Published in final edited form as:

Isr J Chem. 2012 September ; 52(8-9): 767–775. doi:10.1002/ijch.201200005.

Vitamin D Combined with Aminolevulinate (ALA)-Mediated Photodynamic Therapy (PDT) for Human Psoriasis: A Proof-of-Principle Study

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Abstract

We previously showed that select agents (methotrexate or Vitamin D), when administered as a preconditioning regimen, are capable of promoting cellular differentiation of epithelial cancer cells while simultaneously enhancing the efficacy of 5-aminolevulinic acid (ALA)-mediated photodynamic therapy (PDT). In solid tumors, pretreatment with Vitamin D simultaneously promotes cellular differentiation and leads to selective accumulation of target porphyrins (mainly *protoporphyrin IX*, PpIX) within diseased tissue. However, questions of whether or not the effects upon cellular differentiation are inexorably linked to PpIX accumulation, and whether these effects might occur in hyperproliferative noncancerous tissues, have remained unanswered. In this paper, we reasoned that *psoriasis*, a human skin disease in which abnormal cellular proliferation and differentiation plays a major role, could serve as a useful model to test the effects of pro-differentiating agents upon PpIX levels in a non-neoplastic setting. In particular, Vitamin D, a treatment for psoriasis that restores (increases) differentiation, might increase PpIX levels in psoriatic lesions and facilitate their responsiveness to ALA-PDT. This concept was tested in a pilot study of 7 patients with bilaterally-matched psoriatic plaques. A regimen in which calcipotriol 0.005% ointment was applied for 3 days prior to ALA-PDT with blue light, led to preferential increases in PpIX (~130%), and reductions in thickness, redness, scaling, and itching in the pretreated plaques. The results suggest that a larger clinical trial is warranted to confirm a role for combination treatments with Vitamin D and ALA-PDT for psoriasis.

Keywords

Antitumor agents; Fluorescence; Photochemistry; Prodrugs; Vitamins

INTRODUCTION

Photodynamic therapy (PDT) is a therapeutic modality that utilizes an otherwise nontoxic chemical (photosensitizer) to preferentially create oxidative damage in diseased tissue when exposed to light (1, 2). There are four key components to any PDT regimen: photosensitizer, light source, oxygen, and the cellular target; each of these factors can be manipulated to improve the selectivity of the treatment. In particular, excellent selectivity can be obtained when 5-aminolevulinic acid (5-ALA; ALA) is used for photosensitization. ALA is the precursor (i.e., a pro-drug) for the synthesis of endogenous porphyrins via the heme biosynthetic pathway within cells, and amongst the known intermediates, *protoporphyrin IX* (PpIX) is considered the most important for photosensitization during ALA-PDT (3). Because the biochemical pathways that regulate ALA uptake and conversion to PpIX tend to be more active in neoplastic cells than in normal cells, PpIX can accumulate to very high levels in a tumor-selective manner (4). Therefore, ALA-PDT has proven particularly useful for treating large areas of preneoplastic (dysplastic) epithelia of the skin, including carcinoma in-situ (4–7), as well as analogous areas of the cervix (8). ALA-PDT has also proven helpful for image-guided surgical resection of dysplastic epithelial tissues in the urinary tract (9). However ALA-PDT, as a monotherapy, is still unsatisfactory for large and/or deep tumors, with incomplete responses and recurrence rates that are inferior to results obtained from surgical approaches (reviewed in (4)). Yet, ALA-PDT has distinct advantages that make it very attractive when compared to other modalities. For example, unlike chemotherapy or radiation, ALA-PDT has little or no systemic toxicity. Unlike surgery, PDT-treated areas heal without scarring. This tissue-sparing aspect of PDT, combined with the dual targeting provided by photosensitizer and light, argue strongly that ALA-PDT has greater potential for efficacy, and that continued research to develop and improve PDT is highly worthwhile. Although PDT already serves as a useful adjunct to more-established modalities, our goal is to find ways to improve ALA-PDT for current indications and for an expanding list of treatable diseases. Further benefits of successful new combinations with ALA-PDT would be an ability to reduce the ALA dose, thereby avoiding related side effects and complications.

Amongst the major factors that govern ALA-PDT efficacy (photosensitizer, light, oxygen, and cellular responses), the fourth factor in particular has not fully investigated and optimized. Therefore, our approach toward improving the efficacy of ALA-PDT has been to explore cellular biochemistry as a means for developing new combination regimens that might result in enhanced PpIX synthesis and accumulation, particularly in epithelial dysplasia and neoplasia. Regulation of the heme biosynthetic pathway is very complex, and involves eight enzyme-catalyzed steps, from the initial condensation of glycine and succinyl-CoA which forms 5-aminolevulinic acid (ALA), to the final step in which iron is inserted into the mature heme molecule (see (10), or a simplified schematic in Fig. 8 of (11), for a description of these pathways). Initial understanding of heme pathway regulation came from studies of erythropoietic lineages (cells specialized to make hemoglobin). In erythroid cells, it was shown that inducers of cellular differentiation, such as DMSO or butyrate, could upregulate critical enzymes in the heme-synthetic pathway, leading to increased synthesis of protoporphyrins (12, 13). Other differentiation-promoting agents were later shown to increase PpIX in colon carcinoma cells or melanoma cell lines (14, 15).

Because it became increasingly apparent that cellular differentiation might be linked to porphyrin synthesis in many cell types, albeit in a complex and cell-specific manner, we began exploring the possibility of exploiting this phenomenon for ALA-PDT of skin tumors. The first indication that cellular differentiation and PpIX metabolism might be linked in skin cancer cells came from a finding that skin epithelial cells (keratinocytes), when grown *in vitro* and forced to undergo terminal differentiation by raising the calcium concentration,

expressed high levels of *coprophrynogen oxidase* (CPO), a porphyrin-synthetic enzyme that is rate-limiting for PpIX synthesis in this setting (16). PpIX levels were preferentially elevated in these differentiated keratinocytes (16). Methotrexate (MTX) is another agent known to trigger terminal differentiation in normal keratinocytes (17). We showed that MTX, administered as a 3-day preconditioning regimen before ALA-PDT, can enhance PpIX accumulation significantly within skin cancer cell lines, both *in vitro* and *in vivo* (18). MTX pretreatment was also found to increase PpIX in prostate cancer cell lines (11). Both in the skin and prostate tumor cells, the concentrations at which MTX exerts these effects were found to be extremely low, in the subnanomolar range, which is 100- to 1000-fold lower than levels typically employed in the clinic (11, 18). While too low to be tumoricidal, these doses of MTX are sufficient to trigger differentiation in normal keratinocytes (17), choriocarcinoma cells (20, 21), and A431 carcinoma cells (18). The mechanism for the MTX-mediated PpIX elevation was shown to be induction of CPO (18), the same porphyrin-synthetic enzyme shown earlier to be elevated by high calcium in keratinocytes (16).

Vitamin D (VD) comprises a family of steroid hormones that regulate mineral and bone metabolism, calcium homeostasis, and cellular proliferation and differentiation; reviewed in (22, 23). Two major classes of VD exist: VD3 in mammals and VD2 in plants. The initial step in VD3 formation involves production of an early precursor (pre-VD3) from 7-dehydrocholesterol in the skin, a step which requires sunlight (22). Subsequent hydroxylation steps in the liver and kidney produce 25-OH VD3 (calcidiol) and 1, 25-diOH VD3 (calcitriol), respectively. Calcitriol is the final, active form of VD3 that binds to the Vitamin D receptor (VDR), a steroid hormone receptor that translocates to the nucleus of cells and activates gene transcription, thereby eliciting the production of new proteins which modulate cellular physiology (22). Currently, an interesting public debate is playing out in the media regarding widespread Vitamin D deficiency in northern countries, and whether or not VD3 deficiency affects overall health and susceptibility to cancer (24). However, the benefits of using topical VD3 ointment as a treatment for psoriasis is non-controversial. Topical VD3 is utilized for psoriasis by dermatologists worldwide, with an efficacy roughly equivalent to topical glucocorticoids. Although the mechanisms of VD3 action are complex, it is clear that VD3 modulates and normalizes a disordered state of cellular differentiation in psoriatic skin, with effects that include growth arrest and the induction of transglutaminase and involucrin in epidermal cells (25, 26). We were very interested in this ability of VD3 to modulate epithelial cell differentiation in the context of PDT. The first hint that VD3 might be useful for enhancing ALA-PDT was a finding that calcitriol and other synthetic VD3 analogs can stimulate increased PpIX levels in prostate cancer cells (19). Although addition of calcitriol to monolayers of skin keratinocytes did not cause a PpIX-elevating effect (27), the same cells in a tissue-mimetic, organotypic culture setting responded to calcitriol by accumulating significant levels of PpIX (27). The potency of calcitriol (in the picomolar range) for inducing PpIX was quite remarkable (27, 28) and encouraged us to examine these effects further.

As documented above, the idea that a pretreatment with small molecules (MTX, VD3) might be valuable in a new ALA-PDT combination regimen for neoplastic epithelial diseases appears to be firmly established in preclinical animal studies. However, the next step of bringing these new combinations into the clinic presents major obstacles. Most of these difficulties are of a regulatory nature. In the United States, the two FDA-approved versions of ALA-PDT in dermatology, topical 5-aminolevulinate activated by blue light (Levulan®, DUSA Pharmaceuticals), and methyl-aminolevulinate activated by red light (Metvixia®, Galderma Pharmaceuticals), are only permitted for the treatment of non-hyperkeratotic actinic keratoses (6, 29). Use for any other indication is considered to be an "off-label use" and is strictly regulated from a research perspective. To be sure, topical and systemic ALA-PDT have been investigated as treatment for a variety of skin diseases, including cutaneous

malignancies, squamous dysplasia, acne, and inflammatory dermatoses (30–32). However, most of these reports in the USA consist of small case series, with larger studies coming primarily from Europe.

Knowing that the bench-to-beside translation of ALA-PDT combinations for skin cancer will require a long-term effort to overcome regulatory hurdles, we sought other human disease models that might help to validate principles of combination ALA-PDT therapy. In this regard, psoriasis appeared very interesting. Psoriasis is a chronic skin disease characterized by red, scaly plaques located primarily on the knees and elbows, but also involving the entire body in severe cases. These lesions reflect disordered proliferation and differentiation of epidermal keratinocytes. Although a number of effective treatments for psoriasis exist, all have potentially unacceptable side effects, including increased carcinogenesis (ultraviolet B radiation; psoralen plus UVA radiation), liver toxicity (methotrexate), and increased risk of infection (biologicals, such as TNF blockers). ALA-PDT has none of these problems. Attempts to treat psoriasis with systemic and topical photodynamic therapy have been reported previously, but yielded only marginal results in terms of efficacy and patient tolerability (33–43). The tolerability issue refers to severe pain experienced during PDT illumination of the psoriatic plaques (44). Despite a mixed track record for ALA-PDT in psoriasis, our specific goal here was to use ALA-PDT in psoriasis as a model system for asking whether small-molecule pretreatments might lead to elevated PpIX levels in human skin. While efficacy of ALA-PDT for psoriasis was not our primary concern, room for improvement certainly existed, and results might at least point the way toward increased ALA-PDT efficacy.

In deciding which small-molecule agent to try, we noted that several VD₃ analogs (calcitriol and calcipotriol) are approved as topical agents for psoriasis, and are also known to induce keratinocyte differentiation (26, 45, 46). Calcipotriol (calcipotriene, Dovonex®), was the first VD₃ analog approved for psoriasis vulgaris in the U.S., and can be used either as topical monotherapy or in conjunction with corticosteroids, retinoids, or UVB phototherapy (47, 48). Calcipotriol retains the biologic activity of VD₃, but its calcemic activity is approximately 0.5% of its parent compound (48). Here, we describe a small pilot study to test the effect of calcipotriol for its ability to influence PpIX levels during ALA-PDT in human skin, using psoriatic plaques as a model. The study demonstrates the proof-of-principle that pretreatment with topical VD₃ can selectively elevate PpIX levels within psoriatic lesions prior to ALA-PDT.

EXPERIMENTAL SECTION

Materials

Calcipotriol 0.005%, either as a cream or ointment, was purchased from Leo Pharma, Inc. (Parsippany, NJ, USA).

5-aminolevulinic acid (5-ALA) was applied as a 20% solution in a proprietary vehicle (Levulan Kerastick, 20%, DUSA Pharmaceuticals, Wilmington, MA).

Clinical protocol

The study comprised four clinic visits, as outlined in Table 1. Two related protocols were approved by the Institutional Review Boards at Massachusetts General Hospital (red light protocol) and at the Cleveland Clinic (blue light protocol), and conducted according to the guidelines of the Declaration of Helsinki. Patients were recruited with psoriatic plaques (each > 36 cm²) on the arms or legs. Patients could not have taken methotrexate, acitretin, photosensitizing medications, nor received UVB nor PUVA treatment in the previous 3 months. Informed consent was obtained prior to the start of Visit 1. At Visit 1, two

symmetric plaques were chosen in matched anatomic areas (e.g., one on each elbow), and evaluated by clinical examination and photographs. Patients were given two vials to take home, one containing calcipotriol 0.005% cream or ointment (Dovonex; Leo Laboratories), the other containing a matching vehicle (Eucerin cream or Aquaphor ointment; Beiersdorf). Patients were instructed to apply these topicals to the plaques twice daily for six days. Both the investigators and the patients were blinded as to which plaque received the active ingredient.

At Visit 2, on day 7, patients returned for ALA application and fluorescence measurements. For the first three patients, each study plaque was divided into 3 equal regions (2×2 cm) using a template. PpIX fluorescence was measured at the following times: (i) at baseline prior to ALA application, (ii) at 2 hours after ALA application, and (iii) immediately after illumination with the light source. For the last four patients, two areas within each plaque were sampled for fluorimetry. After the baseline fluorescence measurement, 5-ALA was applied topically to all test areas and covered with an occlusive dressing (Tegaderm clear acrylic, 3M Corp., St. Paul, MN, USA) for 2 h. Light treatment, at either a red or blue wavelength (see below), was then administered. After the treatment, only bland topical emollients were allowed until the patients had completed both of the post treatment visits (see Clinical Assessment, below). The clinical evaluator, blind to the identity of the topical pretreatment agents, was the same for each patient at each follow-up visit.

Noninvasive fluorescence dosimetry (PpIX fluorimetry)

Fluorescence was measured with a custom-built device (Aurora Optics, Hanover, NH) in which excitation light (405 nm low-power diode laser) was delivered via a 100 mm diameter fiber optic cable placed against the skin, and fluorescent light emitted by PpIX (at wavelengths >690 nm; long-pass filter) was collected by a photodetector and analyzed using Labview software, as described (49–51). Ten measurements were taken at each of the regions selected within the plaque.

Treatment illumination

Red light was delivered by a 635 nm diode laser (HPD 7401, High Power Devices, Inc., North Brunswick, NJ, USA), directed via an optic fiber and a diffusing tip for a final spot diameter of 2.5 cm. Total light doses of 10, 20, or 40 J/cm², at a fluence rate of 100 mW/cm², were delivered to each of three previously designated areas within the plaque.

Blue light was delivered by a noncoherent 417 nm blue source (Blu-U, Dusa Pharmaceuticals, Wilmington, MA, USA), total dose of 10 J/cm², at a fluence rate of 10 mW/cm².

Clinical assessment of response to treatment

The following tools were employed to evaluate the treatment responses to ALA-PDT.

1. *Photography.*

High-resolution digital photographs of each psoriatic plaque were obtained at each of the four study visits.

2. *Psoriasis severity (TES) score.*

Changes in the severity of individual psoriatic plaques was evaluated using a physician-based, 4-point scoring system in which the thickness, erythema, and scale within each plaque was rated from 0 (none) to 3 (most severe)(52). Scoring at each visit was performed by the same physician, i.e., by A.K. for patients #1–3 and

by G.H. for patients #4–7. Full details of the scoring system are provided in Supporting Information (online).

3. *Itching score.*

At each visit, patients were asked to report their symptoms of itching in the plaques, using a 6-point visual-analog scoring system that ranged from 0 (no itching) to 5 (very severe itching)(53). Details are provided in Supporting Information (online).

RESULTS AND DISCUSSION

PpIX accumulation in psoriasis plaques is specifically enhanced by preconditioning with calcipotriol (VD) ointment

Figure 1 shows the results of an experiment that illustrates a PpIX-elevating effect of topical VD and shows the specificity of PpIX enhancement to psoriatic skin. Relative to the baseline fluorescence before ALA exposure, a marked enhancement of the fluorescence signal is seen at 2 h after ALA application in each of the psoriatic plaques. However, the signal in the VD-pretreated plaque is higher than in the non-pretreated control. The notion that these increases in fluorescence are due to PpIX, rather than to other molecules, is supported by our previous studies that correlated fluorescent readings with biochemical measurements of PpIX in lesional skin (18) and by the observation that the fluorescent increase is almost entirely reversed (photobleached) after illumination (Fig. 1). Strikingly, no change in PpIX is detectable in normal skin, indicating that the ALA-driven accumulation of PpIX occurs only in lesional (psoriatic) tissue. Enhancement of the PpIX signal by VD pretreatment appears to be lesion-specific as well (Fig. 1).

In Figure 2, relative changes in the PpIX signals from psoriatic plaques of multiple patients are pooled and presented. As shown by the third pair of bars, a small but significant enhancement occurred after pretreatment with VD cream, relative to vehicle cream. In contrast, a much larger enhancement (~130%) was observed after VD ointment. These results suggest that the occlusive properties of ointment are important, enabling calcipotriol to penetrate into the cornified tissue and elicit a biological effect.

Preconditioning with VD leads to the recruitment of more tissue regions with high PpIX

Others have reported that a potential problem with the use ALA-PDT for treatment of psoriasis is the nonhomogenous distribution of PpIX within psoriatic plaques; as shown by Kleinpenning, many areas fail to accumulate high PpIX levels (54). This phenomenon was confirmed in the current study (Table 2). At 2 hr after ALA, the amount of PpIX varied widely at different locations in plaques that had not been pretreated with VD, as seen by a relative standard deviation (SD) of 38% (Table 2). However, in plaques receiving VD pretreatment, the relative SD after 2 hr of ALA was only 4%, indicating a more uniform PpIX distribution due to high PpIX levels throughout the plaque. Therefore, VD not only leads to a higher levels of PpIX overall, but also causes more individual areas (cells) within the psoriatic plaque to express high PpIX levels, which should make the tissue more susceptible to PDT.

Preconditioning with VD elevates the amount of PpIX that is available for photochemical activation within psoriatic plaques

Fig. 3A shows that when three different doses (fluences) of 635 nm red light (10, 20, or 40 J/cm²) were used to test the content of PpIX available for photoactivation within psoriasis plaques, PpIX signals were diminished (photobleached) to a lesser extent in VD-pretreated plaques than in vehicle-pretreated plaques. This suggests that more PpIX was available at

the start of illumination, and therefore less susceptible to depletion in the VD-pretreated plaques than in the vehicle controls. Photobleaching using the red laser source, even at our highest fluence of 40 J/cm² (approximating the recommended red light dose for actinic keratoses, 37 J/cm²), was relatively inefficient (< 35%), suggesting incomplete activation/destruction of PpIX in the plaque.

Another problem with our 635 nm laser source was the limited spot size available for illumination (~2.5 cm), too small easily treat a typical psoriatic plaque. To allow us to illuminate a larger area of psoriasis during treatment, a second protocol was developed to provide a broader field of illumination using a 417 nm noncoherent source (Blu-U, Dusa Pharmaceuticals) which is FDA-approved in the USA for treatment of actinic keratoses (AK). Using this source and the standard blue light dose for AK (10 J/cm²), photobleaching appeared to be very efficient (~85%; Fig. 3B). No measurable difference in photobleaching was detectable between VD-pretreated and vehicle-pretreated plaques (Fig. 3B).

Clinical evaluation of bilaterally symmetric psoriatic plaques treated with ALA-PDT shows a trend toward greater clinical improvement in plaques pretreated with VD

Although this investigation was designed as an initial pilot study to look primarily for biochemical changes in photosensitizer (PpIX) accumulation, we did look at clinical response as a secondary endpoint. The first three study patients were given calcipotriol cream formulation (Table 3). Although small to moderate PpIX-elevating effects were observed in the plaques (Fig. 2), no clinical improvement in psoriasis, either with or without VD pretreatment, was noted. During illumination with the 635 nm source, these patients noted very intense stinging pain, alleviated only with vigorous application of ice water (44). Next, three patients (#4 and #5) were pretreated with the VD cream formulation followed by blue (417 nm) light exposure. As with the red light, patients complained of pain during blue light exposure although they were able to tolerate illumination of a much larger lesional area. No objective clinical improvement was observed.

However, when VD was provided as an ointment formulation (Table 3, patients #6 and #7), some clinical improvement due to VD preconditioning become apparent. Clinical results with the calcipotriol 0.005% ointment are illustrated in Fig. 4 and Fig. 5. After ALA-PDT, psoriasis appeared to worsen initially (as was true for all patients in the study), with plaques appearing more inflamed at one week post-treatment (Fig. 4B, 4E). By the second week, inflammation had subsided and lesions were becoming flatter and less red than prior to treatment (Fig. 4C,F). Pretreatment with VD ointment, in particular, appeared to preferentially elicit signs of clearing as evidenced by more thinning of the lesion at the margin (Fig. 4F, *arrows*), and leaving mild post-inflammatory hyperpigmentation at the site of resolution. Two other clinical measures, in addition to photography, showed preferential changes in the VD pretreated lesions of patients #6 and #7 (Fig. 5). First, a standardized psoriasis severity index (physician-conducted scoring of thickness, erythema, and scale, TES) revealed a greater decline in TES score on the VD-preconditioned side than on the vehicle control side, in both patients (Fig. 5, left side). Secondly, a greater improvement in the severity of itching was reported within the VD-pretreated plaques as compared to control plaques (Fig. 5B, right side). Together, these observations indicate a trend towards improved clinical response to ALA-PDT in psoriatic plaques pretreated with VD ointment.

CONCLUSIONS

This pilot study was designed to test the hypothesis that pharmacological agents known to drive cellular differentiation, and known to enhance accumulation of 5-ALA mediated PpIX in epithelial cancers, might also have a PpIX-enhancing effect in a non-malignant disease characterized by abnormal (reduced) differentiation. Psoriasis is such a disease, and the

results of our small pilot study in human volunteers provide preliminary evidence for proof-of-principle, showing that a VD3 analog (calcipotriol) may have a role in the development of improved PDT protocols for psoriasis. The data indicate that if the differentiating agent (calcipotriol) comes in a sufficiently occlusive vehicle to promote drug penetration, then improvements in photosensitizer (PpIX) levels are demonstrable (primary endpoint), along with clinical evidence for early improvement in psoriatic plaques at 2 weeks post-treatment (secondary endpoint). The study was not designed to evaluate responses beyond 2 weeks, so a definitive statement about treatment efficacy is not possible. However, the trend toward improvement is encouraging.

In any larger clinical trial to confirm a role for combination treatment with VD and ALA-PDT of psoriasis, a number of variables should be considered for testing and optimization. These include the drug itself (e.g., calcipotriol versus calcitriol), the photosensitizing pro-drug (e.g., 5-ALA versus methyl-ALA), the length of incubation time with the pro-drug, and the fluence rate of light delivery. One issue that was not formally addressed in our pilot study was the effect of VD pretreatment upon pain during illumination. As mentioned earlier, pain is a major problem with this treatment, and currently represents a major barrier to the use of PDT for psoriasis (reviewed in (44)). Manipulation of the drug-light interval (incubation time) and illumination-related variables (wavelength; fluence rate), in addition to small molecule pretreatment per se, will probably be important for the goal of pain reduction during ALA-PDT of psoriasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded by National Institutes of Health grant P01-CA084203 (T. Hasan).

REFERENCES

1. Brown SB, Brown EA, Walker I. The present and future role of photodynamic therapy in cancer treatment. *Lancet Oncol.* 2004; 5(8):497–508. [PubMed: 15288239]
2. Hasan, T.; Ortel, B.; Solban, N.; Pogue, BW. Photodynamic therapy of cancer. In: Kufe, D.; Bast, R.; Hait, W.; Hong, W.; Pollock, R.; Weichselbaum, R.; Holland, J.; Frei, E., editors. *Cancer Medicine*. 7th edition. Hamilton, Ontario: BC Decker, Inc.; 2006. p. 537-548.
3. Kennedy JC, Pottier RH, Pross DC. Photodynamic therapy with endogenous protoporphyrin IX: basic principles and present clinical experience. *J Photochem Photobiol B.* 1990; 6(1–2):143–148. [PubMed: 2121931]
4. Peng Q, Warloe T, Berg K, Moan J, Kongshaug M, Giercksky KE, Nesland JM. 5-Aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges. *Cancer.* 1997; 79(12): 2282–2308. [PubMed: 9191516]
5. Pariser DM, Lowe NJ, Stewart DM, Jarratt MT, Lucky AW, Pariser RJ, Yamauchi PS. Photodynamic therapy with topical methyl aminolevulinate for actinic keratosis: results of a prospective randomized multicenter trial. *J Am Acad Dermatol.* 2003; 48(2):227–232. [PubMed: 12582393]
6. Piacquadio DJ, Chen DM, Farber HF, Fowler JF Jr, Glazer SD, Goodman JJ, Hruza LL, Jeffes EW, Ling MR, Phillips TJ, Rallis TM, Scher RK, Taylor CR, Weinstein GD. Photodynamic therapy with aminolevulinic acid topical solution and visible blue light in the treatment of multiple actinic keratoses of the face and scalp: investigator-blinded, phase 3, multicenter trials. *Arch Dermatol.* 2004; 140(1):41–46. [PubMed: 14732659]

7. Touma D, Yaar M, Whitehead S, Konnikov N, Gilchrest BA. A trial of short incubation, broad-area photodynamic therapy for facial actinic keratoses and diffuse photodamage. *Arch Dermatol.* 2004; 140(1):33–40. [PubMed: 14732657]
8. Soergel P, Wang X, Stepp H, Hertel H, Hillemanns P. Photodynamic therapy of cervical intraepithelial neoplasia with hexaminolevulinate. *Lasers Surg Med.* 2008; 40(9):611–615. [PubMed: 18951428]
9. Stenzl A, Penkoff H, Dajc-Sommerer E, Zumbraegel A, Hoeltl L, Scholz M, Riedl C, Bugelnig J, Hobisch A, Burger M, Mikuz G, Pichlmeier U. Detection and clinical outcome of urinary bladder cancer with 5-aminolevulinic acid-induced fluorescence cystoscopy : a multicenter randomized, double-blind, placebo-controlled trial. *Cancer.* 2010
10. Bickers, DR.; Pathak, MA.; Lim, HW. The Porphyrins. In: Freedberg, IMea, editor. *Dermatology in General Medicine.* New York: McGraw-Hill; 1999. p. 1766-1803.
11. Sinha AK, Anand S, Ortel BJ, Chang Y, Mai Z, Hasan T, Maytin EV. Methotrexate used in combination with aminolaevulinic acid for photodynamic killing of prostate cancer cells. *Br J Cancer.* 2006; 95(4):485–495. [PubMed: 16868543]
12. Ebert PS, Bonkowsky HL, Deisseroth A. Evidence for multiple sites of regulation of heme synthesis in murine erythroleukemia cells. *J Natl Cancer Inst.* 1979; 62(5):1247–1250. [PubMed: 155751]
13. Fujita H, Yamamoto M, Yamagami T, Hayashi N, Sassa S. Erythroleukemia differentiation. Distinctive responses of the erythroid-specific and the nonspecific deltaaminolevulinate synthase mRNA. *J Biol Chem.* 1991; 266(26):17494–17502. [PubMed: 1894633]
14. Siboni G, Amit-Patito I, Weizman E, Waintraub-Porat M, Weitman H, Ehrenberg B, Malik Z. Specificity of photosensitizer accumulation in undifferentiated versus differentiated colon carcinoma cells. *Cancer Lett.* 2003; 196(1):57–64. [PubMed: 12860290]
15. Ickowicz Schwartz D, Gozlan Y, Greenbaum L, Babushkina T, Katcoff DJ, Malik Z. Differentiation-dependent photodynamic therapy regulated by porphobilinogen deaminase in B16 melanoma. *Br J Cancer.* 2004; 90(9):1833–1841. [PubMed: 15150593]
16. Ortel B, Chen N, Brissette J, Dotto GP, Maytin E, Hasan T. Differentiation-specific increase in ALA-induced protoporphyrin IX accumulation in primary mouse keratinocytes. *Br J Cancer.* 1998; 77(11):1744–1751. [PubMed: 9667642]
17. Schwartz PM, Barnett SK, Atillasoy ES, Milstone LM. Methotrexate induces differentiation of human keratinocytes. *Proc Natl Acad Sci U S A.* 1992; 89(2):594–598. [PubMed: 1731329]
18. Anand S, Honari G, Hasan T, Elson P, Maytin EV. Low-dose methotrexate enhances aminolevulinate-based photodynamic therapy in skin carcinoma cells in vitro and in vivo. *Clin Cancer Res.* 2009; 15(10):3333–3343. [PubMed: 19447864]
19. Ortel B, Sharlin D, O'Donnell D, Sinha AK, Maytin EV, Hasan T. Differentiation enhances aminolevulinic acid-dependent photodynamic treatment of LNCaP prostate cancer cells. *Br J Cancer.* 2002; 87(11):1321–1327. [PubMed: 12439724]
20. Friedman SJ, Skehan P. Morphological differentiation of human choriocarcinoma cells induced by methotrexate. *Cancer Res.* 1979; 39(6 Pt 1):1960–1967. [PubMed: 571758]
21. Hatse S, De Clercq E, Balzarini J. Role of antimetabolites of purine and pyrimidine nucleotide metabolism in tumor cell differentiation. *Biochem Pharmacol.* 1999; 58(4):539–555. [PubMed: 10413291]
22. Bikle, DD., editor. *Vitamin D: Production, Metabolism and Mechanisms of Action.* South Dartmouth, MA: MDTEXT.COM, Inc.; 2009.
23. Bikle DD, Pillai S. Vitamin D, calcium, and epidermal differentiation. *Endocr Rev.* 1993; 14(1):3–19. [PubMed: 8491153]
24. Stechschulte SA, Kirsner RS, Federman DG. Vitamin D: bone and beyond, rationale and recommendations for supplementation. *Am J Med.* 2009; 122(9):793–802. [PubMed: 19699370]
25. Su MJ, Bikle DD, Mancianti ML, Pillai S. 1,25-Dihydroxyvitamin D3 potentiates the keratinocyte response to calcium. *J Biol Chem.* 1994; 269(20):14723–14729. [PubMed: 7910167]
26. Bikle DD. Vitamin D regulated keratinocyte differentiation. *J Cell Biochem.* 2004; 92(3):436–444. [PubMed: 15156556]

27. Sato N, Moore BW, Keevey S, Drazba JA, Hasan T, Maytin EV. Vitamin D enhances ALA-induced protoporphyrin IX production and photodynamic cell death in 3-D organotypic cultures of keratinocytes. *J Invest Dermatol.* 2007; 127(4):925–934. [PubMed: 17068479]
28. Anand S, Wilson C, Hasan T, Maytin EV. Vitamin D3 enhances the apoptotic response of epithelial tumors to aminolevulinate-based photodynamic therapy. *Cancer Res.* 2011; 71(18): 6040–6050. [PubMed: 21807844]
29. Braathen LR, Szeimies RM, Basset-Seguin N, Bissonnette R, Foley P, Pariser D, Roelandts R, Wennberg AM, Morton CA. Guidelines on the use of photodynamic therapy for nonmelanoma skin cancer: an international consensus. International Society for Photodynamic Therapy in Dermatology, 2005. *J Am Acad Dermatol.* 2007; 56(1):125–143. [PubMed: 17190630]
30. Morton CA, Brown SB, Collins S, Ibbotson S, Jenkinson H, Kurwa H, Langmack K, McKenna K, Moseley H, Pearse AD, Stringer M, Taylor DK, Wong G, Rhodes LE. Guidelines for topical photodynamic therapy: report of a workshop of the British Photodermatology Group. *Br J Dermatol.* 2002; 146(4):552–567. [PubMed: 11966684]
31. Ibbotson SH. Topical 5-aminolaevulinic acid photodynamic therapy for the treatment of skin conditions other than non-melanoma skin cancer. *Br J Dermatol.* 2002; 146(2):178–188. [PubMed: 11903225]
32. Akilov OE, Kosaka S, Maytin EV, Hasan T. Prospects for the use of differentiation-modulating agents as adjuvant of photodynamic therapy for proliferative dermatoses. *J Dermatol.* 2008; 35(4): 197–205. [PubMed: 18419676]
33. Smits T, Kleinpenning MM, van Erp PE, van de Kerkhof PC, Gerritsen MJ. A placebo-controlled randomized study on the clinical effectiveness, immunohistochemical changes and protoporphyrin IX accumulation in fractionated 5-aminolaevulinic acid-photodynamic therapy in patients with psoriasis. *Brit J Dermatol.* 2006; 155(2):429–436. [PubMed: 16882185]
34. Radakovic-Fijan S, Blecha-Thalhammer U, Schleyer V, Szeimies RM, Zwingers T, Honigsmann H, Tanew A. Topical aminolaevulinic acid-based photodynamic therapy as a treatment option for psoriasis? Results of a randomized, observer-blinded study. *Br J Dermatol.* 2005; 152(2):279–283. [PubMed: 15727639]
35. Beattie PE, Dawe RS, Ferguson J, Ibbotson SH. Lack of efficacy and tolerability of topical PDT for psoriasis in comparison with narrowband UVB phototherapy. *Clin Exp Dermatol.* 2004; 29(5): 560–562. [PubMed: 15347356]
36. Bissonnette R, Tremblay JF, Juzenas P, Boushira M, Lui H. Systemic photodynamic therapy with aminolevulinic acid induces apoptosis in lesional T lymphocytes of psoriatic plaques. *J Invest Dermatol.* 2002; 119(1):77–83. [PubMed: 12164928]
37. Bissonnette R, Zeng H, McLean DI, Korbek M, Lui H. Oral aminolevulinic acid induces protoporphyrin IX fluorescence in psoriatic plaques and peripheral blood cells. *Photochem Photobiol.* 2001; 74(2):339–345. [PubMed: 11547574]
38. Boehncke WH, Elshorst-Schmidt T, Kaufmann R. Systemic photodynamic therapy is a safe and effective treatment for psoriasis. *Arch Dermatol.* 2000; 136(2):271–272. [PubMed: 10677114]
39. Robinson DJ, Collins P, Stringer MR, Vernon DI, Stables GI, Brown SB, Sheehan-Dare RA. Improved response of plaque psoriasis after multiple treatments with topical 5-aminolaevulinic acid photodynamic therapy. *Acta Derm Venereol.* 1999; 79(6):451–455. [PubMed: 10598759]
40. Collins P, Robinson DJ, Stringer MR, Stables GI, Sheehan-Dare RA. The variable response of plaque psoriasis after a single treatment with topical 5-aminolaevulinic acid photodynamic therapy. *Br J Dermatol.* 1997; 137(5):743–749. [PubMed: 9415234]
41. Stringer MR, Collins P, Robinson DJ, Stables GI, Sheehan-Dare RA. The accumulation of protoporphyrin IX in plaque psoriasis after topical application of 5-aminolevulinic acid indicates a potential for superficial photodynamic therapy. *J Invest Dermatol.* 1996; 107(1):76–81. [PubMed: 8752843]
42. Boehncke WH, König K, Kaufmann R, Scheffold W, Prummer O, Sterry W. Photodynamic therapy in psoriasis: suppression of cytokine production in vitro and recording of fluorescence modification during treatment in vivo. *Arch Dermatol Res.* 1994; 286(6):300–303. [PubMed: 7979545]

43. Boehncke WH, Sterry W, Kaufmann R. Treatment of psoriasis by topical photodynamic therapy with polychromatic light. *Lancet*. 1994; 343(8900):801. [PubMed: 7907764]
44. Warren CB, Karai LJ, Vidimos A, Maytin EV. Pain associated with aminolevulinic acid-photodynamic therapy of skin disease. *J Am Acad Dermatol*. 2009; 61(6):1033–1043. [PubMed: 19925929]
45. Lu J, Goldstein KM, Chen P, Huang S, Gelbert LM, Nagpal S. Transcriptional profiling of keratinocytes reveals a vitamin D-regulated epidermal differentiation network. *J Invest Dermatol*. 2005; 124(4):778–785. [PubMed: 15816836]
46. Kragballe K, Wildfang IL. Calcipotriol (MC 903), a novel vitamin D3 analogue stimulates terminal differentiation and inhibits proliferation of cultured human keratinocytes. *Arch Dermatol Res*. 1990; 282(3):164–167. [PubMed: 2164359]
47. Guilhou JJ. The therapeutic effects of vitamin D3 and its analogues in psoriasis. *Expert Opin Investig Drugs*. 1998; 7(1):77–84.
48. Binderup, L.; Binderup, E.; Godtfredsen, WO.; Kissmeyer, A. Development of New Vitamin D Analogs. In: Feldman, D.; Pike, W.; Glorieux, F., editors. *Vitamin D*. Elsevier Academic Press; 2005. p. 1489–1510.
49. Pogue BW, Burke G. Fiber-optic bundle design for quantitative fluorescence measurement from tissue. *Appl Opt*. 1998; 37(31):7429–7436. [PubMed: 18301577]
50. Lee CC, Pogue BW, Strawbridge RR, Moodie KL, Bartholomew LR, Burke GC, Hoopes PJ. Comparison of photosensitizer (AIPcS2) quantification techniques: in situ fluorescence microsampling versus tissue chemical extraction. *Photochem Photobiol*. 2001; 74(3):453–460. [PubMed: 11594060]
51. Warren CB, Lohser S, Wene LC, Pogue BW, Bailin PL, Maytin EV. Noninvasive fluorescence monitoring of protoporphyrin IX production and clinical outcomes in actinic keratoses following short-contact application of 5-aminolevulinate. *J Biomed Opt*. 2010; 15(5) 051607.
52. Langley RG, Ellis CN. Evaluating psoriasis with Psoriasis Area and Severity Index, Psoriasis Global Assessment, and Lattice System Physician's Global Assessment. *J Am Acad Dermatol*. 2004; 51(563–569)
53. Adams, C. [last accessed on 1/1/2012] Visual Analog Pain Scale adapted and modified from Chris Adam's analog scale. as accessed on the website: <http://ergonomics.about.com/od/ergonomicbasics/ss/painscale.htm>
54. Kleinpenning MM, Smits T, Ewalds E, van Erp PE, van de Kerkhof PC, Gerritsen MJ. Heterogeneity of fluorescence in psoriasis after application of 5-aminolaevulinic acid: an immunohistochemical study. *Brit J Dermatol*. 2006; 155(3):539–545. [PubMed: 16911278]

Fig. 1. Vitamin D exerts a PpIX-elevating effect specifically in psoriatic plaques (PS) and not in normal skin (NL)

Fluorescence attributable to PpIX was measured by noninvasive fluorimetry in two psoriasis plaques located on contralateral legs; one plaque was pretreated with calcipotriol ointment (+) and the other with inert vehicle (–), twice daily for 6 days. On day 7, PpIX fluorescence was measured with a surface dosimeter that excites the PpIX molecule with blue light, then detects PpIX-specific fluorescence at red wavelengths (see Experimental Section).

Fluorimetry measurements were performed at the following times: (i), just prior to ALA application, (ii), at 2 h after ALA application, and (iii), immediately after illumination with 10 J/cm² of blue light. Each bar is the mean $\pm$ SD of 5 replicate measurements. *Dotted line*, average level for fluorescence signal in normal skin.

Fig. 2. Effect of topical formulation upon the ability of calcipotriol to elicit a relative enhancement of PpIX within psoriatic plaques

Calcipotriol 0.005%, formulated as a proprietary cream (Dovonex cream) or ointment (Dovonex ointment), was used. For each study subject, one plaque was treated with the cream (or ointment), and a similar plaque located on the contralateral arm or leg was treated with Eucerin cream (or Aquaphor ointment) twice daily for 6 days. On day 7, fluorimetric measurement of PpIX levels was performed. Two or three regions within each plaque were measured and normalized to the average pre-ALA fluorescence value at baseline; 5 subjects treated with cream, 2 subjects with ointment; mean $\pm$ SD.

Fig. 3. Photobleaching of PpIX in psoriatic plaques, as a function of light dose and of preconditioning with calcipotriol

Photobleaching (%) is expressed as the difference between PpIX fluorescence measured before and after exposure to light at the indicated dose (10, 20, or 40 J/cm²), relative to initial pre-illumination fluorescence values in the same plaque area. **(A)**, Photobleaching observed after red light, using a 635 laser defocused to a 2.5 cm spot size (see Methods). Each bar represents the mean of repeated measurements in 3 different patients, taken before and after illumination at the same plaque region (for a total of 6 readings per condition). **(B)**, Photobleaching after blue light, delivered with a noncoherent light source at 417 nm (Blu-U, DUSA Pharmaceuticals, Inc); mean of 2 patients, dose 10 J/cm², delivered in 1000 sec.

Fig. 4. Clinical appearance of psoriatic plaques, on bilateral elbows of the same patient, before and after PDT

One elbow was mock-preconditioned vehicle ointment (A–C), while the other elbow was preconditioned with calcipotriol 0.005% ointment (D–F). Photographs were taken prior to ALA application at Visit 2 (A, D), one week after PDT (B, E), and two weeks after PDT (C, F). Note the increased swelling and inflammation in plaques at one week after the treatment, followed by thinning of plaques and reduction in the redness (erythema) by two weeks. Better initial clearing of psoriasis on the calcipotriol-pretreated elbow as compared to the contralateral elbow, is suggested by peripheral clearing with hyperpigmentation (*white arrows*).

PSORIASIS SEVERITY SCORING FOR PATIENT #6

PSORIASIS SEVERITY SCORING FOR PATIENT #7

Fig. 5. Improvement in lesion appearance and clinical symptoms in the psoriatic plaques of patients who responded to pretreatment (Pre-Tx) with calcipotriol ointment, followed by ALA-PDT

Physician assessment of plaque morphology (graphs on left) and patient self-reported itching symptoms (graphs on right) were measured over the course of 4 study visits. TES severity index is the sum of scores for thickness (*black*), erythema (*gray*) and scale (*white*). Both the TES scoring system and the scale for itching severity are explained in the Experimental Section.

TABLE 1

Study Protocol, with four clinic visits for each patient subject.

	VISIT 1 *	VISIT 2	VISIT 3	VISIT 4
	Day 0 Baseline visit	Day 7 (± 1) Treatment Day	Day 14(± 1)	Day 21 (± 1)
Purpose of Visit:	Initial assessment. Dispense study medication	Apply ALA; perform fluorimetry; Treat with Light source.	Clinical Assessment	Clinical Assessment
Procedures in clinic:		Apply ALA. After 2 hr, irradiate plaques with red or blue light.		
Noninvasive fluorimetry		Measurements before and after light therapy		
T-E-S scoring of plaques	X	X	X	X
Photography of lesions	X	X	X	X

* In addition, a screening visit at 0–10 days prior to the first visit was conducted, to explain the study and obtain Informed Consent.

FluorImeter Readings (PpIX Signal) in Psoriatic Plaques, as a function of geographic location and history of calcipotriol preconditioning *

TABLE 2

Patient ID	Plaque Region	Pre-ALA No VD	Post-ALA No VD	Pre-ALA After VD	Post-ALA After VD
#1	L1	1.58	6.28	1.80	6.21
	L2	1.69	5.22	2.22	6.14
	L3	2.10	6.38	1.81	6.15
#2	L1	1.55	1.76	2.73	6.40
	L2	1.99	2.39	3.01	6.56
	L3	1.19	3.47	3.26	6.50
#3	L1	5.02	5.21	1.24	6.84
	L2	3.34	6.58	2.08	6.70
	L3	3.37	6.13	2.31	6.72
Mean of 9 locations:		2.43	4.82	2.27	6.47
Std Deviation (SD):		1.24	1.83	0.64	0.26
Relative SD (%)		51%	38%	28%	4%

* Values are averages of 10 readings taken at one of three randomly chosen locations (L1, L2, or L3) within the plaque, immediately before and 2 hr after ALA application.

TABLE 3

Patient characteristics

Patient ID #:	# 1	# 2	# 3	# 4	# 5	# 6	# 7
Gender	M	F	F	F	M	M	F
Age (yrs)	18	51	46	49	40	57	26
Duration of psoriasis (yrs)	1	18	12	>10	>10	>10	7
Plaque location #	Knees	Knees	Elbows	Elbows	Legs	Elbows	Legs
Light source wavelength, nm	635	635	635	417	417	417	417
Dose (J/cm ²)	10, 20, 40	10, 20, 40	10, 20, 40	10	10	10	10
Formulation of calcipotriol	Cream	Cream	Cream	Cream	Cream	Ointment	Ointment
Clinical improvement at 2 wks post-PDT	No	No	No	No	No	Yes	Yes

All patients had minimal disease (< 20% body surface area involved).