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Minireviews

Protoporphyrin IX: the Good, the Bad, and the Ugly

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ABSTRACT

Protoporphyrin IX (PPIX) is ubiquitously present in all living cells in small amounts as a precursor of heme. PPIX has some biologic functions of its own, and PPIX-based strategies have been used for cancer diagnosis and treatment (the good). PPIX serves as the substrate for ferrochelatase, the final enzyme in heme biosynthesis, and its homeostasis is tightly regulated during heme synthesis. Accumulation of PPIX in human porphyrias can cause skin photosensitivity, biliary stones, hepatobiliary damage, and even liver failure (the bad and the ugly). In this work, we review the mechanisms that are associated with the broad aspects of PPIX. Because PPIX is a hydrophobic molecule, its disposition is by hepatic rather than renal excretion. Large amounts of PPIX are toxic to the liver and can cause cholestatic liver injury. Application of PPIX in cancer diagnosis and treatment is based on its photodynamic effects.

Introduction

Protoporphyrin IX (PPIX) is a heterocyclic organic compound, which consists of four pyrrole rings, and is the final intermediate in the heme biosynthetic pathway. Its tetrapyrrole structure enables it to chelate transition metals to form metalloporphyrins, which perform a variety of biologic functions. Chelation of PPIX with iron forms heme (iron PPIX), which is a constituent of hemoproteins that play critical roles in oxygen transport, cellular oxidations and reductions, electron transport, and drug metabolism (Paoli et al., 2002; Kirton et al., 2005; Smith et al., 2010). Amounts of PPIX in cells actively synthesizing heme remain low under physiologic conditions, because the amount supplied does not exceed what is needed for heme synthesis. Thus, PPIX is efficiently converted to heme by the mitochondrial enzyme ferrochelatase (FECH), the final enzyme in the heme biosynthetic pathway. Inherited and acquired diseases and some xenobiotics can disturb heme synthesis and PPIX homeostasis, causing accumulation of PPIX in amounts that are sufficient to produce photosensitivity and liver damage (Magnus et al., 1961; Cox et al., 1998; Meerman, 2000; Chen et al., 2002; Dailey and Meissner, 2013). Clinically useful aspects of PPIX include its use for cancer diagnosis and therapy (Gold and Goldman, 2004; MacCormack, 2008). In this review, we discuss the broad aspects of PPIX, including its biosynthesis and regulation, its toxicity and clinical manifestations when present in excess, and its therapeutic applications.

PPIX Biosynthesis

Biosynthesis of heme is an eight-step process that occurs partly in mitochondria and partly in the cytoplasm (Fig. 1) (Ajioka et al., 2006). All eight enzymes in this pathway are encoded in the nucleus and are synthesized in the cytoplasm. PPIX is the final intermediate in the heme synthesis pathway. PPIX is formed by protoporphyrinogen oxidase (PPOX) and converted to heme by FECH, both of which are mitochondrial enzymes. Biosynthesis of heme initiates in the mitochondrial matrix where one molecule of glycine combines with one molecule of succinyl-CoA to form δ-aminolevulinic acid (ALA). This step is catalyzed by ALA synthase (ALAS; EC 2.3.1.37) in the presence of the cofactor pyridoxal phosphate (Koningsberger et al., 1995; Hunter and Ferreira, 2011). ALA formed in mitochondria is exported to the cytoplasm (Fig. 1). The mechanism of ALA transport is not fully understood, but a

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ABBREVIATIONS: ABCB, ATP-binding cassette subfamily B member; ABCG2, ATP-binding cassette subfamily G member 2; ALA, δ-aminolevulinic acid; ALAD, ALA dehydratase; ALAS, ALA synthase; ALAS1, the ubiquitous form of ALAS; ALAS2, the erythroid-specific form of ALAS; EPP, erythropoietic protoporphyria; FECH, ferrochelatase; IRE, iron regulatory element; IRP, iron regulatory protein; MFRN1, mitoferrin 1; PBzR, peripheral benzodiazepine receptor; PPIX, protoporphyrin IX; PPOX, protoporphyrinogen oxidase; ROS, reactive oxygen species; VP, variegate porphyria; XLP, X-linked protoporphyria.

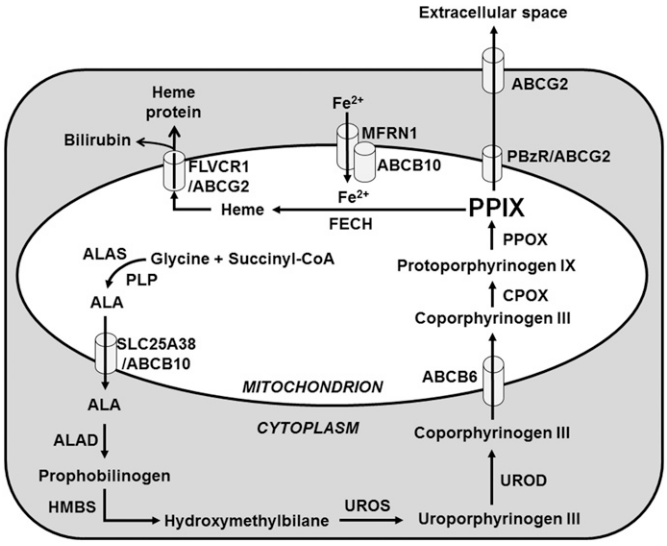

Fig. 1. PPIX/heme biosynthesis and disposition in mammalian cells. Multiple enzymes, cofactors, and transporters are involved in the pathway of PPIX/heme biosynthesis and in PPIX disposition. CPOX, coproporphyrinogen oxidase; FLVCR1, feline leukemia virus subgroup c receptor 1; HMBS, hydroxymethylbilane synthase; PLP, pyridoxal phosphate; SLC25A38, solute carrier family 25 member 38; UROD, uroporphyrinogen decarboxylase; UROS, uroporphyrinogen III synthase.

role for solute carrier family 25 member 38 and ATP-binding cassette subfamily B member (ABCB)10 has been suggested (Guernsey et al., 2009; Bayeva et al., 2013). Two molecules of ALA are condensed by ALA dehydratase (ALAD; EC 4.2.1.24, also known as porphobilinogen synthase) to form the monopyrrole porphobilinogen. Hydroxymethylbilane synthase (EC 2.5.1.61, also known as porphobilinogen deaminase) then assembles four molecules of porphobilinogen to form hydroxymethylbilane (Frydman and Feinstein, 1974). This reactive linear tetrapyrrole can spontaneously cyclize to form uroporphyrinogen I, which is not a heme precursor. The enzyme uroporphyrinogen synthase (EC 4.2.1.75) catalyzes hydroxymethylbilane ring closure with inversion of one of the pyrrole rings to form the heme precursor uroporphyrinogen III (Jordan and Shemin, 1973).

Uroporphyrinogen decarboxylase (EC 4.1.1.37) then removes carboxylic groups from the four acetic acid side chains of uroporphyrinogen III to form coproporphyrinogen III (Lewis and Wolfenden, 2008). This intermediate is then transported into mitochondria through ABCB6 (Krishnamurthy et al., 2006; Krishnamurthy and Schuetz, 2011), where it is decarboxylated to protoporphyrinogen IX by coproporphyrinogen oxidase (EC 1.3.3.3) (Proulx et al., 1993). Protoporphyrinogen IX is oxidized by PPOX (EC 1.3.3.4) with removal of six protons to form PPIX (Proulx et al., 1993). There are two major pathways for cellular PPIX disposition: synthesis of heme or excretion from the cell (Fig. 1). PPIX in most tissues is efficiently converted to heme by FECH (EC 4.99.1.1) in the presence of iron (Dailey, 1977). FECH activity is low in the oviduct of some birds, which allows PPIX to accumulate and serve as a brown pigment on eggshells (Schwartz et al., 1980). Mitoferrin 1 (MFRN1) and ABCB10 are involved in providing iron to mitochondria for heme synthesis (Shaw et al., 2006; Chen et al., 2009). In marrow reticulocytes, most PPIX remaining in small amounts after completion of heme and hemoglobin synthesis is chelated with zinc by FECH, and along with a smaller amount of metal-free PPIX is found in circulating erythrocytes. PPIX remaining in mitochondria may be transported into the extracellular space through ATP-binding cassette subfamily G member 2 (ABCG2) and peripheral benzodiazepine receptor (PBzR) (Jonker et al., 2002; Wendler et al., 2003; Solazzo et al., 2009; Kobuchi et al., 2012) (Fig. 1).

Regulation of PPIX Biosynthesis through ALAS

ALAS is the rate-limiting enzyme in PPIX production and heme synthesis. In mammals, ubiquitous and erythroid-specific forms of ALAS are encoded by separate genes on chromosomes 3 and X, respectively (Bishop et al., 1990). The ubiquitous form (ALAS1) is expressed in all tissues, including the liver. The erythroid-specific form (ALAS2) is only expressed in erythroid cells (Cox et al., 1990; Cotter et al., 1992). Although heme is synthesized in all cells, approximately 85% is produced in the bone marrow, and most of the rest in the liver. Heme is a key regulator of hepatic ALAS1 (Fig. 2). Heme directly binds to the upstream region of the *ALAS1* gene and prevents its transcription (Sassa and Granick, 1970; Kolluri et al., 2005; Zheng et al., 2008). In addition, heme destabilizes ALAS1 mRNA and promotes degradation of mature ALAS1 protein (Cable et al., 2000; Roberts and Elder, 2001; Tian et al., 2011). Furthermore, heme prevents transport of the precursor of ALAS1 protein into mitochondria by binding to the terminal mitochondrial targeting sequence (Lathrop and Timko, 1993; Munakata et al., 2004; Dailey et al., 2005).

In addition, ALAS1 is regulated by certain transcription factors and coactivators (Fig. 2). Fasting and feeding result in up- and downregulation of ALAS1 expression, respectively, by

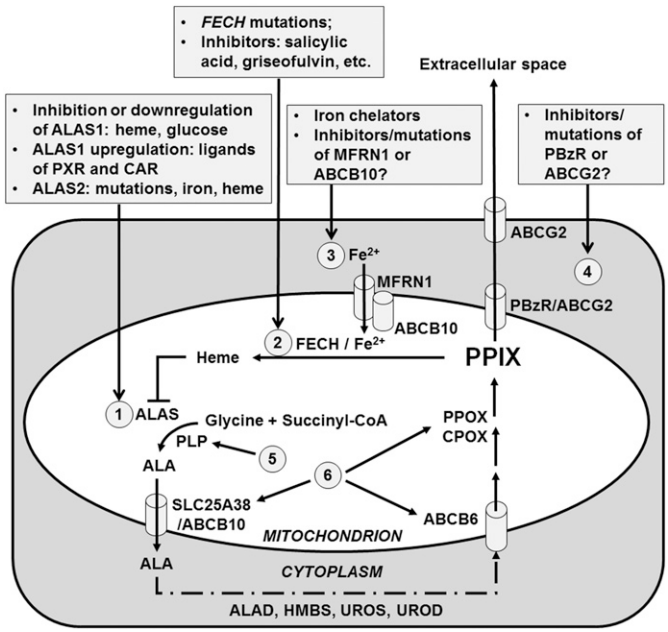

Fig. 2. Regulation of PPIX homeostasis. Genetic factors and xenobiotics can disturb PPIX homeostasis through effects on (1) ALAS, (2) FECH, (3) iron supply, (4) PPIX transporters, (5) pyridoxal phosphate (PLP; the cofactor of ALAS), and (6) the enzymes and transporters downstream of ALAS and upstream of FECH.

modulating the activity of proliferator-activated receptor-γ coactivator 1α (Giger and Meyer, 1981; Scassa et al., 1998; Varone et al., 1999; Wu et al., 1999; Handschin et al., 2005). Expression of proliferator-activated receptor-γ coactivator 1α is also regulated by a circadian oscillator Rev-erbα (Wu et al., 2009; Gerhart-Hines et al., 2013). Thus, ALAS1 expression is modulated by circadian rhythm. Most heme synthesized in the liver functions as a cofactor of cytochrome P450 enzymes (P450s), which are abundant in liver, turn over rapidly, and are critical for metabolism of many endogenous and exogenous chemicals. In response to P450 inducers, ALAS1 is upregulated by depletion of a regulatory heme pool in hepatocytes, and through nuclear receptors, such as the constitutive androstane receptor and the pregnane X receptor (Fraser et al., 2002, 2003; Podvinec et al., 2004). These receptors are important for regulation of both ALAS1 and P450s in the liver by drugs and other chemicals. Therefore, ligands of these nuclear receptors can upregulate ALAS1 expression and increase PPIX and heme synthesis (Fraser et al., 2003; Li et al., 2013).

Transcription of *ALAS2*, unlike *ALAS1*, is regulated by erythroid-specific transcription factors, such as GATA1 (Cable et al., 2000). Regarding the post-transcription of *ALAS2*, it is regulated by iron. ALAS2 mRNA possesses an iron regulatory element (IRE) in the 5′ untranslated region, which responds to cellular iron content (Bhasker et al., 1993; Melefors et al., 1993). In absence of iron, the iron-free form of iron regulatory protein (IRP) binds to the IRE, forming an IRE-IRP complex that prevents translation of ALAS2 (Wingert et al., 2005). This inhibitory effect of IRE-IRP complex is prevented by iron sulfur clusters (Napier et al., 2005; Rouault and Tong, 2005). These intricate regulatory mechanisms of ALAS2 by erythroid-specific transcription factors coordinate PPIX synthesis with iron supply, allowing efficient synthesis of large amounts of heme while avoiding an oversupply of either PPIX or iron, which would have adverse effects. Heme seems not to inhibit transcription of the *ALAS2* or alter ALAS2 mRNA stability (Sadlon et al., 1999). However, heme may inhibit the translation of ALAS2 and the import of ALAS2 precursor into mitochondria (Lathrop and Timko, 1993; Smith and Cox, 1997).

Disregulation of PPIX Homeostasis

ALA Loading to Bypass ALAS1. Because the rate-limiting step is bypassed, intermediates after ALA in the heme synthesis pathway accumulate, including PPIX. PPIX is a photosensitizer, and ALA administration can result in enough PPIX accumulation in lesions in the skin and other tissues to be the basis photodynamic therapy (Kennedy et al., 1990). Tissue selectivity is enhanced by direct application of ALA (e.g., to skin lesions) and by lesion-directed laser treatment. An ALA preparation is Food and Drug Administration approved for this purpose. ALA loading in humans also results in increased urinary excretion of coproporphyrin, particularly isomer III (Shimizu et al., 1978).

Gain of Function of ALAS2. X-linked protoporphyria (XLP) is a recently characterized, rare X-linked disease due to gain-of-function mutations of *ALAS2* (Whatley et al., 2008; Holme et al., 2009). These mutations alter the C-terminal portion of the *ALAS2* gene (Whatley et al., 2008), which enhances the function of ALAS2 such that amounts of PPIX produced exceed that needed by FECH for heme synthesis. Increased metal-free PPIX in erythrocytes and plasma causes a painful, mostly nonblistering type of photosensitivity. Zinc PPIX, a product of normal FECH activity, is also increased in this condition, but mostly remains in circulating erythrocytes and is less photosensitizing.

Decreased ALAD Activity. Deficient ALAD activity leads to accumulation of ALA and other intermediates, including PPIX, in large amounts in erythroid cells. This occurs in ALAD deficiency porphyria, the most rare human porphyria, causing elevations in ALA in plasma and urine, coproporphyrin III in urine (as in ALA loading), and zinc PPIX in erythrocytes. The same findings occur in lead poisoning, suggesting that increases in coproporphyrin III and erythrocyte PPIX in lead poisoning result largely from inhibition of ALAD and a resulting oversupply of ALA. In hereditary tyrosinemia type I, succinylacetone accumulates and potently inhibits ALAD, leading to similar biochemical findings and porphyria-like symptoms (Lindblad et al., 1977). Photosensitivity does not occur in these conditions, because the excess PPIX is predominantly zinc PPIX, reflecting adequate FECH activity. ALAD can also be inhibited by styrene in animals and humans (Fujita et al., 1987).

Decreased PPOX Activity. This penultimate enzyme in the heme biosynthetic pathway catalyzes the oxidation of protoporphyrinogen IX to PPIX with loss of six protons. An inherited partial deficiency of PPOX in variegate porphyria (VP) causes hepatic accumulation of protoporphyinogen IX, which is then oxidized nonenzymatically to PPIX. Chronic, blistering photosensitivity is a common symptom in VP. Phenoxy acid herbicides are potent PPOX inhibitors, and cause PPIX accumulation and phototoxicity in exposed plants (Duke et al., 1990). These chemicals also inhibit PPOX and cause protoporphyrinogen IX and PPIX accumulation in rodent hepatocytes (Sinclair et al., 1994).

Decreased FECH Activity. Loss-of-function *FECH* mutations are found in human erythropoietic protoporphyria (EPP) (Bonkowsky et al., 1975; Thapar and Bonkovsky, 2008). The *FECH* gene is located on the long arm of chromosome 18 on locus 18q21 (Whitcombe et al., 1991). More than 120 different *FECH* mutations have been identified in EPP (Thapar and Bonkovsky, 2008). Most commonly, a severe *FECH* mutation is inherited from one parent and a low expression (hypomorphic) *FECH* alteration that is common in the general population from the other parent (Gouya et al., 1999). FECH deficiency in EPP also impairs zinc PPIX formation, so the excess PPIX that accumulates is mostly metal-free (Nelson et al., 1998; Labbe et al., 1999).

Some xenobiotics and/or their metabolites inhibit FECH and cause PPIX accumulation. Salicylic acid, a nonsteroidal anti-inflammatory drug, binds directly to FECH and inhibits its activity, and can inhibit heme synthesis in cultured cells (Gupta et al., 2013). Certain xenobiotics require P450s to produce metabolites that are FECH inhibitors. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine and the antifungal drug griseofulvin are examples of chemicals that are suicidal substrates of P450s, and their metabolism in mouse liver produces *N*-methyl- and *N*-ethyl-protoporphyrin IX, which are potent FECH inhibitors (Cole et al., 1981; Cole and Marks, 1984). These chemicals are also potent inducers of hepatic ALAS1, which further contributes to PPIX accumulation (Cole et al., 1981). Griseofulvin exacerbates acute hepatic porphyrias

presumably by its inducing effect on ALAS1 and inhibition of FECH (Felsher and Redeker, 1967).

Decreased Iron Availability. MFRN1 imports iron into mitochondria (Shaw et al., 2006), and a deficiency of MFRN1 reduces iron supply for FECH, limits heme synthesis, and leads to PPIX accumulation (Troadec et al., 2011). ABCB10 physically interacts with MFRN1 and increases the half-life of MFRN1 (Chen et al., 2009). Deficiency of ABCB10 impairs mitochondrial iron import and causes PPIX accumulation (Yamamoto et al., 2014). At the same time, ABCB10 deficiency in mice results in iron accumulation in cytoplasm and leads to sideroblastic anemia (Yamamoto et al., 2014).

Iron deficiency impairs heme formation because ferrous iron is a substrate for FECH. However, zinc can substitute for iron, so with iron deficiency FECH catalyzes an increase in formation of zinc PPIX (Braun, 1999), which accumulates in reticulocytes and is increased in circulating erythrocytes. An increase in erythrocyte zinc PPIX is an early indicator of iron deficiency and precedes the development of anemia (Labbe et al., 1999). Iron chelators can bind to ferrous iron in the cytoplasm of cells, such as hepatocytes, and prevent iron from entering mitochondria to reduce FECH function and cause PPIX accumulation (Amo et al., 2009; Blake and Curnow, 2010; Juzeniene et al., 2013). In rodent and chick embryo livers and isolated hepatocytes, iron chelation can limit heme synthesis sufficiently to potentiate induction of ALAS1 by drugs and steroids (Anderson et al., 1982). In lead and other heavy metal poisoning, disturbed iron hemostasis may account in part for zinc PPIX accumulation (Schauder et al., 2010). Impaired iron delivery to reticulocytes accounts in part for anemia of chronic disease, which is also associated with an increase in erythrocyte zinc PPIX (Braun, 1999).

PPIX Accumulation with Normal PPOX and FECH Activity and Iron Supply. Amounts of zinc PPIX under normal and abnormal conditions are higher in younger than older erythrocytes, so increased erythropoiesis and shortened erythrocyte life span will increase the average concentration of PPIX. Therefore, an increase in zinc PPIX in erythrocytes is expected in any erythrocytic disorder (e.g., hemolytic anemias), even if heme synthetic enzymes and iron availability are not impaired (Anderson et al., 1977).

PPIX Transporters in PPIX Accumulation. Excess PPIX synthesized in mitochondria needs to be pumped out to the cytoplasm and then to the extracellular space (Fig. 2). Transporters are required because the two carboxylate side chains of PPIX make the movement of PPIX across a lipid bilayer energetically unfavorable. PBzR locates in the outer membrane of mitochondria and its expression is increased during erythroid differentiation, suggesting that PBzR might be responsible for transporting PPIX (Pastorino et al., 1994; Taketani et al., 1994). Overexpression of recombinant PBzR in *Escherichia coli* supported the role of PBzR in PPIX movement (Wendler et al., 2003). Apart from PBzR, ABCG2 also contributes to PPIX movement (Jonker et al., 2002). ABCG2 is primarily localized on extracellular membranes and responsible for PPIX efflux (Solazzo et al., 2009). ABCG2 also locates in mitochondrial membranes and contributes to PPIX efflux from mitochondria (Kobuchi et al., 2012). ABCG2-deficient mice have a higher amount of PPIX in red blood cells, liver, and the Harderian gland than wild-type mice (Jonker et al., 2002, 2007).

In summary, PPIX homeostasis is maintained by close coordination of multiple factors, including ALAS, FECH, iron supply, and transporters of iron and PPIX (Fig. 2). These are the targets for genetic and environmentally induced disturbances in PPIX homeostasis. PPIX homeostasis can also be disturbed by alterations in enzymes, cofactors, and transporters downstream of ALAS and upstream of FECH (Fig. 2). For example, severe, homozygous, or compound heterozygous deficiency of any enzyme in the heme biosynthetic pathway after ALAS leads to an increase in erythrocyte PPIX, which is zinc chelated if FECH activity is preserved. Heme synthesis is increased in the marrow in an effort to compensate for the severe hemolytic anemia seen in some of these disorders.

Clinical Manifestations and Management of PPIX Toxicity

Normally there is little accumulation of PPIX or other intermediates in cells. Because the bone marrow and liver are most active in PPIX and heme synthesis, under abnormal conditions PPIX accumulation occurs either in the marrow and circulating erythrocytes or the liver (Smith, 1979; Bloomer, 1988; Ajioka et al., 2006; Anstey and Hift, 2007). During erythroid maturation when hemoglobin synthesis is active, excess PPIX can be chelated with zinc, or be pumped out to plasma through ABCG2 (Zhou et al., 2005). In EPP and XLP, metal-free PPIX originates from the marrow and circulating erythrocytes and is delivered to the skin and liver (Brancaleon et al., 2004; Desuzinges-Mandon et al., 2010).

PPIX-Mediated Skin Damage. Metal-free PPIX is an endogenously produced photosensitizer. An acute, painful, and mostly nonblistering type of photosensitivity is the most common and significant clinical manifestation of increased metal-free PPIX in the protoporphyrias (EPP and XLP) (Thapar and Bonkovsky, 2008). To prevent symptoms, patients are forced to avoid light, which interferes with many professional and other everyday activities and impairs quality of life (Thapar and Bonkovsky, 2008). PPIX either in blood in the dermal vessels or after uptake into skin cells absorbs light energy and transfers this energy to oxygen, generating reactive oxygen species (ROS), which can lead to cytotoxicity through proteins, DNA, and lipid damage (Lim, 1989). These ROS can also cause local vasodilatation and edema by complement activation and mast cell degranulation (Baart de la Faille et al., 1991). Patients who remove themselves from exposure as soon as symptoms begin will recover quickly. Longer exposure can lead to more marked reactions with severe pain, redness, swelling, and even blistering, as well as systemic reactions, and may require several days or more for recovery (Dubrey et al., 2015). Repeated reactions to sunlight can have residual effects, such as waxy thickening of the skin, especially over the knuckles, hands, nose, and cheeks, and mild scarring (Horner et al., 2013). Because sunlight exposure is avoided, EPP and XLP patients are often vitamin D deficient (Wahlin et al., 2011a). Mild microcytic anemia with evidence of iron deficiency is common in EPP and XLP and is poorly understood (Holme et al., 2007), because elemental iron absorption is not impaired (Bossi et al., 2015).

Closely woven clothing, hats, and gloves are manufactured for patients with protoporphyria and other photosensitizing diseases. Pharmacological approaches have included pharmaceutical-grade β-carotene, narrow wave UVB phototherapy, cysteine, afamelanotide, antihistamines, and vitamin C (Table 1) (Sawyer et al., 1980; Krook and Haeger-Aronsen,

TABLE 1
Pharmacological approaches for management of PPIX-mediated photosensitivity

Approaches	Mechanisms	Outcomes	References
β-carotene	• Prevents penetration of sunlight	• Effective in some patients	Tintle et al., 2014
	• Acts as a scavenger for reactive radicals	• Causes yellowing of skin	Krook and Haeger-Aronsen, 1982
Phototherapy	• Increases skin pigmentation and thickness	• Effective in some patients	Warren and George, 1998
• Narrow-band UVB	• Induces UV tolerance	• May potentiate erythema and skin photoaging	Collins and Ferguson, 1995
• Psoralen UVA	• Affects Langerhans cells		
Cysteine	• Increases sunlight tolerance	• Efficacy not established	Mathews-Roth et al., 1994
Afamelanotide	• Binds to the melanocortin 1 receptor	• Decreased photosensitivity and improved quality of life	Sawyer et al., 1980; Minder et al., 2009; Fabrikant et al., 2013; Biolcati et al., 2015; Langendonk et al., 2015; Lengweiler et al., 2015; Minder and Schneider-Yin, 2015
	• Induces production of eumelanin that serves as a natural filter to reduce all wavelengths of light, quench UV light, and decrease free radicals		
Antihistamines	• Histamine H-receptor antagonists	• Limited efficacy	Wahlin et al., 2011a
Vitamin C	• Prevent oxidative damage from ROS	• Limited efficacy	Boffa et al., 1996

1982; Mathews-Roth et al., 1994; Collins and Ferguson, 1995; Warren and George, 1998; Wahlin et al., 2011a; Fabrikant et al., 2013; Tintle et al., 2014; Biolcati et al., 2015; Langendonk et al., 2015; Lengweiler et al., 2015; Minder and Schneider-Yin, 2015). Minder et al. (2009) reviewed the treatment options for dermal photosensitivity in EPP in 2009 and concluded that the data were insufficient to prove efficacy of any treatments studied in EPP. Since then, controlled trials have demonstrated improved light tolerance in EPP and XLP with afamelanotide, an α-melanocyte–stimulating hormone analog that increases skin melanogenesis (Harms et al., 2009; Fabrikant et al., 2013; Biolcati et al., 2015; Langendonk et al., 2015).

PPIX-Mediated Hepatobiliary Disease. Because PPIX concentrations in bile are high in EPP and XLP, PPIX-containing gallstones are common and may require cholecystectomy at an early age (Bloomer and Enriquez, 1982; Doss and Frank, 1989; Todd, 1991). PPIX-mediated liver damage, which occurs in less than 5% of patients with protoporphyria, is the most serious clinical manifestation and can be life threatening and require liver transplantation. The marrow is the major source of PPIX in EPP and XLP. Hepatic de novo synthesis is at best a minor contributor (Fig. 3). After uptake from plasma, hepatocytes transport PPIX into bile canuliculi. Bile is stored and concentrated in the gallbladder and delivered to the small intestine during meals, and some may be reabsorbed in the small intestine and delivered back to the liver by an enterohepatic circulation. This is the only route for excretion of PPIX because it is a large, hydrophobic molecule that cannot be excreted by the kidneys (Jonker et al., 2002, 2007).

Large amounts of PPIX are hepatotoxic, damaging both hepatocytes and cholangiocytes. PPIX impairs bile flow when administered in large amounts to bile fistula rodents (Lee et al., 1984). Once liver damage ensues, PPIX elimination by the liver is further compromised and levels of PPIX in plasma and erythrocytes can increase progressively, and bile composition becomes altered (Szechcinski et al., 1986; Meerman et al., 1999b). Increased delivery of PPIX to the liver can lead to further inflammation and accelerated damage to hepatocytes and cholangiocytes. Liver damage can progress slowly, as indicated by mild and often unexplained abnormal liver function tests, or advance rapidly to hepatic failure with evidence of both acute and chronic liver disease (Bruguera and Herrero, 2005; Anstey and Hift, 2007; Ma et al., 2010). Treatment with a combination of plasmapheresis to remove PPIX from plasma, hemin infusions to reduce marrow production of PPIX (Bloomer and Pierach, 1982; Gordeuk et al., 1986), cholestyramine (or activated charcoal) to interrupt the enterohepatic circulation of PPIX (Gorchein and Foster, 1999), bile acids such as ursodeoxycholic acid to enhance bile formation and flow (Van Hattum et al., 1986; Gross et al., 1998), and vitamin E to reduce oxidative stress (Komatsu et al., 2000; Singal et al., 2014) may achieve remission or slow the progression of liver disease and bridge the patient to hepatic transplantation. Such approaches are depicted in Fig. 3. These measures are not always effective and have not been applied in sufficient numbers of patients with hepatopathy to allow rigorous evaluation of efficiency (Anstey and Hift, 2007; Casanova-Gonzalez et al., 2010).

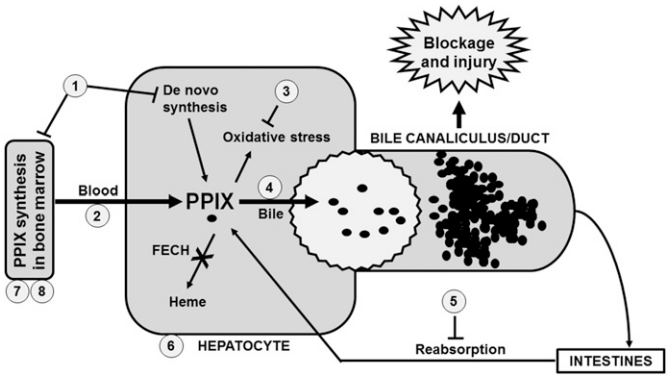

Fig. 3. Mechanisms of PPIX-mediated liver injury and strategies to manage this injury. PPIX-mediated hepatocyte and cholangiocyte damage initiates hepatobiliary injury in EPP. Approaches to manage this condition have included (1) suppression of PPIX biosynthesis by hemin; (2) plasmapheresis; (3) vitamin E or *N*-acetyl cysteine to reduce oxidative stress; (4) ursodeoxycholic acid to increase bile flow and PPIX excretion; (5) activated charcoal or cholestyramine to prevent reabsorption of PPIX from the small intestine; (6) liver transplantation; (7) bone marrow transplantation to restore FECH function; and in preclinical models (8) gene therapy targeting FECH.

New approaches to treatment and prevention of this severe complication of protoporphyria are needed.

Survival after liver transplantation for protoporphyric hepatopathy is comparable to that after other liver diseases (McGuire et al., 2005). However, because overproduction of PPIX by marrow continues, protoporphyric hepatopathy may recur (Samuel et al., 1988; de Torres et al., 1996; Meerman et al., 1999a; Dellon et al., 2002; McGuire et al., 2005). Marrow stem cell transplantation after a temporary remission of hepatopathy or liver transplantation can prevent recurrent liver damage (Rand et al., 2006; Wahlin and Harper, 2010; Dowman et al., 2011; Wahlin et al., 2011b; Singal et al., 2014). At present, identification in advance of patients at risk for development of hepatopathy is impossible. Motor neuropathy, which is characteristic of the acute porphyrias but not EPP and XLP, can develop in patients with severe protoporphyric hepatopathy, suggesting that PPIX or a product of PPIX may be neurotoxic under some conditions (Rank et al., 1993). Gene therapy targeting FECH, which is undergoing preclinical development, will, like bone marrow transplantation, restore FECH activity in the marrow (Poh-Fitzpatrick et al., 2002; Oustric et al., 2014).

PPIX-Based Strategies for Diagnosis and Therapy

PPIX can be activated by light to produce energy in the form of fluorescence and ROS. The photodynamic effect of hematoporphyrin, which is closely related to PPIX and formed by the acid hydrolysis of hemoglobin, was demonstrated in a self-experiment by Meyer-Betz (1913). After self-injection of hematoporphyrin, he developed severe swelling of the face and other sun-exposed areas, similar to the phototoxic reactions seen in EPP and XLP. Use of PPIX as a photosensitizer for photodynamic therapy was explored after observing photodynamic effects in erythrocytes of EPP patients containing excess amount of PPIX (Kosenow and Treibs, 1953; Kosenow, 1954). Administration of ALA, which is more stable and readily taken up in tissues in whole animals, leads to dose-related PPIX accumulation in varying amounts in different tissues (Anderson et al., 1981). Exogenous ALA bypasses the regulatory control of PPIX by ALAS during heme biosynthesis and results in PPIX accumulation. In 1990, ALA was administered locally to generate endogenous PPIX for photodynamic treatment of basal cell carcinomas (Kennedy et al., 1990). Afterward, endogenous PPIX-based strategies have been used for many clinical applications (Karu and Letokhov, 1994; Ishizuka et al., 2011; Rollakanti et al., 2013; Mordon, 2014). Thus, ALA can be administered orally or locally as a stable precursor for PPIX. PPIX-based therapy is now approved by the Food and Drug Administration for treatment of bronchial and esophageal cancers and early malignant lesions of the skin, bladder, breast, stomach, and oral cavity (Henderson and Dougherty, 1992; Pass, 1993; Oleinick and Evans, 1998; Dougherty, 2002; MacCormack, 2008).

Cancer cells accumulate a higher amount of PPIX than normal cells when treated with ALA (Fukuda et al., 1989). Photoactivation of PPIX in cancer cells generates ROS, which can cause apoptosis and necrosis by attacking mitochondrial and cytoplasmic proteins and destroying cell membrane integration (Mroz et al., 2011b; Mfouo-Tynga and Abrahamse, 2015). ROS generated from PPIX also damages blood vessels, leading to vascular occlusions, which deprives the tumor of oxygen and nutrients, thereby impairing tumor growth (Dougherty et al., 1998). ROS can also elicit an immune response that suppresses tumor growth (Castano et al., 2006; Garg et al., 2010; Mroz et al., 2010; Mroz et al., 2011a). Photodynamic effects of PPIX have also been used for cancer diagnosis. PPIX emits red fluorescence when irradiated with light at wave lengths of 400–410 nm, making cancerous lesions that have higher concentrations or less overlying epithelium than surrounding tissue more visible (Ishizuka et al., 2011).

In addition to cancer therapy and diagnosis, the photodynamic effects of endogenously generated PPIX have been used for the treatment of acne, sebaceous skin, rosacea, rhinophyma, Bowen's disease, androgenic alopecia, cosmetic enhancement, and photo rejuvenations (Goldman and Kincad, 2002; Nestor et al., 2006). Furthermore, PPIX-based strategies have been explored against Gram-positive and -negative bacteria, parasites, yeasts, and fungi (Hamblin and Hasan, 2004; O'Riordan et al., 2005; Akilov et al., 2006; EN, 2006; Donnelly et al., 2008; Goslinski et al., 2008).

Conclusions

PPIX homeostasis can be disturbed by effects on enzymes in the heme biosynthetic pathway (e.g., ALAS, PPOX, and FECH), transporters of pathway intermediates, and iron supply. In EPP and XLP, which are caused by loss-of-function *FECH* mutations and gain-of-function *ALAS2* mutations, respectively, PPIX accumulates initially in the marrow and causes painful cutaneous phototoxicity. Excess PPIX in VP is derived from accumulation of protoporphyrinogen IX, the immediate precursor of PPIX. PPIX is water insoluble, and its excretion occurs through the liver and biliary system. An excessive amount of PPIX presented to the liver in EPP and XLP can cause hepatobiliary injury and liver failure. The photodynamic effect of PPIX produced endogenously after ALA administration can be used for cancer diagnosis and

treatment. Approaches for management of EPP and XLP are advancing with emphasis on light protection, such as the development of an α-melanocyte–stimulating hormone that increases skin melanogenesis. Novel therapies are needed to reduce endogenous PPIX levels in these conditions and to treat and prevent PPIX-mediated liver injury.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Sachar, Anderson, Ma.

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