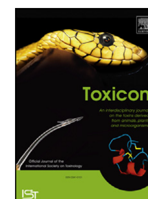
Toxicon 103 (2015) 99–105

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon



Lachesis stenophrys venom reduces the equine antibody response towards *Bothrops asper* venom used as co-immunogen in the production of polyspecific snake antivenom

Cynthia Arroyo, Sergio Solano, María Herrera, Álvaro Segura, Ricardo Estrada, Mariángela Vargas, Mauren Villalta, José María Gutiérrez, Guillermo León*

Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica

A R T I C L E I N F O

Article history:
Received 27 March 2015
Received in revised form
15 June 2015
Accepted 17 June 2015
Available online 19 June 2015

Keywords:
Snake venom
Bothrops asper
Lachesis stenophrys
Immunization
Immunomodulation
Snake antivenom

A B S T R A C T

The anti-bothropic activity of an antivenom prepared from the plasma of horses immunized with *Bothrops asper* venom (anti-B antivenom) was compared with a similar formulation produced from the plasma of horses immunized with a mixture of *B. asper* and *Lachesis stenophrys* venoms (anti-BL antivenom). Likewise, a comparison between the anti-lachesic activity of the anti-BL antivenom and a similar formulation prepared from horses immunized only with *L. stenophrys* venom (anti-L antivenom) was performed. The anti-BL antivenom had lower concentration of anti-bothropic antibodies than the anti-B antivenom. This difference was associated to a lower response towards all components of *B. asper* venom, but particularly towards some D49-phospholipases A_2 (PLA_2s) and PIII-metalloproteinases. Consequently, the anti-BL antivenom was less effective neutralizing lethal, coagulant, defibrinogenating, PLA_2, and myotoxic activities of *B. asper* venom. On the other hand, anti-BL and anti-L antivenoms showed similar concentration of anti-lachesic antibodies, and similar capacity to recognize the HPLC fractions of *L. stenophrys* venom and to neutralize lethal, coagulant, proteolytic, hemorrhagic, PLA_2 and myotoxic activities induced by this venom. It is concluded that, when used as co-immunogens, the venom of *L. stenophrys* reduces the antibody response towards *B. asper* venom, whereas the latter does not affect the anti-lachesic response.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

The parenteral administration of antivenoms is the only scientifically validated treatment for snakebite envenomations (WHO, 2010). Antivenoms are formulations of immunoglobulins purified from the plasma of animals immunized with snake venoms. These immunoglobulins bind to the venom toxins and neutralize their toxic effects (Gutiérrez et al., 2011).

According to the number of venoms used as immunogens, antivenoms can be classified as monospecific (if only one venom is used) or polyspecific (if two or more venoms are used). Polyspecific antivenoms can be produced by immunizing the animals with a venom mixture, or by mixing the plasmas (or purified immunoglobulins) obtained from animals immunized with single venoms (León et al., 2011). The suitable strategy to produce polyspecific formulations depends on the venoms used as co-immunogens and the production yield (dos Santos et al., 2011).

Considering the difficulty to discern between envenomations produced by the 23 species of viperid snakes in Central America (Campbell and Lamar, 2004), and the medical importance of the snakes *Bothrops asper* (fer-de-lance snake), *Crotalus simus* (rattlesnake, formerly *Crotalus durissus*) and *Lachesis stenophrys* (bushmaster, formerly *Lachesis muta*), a mixture of venoms from these species has been used as immunogen to produce a polyspecific antivenom since the end of 1960s in Costa Rica. This antivenom neutralizes the venoms included in the immunization mixture, and cross-neutralize the venoms from most of the other viperids in the region (Bolaños, 1982; Gutiérrez et al., 1996).

Combining various venoms in an immunizing mixture may have unexpected effects in terms of the immune response elicited towards some of these venoms. It has been demonstrated that some snake venoms induce immunosuppressive effects, reducing the magnitude of the immune response towards other antigens used as

* Corresponding author.
E-mail address: guillermo.leon@ucr.ac.cr (G. León).

http://dx.doi.org/10.1016/j.toxicon.2015.06.016
0041-0101/© 2015 Elsevier Ltd. All rights reserved.

co-immunogens (dos Santos et al., 2011). For example, the PLA_2 subunit of crotoxin inhibits the macrophage spreading and phagocytic activities induced by *C. durissus terrificus* venom (dos Santos et al., 1986; Sampaio et al., 2005).

Likewise, immunomodulatory properties have been attributed to *L. muta* venom (Stephano et al., 2005), although the mechanisms behind this effect remain largely unknown. Due to their phylogenetic proximity, venoms from other *Lachesis* sp snakes could also present immunomodulatory properties, a phenomenon that would have practical implications for the production of polyspecific antivenoms. In this work we studied the effect of *L. stenophrys* venom on the antibody response towards *B. asper* venom used as co-immunogen for the production of the Central American antivenom.

2. Materials and methods

All the procedures involving animals in this study were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUA) of Universidad de Costa Rica, and meet the Animal research: reporting *in vivo* experiments (ARRIVE) guidelines (Kilkenny et al., 2010) and the International Guiding Principles for Biomedical Research Involving Animals of the Council of International Organizations of Medical Sciences (CIOMS, 1985).

2.1. Snake venoms

Adult specimens of *B. asper* and *L. stenophrys* snakes were captured in various locations in Costa Rica and maintained in captivity at the serpentarium of Instituto Clodomiro Picado, Universidad de Costa Rica (San José, Costa Rica). Venoms were obtained from individuals anesthetized with carbon dioxide, by mechanical stimulation of their venom glands. Pools of venom prepared for each species were filtered through a 0.45 μm pore membrane (Millipore, USA), freeze dried and kept at $-20\ ^{\circ}C$ until use.

2.2. Immunization and immunoglobulin purification

Groups of four horses were immunized with the venoms of either *B. asper* (anti-B group), *L. stenophrys* (anti-L group) or with a mixture of both venoms (anti-BL group). All groups were immunized following an immunization scheme based on Freitas et al. (1991). Briefly, at different time intervals (0, 24, 32, 35 and 37 days) horses were injected with venoms by the subcutaneous route, in one single injection. The first two boosters consisted of 6.0 mg of immunogen (i.e. 6 mg of a single venom or 6 mg of the venom mixture composed of 3 mg of each venom) emulsified in Freund's complete adjuvant (first injection), or in Freund's incomplete adjuvant (second injection), in a total volume of 10 mL. All emulsions were prepared by using a 1:1 (v/v) ratio of antigen:adjuvant. The three remaining boosters consisted of 2.0 mg of immunogen dissolved in 5 mL of saline solution. Horses were bled 10 days after the last venom injection. Plasma was separated and pooled according to the venom or venom mixture used for immunization. Antivenom immunoglobulins were purified by the caprylic acid method (Rojas et al., 1994) and anti-B, anti-L and anti-BL antivenoms were formulated at 4.5 g/dL total protein, 0.9 g/dL NaCl, 0.25 g/dL phenol and pH 7.0. All antivenoms were freeze-dried without stabilizers following the procedure described by Herrera et al. (2014a), and stored at room temperature (20–25 °C) until use.

2.3. Quantification of antivenom antibodies by ELISA

Polystyrene plates (Nunc MaxiSorp, USA) were coated overnight, at room temperature, with 100 μL/well of a solution containing 3 μg/100 μL of either *B. asper* or *L. stenophrys* venom, dissolved in PBS buffer (0.12 M NaCl, 0.04 M phosphate, pH 7.2). Plates were washed 10 times with water. Then, 100 μL of various dilutions of antivenom (anti-B, anti-L or anti BL), in PBS buffer containing 2% bovine albumin (PBS-2% BSA), were added to wells and incubated for 1 h at room temperature. The plates were washed again. Later on, 100 μL of peroxidase-conjugated anti-horse immunoglobulin (Sigma–Aldrich, MO, USA), diluted 1:8000 with PBS-2% BSA, were added to each well. Plates were incubated for 1 h at room temperature. After a final washing step, color was developed by the addition of 100 μL of substrate solution (2 mg/mL *o*-phenylenediamine, 1 μL/mL hydrogen peroxide in 0.1 M citrate buffer, pH 5.0). Absorbances were recorded at 492 nm (Dynatech MR5000, Denkendorf, Germany). Calibration curves were constructed by plotting the absorbance of dilutions of the homologous monospecific antivenom, as function of their dilution factor. Concentration of anti-bothropic or anti-lachesic antibodies in antivenoms were calculated by interpolating absorbances into the corresponding calibration curve and expressed as percentage, considering as 100% the antibody content of the homologous monospecific antivenom.

2.4. Determination of the immunoreactivity profile

Affinity chromatography columns were prepared by immobilizing 100 mg of antivenoms (anti-B, anti-L or anti-BL) on 10 mL of cyanogen bromide-activated Sepharose 6MB (Sigma; catalog Number C9267), following the manufacturer's instructions. Columns were equilibrated with PBS, and absorbance was recorded at 280 nm. Then, each column was loaded with 6 mg of *B. asper* or *L. stenophrys* venoms and washed with PBS until the non-bound fraction was eluted. The bound fraction was eluted by changing the mobile phase to glycine, pH 3.0, and the pH of the collected fractions was adjusted by the addition of 0.5 M NaOH to reach pH 7.0–7.5. Subsequently, the bound fractions were analyzed by HPLC, using an Agilent Technologies 1100 series system (Santa Clara, CA, USA) equipped with a chromatographic data management system (ChemStation Data Analysis and Reporting, Agilent Technologies) and using a Lichrosphere RP 100 C_{18} column (250 × 4.6 mm, 5 μm particle size). Fractions were eluted at 1 mL/min with a linear gradient of buffer A (95% water, 0.1% TFA) and buffer B (95% acetonitrile, 0.1% TFA) (5% B for 10 min, followed by 5–15% B over 20 min, 15–45% B over 120 min, and 45–70% B over 20 min). Detection was set at 215 nm. Chromatographic runs of whole *B. asper* and *L. stenophrys* venoms dissolved in PBS were used as controls. HPLC fractions of *B. asper* and *L. stenophrys* venoms were identified by comparing the chromatograms with those published by Alape-Girón et al. (2008) and Sanz et al. (2008), respectively. The binding of different venom components to the affinity column was estimated by the following equation: $\%\ Bfx = 100 \times [Bfx/(Bfx + n - Bfx)]$, where Bfx and n − Bfx correspond to the areas of the HPLC fractions in the bound and non-bound affinity fractions, respectively (Herrera et al., 2014b).

2.5. Neutralization of venom-induced toxicity

The standard methods to evaluate the neutralization of venom-induced toxicity at Instituto Clodomiro Picado were used. Briefly, venom/antivenom mixtures were prepared by mixing a constant ‘challenge’ dose of venom, dissolved in PBS, with different dilutions of antivenoms. For the control groups, venom was incubated with PBS instead of antivenom. Then, the mixtures were incubated at 37 °C for 30 min and residual toxicity was assessed in mouse models (CD-1 mice of both sexes from Instituto Clodomiro Picado)

Download English Version:

https://daneshyari.com/en/article/8395425

Download Persian Version:

https://daneshyari.com/article/8395425

Daneshyari.com