dL
	PLT (15.8-34.8×10^4)	15.8×10$^{4/}$ μL
Coagulability	PT (70-130)	112.0 %
	PT-INR	0.96
Biochemical values	Na (138-145)	138 mEq/L
	K (3.6-4.8)	4.2 mEq/L
	BUN (8-20)	9 mg/dL
	Cr (0.65-1.07)	0.55 mg/dL
	eGFR	145
	FBS (73-109)	142 mg/dL
	TP (6.6-8.1)	7.0 g/dL
	Alb (4.1-5.1)	4.0 g/dL
	T-bil (0.4-1.5)	4.0 mg/dL
	D-bil (0-0.4)	2.9 mg/dL
	AST (13-30)	295 U/L
	ALT (10-42)	200 U/L
	ALP (106-322)	388 U/L
	GGT (13-64)	617 U/L
	CRP (0-0.14)	0.155 mg/dL
	TC (142-220)	293 mg/dL
	Fe (40-188)	116 μg/dL
	Ferritin (25-250)	60 ng/mL
Porphyrin metabolism	PP (30-86)	8,500 μg/dL RBC
Immunological test	ANA	(-)
	AMA2	(-)
	IgG (870-1,700)	1,227 mg/dL
Viral marker	HBsAg	(-)
	HCVAb	(-)

WBC: white blood cell, Hb: hemoglobin, PLT: platelet, PT: prothrombin time, BUN: blood urea nitrogen, Cr: creatinine, eGFR: estimated glomerular filtration rate, FBS: fasting blood sugar, TP: total protein, Alb: albumin, T-Bil: total bilirubin, D-bil: direct bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transepeptidase, CRP: C-reactive protein, TC: total cholesterol, PP: erythrocyte protoporphyrin, ANA: anti-nuclear antibody, AMA2: anti-mitochondrial antibody 2, HBsAg: hepatitis B surface antigen, HCVAb: hepatitis C virus antibody
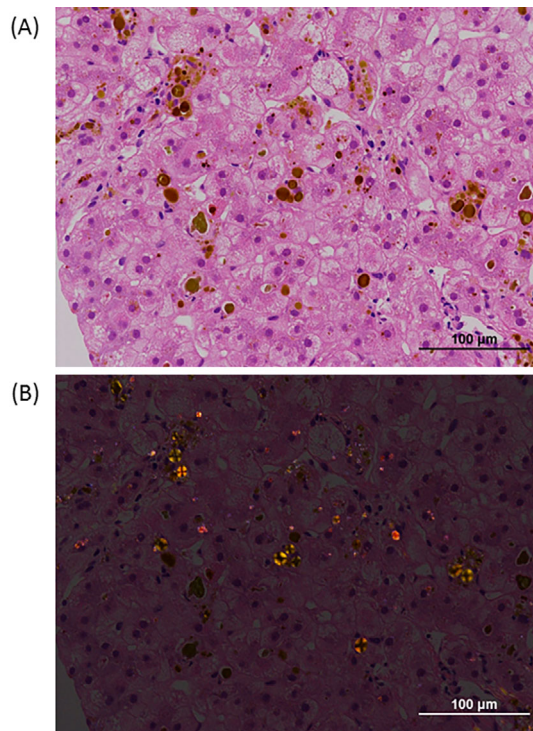
(A)

(B)

Figure 1. **Histology on a liver biopsy at the time of admission. (A) Hematoxylin and Eosin staining shows porphyrin deposition in the liver. Porphyrin deposition was preferentially seen in the hepatocytes rather than in the bile duct. There were no findings suggestive of autoimmune hepatitis, including plasma cell infiltration and rosette formation. (B) Polarizing microscopy confirmed the marked deposition of Maltese-cross-positive porphyrin.**

pathy. Autoimmune-related hepatobiliary disease was unlikely, since the serum levels of anti-nuclear antibody, anti-mitochondrial antibody, and total IgG were normal. Abdominal ultrasonography indicated scattered areas of high brightness, consistent with the deposition of crystalized porphyrin. A liver biopsy was again performed to verify whether or not the abnormalities of hepatobiliary enzymes were due to the exacerbation of EPP-related hepatopathy. Microscopically, the degree of hepatocellular porphyrin deposition was greater than had been noted at the previous biopsy. Although the accumulation of porphyrin is usually observed in the bile duct and hepatocytes (10), porphyrin deposition was preferentially seen in the hepatocytes rather than the bile duct in this case. There were no findings suggestive of autoimmune hepatitis, such as plasma cell infiltration or rosette formation (Fig. 1). Thus, this case was ultimately diagnosed as exacerbation of EPP-related hepatopathy.

Elevated serum levels of total bilirubin in the absence of hemolysis as indicated by normal level of lactate dehydrogenase (LDH) (140 U/L) led us to speculate the presence of severe liver dysfunction despite the normal levels of prothrombin time and albumin. Plasma exchange was selected as the initial treatment, since this treatment is effective not only for the removal of plasma protoporphyrin but also for severe liver dysfunction. Although plasma exchange was repeated for a total of five times, a reduction in the blood levels of AST, ALT, or protoporphyrin was not achieved. Thus, plasmapheresis was not effective for the treatment of EPP-related hepatopathy in this case. We then used phlebotomy to remove red blood cells and plasma protoporphyrin (Fig. 2). Weekly phlebotomy (200-400 mL) was
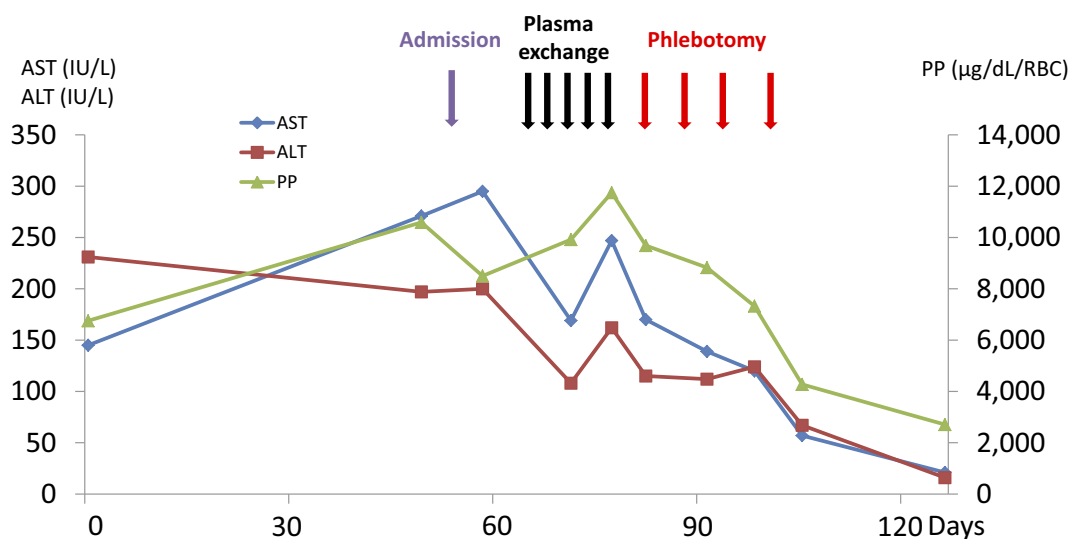

Figure 2. **Clinical course before and after admission. After admission, plasma exchange was performed for a total of 5 times, no decrease in the blood protoporphyrin value was observed, and improvement of liver disorder was not observed either. Therefore, when switching to phlebotomy treatment (200 mL ~ 400 mL) once/week, the blood protoporphyrin value was markedly decreased to 2,710 μg/dL, and AST 21 U/L, ALT 16 U/L, T-bil 0.6 mg/dL and liver function also improved.**

started, leading to a marked decrease in the serum levels of liver enzymes, AST, and ALT, as well as protoporphyrin. As his serum biochemical markers were decreased, symptom relief was obtained. He was discharged and received follow-up at the outpatient clinic via the oral administration of ursodeoxycholic acid, cimetidine, and cholestyramine.

Discussion

EPP is caused by a loss of mutations in FECH, one of the most critical enzymes in the heme biosynthesis pathway (2). The impaired function of FECH in the presence of EPP-associated FECH mutations causes the massive accumulation of protoporphyrin, a substrate for this enzyme, in erythroid cells. Such an accumulation of erythroid protoporphyrin in the bone marrow results in high concentration of protoporphyrin in the circulating blood and skin. High concentrations of plasma protoporphyrin also cause the hepatic accumulation of protoporphyrin, since protoporphyrin is taken up by hepatocytes. Thus, the accumulation of protoporphyrin in the skin and liver plays a critical role in EPP-associated skin disease and hepatopathy, respectively. A reduction in sun exposure is a well-established aspect of managing EPP-related skin disease caused by photosensitivity, since fluorescent protoporphyrin activated by sunlight promotes subsequent inflammatory responses. However, effective treatments for EPP-related hepatopathy have not yet been established. Given that hepatopathy rather than skin disease is associated with the prognosis of EPP (13, 14), the development of a new treatment for EPP-related hepatopathy is important. We described a case of EPP-related hepatopathy successfully treated with phlebotomy. Our present findings strongly suggest that phlebotomy is a suitable treatment option for EPP-related hepatopathy.

Phlebotomy has been shown to be highly effective in patients with another type of erythropoietic porphyria, porphyria cutanea tarda (PCT), which is caused by a reduced activity of uroporphyrinogen decarboxylase (UROD) (1). PCT, which is classified into sporadic and hereditary types, is also characterized by skin lesions in sun-exposed areas and liver dysfunction, as in EPP. A number of factors, such as alcohol (15), hepatitis C virus infection (16), HIV infection (16, 17), estrogen exposure (18), renal failure (19), and lymphoma (20), have been identified as potential triggers for sporadic PCT. However, in contrast to EPP, PCT is characterized by iron overloading, which partially explains the liver dysfunction in this type of erythropoietic porphyria. Dramatic responses to phlebotomy in our case prompted us to consider the co-occurrence of PCT and EPP. In fact, SLE can trigger the development of PCT (21-23). PCT patients exhibit elevated levels of urinary uroporphyrin or fecal coproporphyrin in addition to elevated levels of serum ferritin (24, 25). It should be noted, however, that there was no significant elevation in these urinary, fecal, or serum biomarkers in the present patient. Thus, we deemed the simultaneous occurrence of EPP and PCT-related hepatopathy

unlikely in this case. Furthermore, the dramatic responses to phlebotomy could not be explained by iron overload, which often accompanies PCT.

Plasma exchange was selected as an initial treatment in this case since this treatment can be effective not only in the removal of blood protoporphyrin but also in the restoration of the liver function. However, plasma exchange did not reduce the blood protoporphyrin or ALT levels. Surprisingly, weekly phlebotomy normalized the blood levels of protoporphyrin as well as AST and ALT. The mechanisms underlying the dramatic responses to phlebotomy but not plasma exchange remain unknown at present. Plasma exchange is generally understood to be more effective in patients with hemolysis than in those without hemolysis. We therefore speculate that the absence of hemolysis might have reduced the sensitivity to plasma exchange in this case. In contrast, phlebotomy is expected to be effective regardless of the presence of hemolysis. Given that exposure to sunlight easily induces hemolysis in EPP patients, the presence or absence of hemolysis might be associated with the sensitivity to plasma exchange in EPP-related hepatopathy. Future studies addressing the therapeutic efficacy of plasma exchange in a larger number of patients with EPP-related hepatopathy will be needed to confirm this hypothesis.

Another question arising from the present case is the optimum schedule of phlebotomy for EPP-related hepatopathy. We selected weekly phlebotomy (200-400 mL/week) in this case, using small-volume phlebotomy since the progression of anemia due to massive phlebotomy may promote the accumulation rather than the removal of protoporphyrin as a result of enhancement of the bone marrow function. Careful monitoring of hemoglobin and the reticulocyte count may be required during phlebotomy for EPP-related hepatopathy. Determining the optimum schedule of phlebotomy is absolutely necessary in order to establish phlebotomy as a treatment option in EPP-related hepatopathy.

The pathogenesis of EPP-related hepatopathy has not been fully elucidated, although bile duct occlusion caused by aggregated protoporphyrin is considered to be involved. It has also been suggested that deposition of protoporphyrin may exert direct toxic effects through the induction of hepatocyte apoptosis. In addition to these mechanism, we previously reported on the possible involvement of ATP-binding cassette transporter G2 (ABCG2) in the development of EPP-related hepatopathy (10). We observed a reduced expression of ABCG2, which functions as an important transporter of not only bile acid but also protoporphyrin. Such a reduced expression of ABCG2 may promote the accumulation of protoporphyrin, subsequently occluding the bile duct. Thus, several mechanisms have been proposed for the pathogenesis of EPP-related hepatopathy. In the present case, the protoporphyrin accumulation in hepatocytes was more marked than that in the bile duct. Furthermore, apoptosis was preferentially seen in hepatocytes rather than in the bile duct. These microscopic findings again support the idea that hepatocyte damage rather than bile duct damage is responsi-

ble for the elevated levels of serum hepatobiliary enzymes in this case.

In conclusion, phlebotomy may be effective in some patients with EPP-related hepatopathy. Future studies addressing the efficacy of phlebotomy in EPP-related hepatopathy are necessary to confirm this idea.

The authors state that they have no Conflict of Interest (COI).

References

1. Sassa S, Kappas A. Molecular aspects of the inherited porphyrias. J Intern Med **247**: 169-178, 2000.
2. Bloomer J, Wang Y, Singhal A, et al. Molecular studies of liver disease in erythropoietic protoporphyria. J Clin Gastroenterol **39**: S167-S175, 2005.
3. Pirlich M, Lochs H, Schmidt HH. Liver cirrhosis in erythropoietic protoporphyria: improvement of liver function with ursodeoxycholic acid. Am J Gastroenterol **96**: 3468-3469, 2001.
4. Horie Y, Tanaka K, Okano J, et al. Cimetidine in the treatment of porphyria cutanea tarda. Intern Med **35**: 717-719, 1996.
5. Tu JH, Sheu SL, Teng JM. Novel treatment using cimetidine for erythropoietic protoporphyria in children. JAMA Dermatol **152**: 1258-1261, 2016.
6. Fujimori N, Komatsu M, Tanaka N, et al. Cimetidine/lactulose therapy ameliorates erythropoietic protoporphyria-related liver injury. Clin J Gastroenterol **10**: 452-458, 2017.
7. Bloomer JR. Pathogenesis and therapy of liver disease in protoporphyria. Yale J Biol Med **52**: 39-48, 1979.
8. Gorchein A, Foster GR. Liver failure in protoporphyria: long-term treatment with oral charcoal. Hepatology **29**: 995-996, 1999.
9. Do KD, Banner BF, Katz E, et al. Benefits of chronic plasmapheresis and intravenous heme-albumin in erythropoietic protoporphyria after orthotopic liver transplantation. Transplantation **73**: 469-472, 2002.
10. Hagiwara S, Nishida N, Park AM, et al. Impaired expression of ATP-binding cassette transporter G2 and liver damage in erythropoietic protoporphyria. Hepatology **62**: 1638-1639, 2015.
11. Eichbaum QG, Dzik WH, Chung RT, et al. Red blood cell exchange transfusion in two patients with advanced erythropoietic protoporphyria. Transfusion **45**: 208-213, 2005.
12. McGuire BM, Bonkovsky HL, Carithers RL Jr, et al. Liver transplantation for erythropoietic protoporphyria liver disease. Liver Transpl **11**: 1590-1596, 2005.
13. Doss MO, Frank M. Hepatobiliary implications and complications in protoporphyria, a 20-year study. Clin Biochem **22**: 223-229, 1989.
14. Meerman L. Erythropoietic protoporphyria. An overview with emphasis on the liver. Scand J Gastroenterol Suppl **232**: 79-85, 2000.
15. Yang XM, Zhang Y, Wang T, et al. Sporadic porphyria cutanea tarda induced by alcohol abuse. Chin Med J **130**: 2011-2012, 2017.
16. Quansah R, Cooper CJ, Said S, et al. Hepatitis C- and HIV-induced porphyria cutanea tarda. Am J Case Rep **15**: 35-40, 2014.
17. Aguilera P, Laguno M, To-Figueras J. Human immunodeficiency virus and risk of porphyria cutanea tarda: a possible association examined in a large hospital. Photodermatol Photoimmunol Photomed **32**: 93-97, 2016.
18. Modroño Móstoles N, Pavón de Paz I, Guijarro de Armas G, et al. A case of hypopituitarism and porphyria cutanea tarda in relation to estrogen therapy in a patient with empty sella syndrome. Rev Clin Esp **215**: e11-e13, 2015.
19. Vasconcelos P, Luz-Rodrigues H, Santos C, et al. Desferrioxamine treatment of porphyria cutanea tarda in a patient with HIV and chronic renal failure. Dermatol Ther **27**: 16-18, 2014.
20. Thawani R, Moghe A, Idhate T, et al. Porphyria cutanea tarda in a child with acute lymphoblastic leukemia. QJM **109**: 191-192, 2016.
21. Fritsch S, Wojcik AS, Schade L, et al. Increased photosensitivity? Case report of porphyria cutanea tarda associated with systemic lupus erythematosus. Rev Bras Reumatol **52**: 968-970, 2012.
22. Peitsch WK, Lorentz K, Goebeler M, et al. Subacute cutaneous lupus erythematosus with bullae associated with porphyria cutanea tarda. J Dtsch Dermatol Ges **5**: 220-222, 2007.
23. Clemmensen O, Thomsen K. Porphyria cutanea tarda and systemic lupus erythematosus. Arch Dermatol **118**: 160-162, 1982.
24. Dereure O, Jumez N, Bessis D, et al. Measurement of liver iron content by magnetic resonance imaging in 20 patients with overt porphyria cutanea tarda before phlebotomy therapy: a prospective study. Acta Derm Venereol **88**: 341-345, 2008.
25. Baddams EL, Degg T, Barth JH. Cascade testing of primary care blood samples with hyperferritinaemia identifies subjects with iron overload and porphyria cutanea tarda. Ann Clin Biochem **51**: 499-502, 2014.