



Pain Associated With Photodynamic Therapy Using 5-Aminolevulinic Acid or 5-Aminolevulinic Acid Methylene Ester on Tape-Stripped Normal Skin

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Background: Pain during and after topical photodynamic therapy (PDT) is one of the few severe adverse effects of the new treatment of skin diseases.

Objective: To compare the pain experienced in normal skin treated with 5-aminolevulinic acid (ALA) PDT and 5-aminolevulinic methylester (ALA-ME) PDT.

Design: Double-blind randomized trial.

Interventions: Twenty healthy volunteers were treated randomly with ALA-PDT on one forearm and ALA-ME-PDT on the other forearm after tape stripping of the sun-exposed skin areas.

Main Outcome Measures: Pain was scored using a numerical scale ranging from 0 to 10 during illumination, immediately after illumination, and each day in the following week. In addition, we measured erythema, pigmentation, and protoporphyrin IX (PpIX) fluorescence.

Results: ALA-PDT generated significantly more pain than ALA-ME-PDT during and after illumination ($P = .001$ and $P = .05$, respectively). ALA-PDT induced a larger decrease in PpIX fluorescence than ALA-ME-PDT ($P = .009$). There was no correlation between pain and peak PpIX fluorescence or absolute decrease in peak PpIX fluorescence. Both treatments lead to erythema immediately after illumination and increased pigmentation 1 week after PDT. There was no correlation between pain and degree of erythema or pigmentation.

Conclusions: ALA-ME-PDT was less painful than ALA-PDT when performed on tape-stripped normal skin. The pain scores did not correlate with the intensity of peak PpIX fluorescence in the skin or with the degree of erythema after illumination, suggesting that pain was not caused by activation of PpIX alone. The theory that ALA and not ALA-ME is transported by γ -aminobutyric acid receptors into the peripheral nerve endings may explain the higher pain scores in ALA-PDT-treated areas.

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TOPICAL PHOTODYNAMIC therapy (PDT) is a new method for treatment of skin diseases such as skin cancer, Bowen disease, and actinic keratoses. The therapy is based on activation of light-sensitive molecules (photosensitizers). These molecules form various cytotoxic species, which will damage essential cellular components, causing tissue injury through apoptosis.¹ In PDT that uses topical 5-aminolevulinic acid (ALA), ALA will convert in situ, via the haem cycle, into protoporphyrin IX (PpIX). Protoporphyrin IX is an extremely active photosensitizer, which is activated by red light.¹

The advantage of using ALA is that PpIX and other intermediate photosensitizers are rapidly eliminated from the body, limiting the skin photosensitization to a few days after oral use, whereas topical ALA does not cause any generalized photosen-

sitivity.² ALA-PDT can induce erythema and hyperpigmentation of the treated skin, and importantly patients undergoing ALA-PDT frequently experience itching, pricking, burning, or shooting pain in the treated area during and after PDT.¹

Pain during ALA-PDT is described in several studies.³⁻⁹ In most of the studies, patients experienced mild or moderate pain. Severe pain was reported by approximately one third of the patients in 2 of the studies^{6,8} on the treatment of solar keratoses and plaque psoriasis and was reported in 14% of the lesions in recalcitrant warts treated with ALA-PDT.⁹

Since ALA is a hydrophilic molecule, its penetration through cellular membranes and into the interstitial space of tissues is limited. 5-Aminolevulinic acid methylester (ALA-ME) is an esterified derivative of ALA. Because of the enhanced lipophilicity, ALA-ME should be expected to

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penetrate more easily and deeper into the targeted lesions and may lead to more effective PDT. ALA-ME is deesterified into ALA by intracellular enzymes.¹⁰ Some studies^{2,9,11} have suggested that ALA-ME-PDT is less painful than ALA-PDT. In this study, we compare pain associated with ALA-PDT and ALA-ME-PDT on tape-stripped normal skin.

METHODS

PARTICIPANTS

The protocol was approved by the Ethics Committee of Copenhagen and Frederiksberg and by the Danish Medicines Agency. Written informed consent was obtained from all participants. Twenty healthy volunteers participated in the study. Nine of the volunteers were women and 11 were men. The median age of the participants was 27 years (range, 22-34 years). Skin types were evaluated by the Fitzpatrick method; 7 volunteers (35%) had skin type II, 9 (45%) had skin type III, and 3 (15%) had skin type IV.

DRUGS

The ALA cream was prepared by our hospital pharmacy as δ -aminolevulinic acid hydrochloride (Sigma Chemical Company, St Louis, Mo) in an oil-in-water-based cream. The cream was used within a week after preparation. We used a commercial ALA-ME cream (Metvix; Photocure ASA, Oslo, Norway). Both creams were produced as a 20% (wt/wt) hydrochloride cream with a molecular concentration of 1.19 mmol/g (ALA) and 1.10 mmol/g (ALA-ME).

TREATMENT

A skin area 3 cm in diameter on the dorsal sun-exposed side of the forearm was marked and tape stripped 3 times with occlusive dressing (Tegaderm; 3M Health Care, St Paul, Minn). The application of the ALA cream was randomized to either the right or left arm, and the ALA-ME cream was applied on the opposite arm. The volunteers and the primary investigator (S.R.W.) were blinded to the creams. Approximately 1.5 g of each cream was applied and covered with light, impermeable occlusive dressing (Tegaderm and Hansamed strip; Beiersdorf A/S, Birkerød, Denmark) for 3 hours. Then the remaining cream was removed, and both treatment areas were simultaneously irradiated with red light (570-670 nm; CURELIGHT lamp; Photocure ASA). The illumination time was approximately 13 minutes (total dose of 70 J/cm² and a fluence rate of approximately 90 mW/cm²).

EVALUATION

The volunteers scored the pain in each of the treated areas during and immediately after illumination and daily during the following week. Pain was assessed using a numerical scale ranging from 0 to 10 in which 0 is no pain and 10 is the worst imaginable pain.

SKIN FLUORESCENCE, ERYTHEMA, AND PIGMENTATION MEASUREMENT

Skin fluorescence was measured with a fluorescence spectrometer system (FL3095; J&M, Analytische Mess and Regeltechnik GmbH, Aalen, Germany). The excitation wavelength was 410 nm, and the illumination time was 3 seconds. The instrument was calibrated daily using a fluorescence standard (Uranyl Standard; J&M, Analytische Mess and Regeltechnik GmbH). Curve fitting was applied for spectra analyses to assess the true PpIX fluorescence intensity from PpIX using spectroscopic soft-

ware (Opus, version 3.0; Bruker Analytische Messtechnik GmbH, Hamburg, Germany). The height of the 630-nm peak was used as an objective variable for PpIX intensity.

The PpIX fluorescence spectra from the treatment areas were measured before and 3 hours after cream application (before light exposure and after wiping off the remaining cream) and just after illumination. The percentages of erythema and pigmentation of the skin were measured using a skin reflectance meter (Matic UV-Optimize 555; Matic, Nærum, Denmark). The instrument measures reflectance of skin at 555 and 660 nm and conveys relative content of melanin and hemoglobin in skin in arbitrary units.¹² The measurements were obtained before treatment, after PDT, and at the follow-up 1 week after treatment.

The clinical appearance of skin was evaluated at the end of illumination and at follow-up 1 week later. A 4-point scale was used, with 0 indicating no visible erythema; (+), just perceptible erythema; +, erythema with a well-defined border; and ++, bright red erythema and induration.¹³

DATA ANALYSIS

Aiming for a significance level of .05 and a power of 80% and on the assumption that the smallest clinically important mean difference was 20% and the SD of the difference in response was 30%, we calculated that 17 volunteers should be included (Altman monogram for sample size calculation¹⁴). The Wilcoxon signed rank test for paired data was used to compare the pain scores between ALA- and ALA-ME-treated areas. This test was also used to compare peak PpIX fluorescence, redness, and pigmentation between the 2 treatment areas. The correlation between pain scores and peak PpIX fluorescence, redness, and pigmentation was evaluated by Spearman rank correlation.

To be able to compare peak PpIX fluorescence among the volunteers, we had to correct the fluorescence intensities so each volunteer had the same baseline. This was done by dividing the fluorescence intensity before and after illumination with the fluorescence intensity before application of the cream ($I[\text{before illumination}]/I[\text{normal skin}]$ and $I[\text{after illumination}]/I[\text{normal skin}]$). The decrease in peak PpIX fluorescence during illumination was calculated by subtracting peak PpIX fluorescence intensity after illumination from the intensity before illumination. To be able to compare the redness and pigmentation among the volunteers, we had to correct the measurements in the same way as for the peak PpIX fluorescence due to differences in erythema and pigmentation among the volunteers before the beginning of the treatment (eg, we divided the percentage of redness before illumination by the percentage of redness in normal skin).

RESULTS

PAIN SCORE IN ALA-PDT AND ALA-ME-PDT

The pain scores during, immediately after, and 24 hours after illumination are given in **Table 1**. The reported pain was significantly higher in ALA-treated areas than in ALA-ME-treated areas during ($P=.001$) and immediately after PDT ($P=.01$). There were no significant differences in pain between the 2 arms after 24 hours ($P=.16$). Most of the volunteers described the pain as burning and shooting, and the pain slowly increased during the first minutes of illumination then reached a plateau before the first pain score. Pain intensity decreased immediately after termination of illumination. Half of the volunteers reported mild pain (pain score 1 or 2) in the days following treatment, but no significant difference was found between the ALA- and ALA-ME-treated areas.

Table 1. Number of Treatment Sites With the Indicated Pain Score During, Immediately After, and 24 Hours After PDT

Pain	During PDT*		Immediately After PDT†		24 Hours After PDT‡	
	ALA	ALA-ME	ALA	ALA-ME	ALA	ALA-ME
No pain (score 0)	0	1	4	7	13	18
Mild pain (score >0-3)	9	15	11	10	7	2
Moderate pain (score >3-7)	9	4	4	3	0	0
Severe pain (score >7-10)	2	0	1	0	0	0
Total No. of Treatment Sites	20	20	20	20	20	20
Average (SD) pain score	4.2 (2.1)	2.7 (1.3)	2.3 (2.3)	1.5 (1.8)	0.4 (0.5)	0.2 (0.5)

Abbreviations: ALA, 5-aminolevulinic acid; ALA-ME, 5-aminolevulinic acid methylester; and PDT, photodynamic therapy.

* $P = .001$.

† $P = .01$.

‡ $P = .16$.

Table 2. Comparisons of Objectively Measured Fluorescence, Erythema, and Pigmentation Between the ALA- and ALA-ME-Treated Areas

Variable	Mean (SD), AU		P Value
	ALA	ALA-ME	
Peak PpIX fluorescence			
Before illumination	12.54 (14.41)	10.06 (21.60)	.02
Decrease during PDT	9.36 (12.15)	7.45 (21.01)	.009
Erythema			
Before PDT	16.9 (8.7)	18.8 (8.5)	.19
Immediately after	27.4 (9.6)*	27.3 (10.6)†	.80
One week after PDT	21.4 (14.4)	18.6 (10.4)	.27
Pigmentation			
Before PDT	36.1 (7.8)	35.0 (8.6)	.22
Immediately after	35.9 (7.9)	36.1 (8.6)	.24
One week after PDT	42.6 (7.6)‡	38.8 (8.1)§	.04

Abbreviations: ALA, 5-aminolevulinic acid; ALA-ME, 5-aminolevulinic acid methylester, AU, arbitrary units; PDT, photodynamic therapy; PpIX, protoporphyrin IX.

*Significantly more red than before PDT ($P < .001$).

†Significantly more red than before PDT ($P = .001$).

‡Significantly more pigmented than before PDT ($P < .001$).

§Significantly more pigmented than before PDT ($P < .001$).

PpIX FLUORESCENCE

The mean peak PpIX fluorescence before illumination and the decrease in peak PpIX fluorescence during illumination are given in **Table 2**. The ALA-treated skin had a higher peak PpIX fluorescence than the ALA-ME-treated skin ($P = .02$) before illumination, and ALA-PDT gave a higher decrease in peak PpIX fluorescence during illumination ($P = .009$). No significant correlation was found between pain scores reported during illumination and peak PpIX fluorescence before illumination ($P = .68$ [ALA], $P = .95$ [ALA-ME]). In addition, there was no correlation with decrease in peak PpIX fluorescence during illumination ($P = .81$ [ALA], $P = .25$ [ALA-ME]).

OBJECTIVELY MEASURED ERYTHEMA AND PIGMENTATION

The average percentage of erythema and pigmentation measured in ALA- and ALA-ME-treated skin is given in **Table 2**. The skin was significantly more red immediately after illumination than before in both ALA- and ALA-ME-

Table 3. Number of Volunteers With the Indicated Erythema and Other Skin Changes Graded Visually

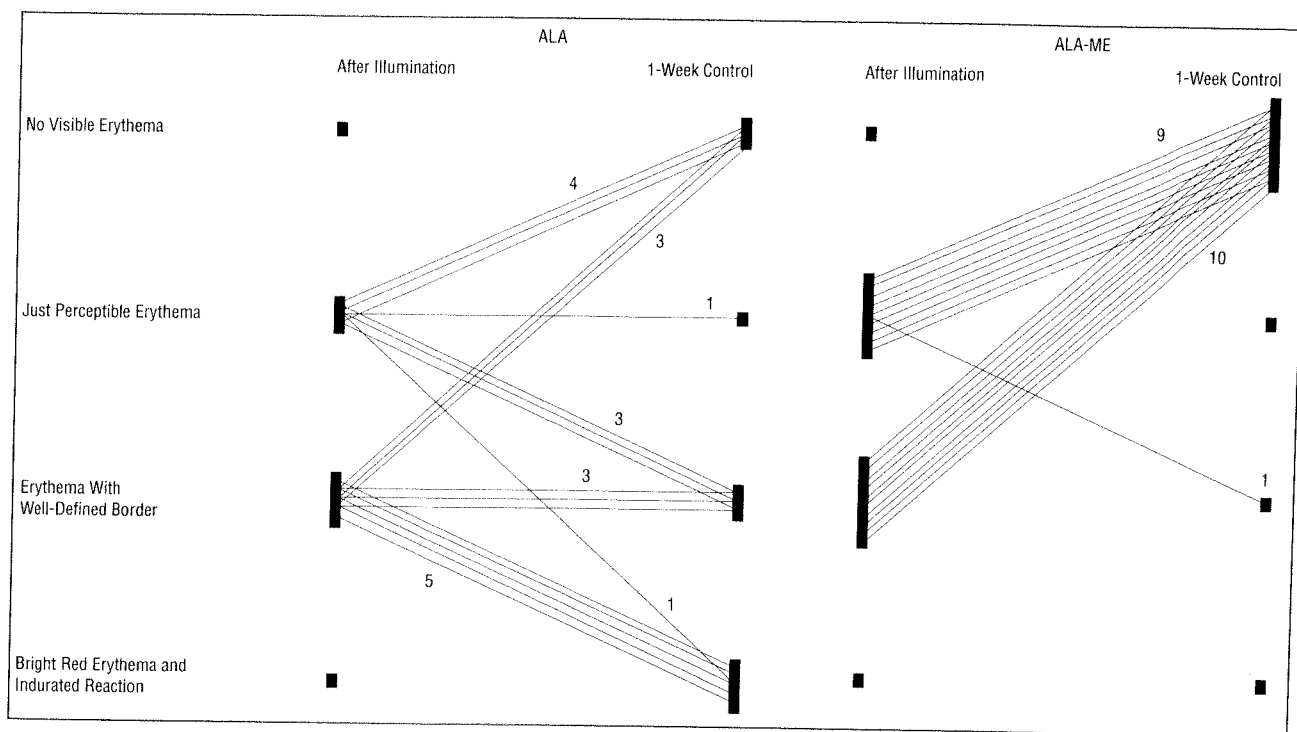
Variable	Immediately After PDT		1 Week After PDT	
	ALA	ALA-ME	ALA	ALA-ME
No visible erythema	0	0	7	19
Just perceptible erythema	8	11	1	0
Erythema with well-defined border	12	9	6	1
Bright red erythema and indurated reaction	0	0	6	0
No pigmentation	NE	NE	2	7
Homogeneous pigmentation	NE	NE	4	1
Follicular pigmentation	NE	NE	1	11
Erythema and pigmentation	NE	NE	13	1

Abbreviations: ALA, 5-aminolevulinic acid; ALA-ME, 5-aminolevulinic acid methylester; NE, not evaluated immediately after PDT; PDT, photodynamic therapy.

treated areas ($P < .001$ and $P = .001$, respectively). One week after treatment all treated skin areas were significantly more pigmented than before PDT ($P < .001$ for both ALA and ALA-ME), and the ALA-treated areas were more pigmented than the ALA-ME areas ($P = .045$). We did not find any significant correlation between pain scores reported during ($P = .95$ [ALA], $P = .42$ [ALA-ME]) and immediately after illumination ($P = .71$ [ALA], $P = .87$ [ALA-ME]) and the percentage of erythema measured just after illumination. We also found no significant correlation between pain scores reported during ($P = .85$ [ALA], $P = .38$ [ALA-ME]) and immediately after illumination ($P = .34$ [ALA], $P = .14$ [ALA-ME]) and the increase in the percentage of skin pigmentation 1 week after PDT. There was no significant correlation between the decrease of the peak PpIX fluorescence during illumination and the percentage of erythema ($P = .28$ [ALA], $P = .09$ [ALA-ME]) and pigmentation ($P = .98$ [ALA], $P = .05$ [ALA-ME]) immediately after illumination and percentage of erythema ($P = .72$ [ALA], $P = .05$ [ALA-ME]) and percentage of pigmentation ($P = .48$ [ALA], $P = .33$ [ALA-ME]) 1 week after PDT.

VISUAL EVALUATION OF THE TREATED AREAS

The results of the visual evaluation are given in **Table 3**. Most volunteers reported that the ALA-treated area had developed a much stronger reaction than the ALA-ME



Visual evaluated degree of erythema in 5-aminolevulinic acid (ALA)- and 5-aminolevulinic acid methylester (ALA-ME)-treated areas in 20 volunteers. Lines connect the erythema immediately after illumination and at the 1-week control (numbers indicating the numbers of volunteers).

area, and the **Figure** shows that 65% of the ALA-treated areas and only 5% of the ALA-ME-treated areas had sustained or developed a more severe degree of erythema 1 week after illumination.

COMMENT

Our study shows that under the variables studied, ALA-PDT is significantly more painful than ALA-ME-PDT on normal tape-stripped skin. All our volunteers reported mild to severe pain during PDT, and the pain decreased immediately after the illumination was terminated. The difference in the pain scores between the 2 creams might be explained by the way ALA and ALA-ME are transported through a cell membrane.¹¹ 5-Aminolevulinic acid has a structure that is similar to the β -amino acids, β -alanine, taurine, and γ -aminobutyric acid (GABA), and might be transported into a cell by the same carrier systems as these amino acids and neurotransmitters. In vitro studies have shown that ALA-ME is transported into the cells by other systems than ALA. Unlike ALA, ALA-ME-induced PpIX is not inhibited by β -alanine, and ALA-ME does not inhibit the transport of ALA.¹¹ Recent studies¹⁵ have shown that ALA-ME might be taken up by more than one active transport mechanism. The relatively greater uptake of ALA into nerve cells via GABA receptors may explain the increased pain experienced by patients during ALA-PDT vs ALA-ME-PDT.

For practical reasons, the study was performed using healthy volunteers with normal skin. The results found in this study may only be valid for pain generated in tape-stripped normal skin and not in diseased skin. During clinical PDT, adjacent normal skin will inevitably be included in the treatment area. It is not possible to differ-

entiate between pain caused by treatment of lesion or adjacent normal skin, which also makes our use of normal skin relevant.

Although the vehicles of ALA and ALA-ME were not identical, the molar equivalents were similar, and any difference in epidermal uptake should have been further minimized by removal of the stratum corneum by tape stripping. The purpose of the study was to compare pain associated with ALA-PDT using the ALA cream routinely used in clinical practice with ALA-ME-PDT using the new commercial cream Metvix. ALA-PDT is performed in a hydroalcoholic solution in the United States, and the results of our study might be different when this solution is used instead of a cream base.

Most volunteers attributed the pain to the heat produced by the lamp. A study by Orenstein and colleagues³ showed that irradiation of normal skin without ALA application was not accompanied by any pain even when the temperature was 44°C to 45°C. Thus, the pain in our study is probably not due to the heat of the lamp. However, as Orenstein et al concluded, if hyperthermia increases tissue damage during ALA-PDT due to enhanced photochemical processes, it could also influence the intensity of pain. This may in part explain why the pain decreased when the illumination was terminated.

By fluorescence measurements, ALA-PDT induced more PpIX in the treatment areas than ALA-ME-PDT. This was not expected since ALA is a hydrophilic molecule, which has limited penetration through the stratum corneum. Therefore, the PpIX formation is often restricted to the superficial layers of the skin. Lipophilic derivatives of ALA, such as ALA-ME, have better diffusing properties and are expected to give a higher PpIX formation.

In a study by Fritsch et al,¹⁰ the PpIX fluorescence in solar keratoses and adjacent normal skin was measured after application of ALA or ALA-ME. They found that ALA induces more PpIX in both the solar keratoses and normal skin than ALA-ME, but ALA-ME more specifically led to PpIX enrichment in the lesion skin.

The use of sun-exposed skin and injury of stratum corneum by tape stripping before application of the cream may not be sufficient to simulate diseased skin. The tape stripping of the skin reduces the penetration barrier, especially for the hydrophilic ALA, and might enhance the formation of PpIX. The difference in the PpIX fluorescence induced by the 2 creams in our study might be explained by the fact that we used healthy volunteers with normal skin to which ALA-ME especially has a lower affinity than diseased skin. The increased PpIX fluorescence may in part explain why ALA-treated areas had sustained or developed a more severe degree of erythema 1 week after illumination and were significantly more pigmented than the ALA-ME-treated areas, since activation of a large amount of PpIX will induce more cell damage during illumination and with that more erythema and postinflammatory pigmentation.

We did not find any correlation between pain and PpIX intensity or decrease in PpIX fluorescence after illumination. We also did not find a correlation between pain and erythema or pigmentation. These calculations were performed by comparing pain among the volunteers and not only between the 2 arms of a volunteer. This may result in higher data discrepancy due to individual differences in pain perception. If we discount the possible difference in pain perception, the result supports the theory that at least some of the pain is caused by other mechanisms than inflammation and cell death, caused by PpIX activation during illumination.¹⁶

Our results show that ALA-ME-PDT is less painful than ALA-PDT performed on normal skin. Since ALA-ME induced less PpIX and resulted in fewer skin reactions after PDT, further studies in diseased skin should be performed to ensure that ALA-ME-PDT is as effective as ALA-PDT.

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