Blistering in Bothrops atrox envenomings: Evidence of antivenom and inflammatory factors in the bite site

Sarah N. C. Gimenes1; Jacqueline de A. G. Sachet2; Mônica Colombini1; Luciana A. Freitas-de-Sousa3; Hicholson N. dos S. Ibiapinã2; Allyson G. da Costa2; Monique F. Santana2; Jeong-Jin Park4; Nicholas E. Sherman5; Luiz C. de L. Ferreira3; Fan H. Wen6; Wuelton M. Monteiro2; Ana M. Moura da Silva1; Jay W. Fox4

1Laboratório de Imunopatologia, Instituto Butantan, São Paulo, SP, Brazil
2Escola Superior de Ciências da Saúde, Universidade do Estado do Amazonas, Manaus, AM, Brazil
3Departamento de Enfermagem e Pesquisa, Fundação de Dermatologia Alfredo da Matta, Manaus, AM, Brazil
4University of Virginia, Charlottesville, VA, USA

Introduction

In Brazil, the northern states present the highest prevalence of snakebite. Bothrops atrox is the species responsible for the majority of snakebites, which consists in an economic impact. The local symptoms (edema, necrosis, inflammation, tissue damage and blistering) are partially responsible for this impact. Inflammatory reactions play an important role in the onset of local tissue damage; and one of the major components of B. atrox venom responsible for blister formation are the snake venom metalloproteinases (SVMPs). SVMPs are responsible for hemorrhetic effect and is also correlated with ability to degrade ECM components. These fragments may potentially act as immunomodulator and Damage-Associated Molecular Patterns (DAMPs), increasing the inflammatory response. Currently, the use of antivenom is recognized as the best approach to systemic effects. However, is not particularly effective in preventing the initial damage triggered by the venom toxins, and consequently we observed the worsening of tissue damage at the site of bite.

Aims

The examination of blister fluids as a window, which could provide important information about the pathophysiological origin of blister formation, considering its biochemical composition. Moreover, to investigate the possible reasons of limited protective efficiency of antivenom to be able to treat the local damage in B. atrox envenoming.

Results

Five patients were attended at Tropical Medicine Hospital, Manaus, Brazil (CAAE: 53192516.8.0000.0065). They were classified according to the clinical data: moderate (patients 1, 2 and 3) and severe (patients 4 and 5) accident. The clinical data provide insights into the local symptoms experienced in this patient cohort. The presence of moderate to severe edema and elevated levels of LDH (=190U/L), which is a marker indicative of tissue damage, was observed during the whole time of hospitalization (Table 1). The histological examination of tissue at the local site corroborated the clinical data, showing an acute inflammatory and hemorrhage in the area around the snakebite (Figure 1).

Table 1: Clinical data of patient whose blister fluid.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Severity</th>
<th>LDH (U/L)</th>
<th>Time 0 hrs</th>
<th>Time 2 hrs</th>
<th>Time 4 hrs</th>
<th>Time 6 hrs</th>
<th>Time 24 hrs</th>
<th>Time 48 hrs</th>
<th>Time 72 hrs</th>
<th>Time 144 hrs</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>M</td>
<td>Moderate</td>
<td>190</td>
<td>88.5</td>
<td>104.5</td>
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<td>2</td>
<td>40</td>
<td>F</td>
<td>Moderate</td>
<td>190</td>
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<tr>
<td>3</td>
<td>25</td>
<td>M</td>
<td>Moderate</td>
<td>190</td>
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<tr>
<td>4</td>
<td>50</td>
<td>M</td>
<td>Severe</td>
<td>190</td>
<td>88.5</td>
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</table>

The blister composition was observed to be similar among the patients regardless of the clinical severity of envenomation. An unprecedented additional finding was that we identified venom (Figure 3A) and antivenom proteins (Figure 3B) in the bite site by ELISA. The venom was quantified in the fluid a significant time after envenomation (up to 135 hours), suggesting a slow clearance of venom at the site of bite, which might have influence on local tissue well after the time of envenomation (Figure 3A).

Antibodies from the administered antivenom identified in the blister fluids were shown capable of binding venom proteins by Western blotting (Figure 4). Thus, blister fluid antibodies should be capable of neutralizing the any venom components in the fluid. However, taken together, these findings suggest that although blistering is a delayed phenomenon of envenomation, its likely pathophysiological origins occur in advance of antivenom administration and venom neutralization at the site of envenomation and continues despite the eventual neutralization of venom.

Conclusion

We demonstrates that antivenom and venom reach and stay even after long time on the local lesions in B. atrox snakebite. However, the presence of antivenin in the blister did not prevent the severity of tissue damage or blister formation. It suggest that the blister is an independent process, and the pathophysiology of blister formation is more related to the presence of proinflammatory molecules released by the toxins action since the first moments after venom inoculation (Figure 4). Our studies underscore the need to develop rapid, in situ therapeutics to eliminate or at least attenuate the local effects of envenomation.