

PHOTODYNAMIC THERAPY IN CONSOLIDATION OF CARAPACE FRACTURE IN GREEN TURTLES (*Chelonia mydas*)

ABSTRACT

Sea turtles have great longevity and exhibit a complex life history that includes pronounced changes in their development. All sea turtles species are on the Red List of the International Union for Conservation of Nature/IUCN, classified as vulnerable, endangered or critically endangered. Among traumatic changes that affect turtles, there are the carapace and plastron fractures that can occur primarily as a result of attacks by predators, boat propellers, among others. The present study aimed to evaluate the effect of photodynamic therapy in consolidation of fracture in a species of green turtles (*Chelonia mydas*).

KEYWORDS: Green Turtle; *Chelonia mydas*; Fracture; Photodynamic Therapy.

A TERAPIA FOTODINÂMICA NA CONSOLIDAÇÃO DA FRATURA DA CARAPAÇA DAS TARTARUGAS VERDES (*Chelonia mydas*)

RESUMO

As tartarugas marinhas têm grande longevidade e apresentam uma história de vida complexo que inclui alterações pronunciadas no seu desenvolvimento. Todas as espécies de tartarugas marinhas estão na Lista Vermelha da União Internacional para a Conservação da Natureza / IUCN, classificados como vulneráveis?, ameaçadas ou criticamente ameaçadas de extinção. Entre as mudanças traumáticas que afetam tartarugas, existem os carapaça e plastron fraturas que podem ocorrer principalmente como resultado de ataques de predadores, hélices de javali, entre outros. O presente estudo teve como objetivo avaliar o efeito da terapia fotodinâmica na consolidação de fratura em uma espécie de tartarugas verdes (*Chelonia mydas*).

PALAVRAS-CHAVE: Tartaruga Verde; *Chelonia mydas*; Fratura; Terapia Fotodinâmica.

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INTRODUCTION

The sea turtles present great longevity and exhibit complex life history that includes pronounced changes in their development, both in habitats and dietary and extensive trans-ocean migrations (LAHANAS et al., 1998). As being species with slow growth and long life cycle, they become extremely susceptible to man action in all their life cycle stages. All species of sea turtles are on the Red List of IUCN, classified as vulnerable, endangered and critically endangered (IUCN, 2013).

Among the traumatic changes which affect the chelonians, fractures of carapaces and plastrons are highlighted, mainly caused by predators' bites, boats' propellers, among others (BARTEN, 2006). The hull of the turtles consists of two parts, a dorsal portion named carapace that covers the back of the animal, and a ventral portion flatter known as plastron, which involves its womb. The skin of these animals is ossified and the epidermis is modified in corneal tissue (DIVERS, 1996). The tegument, the skin and its annexes, presents distinct functions, such as defense against the invasion of micro-organisms, reducing the effects of solar radiation, mechanical protection and aid in thermoregulation and provide camouflage to animal (SOUZA, 2006). The hull acts as a body's natural barrier isolating the internal components of the external environment (KAPLAN, 2002) and when this barrier is partially or totally destroyed the survival of the animal can be compromised.

Photodynamic therapy (PDT-Photodynamic Therapy) is a therapeutic modality used for treatment of superficial tumors and infections sites that combines the use of photosensitizer (FS) and light in specific wavelength. This combination will promote the activation of the FS through the conduct of FS molecules to situations of great chemical instability, which are stabilized by transferring energy from the FS middle molecules. In the presence of oxygen, these reactions can lead to the formation of various free radicals, such as: singlet oxygen, superoxide radical ions, peroxides and hydroxyls, generating a cascade of reactive oxygen species (ROS). The release of ROS in fundamental cellular components changes promotes promoting cytotoxic effect on microorganisms (ARISTEO et al., 2009; MAISCH, 2007). In addition, light which is not absorbed by microorganisms can be scattered and absorbed by chromophores of the tissue, in this way the PDT may act as bio-stimulator agent, accelerating the process of tissue repair (LIMA et al., 2004). The present report aimed to assess the action of PDT in the consolidation of the fracture carapace in a species of green turtle (*Chelonia mydas*).

MATERIALS AND METHODS

A green turtle (*Chelonia mydas*) was examined in the Municipal Aquarium of Santos, SP, presenting a six-month fracture in carapace. The fracture was in third, fourth and fifth vertebral costal shields, fourth right costal shield and eleventh right marginal shield (Figure 1 and Figure 2). After the clinical diagnosis, the animal was exclusively treated with PDT joined with the topical use

of blue of methylene FS for five minutes (aqueous solution, concentration of 60 μ M), followed by irradiation with red laser 100 MW power coupled to optical fiber, wavelength (λ) of 660nm and energy density of 180 J/cm² per point (Figure 3). The treatments were performed with an interval of one application per week until the complete regression of the lesion. The injury was evaluated by descriptive analysis simple for a period of 30 days. The treated animal obtained re-epithelialization and keratinization after four competent treatments in the period of 28 days (Figure 4).



Figure 01: Green turtle presenting fracture.



Figure 02: The initial aspect of fracture in carapace.



Figure 3: Fracture irradiated by laser of diode acopled to optical fiber.

DISCUSSIONS

Several authors have described the ecological impacts from accidents in different parts worldwide (SCHROEDER, 1987; SCHROEDER e WARNER, 1988; ORÓS et al. 2005). Boat collision can be considered one of the main factors which cause fracture in marine turtles, and directly threaten all marine turtle species' survive, and due to the increase of ship and marine

traffic, those accidents tend to occur more intensively. In the event of fracture, the repair depends on factors such as extension of the lesion, age and physical condition of the patient. Recent injuries might be contaminated and not necessarily infected, yet inappropriate treatment of these injuries can cause an infectious process.

These traumas may mainly cause lung lesions, necrotic pneumonia, and most serious cases like provoking fibrinous celomitis, septicemia e death (ORÓS et. Al. 2005). Among the main materials described in the repair of the hull, the use of fiberglass, epoxy resin, polyester colored resins and dental acrylics (WALSH, 1999; KAPLAN, 2002). Although recommended, the use of resins has decreased, because, even stabilizing the lesion, can recover cell fragments and dirt and therefore inhibit recovery. In this way, resins should be removed to maximize recovery, promoting the calcification and normal repair of damaged carapace and, if the stabilization is essential, it must allow access to the lesion for debridement and cleaning (WALSH, 1999), being described the technique of applying sterile bandage transparent film of polyurethane (Tegaderm , 3 M Health care) (WALSH, 1999) and, in the case of single or multiple linear cracks, the use of stainless wire (KAPLAN, 2002).

In recent injuries, it is recommended the administration of antibiotics for at least a week or until the wound is healed (KAPLAN, 2002). According to BARTEN (2006), the use of topical antibiotic can be performed with the cleaning and occlusive bandage of injury, in addition to the systemic antibiotic. In this way, antimicrobial therapies reduce the risk of infection, becoming instrumental in the recovery of affected animals (BARTEN, 2006). Once the conditions for access to the lesion, allowing cleaning and debridement for the treatment, are preserved, the PDT becomes a therapeutic approach that allows, in addition to the promotion of decontamination, maintenance of necessary and fundamental requirements for recovery of the same during the treatments until complete recovery.

Currently, the development of resistance by certain pathogenic bacteria is faster than the ability of industry to produce new drugs (SOUZA, 1998). The use of PDT becomes a therapeutic modality that presents as main advantages the absence of systemic effects and microbial resistance, which are found in most usual treatments that involve the use of antibiotics (GARCEZ et al., 2008).

Among the main surveyed FS surveyed, there are the phenothiazines, like methylene blue. At low concentrations, it does not produce cytotoxic action and the required dose for bacterial death is less than the dose to cause damage to cells, such as the keratinocytes and fibroblasts (SOUKOS et al., 1996).

The present report found no adverse effects of the technique for the treated animal, of so the absence of works in world literature, along with the status of threatened species reinforces the interest for new research. Despite the methodology adopted in this experiment preclude the assessment of all the mechanisms involved, the descriptive analysis of the lesion showed positive development of the clinical frame by means therapy, thus indicating a new possible therapy for

fractures of carapaces in turtles. The researches involving the use of PDT in veterinary medicine have been discussed in recent years (PENG et al., 1996; LUCROY et al., 2000; BIRCHARD and SHERDING, 2003; WITHROW and MACEWEN CRITERIA, 1999; LUCROY, 2002; OSAKI et al., 2012) with great perspective to its clinical application in the near future. The present study proved to be a pioneer, since there are other data referring to the theme in world literature. Further studies should be performed in order to elucidate the mechanisms involved in the repair of fracture of carapaces in turtles by PDT.

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