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Experimental pathology of local tissue damage induced by *Bothrops asper* snake venom

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ABSTRACT

Envenomations by *Bothrops asper* are often associated with complex and severe local pathological manifestations, including edema, blistering, dermonecrosis, myonecrosis and hemorrhage. The pathogenesis of these alterations has been investigated at the experimental level. These effects are mostly the consequence of the direct action of zinc-dependent metalloproteinases (SVMPs) and myotoxic phospholipases A_2 (PLA_2s). SVMPs induce hemorrhage, blistering, dermonecrosis and general extracellular matrix degradation, whereas PLA_2s induce myonecrosis and also affect lymphatic vessels. In addition, the prominent vascular alterations leading to hemorrhage and edema may contribute to ischemia and further tissue necrosis. The mechanisms of action of SVMPs and PLA_2s are discussed in detail in this review. Venom-induced tissue damage plays also a role in promoting bacterial infection. A prominent inflammatory reaction develops as a consequence of these local pathological alterations, with the synthesis and release of abundant mediators, resulting in edema and pain. However, whether inflammatory cells and mediators contribute to further tissue damage is not clear at present. Muscle tissue regeneration after venom-induced pathological effects is often impaired, thus resulting in permanent tissue loss and dysfunction. SVMP-induced microvessel damage is likely to be responsible of this poor regenerative outcome. Antivenoms are only partially effective in the neutralization of *B. asper*-induced local effects, and the search for novel toxin inhibitors represents a potential avenue for improving the treatment of this serious aspect of snakebite envenomation.

1. Envenomations by *Bothrops asper* are characterized by drastic local pathological effects

B. asper is responsible for the vast majority of snakebite envenomations in Central America and in some regions of northern South America (Bolaños, 1982; Gutiérrez, 1995; Otero et al., 2006). This is due to a number of factors, i.e. the widespread distribution of this species in low-land humid areas devoted to agricultural activities, the high capacity of this species to adapt to altered environments, such as agricultural fields and pastures (Savage, 2002; Solórzano, 2004), and the consequent close contact between *B. asper* and humans in rural areas. In addition, *B. asper* is able to deliver a relatively large volume of venom (Bolaños, 1972), thus being capable of provoking severe envenomations. The relevance of *B. asper* goes beyond human medicine, since it is also responsible for a great number of envenomations in domestic animals, particularly cattle, dogs and horses (Villalobos, 2008).

Envenomations by *B. asper* are characterized by a prominent and complex series of local pathological alterations, which appear rapidly after the bite at the anatomical site where venom is injected. Such effects include edema and pain, hemorrhage, myonecrosis, blistering, and dermonecrosis (Picado, 1931; Bolaños, 1982; Gutiérrez, 1995; Otero et al., 2002; Warrell, 2004). Furthermore, local infection by bacteria present in the mouth of the snake or in the skin of

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patients often complicates these cases (Otero et al., 2002), as described in other *Bothrops* sp. envenomations as well (Jorge et al., 1994). The pathogenesis of local pathology induced by *B. asper* venom has been the subject of many experimental studies that have clarified the mechanisms through which toxins present in this venom inflict such a drastic and complex pathological picture. The present work reviews this information and highlights some unsolved issues that demand further investigation.

2. Edema and pain

2.1. The synthesis and release of endogenous mediators play a central role in edema, hyperalgesia and allodynia

Local edema and pain appear very rapidly after injection of *B. asper* venom in experimental animals (Gutiérrez and Lomonte, 2003; Chaves et al., 1995; Chacur et al., 2001). The time-course of edema in rodent footpad assays greatly depends on the dose of venom injected. At low doses, edema peaks within the first hour and drops at later time intervals (Lomonte et al., 1993). However, when higher doses are administered, edema persists for more prolonged time intervals (Gutiérrez et al., 1980a; Lomonte et al., 1993; Chaves et al., 1995). This probably reflects different mechanisms in the genesis of fluid imbalance in the tissues which leads to edema. The pathophysiology of edema formation induced by *B. asper* venom is multifactorial. There is a direct damage on microvessels, i.e. capillaries and venules, by the action of hemorrhagic toxins (see Section 3), with the consequent extravasation. In addition, edema is mediated by the action of inflammatory mediators, released or synthesized in the course of envenomation, which induce increments in the permeability of microvessels (Gutiérrez and Lomonte, 2003; Teixeira et al., 2003a).

Pharmacological studies in mice and rats have identified a number of mediators involved in *B. asper* venom-induced footpad edema, such as prostaglandins, nitric oxide and others (Chaves et al., 1995, 2006; Olivo et al., 2007). This agrees with observations performed with other *Bothrops* sp. venoms (Bonta et al., 1979; Trebien and Calixto, 1989). Similarly, the pharmacological basis of hyperalgesia and allodynia induced by this venom has been investigated, and the involvement of various mediators, such as bradykinin and leucotrienes, evidences a multifactorial mechanism in the onset of pain (Chacur et al., 2001). Myotoxic phospholipases A_2 play a key role in *B. asper* venom-induced hyperalgesia (Chacur et al., 2003). The pharmacological mediation of edema and pain is covered in detail in another contribution in this volume (see Teixeira et al., this issue).

2.2. Pathological effects on lymphatic vessels and their possible role in the pathogenesis of edema

The observation that high doses of *B. asper* venom provoke a long-standing edema suggests that pathological, in addition to pharmacological, mechanisms may be at work in these conditions, i.e. that pathological alterations in the tissues and the vasculature are responsible for a prolonged interstitial fluid imbalance. It is possible that pathological damage to microvessels may be responsible for this phenomenon, since venom drastically affects the integrity of capillaries, venules and small arteries (Arce et al., 1991; Moreira et al., 1992). Recent findings suggest that a direct effect of the venom on collecting lymphatic vessels may be also involved. When *B. asper* venom is directly applied onto exposed mouse mesentery preparations and observed by intravital microscopy, there is a rapid and marked reduction in the lumen of collecting lymphatics, an effect associated with a halting in the flow of lymph in these vessels (Mora et al., 2008).

This effect on lymphatics was not reproduced when a hemorrhagic metalloproteinase or a coagulant serine proteinase was applied, thus evidencing that it is not the consequence of clotting of fibrinogen or extracellular matrix degradation. Instead, the effect is reproduced when a myotoxic phospholipase A_2 (PLA_2) homologue, myotoxin II, is applied (Mora et al., 2008). In agreement, the effect induced by crude *B. asper* venom was abrogated by fucoidan, a polysaccharide inhibitor of myotoxins, but not by the metalloproteinase inhibitor batimastat (Mora et al., 2008). Myotoxin II was shown to lyse smooth muscle cells in culture and it was suggested that the reduction in lymphatic vessel lumen is the result of smooth muscle cell membrane perturbation by myotoxins, with the consequent calcium influx and contraction of muscle cells of the collecting lymphatic wall (Mora et al., 2008). The functional consequences of this phenomenon are obvious, since an adequate lymph flow depends on the periodic contraction of the lymphangions associated with the opening and closure of the lymphatic valves (Schmid-Schönbein, 2006). Thus, if the vessel is permanently contracted, due to the action of venom myotoxic PLA_2s, the lymph flow is halted. Furthermore, if lymphatic smooth muscle cells are irreversibly damaged by the venom, as occurs in cell culture conditions, then the pumping of lymph would be permanently impaired. In agreement with these observations, fucoidan was highly effective in the inhibition of *B. asper* venom-induced mouse footpad edema (Mora et al., 2008).

Venom-induced vascular disturbances leading to edema may contribute to the overall pathophysiology of envenomation in two additional ways: (a) the fluid imbalance resultant from these alterations represents a significant displacement of fluid from the vascular compartment to the interstitial compartment, thus contributing to the hypovolemia and consequent hemodynamic alterations typical of severe envenomations by viperid snakes (Warrell, 1996, 2004). (b) The pronounced increment in interstitial fluid in some muscle compartments, such as the anterior tibial compartment, may result in an increment in intracompartmental pressure. When this pressure increases over 40 mm Hg, a compartmental syndrome may develop (Warrell, 1999), with the consequent effect in the perfusion to distal regions and the onset of ischemic damage (see Section 6.5).

3. Local hemorrhage

3.1. Zinc-dependent metalloproteinases are responsible for local hemorrhagic effects

B. asper venom, and the majority of viperid venoms, are rich sources of zinc-dependent proteinases, a group of