Dermal Damage Promoted by Repeated Low-Level UV-A1 Exposure Despite Tanning Response in Human Skin

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IMPORTANCE Solar UV irradiation causes photoaging, characterized by fragmentation and reduced production of type I collagen fibrils that provide strength to skin. Exposure to UV-B irradiation (280-320 nm) causes these changes by inducing matrix metalloproteinase 1 and suppressing type I collagen synthesis. The role of UV-A irradiation (320-400 nm) in promoting similar molecular alterations is less clear yet important to consider because it is 10 to 100 times more abundant in natural sunlight than UV-B irradiation and penetrates deeper into the dermis than UV-B irradiation. Most (approximately 75%) of solar UV-A irradiation is composed of UV-A1 irradiation (340-400 nm), which is also the primary component of tanning beds.

OBJECTIVE To evaluate the effects of low levels of UV-A1 irradiation, as might be encountered in daily life, on expression of matrix metalloproteinase 1 and type I procollagen (the precursor of type I collagen).

DESIGN, SETTING, AND PARTICIPANTS In vivo biochemical analyses were conducted after UV-A1 irradiation of normal human skin at an academic referral center. Participants included 22 healthy individuals without skin disease.

MAIN OUTCOMES AND MEASURES Skin pigmentation was measured by a color meter (chromometer) under the L* variable (luminescence), which ranges from 0 (black) to 100 (white). Gene expression in skin samples was assessed by real-time polymerase chain reaction.

RESULTS Lightly pigmented human skin (L* >65) was exposed up to 4 times (1 exposure/d) to UV-A1 irradiation at a low dose (20 J/cm²), mimicking UV-A levels from strong sun exposure lasting approximately 2 hours. A single exposure to low-dose UV-A1 irradiation darkened skin slightly and did not alter matrix metalloproteinase 1 or type I procollagen gene expression. With repeated low-dose UV-A1 irradiation, skin darkened incrementally with each exposure. Despite this darkening, 2 or more exposures to low-dose UV-A1 irradiation significantly induced matrix metalloproteinase 1 gene expression, which increased progressively with successive exposures. Repeated UV-A1 exposures did not suppress type I procollagen expression.

CONCLUSIONS AND RELEVANCE A limited number of low-dose UV-A1 exposures, as commonly experienced in daily life, potentially promotes photoaging by affecting breakdown, rather than synthesis, of collagen. Progressive skin darkening in response to repeated low-dose UV-A1 exposures in lightly pigmented individuals does not prevent UV-A1-induced collagenolytic changes. Therefore, for optimal protection against skin damage, sunscreen formulations should filter all UV wavelengths, including UV-A1 irradiation.
Solar UV irradiation causes photoaging, which is characterized by skin wrinkling and laxity. This loss in structural support can be attributed largely to alterations in dermal connective tissue, including type I collagen fibrils, which provide strength and resiliency to skin.

Type I collagen fibrils are synthesized as a soluble precursor (procollagen) by fibroblasts and compose most of the dermal extracellular matrix (ECM). Acute low-level UV-B irradiation (280-320 nm) resulting in slight pinkness, but not sunburn, upregulates matrix metalloproteinase 1 (MMP-1), which initiates type I collagen fibril degradation. Ultraviolet B irradiation also upregulates other MMPs. Together, the actions of these enzymes can completely degrade type I collagen fibrils, impairing dermal ECM structure and function. Ultraviolet B irradiation also suppresses type I procollagen synthesis, thereby promoting further loss of type I collagen fibrils.

Ultraviolet A (320-400 nm) irradiation is 10 to 100 times more abundant in natural sunlight than UV-B irradiation and is not entirely filtered by window glass or clothing. Thus, compared with UV-B irradiation, skin is exposed to more UV-A irradiation, both daily and cumulatively over a lifetime. Fur- thermore, UV-A irradiation penetrates deeper into the dermis than UV-B irradiation and therefore potentially causes more widespread alterations in the dermis.

Despite these observations, the specific role of UV-A irradiation in the molecular pathogenesis of photoaging remains unclear. In the present study, we evaluated the effects of repeated UV-A irradiation on the disruption of dermal ECM integrity. We exposed skin to UV-A1 irradiation (340-400 nm) because these wavelengths penetrate deeper into the dermis than UV-B or UV-A2 (320-340 nm). Additionally, UV-A1 irradiation composes most (approximately 75%) of solar UV-A irradiation and is the primary component of tanning beds. We used low-dose UV-A1 irradiation (20 J/cm²), mimicking UV-A levels from strong sun exposure lasting approximately 2 hours. We exposed participants up to 4 times (1 exposure per day), so that irradiation plus tissue procurement could be completed in 1 week. We found that repeated low-level exposure to UV-A1 irradiation promotes damage to the dermal ECM despite skin darkening (tanning).

**Methods**

The University of Michigan Institutional Review Board approved this study. Participants received compensation for undergoing this study. Participants were aged 18 years or older (range, 22-61 years; mean [SE], 43.4 [2.5] years), provided written informed consent, and received irradiation from a UV lamp (Sallamed 2000; Sellas Medizinische Gerate GmbH) that had a peak output of 360 to 390 nm and power output distribution of 94.4% UV-A1 and 5.6% visible to near-infrared (400-800 nm) (as measured by Robert Sayre, PhD, Rapid Precision Testing Laboratories). To deliver a particular dose, the light source was held 20 cm from the target site and exposed to the skin for an appropriate duration based on the lamp’s irradiance, which was measured by a spectroradiometer (typical irradiance, 55.6 mW/cm²; Sola-Scope 2000, Sol- atel Ltd) and independently verified (Dr Sayre, Rapid Precision Testing Laboratories). Irradiation intensity was monitored before each session by a multimeter (model 8060A; Fluke Corporation). Skin pigmentation was measured by a chromometer (Minolta CR200; Minolta) under the L* variable (luminescence), which ranges from 0 (black) to 100 (white). Gene expression was measured by real-time polymerase chain reaction, as described elsewhere. The housekeeping gene acidic ribosomal phosphoprotein Po (26B4) was used as an internal control. One-way repeated-measures analysis of variance with Tukey adjustment for multiple comparisons was used in data analysis. When appropriate, logarithmic transformation was applied to achieve normality. Logarithmic transformations were performed for all gene expression data. Data were analyzed using commercial software (SAS, version 9.1; SAS Institute Inc), with statistical significance if P < .05 for a 2-tailed paired hypothesis.

**Results**

Participants were exposed to a light source that emitted nearly pure UV-A1 irradiation. There were no adverse events. In lightly pigmented (defined previously as L* > 65) buttock skin of 10 healthy individuals, a single exposure to low-dose UV-A1 irradiation (20 J/cm²) caused slight but significant skin darkening at 24 hours after exposure (P = .004) (Figure 1A). Exposure to higher UV-A1 doses (40 or 80 J/cm²) caused more skin darkening than 20 J/cm² (P < .001) (Figure 1A). Mild erythema occurred after low-dose UV-A1 exposure and was more prominent after exposure to higher doses.

Under these conditions, gene expression of MMP-1 was induced in a dose-dependent fashion, with no significant change after exposure to 20 J/cm² and substantial upregulation after higher doses (P < .001) (Figure 1B). Matrix metalloproteinase 3 (stromelysin) exhibited a similar induction pattern in the 10 participants (Figure 1B). Additionally, types I and III procollagen expression displayed a dose-dependent response, with no downregulation after exposure to 20 J/cm² and significant suppression after higher doses (P < .05) (Figure 1B).

Next, lightly pigmented buttock skin of 12 participants was exposed repetitively at daily intervals to 20 J/cm² UV-A1 irradiation. Skin darkening occurred after 1 exposure and increased incrementally with successive exposures (P < .01) (Figure 2A). Additionally, mild erythema occurred after the first exposure and increased with subsequent exposures.

Under the same conditions, gene expression of MMP-1 was not significantly induced after 1 exposure but exhibited significant and progressive upregulation after 2 or more exposures in the 12 participants (P < .05) (Figure 2B). Furthermore, 3 or 4 exposures caused significantly greater MMP-1 induction than did 1 exposure (P < .01). Matrix metalloproteinase 3 exhibited a similar induction pattern (Figure 2B). Induction of MMP-1 or MMP-3 was not due to a delayed response to the first exposure, because no induction of these MMPs was observed 4 days after a single exposure to 20 J/cm² UV-A1 irradiation (data not shown). In contrast to MMP expression, gene expression of type I procollagen was not significantly altered after multiple exposures to low-dose UV-A1 irradiation in the 12 participants (Figure 2B). Additionally, multiple exposures did not alter the expression of type III procollagen (Figure 2B).
Discussion

In the present study, we exposed lightly pigmented human skin to UV-A1 irradiation at a low dose, simulating UV-A levels from strong sun exposure lasting approximately 2 hours.\textsuperscript{10-12} We found that despite skin darkening, the effects of repeated, daily low-dose UV-A1 irradiation include progressive messenger RNA (mRNA) induction of MMPs that break down the dermal ECM. Of note, the mRNA induction of MMPs correlates with protein expression and enzymatic activity.\textsuperscript{3} This induction does not occur after a single exposure to low-dose UV-A1 irradiation.

Figure 1. Dose-Dependent Effects of UV-A1 Irradiation on Lightly Pigmented Human Skin

Skin pigmentation was measured using a chromometer under the L* variable (luminance), which ranges from 0 (black) to 100 (white). Lightly pigmented (L* >65) buttock skin of 10 healthy humans was exposed to the indicated doses of UV-A1 irradiation. A, Changes in skin pigmentation were measured 24 hours after exposure (L* value). B, Skin samples (4 mm) were obtained 24 hours after exposure and were evaluated using real-time polymerase chain reaction to assess gene expression of matrix metalloproteinase 1 (MMP-1), MMP-3, type I procollagen (COL-I), and type III procollagen (COL-III). Data are presented as mean (SE) fold change. mRNA indicates messenger RNA. \( ^a P < .05 \) compared with no UV-A1 irradiation. \( ^b P < .05 \) when comparing response to different UV-A1 doses.
Further investigation is needed to determine why MMP induction requires 2 or more low-dose UV-A1 exposures. Apparently, the first exposure initiates cellular responses that facilitate MMP mRNA induction after subsequent exposures. Indeed, prior UV-A1 exposure may boost response to the next exposure, as seen not only with the second exposure but also with subsequent exposures beyond the second.

Furthermore, although the magnitude of MMP mRNA induction in response to low-dose UV-A1 irradiation is less than that after mildly erythemogenic doses of UV-B irradiation, our data suggest that repetitive exposure to low levels of UV-A1 irradiation, as commonly experienced in daily life, could promote photoaging. Indeed, accumulation of dermal damage from repeated low-level UV-A1 exposures over a lifetime likely com-

Figure 2. Effects of Repeated Daily Exposure of Lightly Pigmented Human Skin to Low-Dose UV-A1 Irradiation

Skin pigmentation was measured using a chromometer under the L* variable (luminance). Lightly pigmented (L* >65) buttock skin of 12 healthy humans was exposed to low-dose UV-A1 irradiation (20 J/cm²) 1, 2, 3, or 4 times at daily intervals. A, Changes in skin pigmentation were measured 24 hours after each exposure (L* value). B, Skin samples (4 mm) were obtained 24 hours after each exposure and were evaluated using real-time polymerase chain reaction to assess gene expression of matrix metalloproteinase 1 (MMP-1), MMP-3, type I procollagen (COL-I), and type III procollagen (COL-III). Data are presented as mean (SE) fold change. mRNA indicates messenger RNA.

*P < .05 compared with no UV-A1 irradiation.

**P < .05 when comparing response to different UV-A1 exposures.
promise structural support of the skin. As such, regular use of sunscreen may reduce the signs of photoaging. Additionally, we suggest that sunscreens need to filter all UV wavelengths, including UV-A1 irradiation, to protect against skin damage. The Food and Drug Administration has approved many sunscreen ingredients that filter UV-B irradiation and several that filter UV-A2 irradiation, but only 1 chemical sunscreen ingredient (avobenzone) and 1 physical sunscreen ingredient (zinc oxide) are approved to provide UV-A1 protection.

Based on previous studies in which human skin was exposed to UV wavelengths that include the UV-A1 range, we suggest that the MMPs induced by low-dose UV-A1 irradiation may be derived from the epidermis, and, to a lesser extent, the dermis. Additionally, we speculate that one of the chromophores that mediate this induction is DNA.

In lightly pigmented individuals, we also found that progressive skin darkening (tanning) in response to repeated low-dose UV-A1 exposures does not prevent UV-A1-induced collagenolytic changes. Thus, prior exposure to low levels of UV-A1 irradiation from natural sunlight or tanning lamps is unlikely to protect against dermal damage caused by subsequent exposures. One reason for this observation may be that melanin absorbs long wavelengths (eg, UV-A1 irradiation) less effectively than shorter wavelengths (eg, UV-B irradiation). This property of melanin further indicates that optimal sunscreen formulations should provide protection against UV-A1 wavelengths. This property of melanin also suggests that darkly pigmented individuals, who may have adequate UV-B protection, might still benefit from protection against UV-A1 irradiation.

In addition to MMP induction, we considered whether repeated low-dose UV-A1 irradiation may promote photaging by suppressing type I or III procollagen expression, as seen with UV-B irradiation. Type III procollagen is the precursor to type III collagen fibrils, which associate with type I collagen fibrils in the dermal ECM and can be cleaved by MMP-1. In contrast with higher doses, we found no suppression of type I or III procollagen expression after a single exposure or repeated exposures to low-dose UV-A1 irradiation, suggesting that a limited number of such exposures primarily affects breakdown, rather than synthesis, of collagen fibrils. It is possible, however, that more low-dose exposures (beyond the number performed in the present study) may suppress type I or III procollagen expression. Additionally, more low-dose exposures may maintain or further induce MMP-1 and MMP-3 while continuing to darken skin.

Finally, our observations have implications for UV-A1 phototherapy regimens used to treat excessive cutaneous collagen deposition. A previous study reported that substantial skin darkening after high-dose UV-A1 exposures (120-150 J/cm²) prevents sustained MMP induction, potentially limiting the efficacy of high-dose UV-A1 phototherapy for fibrotic skin. In the present study, we found that repeated low-dose exposures allow sustained upregulation of MMP-1 and MMP-3, likely because of less skin darkening compared with higher doses. Thus, our observations provide a rationale for using low-dose UV-A1 phototherapy, which is reported to soften fibrotic skin.

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veins showed through, making them the original “blue bloods.”

Shakespeare’s Othello skin of hers than snow.”

ized again and again in the Western canon: Shakespeare’s Othello.

The Castilian nobles cultivated skin so translucent their
to the world of style—and, unfortunately, to dermatology—may well
be the suntan. For centuries, in a fashion statement freighted with ra-

It was not until 1978 that the US Food and Drug Administration, amid
rising concern about skin cancer, standardized sun protection factor rat-
ings on sunscreen. Tanning booths arrived in the United States the same
year. Sadly, it was a head-to-head battle with a clear victor: since then,
of course, melanoma incidence has skyrocketed. Fortunately, there are
glimmers of pop-culture backlash, from ghostly Twilight vampires to the
ladies of Downton Abbey. In 2006, Cosmopolitan launched a “Practice
Safe Sun” campaign, with instructions on everything from self–skin checks
to “6 sexy sunscreen massages for your man.” Surely, even Chanel would
approve.

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