
CHAPTER 2

Photodynamic therapy based on 5-aminolevulinic acid: Applications in dermatology

This chapter has been adapted from JTHM van den Akker and SB Brown. In *Photobiology for the 21st Century* (Edited by TP Coohill and DP Valenzano) 2001, Valdenmar Publishing Company, Kansas, USA: pp 165-181.

Introduction and context

Photodynamic therapy (PDT) requires three components for success, a photosensitiser, light and molecular oxygen. All three components are necessary if the PDT effect is to occur and the aim of successful PDT is to ensure adequate amounts of each in the target tissue. Most of the development work, both experimentally and clinically, has been aimed at ensuring that there is sufficient photosensitiser and light in the target tissue at the time of treatment. It is much more difficult to control the levels of molecular oxygen but, again, successful PDT is absolutely dependent on having sufficient oxygen present.

Most drugs for PDT have been developed as photosensitisers which are given systemically. However, a quite different approach has been developed over the past ten years in order to produce an adequate quantity of photosensitiser in the target tissue. This involves the administration of 5-aminolevulinic acid (ALA), which is a very simple 5 carbon compound, the structure of which is shown in figure 1. ALA was first used by Kennedy and Pottier (1) to treat various dermatological conditions using a topical preparation of the drug. Since that first demonstration that ALA-PDT was feasible, the approach has been extended a great deal both within dermatology and to other applications in gynaecology, in bladder, in the gastrointestinal tract and in the brain. The drug has been used both topically, in a variety of formulations and systemically through oral administration. This brief review is intended to give an indication of the scope and potential of PDT using ALA and its derivatives, particularly in dermatology, since this was the area which was focused on in the recent Photobiology Congress on which this book is based. It is not intended to be a comprehensive review, even in the dermatology field and references are given to more detailed reviews and papers both in the dermatology area and elsewhere.

Principles of ALA-based PDT

Haem is a vital component of a large number of enzymes and proteins throughout nature. In mammals, it is most abundant as the active centre of haemoglobin but is also a component of myoglobin, cytochromes of the respiratory chain, the cytochrome P450 family of proteins which are essential in oxidative metabolism and many other enzymes. Virtually all mammalian cells have the capacity to synthesize haem, a major exception being the mature red blood cell which has already lost its haem synthesizing capacity during development following synthesis of its complement of haemoglobin. It is this

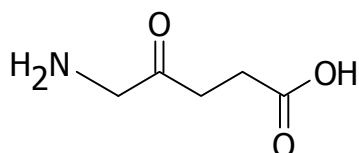


Figure 1 Molecular structure of 5-aminolevulinic acid.

ubiquitous pathway of haem biosynthesis which is utilized in ALA-PDT.

An outline of this pathway is shown in figure 2. Haem is a tetrapyrrolic derivative and the first committed step in the pathway is the condensation of two molecules of ALA to form the first pyrrolic derivative known as porphobilinogen (PBG). Four molecules of porphobilinogen then assemble together to make a linear tetrapyrrolic derivative known as a hydroxymethylbilane which then ring closes to form a porphyrinogen. When this process occurs without enzymatic control, the porphyrinogen formed is a symmetrical derivative known as a Type 1 isomer. However, in the normal biological situation, an enzyme called uroporphyrinogen cosynthetase ensures that there is a rearrangement during the cyclisation to give the Type 3 isomer. Porphyrinogens may be considered as pyrrole rings joined by four CH_2 bridges. They are colourless and not photoactive, because the conjugation does not extend around the macrocyclic ring. Further modification of the side chains is then carried out to convert uroporphyrinogen to coproporphyrinogen and then to protoporphyrinogen. At this point, the side chains are those which are seen in the final product haem. However, the oxidation state of the molecule is still at the level of the porphyrinogen and the molecule is colourless and not photoactive. At this point, under the action of the enzyme protoporphyrinogen oxidase,

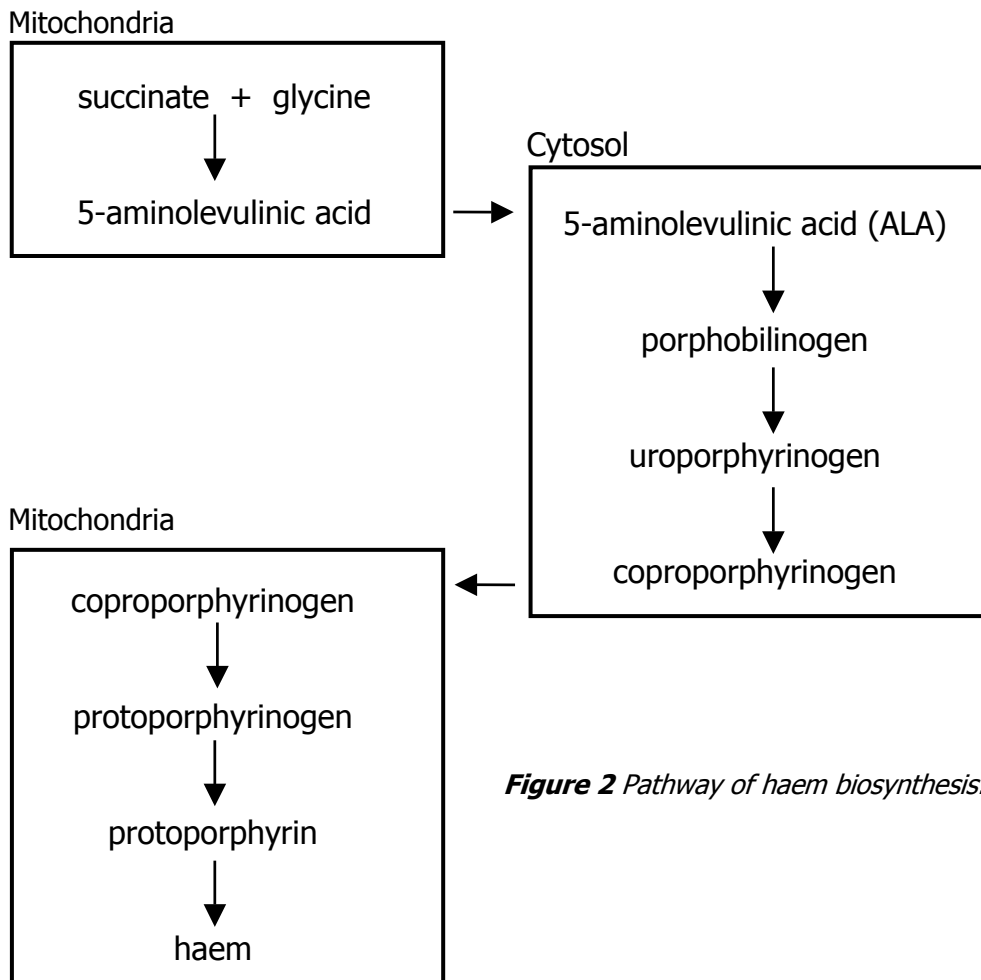


Figure 2 Pathway of haem biosynthesis.

four oxidation steps occur which result in the formation of protoporphyrin IX (PpIX, the IX refers to the particular isomer which is formed based on the order of the side chains, figure 3). Because there is now complete delocalisation of electrons around the macrocyclic ring, protoporphyrin is highly coloured and is a highly photoactive molecule. Indeed, it is a very efficient photosensitiser. The final step in the synthesis involves the insertion of an iron atom into the protoporphyrin ring to make haem. Although haem remains highly coloured because of the delocalisation, it is not a photosensitiser itself because of the presence of the iron atom. Thus the biosynthesis of haem involves the transient formation of a very powerful photosensitiser, PpIX.

This whole process is normally under very tight enzymatic control and there is a strong feed-back control mechanism of haem on ALA synthetase. There exists a group of diseases known as the porphyrias in which one of the enzymes of the pathway is genetically deficient and this can lead to a range of clinical problems. In one particular porphyria, known as erythropoietic protoporphyria, there is a defect in ferrochelatase, the enzyme responsible for inserting iron into PpIX. This leads to a build-up of PpIX in red cells and in other tissues and this can lead to photosensitivity in sufferers.

In ALA photodynamic therapy, an excess exogenous dose of ALA is given to a patient. This dose of ALA temporarily overloads the porphyrin pathway and creates a build-up of PpIX. If this build-up occurs in the target tissue and light is applied at the appropriate time, then a very powerful PDT effect can occur. This is the principle of photodynamic therapy using ALA.

Mechanism of ALA-based PDT

Several factors are involved in the achievement of selectivity of therapy by PDT. Since it is a local technique, clearly a major factor is the ability to direct light precisely to the lesion which is being treated. For the skin, this is usually a relatively easy process but even for internal treatments using ALA (for example in the bladder or in the uterus) this is now a relatively routine procedure because of major advances in lasers and fibre optic

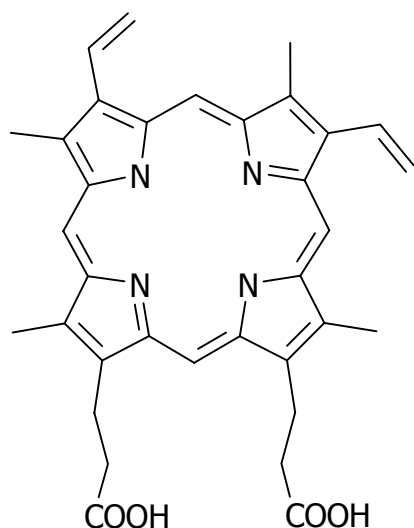


Figure 3 Molecular structure of protoporphyrin IX.

delivery systems in recent years. Detailed consideration of light delivery during PDT is outside the scope of this review.

Selectivity is also determined by preferential accumulation of photosensitiser in the target lesion and, in many cases, ALA is able to induce such localization of the PpIX produced. It should be noted that, whilst increased levels of PpIX in target tissue over surrounding normal tissue is highly desirable for ALA therapy, it is absolutely essential for ALA-based photodiagnosis, which depends upon the observation of increased fluorescence in the lesion compared with the surrounding normal tissue.

The reasons why tumours such as basal cell carcinoma (BCC) in the skin and carcinoma *in situ* in the bladder produce more PpIX than the surrounding normal tissue are not fully understood, though the phenomenon is now extremely well documented both in terms of fluorescence imaging (2-8), fluorescence spectroscopy (2,5,8-11), fluorescence microscopy (4,8,12) and in terms of biochemical analysis (13,14). The localization extends to pre-cancerous dysplastic tissues such as cervical intraepithelial neoplasia (CIN) and Barrett's oesophagus as well as to benign proliferative tissue such as the endometrium. Many theories have been advanced to account for this selectivity including the following: i) tumours may contain a lower level of ferrochelatase (the enzyme responsible for inserting iron into PpIX) than surrounding normal tissue, resulting in a greater build-up of the enzyme's substrate, protoporphyrin; ii) tumours may have less readily accessible iron which is also required for the conversion of PpIX into haem; iii) the lower pH of tumours may result in more PpIX formation; iv) the permeability properties of the skin or epithelium overlying the tumour or lesion are different from the permeability properties of normal skin or epithelium.

Clearly, the reason why tumours and other lesions produce more PpIX must be linked with the reason for which PpIX itself accumulates, rather than other intermediates in the pathway. Whilst it is tempting to use the simple argument that PpIX accumulates because the next enzyme in the pathway, ferrochelatase, is limiting, it is now clear that this represents an oversimplification of the true picture. First, it is possible that other intermediates in the pathway before protoporphyrin do indeed accumulate but that they may be readily removed and only PpIX remains. Alternatively, the immediate precursor of PpIX, protoporphyrinogen IX, may accumulate and may be non-enzymatically oxidized to PpIX. Here, intracellular compartmentation is important, because protoporphyrinogen IX may be released into the cytoplasm. It may be oxidized there to PpIX non-enzymatically, or it may be released into the extracellular compartments and oxidized there to PpIX. In the former case, PpIX may accumulate in subcellular compartments outside of the mitochondria which is its normal organelle of synthesis. A fluorescence microscopy study upon co-incubating cells with ALA and rhodamine 123 (a fluorescent mitochondrial probe), indicated that PpIX is located in the mitochondria (15). Whilst some of these considerations may not be directly relevant to therapy or to

photodetection, they do illustrate the complexity of this apparently simple system and warn against oversimplification.

ALA-based PDT in the skin

Introduction to skin and skin lesions

Healthy human skin consists of a stratified, cellular epidermis and an underlying dermis of connective tissue. The dermis, situated between the epidermis and the subcutaneous fat layer, is a supporting matrix that consists of linked polysaccharides and proteins (mainly collagen and elastin). It supplies nutrition to the epidermis and the cutaneous appendages and is a protective layer for the body against mechanical injury. The epidermis is largely composed of keratinocytes, which are formed by dividing cells in the basal layer of the epidermis, adjacent to the dermo-epidermal junction. The keratinocytes move outwards while differentiating progressively to give several distinguishable layers. They eventually form the stratum corneum, a layer of cells without nucleus and other organelles. These so-called corneocytes, mainly contain keratin and are embedded in a lipid matrix. The rate of keratinocyte production and differentiation (epidermopoiesis) is regulated by a balance between stimulatory and inhibitory signals. Among the stimulating factors are interleukines and other cytokines, and growth factors, such as the epidermal growth factor, transforming growth factor α and fibroblast growth factors. Transforming growth factor β , peptides called chalone, interferons and tumour necrosis factor have a negative feedback on the epidermal growth. One can understand that any disruption in the complex balances or cascades can result in diseases of the skin. We will focus on skin diseases which are currently treated (in clinical trials) with ALA-PDT.

General approach to PDT in the skin

In terms of numbers of lesions treated, there has been more PDT application to the skin than to any other organ. In part this is due to the accessibility of the skin and the fact that cheap, non-laser light sources appear to be as effective as lasers. However, it is also due to the good results obtained, especially the excellent cosmesis as will be seen below. The great majority of skin lesions treated by PDT are non-life threatening – as yet there has been little attempt to treat malignant melanoma. For this reason, there has been a reluctance to use systemic photo-sensitisers, especially if they cause prolonged photosensitivity. This has led to a preference for topical agents, but it has proved extremely difficult to develop topical preparations of most of the photosensitisers which are in use systemically. It is in this arena that ALA makes its major contribution to dermatology, because topical preparations of ALA have been highly successful in inducing photosensitization of target lesions. This was first demonstrated by the pioneering work of Kennedy and Pottier (1) who used typically 20% ALA in an oil-in-water emulsion. Clinical PDT with topical ALA has now become extremely simple

and cheap. First, ALA contained in a cream or other vehicle is spread across the lesion and over a margin of healthy tissue. In some protocols, the skin may be tape stripped or otherwise disrupted to aid penetration. The ALA is left in place for a period of 3 to 4 hours depending upon the particular protocol. During this time, the patient feels no discomfort and the ALA penetrates into the lesion and is metabolised into PpIX. At this time, light is applied in a pre-determined dose, typically for a period of between 10 and 20 minutes. During this phase, the patient may feel effects ranging from discomfort to acute pain and analgesia may be necessary. The treatment is then complete. For some indications (e.g. for Bowen's disease) one treatment is usually sufficient but in other cases (such as in some protocols for BCC) a repeat treatment may be necessary.

The precise mechanism causing the pain during ALA-PDT is not been fully understood. The generally accepted theory is that ALA is taken up in nerve endings and converted to PpIX, which leads to pain when light is applied. Certainly the evidence is that the pain is closely synchronized with the light i.e. the pain begins when the light is switched on and quickly disappears when the light is removed and begins again when the light is reapplied.

ALA-PDT for Bowen's disease

Bowen's disease, actinic keratosis and BCC belong to the group of epidermal non-melanoma (pre)malignancies. Bowen's disease (squamous carcinoma *in situ*) is a premalignant condition located in the epidermis and is characterized as an erythematous, non-elevated scaly or crusted plaque. Atypical squamous cells and individually keratinising cells are present throughout the epidermis, which shows hyperkeratosis, parakeratosis and acanthosis. The atypia and proliferation is limited to the epidermis because of the intact dermo-epidermal junction. The disease can develop into squamous cell carcinoma by invasion into the dermis. Conventional treatments are surgical excision, cryotherapy, laser ablation or topical 5-fluorouracil.

There are now many studies which show that ALA-based PDT can be extremely effective in the treatment of Bowen's disease. The approach is particularly well suited to treatment of the large lesions which sometimes occur in this condition because of the excellent healing which is achieved. Achievement of more than 90% complete response, without recurrence, is now relatively routine for PDT of this condition. Early work carried out by Kennedy (1) and subsequently in Leeds (16,17), Lund (18) and elsewhere (19-22) clearly showed the efficacy of the approach. The treatment is very simple and may be carried out in an outpatient clinic or even in a general practitioner's surgery. Although the formulations in different studies have varied, a 20% preparation of ALA has been typically used. A single treatment is sufficient to cure the Bowen's disease in more than 90% of cases. The only side effect is local pain as described above.

One of the best studies of Bowen's disease has been a clinical trial randomising lesions

to treatment either with cryotherapy or with PDT (19). Cryotherapy produced clearance in 10 of 20 lesions with one treatment, the remaining lesions requiring one or more additional treatments. PDT resulted in the clearance of 15 of 20 lesions with one treatment and all the remaining 5 lesions after a second treatment. The probability that a lesion would be cleared with one treatment was significantly greater with PDT than with cryotherapy. The study also showed that PDT was better for treatment of larger lesions. Finally, the study demonstrated that after 12 months, visible scarring was absent for the PDT-treated lesions, but present in some of the cryotherapy-treated lesions. This excellent healing has come to be accepted as a characteristic benefit of ALA-PDT.

ALA-PDT for basal cell carcinoma

Basal cell carcinoma is a tumour and the lesions are characterized as small, waxy, (semi)translucent raised areas around a central depression which may be ulcerated, crusted and bleeding. Often, the nodules are covered by a thin epidermis through which superficial blood vessels are visible. BCC is generally considered to arise from immature pluripotential cells of the epidermis. Different types of BCC occur with different clinical course and histopathological appearances. Commonly used treatments are surgical and nonsurgical, including Moh's microsurgery, cryosurgery, electrosurgery, curettage, radiotherapy and topical 5-fluorouracil.

ALA-PDT has been applied to the treatment of BCCs in a similar fashion as for Bowen's disease. However, whilst Bowen's is always relatively superficial, BCCs may be nodular or invasive. Not surprisingly therefore, the results achieved with ALA-PDT have depended very much upon the nature of the lesion being treated. For the superficial BCCs, initial data suggested a complete response rate similar to that of Bowen's disease (18,23-27). However, some studies with long follow-up showed a significant recurrence rate, typically up to 50% following a single treatment (17,21,25). Further studies in our laboratory suggested that the failure rate was not due to poor light penetration and was likely caused by poor penetration of the ALA into the deeper parts of the lesion. However, more recent studies have demonstrated that much better results with BCCs may be obtained with repeat treatments (new drug and light) at intervals varying from two days to one week (20-22). Responses of nodular BCCs to ALA-PDT have been much less (18,20,21), probably due to the poor penetration of ALA into the deeper layers of the nodular BCCs (13,28,29). Whether ALA-PDT can compete with alternative therapies if it requires repetition for complete response is an important question. Clearly, there would be major advantages if the technique could be improved with only a single treatment, to give results comparable with alternative therapies.

ALA-PDT for actinic keratosis

Actinic or solar keratosis is found in chronically sun-exposed skin areas. The lesions are small, usually multiple and manifest as rough, scaly, erythematous patches. The

epidermal changes are hyperkeratosis, parakeratosis, dyskeratosis, acanthosis and keratinocyte atypia. Dermatology handbooks describe actinic keratosis as a premalignant condition that can progress into an invasive squamous cell carcinoma. However, other authors state that actinic keratosis is in fact non-invasive squamous cell carcinoma, which has a chance to become invasive (30,31). Approximately 1% of the lesions convert to squamous cell carcinoma. Established treatments are cryotherapy, topical 5-fluorouracil and dermabrasion.

Treatment of actinic keratoses with ALA-PDT has been successful in a number of studies (20,21,23,32). Indeed, this is the lead indication for DUSA Pharmaceuticals with their proprietary product Levulan[®] (ALA) which has recently been approved by the FDA. So far, this is the only approved use of ALA. In 1997, DUSA completed two Phase III studies using Levulan[®]-PDT for actinic keratoses at a total of 16 centres across the USA with over 1500 lesions treated in 243 patients. Treatment was either with a 20% Levulan[®] topical solution or a placebo vehicle, using blue light, which penetrates less than red light. Patients whose lesions did not clear completely were retreated after 8 weeks. In one of the studies (117 patients) after a single treatment with Levulan[®]-PDT, 86% of the actinic keratoses responded completely, with 94% clearing after two treatments. In contrast, after two treatments, only 32% of lesions cleared when treated with placebo and light. In the other study (126 patients), after a single treatment 81% of the lesions gave a complete response with 90% clearing after two treatments, whereas after two treatments with placebo, only 20% cleared. All of these results were highly significant statistically. As expected, a well-tolerated burning or stinging discomfort was experienced during light exposure, but there were no other treatment-related side effects and no systemic photosensitivity. Cosmetic responses were rated by both patients and doctors as good to excellent. 85% of patients said that they would prefer Levulan[®] PDT if they had to be treated again in the future.

ALA-PDT for psoriasis

Psoriasis is a chronic, recurrent, inflammatory and proliferative disease of the skin and it is characterized by round erythematous, red, dry, scaly plaques. The cause is still unknown, but heredity is of significance in some cases. The psoriatic lesions are the result of increased keratin production by the keratinocytes caused by abnormal differentiation and hyperproliferation of the keratinocytes as a response to inflammation. It is not clear whether the increased rate of epidermopoiesis or the increased amount of keratinocytes in the epidermopoiesis process is the basic fault in psoriasis. Established treatment methods are either topical treatment with corticosteroids, tars, dihydroxyanthralin, tazarotene, vitamin D analogues, salicylic acid, or phototherapy with UV-B irradiation or PUVA (psoralen + UV-A irradiation), or systemic treatment with corticosteroids, methotrexate, cyclosporine, retinoids or hydroxyurea, or surgery, or laser clearance of the dermis. Sometimes a combination of above treatments is employed.

ALA-PDT is potentially a good option for psoriasis, because it can treat large areas, the healing is excellent and there are no long-term effects as is the case with PUVA therapy. A number of groups, including our own, have attempted to exploit this potential (33-38). Overall, the results have been disappointing so far. The responses have been variable and the reasons for this are not understood. Certainly adequate levels of PpIX are achieved in at least some of the plaque, but this may not be uniform throughout the lesion. In an attempt to improve response, multiple treatments, up to three times per week, were performed in one study (36). Although clinical efficacy did improve with multiple treatments, unpredictable response and patient discomfort during treatment led to the conclusion that ALA-PDT did not offer advantages over other therapies.

Other photosensitisers may be more suited to treatment of psoriasis. For example, in one study both ALA and methylene blue used topically, gave responses comparable with treatment by dithranol, but methylene blue did not induce the patient discomfort seen with ALA (33). Also, it may be that derivatives of ALA, such as the esters (see below) may be more effective. Finally, it is possible that treatment of psoriasis using a systemic sensitiser may be more successful. A photosensitiser which does not cause skin photosensitivity would be essential and there is evidence that PDT using verteporfin (benzoporphyrin derivative) has potential in this regard (39).

ALA-PDT for acne

Acne vulgaris is a chronic inflammatory disease of the sebaceous glands, resulting in comedos (blackheads), papules, pustules, cysts, nodules and often scars. It is a follicular disease and the comedo formation is caused by keratinous plugs (tightly packed horny cells) in the hair follicles, which fail to be properly discharged from the follicular opening. The formation of inflammatory papules, pustules, cysts and nodules is caused by follicular contents that are discharged into the dermis, permitted by a disrupted follicular epithelium. Another important factor in the formation of papules and pustules is the production of free fatty acids, which induce inflammation, by *Propionibacterium acnes*. Common treatments are either systemic with antibacterials, hormones, spironolactone, dexamethasone, prednisone or isotretinoin, or topical with benzoyl peroxide, retinoids or antibacterials, or use of an abrasive cleanser, or surgery.

The use of ALA-PDT for treatment of acne has been discussed for a number of years, but there are relatively few studies (40,41). There are, however, sound reasons for believing that ALA-PDT will be helpful in this condition. A very recent study by Anderson and co-workers (40) concluded that potentially, ALA plus red light might be useful for some patients with acne. This was based on a study of 22 subjects, each of which was treated in four sites on the back with ALA plus red light, ALA alone, light alone and untreated control. Sebum excretion was eliminated for several weeks and decreased for 20 weeks following PDT with clinical and statistical clearance of inflammatory acne. DUSA Pharmaceuticals are currently beginning a trial to test a non-laser, blue light

source in the treatment of acne.

Other dermatological conditions

A number of other conditions have been treated with ALA-PDT. These include T-cell lymphoma in which a partial response was observed (18) and erythroplasia of Queyrat. However the number of cases is very small and it is not possible at present to make any judgment on the effectiveness of ALA-PDT in these indications.

Improving ALA-based PDT of skin lesions

Problems with topical ALA application

Like other small molecules, ALA can penetrate through skin (42) and into superficial skin tumours and other skin diseases upon topical application. However, high doses of ALA (20% w/w is a standard concentration for a topical formulation) have to be applied in order to achieve sufficiently high PpIX levels in superficial BCC (13,17,29). Fluorescence microscopy studies have shown that there is no or little (inhomogeneous) PpIX fluorescence in the deeper layers of nodular BCC after topical application (3-4 hours) of ALA (13,28,29). Thus, the bioavailability of PpIX is low in deeper layers of the skin (lesions), largely due to poor penetration of ALA into the skin. One reason for the poor penetration of ALA into the skin (and cells) is the fact that ALA is a hydrophilic molecule and at physiological pH a zwitter ion. Hydrophilic and charged compounds poorly penetrate lipophilic barriers, such as cellular membranes and the stratum corneum. In addition, the penetration of compounds is limited by the structure of the skin itself. The stratum corneum, the outermost layer of the epidermis, is the main barrier against the external environment (43-45). This barrier function is attributed to the structure of the stratum corneum, which is often described as a "brick and mortar" structure (43-46). The stratum corneum consists of several layers of dead cells (corneocytes, mainly containing keratin) that are embedded in a lipid matrix. The diffusion of drugs into and through the epidermis is believed to be passive (43,44), mainly across the epidermis and little via the skin appendages (hair follicles and sweat glands). The rate determining step in the epidermal route is the diffusion across the stratum corneum. The diffusion pathway through the stratum corneum can be transcellular (across the corneocytes and the intercellular lipids) or intercellular (via the lipid matrix between the corneocytes). The latter route is believed to be the principal route and thus the major barrier for drug permeation (43,44). Results from animal studies indicate that diffusion of ALA across the stratum corneum is an important limiting factor for ALA penetration into normal skin (47,48).

Penetration enhancement

PDT effects in normal skin of hairless guinea pigs were increased when the thickness of

the stratum corneum was reduced by tape stripping before ALA application (47) and more PpIX was produced in normal nude mouse skin when the stratum corneum was removed by tape stripping the skin prior to ALA application (48).

Besides tape stripping, several other methods to increase ALA penetration into skin and skin lesions have been applied successfully or are presently under investigation. Iontophoretic ALA delivery to normal human skin resulted in increased PpIX fluorescence and PDT effects (49). ALA delivery across the stratum corneum is fast and the amount of ALA in the skin can be increased with increasing charge. Furthermore, the use of ultrasound during ALA application enhances PpIX production in the skin (50). The use of penetration enhancers prior to or during ALA application increases the ALA penetration into the skin. Penetration enhancers are chemicals that improve the permeation properties of the stratum corneum, either by creating disorder in the alkyl chains of the lipids or by altering the solubility characteristics of the lipids. Dimethylsulphoxide (DMSO) has proven to increase PpIX fluorescence in deeper layers of nodular BCC (29) and has been used as a penetration enhancer in several clinical studies with ALA-PDT of BCC (22,24,51). However, one should keep in mind that DMSO is also a potentiator of haem biosynthesis (52-55). ALA- and ALA hexyl ester-induced PpIX production in nude mouse skin was increased by another penetration enhancer, an azacycloalkane derivative (48). Reducing the tumour volume of BCC by curettage of the lesions prior to ALA application has also been used in some clinical studies on ALA-PDT of BCC (51,56,57). The effect of temperature on ALA penetration and PpIX production in cells and skin has also been investigated (11,58,59). *In vitro* ALA uptake into cells is increased when ALA incubation takes place at higher temperatures (59). Higher PpIX fluorescence levels were measured in nude mouse skin (11) and the skin of three healthy volunteers (58) when the skin temperature was increased after, but not during a short-term (10 or 15 minutes) topical ALA application. In the skin of the healthy volunteers, PpIX production could also be increased when the skin was kept at a higher temperature during a 5 to 6 hours topical application of ALA (58). The authors from these two studies therefore concluded that mainly the PpIX production, and not the ALA penetration into the skin, is temperature dependent. However, this is contradictory with the *in vitro* results (59), and therefore more research into this is needed.

ALA esters and other derivatives

ALA prodrugs have been synthesized in order to overcome the limited penetration of ALA into the skin, which is (partly) caused by the hydrophilic and zwitter ionic characteristics of ALA. A prodrug is a chemically inactive derivative of the biologically

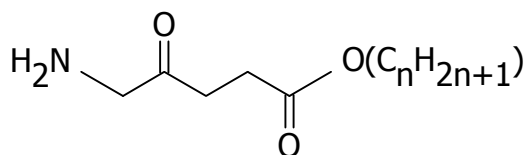


Figure 4 Molecular structure of 5-aminolevulinic acid alkyl esters.

active compound, but is, after administration, converted to the active compound. The most common prodrug linkage is a covalent ester bond (figure 4) which is enzymatically cleaved by intracellular esterases (60). The lipophilicity of ALA can be increased by esterifying the carboxyl group and this higher lipophilicity might then increase the penetration depth, distribution and uptake. Several ALA derivatives, i.e. esters and amide derivatives have been synthesized and tested *in vitro* (61-66), *ex vivo* (67,68), *in vivo* (8,48,63,69,70) and in patients (71).

Several (but not all) ALA esters increased the PpIX production in different cell lines (61-64,66), with the long-chained esters being more efficient than the shorter ones (61,64,66). Some short-chained ALA esters did not increase the PpIX production in cells, dependent on the cell line used in the different studies. ALA methyl ester induced lower PpIX production in all cell lines used (61,63-66), whereas the ethyl ester (64,66) and the propyl ester (66) induced lower PpIX production in some of the cell lines. The differences between ALA and the ALA esters might be explained by the fact that ALA, but not ALA esters, is transported by β -amino acid and γ -aminobutyric acid carriers (59). In a study where *ex vivo* human and rat skin explant cultures were incubated with ALA or ALA esters in the medium, ALA hexyl ester was the only one that increased PpIX production (68). However, in this set-up, ALA and its derivatives are absorbed from the medium through the dermis to reach the epidermis, thus resembling rather systemic than topical administration of the compounds to the skin. After topical application of ALA or esters to *ex vivo* pig skin, there was no difference between ALA and the esters with regard to the amount of PpIX produced in the epidermis. However, in accordance with the previous *in vitro* studies (61-64,66), incubation of freshly isolated pig skin keratinocytes (from the epidermis) with ALA or esters showed that these keratinocytes produce more PpIX when incubated with ALA esters, compared to ALA (personal communication G.M.J. Beijersbergen van Henegouwen).

In vivo PpIX levels in the epidermis and hair follicles of normal nude mouse skin were higher after long-term application (14 hours) of ALA methyl, ethyl or propyl ester than after 14 hours ALA application (69). After 1 or 3.5 hours application, the PpIX levels were similar for ALA and these three short-chained esters.

ALA hexyl ester-induced PpIX fluorescence levels are only slightly higher (compared to ALA) in the normal nude mouse skin during long-term (24 hours) application (48). However, ALA-induced PpIX fluorescence was higher than ALA hexyl ester-induced PpIX fluorescence after short application times (1 to 60 minutes) with a 20% cream and after a 10 minutes application with creams containing 0.5 to 40% ALA (hexyl ester). Removing the stratum corneum by tape stripping the skin prior to application or the use of a penetration enhancer increased the ALA hexyl ester-induced PpIX production more than the ALA-induced PpIX production. Therefore, the results from this study indicate that the stratum corneum is an important barrier for the penetration of both ALA and

ALA hexyl ester into the skin, but to a higher extent for ALA hexyl ester.

In an *in vivo* PpIX fluorescence kinetics study after 30 minutes topical application of ALA and ALA pentyl ester to the back skin of hairless mice with and without UV-B induced (pre)cancerous lesions, it was shown that ALA pentyl ester induced slightly more *in vivo* PpIX fluorescence in the (pre)cancerous lesions, but not in the skin without (pre)cancerous lesions (8). However, microscopic fluorescence images showed these higher ALA pentyl ester-induced PpIX fluorescence levels were in the stratum corneum, but not in the dysplastic layer of the epidermis in which the ALA and ALA pentyl ester-induced PpIX fluorescence levels were the same. This, together with the fact that the PpIX fluorescence levels were also the same in the deeper layers, indicates that ALA pentyl ester does not penetrate deeper into skin than ALA.

In a study with healthy volunteers, ALA and the butyl and hexyl ester were delivered into the skin iontophoretically (70). Slightly higher PpIX levels were reached earlier with ALA hexyl ester, compared to ALA and ALA butyl ester. Furthermore, they observed a higher phototoxicity with ALA hexyl ester. There was no difference in microscopic PpIX levels and depth between the three compounds, but the ALA hexyl ester- and ALA butyl ester-induced microscopic PpIX fluorescence appeared to be distributed more homogeneously than the ALA-induced PpIX fluorescence.

Therapy using ALA esters

So far, ALA methyl ester is the only ALA ester tested in a clinical study for application to skin disease (71). ALA and ALA methyl ester were applied to patients with actinic keratosis and the PpIX levels in the lesions and surrounding normal skin was measured. The PpIX levels for both compounds were higher in the lesions than the surrounding normal skin, with the maximum PpIX levels in the lesions higher after ALA application than after ALA methyl ester. However, the ratio between PpIX in the lesions and PpIX in the surrounding normal skin was higher with ALA methyl ester. ALA methyl ester is being developed as a proprietary product, Metvix, by PhotoCure, a company based in Oslo. PhotoCure ASA are carrying out several pivotal clinical trials to assess the efficacy of Metvix PDT with red light in comparison to placebo and other treatment modalities in AK and BCC. In a phase II trial in primary BCC (nodular and superficial) Metvix PDT with 3 hours application time in one or two treatment sessions gave an overall complete lesion response rate of 95%. In addition, the same Metvix PDT regimen gave an overall cure rate of 90% of AK lesions in a phase II trial. Moreover, in a phase III study in 202 patients with 699 AK lesions, a single PDT session with Metvix showed similar efficacy, better cosmetic outcome, and high patient preference compared to cryotherapy. Based on these finalized phase II and III studies in AK, PhotoCure ASA have prepared and submitted an application for regulatory approval in Europe, with Sweden as reference country.

Other approaches

Several other approaches to the enhancement of ALA-PDT have been looked at. These include the use of agents to influence the PpIX production or breakdown. Iron chelators, like ethylenediaminetetraacetic acid, hydroxypyridones and desferrioxamine, reduce the intracellular conversion of PpIX into haem. This results in greater and longer accumulation of PpIX in cells (*in vitro*) and *ex vivo* skin explants (68,72-74). Iron chelators have been used in the ALA-PDT treatment of skin lesions in patients with Bowen's disease, actinic keratosis and BCCs (21,22,24). Interrupting the illumination for a long period of time (up to several hours) has also shown to be successful in improving the ALA-PDT in hairless mice with or without precancerous lesions (75). After the first light treatment, new PpIX is formed (5,75) which is then used for the second illumination. New formation of PpIX after ALA-PDT was also found to occur in human superficial (3,9) and nodular (3) BCC. Increased ALA-PDT effect in hairless mouse skin with precancerous lesions could also be achieved by decreasing the irradiance or fluence rate (75-77).

Acknowledgements

We thank Yorkshire Cancer Research for financial support.

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