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5-Aminolevulinic acid: A matter of life and caveats

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ABSTRACT

Our mini-review concerns the potential adverse pro-oxidant role of 5-aminolevulinic (ALA), the first protoporphyrin IX (PP-IX)/heme precursor widely used in commercial formulations for endogenous photodynamic therapy (PDT). Our account collates the two sides of (i) ALA aerobic oxidation as a source of H_2O_2 and radicals when accumulated in acute intermittent porphyria (AIP), hereditary tyrosinemia type 1 (HT1), and lead poisoning, thereby triggering similar biochemical damage and symptoms and (ii) the central role of PP-IX as the immediate precursor of the heme prosthetic group of key proteins and enzymes (hemoglobin, myoglobin, cytochromes, catalase, and others). Conversely, PP-IX induces skin photodamage when accumulated in sun-exposed erythropoietic protoporphyria (EPP) patients. Furthermore, we highlight the harnessing of such antithetical ALA and PP-IX properties to design efficient PDT and insecticidal strategies. Research on ALA-based PDT photosensitizers calls for more attention on the deleterious action of excess ALA, whose main target is the mitochondrion.

Introduction

5-Aminolevulinic acid (ALA; 5-amino-4-oxopentanoic acid), the first precursor in the multistep heme biosynthetic pathway, plays a key role in the metabolic map of aerobic organisms. The heme group is an essential prosthetic group of proteins and enzymes involved in dioxygen transport, energy output, detoxification, and catalysis. Inevitably, this group immediately brings to mind hemoglobin, cytochromes, and catalase. Also interesting is the control of feedback of ALA production from succinyl-CoA and glycine catalyzed by ALA synthase (ALAS) and regulated by heme concentration in the mitochondria, since both precursor and final products may also have adverse pro-oxidant properties [1–7]. The fate of accumulated heme is the degradation by heme oxygenase that builds up toxic bilirubin and free ferrous iron, which is a reagent in the hydroxyl radical-generating Fenton reaction [8]. A noteworthy occurrence is an antioxidant heme-binding protein in triatomine insects of the genus Rhodnius and enormous amounts of urate antioxidant in blood-sucking dipterans and hemipterans, such as mosquitoes and barber bugs, to prevent exacerbated indiscriminate iron-catalyzed oxidations in the insect's organism [9,10].

The heme biosynthetic steps can be jeopardized by innate enzyme deficiencies (i.e., insufficient ALAS, ALAD, PBGD, UROS, UROD, CPO, PPO and FECH activities) or inhibited by xenobiotics (i.e., lead, insecticides and other chemicals) [11–14] (Fig. 1). Deficient ferrochelatase (FECH) results in skin-accumulated protoporphyrin IX (PP-IX), which is a powerful photosensitizer of deleterious singlet oxygen and, paradoxically, a useful harnessed tool in photodynamic therapy to treat dermatoses [15]. Both ALA – a source of reactive oxygen species by aerobic oxidation, and PP-IX – a singlet oxygen photosensitizer, are biosynthesized in the mitochondria, and are thus expected to threaten the cell due to failures in energy conservation and signal transduction if overproduced.

In this paper, we present a brief assessment of the double aspect of ALA as a fundamental metabolite for heme biosynthesis and human health, and as a possible cause of disorders when accumulated in liver and brain.

Heme biosynthetic pathway: caveats and diseases

Throughout its pathway (Fig. 1), heme biosynthesis encounters bottlenecks imposed by enzyme downregulation or chemical inhibition,

Abbreviations: ALA, 5-aminolevulinic acid; AIP, acute intermittent porphyria; ALAS, ALA synthase; ALAD, ALA dehydratase; ADP, ALA dehydratase porphyria; CPO, coproporphyrinogen oxidase; DOVA, 4,5-dioxovaleric acid; cAMP, cyclic adenosine monophosphate; EPP, erythropoietic protoporphyria; FECH, ferrochelatase; GABA, γ-butyric acid; cGMP, cyclic guanosine monophosphate; GSH-Px, glutathione peroxidase; HCP, hereditary coproporphyria; HT1, hereditary tyrosinemia type 1; IRP1, iron regulatory protein 1; PBGD, porphobilinogen deaminase; PCT, porphyria cutanea tarda; PP-IX, protoporphyrin-IX; PPO, protoporphyrinogen oxidase; RLM, rat liver mitochondria; SAME, succinylacetone methyl ester; SOD, superoxide dismutase; UROD, uroporphyrinogen decarboxylase; UROS, uroporphyrinogen synthase; VP, variegate porphyria; XLSA, X-linked sideroblastic anemia.

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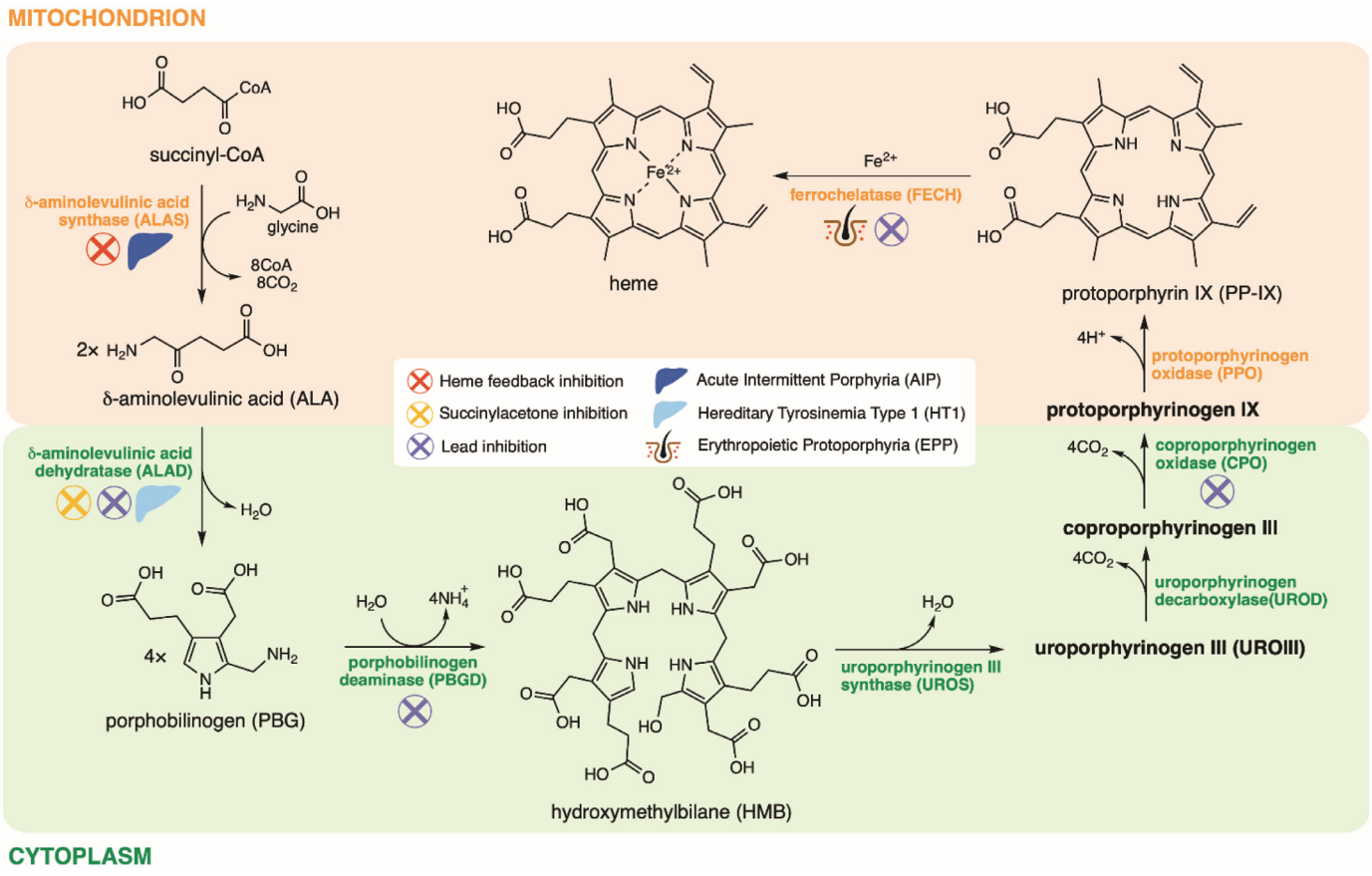

Fig. 1. Schematic overview of the heme biosynthesis pathway and porphyric diseases associated with enzyme deficiency and xenobiotic inhibition [1–3,7,11]. The scheme highlights the hepatic defects of AIP, HT1 and lead poisoning, which are characterized by pro-oxidant ALA overproduction, and the cutaneous EPP attributed to PP-IX photosensitizer accumulation in the skin.

triggering diseases collectively called porphyrias [1–3,7,11]. Eight well-characterized inherited hepatic and cutaneous porphyrias, or both types, correspond to each of the eight defective enzymatic steps in the heme pathway.

Deficient biosynthesis of heme compromises the feedback control of mitochondrial ALA generation from succinyl-CoA and glycine catalyzed by ALAS, resulting in microcytic anemia symptoms, which lead to X-linked sideroblastic anemia (XLSA) [16]. Once ALA is formed and transported to the cytosol, it undergoes bimolecular dehydration by ALA dehydratase (ALAD) to yield porphobilinogen (PBG), triggering neurovisceral symptoms associated with the so-called ALA dehydratase porphyria (ADP). Next, PBG undergoes a tetramolecular combination caused by porphobilinogen deaminase (PBGD), forming hydroxymethylbilane, the first tetrapyrrole of the heme biosynthetic pathway, whose deficiency is called acute intermittent porphyria (AIP), also with neurovisceral outcomes. The name porphyria, from the Greek *porphyrius*, purple, was coined from the typical sun-exposed dark red color of the urine of symptomatic AIP patients, indicative of high PBG concentration. ADP and AIP are typically hepatic disorders that do not result in photodamage. Specific enzymes of heme biosynthesis are affected by the presence of xenobiotics such as lead, cigarette smoke, and agrochemicals, which are serious threats to public health, the environment and the economy [3]. Furthermore, steroid hormones, some medicines, and sugary foods may also precipitate acute neurological and abdominal ADP and AIP crises [2,3,7]. In addition to dehydration to yield uroporphyrinogen III, the tetrapyrrole moiety undergoes further decarboxylation to yield coproporphyrinogen III, which migrates into the mitochondria and undergoes another decarboxylation step to yield protoporphyrinogen IX, which then oxidizes to PP-IX. Chelation of iron by the tetradentate PP-IX pyrrole moiety catalyzed by FECH yields the heme molecule.

The porphyrias comprise a group of eight diseases, each representing a defect in one of the eight steps of heme formation. Defects in enzymes involved in the synthesis of porphyrin intermediates (UROS, UROD, CPO, PPO, FECH) develop both neurovisceral and photocutaneous symptoms such as those in hereditary coproporphyria (HCP) (defects in CPO) and variegate porphyria (VP) (defects in PPO). Erythropoietic protoporphyrias (EPP), caused by deficiency in FECH activity, is one of the most common and severe types of porphyria that cause photodamage, characterized by blisters, scars and deformities in light-exposed face, ears and dorsum of the hands. The mechanism of patient photodamage associated with defects in the heme biosynthetic pathway and its implication in photodynamic therapy (PDT) and photodynamic diagnosis (PDD) will be addressed later in this review. The most prevalent of the porphyrias, porphyria cutanea tarda (PCT), which is estimated to affect 5 to 10 people out of every 100,000, is caused by the inhibition of uroporphyrinogen decarboxylase (UROD), the fifth enzyme in the heme biosynthetic pathway [2,17]. The four types of porphyrias discussed here are the ones most commonly encountered in clinical practice [2,3,7].

ALA, a potential radical weapon in vivo

Over 10,000 research articles, reviews, and letters on ALA have been published [18]. Interest in this subject has increased noticeably in recent years, mostly because of its harmful connections with ALA accumulation in the blood and urine of ADP and AIP patients. Two other porphyric

disorders of great interest, characterized by elevated ALA, are lead poisoning and hereditary tyrosinemia Type 1 (HT1), which are attributed, respectively, to the inhibition of adenosine diphosphate by lead and to succinylacetone derived from the oxidative degradation of tyrosine [19]. Interestingly, albeit not surprisingly from the standpoint of excess of physiological ALA, is that AIP, HT1, and lead poisoning share similar symptoms: abdominal pain, neuropsychiatric alterations, and liver and kidney damage.

The diagnosis, pathogenesis, symptoms and treatment of AIP patients have been reviewed countless times [2,3,7]. Briefly, AIP metabolic disorder may be symptomatic or silent. Medication for acute crisis is limited to a few drugs such as dextrose, antiemetics and hematin, whose administration may provide relief from characteristic abdominal and back pain, nausea, seizures and tachycardia. AIP patients may display aggressive, anti-social behavior and may also experience hallucinations. Tyrosinemia and AIP patients may also be victims of liver cancer [20]. On the other hand, the hematological, abdominal and neurological features of children and adults poisoned by lead at blood levels of less than 25 μmol.L^{-1} resembles those of AIP and tyrosinosis carriers [12,21]. Lead poisoning is still a heavy burden in poor and developing countries today. It afflicts mainly undernourished children and lead-exposed workers in risky environments [12,22–25]. Lead chelation with calcium-EDTA, dimercaptosuccinic acid, D-penicillamine and antioxidants have been administered to alleviate and eventually eliminate the symptoms of plumbism [21,26,27].

Early histopathological work with biopsies of AIP livers revealed extensive mitochondrial structural changes and intramitochondrial ferritin deposits, lipid droplets and lipofucsin [28], while renal mitochondrial accumulation of lead and impairment of energy metabolism was reported in lead poisoning [11]. Severe liver failure and renal dysfunctions associated with DNA fragmentation and reduced mitochondrial transmembrane potential are reported in the literature [29]. Rats subjected to exhaustive swimming tests were found to present lower physical performance upon pre-injection of ALA (40 mg/kg body weight) than treated sedentary rats, while presenting markers of higher glycolic metabolism such as glycogen accumulation in liver and muscle, increased blood lactate, lower mitochondrial antioxidant defenses, particularly MnSOD, and early fatigue during swimming [30].

ALA can indeed act as a neurotoxin after prolonged administration into the brain of rats. Two-fold lower binding of [^{3}H]-muscimol, a γ-butyric acid (GABA) agonist to GABAergic receptors was found in synaptic membranes obtained from distinct areas of the brain [31]. This effect was accompanied by the elevation of oxidative stress: chemical and enzymatic indicators such as iron deposits, carbonyl proteins, and lipid peroxidation products [31]. ALA-activated inhibition of GABAergic receptors were recently confirmed independently elsewhere [32], using synaptic membranes prepared from the cerebral cortex of rats and humans, in the latter case collected less than six hours postmortem from healthy male corpses [33]. In addition, the activity of adenylyl cyclase of rat and human brain samples were significantly inhibited in the presence of ALA, thus suggesting ALA interference in cyclic adenosine monophosphate (cAMP)–mediated cell processes [33].

The neuropsychiatric manifestations of AIP patients were attributed by Brennan and Cantrill (1979) [34,35] to a competition between ALA and GABA for GABAergic receptors, based on the structural similarity of these two metabolites (Fig. 2). The authors also measured ALA-promoted alterations on the fluxes of amino acid neurotransmitters in rat brain synaptosomes that may control GABA release from nerve endings, which might also operate in acute porphyria attacks.

However, the authors were not aware that the presence of a carbonyl group in the vicinal C4 atom of ALA renders the compound prone to enolization to δ-CH(NH$_2$)=C(OH)-(CH$_2$)$_2$-CO$_2$H, similar to other α-aminoketones (aminoacetone and 1,4-diamino-2-butanone) in aerated phosphate buffer at pH 7.4 [1, 5, 36]. The enolic form of ALA is more susceptible to hydrogen abstraction, yielding an enolyl radical and ultimately a highly electrophilic α-oxoaldehyde, namely 4,5-dioxovaleric acid (DOVA). Other by-products include reactive oxygen species (ROS) in aerated medium such as H_2O_2, superoxide radical-anion ($O_2^{\bullet-}$) and hydroxyl radicals (HO$^\bullet$) (Fig. 3).

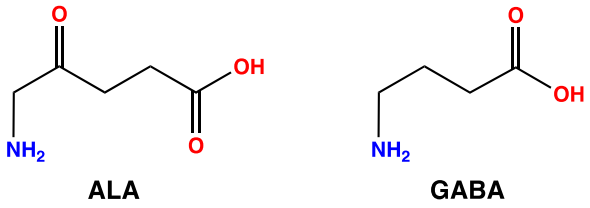

Fig. 2. Structural comparison of ALA and GABA.

If ALA oxidation does indeed occur in the synaptic connections, which is favored by high concentrations of molecular oxygen in the brain, then ALA bound to GABAergic receptors is expected to promote irreversible local protein oxidation and brain damage. Accordingly, radiolabeling and immunohistochemical assays with ALA, succinylacetone methyl ester (SAME), and DOVA treatment of synaptosomes, neurons, and rat brain significantly affected the density, morphology and GABA binding properties of GABA$_A$ receptor (GABA$_A$R) [19,37].

Taken as a whole, these data underpin the hypothesis of involvement of free radical and oxidative stress in the clinical manifestations of human disorders linked to ALA accumulation in brain, liver, muscles and other organs.

Previously, superoxide dismutase (SOD) and glutathione peroxidase (GSH-P$_X$), both acting in the front line against tissue oxidative damage caused, respectively, by $O_2^{\bullet-}$ and H_2O_2, were found to be roughly twofold higher in the blood of AIP patients under acute crisis as in that of healthy individuals [38]. Even latent AIP individuals were found to have significantly elevated antioxidant enzymes. This was then interpreted as a protective response against high levels of deleterious ROS generated by excess ALA oxidation. Moreover, ALA intraperitoneal injection in rats on two alternate days significantly increased their plasma antioxidant capacity and the chemiluminescence of liver, brain and soleus muscle. This is attributed to extensive lipoperoxidation triggered by hydroxyl radical production by iron-catalyzed ALA oxidation [39]. Accordingly, hydroxyl radical was detected through *in vivo* EPR spin trapping experiments in rats in response to intraperitoneal injection of ALA [40]. *In vitro*, ALA was shown to release Fe^{2+} from horse spleen ferritin, the protein that stores iron in cells, which consequently halts hydroxyl radical originating from the Fenton reaction with H_2O_2 [41].

Noteworthy is the treatment of rats with SAME, the inhibitor of ALAD that leads to ALA accumulation. The mobilization of non-heme iron to animal liver and brain was observed, with concomitant increase in plasma iron and transferrin, accompanied by carbonyl proteins, lipid peroxidation, and SOD activity [42]. The cytosolic iron regulatory protein 1 (IRP1), which controls cellular iron homeostasis through post-transcriptional modifications, was also activated in cell cultures in response to the administration of ALA [43]. Overall, these findings support the hypothesis that iron-dependent oxidative imbalance linked to “free” iron contributes to neuropathy and hepatoma in AIP and tyrosinosis.

Experiments with isolated rat liver mitochondria (RLM) have corroborated possible adverse effects of excess ALA in porphyric diseases [1,19,44]. Firstly, in air-equilibrated solutions, RLM undergoes ALA concentration-dependent swelling, collapse of transmembrane potential and decrease of adenosine triphosphate (ATP) production. The addition of a millimolar concentration of calcium exacerbates the decline in these properties, whereas Mg^{2+} ions restore the original RLM properties. Normal intracellular Ca^{2+} ion is reportedly in the nanomolar range (approx. 10 nmol.L^{-1}). Increased calcium ion flux into the mitochondrial matrix leads to organelle permeabilization, cytochrome c release, and ultimately cell apoptosis or necrosis. These findings support the mechanism of ALA-driven opening of mitochondrial transition pores, putatively due to the oxidation of inner membrane thiol proteins by ALA-generated

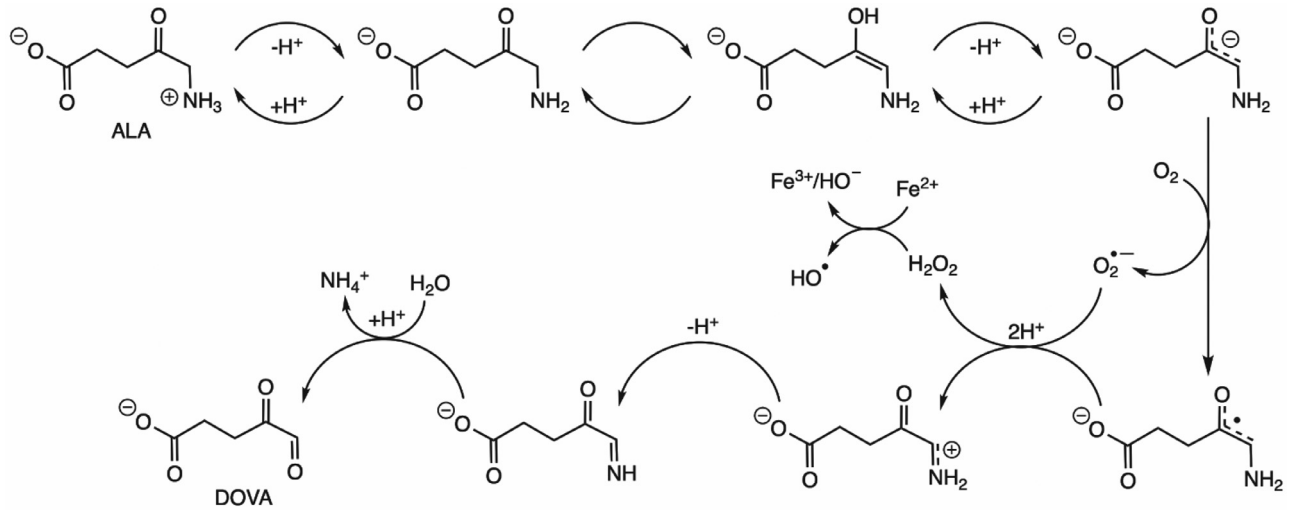

Fig. 3. Proposed chemical mechanism involved in the transformation of ALA into DOVA [1].

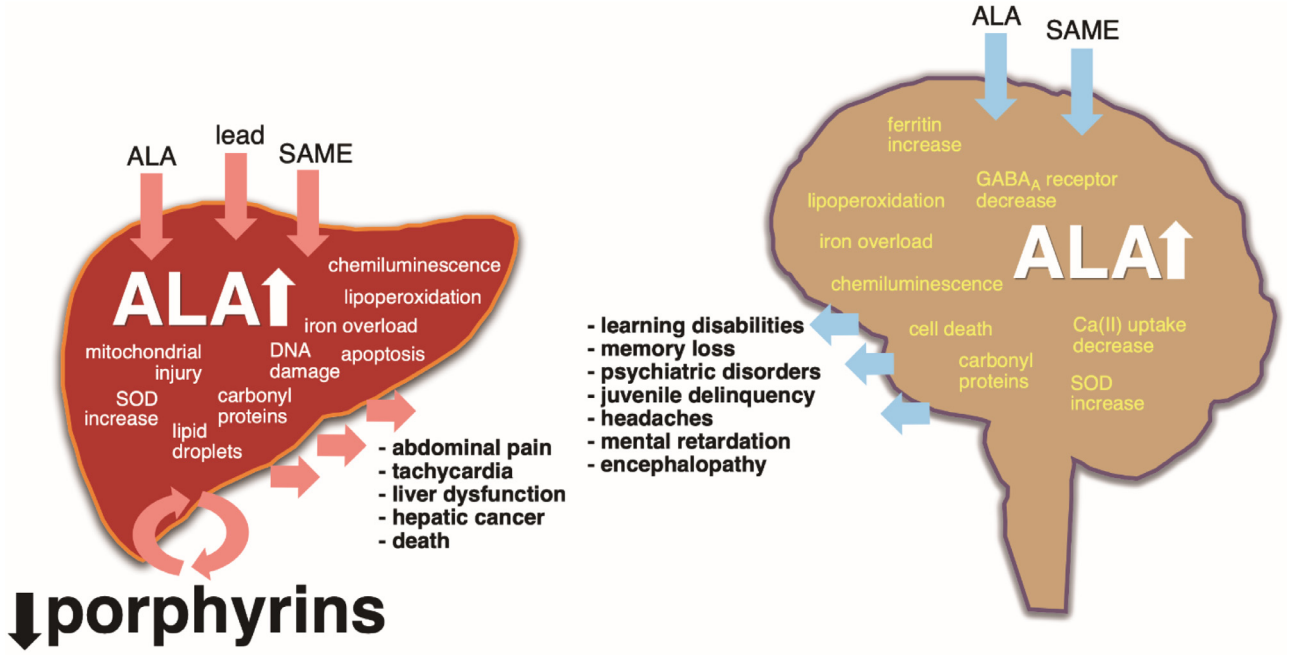

Fig. 4. Schematic overview of hepatic and cerebral oxidative damage and clinical manifestations of ALA overload in AIP, HT1, and lead poisoning. The biochemical and pathophysiological features inserted in the scheme are extracted from *in vitro, in vivo*, and *ex vivo* reports quoted in the present review. Modified from [19].

hydroxyl radicals. Thus, the mitochondria seem to be pivotal targets of ALA accumulated in ADP, AIP, tyrosinemia, and lead poisoning (Fig. 4).

Protoporphyrins, for good and evil

Once biosynthesized in the mitochondria as the final product of ALA through eight enzymatic steps, protoporphyrin incorporates Fe^{2+} in the presence of FECH to form heme: the prosthetic group of key proteins and enzymes that support the aerobic metabolism, such as hemoglobin, myoglobin, cytochromes, catalase, peroxidase, and tryptophan dioxygenase. The heme production rate from ALA is controlled by heme-dependent feedback inhibition of ALAD in conjunction with heme oxidative degradation by heme oxygenase to antioxidant biliverdin, pro-oxidant iron, and carbon monoxide, a signaling molecule linked to cardiovascular functions and inflammatory responses [8].

Deficient biosynthesis of UROD and FECH leads to accumulation of their substrates in the skin. Uroporphyrinogen III (UROIII) and PP-IX, which act as photosensitizers of singlet oxygen in carriers of PCT and protoporphyria, result in dramatic light-exposed skin ulcerations. Upon irradiation, energy transfer from triplet PP-IX to ground state molecular oxygen takes place, yielding oxygen in the singlet state $O_2(^1\Delta_g)$ through the type II reaction (Fig. 5).

Singlet molecular oxygen is a powerful oxidizing electrophile towards double bonds and sulfur and nitrogen atoms in unsaturated biomolecules like membrane polyunsaturated fatty acids and cholesterol, protein histidine, tryptophan, methionine residues, and DNA bases. 1,2-dioxetanes, allyl hydroperoxides, and 1,4-endoperoxides are products from singlet oxygen attack on alkenes, alkadienes, and anthracene derivatives; methionine sulfoxide from methionine; endoperoxides and hydroperoxides from histidine; kynurenine from the indole moiety of tryptophan; and 4-OH-8-oxodGuo, 8-OOHdGuo and 8-oxodGuo from 2'-deoxyguanosine moieties [45]. Thus, necrosis and mu-

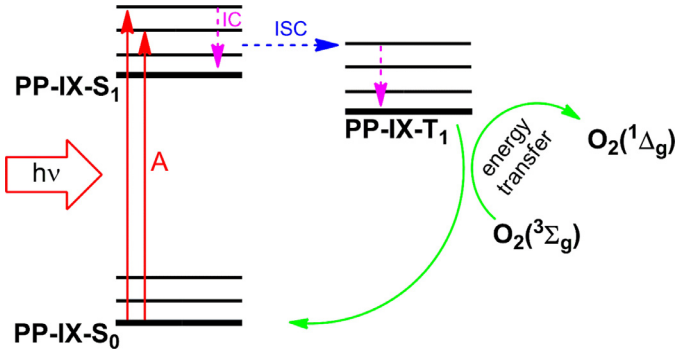

Fig. 5. Simplified Jablonski diagram of the generation of singlet oxygen through PP-IX photosensitization. A – absorption, IC – internal conversion, ISC – intersystem crossing.

tagenesis are expected as adverse outcomes of the reactivity of singlet oxygen. Hydroxy, carbonyl, and cleavage products have been detected *in vitro* and *in vivo* aimed at elucidating the reaction mechanisms and validate biomarkers of cellular oxidative damage caused by singlet oxygen.

The first report of a "diffusible, reactive, metastable state of the oxygen molecule" was made by Kautsky and de Bruijn in 1931 [46,47]. These authors observed the photodegradation of leucomalachite green [4′-benzylidenebis(*N,N*-dimethylaniline)] upon exposure to light in an aerated atmosphere, but not under nitrogen mixed with trypaflavine, a fluorescent donor, both immobilized in silica grains. Decades later, it was discovered that singlet oxygen is produced in biological systems through chemiexcitation reactions promoted by peroxides. For instance, myeloperoxidase present in the macrophage membrane catalyzes the singlet oxygen-generating reaction of H_2O_2 with ClO^- (Eq. 1), previously described and spectroscopically identified *in vitro* [48–50].

$$H_2O_2 + ClO^- \longrightarrow H_2O + Cl^- + O_2(^1\Delta_g) \quad (1)$$

monomol emission: λ = 1,270 nm
dimol emisson: λ = 604 and 703 nm

Various biochemical sources of singlet oxygen have been unveiled, e.g., Russell's reaction of geminal hydrogen-bearing hydroperoxyl reactions, quenching of dioxetane-generated triplet carbonyls by ground state oxygen, and reaction of H_2O_2 and hydroperoxides with the peroxynitrite anion [45,51,52]. Noteworthy is the recent detection of singlet oxygen as an ultimate by-product of the *in vitro* nucleophilic addition of peroxynitrite to glyoxal and acrolein, both carbonyls implicated in the so-called "carbonyl stress" of the ageing process and in several associated human diseases [53–56].

The acute manifestations of EPP in response to sun exposure are skin tingling, itching and burning, which aggravate to erythema, edema, blisters, and scars upon extended exposure to UV rays. Chronic exposure causes lichenified skin on knuckles and around the mouth and further severe skin degeneration leading to face and ear deformities, culminating with poor quality of life and self-social isolation [2,7]. Nevertheless, the elucidation of molecular and biomedical bases of protoporphyria disease enabled the development of photodynamic therapy for skin cancer and other forms of dermatosis, not only with porphyrin derivatives and ALA as a source of PP-IX but also with synthetic and natural photosensitizers [57].

A broad compilation of candidate photosensitizers and their respective quantum yields of singlet oxygen production (Φ_Δ) was provided by Redmond and Gamlin (1999) [57]. These authors measured the highest quantum yields in favorable solvents, some of which deserve to be mentioned here, such as mesochlorin (0.79), ZnPP-IX (0.91), 1,9-dimethyl methylene blue (0.76), hematoporphyrin monomer (0.74), Photofrin® (0.89), and Rose Bengal (0.79). Federal health institutions around the world have approved Photofrin®, a porphyrin derivative drug [57].

The biological responses to PDT depend on the sub-cellular location of the photosensitizer: apoptosis (mitochondria); apoptosis or necrosis (lysosomes); rescue, apoptosis, or necrosis (plasma membrane). The main problems with PDT consist of the difficulty of light delivery, (bio)chemical stability of the sensitizer, tumor selectivity, limited depth of tissue penetration, local oxygen concentration, and extensive skin phototoxicity. More recently, photothermal therapy techniques employ nano-inorganic photosensitizers that absorb NIR light and deliver heat to kill cancer cells [58].

ALA, for good and bad

ALA administration in rats resulted in oxidative damage in lipids, DNA and proteins with associated increases in CuZnSOD, total iron, ferritin, protein carbonyls and Ca^{2+} uptake [31,59]. Notable in this respect is the increase in SOD and GSH-Px found in AIP patients, which has been interpreted as a protective response against ALA-generated reactive oxygen species [38,60].

At the turn of the century, notwithstanding the hepatic and cerebral pro-oxidant properties of ALA, ALA-ester derivatives such as methyl aminolevulinate (ALA-OMe), the active ingredient of the medicine Metvix®, became increasingly investigated for tumor PDT. There were two main reasons for these investigations: (i) controlled ALA administration might act as safe and effective endogenous source of PP-IX; and (ii) ALA esters are more promptly uptaken by cell membranes because they are more lipophilic than ALA, although cellular uptake seems to be actively mediated by transporters of nonpolar amino acids. Reed and coworkers [61] reported that ALA, active ingredient of the Levulan® administration in dysplastic Barrett's esophagus had 5–8 times preferential accumulation in the tumor tissue, where PP-IX was the main porphyrin in the esophageal mucosa (85–90%), whereas uro- and copropophyrin were less that 10% due to excretion.

More recently, new ALA-derivatives have been tested as PDT and PDD drug candidates. Ester derivatives act as prodrugs of ALA and were designed and synthesized for higher hydrophilicity and lipophilicity to optimize tumor cell uptake and metabolism [62,63]. An interesting *N*-modified ALA-OMe with disulfide bond and biotin moieties was recently found to target biotin receptors, cause GSH depletion and deliver ALA-OMe to be used in PP-IX biosynthesis [64]. Depletion of GSH increases redox imbalance of ALA-generated ROS and PP-IX. Furthermore, the use of ALA and ALA-OMe in gold nanoparticles (AuNPs) has been demonstrated to enhance drug toxicity in the dark, which is attributed to intensification of the hydroxyl radical EPR signal. (ALA-OMe)-AuNPs is reportedly more active than (ALA)-AuNPs, and both nanoparticles are more active than ALA alone [65]. In short, the reported findings about ALA-based PDT attest to the involvement of radicals, singlet oxygen and peroxide-mediated imbalance in ALA and PP-IX treatment.

Fewer side effects were reported with 30 mg ALA/kg – headache, photosensitivity, nausea, and vomiting, but liver dysfunction indicated by high ALA and Asp transaminases was also found. Low blood pressure, a frequent adverse effect associated with oral administration of ALA in PDT, has also been reported several times over the years in the clinical literature [66–68]; however, this is not yet fully understood. It has long been known that $NO^\bullet$ radical generated by nitric oxide synthase (NOS) plays an important role in hemodynamics due to its ability to attach and activate guanylate cyclase, thereby facilitating cyclic guanosine monophosphate (cGMP)-elicited blood pressure relaxation. Accordingly, the use of ALA/PP-IX in PDT has been reported to elicit pulmonary artery relaxation and soluble guanylate cyclase activation [69]. On the other hand, $NO^\bullet$ reportedly attacks Fe-enzymes such as FECH [70], thus inhibiting their function leading to PP-IX accumulation. Recent work has demonstrated that after moderate PDT challenge sensitized by ALA-induced PP-IX, NOS upregulation in cancerous cells could also contribute to local vasodilation [71,72]. Importantly, AIP pa-

tients were shown to possess twice the antioxidant GSH-Px and SOD1 activity in blood, which decreases the local concentration of superoxide radical produced by the oxidation of ALA overproduced during an acute attack [38]. Bearing in mind that the reaction of $NO^{\bullet}$ radical with superoxide radical is diffusion-controlled ($k_2 \sim 5 \times 10^9\ M^{-1}.s^{-1}$) [73], it is expected that any abatement of superoxide radical may also contribute to increase $NO^{\bullet}$ and hence be associated with hypotension in Down Syndrome [74] and cancer [75].

Local pain is also a side effect experienced by patients under ALA-treatment, albeit less painful with ALA derivatives [76,77]. ALA, but not methyl- and hexyl-ALA esters, can be uptaken by peripheral nerve endings via the GABA receptors. Thus, the GABA receptors may elicit the most severe abdominal and muscle pain associated with AIP crisis and ALA treatment [78,79]. It has been shown that hexyl- and undecanoyl-ALA esters are not transported by GABA-β receptors but by simple diffusion [80]. Therefore, pain alleviation in ALA ester-treated patients may be attributed to differences in uptake mechanisms.

Using a cell line of murine mammary adenocarcinoma, Battle and coworkers [63] verified that about 50 ng PP-IX/10^5 cell was formed upon treatment with 0.6 mmol.L^{-1} ALA, 1.2 mmol.L^{-1} methyl ALA and 10 μmol.L^{-1} n-hexyl ALA and killed by apoptosis, thereby suggesting higher efficacy of more lipophilic ALA esters for topical PDT. Pharmacokinetic profiles of ALA in humans are scanty in the literature, but low bioavailability after oral and intravenous ALA administration has been reported. This is attested to by an ALA half-life of approximately 45 min and large amounts of unchanged ALA excreted in the urine as consequence of first-pass metabolism [81]. This unfavorable behavior underscores the importance of designing and using ALA derivatives.

Over 500 articles and reviews on the use of ALA and its esters have been published in the past five years, attesting that they are the treatment of choice, directly or combined with surgery, chemotherapy, radiotherapy, immunotherapy of cell carcinomas in dermatology, gastroenterology, head and neck cancer, pulmonology, urology, nephrology, neurology, gynecology and other medical areas. A recent review by Casas (2020) [82] covers some applications of ALA-PDT. Intriguing is the fact that research on ALA-PDT does not examine possible adverse outcomes triggered by ALA-generated reactive oxygen species and mitochondrial damage in tissues surrounding the tumors.

Worth to note from the agriculture point of view is the concept, phenomenology and potential of porphyric insecticides and herbicides based on ALA and coadjutants, namely DOVA, introduced in the 1980s [83]. In this regard, ALA treatment of insects like lepidopterans (e.g., moths, butterflies) and orthopterans (e.g., grasshoppers, crickets) induce porphyrin accumulation, which is lethal both under sunlight and at night, the latter condition possibly reflecting the ALA pro-oxidant activity [84].

Conclusions

ALA occupies a key place on the metabolic map of aerobes for being the precursor of heme, the prosthetic group of molecular oxygen binding and electron carrier proteins and enzymes. Conversely, when administered or endogenously accumulated in cells, ALA exhibits its other Janus face when it reacts with molecular oxygen to yield radicals and an α-oxoaldehyde that can trigger cell oxidative damage and mutagenesis. At first sight, curiously, the adverse responses of abnormally high cell levels of ALA underlie the clinical manifestations in AIP, tyrosinemia, and lead poisoning, respectively, (i) metabolic errors (deficient ALAS and PBGD), directly linked to the heme biosynthetic pathway; (ii) an inborn defect of Tyr catabolism leading to augmented succinylacetone level, a strong inhibitor of ALAD; and (ii) an acquired disease from exposure to xenobiotics. On the other hand, the lack of FECH leads to dermal accumulation of photosensitizing PP-IX and consequent skin lesions and cancer. It is remarkable that both the first step of heme biosynthesis – ALA generation by the ALAS–, and its final product – heme originated by the insertion of iron in PP-IX by FECH – takes place in the mitochondria, where heme can cause feedback inhibition of ALA concentration, enabling it to govern the flux of ALA production and eventually halt deleterious activity. In conclusion, the adverse aspects of both ALA and heme can be circumvented by employing therapeutic approaches to alleviate diseases caused by escape from normal metabolism.

Declaration ofCompeting Interest

The authors have no conflicts of interest to declare.

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