Clinical Dermatology: Research and Therapy

SCIENTIFIC LITERATURE

Research Article

Effectiveness of the Association of Cellulose Membrane Dressing and Photodynamic Therapy Mediated by Curcumin to Reduce Contamination and Prevent Recontamination in Pressure Lesions

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ARTICLE INFO

Received Date: July 28, 2018
Accepted Date: October 02, 2018
Published Date: October 18, 2018

KEYWORDS

Photodynamic therapy
Cellulose membrane
Microorganisms
Pressure lesion

Citation this article: Beatriz Rocha Tanajuraa, Francine Cristina Silva Rosaa, Natalia Mayumi Inadab, Vanderlei Salvador Bagnatob and Luciano Pereira Rosaa. Effectiveness of the Association of Cellulose Membrane Dressing and Photodynamic Therapy Mediated by Curcumin to Reduce Contamination and Prevent Recontamination in Pressure Lesions. Clinical Dermatology: Research and Therapy. 2018; 1(3):121

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ABSTRACT

The aim of this study was to isolate the main contaminating microorganisms from pressure lesions (PLs) and to evaluate the effectiveness of cellulose membrane and Photodynamic Therapy (PDT) mediated by curcumin in its combat and prevention of recontamination in hospitalized patients. Twelve PLs from seven patients were treated with two sessions of PDT, with 7 days interval, mediated by curcumin in 1.5% emulsion and irradiated with blue LED of 450 nm with irradiance of 35 mW / cm², fluence of 25.2 J / cm² for 12 minutes. Cellulose membrane was used as a dressing, replaced every 3 days. Microbiological samples before and after the PDT sessions were seeded in blood agar, Mac Conckey agar; Mannitol agar and Sabouraud agar with chloramphenicol. Plates were incubated for 24 hours at 35 ± 2° C and colony forming units (CFU) counted. Recontamination was assessed over the seven day period between PDT sessions. PDT promoted a significant reduction ($p < 0.05$) in the CFU count for all microorganisms tested in this study. The analysis of the recontamination of the PLs showed that for the enterobacteria there was no significant statistical difference ($p = 0,231$) in the levels of contamination. However, for yeasts, Staphylococcus spp. and total count there was an increase in the CFU count with a statistical significant difference ($p = 0.000$). It can be concluded that the most prevalent microorganisms in the sample studied were Staphylococcus spp., yeast and enterobacteria and that treatment with PDT promoted a significant reduction in the contamination of PLs and did not present any relevant action on the prevention of recontamination.

INTRODUCTION

According to the National Pressure Ulcer Advisory Panel (NPUAP) pressure lesions (PLs) are defined as soft tissue injuries on a prominent bone arising from a prolonged pressure on a rigid surface [1]. This leads to poor blood circulation at the site that can lead to necrosis, ulceration of the skin and other tissues, which favors the growth and development of microorganisms due to secretions, crusty or hemorrhagic material in the lesion. In this context, the contamination is a universal and unavoidable occurrence in PLs [2].

Some factors such as instability of the patient's immune system, the tissue's internal condition and bacterial proliferation may impair wound healing, which contributes to the chronicity of the lesion [3,4].

Prolonged inflammation and colonization of chronic lesions are mostly due in part to the presence of biofilms [5]. Bacteria that live in biofilms are encapsulated by a matrix composed of extracellular polymeric substance separated by water channels that function as a circulatory system, which provide nutrients and removes metabolic residues, being also pointed as a factor of virulence and protection to the microorganisms [6].

In clinical practice, dressings and topical products are used to create and keep a humid and aseptic environment, providing the ideal conditions for wound healing process. Nevertheless, they are often expensive, ineffective and may induce adverse reactions, been necessary the search for new alternative therapy [7]. Recently, the process of wound healing has caught the attention of innumerable researchers, especially concerning the factors that prevent or delay healing, such as the case of mellitus diabetes and infections. Due to the increased number of microorganisms resistant to antibiotics, PDT has been investigated on the inactivation of microorganisms [8].

In this context, the use of Photodynamic Therapy (PDT) has been highlighted how one of the most promising therapeutic choices in eradicating microorganisms, to eliminate the problem of antimicrobial resistance, due to the misuse of conventional antimicrobials [9,10]. PDT is based on the association of a photosensitizer (PS) and visible light radiation, which can be administered locally or systemically to accrue on the desired treatment site. The PS when activated by visible light can produce two types of reactions: Type I when it reacts with hydrogen and produces free radicals, or Type II, when it reacts with oxygen and produces singlet oxygen species which causes damage to the structures of bacterial cells leading to bactericidal action, not being toxic to the host cells [11]. This treatment was initially developed as an alternative cancer treatment, it is being broadly studied for applications in other areas such as, for example, an alternative for the inactivation of microorganisms [12].

Studies have confirmed the efficacy of PDT in the treatment of bacterial infections and their biofilms in wounds acting at multiple points, such as membrane phospholipids, proteins and nucleic acids [13]. Morley et al. [14] determine if PDT in bacterially colonized chronic leg ulcers and chronic diabetic foot ulcers can reduce bacterial load, and potentially lead to accelerated wound healing. For 16 patients with chronic leg ulcers and 16 patients with diabetic foot ulcers with an ulcer duration longer than 3 months, PPA904 treatment for 15 min and red light irradiation at 50 J/cm2 were tolerated with no reports of pain and showed a reduction in bacterial load immediately post-treatment. Lei et al. [15] evaluated the antimicrobial activity and healing-promoting effect of topical PDT ALA mediated on chronic skin ulcers infected with Pseudomonas aeruginosa. The bacteria levels on PDT group was significantly different from the control group.

Numerous photosensitizers have been studied for microbial inactivation, among them toluidine blue O, curcumin, and methylene blue; the lattermost has been widely studied as a photosensitizer in inactivating bacteria with positive results [10,11,16-19]. Curcumin is a polyphenol found in rhizomes of Curcuma longa that has antimicrobial and anti-inflammatory activity [17].

Although there are various types of biological and synthetic dressings available for wound cover, the search for an ideal dressing is still in progress. According to modern methods for wound healing, an ideal system should be structurally and functionally similar to the skin autograft. Recently, advancements in bioengineered materials have led to the development of an artificial skin polymer made from cellulose produced by the bacteria Gluconacetobacter xylinus. These skins have the ability to protect burn wounds and lesions on human skin, allowing gas exchange and respiration of the body and preventing the passage of liquid and impurities. Because of its unique properties, several studies have shown that microbial cellulose has great efficacy in improving the healing process in chronic wounds [20]. Rosa et al. [18] demonstrated in a clinical case that the association of cellulose membrane with PDT can be relevant for the maintenance of the aseptic conditions in pressure lesions, reducing the contamination and directing for wound healing.

Thus, the objective of this study was to evaluate the effectiveness of cellulose membrane and curcumin mediated PDT and 450 nm blue LED on the elimination of microorganisms

isolated from PLs and to evaluate the possible preventive effect of recontamination of the techniques.

MATERIAL AND METHODS

Design, place of study and patient selection

This is a randomized trial. The sample consisted of a non - probabilistic and convenience sample, of which twelve PLs were selected from patients attended by a public hospital in a city of interior of Bahia, Brazil, who presented medical diagnosis of PLs.

The inclusions criteria were: 1) patients aged 18 years or over; 2) presence of PLLs for at least four weeks; 3) Stable or worsening PLs.

Exclusions criteria: 1) presence of comorbidities such as: renal, hepatic, haematological, neurological or immune disease; 2) presence of malignant lesions; 3) use of corticosteroids, immunosuppressant or cytotoxic agents; 4) infection by the Human Immunodeficiency Virus (HIV) or carriers of Acquired Immunodeficiency Syndrome (AIDS); 5) not using antibiotic.

This study was approved by the Committee of Ethics in Research (CEP) with Human Research of the Multidisciplinary Health Institute of the Federal University of Bahia, under registration CAAE 36925714.0.0000.5556, according to the Helsinki Declaration and its subsequent amendments or comparable ethical standards. Informed consent was obtained individually from all patients included in the study.

Isolation of microorganisms

After PLs sanitation with sterile saline solution and under aseptic conditions, biological material was collected from viable granulation tissue using sterile swab (Labor Import, Osasco, Sao Paulo, Brazil). The swab with the biological material was transported, in Stuart's transportation medium, immediately for processing of the sample. In laminar flow (Filterflux, Piracicaba, Sao Paulo, Brazil) , the swabs were introduced into tubes containing 3 mL of sterile saline and agitated for 1 minute in a tube shaker (Phoenix Luferco, Araraquara, Sao Paulo, Brazil). Aliquots of 100 µL of the suspension were seeded in Petri dishes containing the following culture media: blood agar (Difco, Detroit, USA) for total bacterial growth; agar Mac Conckey (Difco, Detroit, USA) for enterobacteria growth; Manitol agar (Difco, Detroit, USA) for Staphylococcus spp. growth and Sabouraud agar with chloramphenicol (Difco, Detroit, USA) for selective yeast growth. The plates were incubated for 24 hours at a temperature of 35 ± 2 ° C. After growth, the colonies were examined for macroscopic morphological characteristics and CFU (Colony Forming Units) count.

Antimicrobial Photodynamic Therapy

The photosensitizer Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione] was used to perform PDT on patient lesions 1,5% emulsion (PDT Pharma Industry and Commerce of Pharmaceutical Products LTDA, Cravinhos, Sao Paulo, Brazil). The UV-Vis (ultraviolet-visible) absorption spectrum of the compound was recorded between 300 and 700nm using quartz cuvette with a wavelength of one centimeter in spectrophotometer UV-Vis (Varian, Darmstadt, Germany), and were characterized by a wavelength of maximum absorption at 430nm. The photosensitizer was applied to the entire surface of the lesion, the region being immediately occluded with PVC (Polyvinyl chloride) film foil (Guarufilme, Guarulhos, Sao Paulo, Brazil) and gauze (Cremer, Sao Paulo, Sao Paulo, Brazil). After 30 minutes of photosensitizer application, the dressing and excess emulsion on the lesion were removed with sterile saline and gauze for further irradiation.

To activate the curcumin, we used the Lince (MMOptics, Sao Carlos, Sao Paulo, Brazil) device that is composed of an active plate (8.0 cm × 7.7 cm) with 30 LEDs of 450 + 10 nm wavelength (visible blue) distributed in 6 rows of 5 LEDs each. The application was continuous during 12 minutes, with illumination intensity of 35 mW/cm2 to total the dose of energy supplied to the tissue of 25.2 J/cm2. The light was applied at a distance of five centimeters from the tip of the equipment in relation to the surface of the lesions.

After seven days of the first PDT session (PDT1), patients received a second PDT session (PDT2), with the same parameters. To prevent cross-infection the equipment was decontaminated before and after each application with 70% alcohol and it was covered by plastic film of PVC substituted in each treatment. Microbiological collections were performed before and after each PDT (methodology and processing described above).

In order to evaluate the effectiveness of decontamination and recontamination of the treatment performed with PDT, after the sessions, the PLs were covered with the cellulose membrane

Nanoskin® (Innovates Biotechnological Products Inc., Sao Carlos, Sao Paulo, Brazil)placed over the entire surface of the ulcer not exceeding the limit with normal skin, and covered with gauze and bandages. The membrane was changed every 3 days.

Recontamination evaluation

The recontamination of PLs was evaluated in the seven-day period comprised between PDT1 and PDT2 by comparing CFU counts obtained after PDT1 and before PDT2, thus, a possible residual effect of PDT could be evaluated.

Statistical analysis

Data on the isolation of microorganisms were expressed as percentage of occurrence. The log of CFU (log 10 CFU) was calculated and adherence to the assumptions of normality and homoscedasticity was verified using the Kolmogorov-Smirnov normality test. Comparisons of the effectiveness of PDT in combating the microorganisms were made through the T-paired test between pre and post-treatment samples, as well as the recontamination analysis that was performed by comparing the CFU values after to PDT1 and before PDT2.

RESULTS AND DISCUSSION

Microorganism isolation

By means of the data obtained from the isolation of the species of microorganisms colonizing PLs, it could be observed that in 100% of the patients there was growth of microorganisms in the blood agar medium, which corresponds to the total microorganism count. 90% of the PLs were contaminated with Staphylococcus spp., and in 70% there was isolation of yeasts and enterobacteria.

Antimicrobial Photodynamic Therapy

In general, there was a statistical difference between the collections made before and after for most of the groups of microorganisms evaluated in PDT1 ($p < 0.05$), except for the group of enterobacteria in which there was no significant reduction ($p = 0.273$). The largest reductions in log10 CFU were obtained for Staphylococcus spp. and total growth of microorganisms (1.1 log10), for yeasts and enterobacteria the reductions were 0.8 and 0.2 log10, respectively, as can be observed in (Figure 1).

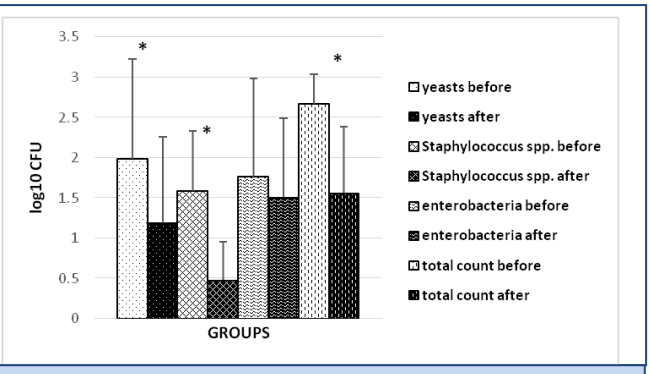

Figure 1: Comparison between the means by means of the paired T test between the CFU count before and after the treatment of PDT1.

* = statistical difference.

The results obtained by comparing the log10 CFU counts of the PLs before and after the PDT2 showed that for all the microorganisms tested there was a statistical significant difference ($p < 0.05$), indicating the effectiveness of the microbial reduction method in PLs. The reductions in log10 were 0.6 for yeast, 0.8 for Staphylococcus spp., 0.5 for enterobacteria and total count of microorganisms (Figure 2).

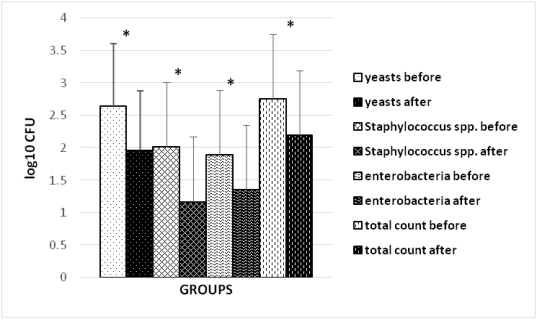

Figure 2: Comparison between the means by means of the paired T test between the CFU count before and after the treatment of PDT2.

* = statistical difference

The analysis of the recontamination of PLs, it was possible to observe that for enterobacteria there was no significant statistical difference ($p = 0.231$) between the log10 CFU count after the PDT1 and before the PDT2, showing that the contamination levels for this microorganisms has not changed. However, the comparison of the contamination after TFD1 and before TFD2 presented a significant increase in the log10 CFU count for yeast, Staphylococcus spp. and total microorganism counts ($p = 0.000$), as shown in (Figure 3).

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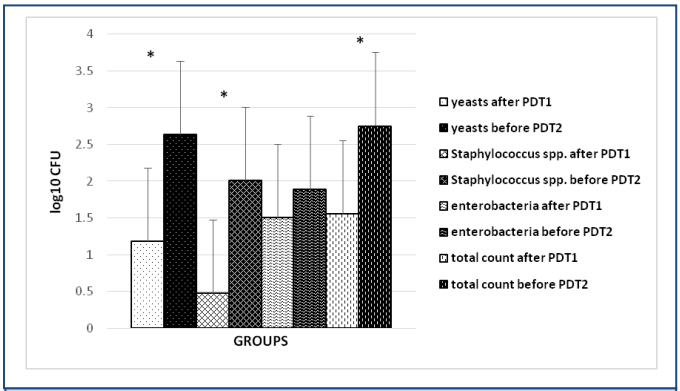

Figure 3: Evaluation of the recontamination by means of the comparison with the paired T test between the means of the CFU values obtained after the PDT1 and before the PDT2.

* = statistical difference

DISCUSSION

PDT is a promising strategy for eradicating pathogenic microorganisms, with a new approach to combat infections, with little possibility of resistant strains generation [8,14]. Studies in the literature in which PDT are used in PLs are scarce, which demonstrates the innovative character of this study [21].

Several procedures have evaluated the presence of microorganisms in cutaneous lesions. Carvalho et al. [22] performed a bacteriological analysis of 141 cases of chronic diabetic foot injuries, where the most frequently isolated pathogens were enterobacteria (83.7%), Staphylococcus aureus (43.3%), anaerobic bacteria (17%), and strains of Streptococcus pyogenes were isolated from only 7.8% of the patients. Discordant results were found in our study, where there was a high growth of microorganisms in blood agar medium, such a culture medium is not selective which allows the growth of various bacteria, for example, Streptococcus spp. alpha, beta and gamma hemolytic.

In our study high contamination by Staphylococcus spp. was found in the PLS evaluated (90%), which is in according with the findings of Martins et al. [23] and Bonfim et al. [24], where they found high levels of contamination with these microorganisms.

Bomfim et al. [24] and Fernandes et al. [25] found high contamination rates with enterobacteria in PLs (67% and 45.6%, respectively), which is compatible with our findings in which a rate of 70% of the respective microorganism was found. A possible explanation for this prevalence of enterobacteria would be the contamination with fecal residues mainly in lesions in the sacral region [26].

However, studies on the contamination of PLs with yeasts are scarce in the literature. In our findings, a mean contamination of 70% with this microorganism was observed, showing an important characteristic that is little known, which can lead to the difficulty of treating contaminated PLs without the use of antifungal drugs.

For the total count of microorganisms, this study showed that PDT was able to considerably reduce the bacteria present in PLs. A similar result was found by Araújo et al., [27], that assessed the overall susceptibility of pathogens of salivary flora to photodynamic therapy after sensitization with curcumin and exposure to blue light at 450 nm. There was a considerable bacterial decline with PDT treatment, with a 68% decrease in bacteria.

In a study done by Lei et al. [15] that evaluated the efficacy of ALA (δ-aminolevulinic acid) mediated PDT in the treatment of chronic ulcers in lower limbs infected with Pseudomonas aeruginosa, in which the results indicated that the levels of bacteria on ulcer surfaces were considerably different before and after PDT. Lopez-Jimenez et al. [28] evaluated the effects of toluidine blue and methylene blue-mediated PDT on Enterococcus faecalis biofilms showing morphological and surface changes in them. In a clinical study, Mannucci et al. [29] evaluated the effectiveness and tolerability of the 689 nm light-activated RLP068 application in infected diabetic foot ulcers. The authors obtained promising results in the reduction of Staphylococcus aureus, Pseudomonas aeruginosa and enterobacteria with a single application of therapy. In our work, although curcumin as a photosensitizer and blue light for irradiation were used, similar results were found regarding the reduction of Staphylococcus aureus and controversial results for enterobacteria after PDT application. This difference can be explained due the Gram-positive strains are more sensitive to the PDT inactivation, than Gram-negative and yeast. This may be explained with differences in cells walls and as a result of these differences the level of uptake of PS to the cell - it is higher for Gram-positive than to the other two types of microorganisms. The more complex character of ceil wall by Gram-negative also contribute to the lowest levels of inactivation do to necessarily of higher concentration of singlet

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oxygen or ROS to destroying membrane to such of degree which leads to bacterial death [30].

Andrade et al. [31] evaluated the effects of different pre-irradiation times of curcumin-mediated PDT in platonics cultures and biofilms of Candida spp. There was complete inactivation of the Candida species with the association of 20.0 μM curcumin after 5, 10 and 20 minutes of PDT. Regarding biofilms, the three Candida species evaluated underwent high reductions in cell viability with the association of 40 μM of curcumin and 20 minutes of PDT. In our study, curcumin was used as a 1.5% emulsion, which diverged from the preparation that was used by the cited authors. However, this type of preparation was preferable, unlike, for example, an aqueous solution for spraying, since it is difficult to access regions, which would make it difficult to maintain the photosensitive agent in the site due to the estimated time in this study , which was 30 minutes. However, similar results regarding the elimination of yeast infection were found in this study, where a reduction of 0.8 and 0.6 log 10 CFU was observed in TFD1 and TFD2, respectively.

The cellulose membrane has been gaining great importance in the medical area due to its use in treatments of skin restoration, in cases of wounds and burns. It is a highly pure, biocompatible and versatile material that can be used in various applications, both individually and in combinations with different components (eg biopolymers and nanoparticles), which provides structural organization and flexible matrices for different purposes, in addition to accelerating the epithelization process and preventing proliferation of microorganisms [32].

Cavalcanti et al. [33] analyzed the efficacy of bacterial cellulose membrane dressings in the treatment of lower limb venous lesions, showing a reduction in the wound area of the studied groups, in which there were no infections, besides the discontinuation of analgesic use. In another study, Shao et al. [34] evaluated the antibacterial properties of the membranes, in which it was found that they had excellent results for the following microorganisms Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Candida albicans, demonstrating great utility and potential. However, in the present study, the application of cellulose membrane in association with PDT contributed to the maintenance of enterobacteria contamination levels, but did not contribute to avoid recontamination with Staphylococcus spp, yeast and total counts of microorganisms, observed in the CFU count between sessions after PDT1 and before PDT2. The fact that there was no statistical difference after PDT1 and before PDT2 for enterobacteria is positive, because the reduction of microorganisms achieved by the technique was conserved during the period, and, in some way, it was impossible to recontaminate the wound. Staphylococcus spp. are part of the normal microbiota of the skin, which facilitates the local recontamination of the ulcers by the proximity to the lesions. The probable hypothesis for the growth of the number of yeasts is due to the fact that they are microorganisms more resistant to PDT, when compared with bacteria, which may be attributed to differences in cell size. Furthermore, as an eukaryotic microorganism, the presence of a nuclear membrane could act as an additional barrier to the photosensitizer. In this way, multiplying and recontaminating the wound [35].

Lopes [36] evaluated the action of PDT in the treatment of stomatitis under prosthesis in total dentures users, in which two sessions of PDT were applied with a seven-day interval, where a significant reduction of the yeast load was observed in the mucosa of the patients in the first session, but not being statistically significant in the second session. Therefore, this study is compatible with our findings, in which it may suggest that PDT has no residual effect. Possible explanations for the increase in microorganism counts after PDT1 and before PDT2 may be the fact that the cellulose membrane does not have the desired antimicrobial activity or care in dressing exchange was not enough, enabling the recontamination of PLs.

Studies using the association of PDT and cellulose membranes are scarce in the literature, showing the need for further research to verify the clinical effectiveness of the combination of these treatment techniques to combat infections, maintenance asepsis conditions and promote the healing of PLs.

CONCLUSION

All the patients in the study showed contamination in the PLs, where the most common microorganisms were Staphylococcus spp., followed by yeast and enterobacteria. PDT was effective to reduce PLs contamination, but the association of cellulose membrane dressing and PDT mediated with curcumin had no effect in the prevention of recontamination. Therefore, PDT

may be an innovative noninvasive tool in the care routine of patients affected by contaminated PLs.

ACKNOWLEDGEMENT

To the National Council of Scientific and Technological Development (CNPq) and to the Institutional Program of Scientific Initiation Grants (PIBIC), for the support and encouragement throughout the study.

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