Photodynamic therapy using chlorophyll-a in the treatment of acne vulgaris: A randomized, single-blind, split-face study

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Background: Chlorophyll-a is a novel photosensitizer recently tested for the treatment of acne vulgaris.

Objective: We sought to evaluate the clinical efficacy and safety of chlorophyll-a photodynamic therapy used for acne treatment.

Methods: Subjects with acne on both sides of the face were included. Eight treatment sessions were performed over a 4-week duration. Half of the face was irradiated using a blue and red light-emitting diode after topical application of chlorophyll-lipoid complex. The other half underwent only light-emitting diode phototherapy. The lesion counts and acne severity were assessed by a blinded examiner. Sebum secretion, safety, and histologic changes were also evaluated.

Results: In total, 24 subjects completed the study. Facial acne improved on both treated sides. On the chlorophyll-a photodynamic therapy-treated side, there were significant reductions in acne lesion counts, acne severity grades, and sebum levels compared with the side treated with light-emitting diode phototherapy alone. The side effects were tolerable in all the cases.

Limitations: All the subjects were of Asian descent with darker skin types, which may limit the generalizability of the study. A chlorophyll-a arm alone is absent, as is a no-treatment arm.

Conclusions: We suggest that chlorophyll-a photodynamic therapy for the treatment of acne vulgaris can be effective and safe with minimal side effects. (J Am Acad Dermatol 2014;71:764-71.)

Key words: acne vulgaris; chlorophyll-a; light-emitting diode; photodynamic therapy; photosensitizer; treatment.

Acne represents one of the most common skin diseases, affects nearly all individuals aged 15 to 17 years, and often persists into adulthood. Conventional treatments such as antibiotics and retinoids may be associated with microbial resistance, irritation, and systemic adverse effects such as hyperlipidemia and teratogenicity. Photodynamic therapy (PDT) has been developed as a treatment alternative for the treatment of acne, and provides potential benefits including better patient compliance and less risk of bacterial resistance. PDT is a therapeutic modality that uses oxygen-dependent photochemical reactions involving a photosensitizer.

Abbreviations used:
ALA: 5-aminolevulinic acid
LED: light-emitting diode
MAL: methyl aminolevulinate
PDT: photodynamic therapy

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and activating light. The most commonly used photosensitizers for the treatment of acne are 5-aminolevulinic acid (ALA) and methyl aminolevulinate (MAL), which can lead to substantial improvement in inflammatory acne by 50% to 80%. However, PDT using ALA or MAL requires strict sun avoidance up to 48 hours, and is associated with common side effects such as pain and inflammatory skin reactions. Chlorophyll-a has recently been used as a novel photosensitizer for PDT, and a pilot study reported that chlorophyll-a PDT showed mild improvement in 4 subjects with acne vulgaris after 3 sessions over 1 month. With appropriate light illumination, chlorophyll can produce various reactive oxygen species leading to antimicrobial activity and tissue damage. As chlorophyll-a has inherent photosensitizing and rapid photodegrading characteristics that may offer several advantages over ALA- or MAL-PDT, we compared chlorophyll-a PDT (activated by light-emitting diode [LED] illumination) with LED phototherapy alone to analyze its efficacy and safety for the treatment of acne vulgaris, via a randomized, single-blind, split-face study.

**METHODS**

**Subjects**

The study was approved by the institutional review board of Hallym University Sacred Heart Hospital, Anyang, Korea, and informed consent was obtained from each subject. The inclusion criteria were an age of 18 to 35 years, acne on both sides of the face (equivalent severity between the 2 sides), and the ability to comply with the study protocol. The exclusion criteria were as follows:

- Use of any topical acne treatment or systemic antibiotics within 6 weeks before study initiation.
- Use of a systemic retinoid within 9 months before study initiation.
- Use of a systemic steroid within 9 months before study initiation.
- History of photosensitivity.
- Recent use of photosensitizing drugs within 6 weeks before study initiation.
- Presence of any other skin disease that could interfere with the assessment of the acne, such as folliculitis or rosacea.
- Presence of any other systemic disease that could affect the acne severity by its presence, such as polycystic ovarian syndrome, or by any medication prescribed for the treatment of the systemic diseases.
- Presence of any change in the use of oral contraceptive pills or anti-inflammatory drugs within 3 months before study initiation.
- Pregnancy or lactation.
- Presence of evidence indicating likely poor compliance with the protocol.

**Materials**

The irradiating device was an LED illuminator (Biolight LT-560, Beautech, Seoul, Korea), of which the treatment heads delivered symmetric peak wavelengths of 430 ± 10 nm for blue light and 660 ± 10 nm for red light. The average irradiance was 600 mW/cm² for blue light and 650 mW/cm² for red light. The radiant fluencies, or doses, during a single treatment of 30 minutes were 1170 and 1080 J/cm² for the blue and red treatment heads, respectively. Chlorophyll-a (Virta-Healer, Aseptica, Moscow, Russia), used as the photosensitizer, is a chlorophyll-lipoid complex derived from the extract of *Spirulina* algae, and consists of chlorophyll-a, carotenoids, lipids, and proteins.

**Treatment**

Subjects were asked to remove sebum from the face by washing with a cleanser (Neutrogena Sensitive Skin Solutions Foam Cleanser, Johnson & Johnson, Los Angeles, CA) including sodium lauryl sulfate, polysorbate, and butylene glycol. According to a predetermined randomization table using a random permuted block method, one side of the face received chlorophyll-a PDT, whereas the other side underwent LED phototherapy as a control. More specifically, sheets of chlorophyll-a were uniformly applied on only one side of the face and incubated without occlusion for 30 minutes, followed by combined red and blue LED irradiation evenly on both sides of the face. The light source was maintained at 5 cm from the nose tip for 30 minutes. A wet dressing of gauze soaked in cooled normal saline was provided for 10 minutes after the irradiation. Subjects were treated in the late afternoon, and instructed to avoid sun exposure until the following morning's
sunlight. There was no restriction placed with respect to other forms of ambient lighting. Subjects were treated at 3- to 4-day intervals (twice per week) for a total of 8 sessions over 4 weeks.

**Assessment**

Subjects were evaluated at 6 time points over the course of the 6-week study period (a 4-week treatment period followed by 2 weeks of follow-up): a baseline visit; follow-up visits after the second (week 1), fourth (week 2), sixth (week 3), and eighth (week 4) sessions; and a final visit 2 weeks after completing the treatment (week 6). Facial photographs were taken at each visit. Assessments were conducted by evaluating the acne severity based on the Cunliffe grading system and acne lesion counts (closed/open comedone, papule, pustule, and nodule or cyst) by a dermatologist who was blinded to the treatment received. Sebum was collected from both cheeks using Sebometer (SM815, Courage+Khazaka Electronic GmbH, Germany) for 30 seconds. For histologic analysis, 2-mm punch biopsies were performed on the cheek of the chlorophyll-a PDT–treated side at baseline and 2 weeks after the final treatment (n = 11), and subjected to routine hematoxylin-eosin staining. Subjects were asked to report any side effects such as burning sensations, pain, and pruritus, and examined by a dermatologist for any adverse events such as aggravation of the acne lesions, erythema, and pigmentation changes at each visit.

**Statistical analysis**

Data were analyzed using software (SPSS for Windows, IBM Corp, Armonk, NY). A paired t test was used to evaluate the significance of the changes in the treatment outcomes during the study. Student t test was used to compare the difference in the effect between the 2 treatments. A P value of less than .05 was considered statistically significant.

**RESULTS**

**Subject characteristics**

See Table 1. Among the 33 subjects screened for eligibility, 24 subjects were enrolled and completed the current study. The mean age of the subjects was 23.4 ± 3.5 years (18-32 years). At the baseline visit, there was no significant difference in acne severity between the 2 sides of the subjects’ faces (P = .821).

**Acne severity**

Representative photographs are shown in Fig 1. A significant improvement in acne severity was observed not only at 1 week after chlorophyll-a PDT (from 3.1 at the baseline to 2.9 at week 1; P = .022) but also at 2 weeks after LED phototherapy alone (control) (from 3.1 at the baseline to 2.6 at week 2; P = .005) (Fig 2). At week 6, the mean acne grade of the sides treated with chlorophyll-a PDT and control LED were 1.8 and 2.2, respectively. The improvement in the acne severity was significantly greater on the chlorophyll-a PDT–treated side compared with the control LED phototherapy–treated side at week 4 (P = .027) and week 6 (P = .022).

**Acne lesion counts**

**Comedones.** Comedone counts were significantly decreased in both sides after 2 weeks. Although LED phototherapy was associated with a considerable improvement of closed comedone counts from 18.4 at the baseline to 13.3 at week 6 (P < .001), chlorophyll-a PDT produced a significantly greater improvement from 18.4 at the baseline to 8.5 at week 6 (P < .001) (P = .033 and P = .014 between the 2 treatments at weeks 4 and 6, respectively) (Fig 3, A). For open comedones, the chlorophyll-a PDT–treated sides showed a significant decrease in lesion counts from 9.0 at the baseline to 4.2 at week 6 (P < .001), whereas those treated by LED alone exhibited decreased lesion counts from 9.1 at the baseline to 6.7 at week 6 (P < .001) (P = .046 and P = .011 between the 2 treatments at weeks 4 and 6, respectively) (Fig 3, B).

**Papules and pustules.** For papule counts, chlorophyll-a PDT led to a significant improvement from 13.0 at the baseline to 5.1 at week 6 (P < .001), whereas LED phototherapy demonstrated a decrease in lesion counts from 13.1 at the baseline to 8.6 at week 6 (P < .001) (P = .047 and P = .030 between the 2 treatments at weeks 4 and 6, respectively) (Fig 3, C).

### Table 1. Demographic data of the subjects

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Age, y</th>
<th>Sex</th>
<th>Fitzpatrick skin type</th>
<th>Acne severity (baseline)</th>
<th>Chlorophyll-PDT side</th>
<th>Phototherapy only side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2</td>
<td>3 (12.5%)</td>
<td>4 (16.7%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>15 (62.5%)</td>
<td>14 (58.3%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>6 (25%)</td>
<td>6 (25%)</td>
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</tbody>
</table>

*PDT, Photodynamic therapy.*

*Equivalent between the 2 sides of the face (P = .821).*
For pustule counts, chlorophyll-a PDT demonstrated a remarkable improvement from 3.8 at the baseline to 1.3 at week 6 (66% improvement; \( P < .001 \)), whereas LED phototherapy resulted in an improvement from 4.2 at the baseline to 3.0 at week 6 (29% improvement; \( P < .001 \)) between the 2 treatments at weeks 2, 3, 4, and 6, respectively (Fig 3, D).

**Nodules and cysts.** In nodule and cyst counts, chlorophyll-a PDT–treated sides demonstrated a
78% improvement at week 4 (\(P = .024\) vs baseline), and its efficacy was sustained until week 6, whereas LED phototherapy treated sides demonstrated a 69% improvement at week 4 (\(P = .026\) vs baseline), but showed an exacerbation at week 6 (\(P = .082\) vs baseline) (Fig 3, E).

There were statistically significant reductions of variable lesion counts after chlorophyll-a PDT compared with control LED phototherapy with regard to comedone and papule counts from week 4, and pustule counts from week 2. However, there was no statistically significant difference in nodule and cyst counts between 2 modalities (Fig 3, E).

**Sebum levels**

The sebum levels were significantly decreased as early as week 2 on the chlorophyll-a PDT treated side. Although the control LED phototherapy-treated side showed a significant decrease from week 4, there was a significant difference in the sebum levels between the 2 treatments at weeks 4 (\(P < .001\)) and 6 (\(P < .001\)) (Fig 4).

**Histopathological findings**

Before treatment, there was horny plugging of hair follicles and moderate infiltration of lymphocytes, histiocytes, and neutrophils around skin appendages such as hair follicles, sebaceous glands, and blood vessels in the dermis (Fig 5, A and C). The biopsy specimens taken after the treatment completion showed a marked improvement characterized by a decrease of dermal pilosebaceous units and perivascular inflammatory cell infiltrates, but showed an increase of normal-looking epidermis (Fig 5, B and D).

**Side effects**

The side effects of the treatments were tolerable in all cases. No reports of pain, burning, or itching sensations were recorded, and no eschar, transient aggravation of acne, or postinflammatory hyperpigmentation was observed.

**DISCUSSION**

Acne is an inflammatory disease caused by excess sebum production and colonization of the anaerobic bacterium *Propionibacterium acnes*.\(^2,4\) *P acnes* intrinsically synthesizes large amounts of porphyrin, which maximally absorb wavelengths of 400 to 430 nm (blue light).\(^22-24\) These shorter wavelengths of light can effectively target shallower structures, but cannot penetrate into a sufficient depth in the skin for clinical efficacy. Although red light (660 nm) targets deeper structures\(^8\) it has less effective photodynamic action on porphyrin.\(^25\) Moreover, red light inhibits sebum production,\(^26\) and demonstrates greater anti-inflammatory properties by affecting cytokine secretion from macrophages, such as fibroblast growth factor.\(^27,28\)

Previous studies indicated that a combination of blue and red light phototherapy is effective in the treatment of acne vulgaris, showing a significant reduction in lesion counts by around 80% at 8 weeks after 8 sessions.\(^29,30\) The absorption peaks of chlorophyll-a are 430 nm and 662 nm,\(^31\) and our study used a combination of blue and red light to maximize the efficacy of chlorophyll-a PDT. In addition to chlorophyll-a PDT, control LED phototherapy was performed using the same light source and fluence in a randomized split-face manner.

Previously, a single pilot study investigated the efficacy of chlorophyll PDT in 4 subjects with acne.\(^18\) Although sebum production was not quantified using sebometry in that study, an actual decrease in the number of acne lesions was observed, and the subjects were satisfied with their treatment.\(^18\) In the current study, chlorophyll-a PDT showed greater improvement in clinical scores, sebum levels, and histologic architecture as compared with LED phototherapy alone, and could be a convenient treatment alternative for subjects who are refractory or who have contraindications to isotretinoin treatment. Histologic changes occurring after chlorophyll-a PDT, including a substantial reduction of pilosebaceous units and dermal inflammatory cell infiltrates, and an increase in normal-looking epidermis, are similar to those from a previous ALA-PDT study.\(^11\) Sustained reductions of pilosebaceous units might at least in part explain decreased sebum secretions which, accompanied by resolution of microscopic inflammation, are thought to contribute to the superior clinical outcomes in chlorophyll-a PDT-treated side.

Although the mechanism of action involved in chlorophyll PDT has not been fully elucidated,
light-activated chlorophyll can produce reactive intermediates such as superoxide and singlet oxygen, leading to direct tissue damage, inflammation, and inactivation of various micro-organisms.\textsuperscript{19,20,32} PDT using chlorophylls or chlorophyll derivatives has been used for the treatment of skin cancer\textsuperscript{33} and for lung cancer and hepatocellular carcinoma.\textsuperscript{34} Chlorophyll has several advantages over other photosensitizers such as ALA or MAL.\textsuperscript{18} First, chlorophyll loses its photosensitizing activity within a few hours. In the presence of visible light and oxygen, chlorophyll not only acts as an effective photosensitizer but also readily and irreversibly degrades into intermediate and end products such as methyl ethyl maleimide with no photosensitizing potential.\textsuperscript{21,31,35,36} Thus, it does not cause a phototoxic reaction when the treated skin is exposed to sunlight after a treatment session. Second, chlorophyll-a has inherent photosensitizing characteristics that can strongly absorb blue and red wavebands, and initiate photodynamic reactions without further metabolism,\textsuperscript{19,32} in contrast to porphyrin precursors such as ALA or MAL.\textsuperscript{5} Thus chlorophyll-a PDT requires a relatively short incubation time (30-60 minutes) compared with the time required with ALA or MAL (3-4 hours).\textsuperscript{8} These features of chlorophyll-a may contribute to the observed diminished frequency and severity of side effects compared with ALA-PDT and MAL-PDT. Third, chlorophyll is much less expensive than ALA; for example, the price of chlorophyll products is less than 10%-20% of that of ALA in Korea.\textsuperscript{18} Because acne is regarded as a chronic disease with a pattern of recurrence or relapse even after improvement,\textsuperscript{4} the cost-effectiveness of therapy is an important issue for selecting acne treatments.

After the completion of chlorophyll-a PDT, there was a significant reduction in the variable acne lesion counts, but not in the cystic and nodular lesion counts, compared with that after control LED phototherapy. Because the total numbers of cysts and nodules were low at the baseline, the range of decrease involving these types of lesions might appear to be smaller than those of comedones, papules, and pustules. It is also possible that the efficacy of chlorophyll-a PDT therapy is comparable
with that of LED phototherapy in such severe acne lesions. The effect of chlorophyll-a PDT in severe inflammatory lesions, including cysts and nodules, is therefore in need of further investigation.

Currently, the therapeutic parameters of chlorophyll-a PDT required for achieving optimal improvements in acne are not fully determined. Thus, we suggest future studies that explore the correlation between treatment effectiveness and treatment-related factors such as incubation time, irradiation time, and treatment intervals. Moreover, further studies that compare the efficacy and tolerability of chlorophyll-a PDT with ALA-PDT or MAL-PDT in the treatment of acne are recommended.

To our knowledge, this study is the first randomized controlled study to evaluate the efficacy and safety of chlorophyll-a PDT. The findings of this study suggest that chlorophyll-a PDT can be an effective and well-tolerated treatment modality for acne lesions.

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