Lasers in Medical Science (2019) 34:1781–1790
https://doi.org/10.1007/s10103-019-02771-y

ORIGINAL ARTICLE

Topical and intradermal delivery of PpIX precursors for photodynamic therapy with intense pulsed light on porcine skin model

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Received: 2 December 2018 / Accepted: 6 March 2019 / Published online: 22 March 2019

Abstract

In order to purposely decrease the time of the photodynamic therapy (PDT) sessions, this study evaluated the effects of PDT using topical and intradermal delivery of two protoporphyrin (PpIX) precursors with intense pulsed light (IPL) as irradiation source. This study was performed on porcine skin model, using an IPL commercial device (Intense Pulse Light, HKS801). IPL effect on different administration methods of two PpIX precursors (ALA and MAL) was investigated: a topical cream application and an intradermal application using a needle-free, high-pressure injection system. Fluorescence investigation showed that PpIX distribution by needle-free injection was more homogeneous than that by cream, suggesting that a shorter drug-light interval in PDT protocols is possible. The damage induced by IPL-PDT assessed by histological analysis mostly shows modifications in collagens fibers and inflammation signals, both expected for PDT. This study suggested an alternative protocol for the PDT treatment, possibility half of the incubation time and with just 3 min of irradiation, making the IPL-PDT, even more, promising for the clinical treatment.

Keywords Photodynamic therapy · Intense pulsed light · Porcine skin · Aminolevulinic acid · Methyl aminolevulinate

Introduction

Photodynamic therapy (PDT) is a therapeutic modality that depends on the activation of a photosensitizer (PS) by light to promote photochemical events of energy transfer that generates reactive species such as free radicals and singlet oxygen. These products can initiate photobiological reactions that induce cell death by apoptosis and necrosis [1–3]. Because of those effects, PDT has been used as a technique for the treatment of several pathological conditions, such as high cell proliferation diseases, particularly cancer or microbiological infections [4–7].

There are benefits of PDT in comparison to other treatments, particularly for cancer treatment. First, it is mostly considered a minimally invasive technique since only a few applications require surgery room or significant infrastructure to be performed. The technique causes selective damage to neoplastic cells avoiding the undesired expressive death of healthy cells.

Currently, among the most relevant protocols for clinical PDT are those involving photosensitization using protoporphyrin IX (PpIX) obtained by the topical administration of its precursor, 5-aminolaevulinic acid (ALA), or its derivatives, such as methyl aminolevulinate (MAL) [8–11].

One of the technical limitations that still challenges clinical PDT refers to the session length. The most common protocol for basal cell carcinoma consists in lesion curettage or debulking before cream application. The drug-light interval (DLI), which is the time between the precursor application and the lesion irradiation, usually varies from 3 to 4 h [11–13], followed by 16 to 20 min irradiation according to the irradiance and fluence choices [14]. This long time interval between lesions' preparation and finalization of PDT delivery becomes a relevant factor for both medical and patient

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adherence to the treatment modality, even further when considered that a PDT session usually is repeated at least once, weekly. Thus, optimization of this treatment length based on an improvement of the light source or drug delivery still demands further investigation.

An alternative approach to the usual light delivery methods for PDT is the use of intense pulsed light (IPL). IPL devices are systems composed by a flash lamp and capacitors able to generate pulses of high energy with polychromatic light and allow for manual setting of relevant parameters, such as pulse number, pulse width, pulse delay, release, and energy per flash [15]. When IPL technology arose, PDT was already in development using lasers and LEDs (light emitting diodes) as light sources. Lasers allow full access to lesions in hollow organs due to the facility of coupling light into optical fibers and may have high power output, while LEDs are sufficiently low costing, reasonable monochromatic, and allow irradiating surface lesions as efficiently as lasers do. IPL instead generates broadband with a large amount of energy being delivered within a short pulse, thus promoting light delivery to deeper layers of tissue and providing different possibilities for PS excitation and a shorter protocol when compared to similar fluences delivered by other light sources. Also, IPL may accomplish less adverse reactions and better tolerance for the patient [16].

IPL also broadens the options for PS available, because it makes possible to irradiate light with different wavelengths (from violet to infrared) depending on the IPL filter type chosen. Therefore, it does not demand one to use only the PS that is suitable for one's device, which for the clinical practice implies reducing the necessity to invest in several devices.

In dermatological and esthetic fields of research, irradiation with IPL has shown to be efficient in hair and tattoo removal, treatment of vascular lesions, spots, and skin photorejuvenation, which are obtained through selective damage based on the choice of wavelength, intensity, duration, and pulse interval [15]. Studies also show that IPL as a PDT light source had promising results for acne treatment [17–19] and actinic keratosis [20]. Piccolo et al. suggested that IPL is a useful alternative light source for PDT in treating non-melanoma skin cancer since further studies for developing protocols are provided [21].

Concerning PS delivery, Rodrigues et al. showed in previous studies that, after mechanically breaking up the skin barriers with microneedles rollers, a more homogenous PpIX formation is observed [22, 23]. High-pressure, needle-free injection systems are also able to decrease the DLI for aminolevulinic acid (ALA) vehicled as a solution, with the advantage of carrying the precursor to deeper layers compared to the topical application [24]. A case report published satisfactory results of BCC treatment using PDT with the PpIX precursor delivered by a needle-free injection system [25]. Li et al. compared the PDT treatment of *condyloma acuminatum* by both topical and intradermal application of ALA, showing that needle-free injection allowed significant improvement in delivery, including faster treatment time, as the DLI successfully used was 90 min [26]. These PS delivery approaches show that the physical barrier for a PS precursor to percolate through skin or other topically accessed tissues is the decisiveness in determining how long it takes to have a PpIX precursor delivered up to the required extent, interfering directly with the DLI.

The main purpose of the article was to propose alternatives of PDT protocols aiming to reduce the amount of time spent on clinical treatment. Since both the PS administration method and the light source may play a role in reducing this time, we decided to explore the IPL approach for either a well-established PS administration method (topical application using cream) or a needle-free injection (intradermal application using solution) approach.

Material and methods

Animals

The studies were performed in porcine healthy skin model because its optical characteristics are the most similar ones to human skin [27–29]. All procedures performed were approved by the National Council for Control on Animal Experimentation (CONCEA) and the Ethics Committee on Animal Use (CEUA) under protocol no. 019546/13. Two 2-month-old male animals of about 20 kg were used to perform the experiments. The animals stayed at the College of Agricultural and Veterinary Sciences from the Sao Paulo State University (UNESP) in Jaboticabal-SP. After induction of anesthesia, the hair of the dorsum was removed by shaving to avoid interference in the collection of widefield fluorescence images of skin. The porcine skin has differences of thickness depending on the body region, breed, age, among other factors [30]. In order to minimize these differences, all the experimental conditions were replicated in the same region of the dorsum for the animals. Further, we have not removed the *stratum corneum* in order to avoid introducing interferences provoked by its removal. The euthanasia was performed 48 h after the PDT experiments.

Intense pulsed light

Commercial equipment (HKS801 Intense Pulse Light, Ningbo Ruipu Medical Equipment Co., Ltd., China) was used as a light source. This equipment consists of a handpiece (containing a crystal and a xenon lamp) coupled to a cooling system. A highpass filter (560–1200 nm) was used to avoid the excitation in the Soret band and to prevent the fast PS's photobleaching. The device allows setting every parameter prior to irradiation. Each flash is a train of pulses which was defined to be composed by 15 pulses with 30-ms duration each pulse apart by a 1-ms delay. It was also set a time of 4 s of release and 50 J of total energy output. For the flashes calibration, after setting these parameters, an automatic optical power and an energy meter specific to IPL devices was used (Fit IPL®, LaserPoint, Italy) and each flash delivered a fluence of 5.7 J/cm^2 (5.7 × 10^4 J/m^2 in SI units).

Photosensitizers

The ALA and MAL were chosen as precursors for PpIX because both have been used clinically, with different results concerning their PDT effects on depth (since MAL has been reported to show deeper average penetration into skin from surface, due to the presence of the methyl group [31]). ALA and MAL powders for manipulation were obtained from PDT-PHARMA (Cravinhos, SP, Brazil). The PpIX precursors were prepared at 20% mass concentration. Both solution and cream vehicles were used, respectively for needle-free and microneedle rollers–based administration. For the topical application as cream, the precursors were incorporated in an oil-in-water emulsion (O/W) based on Blanco et al. and Menezes et al. [32, 33], and the amount applied was approximately 30 mg/cm^2. For the intradermal delivery, the solutions were freshly prepared in sterile 0.9% NaCl [26] and the application was performed using a commercial needle-free injection system with high pressure (INJEX PHARMA®, SAFE INJECT, Germany). The volume injected was 100 μL applied perpendicularly to the skin surface, promoting a hole with around 0.17 mm in diameter.

Photodynamic therapy

The irradiation was conducted setting the handpiece 10 mm away from the skin with a handmade celeron coupler. Celeron was chosen for its low thermal conductivity. Aluminum paper masks having a circular cavity with diameter 8 mm were used as delimiters; three replicates were performed for each animal. The protocol for PDT was set as 15 flashes resulting in a total fluence of 85.5 J/cm^2 (85.5 × 10^4 J/m^2 in SI units) delivered to the tissue. Each flash was triggered manually with 10-s interval between them. For the total fluence to be delivered, about 3 min was necessary in order to reach the total number of flashes to be delivered. These parameters were selected after the pilot test. The DLI was different for each application modality, based on previous studies from our research group: 90 min for the injection [11] and 180 min for the cream application [26].

Widefield fluorescence imaging

For image acquisition, the fluorescence probe of a commercial system device LINCE (MMOptics, São Carlos, SP, Brazil) was coupled to a photographic camera. This probe is composed of 405-nm LED arrays that induce fluorescence emission starting from 450 nm [34]. The epithelial autofluorescence image was collected before the PpIX precursor application, and the PS-induced fluorescence image acquisition was made just before irradiation. The image processing was performed using an algorithm created in MATLAB® (The MathWorks, USA). We defined the PpIX superficial production (Ψ) as shown in Eq. (1). For each sample (j), the ratio between the information from the red (R) and green (G) image components for every pixel (i) was used. The ratios were summed up for each of the autofluorescence (AF) and fluorescence (F) images, and the values for AF were subtracted from values for F.

$$\psi(j) = F_j - AF_j = \left[\sum_i \left(\frac{R}{G}\right)_i\right]_{F_j} - \left[\sum_i \left(\frac{R}{G}\right)_i\right]_{AF_j} \quad (1)$$

This definition was made based on the previous study by Andrade et al., in which the "R" component has been used as referring to the red fluorescence of PpIX [35]. The subtraction removes artifacts represented by the R component from autofluorescence images, and the ratio R/G works as normalization for the red component, since green fluorescence from endogenous skin fluorescence, represented by the G component, should not change. This process is further explored in Fig. 2a in the "Results" section.

Histology

The tissue biopsies were collected after euthanasia. The skin fragments were sliced to undergo the dehydration process. After that, the slices were incorporated into paraffin (Histosec®, Merck, Germany) and histological slides were produced and stained by either Masson's trichrome (MT) or hematoxylin-eosin (HE) techniques. Slide imaging was obtained by optical microscopy (EcIPLse Ti-S, Nikon, Japan).

Results

Figure 1 shows the irradiance spectrum of the xenon lamp between the flashes, the irradiance spectrum of the flash emitted by the xenon lamp during the irradiation, and the PpIX absorbance spectrum. In the IPL flash spectrum, there is a significant emission in the region within 400 and 800 nm, which represents about 80% of the total energy delivered by the flash.

Figure 2 represents the sequence that illustrates the processing steps: (a) original fluorescence image, (b) the original image RGB red component, (c) the original image RGB green component, and (d) the image generated by the ratio between the pixels of the red channel by the ones of the green component. There are also the typical images for the superficial PpIX

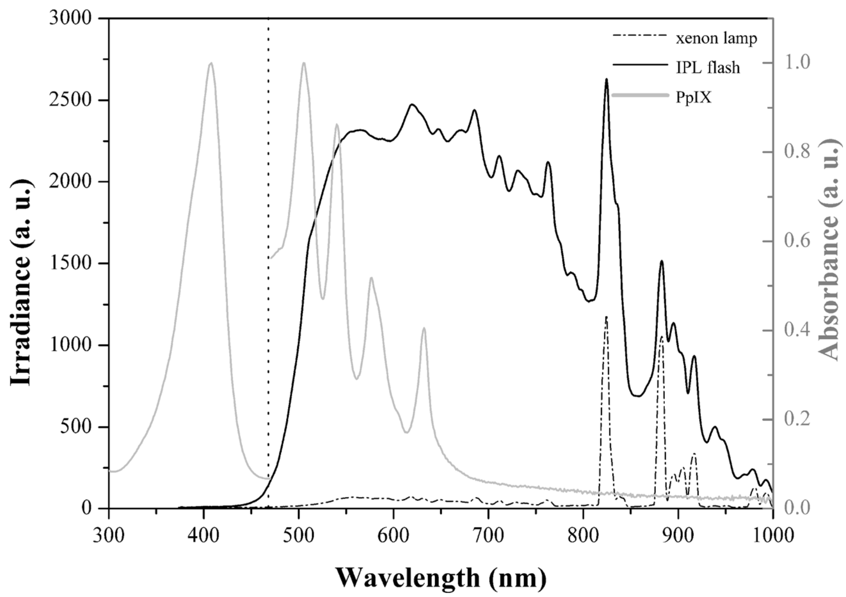

Fig. 1 The irradiance spectra of the xenon lamp between the flashes, of the flash emitted by the xenon lamp during the irradiation, and the PpIX absorbance spectrum

fluorescence, obtained (f, h) 90 min after injection and (e, g) 180 min after cream application. Image collection after 48 h exemplifies the most common macroscopic observation of damage on the surface after (i) light-only irradiation, after PDT using (j, k) ALA and (l, m) MAL by cream and injection application, respectively.

The average between ratios obtained from the widefield fluorescence images is shown in Fig. 3, with error bars representing the standard deviation among animals. The ratio represents the increase of red fluorescence, associated with the PpIX accumulation in tissue.

Figure 4 shows example images of the histological slices of both healthy skin without treatment and healthy skin 48 h after the IPL irradiation without PS. The histological slices from the biopsies collected 48 h after PDT, either using cream or injection, are presented in Fig. 5.

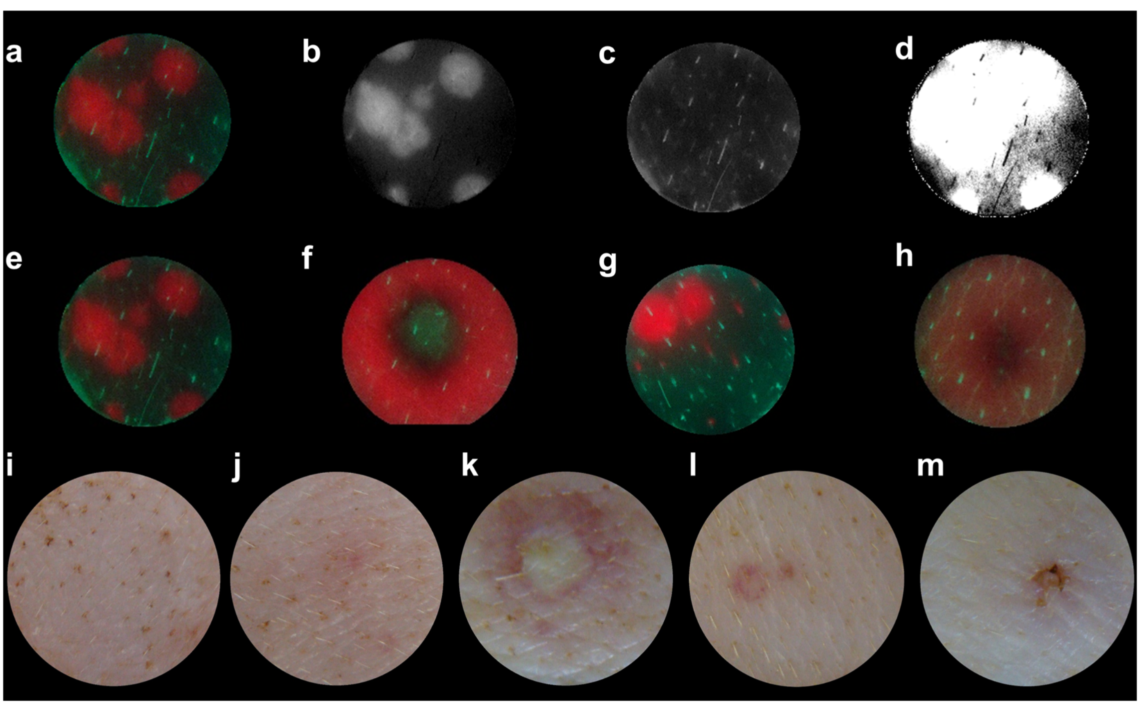

Fig. 2 The sequence of images illustrating the processing steps used: **a** original image, original image **b** red and **c** green components, and the **d** ratio between red and green components. Examples of the superficial PpIX fluorescence after 90 min by **f**, **h** injection application and 180 min after **e**, **g** cream application. The diameter of the circle mask corresponds to 0.8 cm. Image collection after 48 h showing typical damage on the surface after **i** light-only irradiation, after PDT using **j**, **k** ALA and **l**, **m** MAL by cream and injection application, respectively. The diameter of the circle mask corresponds to 1.35 cm

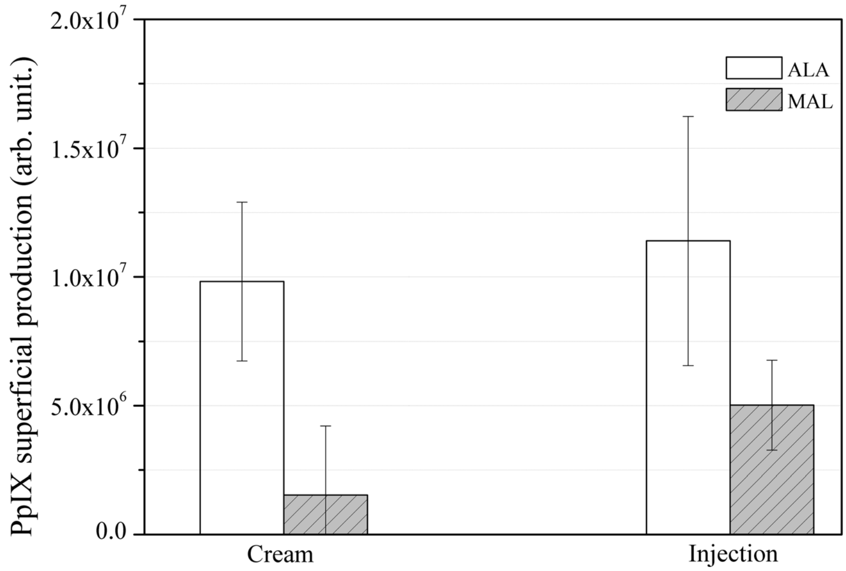

Fig. 3 Estimates of PpIX superficial production by fluorescence assessment (based on red and green channels of fluorescence detection) after 180 min of cream application and 90 min after injection application, using ALA and MAL

All experimental conditions were compared to healthy tissue, which was considered the control group. For clarity, changes in treated groups were represented in Table 1. Every characteristic was ranked from zero to three, with zero being the absence of the characteristic and represented by the symbol (◌), and every rank of the aforementioned characteristic is represented by one (+) mark—more marks represent a more significant characteristic. Aspects like the superficial damage area, classified by macroscopic analysis of widefield images, were taken into consideration. Likewise, evidence of inflammation was observed such as the presence of inflammatory infiltrates (marked by an arrow) and edema signals (spacing between collagen fibers indicating the presence of water in the skin). Also, the characteristics of the proliferation phase were ranked such as the presence of tissue signs of inflammation associated with reepithelialization of skin (characterized as an increase of the thickness of the epidermis and the rise of the connection with the dermis from the increasing amount of dermal papillae) and fibroplasia signals (degradation was observed for changes in conformation and orientation of collagen fibers).

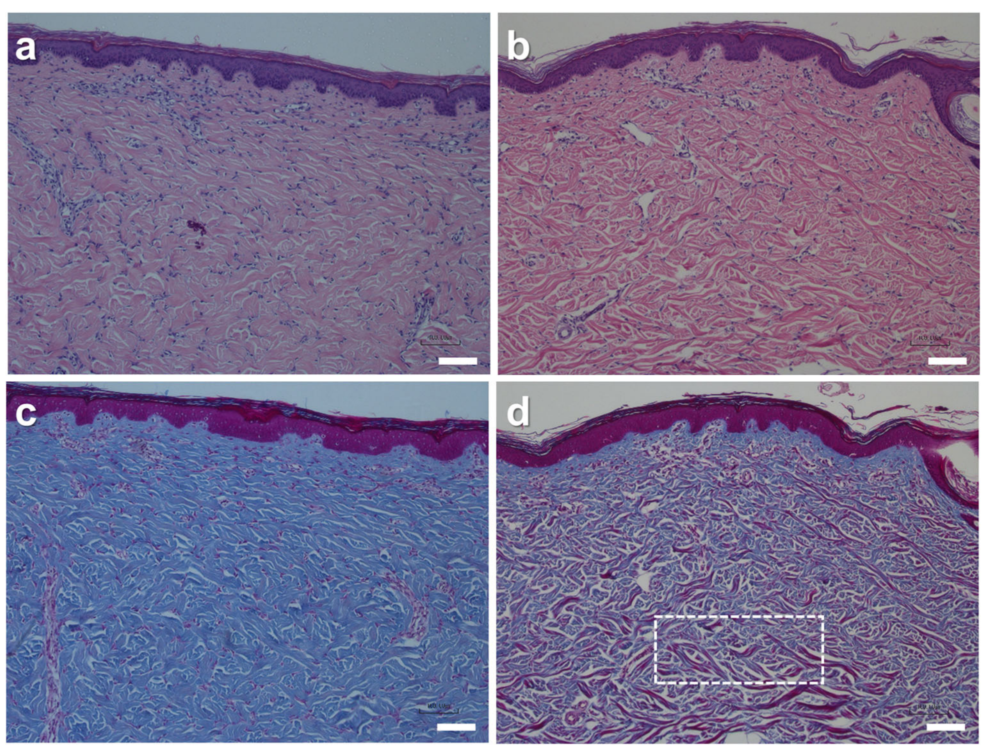

Fig. 4 Histological slices images of **a**, **c** normal skin without treatment and **b**, **d** normal skin 48 h after IPL irradiation without PS, stained with HE and MT. Original magnification of 10 times and scale are illustrated by a yellow bar at the bottom right of the images, equivalent to 100 μm

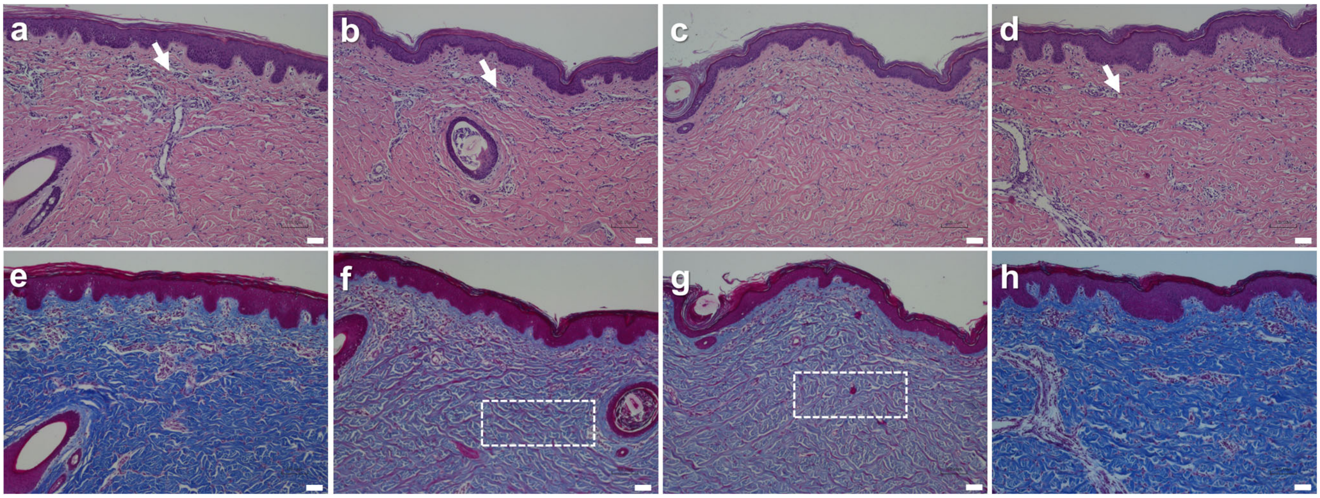

Fig. 5 Histological slice images about 48 h after IPL-PDT, using **a**, **e** ALA and **c**, **g** MAL cream and **b**, **f** ALA and **d**, **h** MAL applied by injection, stained with HE and MT. Original magnification of 10 times and scale illustrated by a white bar at the bottom right of the images equivalent to 100 μm. The white arrows indicate inflammation characteristics, and the white dotted rectangles indicate regions of conformation and cohesion of the collagen fibers

Discussion

The heterogeneity in the superficial PpIX distribution after ALA and MAL cream application was more prominent than when the precursor's solutions were administered using the injection system. Comparing the PpIX production using cream, MAL did not result in a PpIX distribution as homogeneous as that observed with ALA. In some cases, when the photosensitization was performed by injection, a tissue portion that was unable to produce PpIX, as seen for (f) example in Fig. 2. This observation was attributed to the fact that, since the injection breaches into skin layers, mechanical damage promoted to the tissue at the injection spot modifies local cells metabolism and, since metabolism is essential to ALA/MAL conversion to PpIX, this damage interferes with local cells ability to produce PpIX.

The injection system application showed to be more efficient than topically administered cream as seen in Fig. 2, with the advantage of half incubation time [24]. In fact, Gong et al. used a needle-free injection system for ALA delivery (90 min of DLI) to treat nonmalignant skin tumors with PDT. Their study reported useful results, with milder therapeutic pain, which was well-tolerated by patients, and low recurrence rate [36]. Between creams, ALA cream was more expressive than MAL cream concerning superficial homogeneity production. The observed results suggested that the use of needle-free injection systems is more efficient in making PS available in the healthy porcine skin than topical cream application. Figure 2 also shows that, with the light-only irradiation protocol, no superficial macroscopic damage was observed. For ALA cream application, it was possible to see that the region irradiated was erythematous, which is usually expected after PDT application due to damage. Using MAL cream, only part of the irradiated spot (mainly two spots within the treated region) showed any visually noticeable damage. The extension and characteristics of superficial damage using injection, for both precursors, were more expressive than those observed for the cream application.

A reduced red fluorescence was observed at the surface of the tissues sensitized with MAL. As fluorescence is associated

Table 1 Skin modifications about 48 h after PDT with IPL using ALA and MAL, either in cream (C) or with injection (I). The symbol "○" indicates the absence of the characteristic, and the number of repetitions of the "+" symbol indicates the severity of the characteristic when it is present, compared to control (healthy skin) and to the condition using light-only irradiation

Skin modifications			Control	IPL	ALA		MAL	
					C	I	C	I
Superficial damage area			○	○	+	+++	++	++
Inflammation	↑ Inflammatory in filtrates and edema		○	+	+++	+++	++	+
Proliferation	*Reepithelialization*	↑ Epidermal thickness	○	○	+	+	○	+
		↑ Dermal papillae	○	○	+	+	○	++
	Fibroplasia	Collagen fibers degradation	○	+	+	+++	+++	+

to the concentration of PpIX in tissue, this observation represents that the PpIX production associated to MAL was less expressive than the one associated to ALA, both for cream and injection application, which is shown in Fig. 3. This observation was associated to the limitation in the fluorescence detection, as the fluorescence response is obtained mostly from the superficial layers of the skin, and MAL is expected to promote PpIX production in deeper layers of tissue [31].

Based on the fluorescence analysis obtained by widefield imaging, the superficial PpIX production was found to be heterogeneous when the precursor was used via the topic cream. Intradermal administration of the solution by needle-free injection instead resulted in the most homogeneous production of superficial PpIX among the investigated conditions, demanding half of the DLI time when compared to the cream application [24, 26]. This DLI improvement by the use of needle-free injection may allow for the time reduction of the PDT protocol application almost by half, with the important improvement of the clinical practice routine.

The central aspect concerning the histological differences observed was the changes in the arrangement of the collagen fibers (white dashed rectangle), showed by Figs. 4 and 5. HE staining showed that fibers were separated (regarding arrangement and direction) after IPL irradiation, which indicates edema and collagen degradation. Further, MT staining showed that the proportion of skin collagen when the tissue was irradiated is lower than that observed in the skin without IPL irradiation. However, modifications observed for light-only irradiation were not significant compared to the experimental conditions with IPL-PDT. On the other hand, modifications promoted by IPL without PS are due to dermal collagen remodeling which takes place without damaging the epidermis. That is the reason why IPL is a light source of choice for the treatment of telangiectasias, lentigos, and skin texture [37].

After 48 h, the tissue treated by IPL with the application of ALA cream showed critical inflammatory characteristics (such as high amount of inflammatory infiltrate and spacing of the collagen fibers, which indicate edema) represented by the arrows in Figs. 4 and 5. MAL cream application, instead, resulted in evidence of tissue in the proliferative phase, as degradation of the collagen fibers was more relevant than that observed for ALA. In contrast, 48 h after PDT with MAL injection application, the collagen fibers arrangement was observed to be more similar to the one observed in tissue without treatment.

All observations concerning tissue healing and the quality of sensitization and PDT outcome showed that MAL-PDT produced a less inflammatory response and damage than ALA-PDT in either case. The main observation though is that tissue photosensitized via injection resulted in the quickest recovery of the tissue, based on the histology results that showed to be closer to what was observed for intact, untreated tissue. MAL cream histopathology results were consistent with the lower production of PpIX observed when compared to ALA cream results.

Based on the rating adopted for macroscopic evaluation (Table 1), no visible superficial damage was observed after 48 h using light-only irradiation with IPL and histological analysis showed slight changes in the collagen fibers arrangement. After PDT, however, inflammation characteristics were observed for ALA cream, whereas, for ALA injection, more proliferative characteristics were identified, suggesting a more prompt recovery from PDT. Similar results for MAL were observed for cream application and injection, while the lower production of PpIX by MAL led to faster tissue healing after PDT, indicated by proliferative characteristics observed after MAL-cream PDT and by characteristics similar to those observed in the control group for MAL-injection PDT.

Concerning the reduction on the amount of time IPL protocol takes compared to a usual PDT protocol using a continuous light source (for reference, the present study used an irradiance of 0.125 W/cm^2 (1.25×10^3 W/m^2 in SI units) based on the study by Blanco et al. [32], which used the same PpIX precursors), the irradiation time obtained for the same light fluence was about 4.6 times shorter. Aiming to clarify how that is possible, Fig. 6 illustrates both irradiation regimens. The irradiance delivery for each light pulse is several times larger than the one emitted by the continuous light source (each pulse has irradiance of about 190 W/cm^2, or 1.9×10^6 W/m^2 in SI units, in contrast to 0.125 W/cm^2). Thus, one flash composed of 15 pulses is about 0.5 s long and after 15 flashes, the total time of irradiation is 2.5 min. To deliver the same total fluence (85.5 J/cm^2) using the abovementioned continuous light source, it would take about 11 min. Therefore, an improvement in the protocol time was achieved.

This relevant reduction in the length of the protocol has potential to benefit patients who would be subjected to long waiting times when considered a regular PDT session (which usually takes 3–4 h for DLI plus 16–20 min for irradiation) [38], reducing this total time to as short as about 90 min for DLI and about 3 min for irradiation.

The reason for this high output irradiance per flash being possible to be delivered to tissue without thermal damage is because this high power is delivered within a very short time interval, and the time between pulses (and between flashes) allows for tissue molecules to undergo thermal relaxation, i.e., the thermal energy is dissipated before more energy is delivered. Thus, tissue takes longer to increase temperature and therefore thermal damage is mostly avoided [39]. Hence, IPL makes faster treatment protocols possible. Also, such an improvement represents less time for painful stimuli to take place, as IPL flash delivery provides tissue with time for dissipating such stimuli.

It must be said that IPL is not proposed here as a substitute for continuous light sources; it is an alternative option allowing a shorter time for irradiation, with several other advantages, such as reduced cost, larger irradiation areas in comparison to laser devices, and high versatility for the clinical use

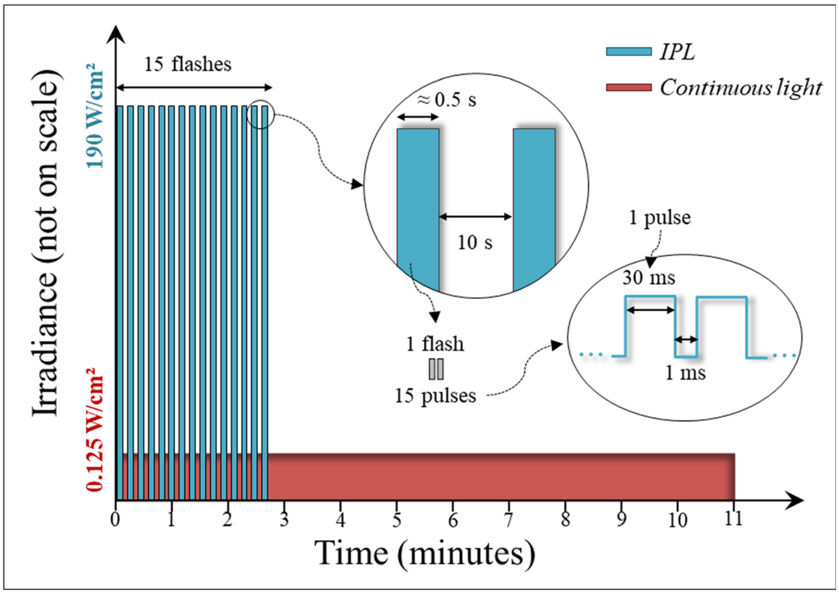

Fig. 6 Irradiance as a function of time for IPL and continuous light sources, represented by blue and red bars, respectively. The irradiance axis is not in scale (it is shown schematically due to the large difference of values), and the time is represented in minutes. For the IPL irradiation protocol, one flash is composed of 15 pulses about 0.5 s long (30 ms of duration and 1 ms apart) with 10-s interval between flashes, for a total of 2.5 min of irradiation. The irradiance delivery for each light pulse is several times larger than the one emitted by the continuous light source (each pulse has irradiance of about 190 W/cm^2, in contrast to 0.125 W/cm^2 used as reference). Thus, to deliver the same total fluence (85.5 J/cm^2) using the continuous light, it would take about 11 min

[40], reducing the irradiation time and, consequently, subjecting patients to shorter-term painful stimuli compared to the standard monochromatic light sources. In comparison to PDT using laser or LED devices, IPL makes it possible to deliver the same fluence with shorter irradiation times, because this system allows delivering many flashes in a few minutes, with high irradiance delivered for each one.

Conclusion

This study proposed to investigate an alternative approach for PDT using IPL as a light source associated to barrier-breaking techniques to improve ALA/MAL penetration in tissue in order to achieve as much as possible reduction in DLI, aiming to optimize time-consuming protocols for clinical PDT. A relevant improvement in DLI was observed, making it possible to produce sufficient PpIX after half of the incubation time. Further, around 3 min of irradiation was necessary using IPL irradiation to obtain satisfactory PDT outcome when compared to usual protocols which are 20 min long, which is a promising result for the clinical approaches. As an additional benefit, optimizing time for healthcare systems may contribute to the reduction of costs, improving the ability to provide treatment to a larger number of patients either with PDT or otherwise. In summary, IPL-PDT associated with barrier-breaking approaches for ALA/MAL delivery may represent an alternative for improvement of the technique by providing time optimization of protocols and must be further explored.

Acknowledgments We acknowledge the support from the Center for Research in Optics and Photonics (CEPOF - FAPESP CePID program grant 2013/07276-1).

Funding This study was funded in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and by grants 2013/07276-1 (CePOF), 2009/54035-4 (EMU) of São Paulo Research Foundation (FAPESP).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

1. Dougherty TJ, Gomer CJ, Henderson BW et al (1998) Photodynamic therapy. J Natl Cancer Inst 90:889–905. https://doi.org/10.1093/jnci/90.12.889
2. Allison RR, Moghissi K (2013) Photodynamic therapy (PDT): PDT mechanisms. Clin Endosc 46:24–29. https://doi.org/10.5946/ce.2013.46.1.24
3. Allison RR, Downie GH, Cuenca R et al (2004) Photosensitizers in clinical PDT. Photodiagn Photodyn Ther 1:27–42
4. Baltazar LM, Ray A, Santos DA et al (2015) Antimicrobial photodynamic therapy: an effective alternative approach to control fungal infections. Front Microbiol 6:1–11. https://doi.org/10.3389/fmicb.2015.00202

5. Robertson CA, Evans DH, Abrahamse H (2009) Photodynamic therapy (PDT): a short review on cellular mechanisms and cancer research applications for PDT. J Photochem Photobiol B Biol 96:1–8
6. Wilson BC, Patterson MS (2008) The physics, biophysics and technology of photodynamic therapy. Phys Med Biol 53:R61–R109. https://doi.org/10.1088/0031-9155/53/9/R01
7. Geralde MC, Leite IS, Inada NM et al (2017) Pneumonia treatment by photodynamic therapy with extracorporeal illumination - an experimental model. Physiol Rep 5:e13190. https://doi.org/10.14814/phy2.13190
8. Rhodes LE, de Rie M, Enström Y et al (2004) Photodynamic therapy using topical methyl aminolevulinate vs surgery for nodular basal cell carcinoma: results of a multicenter randomized prospective trial. Arch Dermatol 140:17–23. https://doi.org/10.1001/archderm.140.1.17
9. Oh SH, Ryu DJ, Han EC et al (2009) A comparative study of topical 5-aminolevulinic acid incubation times in photodynamic therapy with intense pulsed light for the treatment of inflammatory acne. Dermatol Surg 35:1918–1926. https://doi.org/10.1111/j.1524-4725.2009.01315.x
10. Cecília M, Costa D, Keiko A et al (2017) Photodynamic Therapy with 5- Aminolevulinic Acid (ALA) in the Treatment of Acne: A Case Study. Clin Dermatol Res Ther. 2017; 1(1):114
11. Ramirez DP, Kurachi C, Inada NM et al (2014) Experience and BCC subtypes as determinants of MAL-PDT response: preliminary results of a national Brazilian project. Photodiagn Photodyn Ther 11:22–26. https://doi.org/10.1016/j.pdpdt.2013.11.001
12. Matei C, Tampa M, Poteca T et al (2013) Photodynamic therapy in the treatment of basal cell carcinoma. J Med Life 6:50–54
13. Rhodes LE, de Rie M, Enström Y et al (2004) Photodynamic therapy using topical methyl aminolevulinate vs surgeryfor nodular basal cell carcinoma. Arch Dermatol 140. https://doi.org/10.1001/archderm.140.1.17
14. Morton CAA, Szeimies R-MM, Sidoroff A, Braathen LRR (2013) European guidelines for topical photodynamic therapy part 1: treatment delivery and current indications - actinic keratoses, Bowen's disease, basal cell carcinoma. J Eur Acad Dermatol Venereol 27:536–544. https://doi.org/10.1111/jdv.12031
15. Babilas P, Schreml S, Szeimies RM, Landthaler M (2010) Intense pulsed light (IPL): a review. Lasers Surg Med 42:93–104. https://doi.org/10.1002/lsm.20877
16. Zhang L, Wu Y, Zhang Y et al (2018) Topical 5-aminolevulinic photodynamic therapy with red light vs intense pulsed light for the treatment of acne vulgaris : a spilit face , randomized , prospective study. Dermatoendocrinol 9:1–9. https://doi.org/10.1080/19381980.2017.1375634
17. Santos MAV, Belo VG, Santos G (2005) Effectiveness of photodynamic therapy with topical 5-aminolevulinic acid and intense pulsed light versus intense pulsed light alone in the treatment of acne vulgaris: comparative study. Dermatol Surg 31:910–915. https://doi.org/10.1111/j.1524-4725.2005.31804
18. Mei X, Shi W, Piao Y (2013) Effectiveness of photodynamic therapy with topical 5-aminolevulinic acid and intense pulsed light in Chinese acne vulgaris patients. Photodermatol Photoimmunol Photomed 29:90–96. https://doi.org/10.1111/phpp.12031
19. Shaaban D, Abdel-Samad Z, El-Khalawany M (2012) Photodynamic therapy with intralesional 5-aminolevulinic acid and intense pulsed light versus intense pulsed light alone in the treatment of acne vulgaris: a comparative study. Dermatol Ther 25:86–91. https://doi.org/10.1111/j.1529-8019.2012.01427.x
20. Kohl E, Popp C, Zeman F et al (2017) Photodynamic therapy using intense pulsed light for treating actinic keratoses and photoaged skin of the dorsal hands: a randomized placebo-controlled study. Br J Dermatol 176:352–362. https://doi.org/10.1111/bjd.14970
21. Piccolo D, Kostaki D (2018) Photodynamic therapy activated by intense pulsed light in the treatment of nonmelanoma skin cancer. Biomedicines 6:18. https://doi.org/10.3390/biomedicines6010018
22. Gracielli Sousa RP, de Menezes PFC, Fujita AKL et al (2014) Microneedles rollers as a potential device to increase ALA diffusion and PpIX production: evaluations by wide-field fluorescence imaging and fluorescence spectroscopy. Proc. SPIE 8926, Photonic Therapeutics and Diagnostics X, 892614. https://doi.org/10.1117/12.2040618
23. Rodrigues PGS, Campos de Menezes PF, Fujita AKL et al (2015) Assessment of ALA-induced PpIX production in porcine skin pretreated with microneedles. J Biophotonics 8:723–729. https://doi.org/10.1002/jbio.201400081
24. Rodrigues PGS, de Menezes PFC, Requena MB et al (2015) New alternatives to improve the transdermal application of ALA, M-ALA in photodynamic therapy using needle-free injection. Photodiagn Photodyn Ther 12:360. https://doi.org/10.1016/j.pdpdt.2015.07.138
25. Barolet D, Boucher A (2011) No-needle jet intradermal aminolevulinic acid photodynamic therapy for recurrent nodular basal cell carcinoma of the nose: a case report. J Skin Cancer 2011:790509. https://doi.org/10.1155/2011/790509
26. Li X, Wang X, Gu J et al (2013) Needle-free injection of 5-aminolevulinic acid in photodynamic therapy for the treatment of condylomata acuminata. Exp Ther Med 6:236–240. https://doi.org/10.3892/etm.2013.1092
27. Liu J, Kim D, Brown LD et al (2009) Comparison of human, porcine, and rodent wound healing with new miniature swine study data. Journal of the American Association for Laboratory Animal Science: JAALAS.48. 581-581
28. Zamora-Rojas E, Aernouts B, Garrido-Varo A et al (2013) Optical properties of pig skin epidermis and dermis estimated with double integrating spheres measurements. Innov Food Sci Emerg Technol 20:343–349. https://doi.org/10.1016/j.ifset.2013.06.008
29. Lister T (2012) Optical properties of human skin. J Biomed Opt 17:090901. https://doi.org/10.1117/1.JBO.17.9.090901
30. Sandby-Møller J, Poulsen T, Wulf HC (2003) Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits. Acta Derm Venereol 83:410–413. https://doi.org/10.1080/00015550310015419
31. Gold MH (2009) Therapeutic and aesthetic uses of photodynamic therapy part five of a five-part series: ALA-PDT and MAL-PDT what makes them different. J Clin Aesthet Dermatol 2:44–47
32. Blanco KC, Moriyama LT, Inada NM et al (2015) Fluorescence guided PDT for optimization of the outcome of skin cancer treatment. Front Phys 3. https://doi.org/10.3389/fphy.2015.00030
33. Menezes PFC, Requena MB, Bagnato VS (2014) Optimization of photodynamic therapy using negative pressure. Photomed Laser Surg 32:1–6. https://doi.org/10.1089/pho.2013.3670
34. Grecco C, Buzzá HH, Stringasci MD et al (2015) Single LED-based device to perform widefield fluorescence imaging and photodynamic therapy. Proc. SPIE 9531, Biophotonics South America, 953121. https://doi.org/10.1117/12.2185925
35. Andrade CT, Vollet-Filho JD, Salvio AG et al (2014) Identification of skin lesions through aminolaevulinic acid-mediated photodynamic detection. Photodiagn Photodyn Ther 11:409–415. https://doi.org/10.1016/j.pdpdt.2014.05.006
36. Gong Y, Labh S, Jin Y et al (2016) Needle-free injection of 5-aminolevulinic acid in photodynamic therapy for the treatment of

non-melanoma skin cancer. Dermatol Ther 29:255–262. https://doi.org/10.1111/12335
37. Iyer S, Carranza D, Kolodney M et al (2007) Evaluation of procollagen I deposition after intense pulsed light treatments at varying parameters in a porcine model. J Cosmet Laser Ther 9: 75–78. https://doi.org/10.1080/14764170701299313
38. Wan MT, Lin JY. (2014) Current evidence and applications of photodynamic therapy in dermatology. Clinical, Cosmetic and Investigational Dermatology. https://doi.org/10.2147/CCID.S35334
39. Requena MB, Stringasci MD, Pratavieira S et al (2018) Thermographic analysis of photodynamic therapy with intense pulsed light and needle-free injection photosensitizer delivery: an animal study. Proc. SPIE 10489, Optical Biopsy XVI: Toward Real-Time Spectroscopic Imaging and Diagnosis, 104891F. https://doi.org/10.1117/12.2300407
40. Friedmann DP, Goldman MP, Fabi SG et al (2014) The effect of multiple sequential light sources to activate Aminolevulinic acid in the treatment of actinic Keratoses: a retrospective study. J Clin AestheticDermatol. 7:20–25