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Review

Fluorescence evaluations for porphyrin formation during topical PDT using ALA and methyl-ALA mixtures in pig skin models

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ABSTRACT

Background: Photodynamic Therapy (PDT) using Aminolevulinic acid (ALA) and derivative molecules as topical medication and as a precursor of protoporphyrin (PPIX), is limited due to low permeation through skin or efficiency in porphyrin production. This behavior affects the production and homogeneity of PPIX distribution on superficial skin and in the deeper skin layers. Many authors propose alternatives to solve this such as, modification in the ALA and derivativemolecules, modifying the chemical properties of emulsion external phase or incorporating a delivery system to the emulsion. The goal of this study is to discuss what proportion of ALA and Methyl aminolevulinate (MAL) on mixtures increase the amount and uniformity of PPIX formation at superficial skin by fluorescence evaluations.
Methods: The study was conducted in vivo using a pig skin model. PPIX production was monitored using fluorescence spectroscopy and widefield fluorescence imaging on skin surface. 20% of ALA and MAL cream were done mixing the following proportions: ALA, M2 (80% ALA–20% MAL), M3 (60% ALA–40% MAL), M4 (50% ALA–MAL), M5 (40% ALA–60% MAL), M6 (20% ALA–80% MAL) and MAL.
Results: Mixtures M3, M4, and M5 showed the most PPIX production on skin by widefield fluorescence imaging and fluorescence spectroscopy in 3 h of incubation. These results suggest that 50% of ALA and MAL in the same mixture increase the PPIX production in amount, homogeneity and time production when compared to ALA and MAL. This has a positive impact on photodynamic damage optimizing the PDT treatment.

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1. Introduction

Photodynamic Therapy (PDT) is a well-established technique for cancer treatment and shows several advantages over traditional treatment methods. It is possible to define PDT as a photo-induced cytotoxicity of neoplastic cells involving a photosensitizing agent, the light source and molecular oxygen [1,4]. It has been shown to be effective in selective by damage the neoplasia cells with reduced side effects [1,2,8,12]. With PDT, Tumor death by reactive oxygen species (mainly singlet oxygen) production is achieved as a result of illumination in a desired location for an established time interval [7]. The traditional applications of PDT use systemic photosensitizers. The disadvantages of systemic photosensitizers are related to high photosensibility, due to slow elimination from the body at around 1 month [2,8]. The topical application using photosensitizer's precursors as 5-aminolevulinic acid (ALA) and its derivatives arises as one new possibility to improve PDT [8]. The 5-aminolevulinic acid (ALA) and its derivative of methyl aminolevulinate (MAL) are classified as metabolic precursors of protoporphyrin IX (PPIX), an endogenous photosensitizer that are applied on skin usually in an emulsion (cream) [2,8]. The topical and localized application of PDT using the aminolevulinic acid (5-ALA) as the precursor of protoporphyrin IX (PPIX) is common in clinical cases involving cancer treatment and dermatological procedures (diseases and esthetic) [2,8,14]. The penetration and distribution of photosensitizer precursors, through the skin, can limit the PDT efficacy. Many authors have discussed the limitations of ALA and MAL penetration through skin by transdermal application in the literature [15]. It is shown that penetration through skin can be optimized by considering the chemical characteristics of drug and emulsions delivery, techniques for modification of the stratum corneum (tape stripping- mechanical removal, hydration and chemical enhancers), as well as the application of mechanical devices (iontophoreses, ultrasound, Er:YAG.laser, microneedles and injections) [3,9,10,16]. The structure of stratum corneum (SC) has an influence on the transdermal penetration of topical molecules. The transdermal permeation of chemicals through skin involves three pathways: intercellular lipid domain in SC, skin appendages, and keratin bundles in SC [5]. Chemical and pharmaceutical alternatives are being proposed to improve the penetration of ALA and MAL some of which are application of different types of emulsion systems (A/O or O/A) and vehicles, application of microemulsions and nanoemulsions (low particle size of emulsion), and utilization of liposome and other nanostructures [6,15,16]. The differences of ALA and MAL consist in the addition of one methyl radical in the ALA molecule. This addition increases the hydrophobicity of the MAL molecule, increasing the permeation through skin to deeper layers [11]. ALA is a hydrophilic compound making it difficult to cross the biological barriers of the skin, such as cell membranes. However, it has a high efficiency in production of PPIX. On the other hand MAL has a lipophilic character allowing it to be transported by nonpolar amino acids via passive diffusion (does not require driven) facilitating the ability to move across biological barriers reaching higher penetration into the desired tissue, at a cost of a smaller production of PPIX [5,11]. The combination of these two compounds in the same application creates new possibilities to obtain the best at each. In this way, this work has intention to demonstrate that the combination between ALA and MAL can result in different effects optimizing the permeation through skin as well as the PPIX production related to time production and amount. Therefore, we propose here an innovation on PDT procedure using ALA and MAL mixtures evaluating the effectiveness of PPIX production in amount, homogeneity and time of production by fluorescence measurements using fluorescence spectroscopy and widefield fluorescence imaging. We look to understand more about the kinetics of PPIX formation and elimination (time, amount, and distribution) to create new effective protocols in clinical topical PDT.

2. Materials and methods

2.1. Chemicals

The studies were conducted with 5-ALA and MAL (concentration 20%) in different proportions dissolved in an oil-in-water emulsion (O/W) containing: water (~70%), emulsifying agents (O/W) (~20%), preservatives (~0,5%), viscosity agents (10%), humectants and moisturizing agents as urea and propylene glycol(~7%), DMSO (5%), EDTA (0.15%) and BHT (0.05%). The commercial 5-ALA and MAL was obtained from PDT-PHARMA (Cravinhos, São Paulo—Brazil) and were prepared immediately prior to use without previous solubilization, because the drug presents elevated solubility in the used base cream.

Positions at the creams were prepared less than 24 h before performing the experiment and stored in the refrigerator in non-transparent bottles in order to prevent oxidation of the components. Seven cream samples were prepared containing proportions of ALA and MAL as described in Table 1. The overall contained active principles in all samples are kept in 20%.

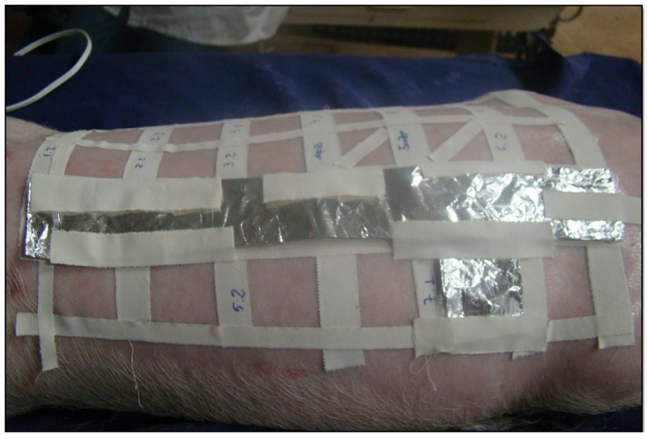

Fig. 1. Dividing in many areas for kinetic and PDT procedures.

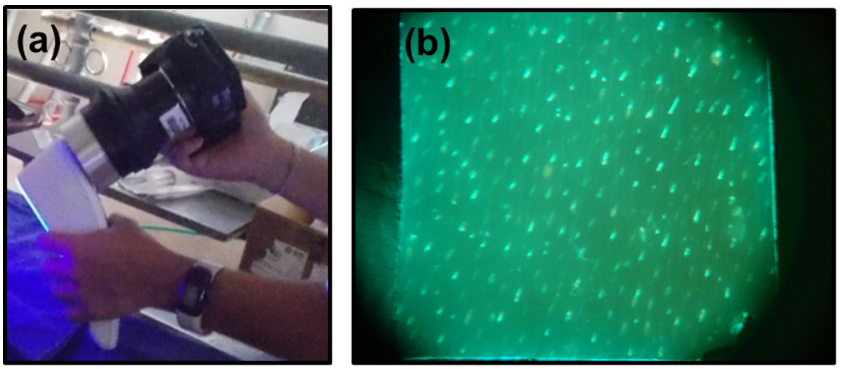

Fig. 2. Widefield fluorescence imaging sytem coupled to a digital camera.

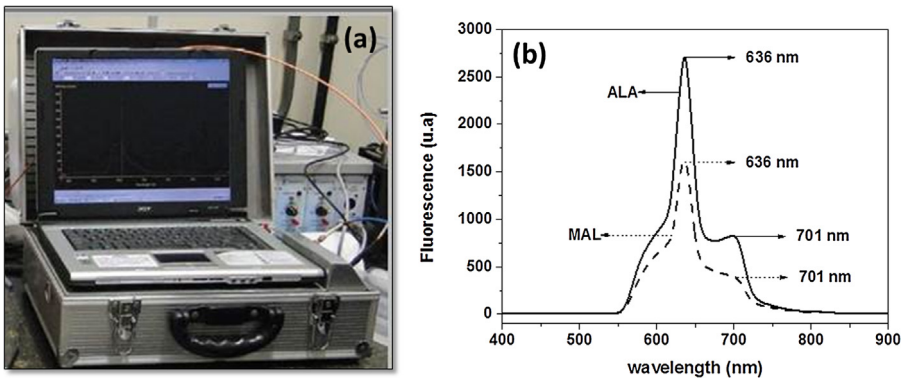

Fig. 3. (a) Fluorescence spectroscopy system (b) Fluorescence spectra of the production of PpIX induced by ALA and MAL, after application 3 h.

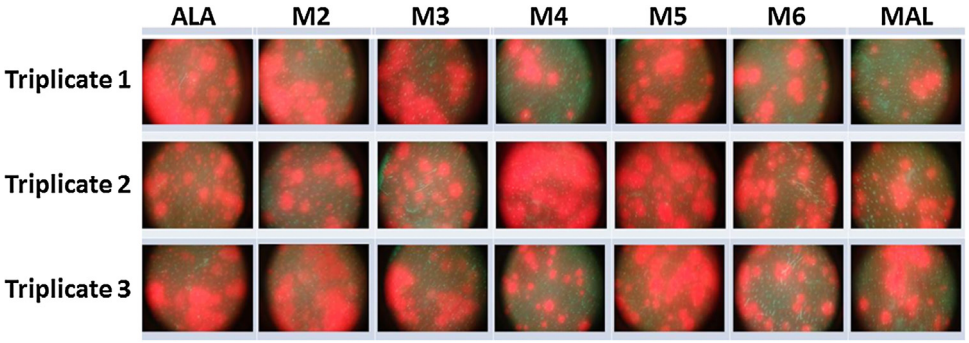

Fig. 4. Widefield fluorescence imaging after 3 h of the photosensitizers topic application in pig skin.

2.2. Animals and study preparation

The study was done *in vivo* using five crossed F1 and Agroceres species pigs (males) in the range of 3–4 months and weighing around 20 ± 2 kg. The animals were kept in adequate facilities. The animal experimental protocol (n° 007344/13) was approved by the Research Ethics Committee of the *Faculty of Agriculture and Veterinary Sciences* – UNESP (Jaboticabal, Brazil).

2.3. Anesthesia and analgesia protocols

Chemical immobilization was carried out as previously described [10]. Preanesthesia was performed by intramuscular administration of ketamine (12 mg/kg), midazolam (0.5 mg/kg) and acepromazine (0.1 mg/kg). Anesthesia was induced with propofol (4 mg/kg IV), animals were intubated with a 6.5-mm endotracheal tube, and anesthesia was maintained with isoflurane (1.0–1.5%) diluted in 100% of oxygen (1L/min) under spontaneous ventilation. We monitored the electrocardiogram, invasive blood pressure, rectal temperature oxyhemoglobin saturation, respiratory rate and end-tidal carbon dioxide. After stabilization of anesthesia (30 min), animals were prepared to PDT procedure. After the end of the procedure, tramadol (4 mg/kg IM) and meloxicam (0.2 mg/kg SQ) were administered for post-treatment analgesia. The Euthanasia was performed after 48 h with the same chemical immobilization protocol previously described and 30 mg/kg of propofol (IV). Tissue samples were removed from the pigs for histological analysis.

2.4. Application of cream on pig skin

The described procedure was carried out in five pigs. They were sedated, intubated and monitored by anesthesiology professionals while we performed the procedures for the study. After the animal was properly anesthetized, its back was shaved using shaving cream and razors. The shaving was done towards the hair avoiding

Table 1
Description of ALA and MAL mixtures evaluated in the research.

Samples	ALA (% relative)	MAL (% relative)
ALA (M1)	100	0
M2	80	20
M3	60	40
M4	50	50
M5	40	60
M6	20	80
MAL (M7)	0	100

cuts in the back, in order to preserve the integrity of the skin, thus retaining the maximum possible stratum corneum.

The animal's back was divided into 4 cm^2 areas. In a fraction of these areas the seven samples were applied in order to perform PDT, and were left occluded for 3 h. In the remaining areas, the samples were also applied and perform kinetic. The kinetics study was to monitor of the PpIX production over 5 h.

Fig. 1 shows the used area of the animal experiment. Each area has received the cream in a quantity sufficient to cover the whole area with a thickness of approximately 2 mm. Before applying the creams autofluorescence spectrum and widefield fluorescence image, were collected to fit as the background values. After applying the creams in each area, a PVC plastic was used together with aluminum foil to protect from ambient light and not degrade the photosensitizer.

In order to evaluate PpIX production, a kinetic test was performed, recording the fluorescence each hour for 5 h. For each fluorescence measurement performed, the photosensitizer was removed and reapplied in the same amount and condition, once the fluorescence data were recorded. We emphasize that no new cream was added to the area of study after each measurement, but rather, the same cream that was applied originally was reapplied. The removal and reapplication steps were done in order to prevent the photosensitizer from blocking light incident onto the skin of the animal during the fluorescence measurements. Moreover, in order to minimize interruption to the kinetic process, the fluorescence measurements were performed in time intervals averaging less than two minutes. The animal dorsum was divided covering experiments for the drugs kinetic and PDT areas. The occlusion for areas that received PDT was at 3 h. The areas chosen for the kinetic study were uncovered each hour for the measurement performance and occluded again.

2.5. Widefield fluorescence imaging system

For the acquisition of the images, a diagnostic system for fluorescence widefield was used. The widefield fluorescence image system is a commercial device produced, MM Optics, Sao Carlos—Brazil, named EVINCE. In brief the imaging system for widefield consists of a lighting device based on LEDs, emitting around 408 nm and coupled to a digital camera for image acquisition [18]. The measurements were taken for each sample and different time of PPIX formation (Fig. 2).

Table 2
IF50 values for the widefield images and fluorescence spectroscopy collected over time (5 h) after applying the creams.

Samples	Widefield image	Spectroscopy fluorescence
	IF_{50} (min)	IF_{50} (min)
ALA	120 ± 10	230 ± 7
M2	134 ± 6	4365 ± 36 × 10^3
M3	40 ± 60	114 ± 16
M4	128 ± 8	17 ± 20
M5	97 ± 7	131 ± 17
M6	120 ± 20	187 ± 25
MAL	70 ± 5	131 ± 9

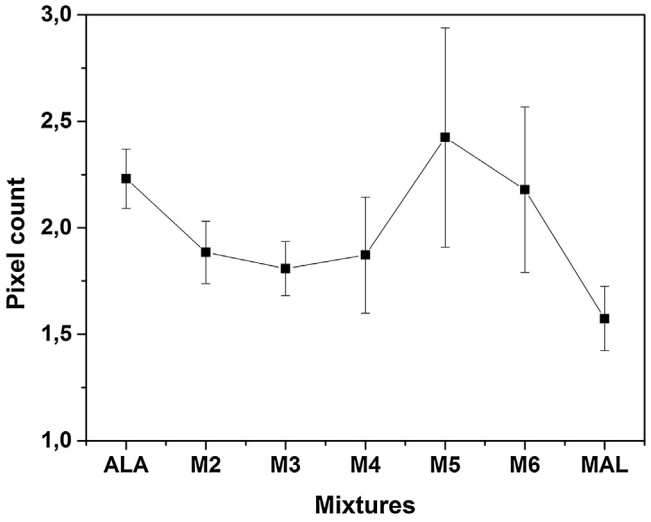

Fig. 5. (a) Analysis of the PpIX formation in 3 h by pixel count, Widefield fluorescence imaging.

The images obtained by widefield fluorescence were assessed quantitatively using a routine written in Matlab program. The program has defined an array separating the channels red, green and blue (RGB) colors. For the pixel count analysis, standardization was obtained dividing the red channel by the green channel. This was done to avoid the effects of different shutter speeds and ISO settings for image acquisition. After all, the fluorescence (pixel count) was determined by summing the values of all red channel divided by the sum of all values of the green channel. With these data it was possible to quantify the PPIX production and evaluated the uniformity the PPIX formation.

The fluorescence measurements obtained by widefield were collected using violet laser excitation (408 nm) that shows information about superficial skin layers (~0.1 mm) in agreement with Menezes [5].

2.6. Fluorescence spectroscopy system

The fluorescence spectroscopy system comprises two lasers for excitation, one emitting at 408 nm and the other at 532 nm. The investigation fiber probe in Y, directs the excitation laser to the tis-

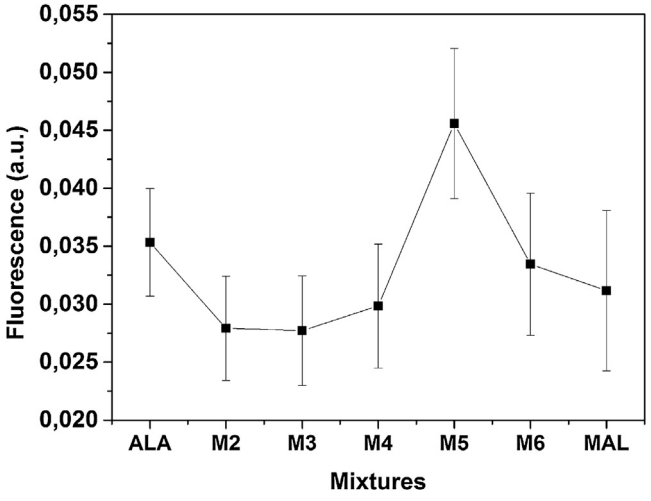

Fig. 6. (a) Analysis of the PpIX formation in 3 h by fluorescence spectroscopy.

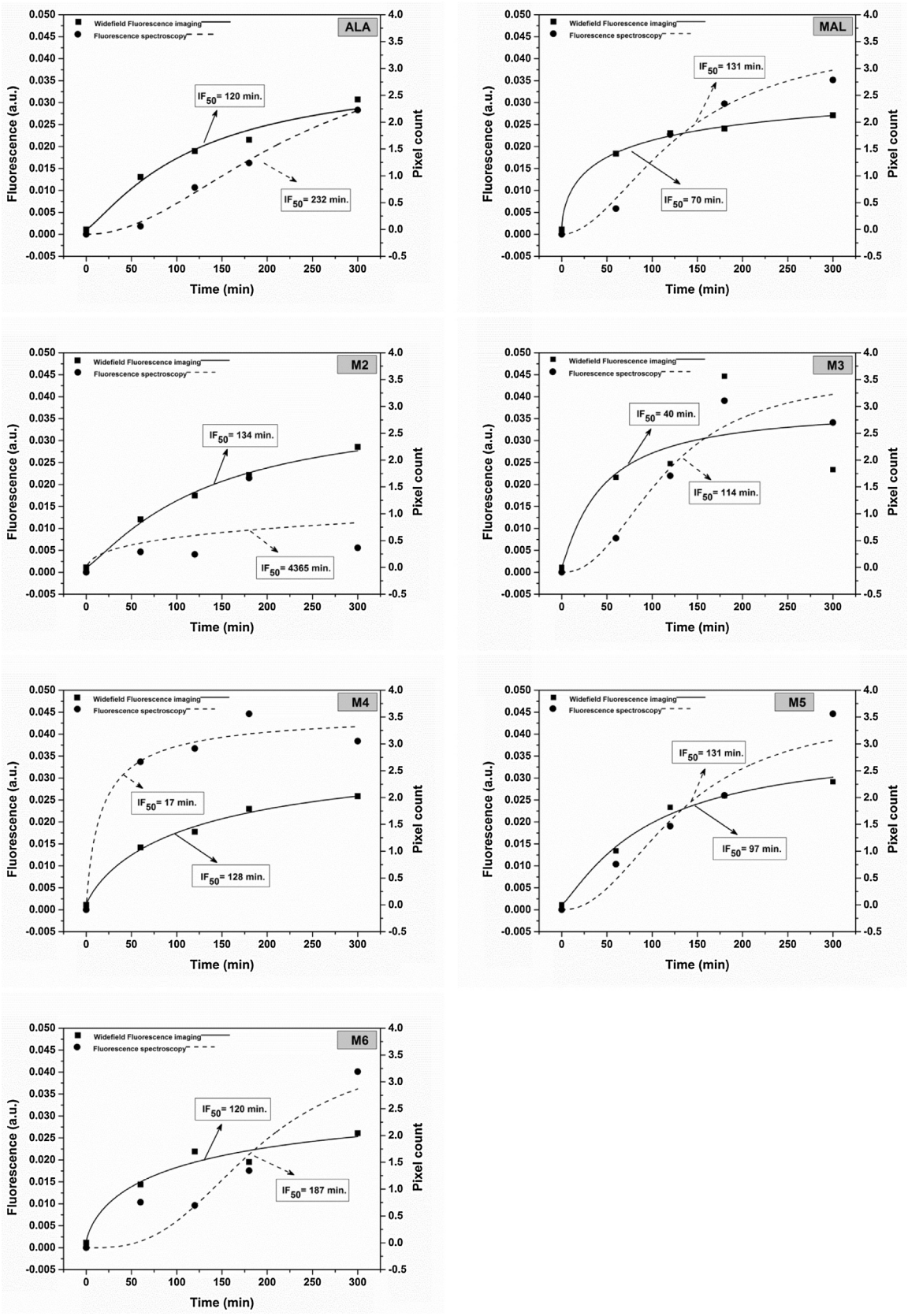

Fig. 7. Kinetics of the PpIX production by spectroscopy fluorescence and widefield fluorescence imaging for samples ALA, MAL, M2, M3, M4, M5 and M6.

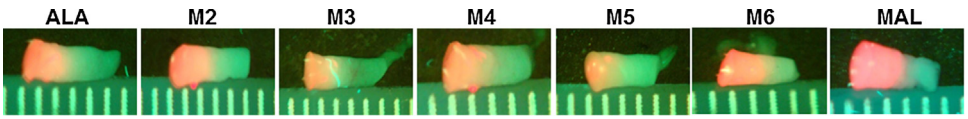

Fig. 8. Fluorescence image obtained by widefield fluorescence imaging to skin biopsies after 3 h of samples application.

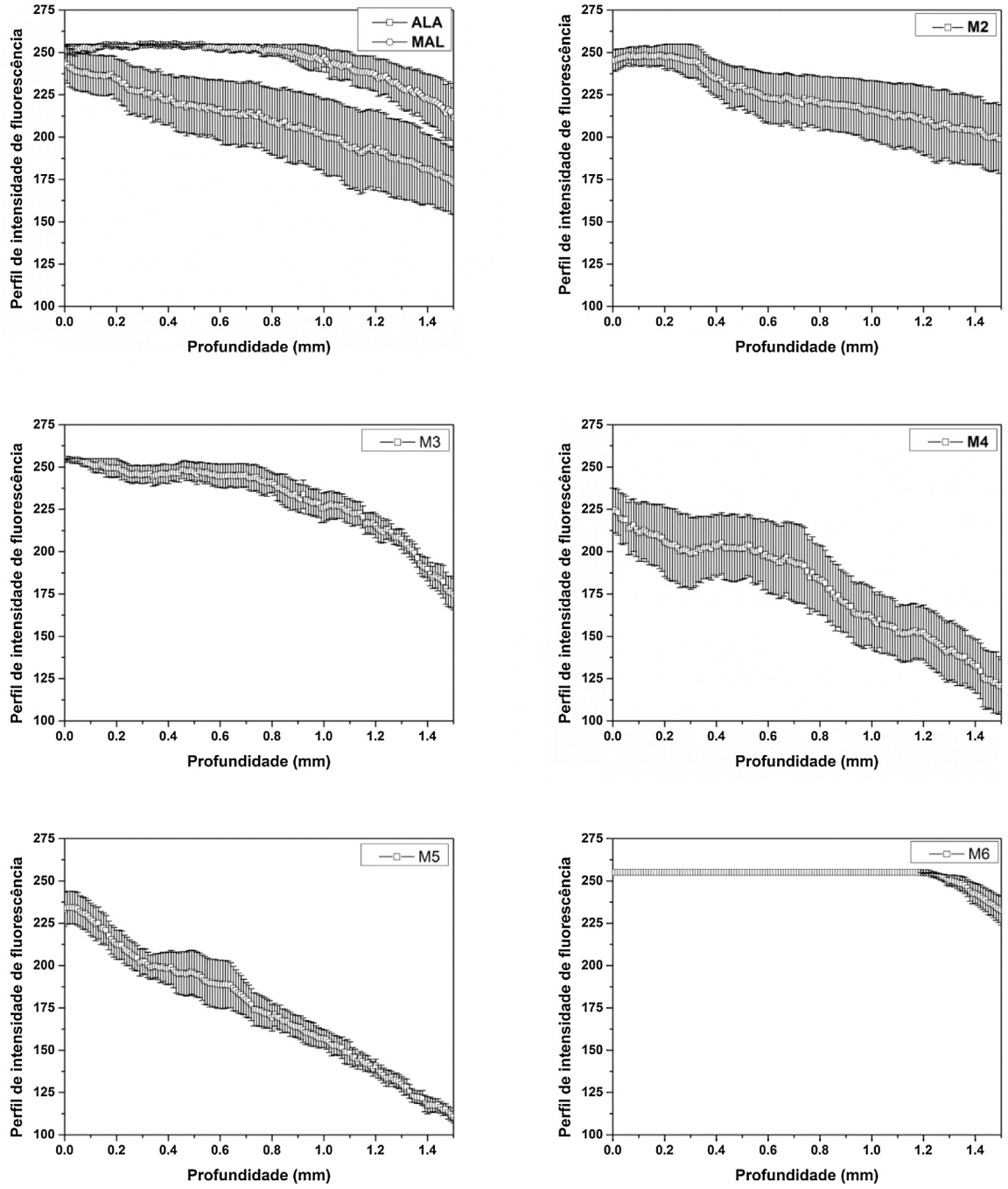

Fig. 9. Widefield fluorescence image profile of PPIX production in the swine skin layers after 3 h of cream application using: ALA, MAL, M2, M3, M4, M5 and M6.

sue while the second arm of the Y-shape collects the re-emitted by the tissue and delivers light to a spectrophotometer. The spectrophotometer is used USB2000 (Ocean Optics®, USA). A filter was used to remove backscattered light so that only the fluorescence was collected [13]. The prototype device as well as the typical spectra are shown in Fig. 3.

Although the fluorescence collects superficial information, there are differences between the data obtained by spectroscopy and wide field fluorescence imaging (more superficial fluorescence data). In fluorescence spectroscopy collection for ALA and its derivative, the observed spectrum containing 2 bands, one with greater fluorescence emission at 636 nm and a smaller band at 700 nm (Fig. 3b).

We recorded the fluorescence spectrum in contact with the tissue at 5 points distributed in the 4 cm^2 area, equivalent autofluorescence collection was performed. The evaluations from spectra analysis were normalized by total area and subtracted from the autofluorescence. The spectrum evaluations were performed using an Origin9 program.

2.7. Biopsies evaluation by widefield image

To evaluating the PPIX accumulation in the different skin layers, a punch biopsy, 3 mm in diameter and ~5 mm deep, of swine skin was taken three hours after application of the ALA and M-ALA mixture at skin.

These skin fragments were evaluated by widefield fluorescence imaging system and the PPIX production profile along the epidermis and Dermis layers was analyzed. The image analyses were performed only counting the red pixels from PPIX formation at skin biopsies.

2.8. Statistics

The statistical analysis between the different treatments was performed using ANOVA test (0.05%).

3. Results

3.1. Widefield fluorescence imaging

The fluorescence image analysis aimed to evaluate the PPIX production and homogeneous distribution in the skin surface. In Fig. 4, we show the images obtained within 3 h after application of the cream on condition of triplicates for 7 mixtures of ALA and MAL in the same animal. It can be seen how the PPIX formation in a healthy skin is heterogeneous for all samples and such formation is quite variable in a same mixture as in different areas. Therefore, although the mixtures are always applied on the animal's back in the same amount, there were differences in the PPIX production between replicas of the same experimental conditions in animals and between animals. This is due to the inherent variability of each animal that has just as the human model alterations in metabolism, skin thickness and natural barriers for penetration in different regions. This may thereby interfere with the permeation of mixtures of ALA and Mal and subsequent the PPIX formation and distribution in the skin.

Fig. 5 shows the quantitative analysis of the PPIX accumulation formed in 3 h according to the widefield fluorescence imaging. The graph is the average of the five animals. We found that in three hours the ALA PPIX accumulated more than MAL (~0.7 points), but the mixtures have accumulated about the same amount that ALA. The M5 sample indicates production over the amount by the ALA alone. The statistical analyzes were performed using ANOVA test that shows a comparison between the samples, and it was observed that the only samples which have a significant difference was M5.

3.2. Fluorescence spectroscopy

The mean fluorescence spectra collected on the samples are shown in Fig. 6. In this graph, we observe that M5 provides indication at producing more PpIX than ALA. In addition, the ALA PPIX accumulated more than the MAL in 3 h, so there are no significant spectral differences between M2, M3, M4 and M6 of ALA and MAL, they are within the standard error.

In the statistical analysis of the fluorescence spectra, there was a greater difference between the mixtures, so the ANOVA test indicates that there are differences of MAL with the mixtures M3, M4 and M5, and between the ALA and MAL.

3.3. Kinetic evolution widefield fluorescence imaging and fluorescence spectroscopy

Through fluorescence, analysis was done a monitoring and training to improve PPIX degradation treatment parameters appropriate time between the application of each photosensitizer and the illumination of the epithelial tissue. By performing the kinetic study with widefield fluorescence imaging and fluorescence spectroscopy, it was possible to analyze the growth behavior of PPIX production in the skin surface over 5 h.

Fig. 7 shows the fluorescence spectrum and widefield fluorescence imaging collected over time for ALA, MAL, M2, M3, M4, M5 and M6 samples, and its PpIX accumulation curve. The data is consistent with faster PPIX production using the samples.

Table 2 shows the index of fluorescence (IF_{50}) values for fluorescence images and spectra collected in the kinetic test.

In case the IF_{50} means the 50% of maximum fluorescence value obtained in 5 h. As we observed in Table 2 the IF_{50} values to ALA (IF_{50} = 231 min) when compared to MAL (IF_{50} = 131 min) shows that the PPIX production is lower to ALA than MAL. However the PPIX production to M5 mixture (IF_{50} = 131 min) is similar to MAL. The mixture M4 (IF_{50} = 17 min) presented the smallest time of PPIX production.

3.4. PPIX production evaluated in biopsies by widefield image

Fig. 8 shows the biopsies removed from pig skin in a scale (~5 mm). Here the proposal is to evaluate the PPIX formation in deep comparing the permeation of ALA, M4 and MAL.

Fig. 9 shows the average fluorescence profile in depth for PPIX production in pig skin biopsies using ALA, MAL and M4 mixture.

The PPIX production in the biopsies indicated that the permeation of MAL in the skin layers remains constant through a depth of approximately 1 mm; while ALA and M4 showed a decaying fluorescence profile, displaying less PPIX production with increasing layer depth. These results suggest that ALA, MAL and Mixture 4 induce the same amount of PPIX formation in superficial skin layers.

4. Discussion

Over the Widefield Image technique, the PPIX production (Fig. 5) obtaining by ALA is a little more than MAL after three hours of cream application (incubation), but according to kinetic analysis (Table 2) MAL (IF_{50} = 70 min) accumulated the same amount of ALA (IF_{50} = 121 min) in half of the time. For mixtures (M2-M6), regarding the PPIX production after 3 h of cream application (Fig. 5), we did not observed significant differences between them. On the other hand the M5 mixture produced a little more PPIX than ALA (Fig. 5). And the other mixtures (M2, M3, M4 and M6) indicated the pixel count greater than the MAL, equaling the production of PPIX from ALA. The data obtained by kinetic analysis (Fig. 7 and Table 2–widefield image), we observed the values of IF_{50}, and MAL (IF_{50} = 70 min) indicated shorter time of PPIX accumulation than ALA (IF_{50} = 120 min). Already the IF_{50} for mixtures M3 (IF_{50} = 40 min), M4 (IF_{50} = 128 min) and M5 (IF_{50} = 97 min) indicated smaller or near time of PPIX accumulation of ALA, but M3 indicated very high error value. Comparing the results mentioned previously the M5 presented higher amount of PPIX production and IF_{50} value near the ALA and MAL.

Regarding the Fluorescence Spectroscopy technique, there was no difference in the PPIX production (Fig. 6) to ALA and MAL after

three hours of cream application (incubation), but the data obtained by kinetic analysis (Fig. 7 and Table 2–spectroscopy fluorescence) showed that the PPIX production induced by MAL (IF_{50} = 131 min) is faster than ALA (IF_{50} = 230 min). Considering that the spectral analysis collect the PPIX production near the dermis, MAL would be the most suitable for use, because its PPIX production was similar to ALA, but IF_{50} was slower than ALA. The data obtained by kinetic analysis of mixtures using different times of PPIX accumulation shows different values of IF_{50} as: M3 (IF_{50} = 114 min), M4 (IF_{50} = 17 min) and M5 (IF_{50} = 131 min) being an indicative of that M4 showed a smallest time of PPIX production between them. The M2 mixture obtained of IF_{50} and error very high due to points no fit the growth curve. Comparing the results to Fluorescence Spectroscopy technique the M4 presented the same amount of PpIX production induced by ALA and MAL but in a smaller time (IF_{50} = 17 min).

The M5 mixture showed large PPIX production evaluated by both fluorescence techniques, and the kinetic analysis showed time of PPIX production similar to MAL. The M3 and M4 mixture presented behavior similar to ALA and MAL. This relative amount of PPIX produced in 3 h. In the kinetic analysis, M4 mixture showed to be the fastest at PPIX production when analyzed by spectroscopy fluorescence, but when analyzed by widefield image the time of PPIX production was similar to ALA and M3 showed to be the inverse of M4.

As expected and previously described in studies, the greatest challenge of our work was also to understand more about the permeation through skin of ALA, MAL and mixtures thereof. Then, the PPIX production is a greater marker on the different skin layers. There are a directly correlation on ALA, MAL and its mixtures permeation with the PPIX formation. Knowing that the stratum corneum acts as a protective barrier of the skin and this is structurally heterogeneous layer consequently the permeation of these precursors differs between animals and inside on the same animal (different skin regions—Fig. 4) [16,17].

There are few studies concerning to comparison of ALA and MAL in healthy skin human, but Andrea Lesar and colleagues [19] compared the formation efficiency of PPIX from these precursors in various parts of the human body (arm, forearm, back and legs) and accompanied fluorescence (4–29 h) after topical application. Then they observed that there were differences in the production of PPIX, which applied regardless of where the ALA accumulated more PPIX, but the location (back) where they applied the tape striping difference was only after 24 h.

According to the work cited, we obtained similar results in pig skin, wherein the ALA indicated to produce more PpIX than MAL, but in some cases the PPIX production was not higher for mixture than ALA and MAL.

As shown by Valentine at all [12], there was no difference for increasing the amount of PPIX using ALA and MAL when analyzed by fluorescence spectroscopy using laser emission in 400 nm. The emission at 400 nm allows measuring the output of PPIX the most skin superficial (epidermis). In the case of our work, this skin superficial analysis was performed by widefield fluorescence imaging. Already the fluorescence spectroscopy technique in the emission at 532 nm allowed evaluating the PPIX formation in the inner layers of the skin, it being an advantage.

The experiments obtained using pig skin biopsies for the fluorescence evaluations showed the profile PPIX production through the skin layers (epidermis and dermis). The results indicated that the MAL and M3 permeation on skin layers remains constant; while ALA, M2, M4 and M5 indicated a decay of PPIX accumulate in deeper skin layers. The result obtained on the M6 mixture can not be interpreted, because it was the saturation of the fluorescence on the images of biopsies to this mixture. In the other hand, the mixture M4 showed similar behavior to ALA.

Comparing all results obtained at superficial and biopsy skin depth by fluorescence evaluation; is possible to suggest that M3, M4 and M5 showed several advantages to optimize the PDT. M3, M4 and M5 presented high PPIX production (amount and homogeneity) in a small incubation time (high speed of PPIX formation) at superficial and deeper skin layers. However, when we calculate the average of the IF_{50} values (widefield image and spectroscopy fluorescence), M4 shows the smallest average; which indicates that M4 samples resulted in the least amount of time needed to produce PPIX.

Therefore observe both the fluorescence measuring used in this study found similar results, and there is correlation between them (correlation coefficient ~ 0.8).

Knowing that the PPIX production at skin is heterogeneous, the fluorescence spectrum collection can reach an area where not PPIX formed in the surface. Then to minimize this error, the fluorescence spectra are subtracted by the autofluorescence.

In this study, it is makes clear that ALA and MAL association can improve PPIX formation in amount (quantity) and uniformity, and this production is still growing. Here we showed that the PpIX production evaluated by fluorescence analyses has a direct correlation with the damage induced by PDT. The results showed that M5 had a greater production of PPIX in for both fluorescence analyses however the M4 shows the same amount of PPIX production induced by ALA in a high speed, optimizing as well the Photodynamic therapy to reducing the time of treatment. In addition, we performed studies using another type of emulsion (water-in-oil emulsion) that had intention to increases the MAL permeation due to lipophilic characteristics of external phase of this emulsion. By fluorescence evaluations, mixtures with more proportion of MAL (M5 and M6) showed high PPIX production. However, the fluorescence of PPIX formation to affect of these emulsion systems in PDT it is necessary to investigate the photodynamic effect of these mixtures in deeper skin layers after PDT by histological analysis.

5. Conclusion

It is known that the fluorescence analysis shows a superficial behavior of PPIX production at skin. However, the evaluations by fluorescence spectroscopy and widefield imaging analyses show differences in the PPIX production to ALA, MAL and mixtures in skin-deep layers as mentioned on text.

Mixture 4 results to PPIX formation (amount and incubation time) obtained by widefield fluorescence imaging is the same to ALA. In the other hand, the fluorescence spectroscopy analyses to mixture 4 shows the same amount of PPIX production than ALA, the incubation time formation is reduced 6 folds (30 min).

As the fluorescence analysis are considerate superficial, the skin biopsies analyses by widefield fluorescence imaging brought us information about superficial and deep skin layers. The results found to mixture 4 prove the best PPIX production along the biopsy. These results confirm that mixture 4 is the best choice to improve the topical PDT.

Here we propose a new alternative to performing topical PDT using ALA and its derivative MAL by using a mixture of both in the same treatment. This mixture improves the best PPIX formation in the amount and homogeneity on skin at superficial and deeper layers. These results suggest that the amount and homogeneity of PPIX formation in the different skin layers is responsible for potential results obtained in the PDT. Therefore, the mixing proportion of ALA and MAL should be changing according to the size and depth of the treatment targets. Another positive impact is the reduction on incubation time on the treatment using the mixture 4 decreasing costs and remains the effectivity of PDT treatment.

Acknowledgments

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