Photodynamic therapy induces epidermal thickening in hairless mice skin: an optical coherence tomography assessment

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ABSTRACT

Photodynamic therapy (PDT) promotes skin improvement according to many practitioners, however the immediately *in vivo* assessment of its response remains clinically inaccessible. As a non-invasive modality, optical coherence tomography (OCT) has been shown a feasible optical diagnostic technique that provides images in real time, avoiding tissue biopsies. For this reason, our investigation focused on evaluates the PDT effect on a rodent model by means of OCT. Therefore, a normal hairless mouse skin has undergone a single-session PDT, which was performed with topical 5-aminolevulinic acid (ALA) cream using a red (630 nm) light emitting diode (LED) which reached the light dose of 75 J/cm^2. As the optical imaging tool, an OCT (930 nm) with axial resolution of 6.0 microns in air was used, generating images with contact to the mouse skin before, immediately after, 24 hours, and 2 weeks after the correspondent procedure. Our result demonstrates that, within 24 hours after ALA-PDT, the mouse skin from the PDT group has shown epidermal thickness (ET), which has substantially increased after 2 weeks from the treatment day. Moreover, the skin surface has become evener after ALA-PDT. Concluding, this investigation demonstrates that the OCT is a feasible and reliable technique that allows real-time cross-sectional imaging of skin, which can quantify an outcome and predict whether the PDT reaches its goal.
Keywords: optical coherence tomography; non-invasive technique; *in vivo* assessment; photodynamic therapy; topical 5-aminolevulinic acid; hairless mouse skin; epidermal thickness.

1. INTRODUCTION

Photodynamic therapy (PDT) is an effective modality that has been reaching inspiring outcomes in dermatological conditions. Superficial basal cell carcinoma (BCC) and actinic keratosis (AK) are skin disorders commonly treated by PDT[1,2], however its on-time assessment, which could define the treatment end-point, remains clinically untouchable. Thus, a non-invasive diagnosis is of extreme importance.

Optical coherence tomography (OCT) is a relatively new optical imaging technology that shares the same principle as an ultrasound technique, except the fact that OCT uses light (infrared radiation) to map the sample and it also gains in term of spatial resolution[3]. As a diagnostic tool in dermatology, OCT imaging can provide real-time *in vivo* cross-sectional images with no need of skin biopsy. Namely, ultraviolet (UV)-irradiated skin and its skin morphology alterations, AK, and non-melanoma skin cancer (NMSC) have been assessed by OCT technique according to some investigators[4-6].

This investigation illustrates a PDT follow-up in SKH-1 hairless mice skin assessed by OCT technology. Hence, ALA-PDT was performed on normal skin using a 630-nm LED as a light source with a fluence of 75 J/cm^2 and a 4-hour incubation time. As a result, the OCT system displayed the mice skin structure, providing qualitative and quantitative outcomes.

2. MATERIAL AND METHODS

2.1. Animals

A well-established animal model for dermatological investigation was used, the albino hairless mouse SKH-1 (Charles River Laboratories, Wilmington, MA). The 8-week-old mice were randomly allocated in different categories according to their correspondent procedure as follow: Control group (n=4): animal treated neither with photosensitizer

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nor light delivery; LED group (n=4): animal treated only with the 630-nm LED (with the same parameters as the PDT group); and PDT group (n=4): animal treated once with topical ALA-PDT.

2.2. Topical ALA-PDT

PDT was performed using 20% ALA cream as the photosensitizer precursor. Before the cream application, a tape-stripping technique was performed on all mice (control, LED and PDT groups) in order to partially remove their stractum corneum, which allows a satisfactory cream penetration on the PDT group, and assuring that this protocol was the same for others animals to maintain the same variability. For this procedure, a commercially tape (Scotch, 3M, Brazil) was cut in 8 pieces and pressed on to the dorsal mice skin and removed right after. Then, the photosensitizer precursor was topically applied on the skin (10 mm diameter) and occluded for 4 hours in order to ensure a properly protoporphyrin IX (PpIX) accumulation. As a light source, we used a red LED (630 nm), 125 mW/cm^2 of fluence rate, reaching a light dose of 75 J/cm^2. Since PDT causes pain, the animals were anesthetized with ketamine and xylazine intraperitoneally before the procedure.

2.3. OCT imaging

For the images, a spectral radar OCT system (OCP930SR, Thorlabs Inc, Newton, NJ) was used, providing images with 2000 x 512 pixels at 4 frames per second. The radiation source was a superluminescent diode centered at 930 nm of wavelength, with axial resolution of 6.0 microns in air. OCT images were acquired with contact to the sample in order to ensure no movements and with a maximal penetration depth of 1.6 mm in mouse skin. Hence, the time points of assessment were defined as follow: before, immediately after, 24 hours and 2 weeks after the correspondent procedure. Formerly, they were check in a scan view in order to detect possible clinical findings, then all mice were assessed by the OCT system to obtain skin cross-sectional images in order to quantify the thickness of their epidermis.

3. RESULTS

3.1. Immediately after PDT

The animals presented no clinical findings immediately after PDT. OCT images detected no significant difference among the groups, however it was possible see a slight epidermal thickening on the PDT group.

3.2. 24 hours post-PDT

Clinically, all mice that underwent the single-session ALA-PDT presented mild edema on the region that was applied the cream, as expected. In contrast, neither erythema nor edema were seen on the control and LED groups. Analyzing the OCT images, it was possible to visualize a moderate thickening of the animals on the PDT group.

3.3. 2 weeks post-PDT

At this time point, clinical findings that have been detected during the follow-up, namely erythema, edema, ulceration and crusting, were not seen anymore, which characterize the mice skin recovery after PDT treatment. Only a slight scar sign was perceived (Figure 1). The OCT images are shown in the Figure 2.

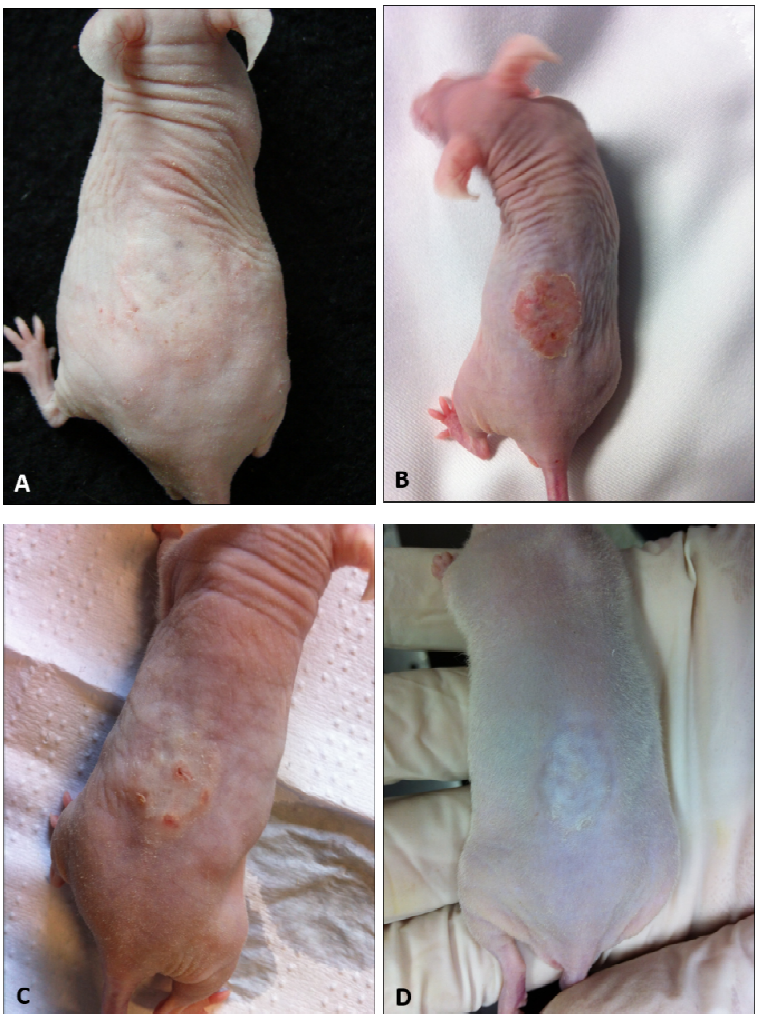

Figure 1. Clinical findings of a representative mouse at different time points: 24 hours (A); 4 days (B); 1 week (C); 2 weeks (D) after the ALA-PDT procedure.

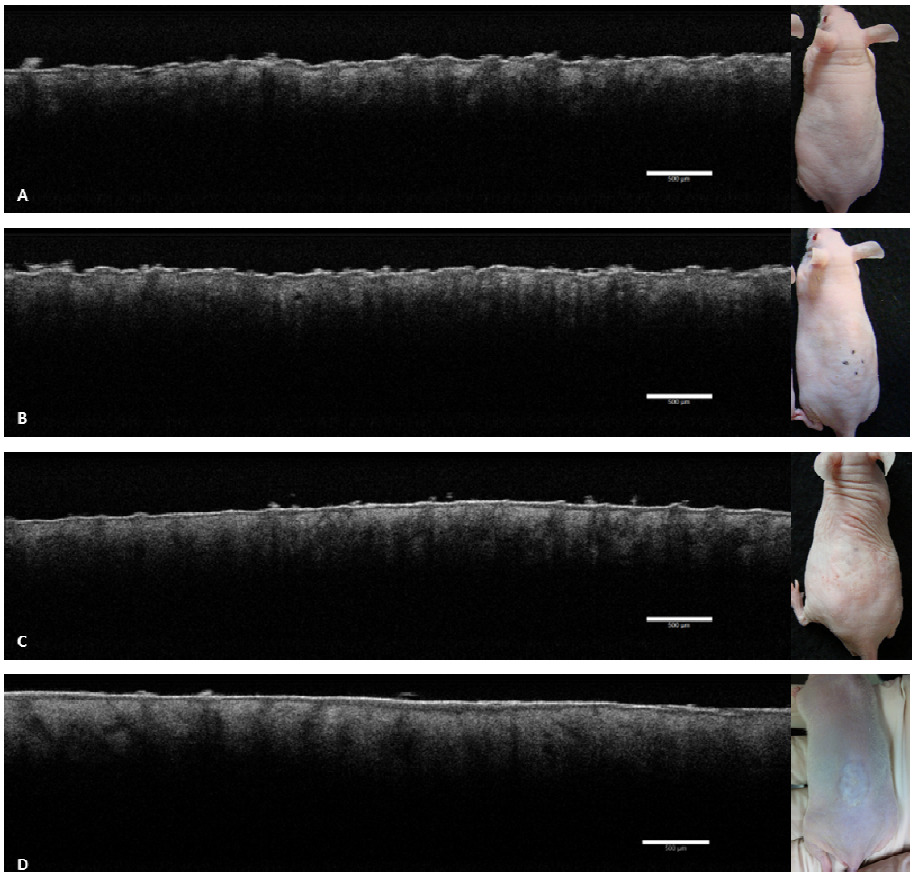

Figure 2. OCT images of the same mouse showed above at different time points: before (A); immediately after (B); 24 hours (C); and 2 weeks after ALA-PDT (D) [scale bar: 500 μm].

Compared to the control and LED groups, OCT examination revealed that the epidermis of all mice from the PDT group appeared thicker immediately after the procedure (Table 1). We could also note a flattened pattern on the PDT group from this time point on, which cannot be seen on the others groups. The Figure 3 illustrates these findings.

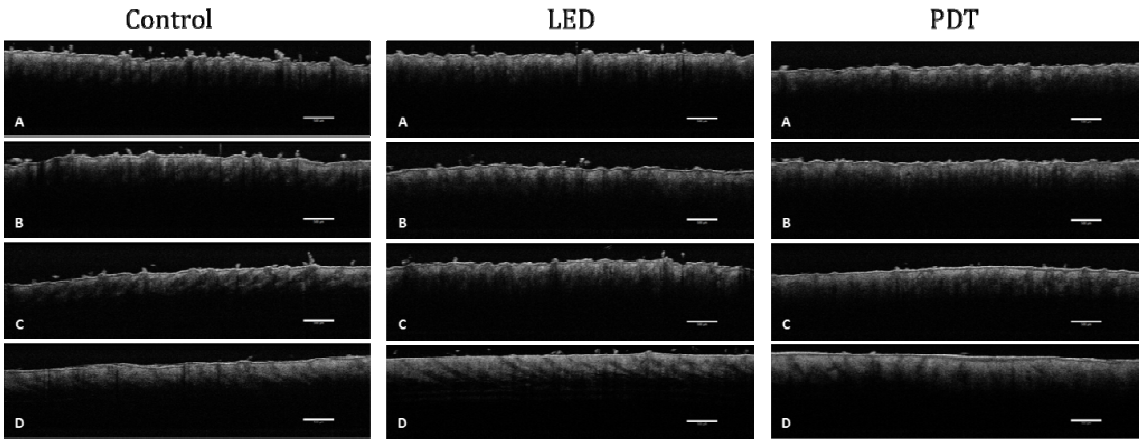

Figure 3. Representative OCT images of all groups at the different time points: before (A); immediately after (B); 24 hours (C); and 2 weeks after ALA-PDT (D) [scale bar: 500 μm].

Table 1. ET expressed as mean and standard deviation (SD) for all groups.

	Epidermal Thickness (μm)			
Groups	*Before*	*Imediately after*	*24 hours after*	*2 weeks after*
Control	41 ± 8	51 ± 10	44 ± 6	38 ± 5
LED	49 ± 8	55 ± 7	41 ± 7	36 ± 5
PDT	48 ± 7	63 ± 10	54 ± 7	88 ± 18

4. DISCUSSION

The OCT is substantially raising interests among scientists and dermatological practitioners due to its reliable, non-invasive and real time *in vivo* assessment[3]. This is possible since it provides cross-section imaging in situ with no need of biopsies. Specifically on ET, Gambichler and co-workers have investigated in a pilot study[7] the ET from the upper back of 16 healthy subjects. In brief, they measured the distance from the entrance to the second peaks of the A-scan, then compared to histological routine. They found no correlation between OCT and histopathology assessment, indicating that the OCT algorithm performed was not a valid method for measuring the ET. In the same vein, however calculating the ET from the OCT images, Mogensen and colleagues[8] have imaged 20 healthy volunteers at 12 anatomical locations in order to measure the thickness of epidermis. As a result, they assessed the skin morphometry by means of OCT technique, which allowed them to correlate the images according to gender, age and skin color.

Regarding the PDT response on skin, epidermal thickening is one of the outcomes detected by histopathological assessment. Buggiani and colleagues[9] have analyzed by means of OCT imaging preliminary data about a PDT response from 25 female patients treated for photodamaged skin. Specifically on dermo-epidermal junction, they noted the reappearance of the undulated pattern after 45 days from the last treatment of a 4-session regimen. Despite this clinical study, the potential role of the OCT technology in PDT monitoring the dermatologic field is still under investigation.

For this reason, we carried out this study using morphometric quantification from the OCT images in order to measure the ET after PDT, which interestingly is among the few studies reporting OCT images following PDT on skin. As described in the literature[10], hairless mice properly resemble the human skin, allowing directly skin manipulation and performance of *in vivo* light-based imaging techniques since they develop neither fair nor pigments on skin. Consequently, this study shows the epidermal response from a single-session PDT on normal mice skin by means of OCT. We observed that the animals treated with ALA-PDT presented a thicker epidermis when compared with the control animals. This is in accordance with a study in which the researchers used healthy hairless mice skin in order to investigate the effect of multi-session of low dose ALA-PDT by means of histopathology and second harmonic generation (SHG). As a result, they proved that after a 12-week period from the last session of ALA-PDT, the epidermis became substantially thicker when compared to the control group[11].

Taking this together, we can conclude that the OCT technology is a reliable and on-time optical technique that may clinically monitor the outcomes from a PDT procedure.

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