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Comparative analysis of newborn and adult *Bothrops jararaca* snake venoms

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ABSTRACT

Different clinical manifestations have been reported to occur in patients bitten by newborn and adult *Bothrops jararaca* snakes. Herein, we studied the chemical composition and biological activities of *B. jararaca* venoms and their immunoneutralization by commercial antivenin at these ontogenetic stages. Important differences in protein profiles were noticed both in SDS-PAGE and two-dimensional electrophoresis. Newborn venom showed lower proteolytic activity on collagen and fibrinogen, diminished hemorrhagic activity in mouse skin and hind paws, and lower edematogenic, ADPase and 5′-nucleotidase activities. However, newborn snake venom showed higher L-amino oxidase, hyaluronidase, platelet aggregating, procoagulant and protein C activating activities. The adult venom is more lethal to mice than the newborn venom. *In vitro* and *in vivo* immunoneutralization tests showed that commercial *Bothrops* sp antivenin is less effective at neutralizing newborn venoms. These findings indicate remarkable differences in biological activities of *B. jararaca* venom over its development. We suggest that not only venom from adult specimens, but also from specimens at other ontogenetic stages should be included in the venom pool used for raising antibodies. Thus, *Bothrops* antivenin can efficaciously neutralize proteins lacking in the adult venom pool, especially those that promote more intense hemostatic disturbances in victims of newborn snakes.

1. Introduction

The jararaca (*Bothrops jararaca*)- is the main agent inflicting snake bites in São Paulo State, Brazil (Ribeiro and Jorge, 1997). This snake is found in southeastern South America, from Bahia State in Brazil to northern Argentina. In such vast area, they inhabit a number of habitats, such as Atlantic forest, semidecidous broadleaf forests, scrubs, cultivated fields, open areas and even in large cities (Sazima, 1992). The mean length of adult *B. jararaca* is 1 m long, but they may reach up to 1.6 m. Litter sizes vary from 5 to 22 neonates, showing a mean of 14. Births usually occur from February to March (Sazima, 1992; Campbell and Lamar, 2004), and the length of newborns is around 20 cm (Melgarejo, 2003). Feeding habits of *B. jararaca* shift during its growth: on the one hand, frogs are the main prey item (75%) for neonates, but birds, lizards, centipedes and small rodents have also been reported; on the other hand, adult snakes preferentially prey rodents (80%), but also lizards, frogs and birds (Sazima, 1991, 1992; Campbell and Lamar, 2004). Noteworthy, in newborn and juveniles of *B. jararaca* the tail tip is usually whitish or yellowish (Fig. 1), and as snakes change their diet to endotherms the tail tip becomes suffused with darker color, and identical to the remaining color pattern. In fact, neonates use the white

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Fig. 1. Specimen of newborn *Bothrops jararaca* snake, depicting an evident whitish tail tip.

tip tail to lure ectothermic preys (Sazima, 1991; Andrade et al., 1996).

Snakebites are a serious health problem, especially in Latin America, Africa and Asia (Warrell, 2010). Victims of bites by *B. jararaca* usually manifest local effects at the site of the bite (edema, ecchymoses, compartmental syndrome, blisters and necrosis) as well as systemic signs of envenomation, such as spontaneous bleeding (gingival bleeding, hematuria and epistaxis) and blood incoagulability (Santoro et al., 2008). Such clinical manifestations have been attributed to the activity of proteins and enzymes found in *B. jararaca* venom, as well as to pharmacological mediators engendered by them (Gutiérrez and Lomonte, 2003; Sano-Martins and Santoro, 2003).

Various toxins contribute to the development of the local inflammatory response evoked by *Bothrops* venoms: bradykinin-releasing enzymes (Vargaftig et al., 1974), lectins (Lomonte et al., 1990; Panunto et al., 2006), phospholipases A_2 (PLA_2) (Soares et al., 2001; Kanashiro et al., 2002; Ketelhut et al., 2003; Rodrigues et al., 2007), serine proteinases (Pérez et al., 2007), metalloproteinases (Gutiérrez et al., 1995; Gutiérrez and Rucavado, 2000; Rodrigues et al., 2001; Zychar et al., 2010) and L-amino oxidases (Stábeli et al., 2004, 2007; Izidoro et al., 2006). Hyaluronidases may also contribute indirectly to the exacerbation of the local reaction, by hydrolyzing hyaluronic acid present in the connective tissue, and potentiating thereby the diffusion and absorption of venom components to the blood stream (Girish and Kemparaju, 2006).

Hemostatic dysfunction evoked by *B. jararaca* venom is a complex phenomenon, eventually leading to consumption of blood coagulation factors and circulating platelets, platelet dysfunction, and secondary activation of fibrinolysis (Maruyama et al., 1990; Santoro et al., 1994, 2008; Santoro and Sano-Martins, 2004). Several *Bothrops* toxins take part on hemostatic dysfunction, e.g., thrombin-like enzymes, factor X and prothrombin activators, toxins that stimulate or inhibit platelet function, and hemorrhagins (Nahas et al., 1979; Maruyama et al., 1990, 1992; Kamiguti et al., 1991; Kamiguti et al., 1994; Santoro et al., 1994; Sano-Martins et al., 1997; Sano-Martins and Santoro, 2003; Santoro and Sano-Martins, 2004; Gutiérrez et al., 2005; Rucavado et al., 2005). In addition, nucleotidases present in *B. jararaca* venom, such as phosphodiesterases and 5′-nucleotidases (Sales and Santoro, 2008; Santoro et al., 2009) may disturb a plethora of physiological functions, including platelet function (Aird, 2002; Santoro et al., 2009) and contribute to local and systemic signs manifested by patients bitten by *B. jararaca*.

Several factors, such as seasonal variation, habitat, age, and sexual dimorphism are known to produce venom variation (Chippaux et al., 1991; Furtado et al., 2006; Menezes et al., 2006; Pimenta et al., 2007), although it is still controversial to which extent the diet influences the protein profile of snake venoms (Daltry et al., 1996; Sasa, 1999). In fact, intraspecies (geographical, seasonal and ontogenetic) variation in venom composition may account for the variability observed in severity and pattern of snakebite envenomation (for review see Warrell (1997)). Differences in the clinical picture and severity of people bitten by adult and young *B. jararaca* have been reported since the pioneer observations by Monteiro (1610), who noticed that patients bitten by "jararaca soatinga" (jararaca with white tail tip, Fig. 1) have intense bleeding and rarely survive. Later on, Casal (1817) reported that "(…) the jararaca, whose strain is the most abundant and fatal: the one with white tail tip is no more than one palm in length, and its venom does not have any known antidote: the being, whom it bit, soon manifests convulsions and bloody sweating, and expires in a short time". Such historic citations demonstrate that hemostatic disturbances are a conspicuous manifestation of the envenomation inflicted by young *B. jararaca*. At a much later time, Rosenfeld et al. (1959b) described that envenomation by young *B. jararaca* snakes did not elicit local signs of envenomation, but evoked blood incoagulability. In fact, a survey of clinical signs observed in patients bitten by *B. jararaca* revealed that a higher incidence of blood incoagulability and a lower frequency of necrosis, blisters and abscess were found in patients bitten by young snakes (Ribeiro and Jorge, 1989, 1990), which are in accordance with preliminary experimental evidence demonstrating discrepancies in coagulant and proteolytic activities of young and adult *B. jararaca* venoms (Rosenfeld et al., 1959a; Kamiguti, 1988; Furtado et al., 1991).

Ontogenetic variation in venom composition has been reported in a number of genera (Minton and Weinstein, 1986; Mackessy, 1988; Gutiérrez et al., 1990, 1991; Saravia et al., 2002), including *Bothrops* snakes (Gutiérrez et al., 1980; Kamiguti, 1988; Furtado et al., 1991; Chaves et al., 1992; López-Lozano et al., 2002; Saldarriaga et al., 2003; Guércio et al., 2006; Zelanis et al., 2007, 2010), but few studies showed in detail what are the changes occurring in the biological activities, chemical composition and immunoneutralization of *B. jararaca* venom during the passage from newborn to adult snakes. Herein, we addressed such question, taking into account a clinical point of view, by studying pooled venoms from newborn and adult *B. jararaca*. We show herein that ontogenetic variation occurs in venom during the development of *B. jararaca*, which are related to the clinical picture observed in patients bitten by either adult or young *B. jararaca* snakes, and that commercial *Bothrops* antivenin does not neutralize the newborn venom as efficiently as the adult one.