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Light emitting diode (LED) therapy reduces local pathological changes induced by *Bothrops asper* snake venom

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ABSTRACT

The therapeutic effect of the Light Emitting Diode (LED) treatment in two wavelengths (635 or 945 nm) was evaluated in the local pathological alterations induced by *Bothrops asper* snake venom. Mice received irradiation of infrared LED (120 mW, 945 nm) or red LED (110 mW, 635 nm) applied immediately, 1 and 2 h after venom injection. LED treatment reduced edema formation in the plantar region and gastrocnemius muscle and significantly reduced neutrophil migration and hyperalgesia after the venom injection. Also, both infrared LED and red LED treatment significantly reduced myonecrosis, as revealed by muscle CK and plasma CK levels. Histological analysis corroborated the reduction in the extent of venom-induced myonecrosis. In conclusion, our data demonstrates that PBM with LED light in both red and infrared wavelengths, when applied after envenomation in mice, reduces the extent of myotoxicity, edema, inflammatory infiltrate and hyperalgesia, suggesting that photobiomodulation is a potential therapeutic approach that should be further investigated for the treatment of local effects of *Bothrops* snakebite.

1. Introduction

The majority of snakebites in Latin America are inflicted by species of the genus *Bothrops*. The snake *Bothrops asper* is responsible for 50–80% of snakebite accidents and 60–90% of deaths secondary to snakebites in Central America and in some regions of Mexico, Ecuador, Venezuela and Colombia (Gutiérrez, 2010; Herrera et al., 2016; Otero-Patiño, 2009; Saldarriaga-Córdoba et al., 2017). In untreated cases, or when antivenom administration is delayed, local necrosis frequently occurs and may lead to permanent tissue damage and, in some cases, leads to amputation (Jorge et al., 1999; Saborío et al., 1998). Additionally, victims of *Bothrops asper* snakebite suffer systemic effects of envenomation, which are responsible for fatalities in severe envenomations (Otero-Patiño, 2009).

The local effects caused by *B. asper* snakebites are characterized by intense reactions that include edema, pain, local hemorrhage, blisters, dermonecrosis and myonecrosis (Chacur et al., 2001; Gutiérrez et al., 2009a; Zamuner et al., 2001). In addition, systemic manifestations in severe cases are associated with thrombocytopenia, platelet hypoaggregation, disseminated intravascular coagulation, cardiovascular shock and kidney injury (Rucavado et al., 2005; Chugh et al., 1975; Gutiérrez et al., 2009b). The mainstay in the treatment of *Bothrops* sp. snakebite envenomation is the parenteral administration of antivenom therapy, which is highly efficient in neutralizing the systemic effects, but has been shown to be partially ineffective in the neutralization of local pathological and inflammatory reactions (Camey et al., 2002; Zamunér et al., 2004; Gutiérrez et al., 1998). In order to circumvent the limitations of antivenom in the neutralization of local effects, various therapeutic alternatives have been explored at the experimental level, such as the use of natural and synthetic inhibitors of venom metalloproteinases (SVMPs) and phospholipases A_2 (PLA_2s) (Rucavado et al., 2000, Lizano et al., 2003, Rostelato-Ferreira et al., 2010, Tribuiani et al., 2017). In addition, photobiomodulation therapy (PBMT) has been explored for it is potential to inhibit local pathological effects of

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venoms (Barbosa et al., 2008; Doin-Silva et al., 2009; Dourado et al., 2003; Nadur-Andrade et al., 2012).

PBMT is a medical technique in which exposures to low level lasers (LLL) or light emitting diode (LED) stimulate cellular function leading to beneficial clinical effects. Several studies have suggested that PBMT could be an effective device for wound healing, anti-inflammation and pain-killing as well as for promoting the processes of regeneration and angiogenesis in many clinical disorders (de Oliveira Melo et al., 2016; Magri et al., 2017, Ruaro et al., 2014). Thus, PBMT may be a significant therapeutic tool in reducing the local effects caused by *Bothrops* sp venom.

Previous studies have demonstrated that LLL treatment decreased considerably the extent of myonecrosis induced by *Bothrops moojeni* (Dourado et al., 2003) and *B. jararacussu* venoms (Barbosa et al., 2009). In addition, LLL treatment also prevented edema formation and leukocyte influx in muscle and paw of mice injected with *Bothrops* venom (Aranha de Sousa et al., 2013, Barbosa et al., 2008, Nadur-Andrade et al., 2012, Souza et al., 2011). Moreover, the anti-edematogenic effect caused by LLL treatment, after *B. jararacussu* venom injection in gastrocnemius muscle, is enhanced when antivenom and LLL were used in association (Barbosa et al., 2009). Furthermore, LLL treatment was effective in reducing the hemorrhage and hyperalgesia induced by *B. moojeni* venom (Aranha de Sousa et al., 2013; Nadur-Andrade et al., 2012, Souza et al., 2011). Additionally, Nadur-Andrade et al., (2014a,b) showed that LED treatment was effective in the reduction of edema, hyperalgesia and hemorrhage induced by *B. moojeni* venom.

Owing to the medical relevance of envenomations by *B. asper* in Central America and parts of Mexico and South America, a comprehensive analysis of the effect of LED treatment on the outcome of *B. asper* venom-induced local pathological reaction was performed. In addition, we compared the near infrared LED and red LED to identify which is the best for envenomation treatment.

2. Materials and methods

2.1. Animal

All animal care was in accordance with the guidelines of the Brazilian College for Animal Experimentation and was approved by the Committee for Ethics in Animal Research of the University of the Vale do Paraíba (UNIVAP) under number A118/2007/CEP. Experiments were performed using 45-day-old male Swiss mice (22–25 g), randomly divided into groups of five animals each. Animals were kept in plastic cages with water and food ad libitum, maintained under controlled temperatures (20–22 °C) and on a 12 h light/dark cycle.

2.2. Venom

Lyophilized crude *Bothrops asper* venom (Bav) was supplied by Instituto Clodomiro Picado, San José, Costa Rica. It is a pool obtained from more than 40 adult specimens collected in the Pacific versant of this country. The venoms were dissolved in 0.9% saline solution only at the moment of its use.

2.3. LED irradiation

A 635 nm red and 945 nm infrared LED devices (Super Bright LEDs, Inc., St. Louis, MO, USA) were employed to irradiate animals. The red LED parameters were 110 mW of power, 44 s irradiation time, and 1.2 cm^2 irradiated area, which corresponded to an irradiation dose of 4 J/cm^2. The infrared parameters were 120 mW of power, 40 s irradiation time, and 1.2 cm^2 irradiated area, which corresponded to an irradiation dose of 4 J/cm^2. Animals were irradiated at 0, 1, and 2 h after Bav injection. The LED irradiation angle was kept perpendicular to the skin surface so that only the point of venom injection was irradiated. The LED dose, low enough to avoid any thermal effect, was chosen on the basis of studies reported in the literature that had shown a beneficial effect of the LED therapy on venom-induced local effects (Nadur-Andrade et al., 2012, 2014a; 2014b).

2.4. Irradiated Bothrops asper venom (iBav)

To verify whether the LED irradiation could change venom toxicity, the lyophilized venom of Bav was diluted in saline solution and irradiated immediately before the injection of the animals, using the same parameters used to irradiate animals. The rationale of this experiment was to elucidate if the LED light can modify the biological activities of the venom.

2.5. Evaluation of paw edema

The ability of the LED to reduce paw edema was studied in mice. To that end, 50 µL of sterile saline 0.9% (w/v) containing Bav or iBav (2.5 µg/paw) were injected in the subplantar region of the right hind paw. The left paw received an equal volume of sterile saline alone and served as control. The volumes of both hind paws were measured plethysmographically (model 7140 plethysmometer, Ugo Basile, Italy) before and at 30 min, 1, 2, 4 and 6 h after venom administration. The edemathogenic effect was calculated as the difference between both paws and expressed as % increase in the volume of venom-injected paw.

2.6. Evaluation of muscle edema

To measure edema in muscle, animals received an i.m. injection of Bav or iBav (50 µg/50 µL) in the central part of the right gastrocnemius muscle and the same volume of apyrogenic saline solution in the contralateral muscle. After 3 and 24 h mice were euthanized and their gastrocnemius muscles were dissected out. Both muscles were weighed, and the edema was expressed as the percentage of the weight increase of the venom injected muscle as compared to the contralateral muscle (Barbosa et al., 2009).

2.7. Leukocyte harvesting and counting

The leukocyte migration into the peritoneal cavity was evaluated 6 h after injection of Bav or iBav (5 µg/cavity dissolved in a volume of 500 µL apyrogenic saline solution) [8]. Leukocytes were harvested 6 h after venom injection by washing the cavity with 2 mL of saline containing heparin (10 U/mL). Aliquots of the washes were collected and used to determine the total cell counts in a Neubauer chamber after dilution (1:20 v/v) in Turk solution (0.2% crystal violet staining in 3% acetic acid). For differential cell counts, cytospin preparations were stained with Instant Prov stain. Differential cell counts were performed by counting at least 100 cells, which were classified as either polymorphonuclear (PMN) or mononuclear (MN) cells, based on conventional morphological criteria.

2.8. Mechanical hyperalgesia

Testing for mechanical sensitivity (von Frey filaments Touch-Test® Sensory Evaluators -North Coast Medical) was based on the method of Chaplan et al. (1994). Mice were placed individually in plastic cages with a wire bottom, which were in contact with the paws. Filaments were applied to the plantar region of the left hind paw of each animal for 5 s. To reduce stress, mice were habituated to the experimental environment one day before the first measurement. Animals were injected with 2.5 µg of crude Bav or iBav diluted in 50 µL of sterile saline into the subplantar surface of the right hind paw. Control group animals received the same volume of sterile saline. The contralateral paw was not injected. The pain threshold was measured at 1, 3, 6 and 24 h after venom or saline injection.