Photodynamic therapy improves the ultraviolet-irradiated hairless mice skin

Ana Elisa S Jorge[1,2,3], Michael R Hamblin[3,4,5], Nivaldo A Parizotto[6], Cristina Kurachi[2], Vanderlei S Bagnato[2]

[1]Programa de Pós-Graduação Interunidades Bioengenharia, Universidade de São Paulo, São Carlos, Brazil
[2]Instituto de Física de São Carlos/USP, Brazil
[3]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA
[4]Department of Dermatology, Harvard Medical School, Boston, MA
[5]Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA
[6]Universidade Federal de São Carlos, Brazil

ABSTRACT

Chronic exposure to ultraviolet (UV) sunlight causes skin photoaging. In light of this fact, photodynamic therapy (PDT) is an emerging modality for treating cancer and other skin conditions, however its response on photoaged skin has not been fully illustrated by means of histopathology. For this reason, the aim of this study was analyze whether PDT can play a role on a mouse model of photoaging. Hence, SKH-1 hairless mice were randomly allocated in two groups, UV and UV/PDT. The mice were daily exposed to an UV light source (280-400 nm: peak at 350 nm) for 8 weeks followed by a single PDT session using 20% 5-aminolevulinic acid (ALA) topically. After the proper photosensitizer accumulation within the tissue, a non-coherent red (635 nm) light was performed at 75 J/cm^2 and, after 14 days, skin samples were excised and processed for light microscopy, and their sections were stained with hematoxylin-eosin (HE) and Masson's Trichrome. As a result, we observed a substantial epidermal thickening and an improvement in dermal collagen density by deposition of new collagen fibers on UV/PDT group. These findings strongly indicate epidermal and dermal restoration, and consequently skin restoration. In conclusion, this study provides suitable evidences that PDT improves the UV-irradiated hairless mice skin, supporting this technique as an efficient treatment for photoaged skin.

Keywords: photodynamic therapy; UV-irradiated skin; hairless mice; histopathology; epidermal thickening; dermal collagen density.

1. INTRODUCTION

Frequent and cumulative sun exposure, mainly ultraviolet (UV) radiation, exacerbates aging of human skin. Shortly, there is an impaired epidermal hyperplasia, a dramatically decrease in dermal collagen fibers and dermal elastosis, which taken together lead to a clinical appearance that includes coarse wrinkles, sallowness, telangiectasia, skin laxity, irregular pigmentation, and in advanced stage, benign and malignant skin tumors[1]. Therefore, PDT is an approved technique that combines light, a photosensitizer (PS) and molecular oxygen in order to treat satisfactorily dermatological conditions, mainly non-melanoma skin cancer (NMSC), actinic keratosis (AK), and recently for photorejuvenation proposal. Addressing, in 2002 Ruiz-Rodriguez and coworkers[2] has named this technique as 'Photodynamic Photorejuvenation' according to its outstanding cosmetic effects on photoaged skin after treating AK with ALA-PDT. Since, many dermatologists have been performed this approach in order to repair the cutaneous photodamage[3-7], and in a consensus conference, the use of PDT in dermatology was well stated, in particular its pretreatment, the commonly used light sources and the proper 5-aminolevulinic acid (ALA) incubation time for cosmetical purposes[8]. In terms of what clinically expect from PDT, it can reverse the main photodamage features, namely sallowness, fine lines, tactile roughness and hyperpigmentation[9].

Nevertheless, since biopsy specimens are not always performed on clinical trials, there still is a necessity of further detailed histological explanation about PDT as a skin photorejuvenation technique in order to reinforce this tremendous dermatological solution. In light of this, the aim of our study is to analyze histologically the effects of PDT on UV-irradiated mice skin. Hence, the hairless mouse model for photoaging was exposed to UV radiation and,

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afterward, the PDT was performed with 5-aminolevulinic acid (ALA) as a photosensitizer precursor. At day 14, skin samples were collected and processed for histopathological analysis.

2. MATERIAL AND METHODS

2.1. Animals

An animal model for skin photoaging was used, the albino hairless mouse SKH-1 (Charles River Laboratories, Wilmington, MA). The animals had access to standard rodent chow and water ad libitum, and they were kept acclimating on the animal facilities 1 week prior to experiments. Thus, female 5-week-old mice were randomly allocated in two different groups: UV Group (2 UV-irradiated mice); and UV/PDT Group (3 UV-irradiated mice treated with one session of ALA-PDT). This study had been approved by the Subcommittee on Research Animal Care (SRAC) of the Massachusetts General Hospital (MGH) prior to the experimental beginning and was ethically conducted.

2.2. UV irradiation

Photodamaged skin was induced by an UV source consisted on a bank of 4 UVA fluorescent lamps (RPR-3500, Southern New England Ultraviolet, Branford, CO), in which wavelength emission was between 315 and 400 nm, with a peak emission at 350 nm. During 8 weeks the mice were exposed 5 days per week to a 5.4 J/cm^2 daily suberythemal dose, assessed by a calibrated spectroradiometer SPR-01/ 235-850 nm (Luzchem Research Inc., Ontario, Canada).

2.3. Topical ALA-PDT

Prior to PDT, a tape-stripping technique was performed in order to ensure a properly ALA cream penetration, in which we removed part of the *stratum corneum* with small pieces of tape (Scotch Brand, 3M, USA) placed 8 times right on skin, pulling it out slightly in order to cause no inflammatory reaction[10]. The PS precursor was prepared with ALA powder (Sigma-Aldrich, Saint Louis, MO) in a 20% concentration on an over the counter emulsion (Eucerin Lotion, Beiersdorf Inc., Norwalk, CT) with EDTA 1 mM (Bio-Rad Laboratories, Life Science Group, Hercules, CA) as an iron chelator, and 3% DMSO (Sigma-Aldrich, Saint Louis, MO) as a penetration enhancer[11]. Then, this prepared solution was applied on the dorsal mice skin in an 8-mm diameter and occluded during the 4-hour incubation time to guarantee a satisfactory protoporphyrin IX (PpIX) accumulation. As a light source, a halogen non-coherent lamp with a 635-nm-fiber optic probe was used ensuring a total light dose of 75 J/cm^2 reaching the back of the mice, assessed by a power meter PM100D equipped with a S310C sensor (Thorlabs, Newton, NJ). Since PDT causes pain, this procedure was handled with ketamine and xylazin as anesthesia procedure.

2.4. Histopathological assessment

For both groups, skin samples were excised at day 14 after treatment, when clinical healing was evident for the UV/PDT group whereas the UV group served as control. The biopsy specimens were fixed in formaldehyde, included in paraffin blocks and their sections stained with hematoxylin-eosin (H&E) for general view and epidermal thickness and Masson's Trichrome (MT) for dermal collagen content assessments. Those microscopic images were obtained by a digital slide scanner, NanoZoomer 2.0RS (Hamamatsu Corp., Bridgewater, NJ) and, in terms of quantification, the epidermal thickness was defined by the mean distance (10 measurements collected each 100 μm within one H&E section) from the basal membrane to the *stratum corneum* measured on the NanoZoomer Digital Pathology software (NDP.view version 1.2.36).

2.5. Statistics

Epidermal thickness outcome from both groups are presented as mean ± standard deviation (SD) and its statistical analysis was performed by the nonparametric Kolmogorov-Smirnov test with a 95% confidence level.

2.6. Results

Clinically, mild phototoxic reaction was seen in all animals at the UV/PDT group followed treatment, represented by slight inflammatory skin reaction in variable degrees. In general, 24 hours after PDT the animals presented no significant signs. Just at hour 48, it was possible to note mild erythema and edema. At day 7, ulceration, crusting and peeling were observed. At day 10, only a discrete erythema could be seen in a wound healing process. At day 14, the

dorsal skin was completely recovered in all mice (Figure 1). The animals from UV group remained with wrinkles perpendicularly to the long axis of the dorsum and sagging skin.

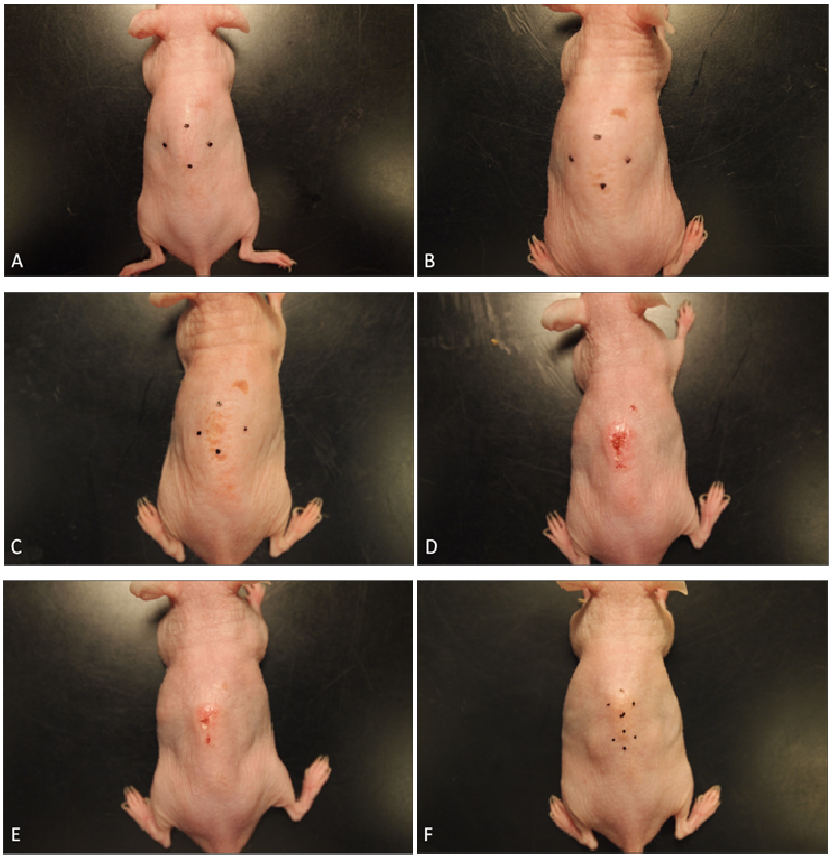

Figure 1. UV-irradiated mouse treated with PDT (light dose 75 J/cm^2) clinical follow-up. Time points: immediately after PDT: no findings (A); 24h: no findings (B); 48h: mild erythema (C); day 7: skin crusting and erythema (D), day 10: wound healing (E); and day 14: skin recovery (F), followed by biopsy.

H&E staining illustrates epidermal thickening of the UV/PDT group when compared to the UV group (Figure 2). In terms of quantification, the animals exposed to UV radiation and treated with PDT showed an epidermis (50 ± 13) significantly thicker than the animals exposed only to UV radiation (32 ± 5) (Figure 3).

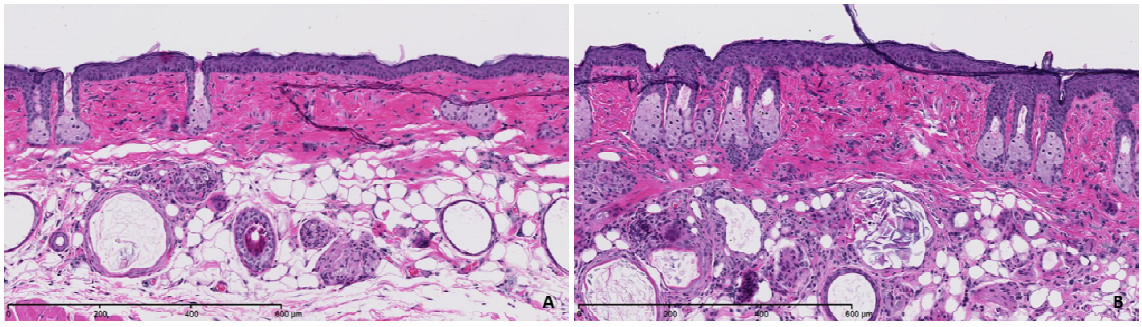

Figure 2. Representative microscopic images of UV (A) and UV/PDT (B) groups at day 14 after PDT. Note a significant epidermal thickness of the mouse treated with PDT when compared to the UV-irradiated age-matched one (original magnification 100x, H&E).

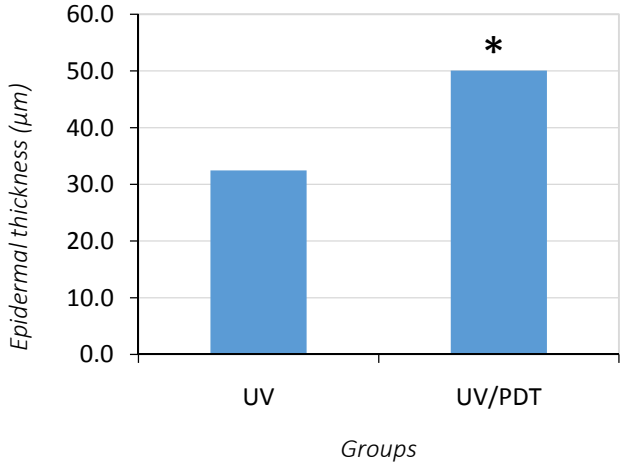

Figure 3. Epidermal thickness of the UV and UV/PDT groups. Means between groups are significantly different (d = 0,78).

The microscopic images from MT-stained sections show an increase in dermal collagen fibers on the UV/PDT group when compared to the UV group (Figure 4).

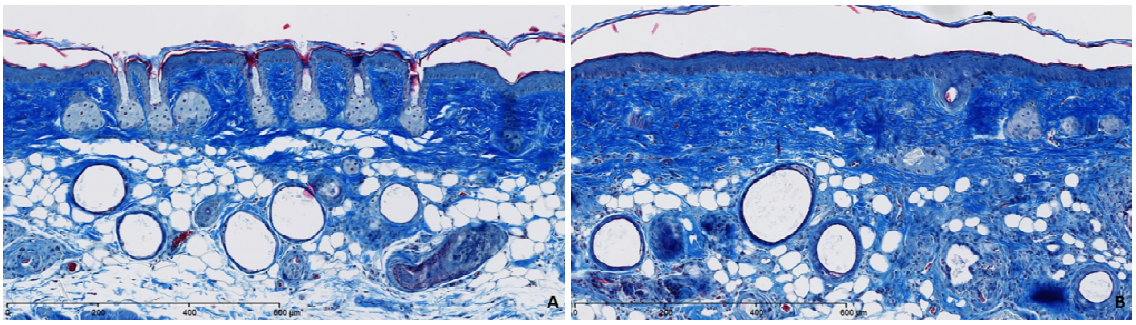

Figure 4. Representative microscopic images of UV (A) and UV/PDT (B) groups at day 14 after PDT. Note a dermal collagen deposition following PDT (B) when compared to the UV-irradiated age-matched one (original magnification 100x, MT).

3. DISCUSSION

PDT treats photoaged skin with outstanding results and many clinical trials have been shown its performance. Moreover, there is an emerging trend in order to investigate different light sources, especially intense pulsed light (IPL), and pretreatment approaches that intensify the PDT effect in actinically damage skin. Addressing, Togsverd-Bo et al. have used ablative fractional laser resurfacing (AFXL) prior to PDT in order to create microholes within the skin that enhanced the methyl aminolevulinate (MAL) delivery, intensifying the PDT efficacy[12].

Although there are sufficient clinical studies in this field, we still need further histopathological comprehension in order to establish this successful photorejuvenation approach. For instance, Issa and co-workers[13] have treated 14 women with two sessions of MAL-PDT using a 635-nm light-emitting diode (LED) with a fluence of 37 J/cm^2. Afterward, they clinically assessed the overall outcome and histologically evaluated the collagen content after 3 and 6 months. Specifically, the increase in the collagens fibers was significantly higher on the sixth month, indicating photorejuvenation. In the same line, another study[14] assessed the induced histological changes of 14 asian patients treated with ALA-PDT. For this, they performed skin biopsies before and 1 month after the treatment and, as a result, observed that the total collagen volume and type I and III procollagen expressions significantly increase.

The hairless mouse is a well-established animal model to assess the histological features of photoaged skin. Experimental studies performed on SKH-1 mice with a long-term UV exposure protocol show epidermal hyperplasia, sebaceous gland enlargement, and collagen damage, which taken together resemble the UV-damaged human skin[15]. For this reason, this investigation assessed the effect of ALA-PDT on UV-irradiated hairless mice skin. As a result of the photoaging protocol, wrinkles and skin sagging were diagnosed and, histologically, epidermal hyperplasia and acanthosis, and connective tissue damage (decrease in collagen content) were features observed in all mice at the end of the UV protocol, which was in accordance with many experimental photoaging studies[16-18].

Although there are many experimental investigations that have been focused on UV photocarcinogenic protocols, mainly whether PDT could treat skin tumour[19, 20] and act as a photochemoprevention of these lesions[21-23], few studies illustrate PDT on photoaged mice skin. Hence, this study demonstrates that the chronically UV-irradiated mice that underwent a single session of ALA-PDT presented epidermal thickening and new collagen fibers deposition. These skin remodeling findings are in agreement with Bruijn and co-workers[24] in a study of the PpIX spatial distribution and its correlation with histological response in normal hairless mice. They showed dermal regions with variable inflammatory responses after ALA and MAL-PDT, in which later could be seen a skin remodeling process. Furthermore, they observed a significant decrease in sebaceous gland, which is in agreement with our results too (data no shown).

In another study, Choi and co-workers[25] have seen a skin improvement with MAL-PDT in haired mice, which included epidermal thickening at day 8 and significant collagen fibers density at day 12 after MAL-PDT. Moreover, they investigated the molecular pathways induced by MAL-PDT, namely matrix metalloproteinases (MMPs) and procollagen expressions in early and later time points, respectively. These molecules are crucial for remodeling the dermal extracellular matrix, mainly collagen, which is related with skin rejuvenation. These molecular and histological data also corroborate with our study, explaining the newly formed collagen fibers.

In conclusion, the present study histologically reinforces the PDT improvement in photodamaged skin, highlighting the substantial benefit of this technique for aesthetic proposals.

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