Temperature-Modulated Photodynamic Therapy for the Treatment of Actinic Keratosis on the Extremities: A Pilot Study

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BACKGROUND The efficacy of photodynamic therapy (PDT) using topical 5-aminolevulinic acid (ALA) for the treatment of actinic keratosis (AKs) is lower on the distal extremities compared with the head and neck areas. The strong temperature dependence of porphyrin synthesis in biologic tissue suggests that heating skin during incubation may improve the efficacy of PDT, particularly in areas where biologic temperatures are naturally lower. The aim of this study was to evaluate the efficacy and tolerability of temperature-modulated PDT for the treatment of AKs on the extremities.

METHODS In this IRB-approved, single-center study, the upper or lower extremities of 20 subjects were treated with 20% ALA under occlusion, followed by 10 J/cm^2, 417-nm blue light. One of the 2 extremities treated was heated during the 1-hour incubation. Outcome measures included lesion counts, tolerability, and global improvement at baseline, 1 week, and 2 and 6 months after treatment.

RESULTS The median temperatures of the heated and control sides were 38.8°C and 29.4°C, respectively. The median clearance for the heated side was significantly greater than the control side at 2 and 6 months ($p < .0001$). Typical PDT side effects were greater on the heated side compared with the control yet were well tolerated by all subjects.

CONCLUSION Warming the skin during incubation of ALA seems to improve the efficacy of PDT in the treatment of AKs on the extremities and is well tolerated when heat application is controlled within the limits of safety.

The efficacy of cutaneous photodynamic therapy (PDT) using topical 5-aminolevulinic acid (ALA) in the treatment of AK has been demonstrated in a large number of clinical trials.[1–10] Emerging literature supporting long-term response rates underscores the potential benefits of PDT in the management of nonmelanoma skin cancer.[11–17] Photodynamic therapy is particularly advantageous for the treatment of large surface areas and is especially suitable for the treatment of multiple actinic keratoses (AKs) and areas of field cancerization.[18,19] Published studies have demonstrated high clearance rates with multiple topical PDT regimens that are comparable with other topical therapies used for the treatment of AKs, particularly on the face and scalp.[8,9,20] However, the efficacy of PDT on the extremities is greatly reduced.[1,2,10,21,22] The strong relationship between temperature and porphyrin synthesis in biologic tissue[23–28] suggests that increasing the temperature of the skin during the incubation of ALA may improve the efficacy of PDT, particularly for areas that are naturally lower in temperature such as the distal extremities.

5-aminolevulinic acid is a precursor drug, whereas its metabolite, protoporphyrin IX (PpIX), is the active

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photosensitizer. Like many metabolic pathways in the body, the conversion of ALA can be modulated by temperature. The aim of this study was to evaluate the efficacy and tolerability of temperature-modulated PDT for the treatment of AKs on the distal extremities.

Methods

Preliminary Determination of Skin Temperature Exposed to Heating Pad in a Single Individual

The temperature of lower extremity skin on a healthy individual with normal vascular tone was measured by a single investigator (A.W.) during a 1-hour exposure to a typical heating pad to determine the appropriate control setting for use during the incubation of ALA. The ambient temperature was recorded as measured with an Acurite temperature monitor (Model 00325; Chaney Instrument Co., Lake Geneva, WI) that measures a range of 0°C to 50°C at 16% to 99% of relative humidity. The skin surface temperature was measured with a calibrated digital thermocouple meter (Traceable model 4233CP; Cole–Parmer, Vernon Hills, IL) with a Type K probe measuring a range of −50°C to 750°C with an accuracy of ±1°C between 0°C and 500°C. The thermocouple probe was taped to the anterior surface of the low leg, which was then wrapped with clear plastic wrap (Saran; SC Johnson, Racine, WI). The extremity was heated by applying a covered Underwriters Laboratories (UL)–tested heating pad (Sunbeam with UltraHeat Technology; Jarden Consumer Solution, Boca Raton, FL). The skin temperature was measured at 1-minute intervals for a duration of 1 hour for each control setting of "low," "medium," and "high." The skin was allowed to return to baseline temperature before measurement at each setting.

The ambient temperature measured 20°C and 65% of relative humidity. The baseline skin temperature of the extremity measured 32°C. When the heat pad was set on "low," the skin measured 35°C at 1 minute and reached a maximum of 38°C at 15 minutes. At a setting of "medium," the skin measured 36°C at 1 minute and a maximum of 39°C at 14 minutes. At a setting of "high," the skin measured 37°C at 1 minute and a maximum of 42°C at 14 minutes. Subjectively, the heating pad was tolerated well on "low" and "medium"; however, it became uncomfortably warm after a few minutes when set on "high," which correlated at a temperature of 40°C. Based on these findings, the setting of "medium" was chosen as for the study in clinical subjects.

Enrolment Criteria

Subjects older than 18 years with at least 10 AK lesions on their arms or legs were enrolled in this IRB-approved, single-center study, conducted by a single investigator (A.W.) at a single center in Fairfield, California. Informed consent was obtained in compliance with the ethical guidelines of the 1975 Declaration of Helsinki and HIPPA regulations.[29,30] Actinic keratosis lesion areas must not have been treated for at least 1 year before enrolment. Subjects were excluded if they met the following criteria during the study period: pregnancy, known history of photosensitivity, sensitivity to ALA or vehicle components, tanning bed exposure, treatment with systemic immunosuppressant, or retinoid medications.

Treatment Protocol

Areas to be treated were swabbed with acetone and gauze and allowed to dry. Topical 20% 5-ALA (Levulan Kerastick; Dusa Pharmaceuticals, Inc., Wilmington, MA) was applied to the entire forearm skin and occluded with plastic wrap (Saran; SC Johnson). One stick (1.5 mL) was used to cover each distal extremity. A covered UL-tested heating pad (Sunbeam with UltraHeat Technology; Jarden Consumer Solution, Boca Raton, FL) set at "medium" was wrapped around 1 randomly selected extremity (right or left) (Figure 1) during the 1-hour incubation. The contralateral extremity (control) was prepared in the same manner, wrapped in plastic, and incubated at room temperature. Control and preheated extremities were randomly assigned by alternating sides at the time of enrolment. After 1 hour, both of the treated sites (heated and control) were irradiated with 10 J/cm^2 blue light (BLU-U; Dusa Pharmaceuticals, Inc.) for 1,000 seconds with the light positioned 2 to 4 inches from the skin surface. During light irradiation, the treated areas were cooled by a small portable fan set on a mayo

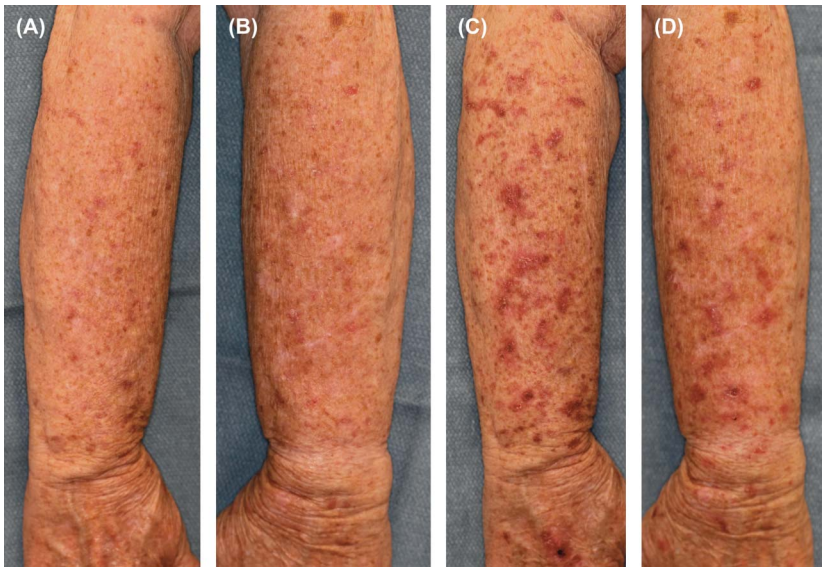

Figure 1. (A) Right arm of Subject 3 at baseline. (B) Left arm of Subject 3 at baseline. (C) Right arm of Subject 3 one week after PDT (heated). Note the increased PDT reaction on heated side compared with control at 1 week, despite similar baseline lesion counts. (D) Left arm of Subject 3 one week after PDT (control).

stand for comfort. The skin temperature was measured through the plastic wrap at baseline and at 15-minute intervals throughout the incubation period (by quickly lifting up the heating pad and replacing it) using an infrared noncontact thermometer (Raytek Minitemp MT4; Fluke Corporation, Everett, WA) that measures temperatures between −18°C and 275°C with an accuracy of ±2°C at ambient temperatures of 0°C and 50°C. Lesions were counted at baseline and at 2 and 6 months after treatment by a single investigator (A.W.). Significant differences in lesion counts were determined by Friedman test and the Wilcoxon signed-rank test using $p = .05$ as the cutoff level.

Standardized photographs of each extremity were taken with a high-resolution digital SLR camera (EOS 5D Mark II; Canon Inc., Tokyo, Japan) with a 21.1 megapixel CMOS sensor, EF 100 mm f/2.8 USM macro lens, and MR-14EX macro ring flash. Global changes in the treatment area were evaluated at baseline, 1 week, and 2 and 6 months by an investigator (F.H.S.) who was blinded to treatment assignments assessing photographs viewed on a 27-inch monitor with 2,560 × 1,440 pixels and RBG color mode (iMac; Apple Inc., Cupertino, CA). For quantitative analysis, photographs were analyzed for the measurement of affected area using the WCIF-ImageJ 1.44 software (Wayne Rasband, National Institutes of Health). To increase the accuracy of photographic analysis, for each image, the treated field was measured and each apparent AK was manually marked by the blinded evaluator (F.H.S.) and measured. Size variations due to slight change in positions of photographs were minimized by collecting a value relative to the treated area. For every photograph, the total area affected was compared with its baseline. Statistical analysis was performed using 2-factor ANOVA to explore the differences in affected area at 1 week, 2 months, and 6 months compared with baseline, and Bonferroni post hoc analysis was used to locate the difference when ANOVA demonstrated a significant interaction. In all evaluations, a significant difference was accepted at a value of $p < .05$.

Adverse effects, including erythema, edema, stinging/burning, blisters/crusting, hyperpigmentation, and hypopigmentation were graded on a scale of 1 to 4 (1 = mild and 4 = severe) and assessed immediately after treatment, 5 minutes, 1 week, 2 months, and 6 months after treatment. At the 6-month follow-up visit, subjects evaluated overall treatment satisfaction and acceptability of treatment time, adverse effects, and duration of adverse effects.

Results

Twenty subjects aged 57 to 90 years (median, 70) were enrolled in this study between June 22, 2010 and June

15, 2011. Subjects included 5 women and 15 men with skin Type II. Areas treated included the low legs of 3 subjects and the forearms and dorsal hands of 24 subjects. All 20 subjects completed the 6-month study.

The temperature of the heated side during the 1-hour incubation period ranged from 36.4°C to 40.6°C (median, 38.8°C). The temperature of the control side (averaged more than the 1-hour incubation period) ranged from 27.7°C to 33.4°C (median, 29.4°C). Throughout the 1 hour incubation period, the heated side increased in temperature on an average of 9.4°C, whereas the control side increased in temperature on an average of 1.4°C.

The median lesion counts and percent differences at baseline and 2 and 6 months are summarized in Table 1. Lesion counts ranged from 6 to 170 on the heated side and 4 to 105 on the control side. The median baseline lesion count on the heated side (29.5) was not significantly different ($p = .9843$) from the median baseline lesion count for the control side (32.0). Compared with baseline, the median lesion counts for both the heated side and the control side was significantly lower at 2 months ($p < .0001$) and at 6 months ($p < .0001$). In comparing the reduction in lesion counts on the heated versus the control side, the median difference from baseline (%) on the heated side was significantly greater than the median difference from baseline (%) on the control side at 2 months ($p < .0001$) and at 6 months ($p < .0001$). The median counts at 2 months and at 6 months did not differ significantly ($p = .5195$), indicating that the PDT-heated treatment effect persisted throughout the follow-up period. Clinical photographs of a typical subject at baseline, 1 week, and 6 months are presented in Figures 1 and 2.

Blinded evaluation of standardized photographs assessing global changes in treatment effect demonstrated a significant difference in the area affected by treatment on the heated side compared with the control side, consistent with an increased PDT reaction on the heated side at 1 week compared with baseline. The global differences in the area affected by treatment in the heated versus control side were significant at 2 months when compared with baseline but not at 6 months.

Adverse Effects

The treatment was well tolerated by all subjects. Adverse effects of the heated and control sides were graded on a scale of 1 to 4 (1 = mild and 4 = severe). Median scores at baseline compared with scores at 0 minutes, 5 minutes, and 1 week after treatment are shown in Table 2.

Significant differences in erythema and stinging/burning were seen on the heated versus control side 5 minutes after PDT ($p = .0107$ and .0039, respectively). At 1 week after PDT, significant differences were seen in erythema and oozing/crusting ($p = .0010$ and .0039, respectively). There were no significant differences in dyspigmentation, scaling, or edema between the heated and control side at any time point.

TABLE 1. Median Lesion Counts and Percent Differences From Baseline at 2 Months and 6 Months

*Median Lesion Counts**						*†Percent Difference‡*			
Heated			*Control*			*2 Months*		*6 Months*	
Baseline	*2 Months*	*6 Months*	*Baseline*	*2 Months*	*6 Months*	*Heated*	*Control*	*Heated*	*Control*
§29.5 (26.8)	§4.0 (5.0)	§4.0 (3.6)	§32.0 (35.0)	§9.5 (10.3)	§7.5 (9.6)	§88.0 (14.8)	§70.5 (28.9)	§88.0 (12.8)	§67.5 (28.8)
$p < .0001$			$p < .0001$			$p < .0001$		$p < .0001$	

*Friedman test.
†([Baseline count − 2-month or 6-month count]/baseline count) × (100).
‡Wilcoxon signed-rank test.
§Median (IQR); IQR, interquartile range, a measure of dispersion; IQR = 75th percentile − 25th percentile.

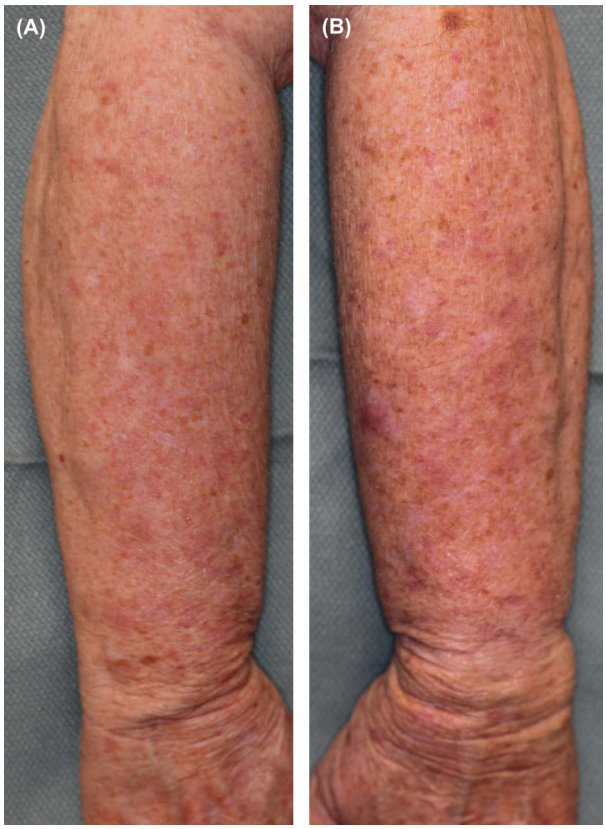

Figure 2. (A) Right arm of Subject 3 six months after PDT (heated). Note increased clearance of AKs on the heated side compared with control. (B) Left arm of Subject 3 six months after PDT (control). Note early recurrence of AK lesions.

The median subject satisfaction score at 6 months was slightly higher in the control side (2 vs 1), but the difference did not achieve significance (p = .0781). Treatment time was acceptable to all 20 subjects who responded. When asked if adverse effects and side effects were acceptable, 18 subjects agreed totally, 1 subject agreed a little, and 1 subject neither agreed nor disagreed. Duration of side effects was acceptable to 18 subjects (totally agree), whereas 2 subjects agreed a little.

One subject developed significant erythema and blisters on both arms (score of 4 on the heated side and 3 on the control side). The blisters resolved with the use of emollients for 2 weeks after treatment. Lesion clearance was near complete at 2 and 6 months after treatment. This subject graded the overall satisfaction with treatment as "excellent," and graded acceptability of side effects as "neither acceptable nor unacceptable" (neutral), and the duration of side effects and the time required for treatment as "totally acceptable." Review of the medical history revealed a long history of iron deficiency, thyroid disease, hypertension, and arthritis. Medications included levothyroxine, hydrochlorothiazide, and diclofenac, which were started 4 days before PDT.

Another subject demonstrated a marked reduction in hair (more prominent on the heated side) 1 week after treatment, which regrew by the 2-month follow-up visit. Although not prospectively evaluated nor graded, subtle reversible hair loss was also noted in the photographs of other subjects.

Discussion

It is no surprise that the synthesis of porphyrins in the heme biosynthetic pathway is a temperature-dependent process. Indeed, biologic processes are highly temperature dependent, with enzymes functioning at ideal temperatures that govern the rate at which chemical reactions occur to support the "biochemistry of life." In general, for a 10°C change in temperature, the metabolic rate in the human body will change by a factor of 2 to 3, increasing with elevated temperatures and decreasing at lowered temperatures. Yet, the strong temperature dependence of porphyrin production and its clinical relevance has only recently been recognized.[23–28]

In 1999, using fluorescence spectroscopy, Moan and colleagues demonstrated that the production of protoporphyrin IX (PpIX) increased nearly twofold when the temperature of normal murine skin in vivo was increased from 37°C to 42°C after a 3-hour incubation of 20% ALA and was increased fourfold after a 6-hour incubation.[23] Although PpIX concentration peaked after 3 hours, the majority of PpIX was produced after 1 to 2 hours. Importantly, no PpIX was produced at 12°C. This group then demonstrated in WiDr cells and in murine skin a twofold to threefold increase in PpIX production at 36°C to 7°C compared with 28°C to 32°C, concluding that conversion of ALA to PpIX is significantly reduced at 30°C.[23–25] Protoporphyrin IX fluorescence was detectible 6 to 10 minutes after incubation of ALA at 37°C.[25] Further study of human forearm skin in vivo demonstrated a 50% increase in PpIX production when the

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