

Clinical Photodynamic Therapy Review and the Brazilian Experience

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ABSTRACT

Photodynamic therapy (PDT) is a simple and promising technique indicated for the treatment of non-melanoma skin cancer. Although multicenter research trials have proved its success, PDT is not as much disseminated as surgery and cryotherapy approaches are. A light-activated substance (a photosensitizer) is usually topically administered. By irradiating the sensitized region with specific wavelengths and appropriated light dose, reactive oxygen species take place, which oxidize molecules and destroy tumor cells. This review article presents not only the base principles of PDT, but also a unique experience Brazil-wide, which reached other countries in Latin America. This initiative was funded by the Brazilian government via the Brazilian Development Bank (BNDES), involving private companies and academia in a validation study of the treatment of both pre-malignant and malignant skin cancer lesions. PDT protocol evolution is presented, including details that made the technique highly efficient and placed this experience in a larger context.

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Key words: Photodynamic therapy (PDT); Non-melanoma skin cancer; Photodynamic Therapy; Brazilian

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INTRODUCTION

PDT is a non-systemic therapy, which has been successful in treating a number of diseases^[1-4]. However, despite favourable results, it has not been the first option to date in the treatment of non-melanoma skin cancer and pre-malignant lesions^[5,6]. This might be associated to the number of variables involved in PDT (photosensitizer, oxygen, light, anatomical site and depth of lesions, as well as other inherent, patient-dependent aspects), which might contribute to variations of the cure rates. Another possible factor is cost, since PDT drugs are not available in variability enough to make prices affordable and, in most cases, the light devices for treatment that are made available for acquisition only by tied sale with drugs. Skin cancer in Brazil is recognized as a public health problem, and at the same time it is considered a social and infrastructural problem, because it affects a great number of people, with important impact in morbidity, mortality, and economy.

A national program (entitled "Photodynamic Therapy Brazil") was started in Brazil in 2012, thanks to the joint efforts of universities, research centers, hospitals, private medical clinics, private companies and research funding agencies. This program made skin cancer treatment available to the Brazilian (including the public health system) and other twelve other Latin America countries' population^[7] in a simple and fast way, resulting in the improvement of patients welfare through the fostering and application of advanced technology, by creating several PDT treatment centers. The program provides photosensitizer, treatment device and training to the upcoming partner centers, making diagnosis and treatment of lesions possible with low cost and shorter time spans when compared to lesions referred to surgery.

One hundred treatment centers were created under this initiative (Figure 1), and approximately 90% of the lesions treated since 2012 were completely cured. When results are assessed by centers, this cure rate is increased when the health professional taking part



Figure 1 Distribution of the upcoming treatment centers established during the first phase of the PDT Brazil program^[89].

in the treatment had previous experience with PDT, due to two main factors: the expertise in referring to PDT treatment lesions that are more appropriate for this approach, and a more adequate preparation of the lesion for the procedure, such as sufficient curettage and administration of the methyl aminolevulinic acid (MAL) photosensitization cream (Figure 2). The results showed that a large-scale program for the treatment of small skin cancer lesions is viable; these results are so positive that new centers are continuously being added to the program, and treatment follow-up data are still being collected for future, more comprehensive analysis. A core result from this approach is that the involved countries currently start to face a new reality concerning skin cancer treatment due to this program^[7].

Photodynamic Therapy

Although light has been used as a curative tool for thousands of years, the first report of the photodynamic effect in cell destruction of protozoa (paramecium) was presented by Oscar Raab in 1900^[8]. However, the first tumor treatment by PDT was reported only in 1903 by von Tappeiner^[8]. Currently, tumor treatment has been the main application of PDT^[9-13], although it is also effective in treating infections by promoting the death of microorganisms^[14-17].

Tumor treatment by PDT is achieved by the interaction of light with the photosensitizer, which promotes the generation of reactive oxygen species that destroy tumor cells. This process takes place when the photosensitizer molecules, which lead molecules from what is called a “fundamental energy state” to a so-called “excited state, absorb light of appropriate wavelength.

Eventually, the excited molecules undergo a relaxation process (which is a very fast process, about 10^{-12} s), releasing energy by vibration processes up to the lowest excited state before the fundamental one. At this point, for photosensitizer molecules, part of them release energy by molecule-molecule interaction, and part emitting a photon (i.e., light), which will have lower energy than the absorbed ones. This process is usually fast (about 10^{-8} s), and is called fluorescence - which can be used for diagnosis^[18]. Part of the molecules, though, during relaxation, undergoes “intersystem crossing” (a change in *spin* configuration), which leads the excited molecule to a triplet electronic *spin* configuration, producing a meta-stable (i.e., long-lived, minimum of 10^{-3} s) excited state.

When at this meta-stable state, the photosensitizer molecule may also emit light (and emission is then called phosphorescence), or may follow two different pathways: interacting with substrate molecules, producing free radicals by the transfer of electronic charges, or interacting with molecular oxygen by energy transfer

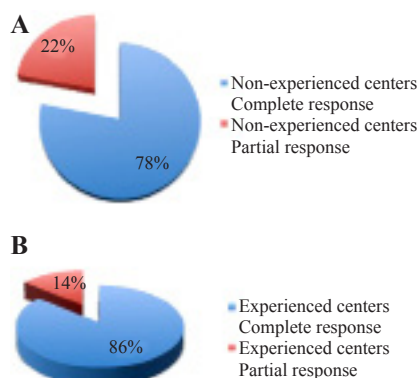


Figure 2 PDT response according to previous experience of the treatment center teams with the technique. (A) Percentual of complete versus partial response to PDT by non-experienced center teams; (B) Percentual of complete *vs* partial response to PDT by experienced center teams.

and producing a special species called singlet oxygen, which is very reactive and, thus, very cytotoxic. One of those processes is usually dominant for each photosensitizer molecule; if free radicals production is dominant, it is called a type-I reaction; if singlet oxygen is more expressive, it is called a type-II reaction.

The wavelengths able to promote the excitation of the photosensitizer molecules are usually within the spectral regions of near ultraviolet^[19,20], visible light^[1,21] or near infrared^[22-24] - these wavelengths avoid the spectral regions able to ionize molecules by ultraviolet and shorter wavelengths, and water absorption of infrared wavelengths. An efficient PDT application requires enough light penetration in biological tissues, determining the choice of wavelengths and photosensitizers. For cancer treatment, efficient photosensitizers must also have a stable formulation^[25], physiological pH^[26], high absorption in wavelengths that favor light penetration at the aimed biological tissue (usually between 600 and 850 nm for most applications)^[27], and show selectivity for malignant cells^[28,29], high quantum yield for reactive oxygen species production^[30,31], no mutagenic effects^[32], low cytotoxicity in the absence of light^[33,34], and fast metabolism - in order to reduce side effects^[35].

The main photosensitizers currently used are haematoporphyrin derivatives, which show efficacy for the treatment of non-melanoma skin cancer^[36]. Among them, the similar first generation drugs Photofrin[®]^[37,38], Photogem[®] and Photosan[®] show unique characteristics, such as differences in toxicity, which make each of them ideally indicated to specific applications.

The second generation of photosensitizers include benzoporphyrin derivatives^[39,40], which are rapidly metabolized and show selective accumulation by endothelial cells^[41], chlorins^[42,43], texaphyrins^[44,45] and dyes^[46,47].

The third generation of photosensitizers conjugate characteristics of the first and second generation molecules, and are not commercially available. The main modifications made to them are related to the use of antibodies and nanoparticles^[48,49].

Photosensitizers can also be endogenous, such as riboflavin (vitamin B12)^[50,51] and protoporphyrin IX (PpIX)^[52,53]. The 5-aminolaevulinic acid (ALA) and its derivatives (such as MAL) can be administered as a pre-drug, converting to PpIX inside cells^[54,55].

Light sources for tumor treatment range from conventional lamp bulbs either with or without optical filters^[56] to light-emitting diodes (LED)^[16,57,58] and lasers^[21,59,60]. The latter ones are the most versatile and thus the most used ones, due to the possibility of coupling

optical fibers to provide light delivery to treatment spots difficult to reach. Lasers are not ideal, though, due to the usual high cost; additionally, less monochromatic light sources may provide multiple excitation of a single absorption band of the photosensitizer molecules. LED devices, on the other hand, are currently largely applied to PDT since they provide an increasing availability of wavelengths with high power output and affordable cost. PDT efficacy has been proved for several applications and our research group currently works toward the development and optimization of protocols and dosimetry^[61], drugs^[62] and instrumentation^[63].

CLINICAL EXPERIENCE

Basal cell carcinoma

“PDT Brazil” program happened as a partnership of the clinical centers with the Sao Carlos Institute of Physics at University of Sao Paulo (IFSC/USP), the device- and drug-manufacturing companies (MMOptics® and PDT Pharma®, respectively) and funded by BNDES. The program provided skin cancer treatment, improving public health system patients’ welfare by making available health treatment with the latest technology^[64,65]. Since then, more than 2 000 lesions have been treated at about 100 treatment centers established by this program, in Brazil - (distribution by country region: North: 1.8%, Central-West: 5.6%, Northeast: 13.2%, South: 30.6%; Southeast 48.8%) - and Latin America (5.2% of them), and particularly by the Phototherapy and Photodiagnosis center at the AmaralCarvalhoHospital Foundation (Jahu-SP, Brazil), a region reference hospital which treats usually more than 70 000 cancer patients a year, and which is a main training center for upcoming treatment centers with the national devices and drug provided under the program^[66-69]. The pharmaceutical ingredient is methyl aminolevulinic acid (MAL, PDTPharma, Cravinhos-SP, Brazil), which was used throughout the research and approved for clinical trials by ANVISA (the Brazilian Health Surveillance Agency). The drug approval (a cream containing 20% MAL, by PDT Pharma®) is in progress by ANVISA, after the end of the Phase II Trial including 600 patients, which compared the efficacy of this drug with surgery, the gold standard method of treatment for non-melanoma skin cancer. The device provided to the centers by the program for the treatments, LINCE® (MMOptics LTDA, São Carlos-SP, Brazil. Figure 3), is composed by a portable console connected to a treatment probe and to a fluorescence imaging probe for visualization of the produced PpIX and the lesions’ endogenous fluorescence. This visualization probe allows for wide field fluorescence imaging and PpIX photobleaching monitoring. Light intensities between 50 and 150 mW/cm² and irradiation periods up to 90 min are available, and can be set up by the console control panel.

Lesions treated under PDT Brazil program are exclusively non-melanoma skin cancer, either superficial or nodular basal cell carcinoma (BCC) type, up to 2 cm in diameter and up to 6 mm infiltration, with histopathology confirmation (Figure 4). Highest incidence among the treated lesions was in elderly patients (60+ years old), in the face - probably due to the accumulative effect of sunlight UV radiation on skin over the years. To increase the cream penetration and favor light absorption into the lesion, a curettage procedure was introduced to the standard protocol, aiming to increase the volume of tissue treated. The three-hours time interval between the cream administration and the illumination is necessary for the PpIX production by the ALA in the cream^[7]. ALA, which is a precursor of PpIX (and a direct by-product of MAL), is a hydrophilic agent that penetrates cells by active transport and



Figure 3 LINCE device by MM Optics. The probe in the physician hand is the treatment probe, composed by LED arrays emitting at 630 nm. The probe resting at the console is the evidencer probe, which is composed by ultraviolet-visible LED arrays and optical filters for adequate selection of the wavelengths delivered to the sample and back to the observer's eye.

depends on pH and temperature to be metabolized^[70].

The fluorescence induced at photosensitized cells is related to PpIX accumulation and its increase to the accumulation time, and can provide evidence about the treatment response^[71]. Due to this, such fluorescence is suggested as a monitoring tool for PDT applications. The main factors associated to the treatment response are MAL absorption, PpIX formation and consumption, adequacy of the curettage and avoidance or proper management of its resulting bleeding (clotting reduces light penetration), the adequate administration of the cream, the photosensitizer incubation time, adequate occlusion of the photosensitized lesion (to avoid premature irradiation), and uniformity of the light delivery to tissue^[72-75].

The energy surface density that is delivered during the application is called light dose, which for this PDT initiative was 150 J/cm². Different light doses can be used, however, since a balance between intensity (which is the light dose delivery rate, and is equivalent to output light power of the device delivered per irradiated area) and the time spent during irradiation; the balance is given mostly by empirical experience and by dosimetry models applied to preclinical and clinical trials^[76]. The light source of choice (lasers, light-emitting diodes, lamp devices) is also not standardized for PDT, because different applications may require different sources.

Among the program results, light penetration was found to be a very important aspect concerning PDT outcome; since nodular BCC show geometrical differences when compared to superficial BCC, lesions thickness may challenge light penetration in PDT. Concerning pigmented BCC, melanin absorbs light, preventing it from being delivered to the full extension of the lesion. In all those cases, geometry and pigmentation is obstacles to light penetration end up reducing PpIX consumption, and thus prevent sufficient photodynamic effect.

Results showed a linear correlation between lesion size and cure rate. Lesions larger than 2-3 cm in diameter which were treated did not show the same cure rate, probably due to the difficulty in light penetration for larger lesions - those lesions demand more light and drug to undergo sufficient PDT effect^[77,78]. The superficial extension of lesions can receive enough cream to suffice adequate sensitization but, since the LINCE® device was aimed for small lesions, the original device was improved during the program development, adapting consoles to the irradiation necessities of larger lesions, up to 10 × 12 cm.

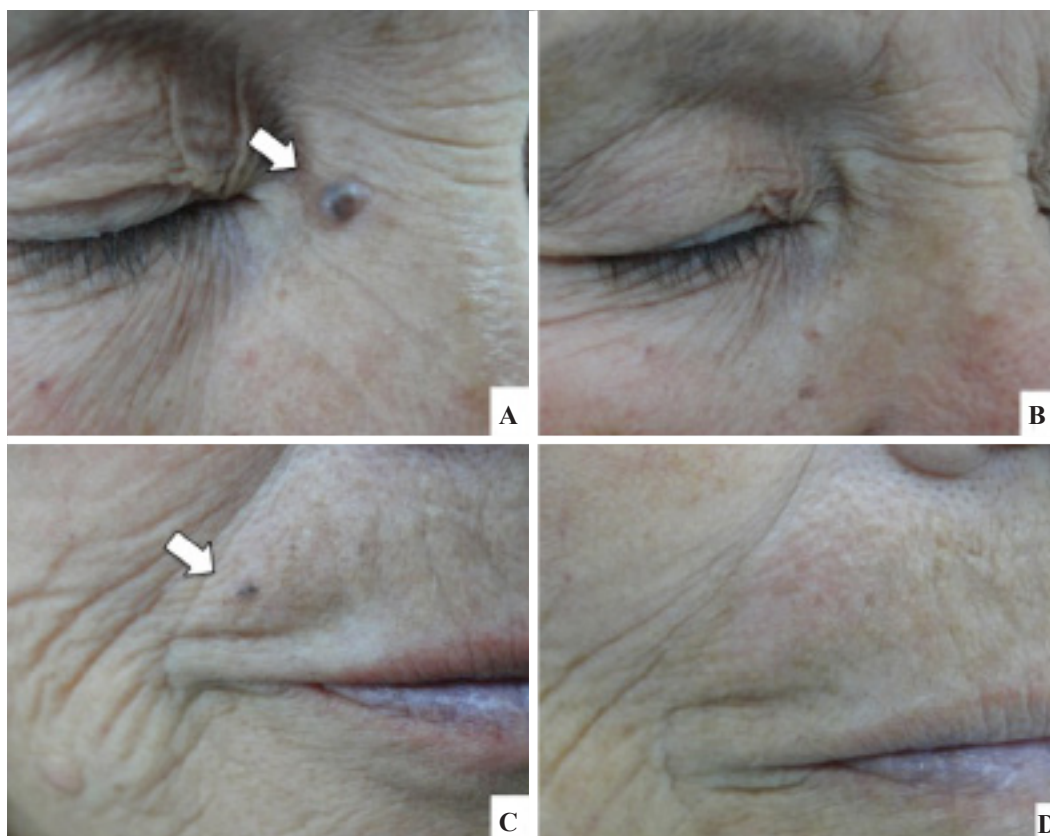


Figure 4 BCC lesions suitable to PDT application under the protocol established for the lesions treated under the PDT Brazil program. At (A) and (C), BCC lesions prior to treatment; at (B) and (D), treatment sites for lesions at (A) and (B), respectively, 6 months after treatment.

Over the clinical trial, the observed PDT side effects were the report of pain during irradiation and cicatrization modifications. Pain is the main expected reaction to the photodynamic effect, due to the PDT-induced inflammatory response. Hyperchromia and hypochromia were observed as cicatrization modifications, but both showed a low rate of incidence, and are both reversible.

The complete response to treatment is greater than 80% and has 1.22% recurrence rate after 6 months from the first PDT session. Patients showed 83% normal healing and 17% abnormal healing (atrophic, hypertrophic, hypochromic and hyperchromic) in the cosmetic results of PDT. The session number and lesion localization influenced in painful intensity during PDT. Patients reported a higher intensity of pain in lesions on the head and neck than on the trunk and limbs in second session^[79].

Premalignant lesions of skin

The use of PDT for the treatment of premalignant lesions such as actin keratosis (AK), including field cancerization, has been approved clinically in several countries^[80,81]. A number of studies showed the efficacy of this treatment modality for field cancerization, since it provides the treatment of subclinical lesions^[82-84].

Our research group also performed trials aiming premalignant lesions^[85], showing that PDT using MAL is efficient in those cases, producing excellent aesthetical results in the treatment of AK using a LED light source named KeratoPDT, developed by the Optics Group at IFSC/USP, by members of the Biophotonics and Technology Support (LAT) laboratories (Patent PI: 1000413-0). KeratoPDT was especially designed to anatomically fit the upper limbs, for the treatment of widespread actin keratosis (DSAP)^[86]. The prototype allows for uniform and simultaneous illumination

of the upper limbs, providing treatment of widespread actin keratosis with relevant cosmetic results, with tolerable pain for the patient^[67,68,86].

Another relevant response to PDT is observed for actinic cheilitis. Actin cheilitis also called lip actinic keratosis, which is an inflammatory pathological condition of the lips that may be caused by the chronic, excessive exposure to sunlight ultraviolet radiation, with potential to become a malignant lesion. Since non-surgical therapeutic protocols are preferable for the treatment of this type of lesion, PDT is a natural candidate as non-invasive treatment option, due to the excellent outcome and aesthetic results. Thus, clinical trials are currently in progress by our research group at IFSC/USP, aiming to define an optimum treatment protocol^[87].

Extensive lesions of skin cancer

One of the great advantages offered by the PDT Brazil program was the opportunity to use PDT for the treatment of both large superficial BCC lesions and Bowen's disease occurring in elderly patients with comorbidities that would prevent treatment involving surgical procedures. Lesions of up to 8 cm in diameter were treated during the program course, with total response observed for more than 70% of the treated lesions. That made possible to provide treatment to patients for which surgery procedures would present risk of death. Even in those cases for which partial response was obtained, lesions showed improvement that reduced surgical complexity and, thus, risk^[69].

Since treating large lesions is a limit due to the cream penetration in bulky skin lesions, large lesions were also treated using Photogem as an intravenously administered photosensitizer, with incubation time of 24 hours and light dose between 200 and 300 J/cm²^[88,89].

Results for this lesions showed improvement of lesions, by reducing them, showing that systemic photosensitization for PDT is a viable alternative for the treatment of bulky lesions.

Finally, one of the most important limitations of PDT - light penetration into biological tissues, which limits the photosensitizer activation - may be actually considered an advantage in some clinical skin cancer situations, in which preserving healthy tissues beyond skin is a must, or at least desirable.

CONCLUSION

Although currently PDT is not the standard method for the treatment of tumors that are responsive to the photodynamic effect, PDT is gaining space among the therapy possibilities for lesions such as skin cancer. Thanks to the opportunity created by the joint public and private funding, and to the partnership between research institutions and hospitals, a well-defined protocol for PDT is made available to patients all over Latin America, which include making available both photosensitizing drug and irradiation devices that make possible to diagnose and to treat premalignant and malignant skin cancer lesions. Our research group is continuously increasing the number of treated lesions, and fostering the improvement of *in vitro* tests, pre-clinical tests, and clinical trials, seek for new possibilities for PDT application and understanding.

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CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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