Photodiagnosis and Photodynamic Therapy 28 (2019) 216–220

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Laser-assisted MAL-PDT associated with acoustic pressure wave ultrasound with short incubation time for field cancerization treatment: A left-right comparison

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ARTICLE INFO

Keywords:
Actinic keratosis
Ablative fractional laser assisted photodynamic therapy
Laser-assisted drug delivery
Skin field cancerization
Incubation time

ABSTRACT

Background: Laser-assisted MAL-PDT has been reported to increase the effectiveness of conventional PDT. Nonetheless, clinical effects of this association when reducing MAL incubation time is poorly discussed. Furthermore, the association of acoustic pressure wave ultrasound with laser-assisted MAL-PDT with short incubation time for field cancerization had not been reported before.

Objectives: To compare clinical effects of ablative fractional laser-assisted MAL-PDT associated with acoustic pressure wave ultrasound (IMPACT US) with 1-hour incubation time and conventional MAL-PDT for skin field cancerization on the forearms, as well as the impact on safety and tolerability.

Methods: Fifteen patients with 638 AK (grade I-III) with field cancerized-skin on the forearms were enrolled in this left-right trial. Two protocols were randomly chosen. One side was treated with conventional MAL-PDT, whereas the other with laser-assisted MAL-PDT associated with acoustic pressure wave ultrasound with 1-hour incubation time. Actinic keratoses were quantitively measured, and the other signs of sun-damaged skin, like pigmentation and texture, in field cancerized skin were qualitatively evaluated before and after six months. Side effects were assessed subjectively during the procedure and one week after.

Results: All patients completed the study. At six months after treatment, both protocols reduced the number of AK (72%; CO2 + PDT, and 65%; MAL-PDT). The difference between these two protocols was not statistically significant ($p = 0.77$). The improvement of pigmentation and texture of field cancerized skin was more significant on the side treated with laser-assisted MAL-PDT associated with acoustic pressure wave ultrasound. Both protocols were well tolerated and without significant difference in adverse events.

Conclusion: Laser-assisted MAL-PDT using CO2 laser and acoustic pressure wave ultrasound with short incubation time of 1 h was as effective as conventional MAL-PDT for field-cancerized skin with actinic keratosis in forearms with better cosmetic outcome.

1. Introduction

Actinic keratosis (AK) it is the most common precancerous cutaneous lesion, affecting predominantly older and fair-skinned individuals, with prevalence in European countries, such as Spain and Austria reported to be approximately 30% in 2011. Prevalence may vary between 0.52% in China and up to 59% in Australia [1]. The prevalence of AK has risen in the past decades, is higher in warmer climates where populations are exposed to more UV radiation [2].

Although the actual risk of an individual AK progressing to an invasive squamous cell carcinoma (SCC) is unclear, estimations vary from as low as 0.1% to as high as 20%, and it is higher in the presence of multiple lesions of AK [2].

Frequently, visible AKs are associated with adjacent subclinical lesions, which has led to the use of the concept field-cancerized skin [3–6]. Recently, it was clinically defined as the anatomical area with or adjacent to AK and visibly sun damaged skin identified by at least two of the following signs: telangiectasia, atrophy, pigmentation disorders, and sandpaper. It is unclear if a visible AK lesion is needed for field cancerization [7].

Photodynamic therapy (PDT) is a widely applied therapeutic modality for AK, some cases of superficial basal cell carcinoma (BCC) and

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https://doi.org/10.1016/j.pdpdt.2019.08.034
Received 9 February 2019; Received in revised form 28 August 2019; Accepted 30 August 2019
Available online 31 August 2019

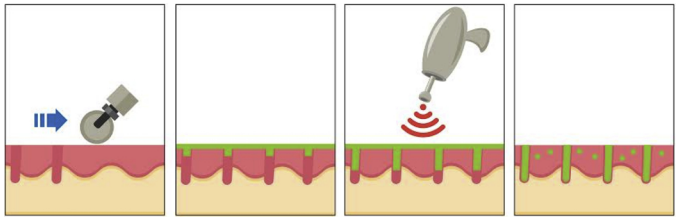

Fig. 1. laser-assisted PDT and IMPACT ultrasound for drug delivery. A single pass of fractional CO2 laser with a roller-type ferrule is performed in the whole area, and immediately after laser, MAL 20% is applied and the acoustic pressure wave ultrasound propels MAL molecules with an acoustic pressure.

Bowen's disease [8–13]. PDT induces cytotoxicity in the proliferative cells by target illumination after application of a photosensitizing agent [8,14].

Lesion thickness is one of the essential factors for treatment outcome, and it's well known that conventional PDT is less effective for hypertrophic AK [8,15]. Therefore, pretreatment with ablative CO_2 fractional laser (AFXL) removes stratum corneum in fractions, and partitions occur between drug and epidermis or dermis and facilitate the delivery and better distribution of topical photosensitizer deeply into the skin [3,16].

Laser-assisted MAL-PDT has been reported to increase MAL penetration and to improve the effectiveness of the treatment [3,15,17,18]. Nevertheless, few studies evaluated the efficacy of this association when shortening MAL incubation time [3,14].

Low-frequency sonic waves (IMPACT US) has been applied to propel molecules in a particular direction with acoustic pressure and was reported to increase treatment effectiveness for areata alopecia [19] and palmar hyperhidrosis [20]. However, the association of laser-assisted MAL-PDT with IMPACT US has not been reported before.

The main objective of this study is to compare the clinical efficacy of laser-assisted PDT associated with acoustic pressure wave ultrasound with short incubation and conventional PDT for field cancerization on the forearms, as an intraindividual left-right comparative trial.

2. Materials and methods

This study was conducted between March 2016 and July 2018 at the Department of Clinical Medicine (Dermatology) at Antonio Pedro University Hospital (HUAP), Fluminense Federal University (UFF), Rio de Janeiro, Brazil. Inclusion criteria were patients with symmetric and more than five AKs on photodamaged skin on each forearm, aged 40–70 years. Exclusion criteria were pregnancy, immunosuppression, photosensitivity, allergy to any constituents of MAL cream, local or systemic active infectious disease and any AK treatment of the area in the previous six months. All patients were provided with verbal and written information on the nature of the study and signed informed consent was obtained in advance. The study was approved by the institutional review board of Fluminense Federal Fluminense University and following the Declaration of Helsinki.

2.1. Study design and protocol

This study was an open, randomized, intraindividual right-left comparative trial. AKs were quantitative measured, whereas signs of field-cancerized skin (pigmentation and texture) were qualitatively graded using a quartile scale (0 = less than 25%, 1 = 25%–50%, 2 = 51%–75%, 3 = more than 75% improvement) as described by Alster et al. [21] and Issa et al. [22]. The clinical dermatological exam was performed before and after a 6-month treatment for all 15 patients.

The forearms were randomized to receive either conventional MAL-PDT (3 -h incubation time) or laser-assisted PDT associated with acoustic pressure wave ultrasound with 1 -h incubation time using Random App. Only one session of each protocol was performed, in the private investigator's office. Clinical and photographic evaluations were carried out at HUAP.

Before treatment, the forearms were cleansed with aqueous chlorhexidine followed by a light curettage in all treated area. No topical or infiltrative anesthesia was used. On the laser-assisted PDT associated with acoustic pressure wave ultrasound protocol, a single pass of fractional CO_2 laser (Pixel CO_2, Alma Lasers, Israel) was performed. The CO_2 laser consisted of a roller-type ferrule, composed of one row with seven fractionating pins (7 × 1), 60 W, 15 mJ/pixel, 3-mm spacing between ablation zones, density < 1%. Immediately after laser, MAL 20% (PDT Pharma, Brazil) was applied with 1 mm thickness over the whole area. The IMPACT ultrasound (Alma Laser Israel) was applied over MAL. It produces low-frequency sonic waves, designed to propel molecules in a particular direction with acoustic pressure (Fig. 1). The last step was the occlusive dressing, which was left for three hours in the conventional PDT protocol, and for one hour in the laser -assisted PDT associated with acoustic pressure wave ultrasound.

After the incubation period, occlusive dressing and residual cream were removed with saline-soaked gauze and the forearms were illuminated with red light (632 nm), a Light-Emitting Diode (LED - Aktilite CL 128; PhotoCure ASA), which was placed 5 cm away from the skin surface for 8 min until a total of 37 J/cm2 dose of light had been delivered. All patients wore protective goggles during illumination.

Side effects, pain, edema, erythema, and crusts were subjectively evaluated in mild, moderate and severe during the procedure and seven days after treatment.

2.2. Statistical analysis

The Wilcoxon test was performed to compare the number of AK lesions in each arm before the procedures to avoid treatment bias. The photodamaged signs and number of AKs (grades II-III) before and after treatment were analyzed using Fisher's exact test and Chi-squared test, respectively. The Mann-Whitney test was used to compare the results between the two different treatments.

3. Results

3.1. Patient demographics at baseline

Fifteen patients with a total of 638 AK lesions were enrolled and completed the study. The study group comprised of 15 patients, seven male and eight female. The mean age was 61 ± 9.8 years; among patients, eight were classified as phototype II, and seven as phototype III

3.2. Efficacy

- *Actinic Keratosis*

Both treatments, the laser-assisted PDT associated with acoustic pressure wave ultrasound protocol and the conventional PDT protocol, were effectively similar in reducing the number of AK lesions.

Nonetheless, the use of the laser associated with acoustic pressure wave ultrasound with reduced incubation time (1 -h) promoted the same benefit when compared to conventional PDT treatment with 3 h incubation (Table 1). The decrease in AK lesions after treatments was observed (PDT: -65%, p = 0.0011; laser-assisted PDT associated with acoustic pressure wave ultrasound: -72%, p = 0.0001) (Fig. 2).

Table 1
Comparison between pre and post-treatment AK numbers. The use of the laser associated with acoustic pressure wave ultrasound with reduced incubation time (1 -h) promoted the same benefit when compared to conventional PDT treatment with 3 h incubation.

ID	No. of AK pre-treatment (PDT)	No. of AK post-treatment (PDT)	% of Improvement (PDT)	No. of AK pre-treatment (laser + US PDT)	No. of AK post-treatment (laser + US PDT)	% of Improvement (laser + US PDT)
1	34	8	76.5%	23	8	65.2%
2	13	2	84.6%	15	4	73.3%
3	15	7	53.3%	30	9	70.0%
4	8	1	87.5%	7	0	100.0%
5	8	2	75.0%	13	4	69.2%
6	6	0	100.0%	6	2	66.7%
7	30	13	56.7%	25	9	64.0%
8	13	11	15.4%	10	7	30.0%
9	38	31	18.4%	31	15	51.6%
10	22	6	72.7%	28	8	71.8%
11	25	9	64.0%	25	7	72.0%
12	7	1	85.7%	6	1	83.3%
13	28	7	75.0%	31	3	90.3%
14	43	8	81.4%	56	11	80.4%
15	24	2	91.7%	18	2	88.9%

ID: identification; AK: actinic keratosis; Wilcoxon test.

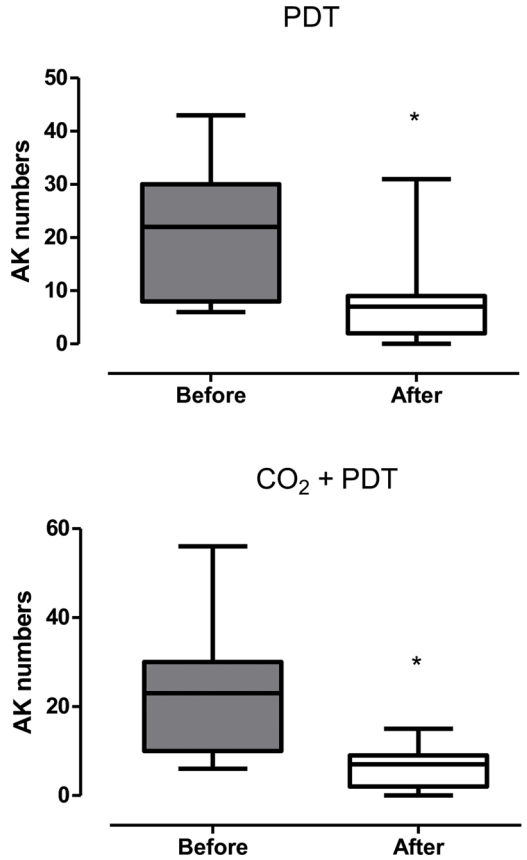

Fig. 2. Comparison between the AK numbers paired according to the treatment used. The decrease in AK lesions after treatments was observed (PDT: -65%, p = 0.0011 and laser-assisted PDT associated with acoustic pressure wave ultrasound: -72%, p = 0.0001).

The AK Olsen grade II-III together had a higher reduction after laser-assisted PDT associated with acoustic pressure wave ultrasound (76.4%) than after conventional MAL-PDT (55.2%, $p < 0.05$) (Table 2).

- *Texture and Pigmentation in Field-Cancerized skin*

The signs of sun-damaged skin, such as pigmentation and texture, in the field-cancerized skin, treated with laser-assisted PDT associated with acoustic pressure wave ultrasound, showed more considerable improvement compared with the other side, treated with conventional PDT. (p = 0.0001) (Table 3).

Table 2
Association between lesion number grade II-III reduction and the treatment used. The AK Olsen grade II-III together had a higher reduction after laser-assisted PDT associated with acoustic pressure wave ultrasound.

Grade II-III	Laser + US PDT	PDT	Total	p-value
Before	72	58	130	0.0479
After	17	26	43	
Total	89	84	173	

AK: actinic keratosis; Chi-squared test.

Table 3
Grade of photoaging improvement. The signs of sun-damaged skin in the field-cancerized skin, treated with laser-assisted PDT associated with acoustic pressure wave ultrasound, showed more considerable improvement.

Grade of improvement	Treatment		p-value
	laser + US PDT	PDT	
Minimal improvement	0 (0.0%)	0 (0.0%)	0.0001
Moderate	2 (13.3%)	3 (20.0%)	
Significant	4 (26.6%)	12 (80.0%)	
Great	9 (60.1%)	0 (0.0%)	
Total	15 (100.0%)	15 (100.0%)	

Fisher's exact test.

3.3. Safety and tolerability

Minimal pain was reported during the laser-assisted PDT associated with acoustic pressure wave ultrasound. All patients experienced mild pain and burning sensation during PDT illumination with complete resolution within 3 h. All patients had mild-to-moderate erythema, edema, and crusts after seven days, with complete resolution after 15 days. Clinical evaluation showed no scarring on follow-up evaluation. There were no significant differences in adverse events between the protocols.

4. Discussion

PDT is an excellent non-invasive therapeutic option with a great

cosmetic outcome for the treatment of AK and field cancerization [4,9,10,15,23]. In the last years, several techniques have been described to increase absorption and create a more uniform distribution of the photosensitizer in PDT [15–18,24]. However, the prolonged time of administration of the standard PDT, with 3 h of occlusion, makes the procedure quite inconvenient for both the patient and the dermatologist.

The use of laser-assisted PDT has been demonstrated in recent years as a promising technique to improve the efficacy of PDT in the treatment of field cancerization and AK [3,16,24–26]. Togsverd-Bo et al. were the first to demonstrate the superiority of AFXL-assisted PDT vs. conventional PDT on AK and field cancerization on scalp and face treatments, but with standard incubation time [26]. Helsing et al. compared the use of AFXL-assisted PDT with AFXL alone for AK treatment on organ transplant patients. A complete response was significantly higher with AFXL-assisted PDT (73%) comparing to AFXL alone (31%) [27]. Song et al. [18] compared efficacy in treatment with PDT associated with conventional PDT in the treatment of 271 AK. All degrees of AK, especially moderate and thick lesions, showed better results after AFXL-assisted PDT. In addition to this satisfactory response, they also found a lower recurrence rate in AK treated with PDT associated with the laser. Our results are consistent with this previous finding, with a more significant response to laser-assisted associated with acoustic pressure wave ultrasound PDT in AK grades II and III togheter.

Despite several studies had demonstrated efficacy in the association of fractional ablative laser with PDT, there are few reports of short incubation time [3,24,28]. Jang YH et al. compared AFXL-assisted PDT to ALA-PDT with a reduced incubation time of 90 min and MAL-PDT with 70 min for the treatment of AK, showing effectiveness in both groups [29]. Song SH [18] compared AFXL-assisted MAL-PDT to treat facial AK with a reduced incubation time of 90 min with conventional MAL-PDT. The clearance rate was similar in both groups. These results are similar to ours, but in our patients, the treatment was performed on the forearms, a region where thick lesions are more common than the face. To our knowledge, our study is the first to report the use of acoustic pressure wave ultrasound associated with laser-assisted MAL-PDT with short incubation time for field-cancerized skin.

The most used MAL concentration is at 16% (Metvix, PhotoCure SA, Norway) [3,13,23,30–32]. The use of MAL 20%, with 3 -hs incubation for superficial and nodular BCC was reported with a clearance rate at 76.5% [33,34]. In our study, we use MAL 20% in both protocols, and until now it seems to be the first study using this MAL 20%, produced in Brazil for field cancerization treatment,

Our results showed that the association of CO_2 laser and acoustic pressure wave ultrasound with PDT with MAL 20% could be used with incubation time reduced to one third (1 h), with a clinical response similar to conventional PDT. According to our findings, there was no statistically significant difference in the number of AK lesions in the treated field-canerized skin but a better improvement on texture and pigmentation was observed on the side where the laser-assisted PDT associated with acoustic pressure wave ultrasound was performed.

5. Conclusion

Our results demonstrated that laser-assisted PDT associated with acoustic pressure wave ultrasound associated with IMPACT ultrasound and short incubation time is equally effective as conventional MAL 20% - PDT for AK lesions on the forearms. The improvement of texture and pigmentation was more evident on the protocol with laser and IMPACT ultrasound, maintaining safety.

Further studies are necessary to evaluate other parameters of CO_2 fractional laser and IMPACT ultrasound, as well as the possibility of shortening MAL incubation time even more.

Disclosures

Maria Claudia Issa: Consultant Doctor – Alma Lasers
The medicaments were donated by São Paulo University (USP)
None of participants and investigators had any financial profit with the research

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