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Single visit PDT for basal cell carcinoma – A new therapeutic protocol

Dora Patricia Ramirez[c,*], Lilian Tan Moriyama[a], Elisângela Ramos de Oliveira[b], Natalia Mayumi Inada[a], Vanderlei Salvador Bagnato[a], Cristina Kurachi[a], Ana Gabriela Salvio[b]

[a] Biophotonics Laboratory, Institute of Physics of São Carlos – University of São Paulo (USP), São Carlos, São Paulo, Brazil
[b] Skin Department, Foundation Hospital Amaral Carvalho, Jau, São Paulo, Brazil
[c] PPGBiotec, Federal University of São Carlos (UFSCAR), São Carlos, São Paulo, Brazil

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ABSTRACT

Non-melanoma skin cancer is the most prevalent type of cancer in Brazil and worldwide. Topical Photodynamic Therapy is a technique that offers advantages as: excellent aesthetic result, possibility of application for outpatients in ambulatory setting, and presenting a minimum functional impact of the treated anatomic site. Fractionated Photodynamic Therapy is a modification of the usual technique in which the full dose of light is delivered in steps separated by a periods of time ("dark intervals"). In Brazil, no studies using this technique for treatment of BCC have been published. Thus, we proposed to evaluate the complete and partial response to the four different protocols of fractional Photodynamic Therapy, when evaluated 30 days after treatment. The study showed a complete response of 65.8%, 67.6%, 72.7% and 95.4% in the groups 1, 2, 3 and 4, respectively. We observed that the dark interval and the irradiated light dose are parameters of great importance for the final response to the treatment. Our results suggest that Fractionated Photodynamic Therapy is a technique with excellent aesthetic result and complete response when evaluated 30 days after treatment. However, a longer follow-up will be necessary for better understanding of the behavior of the lesions treated.

1. Introduction

Topical photodynamic therapy (PDT) has been widely used in Dermatology for basal cell carcinoma (BCC), actinic keratosis (AKs) and Bowen's disease. This therapeutic procedure involves the use of a photosensitizer, a chemical compound that when activated by light at appropriate wavelength produces reactive oxygen species leading to cell death. The topical administration of the aminolevulinic acid (ALA) or methyl aminolevulinate (MAL) cream induces the local production of protoporphyrin IX, an endogenous PDT photosensitizer, resulting in a non-systemic photosensitization of the patient. The possibility of anon-surgical outpatient treatment is the main reason why presently PDT is mostly used in Dermatology, since the systemic photosensitization may represent an important side-effect. On the other hand, topical ALA or MAL-PDT is only indicated for superficial lesions, due to the limitation of the cream transdermal delivery, and consequently the limited PpIX production up to 2 mm in thickness. While in USA, PDT is approved since 1999 by the regulatory agency FDA for treatment of AKs, in the European Union and elsewhere worldwide, the approval expands to the treatment of BCC and squamous cell carcinoma (SCC) in situ [1–4]

According to the guidelines for management, developed by the guideline subcommittee of the European Dermatology Forum, PDT is licensed for the treatment of BCC in several European countries. When using MAL, the present protocol is based on 3 h of cream incubation (drug-light interval or DLI) using an occlusive dressing, followed by irradiation by red light (630 nm, 75 J/cm^2) and the treatment is repeated after 7 days [3,4]. This protocol using two PDT sessions is justified to overcome the limitation of around 2 mm-thickness response, improving the treatment success. The rate of complete response for superficial BCC lesions is about 90%, but for nodular lesions it is about 60–70%, possibly due to the less effective ALA penetration throughout the whole lesion [5,6].

One of the strategies used to improve the outcome of PDT is light dose fractionation, when the total light dose is divided in two or more fractions. The interval between the fractions varies from a few seconds to a few hours. The mechanisms behind the improved PDT result achieved with the fractionation scheme are complex and still not well understood. In *in vitro* and animal model studies show an increased macroscopic and cellular damage using fractionated PDT (fPDT) compared to conventional PDT (cPDT) were observed [7,8]. *in vivo*

* Corresponding author at: Avenue trabalhador São-Carlense 400, São Carlos, SP, Brazil.
E-mail addresses: dorapatriciaramirez@gmail.com (D.P. Ramirez), lili@ursa.ifsc.usp.br (L.T. Moriyama), liicherry42@gmail.com (E.R. de Oliveira), nataliainada@ursa.ifsc.usp.br (N.M. Inada), vander@ifsc.usp.br (V.S. Bagnato), cristina@ifsc.usp.br (C. Kurachi), gasalvio@hotmail.com (A.G. Salvio).

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monitoring of the O_2 has been shown that lower and fractionated irradiance results in slower oxygen consumption, allowing that the oxygen is continuously supplied within the tumor, and enhanced tumor necrosis [7,9,10].

Surgical resection is the present first choice of treatment. Comparing to surgery, PDT has as main advantages the improved cosmetic result and, considering superficial BCC, it can be done using local ALA administration in an ambulatory procedure. The major disadvantage is that the ALA or MAL-PDT is presently performed in 2 sessions, one week apart. Considering the logistics and medical costs involved in a two-step protocol, especially in low-income countries and places where patients have to travel long distances to receive specialized treatment, we propose a PDT protocol for BCC in a single visit.

The aim of this study was to clinically evaluate the short-term response of BCC skin lesions treated by a single visit MAL-PDT protocol. The PDT response using a dual session application at a single visit was evaluated.

2. Materials and methods

2.1. Patient enrollment

In this study, 164 patients with 258 lesions were investigated. The study was approved by the Ethics Committee in human research from the Amaral Carvalho Hospital (N°. 1.507.028) and enrolled patients provided written informed consent. All patients were treated at the Dermatology Department of the Amaral Carvalho Hospital between June 2015 and August 2016, under supervision of a certified dermatologist. All patients presented superficial or nodular BCC lesions with maximum diameter of 2 cm. There were 84 (52%) women and 80(49%) men with mean age of 65.5 years old (ranging from 28 to 92 yo). From all treated lesions, 146 (58%) were located at the head and neck, 48 (18%) at the upper and lower extremities and 64 (24%) at the trunk (Table 1).

2.2. PpIX pro-drug

The target site for PDT was identified by the dermatologist, including the clinically identified lesion and a safe margin of 0.5 cm. The area was cleaned with gauze soaked in alcoholic solution of 0.5% chlorexidine and debulked using a scalpel blade. To induce PpIX production, a 20% MAL cream layer of approximately 1 mm thick was applied over the target site. We used MAL 200 mg/g cream containing: methyl paraben, propylene glycol, EDTA (Ethylenediamine tetraacetic acid), DMSO (Dimethyl sulfoxide), BHT (Butylated hydroxyl toluene), imidazolidinyl urea, water, Polawax, decilaoleate, Nipasol (PDT-Pharma, Cravinhos, SP, Brazil). An occlusive dressing with plastic film, aluminum foil and gauze or bandage was used. The cream was kept in position for 3 h for the first session, and in the groups were a re-incubation was performed the second drug-light interval was of 60 min or 90 min. Tissue fragments from the curettage were sent to histopathological analysis.

Table 1
Distribution of lesions among the experimental groups.

Group /parameter	G. Control	GI	GII	GIII	GIV
Nodular BCC	38	27	18	43	68
Superficial BCC	0	11	16	17	19
Head and neck localization	20	23	22	33	48
Extremities localization	5	1	2	8	7
Trunk localization	13	14	10	20	32

2.3. Photodynamic therapy and fluorescence monitoring

The equipment used in the present study was a dual platform based in LED for both PDT irradiation and fluorescence widefield visualization (LINCE®, MMOptics, São Carlos, SP, Brazil). An array of LED at 630 ± 10 nm is used for the treatment, an array of LED at 405 ± 10 nm for the fluorescence imaging. The fluorescence viewer includes filters that provide excellent contrast between skin auto fluorescence (in greeen) and the red fluorescence of the PpIX.

After the cream incubation, the occlusive dressing was removed and the cream excess cleaned with gauze. The lesion was then evaluated by widefield fluorescence to check the PpIX production, especially considering the relative fluorescence intensity and homogeneity within the target site.

The single visit protocol was divided in two sessions performed at the same day: a) the first one is performed with the same parameters of the conventional MAL-PDT, i.e. 3 h of drug-light interval (DLI) and 150 J/cm^2 at 125 mW/cm^2, and b) second session, the DLI and the light fluence were investigated in three different conditions. In group 1 (G1), the second session was performed with no cream re-application and 100 J/cm^2 at 125 mW/cm^2. In G2, G3 and G4, immediately after the first irradiation, a 20% MAL cream was re-applied and the lesion was occluded (with plastic film, aluminum foil and bandage) to avoid ambient light exposure. The second light fraction in G2 was delivered after 60 min of DLI and the light dose was 100 J/cm^2 at 125 mW/cm^2. In G3, the second light fraction of 100 J/cm^2 at 125 mW/cm^2 was delivered after 90 min DLI. Lesions in G4 received the second light fraction of 150 J/cm^2 at 125 mW/cm^2 after 90 min DLI interval.

As control group, PDT scheme was the present indicated procedure of 2 sessions with an interval of 1 week, in both fractions 150 J/cm^2 was delivered under125 mW/cm^2. Table 1 and 2 summarizes the parameters used for the second PDT fraction in the experimental groups fPDT and the distribution of lesion among Gc, G1, G2, G3, G4.

Since one of the main expected reactions of PDT is the pain sensation, this was monitored during these new PDT protocol. Patients were verbally asked about pain sensation every 3 min during the illumination. A verbal pain scale from 0 to 10 was used for this evaluation: painless = 0; mild = 1–3; moderate = 4–6; severe pain = 7–10.

2.4. Follow-up

Thirty days after PDT, the patients returned to the ambulatory for clinical and histopathological evaluation. If the lesion was considered clinically absent, a 2 mm punch biopsy was performed. If the lesion was clinically considered still present, it was referred to surgery. All tissue materials were stained with hematoxylin and eosin and evaluated by a board pathologist.

3. Results

The present study evaluated 4 protocols of fractionated PDT (fPDT), aiming to improve the complete response rate of the therapy when treating superficial and nodular BCC lesions (nBCC and sBCC, respectively) in a single visit PDT protocol. The proposed fractionation schemes aimed to perform two PDT sessions in a single day. These protocols were compared with the conventional PDT (cPDT) consisted in two sessions separated by 7 days of interval.

Table 2
Experimental groups and respective PDT parameters at the second session.

Group/parameter	G1	G2	G3	G4
MAL re-application	no	yes	yes	yes
DLI	30 min	60 min	90 min	90 min
Light fluence for 2nd session	100 J/cm^2	100 J/cm^2	100 J/cm^2	150 J/cm^2

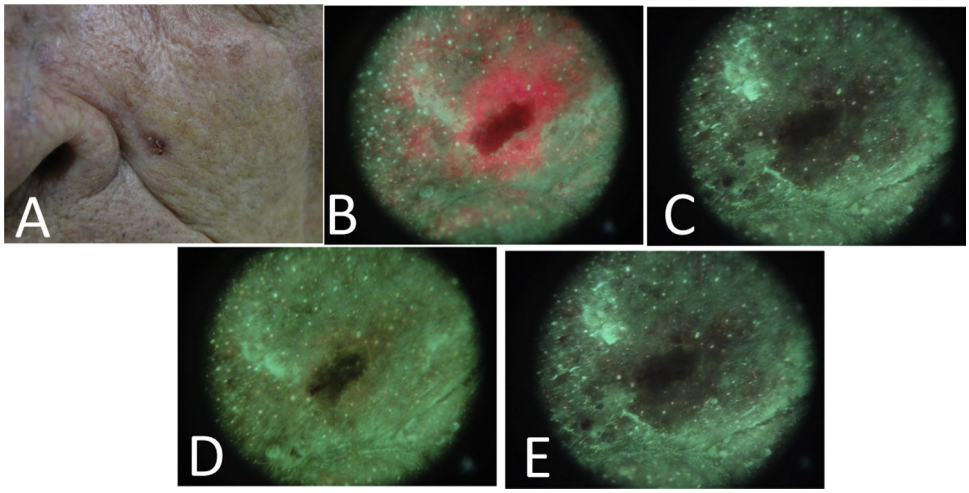

Fig. 1. Patient D.A.S, female, 62 yo. – (A) Clinical presentation of a nodular basal cell carcinoma in perinasal area; (B)PpIX fluorescence after 3 h of cream occlusion; (C) PpIX fluorescence immediately after the first session; (D) PpIX fluorescence 30 min after the first irradiation; (E) PpIX fluorescence after the second irradiation.

In G1, 38 lesions were treated in 27 patients; 27 were nBCC and 11 sBCC. This group, aimed to evaluate if the remaining amount of ALA available after 30 min of DLI would be enough to continuous provide further PpIX production in a minimum amount to induce an effective photodynamic damage and improve the outcome obtained through a single visit scheme. Thirty days after PDT, the complete response rate was of 65.8%. Among the 13 lesions with partial response, 9 were nBCC and 4 sBCC; and 12 were located in head and neck area.

Fig. 1 shows an example of the observed PpIX fluorescence in G1in different times. (A)high amount of PpIX production is observed before the first irradiation (B). It is possible to observe a low red emission after the first irradiation(C) and a very low production of PpI X 30 min after the first irradiation (D) and low red emission before the second irradiation, and mostly at the lesion periphery. This weak red emission is potentially due to a short time for PpIX production, low availability of ALA to produce more PpIX, and irradiated cells suffered PDT damage and were not sufficiently metabolic viable to produce PpIX.

Considering this poor PDT response (65.8% of complete response), we decided to re-apply the MAL cream, increasing the availability of the active compound to produce a higher amount of PpIX. In this sense, the other experimental groups incorporated a second incubation of MAL immediately after the first irradiation. In G2, after MAL was reapplied, the second DLI was of 60 min, and then the second light fluence fraction of 100 J/cm^2 was delivered. In this group, 34 lesions in 25 patients were treated. Thirty days after PDT, the complete response rate was of of67.6%. Among the 11 lesions with partial response, 8 were nBCC and 3 sBCC; and 9 were located in head and neck area.

Fig. 2 shows an example of the observed PpIX fluorescence in G2 before the first irradiation (B), after the first irradiation (C), after 60 min of second cream incubation (D) and after the second irradiation (E). A low red emission is still observed before the second irradiation, indicating a still low production of PpI X 60 min after the reapplication of MAL. We observed a relative higher PpIX emission when compared to G1, but still weaker than the ones observed with the first cream incubation. The weak red emission could be due to the short timefor PpIX reappearance or intense damage induced by the first light fraction that diminishes the capacity of the tissue to produce PpIX.

In G3 the 61 lesions in 37 patients underwent the same procedure as G2, however the DLI was increased up to 90 min. Thirty days after PDT, the complete response rate increased to 82%. Among the 11 lesions with partial response, 8 were nBCC and 3 sBCC; and 8 were located in head and neck area.

Comparing G3 and G2, there was an improvement of 14.4% in the complete response rate when increasing the DLI from 60 to 90 min.

Fig. 3 shows an example of the observed PpIX fluorescence in G3 before (B) and after the first irradiation (C), after 90 min of the second MAL incubation (D), after the second irradiation (E). Clinical aspects before and after 30 days of treatment are showed in Fig. 3A and F, respectively. It is possible to observe a lower red emission before the second irradiation when comparing to the PpIX emission resulted from the first cream incubation, however it was comparatively higher than the PpIX emission in groups 1 and 2 at the same evaluated time.

In G4, we decided to test an increase of the delivered fluence of the second PDT irradiation, because any further increase of the second DLI, in our opinion, would make this single visit PDT clinically unfeasible, considering the total treatment time. In this way, the second DLI second incubation was of 90 min and the second light fraction was of 150 J/cm^2. In this group, 87 lesions in 53 patients were treated and thirty days after PDT, the complete response rate was 95.4%. The four lesions presenting partial response were classified as nBCC and 2 of them were located in head and neck area.

Fig. 4 shows an example the observed PpIX fluorescence in G4 before (B) and after the first irradiation (C), after the second MAL incubation during 90 min (D), and after the second irradiation. It is possible to observe that the red emission before the second irradiation is slightly lower to the emission before the first irradiation.

In Gc (control group), 38 lesions in 22 patients were treated using the present indicated PDT protocol for nBCC. PDT scheme was a double session with an interval of 1 week. In each session the patient received topical MAL cream 20%, the DLI was of 3 h and 150 J/cm^2 was delivered under125 mW/cm^2. Thirty days after PDT, the complete response rate was of 81.6%, being the partial response with 7 lesions classified as nBCC presenting partial response and 85% them were located in head and neck region.

From the graph of Fig. 5 it is possible to observe that the complete response rates obtained in G1 and G2 were lower than in the conventional procedure (control group), and the best results were obtained in G3 and G4. The CR was higher in G4, when light dose was increased by 50% in the second light fraction and this was crucial to achieve approximately 95% of CR.

The results show that all the varied parameters (DLI, MAL re-application and irradiation fluence) influence the outcome of the fPDT. In order to better compare the proposed fractionated schemes of the second irradiation, a figure of merit, *P*, is proposed as

$$P = T_d . D_t$$

where T_d is the DLI (in minutes) and D_t is the total delivered fluence. The percentage of the achieved CR as a function of the figure of merit *P* is presented in Fig. 6

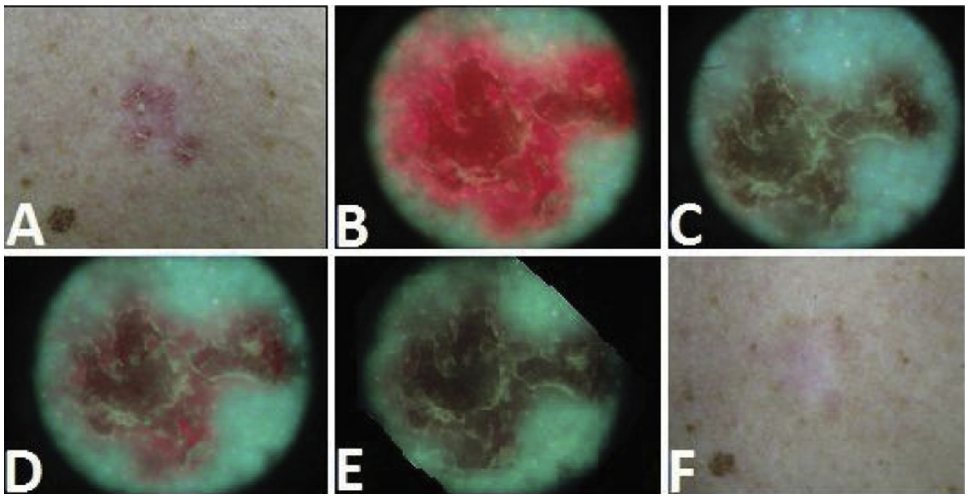

Fig. 2. Patient V.C.G, 52yo female: A) Clinical presentation of a superficial basal cell carcinoma on scapular area; B)PpIX fluorescence after 3 h of cream occlusion; C) PpIX fluorescence immediately after the first session; D) PpIX fluorescence after 60 min of second DLI ; E) PpIX fluorescence after the second session; F) Complete response 30 days after treatment showing an hypochromic area.

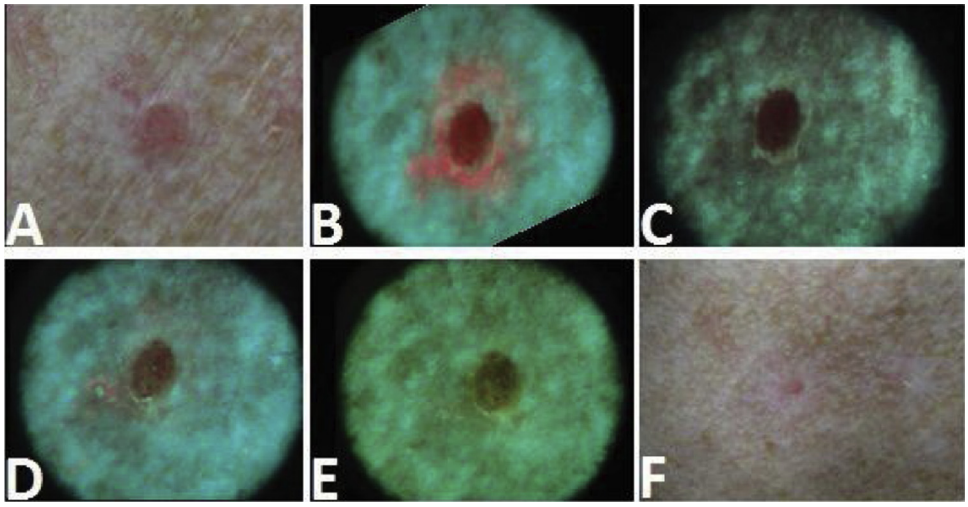

Fig. 3. Patient M.P., 60 yo female: A) Clinical presentation of a nodular basal cell carcinoma on trunk; B)PpIX fluorescence after 3 h of cream occlusion; C) PpIX fluorescence immediately after the first session; D) PpIX fluorescence after 90 min of DLI before the second irradiation; E) PpIX fluorescence after the second session; F) Complete response 30 days after treatment showing a central erythematous area.

One can observe from Fig. 6 that both DLI and delivered fluence are important for the treatment outcome. G4 presented the best treatment response even when compared to the conventional protocol PDT (Gc).

Most part of the lesions treated in this study was classified as nBCC (n = 195, or 76%), and this was also the type of lesion which showed the highest percentage of partial response to the treatment after 30 days of follow up. Fig. 7 shows the distribution of nodular and sBCC in the groups and the numbers of complete response (CR) and partial response (PR) in each group.

The cosmetic outcome obtained in two patients participating in G4 is presented in Fig. 8. From the images it is possible to evaluate the lesions before the treatment (Fig. 8A and C) and 30 days after the treatment (Fig. 8B and D). A hypochromic area is observed on Fig. 8 B and a mild erythema on Fig. 8 C, with no scars formation.

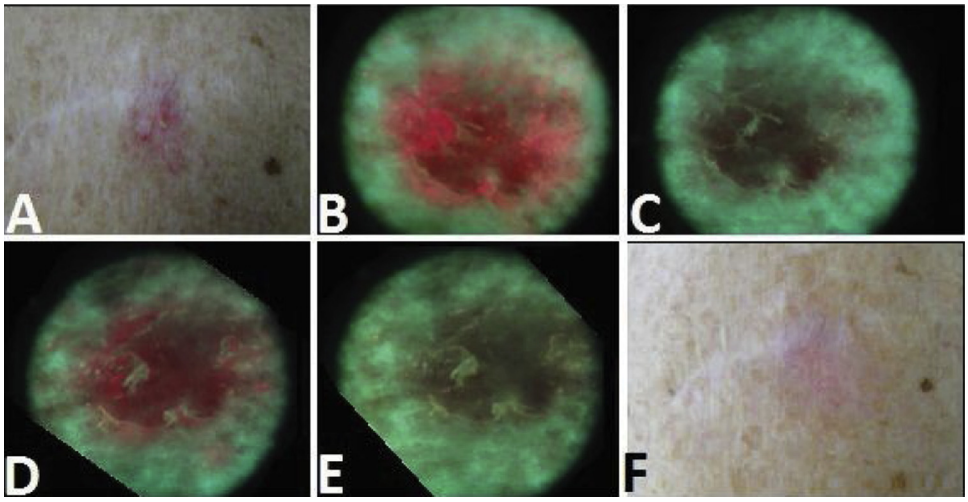

Fig. 4. Patient M.F.C.G., 54 yo female: A) Clinical presentation of a superficial basal cell carcinoma on left arm; B)PpIX fluorescence after 3 h of firs cream occlusion; C) PpIX fluorescence immediately after the first irradiation of 150 J/cm^2; D) PpIX fluorescence after 90 min of second DLI; E) PpIX fluorescence after the second irradiation of 150 J/cm^2; F) Mild erythema showing complete response 30 days after treatment.

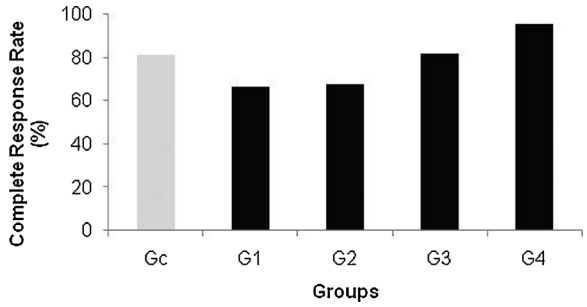

Fig. 5. A comparison of the complete response rates obtained for investigated experimental groups.

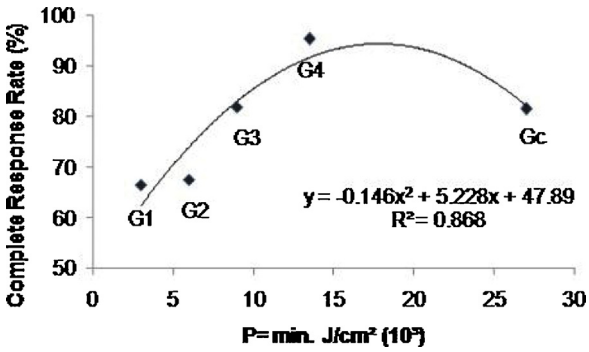

Fig. 6. Response to treatment with different PDTprotocols at the second session. G1, DLI 30 min, fluence100 J/cm^2; G2, 60 min, 100 J/cm^2; G3, 90 min, 100 J/cm^2; G4, 90 min, 150 J/cm^2; Gc, 180 min, 150 J/cm^2.

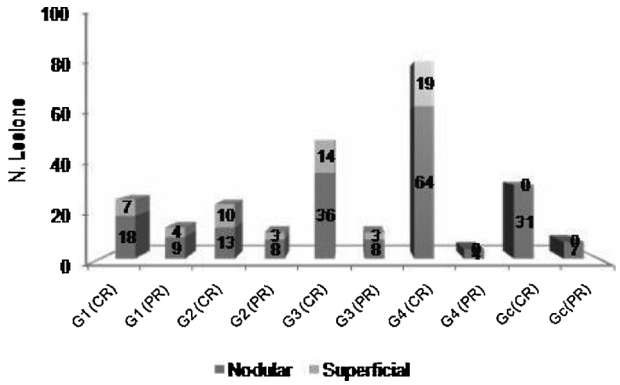

Fig. 7. A comparison of the number of lesions with complete response (CR) or partial response (PR) for each BCC subtypes (nodular or superficial).

Figs. 9 and 10 present the percentage of severe pain reported by the patients during the course of treatment. In both conventional (Fig. 9) and fractionated (Fig. 10) PDT, severe pain scores (levels 7–10) reported in the second session were lower than in the first session.

4. Discussion

The re-appearance of PpIX in cells, animal models and patients after PDT irradiation has been reported by a few authors [11–15,12,16,17] and it is an indicative that even after receiving a certain light dose that is capable of inducing tissue damage that leads to cell death, there are some cells that remain metabolic viable to synthesize PpIX. The mechanisms involved in this process are not completely understood, but this PpIX generated after a first PDT irradiation can be further photoactivated in order to improve the tissue response.

The protocol treatment whit cPDT performed as 2 sessions one week apart is approved in Europe for MAL-PDT treatment of superficial and nodular BCC [3,4]. In this scheme, the pro-drug cream remains in occlusion for 3–4 hours, and the lesion is irradiated most often using a red LED light source (630 nm) using a delivered fluence of 37 J/cm^2. The lower rates of complete response obtained with PDT compared to surgery makes several groups in the world seek for optimized protocols [18,19].The fPDT seems to be one of the main possibilities for this optimization.

In our study, the re-appearance of PpIX 30 min after irradiation with 150 J/cm^2 at 125 mW/cm^2 did not presented a satisfactory fluorescence emission as observed in Fig. 1C, and this can be a reasonable explanation for the low rate of complete response obtained in G1. The 30 min DLI between the first and second light fractions is likely short for the cells to synthesize more PpIX, as well as the remaining amount of ALA may not be sufficient for further PpIX production at this DLI.

In an *in vivo* study, Bruijim et al. 2006 treated mouse skin with different fPDT schemes and reported that after 30 min of DLI, the increase in fluorescence was not significant. When the DLI was 1, 1.5 or 2 h, there was a significant increase in fluorescence comparing to the control, but no significant difference was observed between these three different DLIs [20]. These reports agree with our study, showing that 30 min is not enough for a significant PpIX reappearance. Other authors reported that the reappearance of PpIX after irradiation is detectable through wide field fluorescence imaging after 1 h [21–23,20]. Studies on fPDT has been shown that a DLI of 2 h should be waited until delivering the second light fraction in order to induce more tissue damage [20].

Comparing the CR of G1 and control, we see that the control had a better outcome indicating that the second treatment is important to guarantee a higher level of CR.

Our strategy was not to wait longer time between first and second irradiation, in this way, we chose to re-apply the MAL cream for a second incubation of 60 min. When we compare the results from G2 and G1, was observed that their percentage of complete response were close, meaning that the fractionation scheme adopted in G2 was still not efficient. The red fluorescence emission of PpIX 60 min after MAL reapplication (Fig. 14C) may indicate that 60 min is not enough for the tissue to synthesize PpIX as it could be observed by wide field fluorescence imaging system. Another possibility is that the tissue damage caused by the first light fraction was severe and compromised the capacity of the cells to synthesize PpIX even after providing extra ALA. The result indicates that a DLI shorter than 1 h after MAL re-application has results similar to when MAL is not re-applied.

Based on these results, we further decide to a DLI of 90 min at the second MAL application (G3). In this group, we achieved a higher CR when compared to the G1 and G2, similar to the cPDT of approximately 82%, but still not reaching the CR values of surgical resection of 95%. The better result obtained in G3 shows that the damage induced by the first irradiation did not compromise completely the production of PpIX, however, the DLI had to be over 1 h in order to increase the complete response rate.

With the observed results of G3, we decided not to increase the DLI of the second session, since it would result in a prolonged treatment session that would not be clinically feasible. Another issue is that based on the reported studies, in skin of hairless mice [20], the increase on the incubation time would not result in a much higher PpIX production. Our strategy was then to increase the delivered light fluence, since the photodynamic effect needs a minimal amount of the three components (photosensitizer, light, and oxygen) and it can be improved increasing at certain level each one of those elements.

In order to evaluate if the outcome could be improved by increasing the delivered light fluence, the second light fraction was 50% higher in G4. The CR rate in this group was of 95.4%, the best rate of success obtained in our studies among the different protocols tested and it is similar to the one obtained with the surgical resection. This result indicates that the DLI and also the light fluence are important parameters for the success of fPDT.

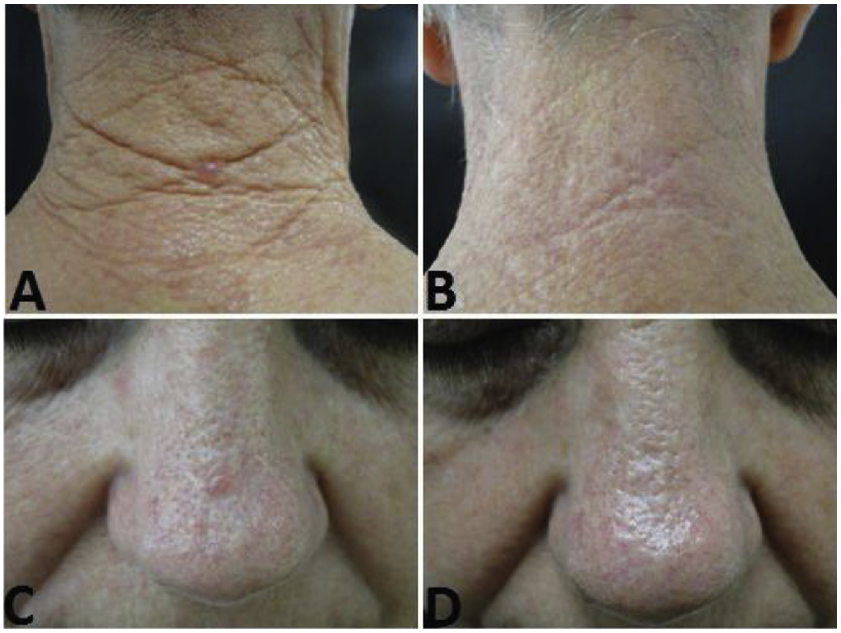

Fig. 8. Patient J.P. A., male, 71 yo. - A) A nodular basal cell carcinoma on neck area before treatment B) 30 days after treatment. Patient E.A.G., female, 59 yo. – C) A nodular basal cell carcinoma in nasal area before treatment; D) Hypochromic area 30 days after treatment.

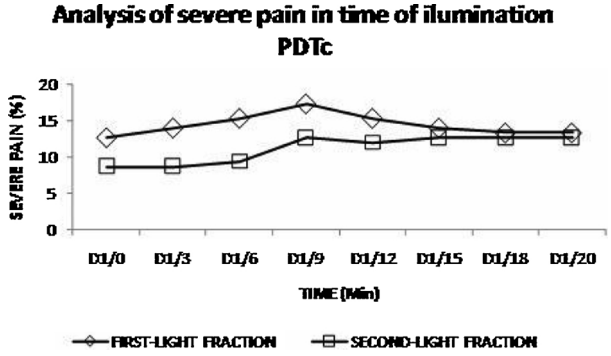

Fig. 9. Percentage of severe pain reports during the first and second sessions of photodynamic therapy over time for the conventional protocol.

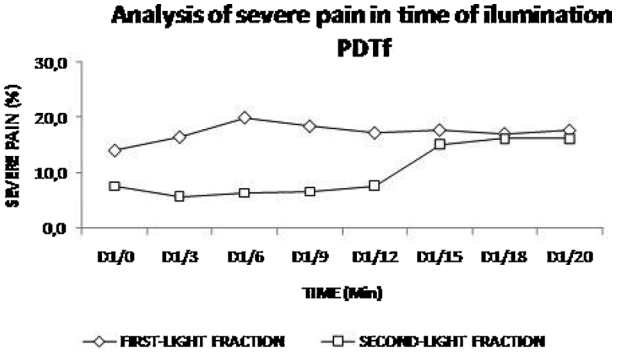

Fig. 10. Percentage of severe pain reports during the first and second sessions of photodynamic therapy over time for the fractionated protocol.

When delivering a higher light dose, the treatment is more effective in depth and it can be explained by the concept of photodynamic threshold dose, D_{th} [24]. If we consider that the tissue has enough amount of photosensitizer and oxygen available during the treatment, the induced depth of necrosis (d_{nec}) is related to the total light dose delivered to the tissue surface (D_0), to the tissue intrinsic threshold dose and to the effective penetration light depth (δ, which depends on the tissue optical properties and light wavelength). The relation between light fluenceand necrosis depth can be obtained from Beer-Lambert's Law, since light fluence (in J/cm^2) and irradiance (in W/cm^2) are related to each other by the irradiation time, then

$$d_{nec} = \delta ln\frac{D_0}{D_{th}}$$

If we consider that all cells have the same D_{th}, increasing D_0 by 50%, means that the depth of necrosis will be increased by a factor equivalent to 0.4δ. In this way, the higher CR obtained in G4 would be due to an increased necrotic volume induced by a higher light fluence. Another possibility for the better outcome is that if we consider that not all cells has the same susceptibility to PDT, meaning that the D_{th} is slightly different from cell to cell, when we increase D_0 by 50%, we are able to kill cells with D_{th} up to 50% higher. It means that there will be more chances to kill cells that are more resistant to PDT.

Despite the results of our study correspond to a short term follow up, of only 30 days, and a longer follow up would be necessary, the proposed fPDT scheme of G4 seems to be very promising. The control group with two PDT sessions one week apart, with equivalent light fluence, the complete response 30 days after the treatment was 13.8% lower.

The worse response of nodular lesions to PDT when compared to superficial lesions was already expected, since this response has been reported in several studies [5,6,25]. Then BCC, based on histological analysis, is defined as basaloid cell aggregates that eventually may present acystic pattern, with central necrosis or cell pigmentation. The macronodular BCC can be even less uniform than the micronodular one, and compared to the sBCC, it can reach deeper tissues [26]. These characteristics justify why the nBCC subtype shows a lower complete response of the topical PDT, especially when the lesion presents a thickness of over 2 mm.

Another factor that has been shown by our previous studies as crucial for the response to PDT is the medical doctor experience with the technique [5]. We have reported that higher rates of complete response were obtained by doctor that already had more than one year of clinical experience with PDT. We believe that as more experienced the doctor, better is the selection of the proper lesions for the therapy.

The response obtained in G4, shows that the used fractionation scheme achieve higher CR and lower rates of partial response for

nodular lesions. Only 4 of the 68 nodular lesions treated in this group did not present complete response (clinical and histological) after 30 days of follow up. The CR obtained in G4 is comparable to the gold standard technique, the surgical resection [27]. Another interesting observation in the present study is the response related to the lesion site. In all experimental groups, head and neck lesions showed the worst response. Basal cell carcinoma appears in regions of chronic sun exposure. Thus, the highest incidence of BCC is on the face with 80% of all BCC lesions. It is well known that face H region is more subjected to lesion recurrence and aggressiveness [28]. The irradiation scheme we used in G4 resulted in lower PR in head and neck; only 2 of 48 head and neck lesions did not present complete response 30 days after the treatment.

Pain sensation is an expected adverse effect of topical PDT and it has been shown to depend on the lesion site, treatment area, light irradiance, and other factors related to individual patient characteristics. The pain sensation during the fractionated scheme showed that the patients reported lower scores during the second treatment. This result is the opposite of the one reported in a previous publication of our group when the conventional PDT scheme was used [29]. With the conventional protocol, the pain sensation reported was higher for the second treatment. A possible explanation for this is associated with tissue characteristics during the second treatment, which in the conventional scheme, one week after the first treatment, the skin is undergoing an inflammatory process. The damaged tissue can be more sensible to pain, resulting in a higher pain score during the second irradiation. Anyway, the present pain monitoring at our fPDT protocols did not show higher patient percentage with severe pain scores when compared to the conventional PDT (control group).

Optimizing the PDT protocol for a single visit appointment has a great impact for the public health system, especially for those countries where the patients have to travel long distances to receive cancer medical care. In Brazil, cancer hospitals are centralized at bigger and higher-income cities, so patients living outside of those regions have to travel, in some cases hundreds of km, this is why patient compliance for any treatment that require more than one session is a relevant issue. If the CR of approximately 95% is sustained in the longer follow up assessments, our single visit PDT protocol (G4) may constitute a highly attractive treatment option for BCC. Based also on this scenario, comparing to surgery, PDT does not require high complexity facility, so it has the potential to contribute for improve public medical care for BCC, considering that the patients could be treated at ambulatory clinics and with overall lower costs.

In summary, the present clinical study showed that the reapplication of MAL is an important step of fPDT when 150 J/cm^2 at 125 mW/cm^2 is delivered in the first irradiation fraction. The DLI between the first and second irradiation has to be longer than 60 min and 90 min seems to be enough.

5. Conclusions

A clinical study was conducted and the achieved results indicate that the PpIX reappearance do not occur in 30 min after irradiation without cream re-application. However, the irradiated cells could still synthesized PpIX. MAL reapplication after the first irradiation is important for the success of the fPDT as proposed in this study. The DLI after MAL reapplication is an important parameter and 90 min was enough to result in a CR of 95.4% when the two irradiations are performed with 150 J/cm^2 at 125 mW/cm^2, in a 30 days follow up evaluation. This CR is higher than the one obtained with 2 MA L-PDT sessions one week apart (with the same irradiation parameters) without compromising the cosmetic outcome for the same follow up period. The rate of partial response was higher for nodular lesions and for those located in head and neck. The pain monitoring showed that the second irradiation is less painful than the first one when the single visit of a fPDT is performed. All the results reported here considered a short term follow up of 30 days and a long term evaluation is necessary in order to consolidate the single visit fPDT scheme.

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Conflicts of interest

None declared.

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