

Molecular Effects of Photodynamic Therapy for Photoaging

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Objective: To quantitatively examine the epidermal and dermal cellular and molecular changes that occur after photodynamic therapy of photodamaged human skin.

Design: Serial in vivo biochemical and immunohistochemical analyses after photodynamic therapy using topical 5-aminolevulinic acid (5-ALA) and pulsed-dye laser treatment.

Setting: Academic referral center, Department of Dermatology, University of Michigan, Ann Arbor.

Patients: A volunteer sample of 25 adults, 54 to 83 years old, with clinically apparent photodamage of the forearm skin.

Interventions: Three-hour application of 5-ALA followed by pulsed-dye laser therapy using non-purpura-inducing settings to focal areas of photodamaged forearms and serial biopsy specimens taken at baseline and various times after treatment.

Main Outcome Measures: Immunohistochemical analysis was used to assess levels of markers of epidermal proliferation (Ki67), epidermal injury (cytokeratin 16), and photodamage (p53), as well as various markers of dermal collagen production (including prolyl 4-hydroxylase and heat shock protein 47, and type I procollagen). Real-time reverse transcriptase-polymerase chain reaction technology was used to quantify type I and type

III collagen. Type I procollagen protein was quantified with enzyme-linked immunosorbent assay.

Results: Epidermal proliferation was stimulated as demonstrated by increases in Ki67 (more than a 5-fold increase; $P < .05$) and epidermal thickness (more than a 1.4-fold increase; $P < .05$). Epidermal injury was produced with increased cytokeratin 16 levels demonstrated (to nearly 70-fold of baseline levels; $P < .05$). Upregulation of collagen production was demonstrated with increases in procollagen I messenger RNA (2.65-fold; $P < .05$), procollagen III messenger RNA (3.32-fold; $P < .05$), and procollagen I protein (2.42-fold; $P < .05$) levels detected. The baseline epidermal p53 level correlated with cytokeratin 16 levels at acute time points, and the latter were found to correlate with peak collagen production.

Conclusions: Photodynamic therapy with the specific treatment regimen employed produces statistically significant quantitative cutaneous molecular changes (eg, production of types I and III collagen) that are associated with improved appearance of the skin. Baseline epidermal p53 immunostaining levels may be predictive of dermal responses to this therapy. Comparison with historical data using pulsed-dye laser therapy alone suggests that use of the photosensitizer may enhance dermal remodeling. The quantitative in vivo molecular data presented herein are in keeping with an evolving model to potentially predict the efficacy of new techniques for the treatment of photoaging.

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THE DELETERIOUS EFFECTS OF exposure of the skin to UV irradiation are well established. Alternatively, several visible and infrared lasers and light sources have been reported¹⁻¹⁰ to produce various positive changes in the clinical and histologic appearance of the skin. In recent years, the concept of employing a photosensitizing compound to enhance the effects of some light-based therapies has been espoused. In the context of aesthetic treatments, such photodynamic therapy (PDT) typically involves application of a topical photosensi-

tizer, such as 5-aminolevulinic acid (5-ALA), which is activated by exposure to a visible light source. It is known that topical 5-ALA is converted to protoporphyrin IX (PpIX) in the skin, which then preferentially accumulates in more rapidly

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proliferating cells. Free radicals are produced when PpIX is irradiated with visible light, causing injury to various cutaneous targets. Precisely how this translates to im-

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improvements in the appearance of sun-damaged skin has not been fully elucidated.

Photodynamic therapy has been examined as a treatment for conditions ranging from acne to superficial skin cancers to the signs of photoaging.¹¹⁻¹⁷ Relatively few studies^{18,19} have focused on the latter, with most of the research in this specific area centered on clinical assessments of treated patients. Despite a growing body of primarily clinically oriented literature, there remains a paucity of quantitative data that directly examine the biochemical alterations that result from PDT for photoaging beyond the effects of the treatment for actinic keratoses. Prior work has delineated the molecular events that follow ablative laser resurfacing and "traditional" nonablative laser therapy without the use of a photosensitizer.^{20,21} Given that such biochemical changes are likely to mechanistically underlie many of the clinical improvements noted with laser therapy, we sought to examine and quantify epidermal and dermal matrix changes that result from PDT on both cellular and molecular levels.

METHODS

This study was approved by the institutional review board of the University of Michigan Medical School, and informed consent was obtained from all study subjects prior to entry into the study. Patient recruitment occurred from September 8, 2004, through September 27, 2006. Patients included in the study were those of either sex who were at least 40 years of age with clinically evident photodamage of the forearm skin globally rated by investigators as at least moderate in severity. All included subjects were in generally good health and were thought to be able and willing to understand and comply with the requirements of the study protocol. Exclusion criteria included patients with a history of oral retinoid use within 1 year of study entry; a history of photosensitivity-related skin disorders; those who had undergone any form of prior laser therapy, dermabrasion, or chemical peels at the specific sites to be treated; and those with an allergy to 5-ALA or lidocaine. Also excluded from study participation were pregnant or nursing patients, those with a history of keloid scar formation, patients with evidence of an active infection of the forearm skin, and those with a history of herpes simplex or zoster in this area. Noncompliant patients and those with any clinically significant medical history or concurrent conditions deemed by investigators to be unsafe for study participation were also excluded.

All patients underwent a medical history and cutaneous physical examination of the forearm skin to be treated. The severity of subjects' photodamage was rated and recorded using an ordinal photodamage scale. The specific focal site to be treated, measuring approximately 2.5 × 8.0 cm, was outlined by an investigator and marked with a pen. Measurements from anatomic landmarks (eg, the wrist crease) were recorded to ensure that subsequent biopsy specimens were obtained from the laser-treated area. Hair in the relevant area was shaved prior to treatment. A baseline punch biopsy specimen (3 mm in diameter) from untreated forearm skin was obtained under sterile conditions after anesthesia was achieved with locally injected lidocaine, 1%. Following an acetone scrub of the forearm skin, Levulan (20% 5-ALA solution; DUSA Pharmaceuticals Inc, Wilmington, Massachusetts) was applied to the chosen treatment site and left in place for 3 hours. The treatment site was washed with Cetaphil cleanser (Galderma, Fort Worth, Texas) and then treated with a pulsed-dye laser (PDL) (Vbeam; Candela Corp, Wayland, Massachusetts) for a single pass at the fol-

lowing treatment parameters: 595-nm wavelength, 10-mm spot size, 10-millisecond pulse duration, and a fluence of 7.5 J/cm². Following laser therapy, the treatment site was again washed with Cetaphil cleanser, and the treated skin was covered with a nonstick bandage. Patients were instructed to prevent exposure of the treated skin to direct light for 48 hours after treatment. Subjects returned for reevaluation and to provide additional skin biopsy samples (3 mm in diameter) on 4 to 5 additional occasions during the first 6 months following the treatment. All biopsy sites were sutured with simple interrupted 4-0 Prolene sutures, and individual skin biopsy samples were spaced a minimum of 0.5 cm apart to eliminate the possibility that molecular changes resulting from wound healing at other biopsy sites would affect our measurements. Tissue biopsy specimens were examined using previously published immunohistochemical techniques and image analysis (Image-Pro Plus; Media Cybernetics, Silver Spring, Maryland) as well as real-time reverse transcriptase-polymerase chain reaction technology to quantify the resulting molecular changes.²² The following antibodies were used for the immunohistochemical analysis: Ki67 (AM297-5M; Biogenex, San Ramon, California), cytokeratin 16 (NCL-CK16; Novacastra, Norwell, Massachusetts), p53 (AM239-5M; Biogenex), prolyl 4-hydroxylase- α (63163; MP Biomedicals, Solon, Ohio), and HSP47 (SPA-470; Stressgen, San Diego, California). Serial frozen sections were prepared from optical cutting temperature medium-embedded skin biopsy samples. Soluble protein extracts were prepared and assayed for procollagen I using a commercial enzyme-linked immunosorbent assay kit (Panvera, Madison, Wisconsin) using previously described techniques.²² This assay is known to be a measure of newly synthesized collagen.

Biomarkers derived from the analysis of skin biopsy samples obtained at various time points throughout the study were compared with baseline levels with the repeated-measures analysis of variance and Dunnett test for specific pair-wise comparisons. The degree of correlation among biomarkers was assessed with Pearson product-moment correlation analysis. Descriptive statistics include means, ranges, standard deviations, and standard errors (not all data shown). The data were analyzed with SAS analytic software (version 9.1; SAS Institute, Cary, North Carolina). Statistical significance was considered reached where $P < .05$ in all statistical analyses performed.

RESULTS

CLINICAL FINDINGS

A total of 25 patients aged 54 to 83 years (15 women and 10 men) with clinically significant photodamage of the forearm skin were enrolled in the study, and 24 provided at least 4 skin biopsy samples. One patient was excluded from the study after developing contact dermatitis from the adhesive used in a bandage placed after treatment. Patients generally tolerated the procedure well. As expected, treated skin was erythematous in all patients at their initial follow-up visits. Some patients also developed mild scaling of the treated skin, and a few had mild focal crusting that resolved without additional sequelae after applications of a bland emollient.

EPIDERMAL FINDINGS

Ki67 is a protein localized to the nucleus that is thought to play a fundamental role in cellular proliferation.²³ As such, it is a known immunohistochemical marker of ke-

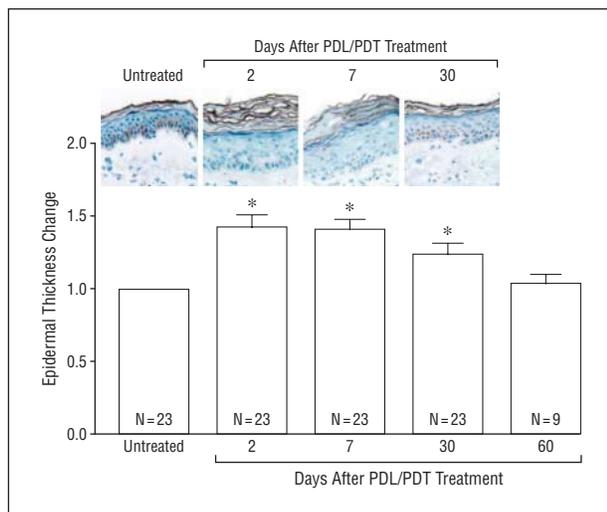


Figure 1. Photodynamic therapy with pulsed-dye laser (PDL/PDT) increases epidermal thickness of photodamaged human forearm skin. The indicated numbers (N) of subjects were treated on the photodamaged forearm skin with 20% aminolevulinic acid for 3 hours, followed by a single pass of PDL (595-nm wavelength, 10-mm spot size, 10-millisecond pulse duration, and a fluence of 7.5 J/cm²). Skin biopsy samples were obtained at the indicated times, and frozen sections were stained with hematoxylin-eosin. Epidermal thickness was determined by image analysis using Image-Pro Plus software (Media Cybernetics, Silver Spring, Maryland). Epidermal thickness was significantly increased (denoted by an asterisk; $P < .05$) within 2 days of treatment and remained increased for at least 30 days. Bars indicate means and standard errors of the mean. Insets are representative stained sections (original magnification $\times 120$).

keratinocyte proliferation. Using image analysis, its levels were quantified and found to be significantly elevated with a 5.32-fold change detected ($P < .05$) on day 2 following PDT with PDL (PDL/PDT). This rise in Ki67 continued on day 7 after treatment with a 5.28-fold change ($P < .05$) over pretreatment values. Concomitant with increased keratinocyte proliferation, changes in epidermal thickness were observed histologically (**Figure 1**). These changes were measured and found to be significantly increased ($P < .05$) on days 2 and 7 after the laser therapy (1.42-fold and 1.41-fold changes, respectively). A positive effect on epidermal thickness was demonstrated to be sustained 1 month after PDL/PDT with a 1.24-fold change ($P < .05$) noted (Figure 1).

Epidermal expression of cytokeratin 16 is a well-characterized marker of epidermal irritation and injury.²⁴ Minor staining for cytokeratin 16 was often observed on day 1 after treatment. Detection of cytokeratin 16 protein peaked 2 days after treatment and was still present 7 days after treatment (**Figure 2**). At baseline, its levels were negligible (0.06% of the epidermal area stained), but on day 2 after PDL/PDT, there was a sharp and statistically significant ($P < .05$) rise in cytokeratin 16 level to 4.25% of the epidermal area stained, a 70-fold increase (Figure 2).

We also assessed levels of p53, a well-established marker of epidermal photodamage.²⁵⁻³⁰ In prior work,²⁵ we established that ablative laser therapy produces a marked and sustained decrease in p53 staining in sun-damaged skin. However, no significant changes in p53 staining were found to result from PDL/PDT at any time point studied (data not shown).

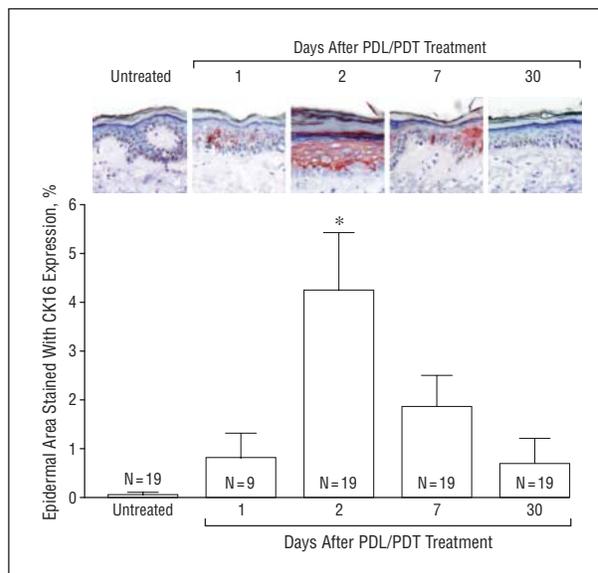


Figure 2. Photodynamic therapy with pulsed-dye laser (PDL/PDT) induces cytokeratin 16 (CK16) protein expression in photodamaged human forearm skin. The indicated numbers (N) of subjects were treated on photodamaged forearm skin with 20% aminolevulinic acid for 3 hours, followed by a single pass of PDL (595-nm wavelength, 10-mm spot size, 10-millisecond pulse duration, and a fluence of 7.5 J/cm²). Skin biopsy samples were obtained at the indicated times, and frozen sections were immunostained for CK16. Immunostaining was quantified by image analysis using Image-Pro Plus software (Media Cybernetics, Silver Spring, Maryland). Cytokeratin 16 expression was significantly increased (denoted by an asterisk; $P < .05$) 2 days after treatment. Bars indicate means and standard errors of the mean. Insets are representative stained sections (original magnification $\times 120$).

DERMAL FINDINGS

Dermal remodeling that occurs in response to wounding involves initial breakdown of the collagenous extracellular matrix followed by deposition of new collagen, and prior in vitro work suggests that PDT may induce matrix metalloproteinases.^{21,31,32} Matrix metalloproteinase-1 (MMP1) initiates collagen breakdown, and we found that MMP1 gene expression 1 day after PDL/PDT was acutely elevated approximately 20-fold ($P < .05$; $n = 6$) and then returned to baseline levels within 24 hours. Prolyl 4-hydroxylase- α is an enzyme involved in posttranslational modification of collagen and is expressed in collagen-synthesizing cells. It catalyzes hydroxylation of proline residues in procollagen protein, a step that is required for collagen synthesis.³³ Thus, dermal fibroblasts that are actively producing collagen express high levels of prolyl 4-hydroxylase and stain positively for this marker.³⁴ Prolyl 4-hydroxylase- α immunostaining was statistically significantly elevated 1 month after PDL/PDT, its levels having approximately tripled compared with baseline ($P < .05$) (**Figure 3**). Heat shock protein 47 (HSP 47) is a chaperone protein thought to be vital for procollagen production in dermal fibroblasts. As such, it has been used as a marker for cutaneous collagen production.³⁵ Although no statistically significant increase in HSP 47 levels was demonstrated, there was a trend toward elevation of HSP 47 levels (data not shown). Furthermore, levels of HSP 47 were found to correlate with those of prolyl 4-hydroxylase- α at days 1 and 2 and months 1 and 2 after treatment. The highest degree of correlation

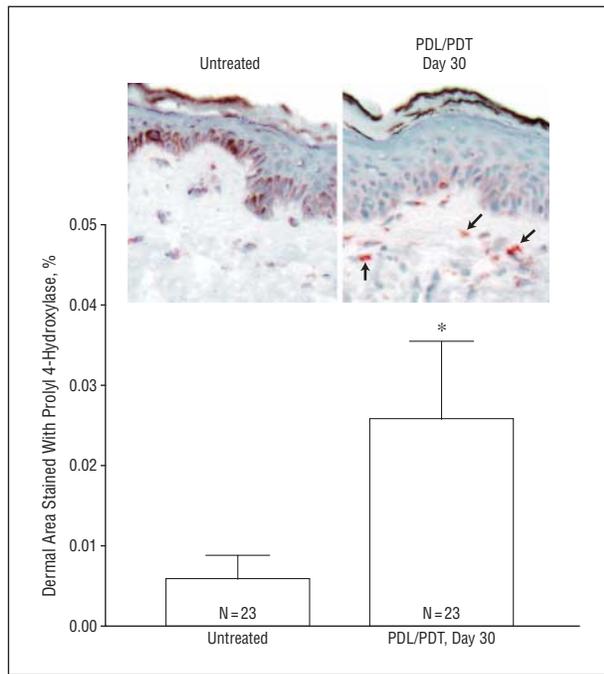


Figure 3. Photodynamic therapy with pulsed-dye laser (PDL/PDT) induces prolyl 4-hydroxylase immunostaining protein expression in photodamaged human forearm skin. The indicated numbers (N) of subjects were treated on photodamaged forearm skin with 20% aminolevulinic acid for 3 hours, followed by a single pass of PDL (595-nm wavelength, 10-mm spot size, 10-millisecond pulse duration, and a fluence of 7.5 J/cm²). Skin biopsy samples were obtained at the indicated times, and frozen sections were immunostained for prolyl 4-hydroxylase. Immunostaining was quantified by image analysis using Image-Pro Plus software (Media Cybernetics, Silver Spring, Maryland). Prolyl 4-hydroxylase expression was significantly increased (denoted by an asterisk; $P < .05$) 30 days after treatment. Bars indicate means and standard errors of the mean. Insets show representative stained sections (original magnification $\times 120$). The arrows are pointing to dermal fibroblasts that have stained positively for prolyl 4-hydroxylase.

($r = 0.92$; $P < .001$) occurred 1 month after PDL/PDT. Assessment of procollagen gene expression indicated statistically significant increases in both type I and type III procollagen messenger RNA (mRNA) levels following PDL/PDT (**Figure 4**). Type I procollagen mRNA levels peaked 1 month after treatment at a 2.65-fold increase above baseline values ($P < .05$), with a similar increase in type III procollagen mRNA levels to a 3.32-fold increase above pretreatment levels ($P < .05$) at this time point (Figure 4). We also found a time-dependent increase in type I procollagen protein levels, peaking 1 week after PDL/PDT. This 2.42-fold increase above baseline levels 1 week after treatment was statistically significant ($P < .05$) (**Figure 5**).

CORRELATIONS

We also sought to demonstrate the correlation between various markers studied to provide additional evidence of the molecular mechanisms associated with PDL/PDT. Noteworthy is the fact that baseline p53 staining levels correlated with cytokeratin 16 levels on day 7 following PDL/PDT ($r = 0.47$; $P = .04$). Furthermore, levels of cytokeratin 16 determined shortly after the therapy (on days 2 and 7 after treatment) correlated with peak collagen I levels 1 month after PDL/PDT ($r = 0.51$, $P = .03$; and $r = 0.50$, $P = .03$,

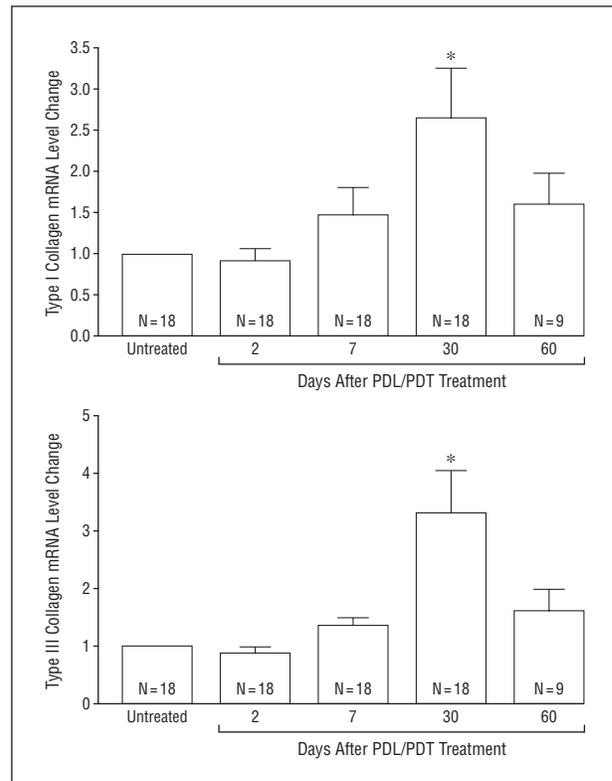


Figure 4. Photodynamic therapy with pulsed-dye laser (PDL/PDT) induces type I and type III procollagen messenger RNA (mRNA) levels in photodamaged human forearm skin. The indicated numbers (N) of subjects were treated on photodamaged forearm skin with 20% aminolevulinic acid for 3 hours, followed by a single pass of PDL (595-nm wavelength, 10-mm spot size, 10-millisecond pulse duration, and a fluence of 7.5 J/cm²). Skin biopsy samples were obtained at the indicated times, and the total RNA was extracted. Type I and type III procollagen and *36B4* (internal control housekeeping gene) mRNA levels were quantified by real-time reverse transcriptase–polymerase chain reaction. Type I and type III procollagen mRNA levels were normalized to *36B4* mRNA levels and expressed as fold change relative to untreated skin. Type I and type III procollagen mRNA levels were significantly increased (denoted by an asterisk; $P < .05$) 30 days after treatment. Bars indicate means and standard errors of the mean.

respectively). In addition, peak levels of collagen I and collagen III 1 month following PDL/PDT were highly correlated as expected ($r = 0.88$; $P < .001$).

COMMENT

Although PDT for actinic keratoses and superficial non-melanoma skin cancer has been studied rather extensively, its use in appearance-oriented dermatology is comparatively new. Despite the often somewhat subjective assessments of PDT for photoaging provided to date, there is substantial and growing clinical evidence for the efficacy of such therapy. What is less well understood are the molecular changes that occur in the skin in response to PDT. To date, relatively little quantitative data have been available to support the role of PDT in the treatment of photoaging, despite the positive clinical experiences of some patients treated in this fashion. Our study represents an attempt to quantify, on a molecular level, epidermal and dermal matrix changes following PDT.

Previous work involving carbon dioxide laser resurfacing, a gold standard cosmetic intervention, has dem-

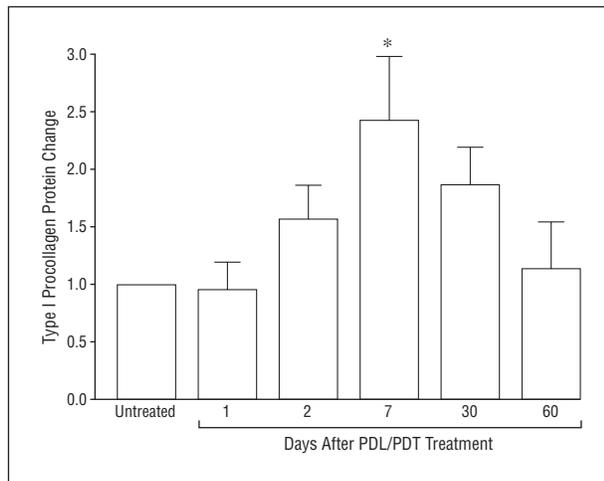


Figure 5. Photodynamic therapy with pulsed-dye laser (PDL/PDT) induces type I procollagen protein in photodamaged human forearm skin. Subjects (N=9) were treated on photodamaged skin with 20% aminolevulinic acid for 3 hours, followed by a single pass of PDL (595-nm wavelength, 10-mm spot size, 10-millisecond pulse duration, and fluence of 7.5J/cm²). Skin biopsy samples were obtained at the indicated times, and procollagen protein levels were quantified by enzyme-linked immunosorbent assay. Type I procollagen protein was significantly increased (denoted by an asterisk; $P < .05$) 7 days after treatment. Bars indicate means and the standard errors of the mean.

onstrated that this procedure is associated with considerable dermal matrix remodeling and collagen production.²¹ Although the current study did not produce molecular changes as pronounced as those following ablative resurfacing, statistically significant changes were demonstrated. Specifically, PDL/PDT was found to substantially induce production of type I and type III procollagen, a factor known from prior work to be associated with an improved appearance of the skin.^{21,36} Evidence of enhanced collagen production included increases in immunohistochemical markers of neocollagenesis, upregulation of procollagen I and procollagen III mRNA, and increased procollagen I protein level. Interestingly, our data suggest that statistically significant increases in procollagen I protein level preceded increases in procollagen I mRNA level in the skin samples analyzed. To the extent that this might hold true for other patients, these data indicate that PDL/PDT initially stimulated procollagen I protein production in our patients followed by increased mRNA levels. Procollagen protein production is complex and known to be regulated via both transcriptional and posttranscriptional mechanisms.^{33,37,38} Our finding of increased collagen production is in keeping with the results of a pilot study by Marmur et al³⁹ that examined ultrastructural changes produced by 5-ALA applications followed by exposure to a noncoherent intense pulsed-light source. Electron microscopic findings of that study suggested increased type I collagen.

We believe that the quantitative *in vivo* molecular data presented herein are in keeping with an evolving model to potentially predict the efficacy of new techniques for the treatment of photoaging. We have previously examined the molecular alterations that result from other popular interventions for photoaged skin. For example, in prior work⁴⁰ regarding microdermabrasion, we found that this procedure did produce measurable increases in type I pro-

collagen gene expression, but it did so in only 2 of 11 patients studied. In addition, even among those 2 subjects who did show upregulation of type I procollagen gene expression, corresponding increases in type I procollagen protein immunostaining were quite modest. The molecular findings from this treatment, which is not generally thought to produce clinically consistent long-term benefits, stand in sharp contrast to those that result from carbon dioxide laser resurfacing, as we have previously reported.⁴⁰ In the latter study,²¹ not only were a series of complex changes in various cytokines and matrix degrading enzymes measured, but we also demonstrated clear, consistent, and marked elevations in types I and III procollagen mRNA levels. These were found to persist for at least 6 months, with levels at that relatively long time point still several times higher than any seen following microdermabrasion. These molecular data are very much in keeping with the marked clinical improvement seen after ablative laser resurfacing. Thus, the magnitude and consistency of the molecular changes reported in those studies^{21,40} were very different indeed.

The results we later reported²⁰ using a similar *in vivo* quantitative model with respect to nonablative laser therapy were somewhere in between the 2 extremes described, again in keeping with the generally accepted relative clinical benefits of such a treatment. In that study, both a PDL and an infrared laser were found to produce statistically significant but relatively minor and inconsistent increases in procollagen gene expression in treated sun-damaged skin.

In contrast, the data reported herein show a more consistent and quantitatively greater change after PDL/PDT than after PDL alone compared with our prior data²⁰ using a PDL without pretreatment of the skin with a topical photosensitizer. The peaks in types I and III procollagen gene expression reported herein are roughly twice those produced with PDL treatment alone, suggesting that the photosensitizer may enhance results—just as many have reported on clinical grounds. Although our prior work did not include precisely the same laser treatment parameters, non-purpura-inducing settings were employed in each study. Thus, although our molecular measurements cannot yet precisely predict clinical outcomes for a single given patient, taken together they are very much in keeping with the bulk of the clinical literature and thus lend substantial support to the conclusions reached by other researchers who have published purely clinically oriented work in this field. We believe that the quantitative amount of dermal repair and regeneration induced by a specific therapeutic intervention very likely underlies the degree of clinical rejuvenation produced. Thus, it is our hope that, with further development, our working molecular model may one day be used to predict the clinical value of new technologies in aesthetic dermatology.

Of note, many of the cutaneous changes detailed in the “Epidermal Findings” and “Dermal Findings” subsections in the “Results” section, such as epidermal thickening and collagen upregulation, are qualitatively similar to those described in response to topical tretinoin therapy.^{36,41,42} It is possible that PDL/PDT may provide some “retinoidlike” benefits to the appearance of the skin,

with these changes likely occurring more rapidly than with topical retinoid therapy. Because measurements of epidermal thickness returned to baseline values and collagen I production also moved back toward baseline levels within a few months of PDL/PDT, ongoing maintenance therapy would likely be required to maintain any clinical benefits achieved. Although this would not require as great a level of compliance on the part of the patient as would daily topical retinoid use, some level of compliance with follow-up care would still be required, and financial considerations might also come into play when making therapeutic recommendations. Improvement in cutaneous pigmentation, another known benefit of tretinoin, was not assessed in this study.

Also of interest is the fact that baseline p53 levels were found to correlate with cytokeratin 16 levels at early time points, which in turn were found to correlate with the ultimate peak production of type I procollagen 1 month after PDL/PDT. Because the degree of p53 immunostaining is believed to be a marker for the severity of photodamage and cytokeratin 16 levels are a marker of epidermal injury, these results suggest that skin that is more profoundly sun damaged may be more susceptible or responsive to a controlled injury from a given stimulus (in this case, PDL/PDT) and that such photoaged skin may then respond particularly well in terms of neocollagenesis and dermal remodeling. Although the correlations noted regarding these markers do not specifically delineate the potential molecular mechanisms involved, they may have implications with respect to patient selection for PDL/PDT because they suggest that those with considerable, clinically apparent sun damage may respond particularly well to the treatment. In addition, the finding that a marker of epidermal injury (cytokeratin 16 level) correlated with the peak production of collagen in response to PDL/PDT implies that those patients whose skin was more profoundly injured or irritated by the therapy ultimately responded best. This fact tends to validate the intuitive concept that "one gets out of a procedure what one puts into it"—meaning that a variety of interventions may cause dermal remodeling and collagen formation, but those that are "stronger" treatments may in general prove to produce the best results. An alternative interpretation of this finding is that photodamaged skin may allow for greater penetration of 5-ALA owing to altered barrier function. As a marker of photodamage, elevated p53 might also imply greater loss of normal barrier function, resulting in a greater conversion of 5-ALA to PpIX in the skin and thus leading to a "stronger" PDT effect.

Previous work²⁵ has demonstrated that ablative laser resurfacing results in the near-complete elimination of p53 immunostaining for a period of at least 6 months, helping provide the rationale for using p53 positivity as a marker of residual photodamage after a therapeutic intervention. Furthermore, PDL/PDT has been reported to be successful in the clinical eradication of actinic keratoses and in the treatment of actinic cheilitis.^{43,44} Thus, it was somewhat surprising that a similar treatment did not alter p53 immunostaining in our study. It is possible that more rapid uptake of the 5-ALA by the more quickly proliferating cells within the actinic keratoses

treated in the prior study⁴⁴ resulted in more efficient alteration of these clinically apparent lesions compared with the simply sun-damaged (but nonlesional) skin of our patients. Our data suggest that additional research regarding the possible role of PDL/PDT in the treatment of such premalignant conditions may be warranted.

It is important to note that our study has some limitations. The current results reflect only PDT using a specific set of treatment parameters. For example, we used a 3-hour ALA incubation time, whereas many clinicians use shorter contact application times of 60 minutes or less. Furthermore, prior work⁴⁵ has suggested that continuous-wave blue light is a substantially more potent PDT activator than are pulsed-light sources. It is likely that by altering variables such as the photosensitizer used, its application time, the laser or light source used, and the fluences employed, both biochemical and clinical cutaneous responses may vary widely. Also, this study was not designed to directly compare the PDL/PDT effect with that of PDL alone. Although we believe that the comparison of our current results with those of our prior work²⁰ with nonablative laser therapy as discussed herein is valid, a randomized, controlled trial of this particular subject would be required to more definitively assess this particular issue. In addition, although it is generally believed that photodamaged forearm and facial skin employs fundamentally similar wound healing responses, it remains possible that treatment of facial skin (the site of most clinical PDL/PDT procedures) might produce somewhat different results. Currently, we are performing additional research examining PDL/PDT treatments of sun-damaged facial skin with varying therapeutic parameters to optimize the clinical responses of our patients to this therapy.

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Author Contributions: Dr Orringer had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Orringer, Kang, Fisher, and Voorhees. *Acquisition of data:* Orringer, Hammerberg, and Fisher. *Analysis and interpretation of data:* Orringer, Hammerberg, Hamilton, Kang, Sachs, Fisher, and Voorhees. *Drafting of the manuscript:* Orringer and Hamilton. *Critical revision of the manuscript for important intellectual content:* Hamilton, Sachs, Fisher, and Voorhees. *Statistical analysis:* Hamilton and Kang. *Obtained funding:* Voorhees. *Administrative, technical, and material support:* Orringer, Hammerberg, Kang, Fisher, and Voorhees. *Study supervision:* Orringer, Hammerberg, Kang, Sachs, and Fisher.

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