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Abstract

Background: Photodynamic therapy has been investigated in different areas of health through experimental conditions. Its action can alter fundamental structures for the survival of microorganisms without any development of microbial resistance.

Materials and Methods: Young sheep presenting with abscess in the left forelimb caused by *Streptococcus* spp. was previously treated with antibiotics. There was no clinical improvement with the treatments, and the bacteria presented sensitivity *in vitro*. Therefore, Photodynamic therapy associating methylene blue and red laser (660 nm) was used to treat the abscess.

Results: After a day of treatment, complete healing was witnessed with no recurrence was observed during the 3-month follow-up period.

Conclusion: The scientific results of the antimicrobial effect of PDT proved to be a therapeutic option with great potential for clinical application.

Keywords: Photoinactivation, Laser, Sheep, *Streptococcus* spp.

Introduction

The control of pathogenic microorganisms is one of the most challenging fields in the pharmacological scope. The pharmaceutical industry is constantly alert, mainly due to the rapid adaptability and diversity of pathogens found. The emergence of wide range of resistant microorganisms means that there is great increase in morbidity from infections that were easily treated in the past. Therefore, systemic antibiotics should be used with greater caution, and especially with specific target, thus preventing further development of bacterial resistance, being recommended that local infections should not be treated systemically if there were viable alternative (Conly and Johnston, 2000).

Thus, the topical photodynamic therapy or PDT, becomes a therapeutic modality that meets most of the advantages, especially because it doesn't have undesirable systemic and microbiological effects which can be found in the main standard treatments involving antibiotics (Hamblin and Hasan, 2004).

Recently, PDT has been one of the most studied techniques in health sciences. This therapy has been employed in the treatment of superficial tumors, local infections caused by bacteria, fungi, virus and parasites. Its mechanism of action is a combination of a photosensitizing substance that is activated by a light source of a specific wavelength and resonant to the photosensitizer, inducing an oxidative process capable of causing the death of microorganisms without harming host cells such as fibroblasts, keratinocytes and neutrophils (Stashak and Theoret, 2008; Tanaka et. Al., 2012).

The photodynamic effect occurs when photosensitizer molecules absorb light, which will be changed from the fundamental singlet state to the excited state. In the singlet state, the excited molecules can return directly to the fundamental state (through fluorescence) or can change from the singlet excited state to the triplet excited state. In this state, the molecule can return to the fundamental state (through phosphorescence), or, by an oxi-reduction reaction with an additional neighbor molecule (type I mechanism), will result in the formation of reactive oxygen species (ROS), such as peroxide, superoxide anion, hydroxyl radical and hydrogen peroxide, which can cause destruction of the cellular membrane. On the other hand, the photosensitizer in the triplet state can transfer energy to oxygen in its fundamental state (type II mechanism), producing singlet oxygen. Almost all cellular components react with singlet oxygen, since the unsaturated organic compounds are, in general, susceptible to its action. Factors that determine the selective death of the host cells or microbial inactivation are strictly related to the characteristics of the employed photosensitizer molecules and the chemical and light dosimetry (Hamblin and Hasan, 2004).

First described over a century ago, PDT application appeared by the 80s in Veterinary Medicine when several treatments were performed in tumors of dogs and cats, and despite the wide variety of treated tumors, most of them showed responsive (Cheli et al., 1984). However, unlike other areas of health, such as human medicine and dentistry, few studies have been published about antimicrobial PDT in Veterinary Medicine (Sellera et al., 2013).

Materials and Methods

A young male East Friesian ovine was attended presenting lameness in the left forelimb caused by a swelling with floating consistency. After the physical examination, the presence of an abscess on the lateral side of the elbow joint, with approximately five centimeters in diameter but, after radiographic exam, there was no bone involvement. A sample of the abscess was collected through a sterile swab and sent to the laboratory of bacteriology, in order to identify the microorganism and to perform an antibiogram test.

The etiologic agent was identified as a *Streptococcus* spp. These bacterial strains were subject to *in vitro* susceptibility tests for different antibiotics and chemotherapy drugs, namely: amikacin (30 µg), ampicillin (10 µg), cephalixin (30 µg), cephalothin (30 µg), cefepime (30 µg), ceftiofur (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), neomycin (30 µg), norfloxacin (10 µg), oxacillin (1 µg), penicillin (10 IU), sulfamethoxazole + trimethoprim (25 µg), tetracycline (30 µg), and vancomycin (30 µg).

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The results of antibiogram test showed that *Sreptococcus* spp. was sensitive to the following antibiotics: norfloxacin, ampicillin, ceftiofur, gentamicin, amikacin and enrofloxacin, and resistant to 13 of the 19 active substances tested: cefepime, neomycin, oxacillin, cephalixin, penicillin, cephalothin, ciprofloxacin, clindamycin, chloramphenicol, erythromycin, sulfamethoxazole + trimethoprim, tetracycline and vancomycin. The tests were evaluated based on the measured halo diameter, which reflects the degree of inhibition on microorganism growth. The concentrations used and interpretation criteria adopted were chosen based on recommendations of the NCCLS (2008).

First performed a treatment with penicillin (0.15 mL, IM) associated with gentamicin (1.5 mL / SC) for 7 days. As there was no clinical improvement, we opted for the administration of ceftiofur (0.2 mL / SC) and use of gauze dipped in a solution of 2% iodine to aid healing for 7 days. However, this second treatment was also not effective, so we opted for treatment with PDT.

In our procedure, methylene blue (aqueous solution at a concentration of 0.01%) was applied topically and after 5 min of incubation in dark to allow microbial uptake, the lesion was irradiated using a diode laser (Laser Hand, MMOptics, São Carlos, Brazil), emitting 100mW at 660 nm of wavelength, coupled to the optical fiber during 180 seconds. The total energy used was 0.4 J per point and the energy density was 10 J/cm² per point distributed in five equidistant points of 0.02 cm² (Fig.1). After irradiation, the lesion was covered with sterile gauze and then covered by a bandage to protect the site. The daily treatment routine was performed by cleaning the abscess with NaCl 0.9% followed by topic repellent and healing ointment on the wound.

Analysis of injury recovery was made by descriptive analysis and after one day the complete healing has occurred with a single treatment using PDT (Fig.2). After PDT treatment no recurrence was observed during the 3-month follow-up period.



Figure 1: Irradiation of the wound with diode laser coupled to the optical fiber.



Figure 2: Wound healing after one day of treatment.

Results and Discussion

The use of antiseptics has the intention to control the proliferation of microorganisms, and although this reduction occurs, these solutions must be analyzed in relation to their toxicity. According to Stashak (2008), the use of iodine solutions above 0.2 % is cytotoxic to neutrophils, being contraindicated in the treatment of wound healing. In contrast, Dealey (2012) stated that iodine solutions diluted to 0.001% do not become cytotoxic to fibroblasts, slowing tissue re-epithelialization and decreasing the tensile strength of the wound, but these solutions at low concentration has compromised its antiseptic efficacy. Furthermore, administration of topical antibiotics in wounds is not recommended unless there is a specific indication, mainly due to disadvantages such as skin sensitization, development of microbial resistance, inhibition of the healing process and inactivation of antibiotics by organic matter from the wound (Degreef, 1998).

In recent years, phototherapy by coherent (lasers) and no coherent (Leds) light stands out as a biostimulator method for tissue repair, increasing local circulation, cell proliferation and collagen synthesis, and thus accelerating the process of tissue repair (Minatel et al., 2009).

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However, studies have investigated the use of PDT in the treatment of wounds. The light that is not absorbed by microorganisms can be absorbed by the tissue chromophores, triggering a similar response to the biomodulation process stimulated by light. Thus, PDT may present two synergistic effects: control of microorganisms and accelerate the tissue repair process (Brown, 2012).

According to the studies performed by Nafee et al. (2013) the PDT promoted healing in less time, better re-epithelialization and keratinization, and better development of collagen fibers compared to the untreated control group. Such findings confirm studies by Brown (2013), which concluded that PDT inactivated a wide variety of microorganisms, accelerated wound healing and prevented clinical infection, especially in patients with chronic wounds.

Moreover, in a study performed by Sperandio et al. (2010) laboratory animals were submitted to surgical procedure and evaluated by histopathology exams. They compared four treatment groups: control (no treatment); topical application of methylene blue; laser irradiation and topical application of methylene blue followed by irradiation with laser. It was found that the laser-treated group alone showed complete re-epithelialization of the wound between 5 and 7 days after surgery, whereas in the group treated with PDT and this time the control group was 14 days, indicating that the laser effects were altered when exposed to methylene blue. However, it was concluded that the application of PDT mediated by methylene blue was not detrimental to wound healing even with delay in the healing time when compared to the control group.

Diseases caused by microbiological agents continue to be a serious global health problem in humans and animals. Effective alternative treatments not susceptible to microbial resistance are extremely necessary, and the killing of bacteria by PDT could emerge as an option (Brown, 2013).

Several studies have been demonstrating the antimicrobial effect of PDT *in vitro* against various microorganisms, including *Streptococcus* spp. (Melo et al., 2013). However, most of these experiments are limited to experimental conditions; therefore the present report encourages future studies involving PDT for microbiological control.

This experiment demonstrated the application of PDT in the treatment of an abscess caused by *Streptococcus* spp. in sheep, in order to encourage future research, since the procedure was considered responsive and no other studies were found about the subject.

Conclusion

PDT was shown to be a therapeutic option, showing great potential for clinical application. More studies are needed so that we can better understand its mechanisms and also their possible applications in Veterinary Medicine.

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