Topical ALA-Photodynamic Therapy for the Treatment of Acne Vulgaris

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Topical aminolevulinic acid is converted into a potent photosensitizer, protoporphyrin, in human hair follicles and sebaceous glands. Photodynamic therapy with topical aminolevulinic acid was tested for the treatment of acne vulgaris, in an open-label prospective human study. Each of 22 subjects with acne on the back was treated in four sites with aminolevulinic acid plus red light, aminolevulinic acid alone, light alone, and untreated control. Half of the subjects were treated once; half were treated four times. Twenty percent topical aminolevulinic acid was applied with 3 h occlusion, and 150 J per cm^2 broad-band light (550–700 nm) was given. Sebum excretion rate and auto-fluorescence from follicular bacteria were measured before, and 2, 3, 10, and 20 wk after, treatment. Histologic changes and protoporphyrin synthesis in pilosebaceous units were observed from skin biopsies. Aminolevulinic acid plus red light caused a transient acne-like folliculitis. Sebum excretion was eliminated for several weeks, and decreased for 20 wk after photodynamic therapy; multiple treatments caused greater suppression of sebum. Bacterial porphyrin fluorescence was also suppressed by photodynamic therapy. On histology, sebaceous glands showed acute damage and were smaller 20 wk after photodynamic therapy. There was clinical and statistically significant clearance of inflammatory acne by aminolevulinic acid plus red light, for at least 20 wk after multiple treatments and 10 wk after a single treatment. Transient hyperpigmentation, superficial exfoliation, and crusting were observed, which cleared without scarring. Topical aminolevulinic acid plus red light is an effective treatment of acne vulgaris, associated with significant side-effects. Aminolevulinic acid plus red light causes phototoxicity to sebaceous follicles, prolonged suppression of sebaceous gland function, and apparent decrease in follicular bacteria after photodynamic therapy. Potentially, aminolevulinic acid plus red light may be useful for some patients with acne. *J. Invest Dermatol 115:183–192, 2000*

Acne vulgaris is a skin disease affecting more than 80% of young people. *Propionibacterium acnes* and sebum secretion play major roles in the pathogenesis of acne. Topical and systemic antibiotics are mainstays for treatment of acne, but the success rate varies in part due to the gradual resistance to antibiotics. Sun exposure has a well-known beneficial effect on acne, which is not the case for ultraviolet exposure (Sigurdsson *et al*, 1997). Studies show that the bacteria produce porphyrins as a by-product of their metabolism. Visible light is known to activate the porphyrins, inducing a photodynamic reaction that subsequently kills the pathogenic bacteria (Kjeldstad, 1984). Furthermore, photodynamic reactions can kill all strains of bacteria (Soukos *et al*, 1998).

Photodynamic therapy (PDT) with topical aminolevulinic acid (ALA) has been used to treat nonmelanoma skin cancer, actinic keratoses, and psoriasis (Szeimies *et al*, 1996). Topically applied ALA is taken up by epithelial cells and metabolized via the porphyrin pathway to protoporphyrin *IX* (*PpIX*), the precursor of heme (Kappa *et al*, 1989; Iinuma *et al*, 1994). *PpIX* is a photosensitizer that accumulates not only in the epidermal cells but also in the pilosebaceous units (Divaris *et al*, 1990; Kennedy and Pottier, 1992). When intense visible light is delivered on the ALA-treated skin, *PpIX* is excited into a triplet state, which reacts with oxygen to produce singlet oxygen, causing membrane damage and cell destruction. Topical ALA may directly enter hair follicles, where sebaceous glands actively synthesize and retain *PpIX*. We conducted this pilot study to test the hypothesis that photodynamic destruction of *P. acnes*, sebaceous glands, or both would occur in human skin, improving acne vulgaris.

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Abbreviations: ALA, aminolevulinic acid; PDT, photodynamic therapy; *PpIX*, protoporphyrin *IX*; SO, sebum output.

MATERIALS AND METHODS

Subject selection Twenty-two subjects of both sexes with mild to moderate acne vulgaris (grades 1–4) (Burke and Cunliffe, 1984) on their backs were enrolled between October 1998 and March 1999. People were excluded if they had used any topical acne treatment, systemic antibiotics in the past 2 wk, or systemic retinoids in the past year. People were also excluded who were using medication that may exacerbate or alleviate acne, who were planning to have excessive sunlight exposure, who had a history of keloid or photosensitivity disorder, or who had Fitzpatrick's skin phototype V-VI; pregnant and lactating women were also excluded.

Table I. Subject characteristics in both groups

	Single-treatment group	Multiple-treatment group
Age (y, mean + SD)	30 + 8.74	27 + 4.56
Gender (M/F)	9/2	8/3
Skin phototype	Type I 9.1%, Type II 27.3% Type III 54.5%, Type IV 9.1%	Type I 18.2%, Type II 36.4% Type III 27.3%, Type IV 18.2%
Disease history (y, mean + SD)	11.45 + 8.38	11.27 + 4.24
Previous systemic antibiotic treatment (number of subjects)	3 (27%)	4 (36%)
Previous topical antibiotic treatment (number of subjects)	3 (27%)	4 (36%)
Previous systemic isotretinoin treatment (number of subjects)	3 (27%)	2 (18%)
Number of baseline comedones (median, range)	3.0, 30	3.5, 34
Number of baseline inflammatory comedones (median, range)	3.0, 41	2.5, 17
Number of baseline papules (median, range)	4.5, 22	6.5, 33
Number of baseline pustules (median, range)	0.0, 3	0.0, 2
Number of baseline nodules (median, range)	0.0, 3	0.5, 13
Number of baseline cysts (median, range)	0.0, 0	0.0, 0

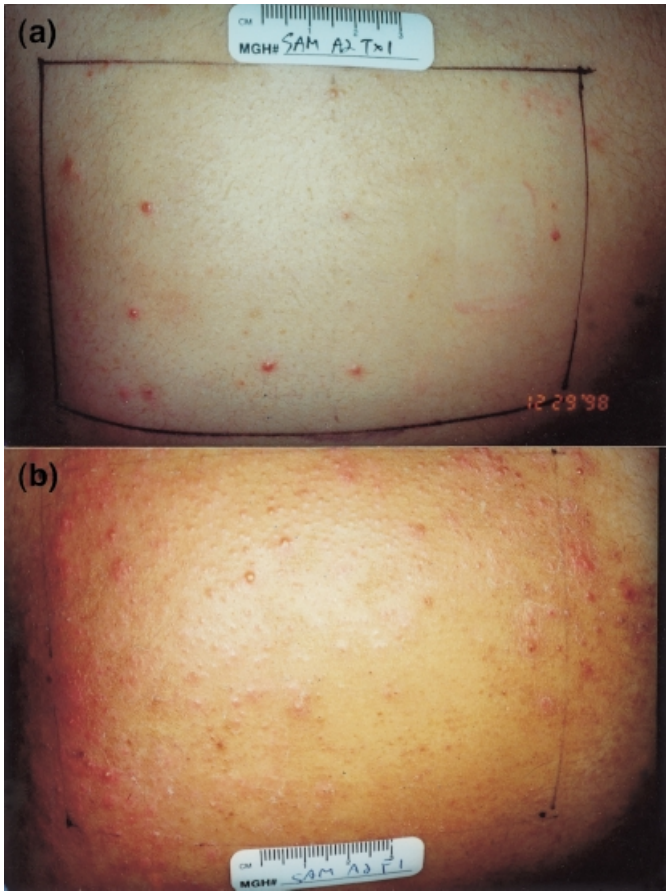

Figure 1. Transient acneiform eruption caused by a single PDT treatment. (*a*) Baseline; (*b*) one week post-treatment.

Study design Subjects were randomly divided into single-treatment and multiple-treatment groups. Each patient's back was equally divided into four 7.5 × 10 cm areas for ALA plus red light (ALA-PDT), ALA alone, light alone, and untreated control. Sites were marked with templates to precisely relocate each test area. At baseline, clinical evaluations, natural bacterial porphyrin fluorescence photography, and sebum output (SO) evaluation were performed. Before application of ALA, the skin was cleaned with 70% isopropyl alcohol. Then, 20% topical ALA in a

In the multiple-treatment group, subjects were treated once a week for four consecutive weeks. In this group, if severe exfoliation, erosions, or purpura occurred, treatment was postponed to the following week. In both groups, subjects returned 1 wk after treatment for clinical evaluation and at weeks 2, 3, 10, and 20 for clinical, fluorescence, and SO evaluations.

Clinical evaluations Each subject's acne was visually assessed using an inflammatory acne score modified from that previously described (Michaelsson *et al*, 1977). The modification we used in this study accounted for both number and size of acne lesions. The numbers of comedones, inflammatory comedones, papules, pustules, nodules, and cysts in each test area were recorded. Each type of lesion was given a severity index as follows: 0.5 for comedo (<1 mm), 0.75 for inflammatory comedo, 1 for papule (1-5 mm), 2 for pustule, 3 for nodule (>5 mm), and 4 for inflammatory cyst.

Clinical improvement was globally assessed by three dermatologists unaware of the status of treatment, who blindly graded changes in acne from fixed-magnification clinical photographs, after being shown a small set of standardized series of training slides not used in the data evaluation. The grading scale was defined as –3 for >50% exacerbation, –2 for 25%$^+$-50% exacerbation, –1 for 1%$^+$-25% exacerbation, 0 if unchanged, 1 for 1%$^+$-25% improvement, 2 for 25%$^+$-50% improvement, 3 for 50%$^+$-75% improvement, 4 for 75%$^+$-99% improvement, and 5 for 100% improvement, compared with the baseline.

Fluorescence photography A Nikon E2N digital camera body with a Nikon 105 mm macro lens was used. A filter (Corion LL-550S) was placed on the lens to block light below 550 nm. The excitation light source was composed of two synchronized photoflashes with Norman 400 W s lampheads (FT400/FT6), mounted on a stationary tower with angles of incidence of 60° bilaterally. Two 400 nm bandpass filters with 5 nm bandwidth (Corion S40–400S) were placed on the flashes. By this method, the punctate orange-red fluorescence of hair follicles populated with *P. acnes* was seen (Lucchina *et al*, 1996). Fluorescence emission has been attributed to bacterial coproporphyrin III and protoporphyrin IX (Cornelius and Lugwig, 1967; Lucchina *et al*, 1996), and intensity of fluorescence is related to the *P. acnes* population (Cornelius and Lugwig, 1967; Lucchina *et al*, 1996). Fluorescence photography was performed at weeks 0, 2, 3, 10, and 20 in all sites. The number of punctute red fluorescent dots was counted blindly for each test area.

SO measurement Sebum-absorbent tape (Sebutapes, CuDerm, Dallas, TX) is a noninvasive, easy, and reproducible method to evaluate human SO (Pagnoni *et al*, 1994a). The subject's skin was shaved and then cleansed for 15 s with cotton pads soaked in 70% ethanol. When the skin was completely dry, a strip of Sebutape was adhered to each test site for an hour. After removal from the skin, the white tape was placed on a black card for image analysis. Small transparent spots due to sebum excretion from follicles were visualized as a black spot on the white background. A CCD camera and digital frame grabber were used to capture images of the Sebutape, which were then examined using a computer-assisted image analysis (IP-

area correlates directly with the SO (Pierard, 1987). Sebutape assays of SO were done this way, at weeks 0, 2, 3, 10, and 20, in all sites.

Adverse effects Adverse effects were scored by clinical evaluation of erythema, edema, loss of epidermis, hyperpigmentation, hemorrhage, vesiculation, and exfoliation on a visual analog scale from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe) for each finding. Subjective sensation of pain, burning, and itching was generally maximum about 10 min into light exposure, and was ranked at that time and at the end of treatment (1 h) by subjects on a scale from 0 to 3 similar to above.

Histologic examination Punch biopsy specimens (4 mm) were taken immediately after PDT, a few weeks after PDT, and at 20 wk, from both the untreated control and ALA-PDT areas. Specimens were sectioned in either vertical or horizontal fashion and stained with hematoxylin and eosin, Fontana-Masson, and Masson-trichrome stains. Histologic examination was performed. Cross-sectioned areas of sebaceous glands, representative sebocytes, and the sebocyte nuclear area were measured from planimetric analysis of serial sectioned specimens of the skin using a computer-assisted planimetry system (Weissmann *et al*, 1984). To minimize the variation of sebaceous gland and sebocyte areas due to huge differences in cross-section and to provide a representative estimate of sebaceous gland and sebocyte areas, the largest sebaceous area of each follicle and largest sebocytes near the center of follicles from each serial sectioned specimen were measured. The area of sebaceous gland and the cytoplasm/nuclear area ratio in sebocytes were calculated and compared between control and PDT areas at each follow-up. To determine the level of *PpIX* converted from ALA in the pilosebaceous units, punch biopsy specimens were also taken from ALA-treated areas after 3 h occlusion as described above. A series of horizontal cross-sections of fresh-frozen specimens was obtained, and localization of *PpIX* production was noted by fluorescence microscopy.

Histologic examinations were performed to get a qualitative picture of reactions to PDT. A total of 15 specimens were obtained. Eight biopsies of PDT-treated areas were taken with accompanying specimens from the nontreatment area: four from multiple PDT-treated areas at follow-up 5, one from a multiple PDT-treated area at follow-up 3, one from a single PDT-treated area immediately after PDT, one from a single PDT-treated area at follow-up 3, and one from a single PDT-treated area at follow-up 5. Seven biopsies were obtained without an accompanying specimen from control areas, and were analyzed for morphologic changes due to PDT: two from single PDT-treated areas immediately after PDT, one from an acneiform lesion appearing 3 d after PDT, one from a single PDT-treated area at follow-up 2, one from a single PDT-treated area at follow-up 5, and one from a multiple PDT-treated area at follow-up 3.

Statistical analysis Treatment effects were determined based on the following analyses: (1) comparing the scores from each follow-up visit to the baseline scores using paired *t* tests; (2) comparing the change from baseline among the four treatment sites using paired *t* tests; (3) comparing the change from baseline between the single-treatment and multiple-treatment groups using two-sample *t* tests; and (4) comparing the change from baseline between the single-treatment and multiple-treatment groups using a repeated measures analysis to combine data from all follow-up visits. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

Of the 23 subjects enrolled, 22 (17 males and five females) completed the study. One was dropped from the study because his asthma necessitated systemic steroid treatment, which is one of the exclusion criteria. The age of patients completing the study ranged from 18 to 44 y. Characteristics of the subjects in both groups are shown in **Table I**.

An impressive, acute eruption of inflammatory acneiform lesions was observed in the ALA-PDT sites only, in all patients (100%) in both groups, starting approximately 3–4 d post-treatment (**Fig 1**). The induced lesions were papules, pustules, and nodules that lasted for 4 d to 3 wk in the single-treatment group. In the multiple-treatment group, subsequent treatments induced progressively less inflammatory acne, such that almost no new acneiform lesions were observed after treatment 4.

Inflammatory acne score (Figs 2*a* and 3)

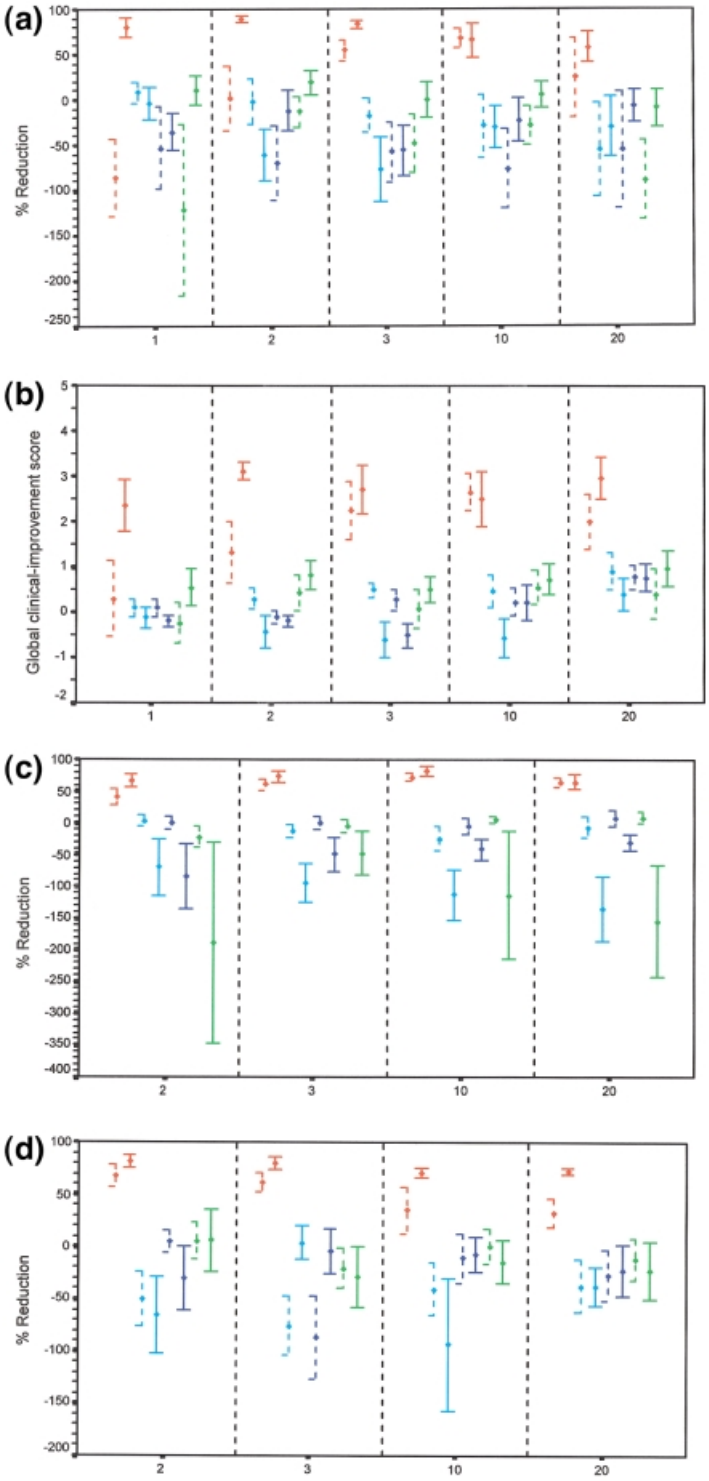

Figure 2. The mean improvement (+ SEM) by treatment sites, treatment groups, and follow-up visits. (*a*) Reduction in inflammatory acne score; (*b*) global clinical-improvement grading; (*c*) reduction in autofluorescence of follicles, related to *P. acnes*; (*d*) reduction in sebum excretion rate. - - -, single treatment group; ——, multiple treatment group; red, PDT; blue, untreated; deep blue, light alone; green, ALA alone.

3 wk after treatment. The other three areas (ALA alone, light alone, untreated) showed slightly worse acne not significantly different from baseline, for all visits. When comparing the change from

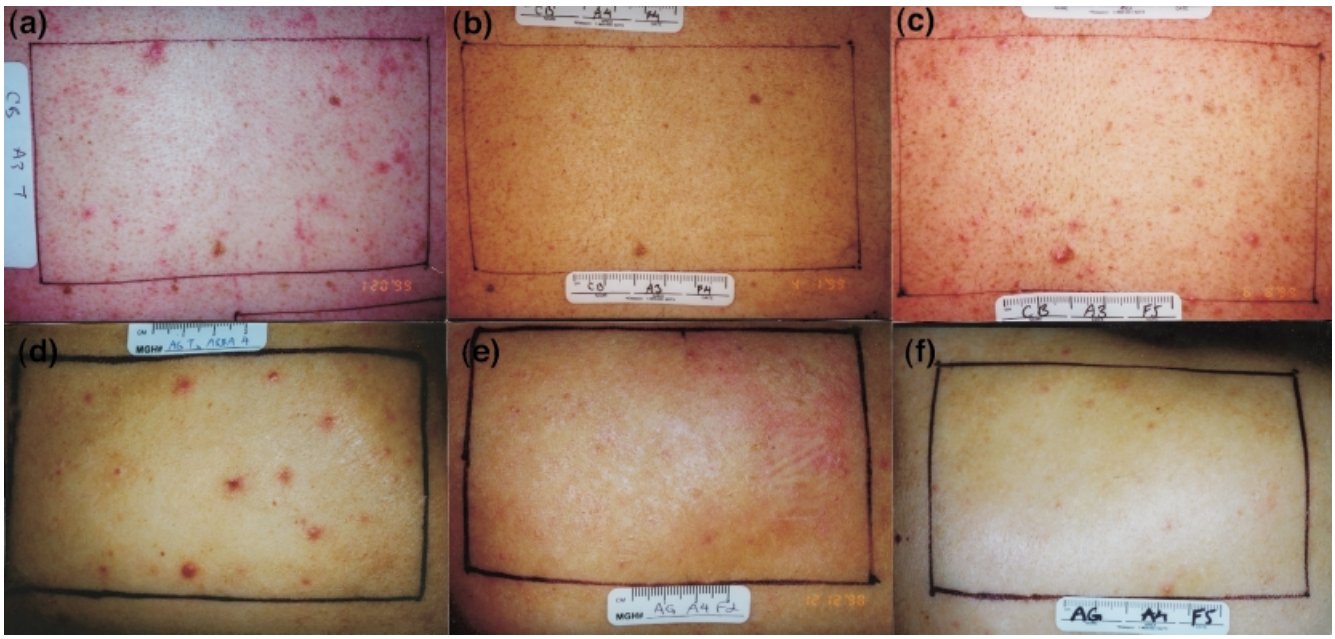

Figure 3. Inflammatory acne improved by a single PDT treatment. (*a*) Baseline. (*b*) Ten weeks post-PDT. (*c*) Acne starts to resume 20 wk after PDT. Long-term remission of acne after multiple PDT treatments. (*d*) Baseline. (*e*) Two weeks post-PDT (an irritation reaction to Sebutape is seen on the right-hand side in this subject). (*f*) Twenty weeks post-PDT.

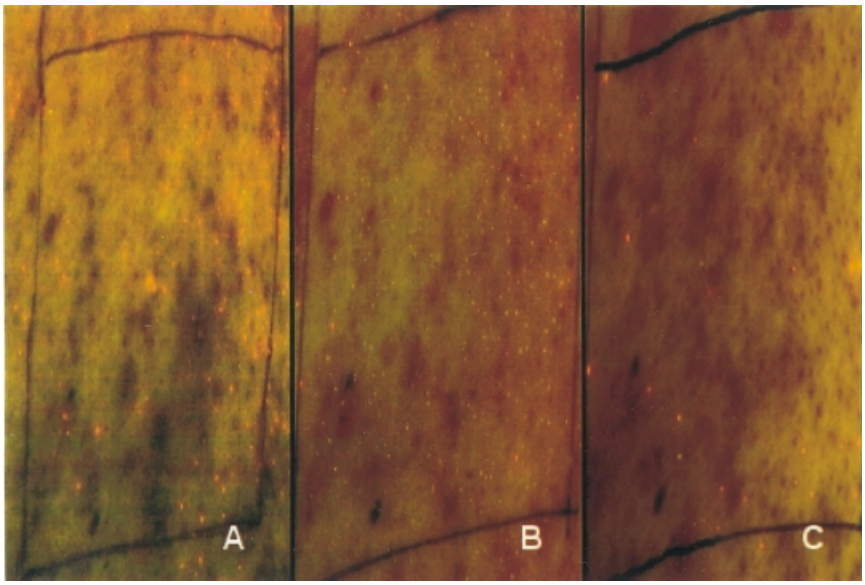

Figure 4. Fluorescence of porphyrin from bacteria in follicles (red dots) decrease after a single PDT. Photographs were taken as described at baseline (*A*), week 3 (*B*), and week 20 (*C*) post-PDT.

Multiple-treatment group There was obvious and statistically significant improvement in acne at all follow-up visits after multiple PDT treatment. There was no improvement in ALA-alone, light alone, or untreated sites. Change from baseline was significantly greater at sites of multiple PDT compared with the other three sites for all visits ($p < 0.05$). At visit 2 only (week 2), there was a barely significant improvement in the area treated with ALA alone compared with the untreated area ($p = 0.046$).

Comparison between single- and multiple-treatment groups The multiple PDT treatment group showed significantly more improvement than the single PDT treatment group at the first three follow-up visits. This difference diminished after week 3. No significant

multiple PDT and multiple ALA alone treatment sites showed more improvement than the single-treatment group ($p < 0.001$ and $p = 0.007$, respectively).

Global clinical-improvement score (Fig 2*b*)

Single-treatment group The PDT site showed significant global improvement starting week 3 and extending through week 20. The area without treatment, and the area treated with light alone, also showed improvement reaching statistical significance at weeks 3 and 20 ($p = 0.017$ and 0.018, respectively). The difference between PDT and the other three treatment sites was statistically significant at weeks 3 and 10.

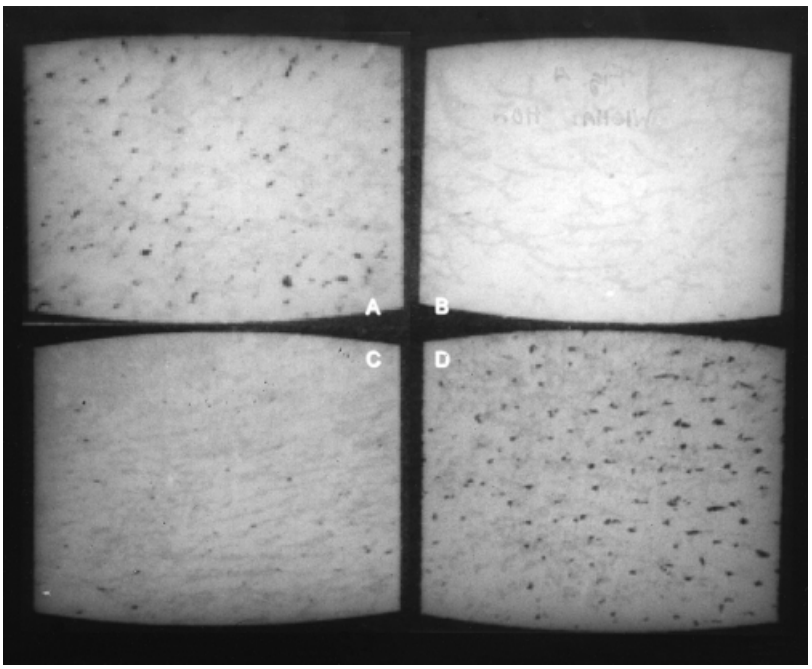

Figure 5. Sebum excretion is suppressed by a single PDT, then gradually recovers. (*A*) At baseline, (*B*) week 2 post-PDT, (*C*) week 10 post-PDT, (*D*) week 20 post-PDT.

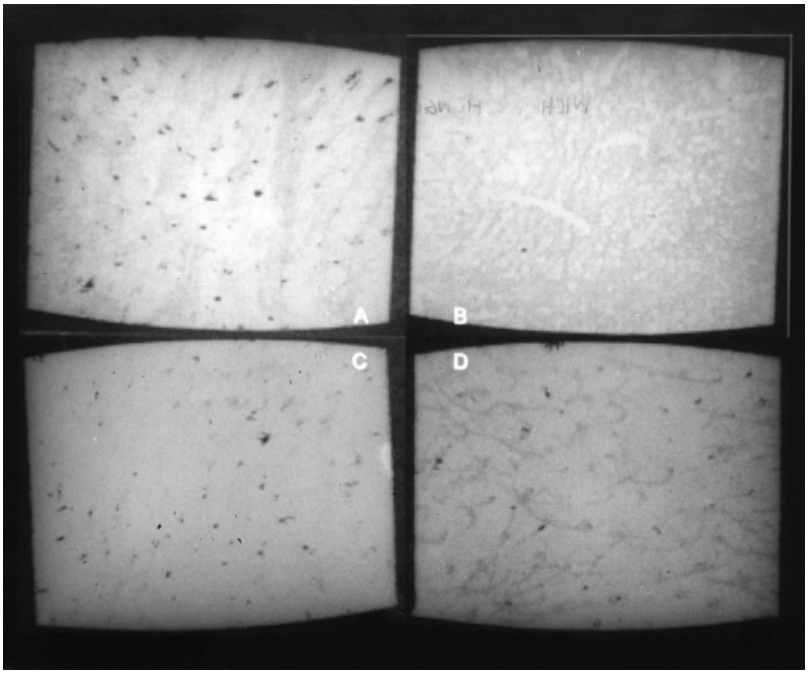

Figure 6. Sebum excretion remains suppressed after multiple PDT treatments, for at least 20 wk. (*A*)–(*D*) as in **Fig 5** above.

improvement persisted throughout all the four follow-up visits (up to 20 wk at least). The area treated with ALA alone at visit 2 and the area treated with light alone or ALA alone at visit 5 also showed improvement reaching statistical significance. There was significantly more improvement, however, in the PDT treated site than the other three sites, at all follow-up visits.

Comparison between single- and multiple-treatment groups The multiple PDT treatment group showed significantly more improvement than the single PDT treatment group when evaluated at the first two follow-up visits (weeks 1 and 2). The single-treatment group did not have significantly more acne improvement than multiple

Fluorescence photography evaluation (Figs 2*c* and 4)

Single-treatment group Only the PDT treated sites showed significant loss of fluorescence related to *P. acnes*, which lasted for all four follow-up visits. The differences between PDT and the other three test sites were also statistically significant for all visits.

Multiple-treatment group Again, only the PDT sites showed significant loss of *P. acnes* fluorescence, starting at follow-up visit 2. The sites treated with ALA alone or untreated had significantly greater fluorescence than baseline, at weeks 10 and 20. The differences between the PDT area and the other three test sites were statistically significant for all visits.

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