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Dexamethasone antagonizes the *in vivo* myotoxic and inflammatory effects of *Bothrops* venoms

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ABSTRACT

In the present work we investigated the toxic activities of two *Bothrops* snake venoms using *in vivo* and *in vitro* experimental protocols in mice and tested the protective effect of dexamethasone (DEXA) in different conditions, comparing it with the polyvalent anti-venom. We also expanded the investigations on the antiophidic effect of the *Eclipta prostrata* (EP) crude extract.

The administration of *Bothrops jararaca* and *Bothrops jararacussu* snake venoms induced muscle damage demonstrated *in vivo* by the elevation on plasma creatine kinase (CK) activity in mice and by the decrease in CK content in the *extensor digitorum longus* (EDL) muscle of these animals, and *in vitro* by the increase in the rate of CK release from the isolated EDL muscle. We also observed inflammatory response following perimuscular injection of *B. jararacussu* venom (1.0 mg/kg). Treatment with DEXA (1.0 mg/kg) preserved over 50% of the EDL muscle CK content *in vivo* when evaluated 24 and 72 h after the injection of *B. jararacussu* venom in mice, and likewise reduced about 20% of the edema induced by this venom. DEXA reduced in 50% the presence of inflammatory cells and their activity in EDL muscle. The EP extract (50 mg/kg) showed similar ability in preventing the induction of edema and the decrease in muscle CK content, and its association with DEXA showed additive effect. EP reduced over 77% of the plasma CK activity induced by the *B. jararacussu* venom. In the *in vitro* experiments, DEXA was not able to change the rate of CK release from EDL muscles exposed to 25 μg/mL of *B. jararacussu* venom, neither to prevent the fall in the amplitude of the indirectly evoked twitch at the phrenic-diaphragm preparation. EP extract showed otherwise a protective effect on these protocols, reaching up to 100% of protection when concentrations of 50.0 and 100.0 μg/mL were used.

Altogether our results show that inflammation is at least in part responsible for the tissue damage induced by *Bothrops* snake venoms, once the steroidal anti-inflammatory drug dexamethasone was able to decrease the myotoxic effects of these venoms, by reducing the inflammatory response to the venom injection.

1. Introduction

Envenomation induced by snakebites occurs in many countries around the world, and although it has been

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present since the human being started reporting the history, it was not until recently that they have been considered a public health problem (Williams et al., 2010; Gutierrez, 2012). Despite being globally neglected, the relevance of snakebite envenoming is due to the great incidence, morbidity and mortality, which is estimated to be around 85,000 deaths per year affecting mainly the poor rural inhabitants (Chippaux, 1998; Gutierrez et al., 2010; Gutierrez, 2012). In the American continent, especially in Brazil, the majority of these accidents is caused by *Bothrops* genus snakes, which induce prompt local injury characterized by hemorrhage, myonecrosis and edema (Kamiguti et al., 1986; Sanchez et al., 1992; Moreno et al., 2005; Gutierrez et al., 2007; Santoro et al., 2008). These different tissue responses have been investigated under different *in vitro* and *in vivo* models in order to understand the local cytotoxicity and the systemic effects of the complex mixture of snake venoms (Gutierrez et al., 1986; Sanchez et al., 1992; Melo et al., 1993; Melo and Ownby, 1999; Murakami et al., 2005; Teixeira et al., 2009; Escalante et al., 2011).

The recommended therapy to snakebite envenomation has been based on the administration of animal-derived antivenom that can ameliorate and stop many of the venom effects (da Silva et al., 2007; Gutierrez et al., 2007, 2011a,b). However, the local response induced by *Bothrops* snake venoms is described as being only partially neutralized by either the specific or the polyvalent antivenom even if the antivenom is locally injected (Chaves et al., 2003; da Silva et al., 2007; Gutierrez et al., 2007, 2011a). The problem is bigger when the therapy is delayed for many different reasons, such as geographical problems or lack of accessibility to the antivenom (Chippaux, 1998; Pardal et al., 2004; Gutierrez et al., 2007). In many rural areas in Brazil or elsewhere in the world where the antivenom is not easily available, local people use folk medicine such herbal preparations in the snakebite treatment, trying to interrupt the venom effects (Martz, 1992; Mors et al., 2000; Coe and Anderson, 2005). When it is available, the use of antivenom can still elicit different reactions once they are animal-derived products.

The local venom effects are poorly understood, and although many studies have been trying to develop new substances able to stop or antagonize the powerful local inflammatory response induced by *Bothrops* venoms, which involves cytokines and white blood cells, it is still a challenge (Lomonte et al., 1993; Olivo et al., 2007; Gutierrez et al., 2007; Melo et al., 2010). It has been difficult to develop new drugs for snakebite envenoming treatment, either from plants or from new planed synthetic molecules, because they are not attractive to developed countries nor to big companies once they will not return the investment and the endeavor (Gutierrez et al., 2007; Lomonte et al., 2009).

The local myonecrosis and inflammatory response are critical to late disabilities (Gutierrez et al., 1986; Rucavado and Lomonte, 1996; Teixeira et al., 2009), but even the well-known substances used for the treatment of allergic reactions induced by antivenom treatment are not frequently investigated for their anti-inflammatory activities (Chen et al., 2007; Olivo et al., 2007; Thiansookon and Rojnuckarin, 2008; Nascimento et al., 2010). Nascimento et al. (2010) described that dexamethasone decreased the acute inflammatory response induced by *Bothrops moojeni* in mice, and this observation is ascribed to the ability of dexamethasone to decrease the formation of eicosanoids in the presence of the venom. Dexamethasone is a corticosteroid widely used in medical practice that has not been investigated regarding its ability to preserve muscle tissue exposed to *Bothrops* venoms in mice, although its ability to interfere with prostaglandin synthesis has been largely investigated (Goppelt-Struebe, 1997).

In this investigation we have tested the myotoxic and edematogenic effects of *Bothrops jararaca* and *Bothrops jararacussu* venom in mice under different *in vitro* and *in vivo* approaches, and the anti-inflammatory and anti-myotoxic effects of dexamethasone.

2. Material and methods

Male Swiss mice (25.0 ± 1.0 g) used for the study received water and food *ad libitum* and were kept under a natural light cycle. Euthanasia and all the procedures that could cause pain were performed under diethyl-ether anesthesia according to protocols approved by the Ethics Committee for the Use of Animals of the Federal University of Rio de Janeiro (CEUA-UFRJ). *B. jararaca* and *B. jararacussu* venoms, and polyvalent antivenom (PAV) serum were obtained from Instituto Vital Brasil, Rio de Janeiro, Brazil; dexamethasone was obtained from Hypofarma, Brazil; dry ethanolic extract of *Eclipta prostrata* was prepared as previously described (Mors et al., 1989; Melo et al., 1994) and fresh solutions were made from the lyophilized plant prior to each experiment; creatine kinase (CK) activity was determined using a CK NAC® kit from BIOCLIN, Brazil; hexadecyltrimethylammonium bromide (HTAB) and O-dianisidine dihydrochloride were purchased from Sigma–Aldrich Co, USA.

2.1. In vivo *experiments*

Perimuscular injections of *B. jararaca* and *B. jararacussu* venoms (1.0 mg/kg), dissolved in PSS to final volume 50 μL, were performed in mice at their legs over the *extensor digitorum longus* (EDL) muscle, not directly into the muscle, but under the *tibialis anterior* muscle and next to the tibia, close to the external surface of EDL muscle, in order not to cause mechanical damage to this muscle, as previously described (Melo and Ownby, 1999; Calil-Elias et al., 2002). Negative controls consisted of mice injected with the same volume of physiological saline solution (PSS) composed of (mM): NaCl, 135; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; $NaHPO_4$, 1; $NaHCO_3$, 15; and dextrose, 11. The pH of this solution was equilibrated to 7.3 with 5% CO_2/95% O_2. Treatment groups consisted of: intraperitoneal dexamethasone (1.0 mg/kg) in a final volume of 100 μL, injected simultaneously with the venoms; *E. prostrata* (50.0 mg/kg) pre-incubated with the venom for 15 min (Melo et al., 1994) prior to perimuscular injection; and the association of DEXA and EP protocols. We also used intravenous PAV (0.2 mL/mg of venom, once each milliliter of PAV is ascribed to neutralize 2.5–5.0 mg of the *Bothrops* crude venoms according to the producers' recommendations) injected simultaneously with the venoms. Finally, PAV was associated with DEXA in protocols that

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