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Influence of thyroid states on the local effects induced by *Bothrops* envenoming

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ABSTRACT

Bothrops leucurus venom causes significant local effects, such as necrosis, pain, hemorrhage and edema. These effects are important because of their high frequency and severity. The treatment of these local effects is not simple because of their quick triggering and a variety of components that induce these effects. Myonecrosis, dermonecrosis and edema are primarily caused by the action of hemorrhagins and myotoxins. A number of investigators have demonstrated the influence of thyroid hormones on inflammatory processes, particularly on wound healing. We investigated the edematogenic, hemorrhagic and necrotic activity of the *B. leucurus* venom in the hypothyroid, hyperthyroid and euthyroid of rats. The CK (creatine kinase) plasma level decreased in the animals in a hypothyroid state. The hypothyroid condition also significantly reduced the hemorrhagic and dermonecrotic area compared to the euthyroidism and hyperthyroidism states. It also mitigated the rat paw edema compared to that found in the euthyroid and hyperthyroid animals. The hyperthyroid animals showed no significant differences in the three treatments compared to the euthyroid animals. Our results suggest that the triggering of local effects induced by envenomation by *B. leucurus* is attenuated in hypothyroid animals, possibly by the effect of hypothyroidism on the immune system and blood flow.

1. Introduction

Snakebite envenomation is a remarkable public health problem in tropical countries. *Bothrops* species are responsible for more than 90% of the snakebites reported in Brazil. In the Northeast, *Bothrops leucurus* is the main cause of ophidic accidents. These envenomations are characterized by severe local tissue damage, such as necrosis, hemorrhage, neutrophil migration and edema with high occurrence, severity and rapid development (Lira-da-Silva, 2009).

The local manifestations induced by snakebite are characterized by highly complex pathophysiological phenomena and involve the combined actions of a variety of venom components, such as myotoxins, phospholipases A2 and hemorrhagic metalloproteinases. Their action culminates in the appearance of wound, pain and an early and progressive edema, which reaches the entire affected limb. In addition, there are blisters, ecchymosis, necrosis, and enlarged lymph nodes (Mise et al., 2007). The administration of antivenoms is the major support for the treatment of ophidic accidents. Despite its high effectiveness in the neutralization of systemic alterations, the neutralization of local effects is difficult. As a consequence of envenoming, a large number of victims survive with permanent sequelae, such as tissue loss that may require amputation (Homma and Tu, 1971; Gutiérrez et al., 1981, 1998; Lira-da-Silva, 2001; Warrell, 2010).

Both hypothyroidism and hyperthyroidism cause problems in a large percentage of the population worldwide, affecting approximately 300 million people (Smyth, 2009). Thyroid hormones modulate important mechanisms for controlling body energy expenditure, e.g., by playing important roles in the regulation of mitochondrial oxygen consumption, thereby acting on the control of the basal metabolic rate (Brent, 1994; Yen, 2001; Kim, 2008). Thyroid hormones significantly depress immune responses via their action on the synthesis, storage, release and degradation of other hormones, cytokines and neurotransmitters, which regulate inflammatory reactions (Rodriguez et al., 2003). There are clear

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examples of the existence of bidirectional integration between thyroid hormones and the inflammatory mediators. For example, T3 receptors are present on the surface of granulocytes, mast cells and other cells of the immune system, and there is increased production and release of cytokines in the presence of physiological concentrations of T4 (Duarte et al., 2000; Csaba and Pállinger, 2009). The relationship between thyroid hormones and wound healing is also well known (Natori et al., 1999; Ekmektzoglouw et al., 2006; Safer et al., 2013). Thyroid hormones stimulate epidermal proliferation, which is a stage of the wound healing process; therefore, abnormal serum thyroid hormone, as observed in hyperthyroidism and hypothyroidism, also affect wound healing (Safer et al., 2013). There are no reports that investigate the influence of endocrine dysfunction on the development of snakebite symptoms, but some that refer to the effect of some snake venoms on the pituitary gland (Wolff, 2013). The objective of the present study was to investigate the development of local effects in hypothyroid, euthyroid and hyperthyroid rats after the injection of *B. leucurus* venom to determine whether the change of serum thyroid hormone alters the local effects induced by this venom.

2. Materials and methods

2.1. Animals and venom

Adult male Wistar rats weighting 180–220 g were housed in conventional plastic cages with a controlled light period (12:12) at 24 °C with free access to food and water. Pooled *B. leucurus* venom was obtained from adults specimens captured in the Salvador Metropolitan Area provided by the Regional Center for Ophydiology and Venomous Animals of Bahia (NOAP-UFBA). The samples were obtained by manual extraction, dried under vacuum and immediately stored at −20 °C until used. The experiments were approved by the Experimental Animals Committee of the Institute of Health Sciences (CEUA-ICS; Protocol number 033/2012) in agreement with the recommendations of the National Council for the Control of Animal Experimentation of Brazil (CONCEA) and the International Guiding Principles for Biomedical Research Involving Animals of the Council of International Organizations of Medical Sciences (CIOMS).

2.2. Hypothyroidism

Hypothyroidism was induced by adding 0.05 g/100 mL PTU (6-n-propyl-2-thiouracyl; Sigma Chemicals, St. Louis, MO) to the drinking water for 4 weeks as described by Ghenimi et al. (2010). Exposure to this regimen of PTU is sufficient to induce short-term hypothyroidism and a significant and effective T4 deficit, and it is even more effective than the other effective doses presented in the literature (Sawin et al., 1998; Gilbert, 2011; Afzal et al., 2014). The control group received filtered water.

2.3. Hyperthyroidism

Experimental hyperthyroidism was induced in animals for 7 days by daily subcutaneous injections (300 μg/kg) of L-thyroxine (T4; Sigma Chemical Company, St. Louis, MO, USA), resulting in a thyrotoxic state (Basset et al., 2000; Rastogi et al., 2008; Ribeiro et al., 2012). The control animals received daily subcutaneous injections of saline for 7 days.

2.4. Serum hormone levels

Blood samples were collected from anesthetized rats via jugular puncture and were immediately separated by centrifugation at 3000 rpm/min for 10 min. Serum concentrations of T4 and T3 were determined by chemiluminescence immunoassay using the Architect kit. The assay procedures were followed as instructed in the kit manual. The detection limit of the assay was 0.25 ng/mL for T3 and 1 pg/dL for T4.

2.5. Edema

To induce paw edema, the animals were injected with 100 μL saline solution containing *B. leucurus* venom (50 μg/paw) into the subplantar region of the left footpad. The control group received the same volume of sterile saline as described. The volume increase of the paw (edema) was measured with digital caliper at several intervals (before venom injection and at 15 min, 30 min, 60 min, 120 min, 180 min, 6 h and 24 h after venom administration). Edema was expressed as the percentage increase above the initial paw volume.

2.6. Myotoxicity

The myotoxic activity was performed using the serum creatine kinase (CK) level as a quantitative marker of myotoxicity as previously described by Gutiérrez et al. (1980). The rats received an intramuscular (i.m.) injection of 50 μg of *B. leucurus* venom (50 μL) in the right gastrocnemius muscle. The control group received injections of sterile saline solution by the same route and volume. Three hours later, the animals were anesthetized i. p. with xylazine (14 mg/kg) and ketamine (100 mg/kg), and the blood (1 mL) was collected. After centrifugation, the enzyme CK activity was determined using a commercial kit (CK-NAC; Doles Chemical Industry, Goiania-GO, Brazil) according to the manufacturer's protocol. The CK activity was expressed in units/L.

2.7. Hemorrhage

The effect of thyroid states on hemorrhage induced by *B. leucurus* venoms was assessed by measuring the formation of the hemorrhagic halos into dorsal skin. The assay was performed according to the rodent intradermal method described by Theakston and Reid (1983). Groups of rats received intradermally (i.d.) *B. leucurus* venom (300 μg/application site) diluted in 100 μL of sterile saline solution at room temperature into their previously shaved dorsum skin. After 3 h the animals were killed with using an overdose of thiopental sodium, the dorsal skin was removed and the axis of the hemorrhagic halos were measured on the inner surface of the skin in two directions at right angles using a caliper (cm^2).

2.8. Dermonecrosis

This activity was determined using the rodent intradermal method (Theakston and Reid, 1983). Briefly, aliquots of 100 μL of saline solution, pH 7.2, containing 300 μg of *B. leucurus* venom were injected into the shaved dorsal skin of the rats. After 72 h the animals were killed with using an overdose of thiopental sodium, the dorsal skin was removed and the diameter of the necrotic lesion was measured on the inner surface of the skin in two directions at right angles using a caliper (mm^2).

2.9. Statistical analysis

The results were expressed as the mean ± S.D. The statistical significance was determined using the Kruskal–Wallis test for non-parametric measures followed by the Dunn post-test for multiple comparisons. Probability values (p) lower than 0.05 were