Experience and BCC subtypes as determinants of MAL-PDT response: preliminary results of a national Brazilian project
Experience and BCC subtypes as determinants of MAL-PDT response: Preliminary results of a national Brazilian project

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Summary Non-melanoma skin cancer is the most prevalent cancer type in Brazil and worldwide. Photodynamic therapy (PDT) is a noninvasive technique with excellent cosmetic outcome and good curative results, when used for the initial stages of skin cancer. A Brazilian program was established to determine the efficacy of methyl aminolevulinate (MAL)-PDT, using Brazilian device and drug. The equipment is a dual device that combines the photodiagnosis, based on widefield fluorescence, and the treatment at 630 nm. A protocol was defined for the treatment of basal cell carcinoma with 20% MAL cream application. The program also involves the training of the medical teams at different Brazilian regions, and with distinct facilities and previous PDT education. In this report we present the partial results of 27 centers with 366 treated BCC lesions in 294 patients. A complete response (CR) was observed in 76.5% (280/366). The better response was observed for superficial BCC, with CR 160 lesions (80.4%), when compared with nodular or pigmented BCC. Experienced centers presented CR of 85.8% and 90.6% for superficial and nodular BCC respectively. A high influence of the previous doctor experience on the CR values was observed, especially due to a better tumor selection.

Introduction

The non-melanoma skin cancer (NMSC) represents the most common type of cancer worldwide. In the United States, it accounts for more than 2 million cases per year and, in Brazil, more than 130,000 new cases were estimated
for 2012, with the basal cell carcinoma (BCC) representing approximately to 80% of all NMSC lesions [1].

PDT has been investigated for several applications, being widely used for the treatment of NMSC [2,3]. Three elements are fundamental in PDT: the presence of a photosensitizer (PS), a molecule which absorbs light to initiate a series of photochemical reactions; light at a specific wavelength to be absorbed by the PS; and the availability of molecular oxygen in the tissue to be treated [3,4]. The combination of drug, light and oxygen leads to the production of oxidative cytotoxic agents, leading the treated tissue to death by inducing necrosis, apoptosis or autophagy, when a irreversible damage is achieved [4]. Methyl aminolevulinic acid (MAL) is topically administered to the patient, using a transdermal delivery cream. MAL penetrates in the cells and the aminolevulonic acid induces in the mitochondria, the protoporphyrin IX (PpIX) production [4].

For a decade, our group has been introducing PDT as an experimental therapeutic option in Brazil [5]. After several years of local experience, we have organized a national initiative with the main goal of installing about 100 centers and treatment of approximately 2000 lesions to evaluate the efficacy of the proposed protocol and a new device that combines the fluorescence visualization and treatment.

The low accessibility to imported drug and equipment, mainly due to the high costs, prevents a large clinical implementation of PDT among Brazilian dermatologists. Brazil is a country with a large territorial area; the patient access to specialized facilities is difficult and inefficient considering the long distances and waiting list, which in some regions can achieve 12 months.

With the disponibility of Brazilian drug and equipment, the costs of a PDT procedure decrease significantly, allowing a high technique implementation. First of all, these components must be tested before its disponibilization by the public health system. This program has as a major aim to evaluate all these at different medical levels, from simple general facility to highly qualified cancer centers, and Brazilian regions. For the validity of the program, as well as to promote adjustments that guarantees a full success, it is necessary the analysis of different response aspects. Therefore in this communication, we present some aspects concerning the PDT clinical outcome based on partial results (with about 20% of the expected number of patients) already collected. The complete response depending on a previous professional experience and the BCC subtypes is presented.

Materials and methods

Training the centers

This is a multicenter prospective study involving 27 dermatological centers in Brazil. Each center was classified as experienced (EC) or not experienced center (NEC) according to the spontaneous physicians report, regarding their own previous knowledge and/or practice with PDT for more than one year.

Regardless of this experience, all teams received the same training, lasting 8h, which included a lecture with the concepts of photodynamic therapy, pharmacology and all clinical protocol followed by training with practical application of the protocol in patients previously scheduled. A training team was composed of doctors, pharmacists, physicists and related fields toward a more comprehensive approach to the concepts involved.

The presented data are from 14 EC and 13 NE. The study was approved by local Internal Review Board, and by the Brazilian Federal Council on Ethics. All patients provided a written informed consent before enrollment into the study.

Clinical protocol

Eligible patients were subjects of both genders, over 18 years old with primary superficial (sBCC), nodular (nBCC) and pigmented (pBCC) BCC with diameter less than of 20 mm, estimated clinically immediately prior to treatment. The maximum thickness of the tumor was 2 mm. This evaluation was done for histopathological or clinical analysis. Pregnant women and subjects presenting porphyria or any type of photosensitivity were excluded.

All lesions were measured and photographed. Then, the lesion was investigated using the fluorescence visor —(LINCE®, MMOptics, Brazil) that emits an ultraviolet-blue light centered at 400 ± 10 nm, from an LED array and with irradiance of 40 ± 8 mW/cm². Autofluorescence visualization improves the physician ability on discrimination of altered tissues, by highlighting lesion structures. Superficial BCC lesions were prepared by surface debridement to remove scales and crusts and to facilitate penetration of the MAL cream. For nBCC, a curette without local anesthetics was performed to reduce the tumor thickness, resulting in a flat surface. Was used MAL 200 mg/g cream containing: methylparaben, propylene glycol, EDTA (ethylenediaminetetraacetic acid), DMSO (dimethyl sulfoxide), BHT (butylated hydroxytoluene), imidazolidinyl urea, water, Polawax, decila oleate, Nipasol (PDT-Pharma, Cravinhos, SP, Brazil) was placed over the lesion, including an extension to 5 mm of normal surrounding skin, with at least 1 mm thickness. An occlusive dressing, using plastic film and aluminum foil was placed for 3h. After that, the dressing was removed, the cream was washed off with saline solution. The presence of PpIX, a highly fluorescent molecule, was verified by using the fluorescence visor to observe the characteristic red fluorescence under 405 nm excitation. The uniformity and intensity of red fluorescence give indication to evaluate the PpIX production. If there is lack or dimmed red emission, the photodynamic response will not take place. If there is a non-uniform PpIX distribution, the PDT will not properly occur, potentially resulting in remaining untreated cancer cells. After checking the PpIX, the lesion was immediately illuminated with a red (630 ± 10 nm) LED light source (LINCE®, MMOptics, Brazil), with irradiance of 125 mW/cm², and fluence of 150 J/cm². Subsequently to treatment, PpIX photobleaching was verified by fluorescence imaging in order to evaluate consumption of PS and certification of an expected photodynamic reaction. We believe that a fluorescence visor is a relevant device to enable an improved lesion examination, and analysis of the PDT steps, giving clinical information to predict the individual response.

The same protocol was repeated, with an interval of 1 week between sessions.
**Evaluation of response**

Thirty days after the second PDT session, the patient was clinically evaluated by the physicians with digital pictures to help the macroscopic analysis and a punch biopsy was performed for histological analysis. The final response, based on the clinical and histological analyses, was classified as complete response (CR), partial response (PR) or interrupted treatment (IT).

CR was used to classify results when no lesion was observed in clinical and/or histological analysis. PR was used for lesions that have regressed, but not enough to eradicate the lesion. IT was used when there were external causes for the patient. There was no reported allergic reaction.

**Statistical analysis**

Statistically, data were reported as numbers and/or percentages. The chi-square statistic was used to assess predetermined bivariate comparisons, and the equal proportions hypothesis was rejected when the $P$ values were less than 0.05.

**Results**

In this multicenter study, 294 patients with a total of 366 BCC lesions were treated in 27 different centers. The skin type, I–VI rated according to the Fitzpatrick’s classification, of each patient was recorded. From all lesions, 296 (80.8%) were located at the head and neck, 24 (6.5%) at upper and lower extremities, and 46 (12.5%) at the trunk. Two hundred and twenty-two lesions (60.6%) were diagnosed in women, and 144 lesions (39.3%) in men. CR was observed in 76.5% (280/366), PR in 20.2% (74/366), IT in 3.2% (12/366) of the cases (Table 1). The better response was observed for sBCC, with 160 CR (80.4%), 29 PR (14.5%), and 10 IT (5%) of treated lesions ($P<0.01$). For nBCC, the results were: CR at 112 lesions (74.1%), PR at 37 (24.5%), and IT at 2 (1.3%) ($P<0.01$). Only 16 pBCC lesions were included in the present study, showing CR at 50%, and PR at 50% of the cases (Fig. 1). In Fig. 2, a comparison between the results obtained at experienced centers (EC) and non-experienced centers (NEC) is presented. EC corresponds to centers where the professionals self-related previous experience in PDT, while NECs are those introduced to PDT. There is an evident higher CR rates for the EC, showing the importance of the previous education and experience of the professionals on the therapy. In general, for BCC lesions, a CR of 86% was observed at ECs, when compared to 69.2% of the NECs. For sBCC, was no statistically significant association between CR for ECs and NECs (85.8% versus 74.1%) respectively ($P=0.053$). Nodular lesions showed CR of 90.6% (39/43), and PR of 6.9% (3/43) ($P<0.01$) for ECs, and NECs obtained CR of 67.5% (73/108), and PR of 31.4% (34/108) ($P<0.01$).

**Discussion**

This report showed the preliminary results of a multicenter clinical study that included 27 dermatological centers in different regions of the Brazilian territory. One of the main aims of this project is to evaluate the proposed PDT protocol in facilities of different levels, and with medical professionals of distinct training and educational. A treatment technique for cancer to be diffusely implemented in Brazil must first be
tested and show satisfactory results in all levels of Medicine services. In this study, it was possible to verify the influence of the previous experience on PDT in the treatment response. A CR was observed in 76.5% of the treated cases (Fig. 3), which is in the range of the rates reported in scientific literature, from 52.2% to 100% [2,6–8]. Horn et al. in 2003 observed that for BCC lesions a better response was observed for superficial lesions when compared to nodular lesions (85% versus 75%) of 47 and 51 respectively investigated cases [9]. In our study, sBCC responded better to PDT when compared to nodular lesions, with CR rates of 80.4%, 74.1%, respectively but it was no statistically significant (P = 0.053) (Fig. 1). In BCC, different factors can affect the response to the technique [10–13]. BCC lesions thicker than 2 mm present limited response to topical PDT [14], with efficacy decreasing as thickness increased. In our study, a limit of 2 mm of thickness was set; however, in some cases, especially at centers not specialized in Oncology the definition of the lesion thickness could not be properly accessed. A limited penetration depth of MAL cream and of visible light into the deeper layers of the tissue also explains the resulted response rates. Pigmented lesions are evidently more difficult to be treated with PDT due to higher light absorption at the presence of melanin, preventing deeper illumination with enough energy, resulting in lack of PDT response at tumor bottom margin.

Fig. 4 shows PpIX fluorescence in the photosensitized tissue. It is possible to observe that the pigmented region absorbs both the excitation and the fluorescence emission from PpIX. In the same way, these absorbing regions produce shield effect during irradiation, avoiding that all the lesion receive the proper amount of light, reducing PDT efficacy [15].

Pre-PDT curettage and/or debulking of nBCC are procedures proven to increase the success of the technique. Christensen et al., in 2009, attempted to use curettage only to remove the superficial layers of epidermis, and have observed that it contributed to high success rates in their results [16]. However, the execution of these procedures depends on the skills of the physician. In our study, we have observed that ECs presented better results when compared to the NECs. Even though, PDT is a simpler treatment procedure that requires less complex training, when compared to surgery, the resulted response may derive from the fact that an EC do not require an adjustment period to properly treat these lesions using PDT. This experience reflects in an improved selection of the indicated lesions, and also in their ability in performing proper lesion curettage for PDT. An example of that is that the lowest number of nodular BCC lesions was observed at the ECs.

**Conclusion**

MAL-PDT is a noninvasive, safe, moderately effective, and well-tolerated option for treating superficial BCC lesion. Moreover, we can anticipate that the BCC subtype and medical experience influence the final result. The fact that the lack of previous knowledge on PDT results in a lower degree of success, indicates that a closer interaction of the professionals involved in the multicenter study should provide an improved education on the PDT principles and clinical case discussions. As the study progress, we expected that the acquire experience will improve considerable outcome results.
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