

Photochemical predictive analysis of Photodynamic Therapy with non homogeneous topical photosensitizer distribution in dermatological applications

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ABSTRACT

Photodynamic Therapy (PDT) is a therapeutic technique widely used in dermatology to treat several skin pathologies. It is based in topical or systemic delivery of photosensitizing drugs followed by irradiation with visible light. The subsequent photochemical reactions generate reactive oxygen species which are considered the principal cytotoxic agents to induce cell necrosis.

In this work we present a PDT model that tries to predict the photodynamic effect on the skin with a topically administered photosensitizer. The time dependent inhomogeneous distribution of the photoactive compound protoporphyrin IX (PpIX) is calculated after obtaining its precursor distribution (Methyl aminolevulinate, MAL) which depends on the drug permeability, diffusion properties of the skin, incubation time and conversion efficiency of MAL to PpIX. Once the optical energy is obtained by means of the Beer Lambert law, a photochemical model is employed to estimate the concentration of the different molecular compounds taking into account the electronic transitions between molecular levels and particles concentrations. The results obtained allow us to know the evolution of the cytotoxic agent in order to estimate the necrotic area adjusting parameters such as the optical power, the photosensitizer concentration, the incubation and exposition time or the diffusivity and permeability of the tissue.

Keywords: Photodynamic Therapy, skin pathology, optical dose, photochemical model, topical photosensitizer

1. INTRODUCTION

Within the area of light activated therapy, Photodynamic Therapy (PDT) has become very important. This is a technique that uses light to activate a photosensitive substance known as photosensitizer that allows the destruction of cancer cells, [1]. Three critical elements are required for the initial photodynamic processes to occur: photosensitizer, light and oxygen.

This therapeutic optical technique has application in many clinical fields and especially in dermatology, due to the radiation facility of the affected area without using invasive methods, such as endoscopes. The current use of PDT in dermatology has experienced an upturn, especially in the treatment of premalignant and malignant lesions as well as inflammatory and infectious diseases. Some of these lesions are basocellular carcinoma, Bowen disease, Paget's disease, lichen sclerosus et atrophicus, acne, rosacea, photoaging, photosensitizeroriosis and actinic keratosis, [2]. The clinical application is based on a strict protocol for all the patients, that generally produces a good response to treatment. However, the photodynamic efficiency is not complete in all the cases, obtaining different responses depending on both the type of pathology and the patient. Furthermore, the cytotoxicity depends on several parameters, such as the concentration of the photosensitizer, intracellular location, time of incubation, fluence rate, irradiation time or type of cell. This large number of parameters reflects the complexity of the photodynamic procedure and therefore its modeling and characterization.

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In recent years there have been different works relating to the improvement of light sources, techniques for the photosensitizer delivery, new photosensitizers developing, improved oxygenation of the treated tissue, cellular targets, and so on. However, there is a limited number of works related to the development of mathematical models that characterize the processes that take place during PDT, [3]. Among these studies, it is noted mainly the development of photochemical, [4], [5], and optical propagation models [6]. Works linking these types of models to consider the whole process usually suppose the systemic application of the photosensitizer that is distributed uniformly in the target tissue. However, there are various studies which show that in the case of topical photosensitizer, the distribution of the drug through the skin occurs in an inhomogeneous way, [7], [8], which can definitely influence the outcome of treatment, where the accumulation of photosensitizer needed to achieve the desired cell destruction could be not enough.

The development of accurate models could allow a future adequacy of treatment to individual patients in order to maximize the treatment efficiency. This work provides a PDT predictive model applied to skin disorders with a topically applied photosensitizer. In section 2 a clinical study of applying PDT in dermatology is presented, then the complete PDT model is explained in the next section and finally obtained results are presented. The conclusions drawn from this work are listed in the section 5.

2. CLINICAL PDT RESULTS

PDT can be applied as a potential cancer therapy (endobronchial lung tumor, breast cancer, skin cancer, brain tumor, gynecologic malignancies) and for other diseases (macular degeneration, cardiovascular pathologies, endometriosis, chronic skin diseases). This work focuses its attention on the dermatology area where PDT has been widely used due to the facility that offers the direct irradiation of the diseased area without employing invasive methods.

In clinical practice, 3 h after the photosensitizer (Methyl aminolevulinate, MAL) administration, the lesion is illuminated with a LED light source with a wavelength of 630 nm and a light dosage of 37 J/cm^2 . In the time period between April 2006 and January 2009, 71 skin pathologies were treated with PDT following these indications, in the Dermatology Department of Marqués de Valdecilla University Hospital. Most of them were actinic keratosis (53 %) and basocellular carcinoma (47 %). It offers good response results for the majority of the patients although it cannot achieve an optimal efficiency in all of them, especially in certain types of lesions. Figure 1 show the data related to treatment response for the patients with actinic keratosis and basocellular carcinoma which show the great influence of the type of pathology and patient in the treatment efficiency when all of them have been treated following the same clinical protocol.

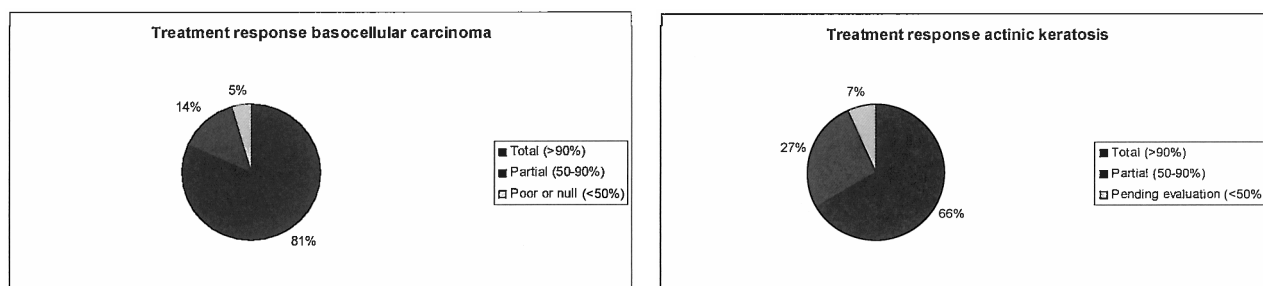


Figure 1. PDT treatment response of patients with basocellular carcinoma (graphic on the left) and actinic keratosis (graphic on the right).

The standard clinical PDT protocol produces different outcomes depending on the type of disease and the patient, so the predictive mathematical models may constitute a first attempt to adjust the treatment to each individual case.

3. PDT MODEL

The photosensitizer MAL, is a prodrug that is metabolized to the photoactive element Protoporphyrin IX (PpIX). After application of MAL on the skin surface, there is a diffusion process through the different skin layers and a conversion process to PpIX. There are several studies that have evaluated the PpIX content of the skin using fluorescence techniques

[7] and the influence of the stratum corneum as the main barrier to the diffusion of the photosensitizer to deeper layers of skin, [8].

The inhomogeneous distribution of a topical photosensitizer through the different layers of the skin plays an important role to determine the concentration of photosensitizer to be accumulated during the incubation period. PDT process modeling using systemic photosensitizer usually assumes a uniform distribution. However, this is not valid in the case of a topical photosensitizer that has an uneven distribution.

So far, there are few jobs relating to the photodynamic procedure modeling that consider an inhomogeneous distribution of the photosensitizer in the cancer tissue to be treated. However, a more accurate model should consider an uneven distribution in the case of topical photosensitizers. For this reason we used the Fick's law to characterize the inhomogeneous photosensitizer distribution and calculate the concentration reached at each point of the tissue during the incubation period. According to Fick's law, if there are differences of concentration of a substance, its molecules move from regions with higher concentration to those of lower, so that the flow of substance goes in the opposite direction of the concentration gradient.

$$J = -D \frac{\partial M}{\partial z}, \quad (1)$$

Where J is the flux vector indicating the direction and magnitude of substance, D is the diffusion coefficient, M is the prodrug concentration and z is the depth in the tissue.

The distribution of the photosensitizer in the skin is limited by several factors, including the stratum corneum which acts as a diffusion barrier and is characterized by the permeability, K , the diffusion coefficient through the epidermis and dermis, D , the elimination time of the drug, τ , and the conversion rate of photosensitizer in its active compound PpIX, [9]. The temporal evolution of the photosensitizer concentration for each depth in the tissue sample can be calculated as

$$M(t) = M_o \int_0^t \left(\frac{K}{\sqrt{D\pi t'}} e^{-\frac{z^2}{4Dt'}} - \frac{K^2}{D} e^{\frac{K}{D}z} e^{\frac{K^2}{D}t'} \operatorname{erfc} \left(\frac{K}{\sqrt{D}} \sqrt{t'} + \frac{z}{2\sqrt{Dt'}} \right) \right) e^{-\frac{t'}{\tau}} dt', \quad (2)$$

where M_o is the concentration of photosensitizer in the skin surface at $t = 0$ and z is the distance from the corneal layer located at $z = 0$. Once known the concentration of MAL in each point, the tissue concentration of active substance, S_o , which accumulates in the tissue during the incubation period is calculated. This lets us know the amount of photosensitive substance in every point of the cancerous tissue at the beginning of the interval of radiation. In this case it is assumed that the time of removal of the active compound is fast compared to the transmission time of the photosensitizer ($\tau_p \ll t$), and therefore the concentration of PpIX, is proportional to the instantaneous value of MAL concentration that can be calculated by the expression 3, where ε_p is the yield of the conversion process and $\tau_{a \rightarrow p}$ the time spent in the generation of PpIX.

$$S_o(t) = \varepsilon_p \frac{\tau_p}{\tau_{a \rightarrow p}} M(t), \quad (3)$$

The corneal layer reduces the permeability of the skin, so that its value can be adjust to characterize different skin conditions. Thus, in the case of certain skin diseases the corneal layer is damaged or reduced, in which case a value of permeability greater than in the case of skin with an intact cornea can be taken.

Regarding light distribution, there are different optical models to obtain the distribution of light in the tissue, such as the Radiation Transport Theory (RTT) or the Kubelka-Munk model. However, in this work we have used the Beer Lambert law shown in equation 4 in order to simplify calculations. Where I_0 is the irradiance in the tissue surface, μ_t is the attenuation coefficient and z is the depth in the tissue sample.

$$I(z) = I_0 e^{-\mu_t z}, \quad (4)$$

The attenuation coefficient μ_t combines absorption and scattering effects as follows:

$$\mu_t = \mu_a + \mu_s, \quad (5)$$

where μ_a and μ_s are the absorption and scattering coefficients respectively.

Interaction of light at the appropriate wavelength with a photosensitizer produces an excited triplet state photosensitizer that interacts with ground state oxygen via two types of reactions, known as Type I and Type II. The Type II reaction is believed to be predominant and responsible for singlet oxygen production which is considered as the cytotoxic element in charge of killing carcinogenic cells.

The photochemical reactions are characterized by means of a photochemical model, [4], [5], which takes into account the transitions between states of the particles involved, such as the photosensitizer and oxygen. The solutions of the stiff differential equations system employed, (6) to (11), are obtained by means of a differential equation solver within the Matlab platform. In order to obtain coherent results, we had to adjust relative and absolute error tolerances. These solutions provide the temporal evolution of the different molecular concentrations of the compounds involved in a Type II reaction everywhere in the tissue sample.

$$\frac{d[S_0]}{dt} = -\nu\rho\sigma_{psa}[S_0] - kpb[{}^1O_2][S_0] + \frac{\eta_{10}}{\tau_1}[S_1] + \frac{\eta_{30}}{\tau_3}[T] + \frac{\alpha s}{\tau_3}[T][{}^3O_2] \quad (6)$$

$$\frac{d[S_1]}{dt} = -\frac{1}{\tau_1}[S_1] + \nu\rho\sigma_{psa}[S_0] \quad (7)$$

$$\frac{d[T]}{dt} = -\frac{\eta_{30}}{\tau_3}[T] - \frac{\alpha s}{\tau_3}[T][{}^3O_2] + \frac{\eta_{13}}{\tau_1}[S_1] \quad (8)$$

$$\frac{d[{}^3O_2]}{dt} = -\frac{\alpha s}{\tau_3}[T][{}^3O_2] + \frac{\eta_0}{\tau_0}[{}^1O_2] + P \quad (9)$$

$$\frac{d[{}^1O_2]}{dt} = -kpb[S_0][{}^1O_2] - kcx[R][{}^1O_2] - ksc[C]i[{}^1O_2] - \frac{\eta_0}{\tau_0}[{}^1O_2] + \frac{\alpha s}{\tau_3}[T][{}^3O_2] \quad (10)$$

$$\frac{d[R]}{dt} = -kcx[{}^1O_2][R] + U \quad (11)$$

In these equations, $[S_0]$ is the concentration of the photosensitizer in ground state, $[S_1]$ is the concentration of the photosensitizer in singlet excited state; $[T]$ is the concentration of photosensitizer in triplet excited state; $[{}^3O_2]$ the concentration of oxygen in ground state; $[{}^1O_2]$ is the concentration of singlet oxygen; $[R]$ the concentration of singlet oxygen receptors; $[C]_i$ is the scavenger concentration; τ_1 is the relaxation time from state S_1 to S_0 ; τ_3 is the relaxation time from state T to S_0 ; τ_0 the relaxation time from state 1O_2 to 3O_2 ; η_{10} is the quantum yield of the transition from state S_1 to S_0 ; η_{13} is the quantum yield of the transition from S_1 to T ; η_{30} is the quantum yield of

T transition to S_0 ; η_0 is the quantum yield of 1O_2 transition to 3O_2 ; α_s is the efficiency factor for energy transfer from T to 3O_2 ; kpb stands for the biomolecular photobleaching rate; kcx is the biomolecular cytotoxicity rate; ksc is the rate of reaction of 1O_2 with various oxygen scavengers; ν is light speed in tissue; ρ is the photon density; σ_{psa} is the absorption cross-section of S_0 molecules; P is the rate of oxygen diffusion and perfusion; and U is the cell damage repair rate.

4. RESULTS

Figure 2 displays the spatial and temporal distribution of a topical photosensitizer in the case of a damaged tissue sample which presents a strongly reduced diffusion barrier. As it can be observed, during the first part of the photosensitizer incubation process the barrier blocks the diffusion to deeper layers. This phenomenon causes an uneven distribution of the photosensitizer in the skin beyond the period of incubation. So, the initial photosensitizer concentration at the beginning of the radiation period will not be the same at all the points of the tissue and therefore there will be differences in the results of the photodynamic procedure. This is an important aspect to consider when dealing with topical photosensitizer, since in the case of systemic photosensitizer is generally considered a homogeneous distribution throughout the tissue to begin radiation.

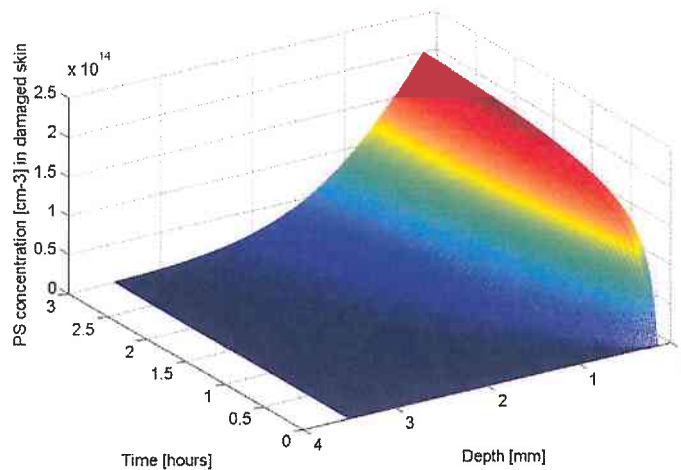


Figure 2. Photoactive substance distribution depending on the incubation time and depth in the tissue sample with a damaged stratum corneum, $K = 10^{-6} m/s$.

By different tests we have checked the effect of the diffusion barrier and the results have shown to have a great influence on the final accumulation of the photosensitizer. Thus, considering a reduced diffusion barrier in the case of a damaged tissue sample produces a higher photosensitizer accumulation than in normal skin whose permeability is lower. This effect can be observed by varying the permeability of the stratum corneum. Figure 3 displays graphically the photoactive component concentration at different depths for different types of tissue, from skin with a high diffusion barrier in the case of normal skin to damaged skin with a low diffusion barrier.

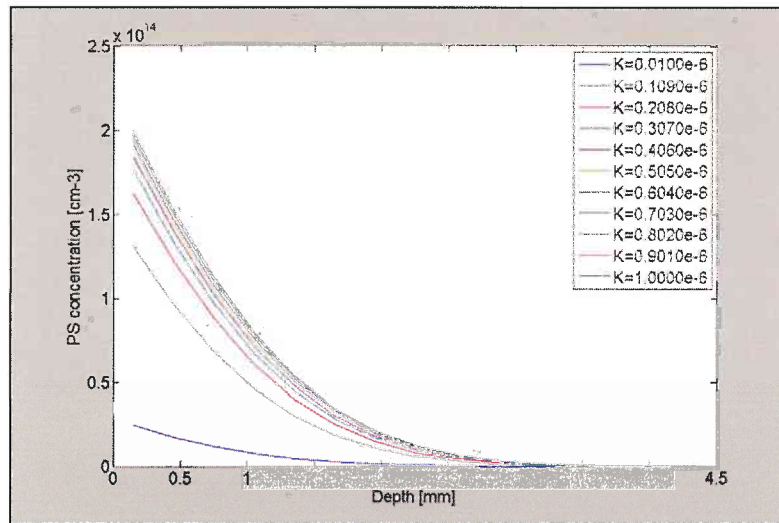


Figure 3. Effect of the stratum corneum permeability in the photosensitizer concentration at different depths in the tissue

In Figure 4, it is shown the temporal evolution of the photosensitizer concentration for different depths in the tissue. This type of graphics permit to observe and estimate the time that stratum corneum blocks the photosensitizer diffusion at different depths. It can be observed an increase in the block time as deepen into the skin sample, which limits the accumulation of photosensitizer in the deeper layers at the end of the incubation period.

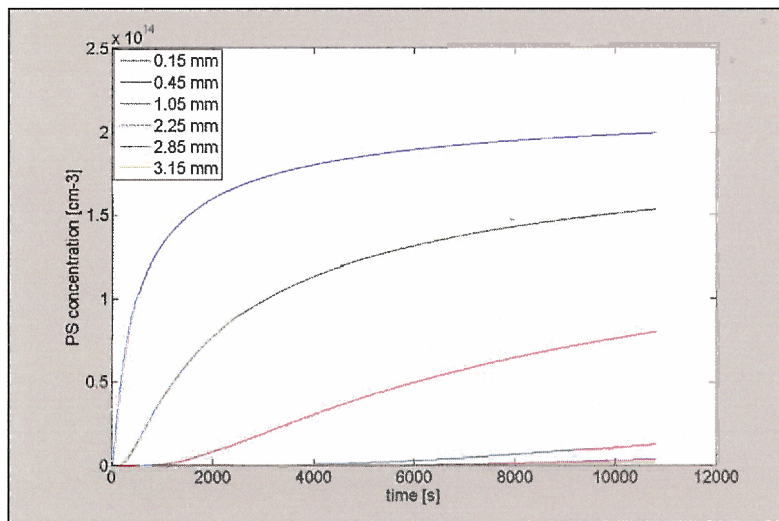


Figure 4. Temporal evolution of photosensitizer at different depths in the skin sample with a damaged stratum corneum.

Finally, we show the great importance of the photosensitizer distribution in the PDT modeling process. Specifically, the influence of considering an inhomogeneous photosensitizer distribution in the maximum singlet oxygen concentration produced. To do this, we used the model presented to simulate the standard clinical procedure used nowadays (37 J/cm^2 during a radiation period of 8 or 9 minutes after 3 hours of photosensitizer incubation). The result is shown graphically in Figure 5, where it can be observed a significant difference in such maximum singlet oxygen concentration as deepen into the skin and depending on the type of the tissue sample. The difference in singlet oxygen concentration reached will produce differences in the treatment outcome. So that, if the amount of reactive oxygen produced is not enough, cell destruction processes will not occur and therefore there will be no tissue necrosis in certain parts of the tissue.

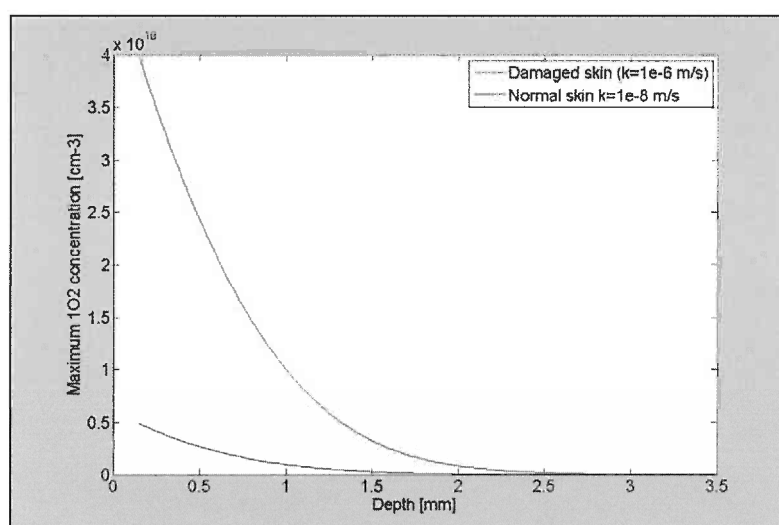


Figure 5. Maximum $[^1O_2]$ reached at different depths in the tissue sample in the cases of damaged and normal skin.

These results provide a first approximation for the future development of cellular damage models, to determine the extension of the necrotic area after applying the PDT procedure under different conditions of application such as the light source characteristics, properties of the photosensitizer or type of tissue.

5. CONCLUSION

In the future, the development of accurate models could help to predict the photodynamic process efficiency depending on different dosimetric parameters related to illumination, photosensitizer characteristics, type of pathology and even patient.

The predictive model proposed takes into account the inhomogeneous distribution of the photosensitizer through the layers of the skin during the incubation period. Once calculated the concentration of photosensitizer accumulated in the tissue sample during this period, optical propagation is obtained by Beer-Lambert law. During the exposure of the tissue sample to optical radiation, a photochemical model is used to take into account the electronic transitions between molecular levels and particles concentrations.

The results obtained in this work, constitute an approach to predict the temporal and spatial evolution of the different components involved in PDT. The model permits to simulate different treatment situations by adjusting several parameters (photosensitizer concentration and incubation time, type of pathology by varying permeability of the stratum corneum, power and time of illumination, and so on) and to observe their influence in the process outcome. Thus, the results show the temporal and spatial distribution of the photosensitizer concentration through the layers of the skin in order to know the final amount of photosensitizer accumulated during the incubation period as well as the diffusion blocking effect produced by stratum corneum in deeper layers. The results show the great importance of knowing the amount of photosensitizer accumulated in every tissue sample point, since its value will greatly influence the amount of reactive oxygen generated and therefore the intracellular oxidation produced by treatment.

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