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Acute Tonsillopharyngitis: Photosensitizer Fluorescence-Guided in Topical Therapy

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Abstract: Background: As is observed in acute respiratory pandemics characterized by inflammation of the oropharynx, microbial transmission caused by viruses and bacteria through the upper respiratory tract can quickly affect the lower respiratory tract due to its ease of transmission, The challenges are presented in the development of an adequate lighting system to prevent infections of the upper respiratory treatment and highlight the importance of following the procedure due to fluorescence decay from the given light dose. Methods: for this purpose, our study evaluated the lighting distribution on the oropharynx established and determined under simulation by TracePro software. The photobleaching (PB) is obtained by different fluorescence analysis methods of photosensitizer (PS) in formulation located in the oropharynx and tonsils. A blue light emission system at 450 nm was studied to improve the distribution, uniformity, and ray's incidence. The PB of the PS was investigated using two different methods for detecting fluorescence in the oropharynx by quantifying the active portion of the PS assessed by fluorescence spectroscopy for patients with pharyngotonsillitis by consuming 2.25 mg of curcumin in formulation after applying light by analysis of images in Software MatLab. Results: The lighting device showed a uniformity of 82% in the oropharynx. In healthy volunteers, the minimum energy of photodegradation of curcumin is 0.2 and the maximum energy is 17.4 J/cm^2. We observed intensities of blue and green emission of the analyzed fluorescence images related to PS through fluorescence measurements. Conclusions: We conclude that the pharyngotonsillitis infections can be treated by using an adequate distribution of light in the oropharynx and by activating photosensitizing agents in the infection sites supported by the fluorescence of the compound.

Key words: Curcumin, photodynamic therapy, infection, oropharynx, lighting.

1. Introduction

The upper respiratory tract is colonized by different microbial strains that present virulence factors to multiply their genetic material and enable the proliferation of pathogenic species, which can affect different human body locations, developing diseases due to the toxicity of cells in tissues adjacent to microorganisms or even their products [1]. The colonization of the upper respiratory tract by microbial cells can be influenced by people's intrinsic factors or even by the environmental conditions they are subjected to [2]. Microorganisms can be present on the surfaces and inside the tonsils, the surface being colonized predominantly by bacteria from the oral cavity microbiota and the interior mainly by pathogens [3].

The microbial biofilm can appear in the tonsils from the fixation of microbial cells forming microcolonies that are dispersed and can colonize new areas maintaining their primary matrix [4]. Viruses release DNA that is often present in these microbial communities after their cellular invasion, thus often becoming one of the components of the microbial biofilm matrix [5]. In addition to containing cellular components for localized microbial proliferation, these microbial multispecies systems can reach the lower aero system, causing severe diseases. To decrease the rate of colonization and reproduction of these microorganisms in the airways and avoid complications such as acute respiratory syndrome,

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optical treatments such as photodynamic antimicrobial therapy can help prevent penetration and thus the development of microorganisms.

The wavelengths in the ultraviolet or blue region have little penetration, implying possible applications in localized surface treatments [6]. Curcumin in the oral mucosa, primary entrance ports of the respiratory systems, human irradiated with blue light-emitting diode (LED) at 455 nm, 400 mW has shown antibacterial effects without mentioning local adverse effects in photodynamic therapy (PDT) [7]. However, treatment can become ineffective when the lighting is not uniform or does not even reach the desired region. The oropharynx includes the base of the tongue, the soft palate, the tonsils, and the lateral and posterior parts of the throat that can alter the distribution of light in the tonsils where there is infection.

For this, the study of lighting systems that guarantee uniformity of oropharyngeal illumination [8] and synergy with PS in clinical trials was performed using curcumin fluorescence detection, evaluating similarities in spectral characteristics. Curcumin is an orange-yellow PS ($HOC_6H_3(OCH_3)CH = HCO\,|\,_2CH_2$) with two main chemical configurations: keto and enol, which presents possibilities for the treatment of diseases located by PDT due to optical absorption in the blue-green region of the electromagnetic spectrum [9]. The degradation of curcumin in the solution can be induced by lighting, which is studied considering different factors such as light sources and irradiation times by absorption and fluorescence spectroscopy [9].

2. Material and Methods

2.1 Treatment Device

The lighting device has a 50 mm polished acrylic diffuser fiber for light output from the sides. The light intensity of the LED-based device was estimated according to the brightness of the light module's surface. A LED lighting model in the TracePro software (Lambda Research Corporation) was established to assess its lighting distribution in the oropharynx. Isometric and lateral light scattering was evaluated in the treatment and diffuser tips.

The lighting was established using a cavity thickness of 20 mm, using the blue LED as a light source with a spacing of 20 mm. The illumination probe for PDT treatment constituted with light emission centered at 450 nm at a distance of less than 50 mm between the light source and the throat.

2.2 Fluorescence Collection

The quantification of the active portion of PS was evaluated by fluorescence spectroscopy in the irradiated region of the oropharynx immediately before and after the PDT procedure, with the spectra collected through the positioning of the fiber in contact perpendicularly with the oropharynx mucosa. The total exposure time to fluorescence excitation light for each measurement step includes the collection of five spectra (mean time 10 s).

Degradation was observed in healthy patients by fluorescence during irradiation. Fluorescence spectra from 350 to 900 nm were recorded before and after illumination. An initial assessment was carried out on 20 patients older than 18 years (healthy volunteers), considering the tolerance of the highest tolerable dose, 17.4 J/cm^2 and the lowest effective dose, 0.2 J/cm^2. The autofluorescence spectrum obtained before photosensitization was subtracted from the spectra after sensitization.

2.3 Image Methods and Analyses

The fluorescence visualization of the oropharyngeal tissues was performed using a wide-field image system (EVINCE -MMOptics, São Carlos, SP, Brazil) composed of LEDs at 405 nm, in the violet-blue region of the electromagnetic spectrum, and optical filters with transmission over 450 nm. A black lingual lower was adjusted to the enhancer for the mouth opening.

The fluorescence images were obtained after consuming 2.25 mg curcumin in formulation and after

the light application for 8 minutes. The image processing and analyses were performed in MatLab (R2014b, Mathworks, Natick, MA, USA).

3. Results

In PDT, the scattering of light in the illumination tip has been adjusted for its use. Lighting efficiency must be guaranteed with an adequate design to reach the light in the oropharynx, especially the tonsils. Figure 1 shows the modifications made to the light-conducting fiber. The light dispersion was obtained with the "standard" tip and the diffusing tip, as shown in Figure 1. The mapping of incident light is compared to the "standard" lighting tip (A) and diffuse tip (B). Uniform illumination is observed with a diffuse tip with a localized intensity only in the retropharyngeal, which is considerably reduced. The standard tip provides an incidence of 52% of the rays that do not illuminate the tonsils, positioned more laterally. On the other hand, the diffuse tip promotes the incidence of 29% of the rays of the retropharyngeal. However, it presents a more uniform illumination, reaching the simulated tonsils on the sides of the sphere.

The light dose (considering 50 mm of distance) of standard device 1.44 J/cm^2 (lateral) and 8.4 J/cm^2 (tip) has not been modified to guarantee the safety of the procedure. However, the diffuse tip divides the dose of light that reached only the retropharyngeal and also reached the tonsils with 9.6 J/cm^2 (lateral) and 1.2 J/cm^2 (tip).

Fluorescence spectra were collected to assess the variation in PS emission during treatment. These spectra were collected both within the irradiated region and in the region adjacent to it (which was not illuminated). The variation in fluorescence observed in the unlighted region (after subtracting the autofluorescence) represents the variation related to the natural pharmacokinetics of PS. At each interval, during irradiation, the treatment light was interrupted to acquire the fluorescence spectra. Figure 2 shows five different regions of fluorescence within the irradiated oropharynx after photosensitization with curcumin in the formulation (Fig. 2A). The average curcumin degradation in the formulation was observed by fluorescence for 20 patients with increased fluency (Fig. 2B). The autofluorescence spectrum obtained before photosensitization (Fluence 0) was subtracted from the spectra after sensitization with a maximum of 11.7J/cm^2.

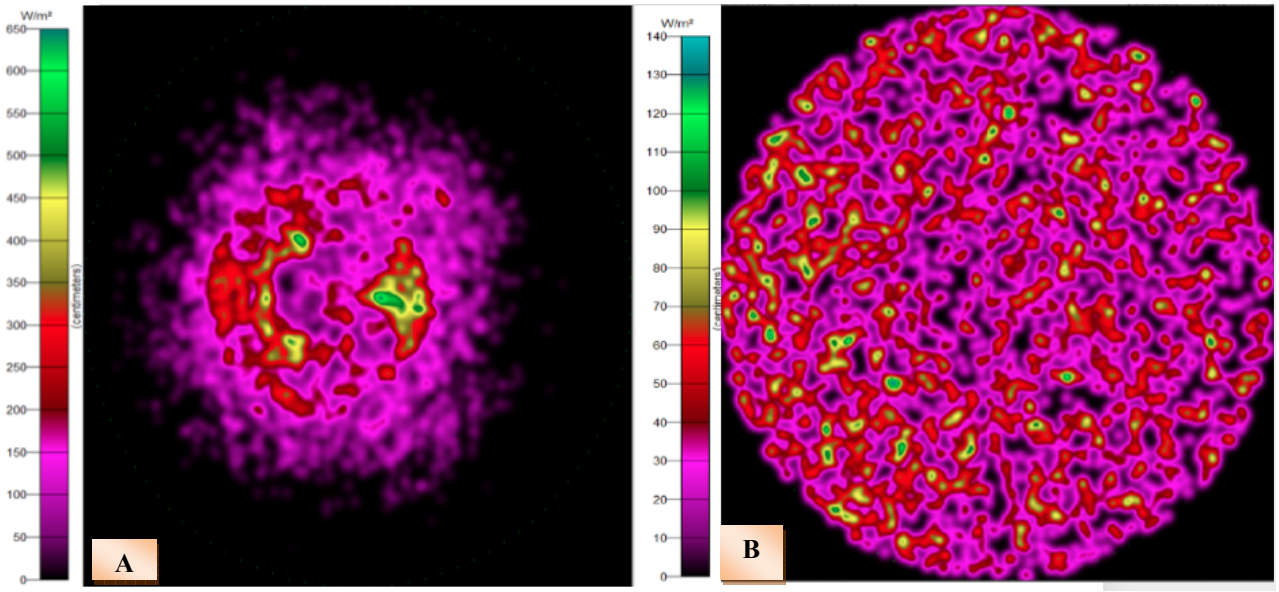

Fig. 1 Simulation of lighting distribution in the oropharynx using standard (A) and diffuse tip (B), and diffuse (B) using scale in W/m^2.

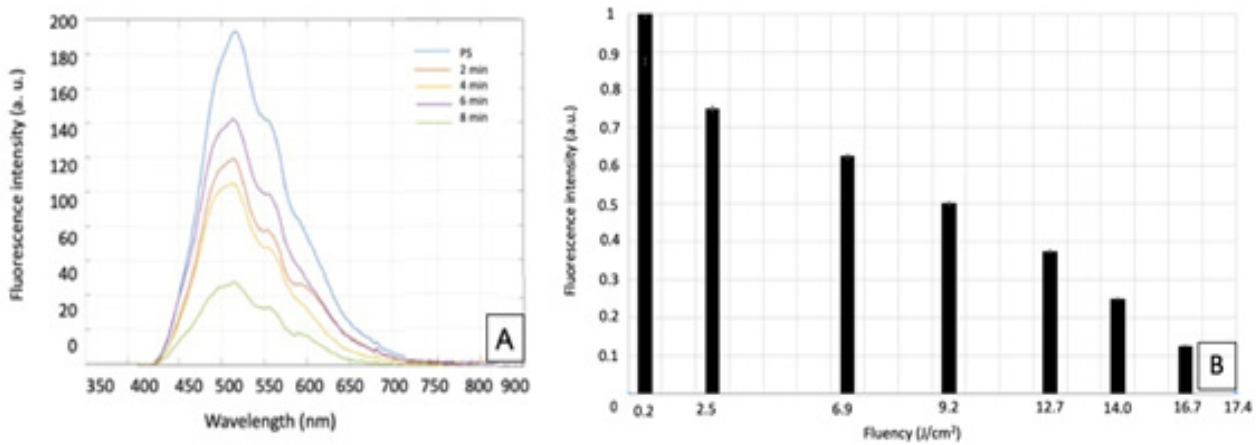

Fig. 2 (A): Fluorescence intensity spectra of curcumin in formulation on oropharynx of a healthy patient with 8 minutes of lighting. (B): Average fluorescence intensity of volunteer patients after fluency of 0, 0.2, 2.5, 6.9, 9.2, 12.7, 14.0, 16.7, 17.4.

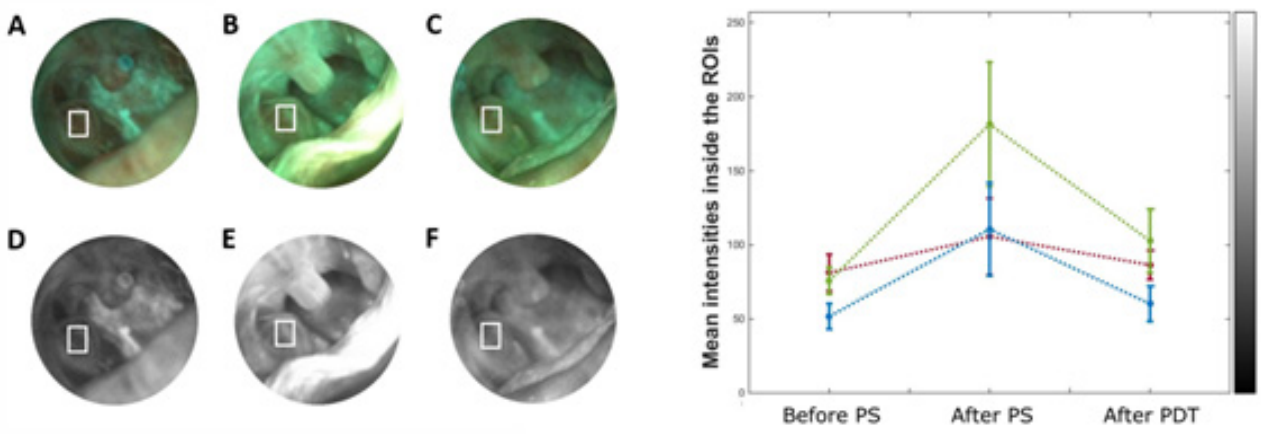

Fig. 3 Images of a patient related to conditions before and after application of PS and after PDT can be seen in A, B, C, and D, E, F, respectively. Plot of mean intensities and standard deviations of histograms of the red, blue, and green channels of each ROI intensities value.

The dose/effect relationship considers the dose of light related to the fluorescence decay during treatment. The absorption of light by the PS produces fluorescence. The longer the light exposure time, the greater the fluorescence decay, indicating that PS is absorbed, and a photodynamic reaction occurs. Fig. 2A shows that the photodynamic reaction occurs due to the presence of PS in up to eight minutes of illumination. However, in Figure 2B, lighting was tested for up to 12 minutes, which proves the total PB of PS in the oropharynx.

The visualization of fluorescence images of the tonsils performed using LEDs at 405 nm after photosensitization with 2.25 mg of curcumin in the formulation are represented in Figure 3 before and after 12.7 J/cm^2 of illumination.

For each patient, three images were analyzed, each one representing one condition: 1-Before the PS application (Before PS); 2-After PS application (After PS); 3-After PDT. Thus, as we use curcumin as a PS, we expect an intensity increase in the images' green values after the curcumin application and the same behavior, but with less intensity, in the blue region of the spectra, as a result of PB occurrence after PDT.

To evaluate images at each condition, we delimit a region of interest (ROI) corresponding to a selected area of one of the exposed tonsils. Histograms of the red, blue, and green channels of each ROI were

analyzed, and their respective mean intensities plotted in the graphic of Fig 3 confirm the expected behavior for intensities values.

It is important to note that, as expected, the condition with more intensity in the green and the blue channel is after PS application and, after PDT application, these intensities reduce and become similar to the first condition, when the PS was still not present; indicating that the fluorescence intensity after PDT is mainly due to tissue autofluorescence.

4. Discussion

Here, the light diffusion from the source was carried out considering protocols that consider the dispersion of light by optical fiber inside the mouth for treatments of the oropharynx with PDT [10-12]. Optical fiber diffuse provided light for the total coverage of the oropharynx's volume, considering the minimum treatment diameter to optimize the treatment, which is relevant to any clinical protocol that involves lighting [13]. The fiber of diffused light studied promoted an incidence of rays in the oropharynx and a more uniform illumination in the oropharynx, reaching the simulated tonsils on the sides of the sphere.

For PB evaluations, the accumulation of curcumin PS in the oropharynx mucosa was considered. For the study of the photodynamic interactions that begin with the activation of curcumin by 450 nm light considering the penetration into the mucosa tissue, fundamental interactions of the light of the studied equipment were determined, including parameters of reflection, refraction, dispersion, and absorption from the study of the simulation of the incidence rays with 82%. Penetration beyond wavelength may depend on characteristics of the tissue, with the scattering of light described by Rayleigh or Mie often used to describe this process [14].

The curcuminoids formulation used in this work contributes to the study of fluorescence spectra during the photodegradation of this PS. The PB of curcumin in the oral mucosa was obtained by excitation of the molecule due to the observation of fluorescence decay shown using different techniques. The use of light at 450 nm studied together with the device carefully considered for the effectiveness of clinical PDT, therefore considering the time and type of exposure to light. The clinical efficacy in PDT of infections may depend on the shape of the surface of tissue as well as on the total light dose and the design of the lighting fiber to be performed. Therefore, the monitoring of curcumin fluorescence can be a safe alternative to determine the effectiveness of treating infections based on the inactivation of microorganisms contained in the oropharynx, mainly tonsils, which is obtainedwith adequate lighting.

5. Conclusions

The light sources of the application device for treating oropharyngeal infections used for PDT are considered in this study. The tip of the light-emitting device was modified and studied considering changes in the distribution of light, without optimizing the tonsils' lighting, however, changing the light dose properties to be applied to the oropharynx mucosa. Clinical PDT was realized by dosimetry and light source optimization combined with PS&PB study. The fluorescence of curcumin through its light-dose related PB showed us the importance of monitoring the fluorescence of the PS related to the effectiveness of the treatment. PDT optimization in oropharyngeal tissue proved to be related to dynamics between light and the tissue and, the light and the PS. The clinical efficacy in PDT of infections may depend on the shape of the surface of tissue as well as on the total light dose and the design of the lighting fiber to be performed.

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Conflicts of Interest

The authors declare no conflicts of interest.

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