Antimicrobial Efficacy of Curcumin Formulations by Photodynamic Therapy

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Abstract: URTI (upper respiratory tract infections) are caused by acute, chronic or recurrent infections including pharyngitis and tonsillitis. The etiologic agents involved are bacteria, virus, and fungi. The standard treatment for bacterial infections is the use of antibiotics. The antibiotic resistance, side effects of drugs and URTI complications indicate the importance of new therapies. PDT (photodynamic therapy) is a treatment option with a fast onset of action, low side effects, and easy administration of PS (photosensitizer) based on this study. The main aim of this study was to develop a new modality for the treatment of infectious pharyngotonsillitis by photodynamic therapy. In addition, this study aims to evaluate components of the therapy as the PS, curcumin, in two formulations and irradiation conditions in the effectiveness of treatment. The procedure consists of two steps: oral administration of CFs (curcumin formulations) containing a PS and illumination (450 nm and 0-60 J/cm²). Bacterial isolates were obtained from patients in a routine clinical pratice. The new methodology has been developed from the principle of PI (photodynamic inactivation). The strain identification number of colony forming units determined the efficacy of PI using each component, lighting and PS. The use of curcumin formulations in PI presented microbial reduction of 5 log.

Key words: Upper respiratory tract infections, pharyngitis, tonsillitis, curcumin, photodynamic therapy, clinical isolates, syrup, gum.

1. Introduction

URTI can involve multi-organs such as pharynx and tonsils. Staphylococcus ssp. and Streptococcus spp. are the main bacterial etiological agents of URTI. In particular, Streptococcus pyogenes of Group A may affect up to 30% of all PTs (pharyngotonsillitis) cases in children between 5 and 15 year old [1]. PT has been identified with high-indice by hospital emergency departments [2, 3]. The standard treatment of infectious PTs consists in the administration of antibiotics, including penicillin. However, some bacteria are resistant to antibiotics, as penicillin, due to their frequent use [4]. Microbial genetic mutations can modify the drug metabolism through protein binding and cell permeability [5]. The RF (rheumatic fever) is a complication after repetitive episodes of PT with the conventional treatment. RF presents clinical difficulty in treatment of congestive heart, which can lead to death.

The development of therapies for new application, as PDT could solve problems, as drug toxicity and antibiotic resistance. Antimicrobial PDT is an oxygen-dependent photochemical reaction that occurs with the activation of a PS by a light source leading to the generation of reactive oxygen species as singlet oxygen, toxic to microorganisms [6, 7]. It is effective against a wide variety of strains of bacteria, fungi, parasites, vectors, and virus.

Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] from Curcuma longa rhizome, is a yellow pigment sensitive to light, insoluble and unstable in alkaline solutions. The beneficial effects of curcumin on health against pathologies such as Alzheimer’s, Diabetic, Cataract, Malaria has been proven. In addition to curcumin, there are two components in Curcuma longa, the curcuminoids demetoxicurcumin and bis-demethoxycurcumin. Studies related to photosensitive action of curcumin and synergistic actions of the curcuminoids has been performed in...
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microbial control. Several studies with nanoparticles to improve the solubility of curcumin have been carried; however the clinical use is still limited. The sugar formulations may increase the solubility of the curcumin molecule in water. The hydroxyl groups of sugar may participate in competitive hydrogen bonds and maintain the characteristics of curcumin [8].

Considering the challenge of curcumin delivery in different clinical conditions, the incorporation of curcumin in two different pharmaceutical vehicles has been performed in this study. The aim of the present study is to use sugar formulations as a transport system to increase the solubility of curcuminoids and improve the therapeutic efficacy of PDT of bacterial URTI.

2. Materials and Methods

2.1 Curcumin Syrup

The PS used in this study was a mixture of 46.6% bisdemethoxycurcumin and demethoxycurcumin and 53.4% curcumin (PDT Pharma, Cravinhos-SP) purified from Curcuma Longa. A diluted water (70%) and sugar (30%) mixture (phase A) was prepared and boiled until syrup is obtained. A curcumin (0.75g/L) and ethanol (2%) mixture (phase B) was performed and added to syrup. The curcumin syrup was placed in appropriate flasks protected from light.

2.2 Curcumin Gum

The gum was prepared in two phases: (A) aqueous phase and; (B) dissolved curcumin. To prepare phase A, 20% gelatin, 50% sugar and distilled water (q.s.p) mixture was heated to 100 °C. To prepare phase B, ethanol (0.6%), pineapple flavor (0.6%), citric acid (0.12%), crushed curcumin (15 mg/g) mixture was performed. The aqueous phase was inserted into B phase and stirred for few minutes. The curcumin gum was placed in appropriate flasks protected from light.

2.3 Formulation Characterization

2.3.1 Stability

The samples of gum and syrup were kept at 8 °C, 25 °C and 37 °C exposed and protected to light. Variables as macroscopic characteristics, pH and fluorescence intensity were analyzed in time intervals of 1, 3, 8, 10 and 15 days. Organoleptic properties of CF (curcumin formulations) as appearance, taste, aroma, color and consistency were analyzed according to the Brazilian Pharmacopeia [9].

2.3.2 Fluorescence

The presence of curcumin in both syrup and gum was identified by fluorescence spectroscopy (408 nm). A portable system consisting of a Y-type investigation probe (Ocean Optics, USA) [10] with two optical fibers (600 nm) for delivery of the excitation laser and another that re-emits light from the target tissue (USB2000 spectrometer (Ocean Optics)) operating between 350 nm and 1000 nm.

2.3.3 pH

The pH values of both CFs (syrup and gum) were determined at room temperature during a total of 15 days. The samples were homogenized for 15 min and measurements were taken by a pH meter previously calibrated.

2.4 Antimicrobial Photodynamic Inactivation

2.4.1 Microorganism

Staphylococcus aureus (ATCC 25923) was acquired from the culture collection of the Biophotonic Laboratory of São Carlos Institute of Physics (SP, Brazil) and originally purchased from the American Type Culture Collection. Suspensions (500 μL) of S. aureus and clinical isolates were incubated in 24-plates for 30 min in the presence of 500 μL of curcumin in syrup and gum. The wells were illuminated at 450 nm by Biotable [11]. Fluencies ranged from 0 to 60 J/cm² at 35 mW/cm² of irradiance. The experiments were performed under the following conditions: a) absence of irradiation and PS; (b) absence of irradiation; (c) absence of PS; (d) presence of irradiation and PS. The aliquots were serially diluted in PBS for the determination of CFU (colony-forming units) of bacteria.
2.4.2 Clinical isolates
Clinical isolates from five patients diagnosed with acute PT were obtained for in vitro experiments. Stuart Transport Medium was used for the transport of bacterial samples, which were cultivated in TSB (tryptic soy broth) in an orbital shaker at 37 °C for 18 h. TSB was added to the culture at optical density (DO600) of $10^7$ bacteria per milliliter.

3. Results and Discussion

3.1 Stability of Curcumin Incorporated in the Syrup and Gum

It was observed that up to 15th day of study there was significant loss of drug at temperatures above room temperature and no protected from light. It is observed with a change in the fluorescence intensity of PS with higher variations in gum. The organoleptic and physicochemical characteristics of formulations as color and pH change with increasing temperature. The rates of thermodynamic reactions change with increase of temperature and it may be degraded the PS. These results show the importance of CFs preservation at 4 °C and protected from light.

3.1.1 Fluorescence spectrophotometry
After preparation of the formulations the presence of curcumin was identified by fluorescence spectroscopy with excitation at 408 nm. The fluorescence spectrum was collected and both formulations showed fluorescence peak related to the presence of curcumin with emission at 560 nm (Fig. 1). The fluorescence peak of curcumin shows no change in the formulations.

The incorporation on the curcumin into the formulations was analyzed by fluorescence peak at 560 nm. The fluorescence intensity to analyze the presence of curcumin was along a total of 15 days in several conditions (temperature, pH, and light). The formulations showed fluorescence related to the presence of curcumin at 560 nm (Figs. 2 and 3).

The fluorescence intensity of PS is proportional to the concentration of fluorescent component of the photosensitive substance, the fluorophore. To ensure the stability of curcumin whereas its fluorescent properties, the fluorescence intensity of CF under different light and temperature conditions were observed (Fig. 2).

Changes in fluorescence intensity of curcumin under different exposure conditions were observed (Fig. 2). In photodecomposition the light changes the chemical properties of the formulations and the spectrum of absorption and emission of the fluorophore of fluorescent molecule. Like light, the temperature also modifies the stability of the formulations.

The loss of function of molecules may occur due to changes in temperature. The curcumin gum is more unstable curcumin syrup with temperature range. The collagen proteins contained in the gum may not withstand temperature variation, causing a faster degradation of PS. It was possible to observe that the gum presented less fluorescence intensity than the syrup in all light and temperature conditions. However, the colagen is characterized by low antigenicity, biodegradability, biocompatibility in the drug formulations.

The CF exposed to light under all conditions tested showed lower fluorescence intensity. PS are photosensitive substances that expose to light are degraded.

![Fig. 1](image_url) Fluorescence spectroscopy of curcumin in gum and syrup.
3.1.2 pH

In order to determine the stability of CF, it was monitored the pH of solutions for 15 days. Fig. 3 shows the pH values of syrup (A) and gum (B) at different temperatures with and without light exposure.

The pH of formulations changed with the temperature. The pH of water is 7 at 25 °C and with increase of temperature decreases. The pH of syrup is more stable than gum after 15 days of exposure at temperatures greater above 4 °C. In addition to temperature and light exposure, the pH may influence on the organoleptic characteristics of CFs. In the first day of preparation, both gum and syrup showed a characteristic orange color. No change as color was observed in syrup formulation and however changes in gum appearance were observed along the days.

3.2 Antimicrobial Photodynamic Inactivation

The present study investigated the antimicrobial action and the stability of curcumin in two pharmaceutical formulations for treatment of URTI by PDT. However, pathogenic microorganisms can be bound to oral epithelial cells. The oral mucosa is a non-uniform and highly permeable tissue, which make the adhesion of medicine more difficult. Setthacheewakula et al. [12] developed and characterized drug delivery systems that improved the...
solubility, dissolution, and oral absorption of curcumin, a poorly water-soluble compound. The antimicrobial activity of PDT is probably connected to the curcumin stability in the formulations, therefore it could determine the efficacy of the treatment. The use of syrup or gum is an alternative for carrying PSs to oral mucosa. The antimicrobial properties of CFs in PI conditions were evaluated with *S. aureus* strain ATCC 25923 (Fig. 4A). PI tests were conducted in the presence of curcuminoids syrup and gum with light fluence (0-65 J/cm²).

The use of curcuminoids at 60 J/cm² and curcuminoid syrup were the most effective PI protocol. The antimicrobial activity of curcuminoids has been observed in *S. aureus* strains [13-15]. Despite being part of normal microbiota of upper respiratory tract, *S. aureus* can colonize the oral mucosa causing diseases. Santezi et al. [16] showed the antimicrobial inactivation of 2.54 log₁₀ of salivary pathogens using a water-soluble mixture of curcuminoids (3 g/L) and 9.0 J/cm² by PI, however presented similar effects using the curcumin without light. CF can promote adherence on oral mucosa and improve the photobleaching during PDT.

The antioxidant activity of such curcuminoids and their effect on the cleavage of plasmid DNA were examined by Haseeb Ahsan and showed a similar rate of formation of hydroxyl radicals. Safety studies were conducted with curcumin and its derivatives, which showed nontoxic effects on the animal cell [17]. Fig. 4B shows the antimicrobial effects of PI with curcuminoid syrup (0.75 mg/mL) in clinical samples of five patients at 60 J/cm². The results showed the antimicrobial efficiency of CF in clinical samples by PI, which demonstrated the possibility of use of this protocol to treat pharyngitis. Studies on the effects of CFs on the treatment of cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases have been developed. Lüer et al. [18] concluded that synthetic curcumin proved to be safe, palatable and an excellent option for treatment of oral mucositis.

4. Conclusions

In this study, CFs developed to improve the action of PS in PDT of pharyngitis. The stability studies of formulations determine the manufacturing process and storage conditions for the use of these drugs for future clinical applications of oral infections. In vitro studies revealed the use of PDT with CFs is effective against *S. aureus* and clinical isolates of bacteria from acute pharyngitis. The results are promising for Clinical Translation of topical use of CFs in the treatment of ITRS.

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References


