Current Advances in 5-Aminolevulinic Acid Mediated Photodynamic Therapy

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Abstract

Kennedy and Pottier discovered that photodynamic therapy (PDT) could be carried out using a procedure consisting of topical application of the porphyrin-precursor, 5-aminolevulinic acid (ALA) to the skin, followed after some time by illumination with various light parameters in the 1980s. Since then, ALA-PDT has expanded enormously and now covers most aspects of dermatological disease. The purpose of this review is to discuss a range of ingenious strategies that investigators have devised for improving the overall outcome (higher efficiency and lower side effects) of ALA-PDT. The big advance of using ALA esters instead of the free acid to improve skin penetration was conceived in the 1990s. A variety of more recent innovative approaches can be divided into three broad groups: (a) those relying on improving delivery or penetration of ALA into the skin; (b) those relying on ways to increase the synthesis of protoporphyrin IX inside the skin; (c) those relying on modification of the illumination parameters. In the first group, we have improved delivery of ALA with penetration-enhancing chemicals, iontophoresis, intracutaneous injection, or fractionated laser. There is also a large group of nanotechnology-related approaches with ALA being delivered using liposomes/ethosomes, ALA dendrimers, niosomes, mesoporous silica nanoparticles, conjugated gold nanoparticles, polymer nanoparticles, fullerene nanoparticles, and carbon nanotubes. In the second group, we can find the use of cellular differentiating agents, the use of iron chelators, and the effect of increasing the temperature. In the third group, we find methods designed to reduce pain as well as improve efficiency including fractionated light, daylight PDT, and wearable light sources for ambulatory PDT. This active area of research is expected to continue to provide a range of intriguing possibilities.

Introduction: PDT with ALA and ALA Esters

The birth of ALA-PDT can be traced back to studies that were concerned with the biochemistry of porphyria [1••]. The term "porphyria" describes a range of rare genetic diseases that are characterized by disturbances to the heme biosynthetic cycle (see Fig. 1) that provides all the tetrapyrrole molecules required, not just for the oxygen carrying capability of hemoglobin and myoglobin, but for a myriad of enzymes and pathways that require heme-based co-factors and cytochromes. Since all mammalian cells rely on oxidative phosphorylation for (at least part) of their energy supply, the heme biosynthesis pathway must be present to some degree in all the tissues of the body. There are many varieties of the inherited disease called "porphyria," but the one that is relevant to this review is erythropoietic protoporphyria (EPP) caused by a deficiency (genetic or acquired) in the last enzyme in the heme synthesis chain, ferrochelatase. Since the immediate precursor of heme, protoporphyrin IX (PPIX) is a photosensitizer, sun-exposed areas of the skin can suffer severe photodamage in this disease. 5-Aminolevulinic acid (ALA) is a stable small molecule precursor of the pathway, and if this compound is added exogenously to cells in culture, or to experimental animals (either systemically or topically), some degree of additional free porphyrin synthesis is observed (frequently assessed by the characteristic red fluorescence of PPIX). In the 1950s, four human volunteers orally consumed an ALA solution and suffered from dose-related phototoxic skin reactions especially after sun exposure [2]. These observation provided Kennedy and Pottier with the inspiration to test whether topical application of ALA to the skin followed by illumination could carry out photodynamic therapy (PDT) [3•]. The idea was that there was a feedback inhibition loop involving excess heme inhibiting ALA-synthetase that serves to prevent the heme biosynthetic cycle from producing more porphyrins than were required to make the amounts of heme the cell needs. However, the addition of exogenous ALA bypasses the feedback control, and an excess of free PPIX accumulates since ferrochelatase (which insets the ferrous iron into heme) is the slowest acting enzyme in the cycle, and is therefore rate limiting.

The principle of PDT had been discovered as early as 1900, but in 1978, Dougherty et al. published striking results in which they treated 113 cutaneous or subcutaneous malignant tumors by intravenous injection of a hematoporphyrin derivative and observed a total or partial response in 111 cases [4••]. Photofrin-PDT was first approved in the USA in 1993 for treatment of bladder cancer [5]. Kennedy and collaborators in the 1990s treated superficial skin cancers (mainly basal cell carcinomas) with topically applied ALA and subsequent illumination using broad-band white light from a slide projector [3•].

ALA-PDT was first used on a large scale for treating actinic keratosis (AK) when activated by blue light and received US approval as Levulan Kerastick with BLU-U activation in 1999 [6]. One big advance in ALA-PDT was made when the use of ALA esters was introduced.

The idea here was that the more lipophilic structure of the esters (compared to the free carboxylic acid) would increase the speed and amount of uptake into cells, and therefore, more PPIX would be formed faster [7]. The methyl ester of ALA was the first to be approved for treating BCC and AK in 2001 as Metvix or MAL [8]. MAL-PDT when activated by red light was approved in the USA for treating AK. The hexyl ester of ALA was approved for use in the bladder (for fluorescence diagnosis) as Hexvix (now Cysview) in 2010. Numerous other esters of ALA have been investigated in the laboratory setting [9].

Improvements in ALA Penetration

Since the most important limiting factor in ALA-PDT is the penetration of the relatively polar molecule ALA through the permeability barrier of the skin, the lipophilic *stratum corneum*, many approaches have concentrated on increasing penetration. The approaches tested for increasing ALA penetration are schematically illustrated in Fig. 2.

Intracutaneous ALA

The simplest way to get more ALA into the skin is simply to inject it into the skin where it is meant to be. This seemingly obvious approach was not actually tested until 2001. De Blois et al. [10] administered ALA to the normal skin of 6-week-old female Dutch pigs by intracutaneous injection of an aqueous solution of ALA (pH 5.0) in volumes of 0.1–0.5 ml and concentrations ranging from 0.5 to 2 % and by topical administration of a 20 % ALA cream. All injected doses of 3 mg ALA or more led to a faster initial increase in the rate of PpIX synthesis and significantly greater fluorescence than that measured after topical administration of ALA. Irradiation (60 J/cm^2 of 600–730 nm) of the spots was performed at 3.5 h after ALA administration. Injection of 2 mg ALA or more followed by light gave more PDT damage than after topical application of 20 % ALA.

Sakamoto et al. [11] in 2011 obtained similar data in a pig model looking at intracutaneous injection of ALA and illumination with a 635 nm LED array at a fluence of 200 J/cm^2.

Combination with Fractional Laser

A different approach to ALA delivery into the skin is through manipulating the skin surface with lasers. Applying ALA in combination with ablative and non-ablative fractional laser treatment helped increase the penetration of ALA into deeper structures of the skin. Ablative fractional laser is used as a pretreatment before ALA-PDT by removing skin material through irradiating the skin surface with a small scanned laser spot. This process creates small holes (see Fig. 2) in the skin in which ALA can more easily pass through. Non-ablative fractional laser is also used as a precursor to ALA-PDT but is less invasive and instead of removing the skin layer to create holes; it simply heats the skin (thermal denaturation) to increase penetration of ALA. The beneficial feature of fractional laser treatment is the fact that healing of the microscopic lesions in the skin is much faster than comparable amounts of damage produced in macroscopic lesions.

Haedersdal et al. [12] showed the success of ablative fractional laser (AFXL) first in an animal model. They showed that in Yorkshire, swine skin pretreated with the CO_2 AFXL (10.6 μm, 1850 μm ablation depth) produced increased porphyrin fluorescence versus skin

that was left intact after the topical application of ALA and MAL cream (20 %). This increase in topical drug uptake was also seen for non-ablative fractional laser pretreatment. Lim et al. [13] pretreated 12 (1 cm^2) areas on the backs of 10 healthy male patients and followed the pretreatment with application of ALA. Sites pretreated with non-ablative fractional laser showed a significant increase in porphyrin fluorescence when compared with non-pretreated areas. The results also showed that laser energy strength and ALA incubation time were positively correlated with ALA absorption in the skin.

Fractional laser treatment in combination with ALA and MAL was also shown to have clinical effectiveness. Previously, ALA-PDT could not be used as a treatment for nodular basal cell carcinoma because these tumors were thicker than 2 mm. However, Lippert et al. [14] showed that ALA-PDT with ablative laser therapy could overcome the limit caused by tumor thickness in patients with nBCC as the increased penetration allowed ALA to reach deep enough into the tumor layers. Yin et al. [15] evaluated the effects of erbium: yttrium-aluminum-garnet (Er:YAG) ablative fractional laser resurfacing for treatment of severe acne. Severe acne is known to be difficult to treat by conventional methods, but with fractional laser resurfacing combined with ALA-PDT, the depth of penetration and unique ability to stimulate wound healing is promising as a new treatment approach. Forty subjects with severe acne were treated with 15 % ALA-PDT, 4 times over 40 days (10 day intervals) and then received ablative fractional Er:YAG laser treatment five times at 4 week intervals. After 6 months post-treatment, all subjects showed an overall improvement in acne inflammatory lesions and an 80 % improvement in acne scars.

Forster et al. [16•] investigated how ALA and MAL treatments were affected when combined with continuous versus fractional ablation lasers. The stratum corneum (SC) was irradiated with continuous and fractional ablation with 2940-nm Er:YAG laser systems (Continuous: 0.5–1.5 J/cm^2; Fractional: 4–24 J/cm^2). The mean fluoresces intensity enhanced 13.8-fold with continuous ablation and 7.3-fold with fractional ablation when compared against skin without laser pretreatment showing that continuous ablation increased ALA and MAL penetration by the greatest amount.

Lee et al. [17] looked at another parameter of fractional laser pretreatment with ALA-PDT. Lee and colleagues examined how the magnitude of fluences of fractional laser affected ALA permeation in nude mouse skin and porcine skin and found that laser-treated nude mouse skin versus intact skin had a 27–124-fold increase in ALA flux while porcine skin versus intact skin had a 3–260-fold increase. Compared to a conventional laser, the low fluence lasers showed less severe skin disruption, a shorter recovery duration and were overall safer.

Penetration Enhancement

Methods of increasing the permeability of ALA through the skin encompass a much wider variety than just ablative and non-ablative fractional laser treatments. In fact, subtleties exist between the different combinations of therapies with laser treatments, how to most effectively use these lasers as well as a plenty of other techniques that all help enhance the transdermal penetration of ALA.

Combinations of multiple different penetration enhancement techniques were examined by Fang et al. [18]. They investigated how electrically assisted methods of iontophoresis and electroporation used on laser- or microdermabrasion-treated skin affected ALA permeation. First, Fang et al. measured how Er:YAG laser and microdermabrasion affected ALA permeation through skin separately from other techniques. Microdermabrasion increased ALA permeation 5–15 times more than intact skin, while Er:YAG laser-treated skin had the greatest enhancement of ALA permeability by enhancing penetration by 4–246 times versus controls. In combination with iontophoresis, ALA permeation increased by another 15-fold and electroporation increased permeation by 2-fold. This combination of electrically assisted techniques along with resurfacing methods caused a strong synergistic effect on ALA permeation.

There exist other penetration enhancers that work in ways other than physically damaging the skin structure. Delivery vehicles and ALA mixtures are an alternative to combination therapies listed above. De Rosa et al. [19] studied how ALA emulsions containing dimethyl sulfoxide (DMSO) and ethylenediaminetetraacetic acid disodium salt (EDTA) affected ALA-induced protoporphyrin IX. De Rosa et al. used a 20 % DMSO oil-in-water emulsion that increased the in vitro permeation of ALA, while an oil-in-water emulsion contain 10 % ALA, 3 % EDTA and 20 % DMSO significantly increased PpIX. However, an EDTA emulsion had no effect on short-term protoporphyrin IX accumulation in skin. Maisch et al. [20] found similar data when analyzing the effects of a nanoemulsion-based formulation of ALA (BF-200 ALA) for increased penetration of ALA. ALA penetration was 4.8-fold and 5.0-fold stronger after 8 and 12 h, respectively, for BF-200 ALA use than after use of the control ALA cream. These delivery formulations have clinical applications, just as the laser therapies did. Fang et al. [21] looked at how ALA-PDT in tandem with acid-encapsulated ethosomes could help treat the inflammatory skin disease of psoriasis. Because the ALA precursor is hydrophilic, it has limited skin penetration. However, by using an ethosomal carrier for ALA, this hydrophilic barrier can be overcome and thus increase the penetration of ALA into the skin. Results of this paper found that the application of this ethosomal carrier produced increases of cumulative PpIX by 5–26-fold and ultimately lead to better outcomes in patients with psoriasis.

Iontophoresis

Iontophoresis is the technique for pushing or driving charged compounds into the body through the skin by applying a local electric current and creating a repulsion force. As mentioned earlier, iontophoresis can be an effective method for increasing the penetration of ALA through the skin. Krishnan et al. [22] first looked at using iontophoresis to deliver small peptide compounds (ALA and L-alanine-L-tryptophan) across human skin. Compared to passive diffusion, iontophoresis increased the rate of transport of L-alanine-L-tryptophan and ALA by 15- and 22-fold, respectively. Similar to the nanoemulsions discussed previously, Merclin et al. [23] investigated how iontophoresis interacted with ALA dissolved in a lipid sponge phase of monoolein, propylene glycol, and an aqueous buffer. The sponge phase mixture resulted in a higher passive flux (140 nmol/cm^2/h at 5 h) and a lower iontophoretic flux (800 nmol/cm^2/h at 5 h). These numbers were comparable to passive

fluxes from more concentrated vehicles and show the potential this method has to be a transdermal drug delivery vehicle.

Improvements in ALA Delivery Using Nanotechnology

One of the most important limitations of ALA-PDT is the low permeability of ALA through the *stratum corneum* (SC) of the skin. The hydrophilicity of ALA prevents meaningful diffusion through the highly hydrophobic *stratum corneum*, which is known to be a major barrier against transcutaneous drug delivery. To overcome this hydrophobic barrier and to enhance ALA penetration, the new field of nanotechnology and drug delivery has been harnessed. New synthetic and lipophilic vehicles such as liposomes, ALA dendrimers, and niosomes along with other nanotechnology-based vehicles are being investigated. However, despite the promising future of nanoparticles as delivery vehicles for ALA (and in nanomedicine in general), we must not forget the concerns about the possible toxicity to both the patients themselves and to the wider environment that we all inhabit. Table 1 lists some of the nanodrug-delivery vehicles that have been investigated with ALA.

Improvements in Cellular PPIX Synthesis

Another approach to improve ALA-PDT involves manipulation of the biochemical pathways of the heme biosynthetic cycle within the cells themselves (see Fig. 3).

Combination with Iron Chelators

Due to its unique biochemical and biophysical properties, iron plays a role in most important processes within cells. Its availability is crucial for rapidly proliferating tumor cells to convert PPIX into heme. After the exogenous ALA has induced the PPIX production, ferrous iron (free Fe^{2+}) is inserted into PPIX by the action of the ferrochelatase enzyme, which converts PPIX into heme. Iron chelators can combine with the ferrous iron within the cells and remove it from the heme synthesis system. Moreover, iron chelators also have the ability to inhibit ferrochelatase activity. Employing iron chelators prevents the formation of heme, and as a result, more PPIX accumulates inside the cells [42]. Up to now, the following iron chelators including ethylenediaminetetraacetic acid (EDTA), desferrioxamine (DFO), 1,2-diethyl-3-hydroxypyridin-4-one hydrochloride (CP94), and dexrazoxane (ICRF-187) [43•] have all been proved to increase PPIX accumulation and photosensitization in PDT. They can be divided into specific and nonspecific iron chelators. EDTA is a nonspecific chelator of metals, which has been found to have the potential to increase accumulation of endogenous porphyrins in the skin and ALA-induced PPIX fluorescence and phototoxicity in the Hep-2 cell line. By treating non-melanoma skin cancer patients with ALA-PDT combined with $Ca^{2+}Na^{+}_2$ EDTA, Liu et al. found greater depth of PPIX production and PDT necrosis compared to ALA-PDTalone [44]. This observation suggested the clinical outcome could be improved. Desferrioxamine (DFO) is an iron-specific chelator which has greater specificity for iron than EDTA and has higher affinity to bind iron, with a large preference over other metal ions. DFO has the potential to accumulate in high concentrations in the lysosomes and to complex with intra-lysosomal iron. Yang et al. investigated the effectiveness of DFO in enhancing PPIX-based photodynamic reaction in different skin targets, including fibroblasts, HaCat cells, and Hep-2 cells, and found that DFO showed

different effects on PPIX accumulation and PDT reaction in different cells lines [45]. CP94 is a novel hydroxypyridinone iron-chelating agent that was proved to be superior to DFO in the enhancement of PPIX accumulation using ALA and MAL in fetal lung fibroblasts, squamous carcinoma cells, and human glioma cells [46]. Dexrazoxane (ICRF-187) is a clinically approved cardioprotective agent against anthracycline-induced heart damage. Dexrazoxane can readily penetrate cell membranes and is converted into two one-ring open intermediates in cells followed by the formation of ADR-925, which can either remove iron from the iron-anthracycline complex that causes cardiotoxicity or else can bind to free iron. It has been reported that dexrazoxane and its hydrolysis products are all good iron-chelating agents that could increase PPIX formation [47].

Combination with Differentiating Agents

However, the cure rates of superficial skin carcinomas using ALA-PDT are still not sufficient, based on the fact that ALA-induced PPIX formation is related to the differentiation state in an erythroleukemia cell line [48•]. Ortel and his colleague studied how the effectiveness of ALA-dependent PDT depends on the differentiation state of the cell. They found that the more differentiated tumors with slowly cycling cells could actually accumulate more PPIX that less differentiated tumors with rapidly cycling cells and therefore could be better targets for ALA-dependent PDT [49•]. Several factors such as increased ALA uptake, enhanced PPIX production, and decreased PPIX export from the cell could all possibly contribute to the elevated level of PPIX, which results in an enhanced lethal PDT effect in differentiated cells. The conclusion was made that differentiation therapy, which is an approach to reverse the lack of differentiation in tumor cells using pharmacological agents, may play an important role in the enhancement of ALA-PDT. In a further study, Ortel and colleagues used the analogues of vitamin D, synthetic androgen R1881, and isomers of retinoic acid to produce a short-term differentiation therapy with 3 to 4 days in LNCaP cells. They found that not only the overall PPIX production was increased but also the fraction of cells that contained low PPIX levels was reduced after the treatment [50]. Until now, the following agents have been shown to improve PPIX accumulation and enhance the therapeutic response: (i) In patients, various carcinomas and psoriasis lesions pretreated with vitamin D and its derivatives [51, 52]; (ii) Skin or prostate carcinoma cells pretreated with methotrexate; (iii) Actinic keratosis lesions pretreated with retinoids [53]; (iv) Skin keratinocytes pretreated with calcium; (v) Hormone-responsive prostate cancer cells pretreated with androgens [54]. The molecular mechanism of combining differentiation therapy and ALA-PDT in cancer involves C/EBP (a transcription factor)-mediated cellular differentiation. Previous studies proved that differentiation-promoting agents upregulate CCAAT/enhancer-binding proteins (C/EBP) and increase expression of coproporphyrinogen oxidase (CPO). C/EBP transcription factors are powerful regulators of cellular differentiation. CPO is a heme-pathway enzyme responsible for the conversion of coproporphyrinogen into coproporphyrin and then into PPIX. The cooperative interactions between regularly spaced C/EBP sites is critical for CPO transcription, which regulated by differentiation [54].

Temperature Modulated ALA-PDT

Because the biosynthesis of PPIX is a metabolic process, it appeared reasonable to ask whether the amount of PPIX that was synthesized could be affected by the temperature of the skin. It had been shown as early as 1999 that both in cells [55] and in mouse skin [56], there was a strong dependence of the amount of PPIX synthesized on temperature during the incubation. Willey et al. carried out a clinical trial in 20 patients with actinic keratoses (AK) on the arms or legs. They found that by raising the temperature of the skin on one limb from 29.4 to 38.8 °C during 1 h of application of ALA under occlusion, the response of the skin (reduction in number of AK lesions) to blue light-mediated PDT was significantly improved in the heated limb without a concomitant increase in side effects [57]. It is at present unclear what the exact mechanism of temperature modulated ALA-PDT actually is. It is possible that temperature increases ALA uptake, increases PPIX production inside the cells, or increases the oxygen supply during PPIX synthesis or during PDT. A recent paper suggests that increased temperature can produce increases in total apoptosis and elevate superoxide ROS generation between 33 and 42 °C [58].

Improvements Relying on Illumination

The last group of methods of improving the effectiveness of ALA-PDT that will be discussed focuses on manipulating the parameters of light delivery used to excite the PPIX formed in the skin after ALA application. These approaches are graphically illustrated in Fig. 4.

Light Fractionation

The use of fractioned light delivery for ALA-PDT has been developed over the years by investigators in Rotterdam in the Netherlands [59]. The first report was as early as 1994 where it was shown that PDT damage in a mouse tumor was increased when the light was fractionated [60]. A clinical trial was then conducted in which 91 patients with 299 superficial BCC lesions were treated with a 2-fold illumination scheme with 2 light fractions of 20 and 80 J/cm2 delivered 4 and 6 h after a single application of 20 % ALA, and 106 patients with 274 lesions were treated with a single illumination of 75 J/cm^2 4 h after ALA. The complete response rate was significantly greater following the 2-fold illumination than the single illumination [61•]. Similar results were obtained in a clinical trial of actinic keratoses (AKs) using the same parameters [62].

In order to understand the mechanism by which a fractionated light dose is superior, de Bruijn et al. [63] investigated PAM 212 cells in vitro. With a low (0.2 mM) ALA concentration (but not a high 2 mM ALA concentration), there was approximately an order of magnitude greater reduction in cell survival when two light fractions were delivered at 2 and 4 h compared to a single illumination with the same cumulative fluence at either time point. This data suggests that there is a mechanism by which cells are rendered more sensitive to light 2 h after the first sub-lethal light delivery. Very recently, de Bruijn et al. reported that light fractionation increased the PDT effect found with BF-200 (a nanoemulsion-based gel formulation of ALA) on mouse skin [64•].

Daylight ALA-PDT

The use of daylight PDT originated in Denmark [65]. The idea was that using daylight exposure to activate the PPIX during the incubation period would reduce the total amount of PPIX that accumulated within the skin at any one time, and this reduction would have a big effect on the level of pain suffered by patients during the procedure. Moreover for patients with large areas of the skin affected by AKs (field cancerization), daylight exposure would be much more practicable and convenient than using a medical light source to illuminate multiple sites on the skin. Rubel and colleagues [66] carried out a 24-week randomized, controlled, investigator-blinded, multi-center clinical trial of 100 subjects with AKs on the face/scalp. Patients received daylight (DL-PDT) on one side and conventional (c-PDT) on the contralateral side. At week 12, the complete lesion response rate with DL-PDT was non-inferior to c-PDT (89.2 vs. 92.8 %). For DL-PDT, subject-reported pain was significantly lower (0.8 vs. 5.7, respectively; $p < 0.001$), with better tolerability and significantly higher subject satisfaction regarding convenience and outcome.

Wearable Red Light Sources

With similar motivation to using daylight ALA-PDT, Moseley et al. came up with a new concept called "ambulatory PDT" [67]. Again, the idea was to allow long illumination times which would reduce total accumulation of PPIX in the skin and thereby reduce pain.

A prototype diode array, comprising 37 AlGaInP LEDs cast in epoxy with a diffuser, and driven by a battery pack, was designed to be attached to the patients' skin with microporous tape. A pilot study was carried out in five patients with histologically proven Bowen's disease who were referred for ALA-PDT. In accordance with the usual practice, patients received two treatments at a 4-week interval. Each treatment lasted for 1 h 40 min to deliver 75 J/cm^2 at an irradiance of 12 mW/cm^2. Pain levels were reduced to mild (3) or moderate (2) at the first treatment and mild (4) or none (1) at the second treatment. The same group went on to test a low-irradiance (5 mW/cm^2), potentially disposable, lightweight, organic light-emitting diode (OLED), which is a light source (2 cm diameter), suitable for ambulatory PDT [68].

Cochrane and colleagues reported the design of flexible light sources that could be worn as fabrics [69]. The integration of plastic optical fiber (POF) and polyester yarn were woven into textile structures using a hand-loom, then both ends of the POF were coupled to a laser diode (5 W, 635 nm). Predetermined POF macrobending led to side emission of light when the critical angle is exceeded. The illumination was homogeneous over a fabric area of 100 cm^2 with a fluence rate of 18 ± 2.5 mW/cm^2. Optical losses were very low and the temperature elevation was only 0.6 °C after 10 min of illumination.

Inhibitory PDT with an Initial Blue Light Irradiation

The pain and swelling associated with ALA-PDT make it a poor choice for acne patients, particularly those with facial acne. In an effort to maintain efficacy but reduce pain, Sakamoto et al. [70] performed a randomized, double-blind controlled study to compare conventional ALA-PDT to a new method called "inhibitory PDT" (iPDT) that involves an initial irradiation with low levels of blue light during the incubation period. They found that

low-level blue light exposure during ALA metabolism prevented the accumulation of PPIX in the superficial epidermis, while allowing for accumulation in deeper tissues, including the sebaceous glands. Both methods (conventional and iPDT) demonstrated significant improvement in the 18 subjects that finished the study (out of 29 original participants), but the intense pain and immediate inflammatory side effects were reduced in iPDT-treated sites.

Conclusion

ALA-PDT continues to fascinate both clinical dermatologists, alongside biomedical researchers and nanotechnologists because of the ingenuity of the approach. To take advantage of the body's own intrinsic biochemical metabolic pathways to make a light-activated therapeutic molecule localized within the tissue where the ALA is actually applied is considered intellectually satisfying. As can be seen from our review, there is also no shortage of ingenious ways of improving its effectiveness. These range from methods familiar to all dermatologists such as fractionated laser, intracutaneous injection, and iontophoresis to approaches that are common in nanotechnology and drug delivery laboratories such as various liposomes, gold or silica nanoparticles, nanocarbon allotropes, etc. Because the cells are actually making the molecule that will eventually kill them, there is the opportunity to modulate the actual enzyme activity levels inside the cells to increase the amount of PPIX that accumulates inside the target cells. Finally, since nothing happens in PDT without the final *coup de grace* of light delivery, there is also an opportunity to play with the illumination parameters. This can help to reduce the pain suffered on light delivery, which is by far the most dominating side effect of ALA-PDT, to say nothing of improving the patient friendliness of the procedure. We expect that efforts will continue to be made to improve the effectiveness of ALA-PDT, which will become an even more fertile field for researchers.

Acknowledgments

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•• Of major importance

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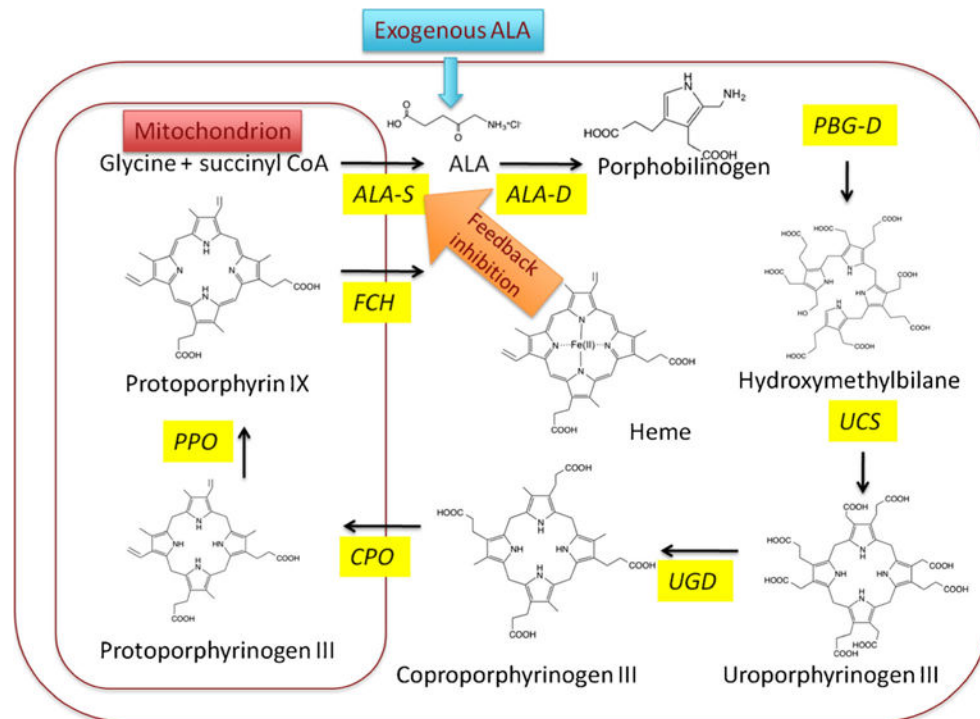
Fig. 1.
Heme biosynthetic cycle in eukaryotic cells. Exogenously administered ALA bypasses the feedback control mechanism whereby heme inhibits ALA synthase which is designed to prevent excess accumulation of PPIX in normal cells. Consequently, excess free PPIX accumulates in the cells since ferrochelatase is now rate limiting. *ALA-S* 5-aminolevulinic acid synthase, *ALA-D* 5-aminolevulinic acid dehydratase, *PBG-D* porphobilinogen deaminase, *UCS* uroporphyrinogen co-synthase, *UGD* uroporphyrinogen decarboxylase, *CPO* coproporphyrinogen oxidase, *PPO* protoporphyrinogen oxidase, *FCH* ferrochelatase
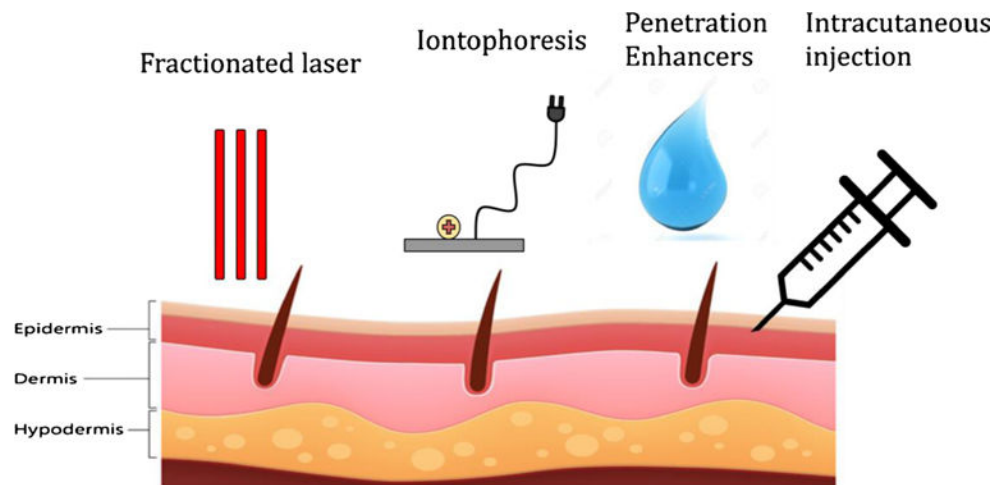
Fig. 2.
Methods of increasing ALA penetration into the skin. Fractional laser, iontophoresis, penetration enhancers, and intracutaneous injection are all methods/techniques to either improve the permeability of the skin or increase the penetration ability of ALA. Fractional laser irradiates the skin to remove skin layers in microscopic holes so ALA can reach deeper structures underneath. Iontophoresis is a technique to push compounds into the body through the skin by applying a local electric current and creating a repulsion force. Penetration enhancers work in combination with ALA (usually as an emulsion of other substances) to increase the ability of ALA to move transdermally across the lipophilic *stratum corneum*. Intracutaneous injection is the simple approach to inject ALA into the skin
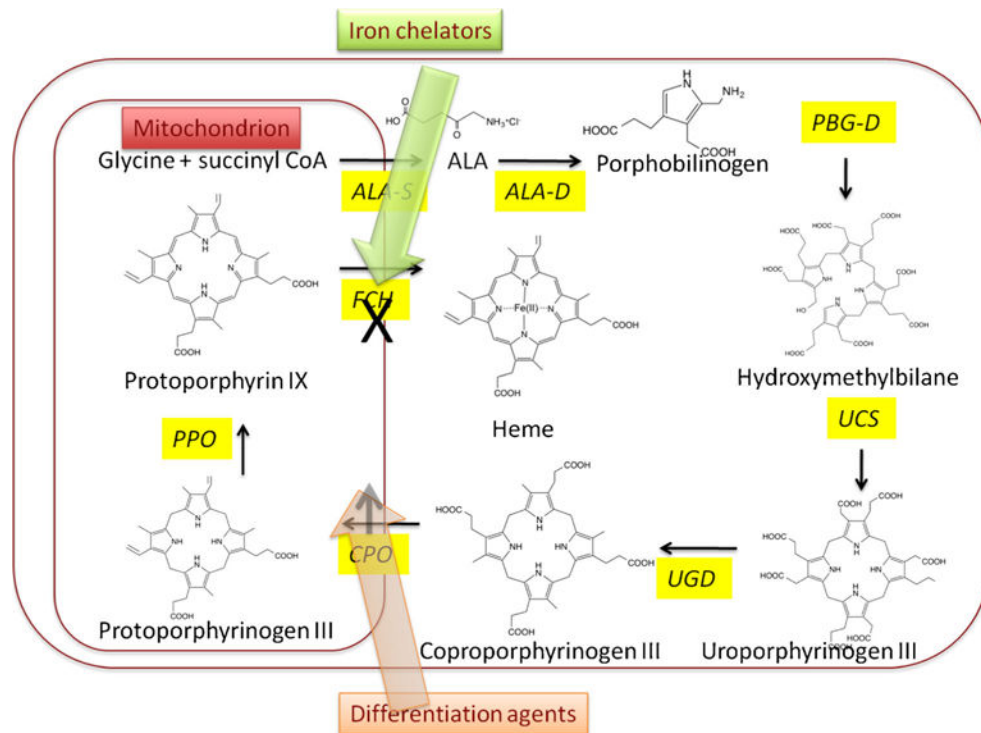
Fig. 3.
Methods of biochemically enhancing PPIX accumulation by modulating enzymes in heme biosynthesis cycle. Iron chelators can enhance PPIX accumulation by depriving the enzyme ferrochelatase of the ferrous iron it needs to form heme. Differentiation agents (vitamin D, retinoids, methotrexate, calcium, or androgens) can increase expression of coproporphyrinogen oxidase producing more PPIX
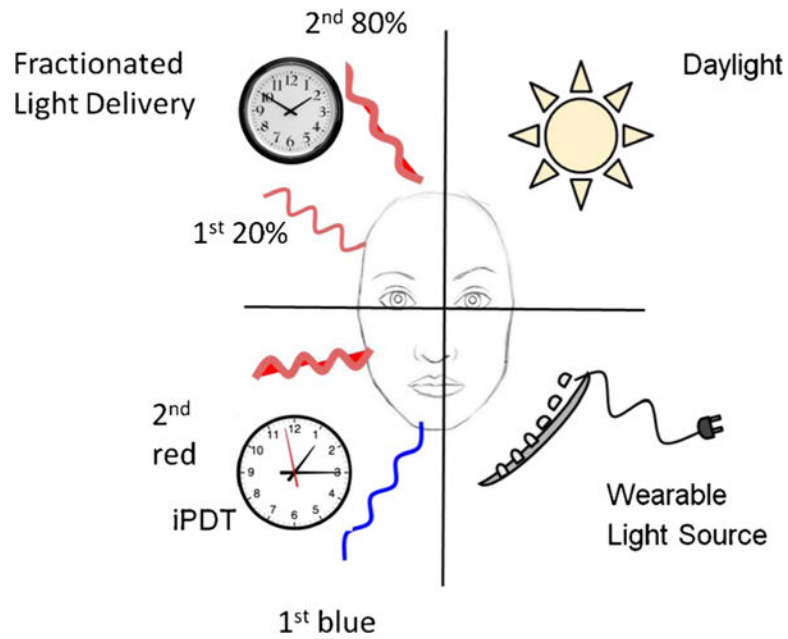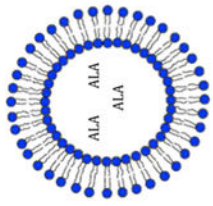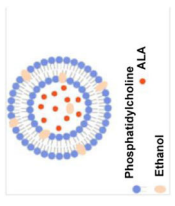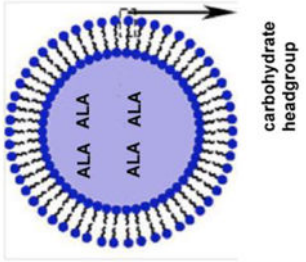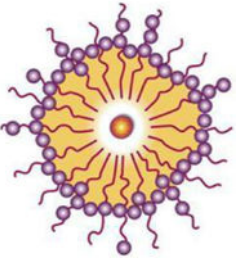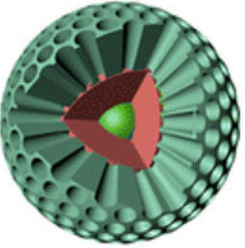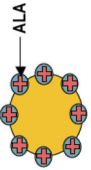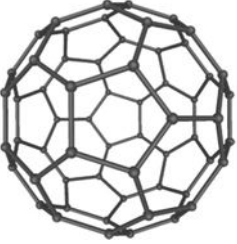
Fig. 4.
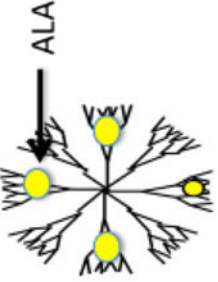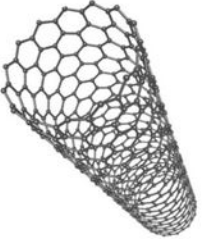Methods of improving ALA-PDT by manipulating the light delivery parameters. Fractionated light delivery applies 20 % of the calculated red light dose after 4 h of ALA, incubation followed after 2 h by the remaining 80 % of the light dose. Daylight PDT involves exposing the patient to daylight for several hours as soon as the ALA has been applied. Wearable light sources allow a very low irradiance of red light to be applied for a longer time to reduce pain and make the treatment more patient friendly. iPDT uses an initial low dose of blue light exposure followed by red light to reduce the pain of treatment

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Table 1

Reports of improved ALA delivery using nanotechnology and nanomedicine

Nanoparticle	Structure	Type of Experiment/Animal Model	Advantages	Clinical Application	Ref
Liposomes		In vitro and in vivo in rats	Compatible with lipid bilayer structure May employ surface ligands to attach to tissue	Drug delivery to treat: Skin cancer, Acne, Wrinkles	[17, 24, 25]
Ethosomes		Mice	Greater penetration ability than liposomes, smaller particle size	Skin cancer treatment	[21, 26]
Niosomes		Excised human skin	80 and 40% increased permeation of drug and 50 to 100% of the drug retained into the skin when compared to aqueous drug solution	Therapeutic treatment for skin malignancies, hidradenitis suppurativa	[27]

Nanoparticle	Structure	Type of Experiment/Animal Model	Advantages	Clinical Application	Ref
Polymeric nanoparticles		HeLa cancer cells Ex vivo skin transport	10× higher transdermal transport Increased cell killing by PDT		[28–30]
Mesoporous Silica		Mice	Bypasses the lipophilic barrier to directly enter into cancer cells Exhibited high photocytotoxicity to cancer cells *in vitro*		[31]
Conjugated Gold Particles		Human chronic myeloid leukemia K562 cells, biodistribution in rabbits Human fibroblast and A431 cells	Used to circumvent multidrug resistance mechanisms Singlet oxygen generation by localized surface plasmon resonance of GNPs	Early diagnosis and therapy of atherosclerosis	[32–36]
Fullerene Nanoparticles		Mice	No detectable toxicity Compared to ALA, significant improvement in antitumor efficacy		[37]

Nanoparticle	Structure	Type of Experiment/Animal Model	Advantages	Clinical Application	Ref
ALA Dendrimers	ALA	LM3 mammary carcinoma cells, PAM 212 murine keratinocyte and A431 human epidermoid carcinoma cell lines	More efficient at low concentrations compared to equimolar ALA for porphyrin production, most lipophilic, minimal dark toxicity, high drug payload		[38–40]
Carbon Nanotubes		MGC-803 tumor cells	Increased PPIX formation Strong optical absorbance in NIR (near-infrared) light		[41]