Research Article

Topical Photodynamic Therapy Using 5-Aminolevulinic Acid (Ala) for Cosmetic Purposes on Aesthetic Procedures

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ABSTRACT

Topical Photodynamic Therapy (PDT) applications have been increasing on dermatological fields on skin diseases as well as in cosmetic procedures. Currently, PDT in dermatology for cosmetic procedures using 5-aminolevulinic acid (ALA) for application is done in elevated concentrations and often is followed by several side effects due to elevated inflammatory process. However the inflammatory response level depends not only of ALA concentration but incubation time (that increases the PPXI production) and fluencies of laser/LED irradiation. The photodynamic reaction follows when light interacts with protoporphyrin IX (PPIX), which is produced on skin through ALA application in the presence of oxygen on tissue. After that oxygen's species formation resulting in an increase of the inflammatory response characterized by additional side effects. The goal of study is to demonstrate an alternative procedure to perform topical PDT using ALA in small concentrations for cosmetic purposes on aesthetic procedures now defined by us as Photodynamic Cosmetic Therapy (PDTC) that combines both benefits from Phototherapy and PDT with controlled adverse effects. The studies were performed in human volunteers using ALA in different concentrations (2% and 20%) and fluencies using laser/led red irradiation ranging from 25 to 150J/cm^2 with the same incubation time of 1 h after micronnedling procedure. The inflammation process after procedure was monitored by grade of severity as well as its benefits on wrinkles reduction and quality of skin. The results show that there is a threshold light dose to perform Photodynamic cosmetic therapy, using low concentrations of ALA and fluencies, where the side effects due to inflammatory process are controlled and the results on skin rejuvenation can be observed. These results can be useful to optimize topical PDT for cosmetic purposes on aesthetic procedures applications with safety and efficacy given opportunities to other non-medical professionals to work with PDTC.

INTRODUCTION

Topical PDT application using ALA for cosmetic purposes on dermatology is increasing and promoting great results in the skin treatment of acne, scars, wrinkles, and flaccidity reduction. In addition, this treatment has a positive impact on pathologic skin

conditions such as psoriasis, atopic dermatitis, actinic keratosis and others On oncological and dermatological areas, topical PDT using ALA shows many side effects because of the elevated inflammatory process on skin; and its inflammatory response level depends on aminolevulinic acid (ALA) concentrations and fluencies [1-5].

The inflammatory process occurs in response to the photodynamic reaction, and then the cutaneous photosensitization, dark crusts, sensitive red skin and the peeling effect can be observed at different levels. It is important to emphasize that; in any case, hyperpigmentation after the procedure (phototypes IV-VI) may also occur. Mainly in cases of elevated inflammation process on elevated skin phototypes [6-9].

Photodynamic Therapy (PDT) applied for cosmetic purposes on aesthetic procedures using ALA in small concentrations is now defined as Photodynamic Cosmetic Therapy (PDTC) by us and combines both skin benefits from Phototherapy and PDT with controlled adverse effects. In a previously paper we introduced the Photodynamic Cosmetic Therapy concept for alopecia treatment, using photoctives to cosmetic proposes as curcumin and methylene blue, optimizing the growth hair [10].

Although Photobiomodulation and PDT are both light-based treatments and may be applied alone or in combination with cosmetic and dermatological treatments, they are described as distinct techniques. Photobiomodulation is based on the interaction of light with the endogenous skin biomolecules (i.e., photoreceptors or endogenous chromophores) such as melanin, hemoglobin and others using low power light radiation (mW) and low fluencies (J/cm^2) acting on mitochondria promoting differentiation and proliferation of cell and biomolecules acting on rejuvenation process [11-16]. PDT is based on laser/led light interaction with a photosensible substance or photosensitizer (i.e., photoreceptor or exogenous chromophores) or its precursor as ALA, which is capable of promoting the photophysical and photochemical processes of absorption and energy transference. Thereby, reactive oxygen species and singlet oxygen are produced and consequently, the inflammatory response occurs promoting tumor cells destroy (apoptosis and necrosis pathways), microorganism control (bacteria and fungi) as well as induce the production of a new tissue, that replaces the damage tissue due to the activation of the healing process, and the rejuvenating effect on skin can be observed [17-21].

Topical PDT on oncological and dermatology field using ALA is often applied on concentrations from 8 to 20 % using Incubation times around three hours and fluencies using red light irradiation, at 630nm from 37 to 150J/cm^2 , and blue light irradiation, at 450nm around 10 J/cm2, for deeper and superficial interaction on skin respectively [1,17-20].

However, there is a narrow relation between ALA concentration, fluencies and incubation time on PDT procedure. Furthermore, ALA is the precursors of the endogenous protoporphyrin IX (PPIX) produced at skin and the red light interaction with PPIX depends on the direct correlation between ALA permeation through the skin as well as its conversion on PPIX at skin [2-4]. ALA presents hydrophilic properties showing low permeation trough skin and when associated to microneedling technique firstly on PDT procedure optimizes the ALA permeation through skin [22-25].

In a deal with the literature the elevated photodynamic reaction on Photodynamic Therapy (PDT) procedure is related to concentration of ALA and fluencies, high fluencies using laser/led red irradiation (above 50J/cm^2) and small concentrations of ALA (below 5%) as well as low fluencies using laser/led red irradiation (above 50J/cm^2 or around 37J/cm^2) using high concentrations of ALA (above 5%) the side effect due to elevated inflammation response increases.

The goal of study is to demonstrate an alternative procedure to perform topical PDT using 5-Aminolevulinic Acid (ALA) in small concentrations for cosmetic purposes on Aesthetic procedures now defined by us as Photodynamic Cosmetic Therapy (PDTC) that combines both benefits from Phototherapy and PDT with controlled adverse effects. Here we presented of the threshold light dose to perform the Photodynamic Cosmetic Therapy, using small concentrations of ALA (from 2 to 5%) and fluencies using laser/led red irradiation on range from 25 to 50J/cm^2, where the photodynamic activity reaction is controlled on Aesthetic procedures, and the results on skin rejuvenation can be observed.

MATERIALS AND METHODS

Chemicals: The studies were done using 5-Aminolevulinic acid hydrochloride (5-ALA) using different concentration (from 2 to 20%) concentration, in an oil-in-water emulsion (O/W)

obtained from PDT-PHARMA (Brazil). The formulation was evenly applied on skin (face) and an occlusive mask to protect it from light was dressed. The ALA formulation was done using one oil /water emulsion with 2 % of ALA.

Treatment procedure: Twenty one patients, around 25 and 40 years of age with a clinical diagnosis of normal skin were recruited for this clinical study. The protocol used in this study followed the procedures establish by Human Research Ethics Committee of Brazil (n0 73603317.0.0000.5504). Written informed consent was obtained from all participants. In this study we have 16 groups of conditions considering 4 concentrations of ALA (2,5, 10 and 20%) and 4 fluencies (25,50,75 and 150J/cm^2) as described as: GROUP 1: 1A) ALA 2%/D=25J/cm^2, 1B) ALA 2%/D=50J/cm^2, 1C) ALA 2%/D=75J/cm^2, 1D) ALA 2%/ D=150J/cm^2; GROUP 2: 2A) ALA 5%/D=25J/cm^2, 2B) ALA 5%/D=50J/cm^2, 2C) ALA 5%/D=75J/cm^2, 2D) ALA 5%/ D=150J/cm^2; GROUP 3: 3A) ALA 10%/D=25J/cm^2, 3B) ALA 10%/D=50J/cm^2, 3C) ALA 10%/D=75J/cm^2, 3D) ALA 10%/D=150J/cm^2; GROUP 4: 4A) ALA 20%/D=25J/cm^2, 4B) ALA 20%/D=50J/cm^2, 4C) ALA 20%/D=75J/cm^2, 4D) ALA 20%/D=150J/cm^2.

In case all volunteers was submitted to the same protocol as described: 1) Cleaning the face to remove dirt; 2) Evaluate skin autofluorescence (green) using the EVINCE equipment (MMOptics); 3) perform microneedling on the skin with the dermarroler equipment; 4) Apply the ALA cream to the skin surface (2 to 20%); 5) occlusion of the skin with film paper; 6) After 1 hour, evaluate the production of PPIX in the skin by assessing the fluorescence (red) by the EVINCE equipment (MMOptics); 7) Remove the ALA cream from the skin; 8) Carry out skin irradiation with red light using different irradiation doses from 25 to 150J / cm2; 9) Apply the sunscreen to the patient's skin. In previously paper we discussing about the widefield fluorescence imaging technique on fluorescence imaging evaluations with the EVINCE equipment (MMOptics) using ALA [20]. On Figure 1 is possible to see the details of the PDT procedure.

The evaluations of Photodynamic reaction were done comparing the initial time (t 0), 3, 7, 10 and 15 days after procedures in the same volunteer and between the same treatments groups. The measurements related to PPIX formation at skin (face) was compared between all 21 volunteers in the different conditions: ALA concentration (from 2 to 20%), fluencies (from 25 to 150J/cm^2) at same incubation time (1h) using previously microneedling technique optimizing the ALA permeation as well as the PPIX production in 1h of incubation time at skin.

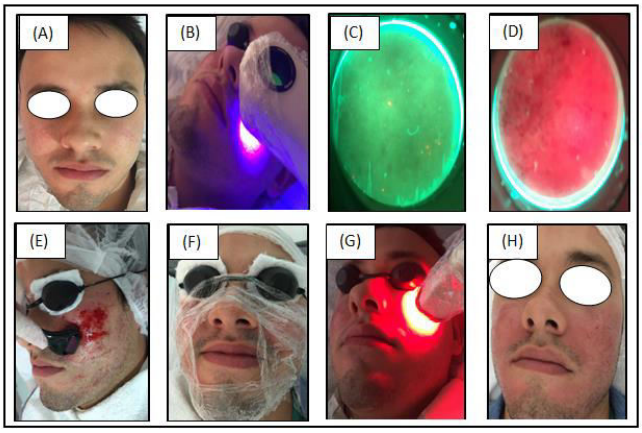

Figure 1: Photodynamic Therapy (PDT) procedure using 2% ALA: A) initial skin, B) evaluation autofluorescence at superficial skin by EVINCE (MMOptics-Brasil), C) green autofluorescence at superficial skin, D) RED Fluorescence of PPIX at superficial skin, E) Microneedling technique, F) occlusion mask on skin after ALA cream application, G) Lince equipment- illumination system (MMOptics-Brasil) with red light and fluence of 150J/cm2, H) skin after PDT procedure.

Inflammatory response level measurements: The photodynamic reaction grade, measured by inflammatory response level, was classified by investigator on 5-point scale [l=minimal][2=mild][3=moderate][4=elevated], [5 severe]). For each photodynamic reaction grade (from l to 5) a punctuation scale was applied to volunteers based on specific skin observations as: erythema, blisters, edema, pain, burn sensation, photosensibilisation days, dry skin, dark crust, sensible skin and peeling trying to demonstrating the variability between the volunteers in the same group grade on 24, 48, 72 hours and 7, 10 and 15 days after procedure. The Figure 7 shows the threshold light dose to different concentrations of ALA and fluencies of Light irradiation where the data were expressed by mean ± Standard Error of Mean (SEM) of the twenty one participants.

DIAGNOSIS AND EQUIPMENT FOR IRRADIATION PROCEDURE

The light irradiation procedures using red laser/LEDs were done using tree equipment's from MMOptics (São Carlos, SP –

Brazil) a laser company from Brasil. For PDT procedures the commercial equipment called Lince (MMOptics) were used using high fluencies of light irradiation above 50J/cm^2 and for and Venus equipment using fluencies from 25 to 150J/cm^2. This system provides a dual function: optical diagnosis of Protoporphyrin IX and illumination system using high fluencies of light irradiation to be applied on PDT procedure. The evince equipment is another equipment, derivate from Lince, capable to perform the widefield fluorescence imaging and in case; its advantage is that is a portable optical diagnosis. In addition, another commercial equipment was used called Venus (MMOptics, São Carlos, SP - Brazil). This system shows a dual platform with LED (blue in 450nm and yellow in 590 nm) and laser system (infrared in 850nm and red in 630nm). Both equipment's were applied using red light.A special device was developed presenting a dual platform: a widefield fluorescence imaging system that is optically based on 405 nm LED (Light Emitting Diodes) arrays that allows observing the fluorescence emission and an illumination probe for PDT treatment constituted with 630nm LED arrays.

RESULTS AND DISCUSSION

Many techniques are being proposed as being capable to increase the permeation of ALA and MAL through skin. Among a variety of proposed procedures [22,25], microneedling is a promising technique for transdermal drug delivery. The latter shows interesting results on PDT procedures by optimizing the PPIX production extensively and also homogeneity [22,25]. In a previous study, we observed an incubation time reduction from three hours to one hour when considering ALA application as a way to improve PPIX formation at the skin using microneedle technique [25]. The microneedle technique has two functions on skin: 1) it promotes wound healing by increasing the collagenases and angiogenesis processes, and 2) it enhances the best well-known transdermal drug delivery on the skin. For the aforementioned reasons, this technique has been regularly applied on Photodynamic therapy to aesthetic protocols. In the current study, researchers have discovered that in the presence of microneedles even in small concentrations of ALA and MAL, the PPIX formation is greater in amount and homogeneity under a small incubation time (1h) [25].

In Figure 1 is possible to see the PDT procedure using Microneedling technique before 2% ALA cream application, with 1h of incubation time previously, irradiated with red light in 630nm and fluence of 150J/cm^2 using the Lince equipament (MMOptics-Brasil). It is observed that the PPIX formation is greater in amount and homogeneity (red fluorescence). The microneedling technique prior to the ALA cream application at skin results in a greater amount and homogeneity of PPIX formation after one hour of incubation time.

On Figure 2 the photodynamic reaction after 24h of PDT procedure, presented on Figure 1, shows elevated Inflammation response level and adverse effects as erythema, blisters, edema, pain and burn sensation, photosensibilisation days, dry skin, dark crust, sensible skin and peeling. Also is evident that the skin remains sensible over 7 days.

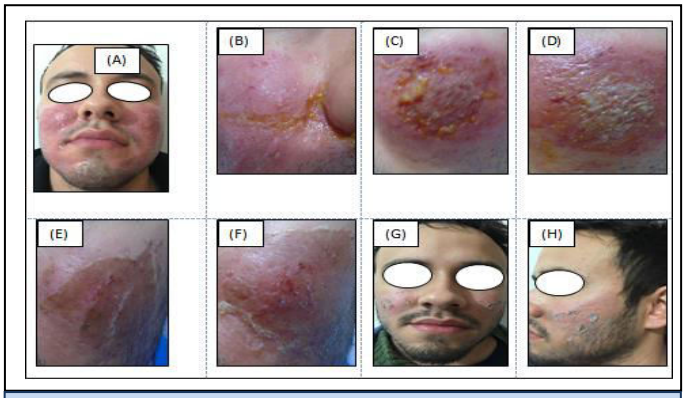

Figure 2: Evaluation of photodynamic reaction on PDT procedure done on Figure 1: A) 24h after PDT procedure, B-C-D) 24h after PDT procedure: elevated inflammation response level (erythema, blisters and edema), E-F) 7 days after PDT procedure (dark crust, dry skin, sensible skin and peeling), G-H) 10 days after procedure.

The Photodynamic reaction on PDT is associated with elevated Inflammatory Response Level firstly characterized by erythema, blister, edema, pain and burn sensation, skin photosensibilisation and as its consequences dark crusts, peeling and hyperpigmentation after procedure. High inflammatory response level increases the inflammatory phase time on healing process and the side effects as elevated. In a deal with our clinical observations the dark crusts and peeling will appears over 3 days in cases of elevated Inflammatory Response Level.

On Figure 3 the evolution of photodynamic reaction on PDT procedure can be observed using 2% ALA with fluencies of 75J/cm^2 (3A;3B;3C;3D). The elevated inflammation response

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level is presented (erythema, blisters and edema) and the followed alteration on skin as dark crust, dry skin, sensible skin and peeling is besides observed. Also in the same patient, the influence of different fluencies, related to inflammation response level, on Photodynamic Cosmetic Therapy – PDTC procedure was evaluated.

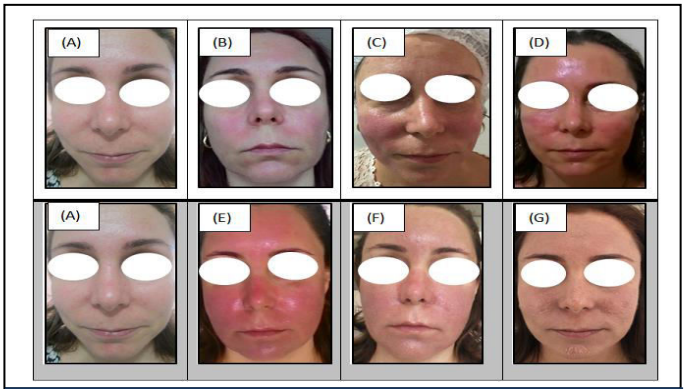

Figure 3: Photodynamic Therapy (PDT) and Photodynamic Cosmetic Therapy (PDTC) procedures using 2% ALA in different fluencies of light irradiation (25-75J/cm²). Photodynamic Cosmetic Therapy (PDTC) procedure using 2% ALA with fluence from 25 to 50J/cm²: A) initial skin, B) 24h after PDC procedure with fluence of 25J/cm², C) After 24h of PDC procedure with fluence of 37J/cm² , D) After 24h of PDC procedure with fluence of 50J/cm². Photodynamic Therapy (PDT) procedure using 2% ALA with fluence of 75J/cm²: A) initial skin, E)24h after PDT procedure (elevated inflammation response level), F - G) 7 and 10 days after PDT procedure (dark crust, dry skin, sensible skin and peeling).

The Figure 3 (A-B-C-D) shows that using 2% ALA with fluencies of light irradiation around 25, 37 and 50J/cm² the inflammatory process effect is controlled since that the adverse effects are minimized. The results suggest that the inflammation response level decreases when using low fluencies of light irradiation (≤50J/cm²). On Figure 3 (A-E-F-G) it is possible to see that high fluencies of light irradiation (≥50J/cm²) increases the inflammatory process effect shows elevated inflammation response level after procedure (7 and 10 days) where it is possible to see the adverse effects as dark crust, dry skin, sensible skin and peeling. Using fluence of 25J/cm² is possible to see that the inflammatory response is low and controlled.

The skin photosensibilisation and its level (from slow to high) is associated to amount of PPIX produced and its faster elimination from skin which can be occurs during 7 days using small concentration of ALA (~2-5%) increasing over 7 days using ALA in high concentrations from 10 to 20% (results not shown here). As known on literature topical application of ALA on PDT, using elevated concentration of ALA from 10 to 20% and fluencies of light irradiation from 37 to 150J/cm², shows high side effects at skin, as mentioned before, due to elevated inflammatory response level. But in a deal with our results presented here when applied in small concentrations around 2% (i.e., ten folds lower), these side effects can be easily controlled.

The dark crusts and the peeling effect will appears around 3 days after procedure and in cases of elevated inflammatory response level can be occurs over 3 days. Depending on inflammatory response level the healing process progress can be slowest or fastest. In case of elevated photodynamic reaction (elevated inflammatory response level) the healing progress occurs over 7 days and in cases of moderated to slow photodynamic reaction the healing process progress occurs early (around 7 days).

The ALA cream shows pH level ranging around 3-3.5 and acts promoting a superficial skin peeling and also stimulates the PPIX production at skin. Then, both functionality ensures one action at epidermis and dermis, promoting the reepithelization and dermis structuration (neocollagenesis) simultaneously; such impact differs from others acids which normally only promote peeling at superficial skin layers [15]. In this case ALA can be considered as a chemical active that promotes peel on skin surface (Chemical Peel) and the PPIX (endogenous Peel produced after ALA application) can be defined as a Photoactive Peel that promotes peel on skin surface when activated by light.

Cosmetic products using ALA, as PPIX precursors (Photoactive Peel), can be defined as photocosmetics, shows the following characteristics: 1) absorption bands on the visible light, 2) high photostability (low photodegradation), 3) Act as guide light on different skin layers and 4) acting stimulating the pro-inflammatory mechanisms (neocollagenesis, angiogenesis) as well as photobiomodulation mechanisms.

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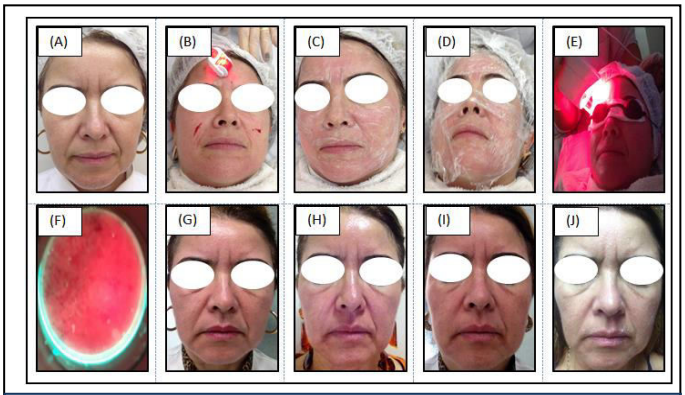

Figure 4: Photodynamic Cosmetic Therapy (PDTC) procedure using 2% ALA: A) initial skin, B) Microneedling technique C) 2% ALA cream application, D) occlusion mask on skin, E) Venus -illumination system (MMOptics-Brasil) with red light and fluence of 25J/cm^2, F) red fluorescence of PPIX at superficial skin, G-H-I) 24,48 and 72h after PDTC procedure: low inflammation response level (erythema, blisters and edema), J) 7 days after PDTC procedure: controlled adverse effects (dark crust, dry skin, sensible skin and peeling).

Figure 4 shows the evolution of photodynamic reaction on PDTC procedure using 2% ALA with fluence of 25J/cm^2 and is evident witch the inflammation response is controlled. It is possible to see on Figure 4 that after 7 days of PDTC procedure the adverse effects as dark crust, dry skin, sensible skin and peeling effect is controlled.

The Figure 5 shows the Photodynamic Cosmetic Therapy procedure using 2 and 5% of ALA in the same fluence of light irradiation (25J/cm^2) and it is observed that the inflammation response level is greater increasing the ALA concentration.

In Figure 5 the Photodynamic Cosmetic Therapy procedure was done using 2 and 5% of ALA in the same fluence (25J/cm^2) and it is observed that the inflammation response level is greater increasing the ALA concentration (from 5 to 2%).

Then we suggest that in a deal with results presented before (Figure 3,4 and 5) that the PDTC can be applied with safety using 2% ALA with fluencies of light irradiation from 25 to 50J/cm^2 leading to low and moderated inflammation response respectively.

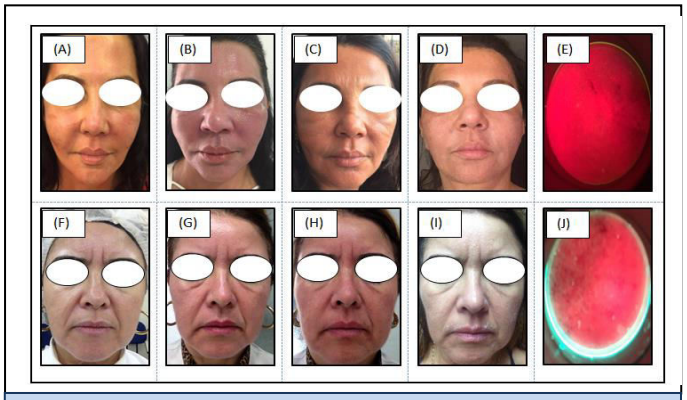

Figure 5: PDTC procedure using 2 and 5% ALA with fluence of 25J/cm^2. A) and F) skin before PDTC using 2 and 5% of ALA respectively. B) and G) after 24h of PDTC procedure. C) and H) after 72h of PDTC procedure. D and I) After 7 days of PDTC procedure. E) and J) Red fluorescence of PPIX at superficial skin respectively after 1h of 2% ALA cream application using microneedling technique previously.

The graph on Figure 6 shows the curves to Inflammation Response Level versus Fluencies of light irradiation as a function of ALA concentration. Using an exponential grow fitting we found values to threshold dose to ALA cream application (in different concentrations and fluencies). To 2% ALA the threshold dose is around 42J/cm^2, increasing ALA concentration to 5% the threshold Light dose decreases to 34J/cm^2, and to 10 and 20% of ALA the Threshold Light Dose decreases more yet around 25 J/cm^2. Then we can conclude that using ALA concentration above 2% and fluencies from 25 to 50 J/cm^2 the inflammatory response level will be low to moderate and the adverse effects can be controlled.

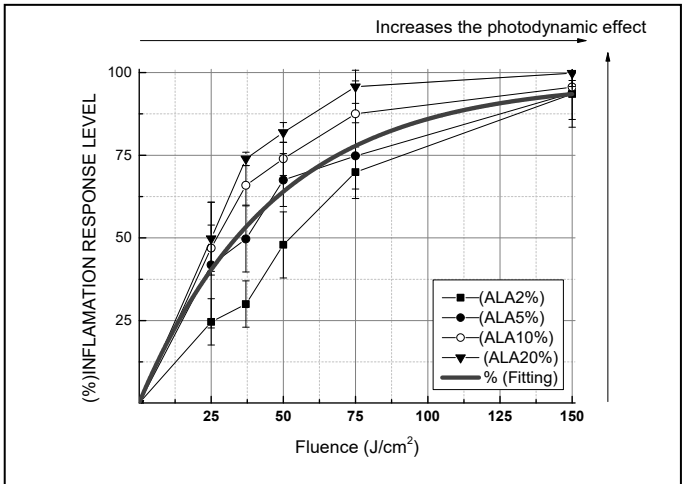

Figure 6: Threshold light dose evaluation to PDT and PDC.

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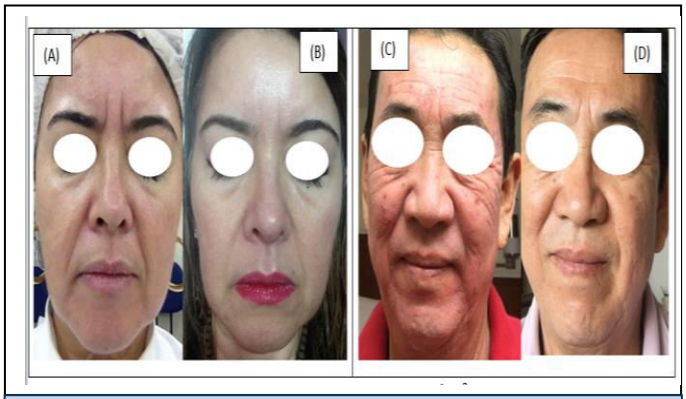

Figure 7: PDTC procedure using 2% ALA with fluence of 25J/cm² (A) before and (B) after 30 days of treatment. PDT procedure using 2% ALA with fluence of 150J/cm² (C) before and (D) after 30 days of treatment.

The results show that there is a threshold dose to perform PDTC given that when using small concentrations of ALA ranging from 2% to 5% and fluencies of light irradiation from 25 to 50J/cm², the side effects are controlled. The side effects increase when ALA concentration is around 5% or up and fluencies up to 50J/cm². Using high fluencies up to 100 J/cm², the concentration effect of ALA is not impacted.

The Figure 7 shows the PDT and PDTC results using 2% ALA and fluencies of 25 and 150J/cm² on aging treatment.As we can see on the figure 7 for both treatments PDT and PDTC the results obtained on aging treatment are positives. Then we can suggest that the photodynamic cosmetic therapy for cosmetics purposes can be useful on aesthetical applications using 2% ALA and fluence of light irradiation around 25J/cm² on skin previously prepared by microneedling technique. Knowing about the elevated inflammatory response level to PDT on skin, increasing the healing process time and consequently the side effects, the PDTC offers new possibilities of skin care techniques for skin rejuvenation without side effects.

CONCLUSION

Here we presented of the threshold light dose to perform the Photodynamic Cosmetic Therapy and PDT. The Photodynamic Cosmetic Therapy can be utilized safely and efficiently in aesthetic applications using small concentration of ALA around 2% and fluencies of light irradiation until 50J/cm². These results can be useful to optimize the topical applications of PDT using ALA for cosmetics purposes on aesthetic applications (PDTC).

AUTHOR DISCLOSURE STATEMENT

The authors inform that there is not conflict of interest.

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REFERENCES

1. Gold MH. (2016). Cosmetic photodynamic therapy. 1. ed. New York: Karger. 147: 118-119.
2. Uebelhoer NS, Dover JS. (2005). Photodynamic therapy for cosmetic applications. Dermatologic Therapy. 18: 242-252.
3. Pinto MCC, Fujita AKL, Menezes PFC, Bagnato VS. (2018). Photodynamic Therapy with 5-Aminolevulinic Acid (ALA) in the Treatment of Acne: A Case Study. Clin Dermatol Res Ther. 1: 114.
4. Babilas P, Landthaler M, Szeimies RM. (2006). Photodynamic therapy in dermatology. European Journal of Dermatology. 16: 304-348.
5. Ibbotson SH. (2010). An overview of topical photodynamic therapy in dermatology. Photodiagnosis and Photodynamic Therapy. 7: 16-23.
6. Agostinis P, Berg K, Cengel K, Thomas H Foster, Albert W Girotti, et al. (2011). Photodynamic Therapy of cancer: an update. CA Cancer J Clin. 61: 250-281.
7. Sibata C, Colussi V, Oleinick N, Kinsella T. (2001). Photodynamic therapy in oncology. Expert Opin Pharmacother. 2: 917-927.
8. Allison R, Sibata C. (2010). Oncologic photodynamic therapy photosensitizers: a clinical review. Photodiagnosis Photodyn Ther. 7: 61-75.
9. Allison RR, Moghissi K. (2013). Photodynamic Therapy (PDT): PDT Mechanisms. Clin Endosc, v. 2013; 46: 24-49.
10. Menezes PFC, Mauricio Junior N, da Mata R, Urbaczek AC, Pinto MCC, et al. (2018). Photobiomodulation and Photodynamic Cosmetic Therapy on Hair Growth: Case Report. Clinical Dermatology: Research and Therapy. 1: 123.

11. Hamblin MR. (2016). Photobiomodulation or low-level laser therapy. J Biophotonics. 9: 1122-1124.
12. Ying-Ying Huang, Sulbha K Sharma, James Carroll, Michael R Hamblin. (2011). Biphasic Dose Response in Low Level Light Therapy – an Update . Dose-Response. 9: 602-618.
13. Avci P, Gupta A, Sadasivam M, Daniela Vecchio, Zeev Pam, et al. (2013). Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. Semin Cutan Med Surg. 32: 41-52.
14. Vladimirov YA, Osipov AN, Klebanov GI. (2004). Photobiological principles of therapeutic applications of laser radiation. Biochemistry (Mosc). 69: 81-90.
15. Hamblin MR. (2008). The role of nitric oxide in low level light therapy. Proceedings of SPIE. 6846: 684602-684614.
16. Menezes PFC de, Requena MB, Lizarelli R de FZ, Bagnato VS. (2013). Blue LED irradiation to hydration of skin. (Cristina Kurachi, K. Svanberg, B. J. Tromberg, V. S. Bagnato, Eds.) In: Proc. SPIE 9531, Biophotonics South America, Anais.
17. Blanco KC, Moriyama LT, Inada NM, Ana G Sálvio, Priscila FC Menezes, et al. (2015). Fluorescence guided PDT for optimization of the outcome of skin cancer treatment. Frontiers in Physics. 3: 1-7.
18. Fujita AKL, Rodrigues PGS, Requena MB, Escobar A, Rocha RW da, et al. (2016). "Fluorescence evaluations for porphyrin formation during topical PDT using ALA and methyl-ALA mixtures in pig skin models. Photodiagn. Photodyn. Ther. 15: 236-244.
19. Requena Michelle Barreto, Cury Thereza Fortunato, Vollet-Filho José Dirceu, Grecco Clóvis, Kurachi Cristina, et al. (2015). The effectivity of ALA-PDT using IPL evaluated by image and histological analysis using porcine skin models in vivo. Photodiagnosis and Photodynamic Therapy. 12: 347-348.
20. Menezes PFC de, Requena MB, Bagnato VS. (2014). Optimization of photodynamic therapy using negative pressure. Photomed Laser Surg. 32: 296-301.
21. Macedo Paula Delello, Corbi Sâmara Tfaile, De Oliveira Guilherme José Pimentel Lopes, Perussi Janice Rodrigues, Ribeiro Anderson orzari, et al. (2018). Hypericin-glucamine antimicrobial photodynamic therapy in the progression of experimentally induced periodontal disease in rats. Photodiagnosis and Photodynamic Therapy. 25: 43-49.
22. Zhang LW, Fang YP, Fang JY. (2011). Enhancement techniques for improving 5-aminolevulinic acid delivery through the skin. Dermatologica Sinica. 29: 1-7.
23. Park J, Choi S, Seo S, Choy Y, Prausnitz M. (2010). A microneedle roller for transdermal drug delivery. Eur J Pharm Biopharm. 76: 282-289.
24. Fabbrocini G, Padova MP, Vita V, Fardella N, Pastore F, Tosti A. (2009). Periorbital wrinkles treatment using collagen induction therapy. Surgical & Cosmetic Dermatology. 1: 106-111.
25. Rodrigues PG, Campos De Menezes PF, Fujita AK, Escobar A, Barboza De Nardi A, et al. (2015). Assessment of ALA-induced PpIX production in porcine skin pretreated with microneedles. J Biophotonics. 8: 723-729.