Neutralization of the hemorrhagic and edema–forming activities from venom of *Bothrops alternatus* offsprings from Argentina

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Abstract

Zeinsteger, P.A.; Maruñak, S.L.; Acosta de Pérez, O.: ***Neutralization of the hemorrhagic and edema–forming activities from venom of Bothrops alternatus offsprings from Argentina.*** Tests of neutralization of hemorrhagic and edema–forming activities from the venom of *Bothrops alternatus* (víbora de la cruz) one month–old offsprings were performed using a bivalent antivenom, produced by Laboratorio Central de Salud Pública in La Plata, Buenos Aires, Argentina. We determined that 172 µl of the antivenom neutralize 50 % of the hemorrhagic activity, induced by 9 µg of the venom (challenge dose). Neutralization of edema–forming activity was more effective when the antivenom was administered previous to the venom. We conclude that the antivenom neutralizes the studied toxic activities, taking into account that for its production horses are immunized using venom from adult snakes.

Key words: *Bothrops alternatus*, neutralization, hemorrhagic activity, edema–forming activity.

INTRODUCTION

In Argentina there are snakes from many species of the *Viperidae* family. *Bothrops alternatus* and *Bothrops neuwiedii diporus* are widespread all along the country. The northeast region presents a subtropical climate; *B. jararacussu, B. jararaca, B. cotiara* and *B. moojeni* and the two species mentioned above are common there. *B. ammodytoides* inhabits the south area, which is arid and cold. In this country, 90% of the envenomings are caused by *Bothrops* genus [5].

Previous studies demonstrated that *Bothrops alternatus* venom from the northeast of Argentina has a prominent local effect, characterized by intense pain, edema, ecchymosis, hemorrhagic phlyctema and necrosis of the muscular and connective tissues [2, 3]. The systemic effects lead to hepatic and renal alterations [3], as well as coagulation abnormalities [1].

The coordinated activity of the different components of the venom causes a complex envenomation that must be treated using a specific antivenom, commonly obtained from horse immunization.

Several studies state that there are considerable differences in the composition of venoms from the same species of snakes that inhabit different geographic areas [8, 9].

A satisfactory effect has been obtained with the use of antivenoms in cases where the involved species were not used for horse immunization [11]. An example of this would be the neutralization activity of a polyvalent antivenom from South–African snakes which neutralizes the toxic activities from venoms of North–American species [13].

The purpose of the present study was to evaluate the neutralizing activity of a bivalent antivenom on the hemorrhagic and edema–forming activities induced by venom from *Bothrops alternatus* offsprings, taking into account that this venom in particular is not used for the immunization of the horses destined to antivenom production.

MATERIAL AND METHODS

Venom. A venom mixture from one month–old offsprings *Bothrops alternatus* was used. After the extraction of the venoms, the pool was homogenized, dehydrated and maintained at –20 ºC.

Antivenom. A bivalent hyperimmune antivenom obtained by horse immunization was used. It neutralizes 25 mg of *Bothrops alternatus* venom, and 15 mg of *B. neuwiedii* venom (Lote Nº 021, Laboratorio Central de Salud Pública, La Plata, Buenos Aires, Argentina).

Neutralization of the hemorrhagic activity (ED_{50}). Our laboratory previously determined the hemorrhagic activity from the venom of *Bothrops alternatus* offsprings (9 µg), expressed as minimum hemorrhagic dose (MHD) [12]. For this determination, we applied the Kondo *et al.* 1960 method [10], modified by Gutiérrez *et al.*, 1985 [7]. For the neutralization of the hemorrhagic activity, a challenge dose equivalent to 10 times MHD was used. Different proportion mixtures of antivenom and venom were incubated for 30 minutes at 37ºC, and from each of them 0.1 ml were injected to mice. Animals were grouped in 5 individuals (weight varied from

18 to 20 g). Each inoculation had 9 μg of the venom in 0.1 ml phosphate buffer solution (PBS) and was injected intradermically. Two hours later, animals were euthanized with ether and skin was removed to measure the hemorrhagic area. Controls were injected with 0.1 PBS. The effective dose 50% (ED_{50}) is the antivenom/venom reason that reduces 50% of the hemorrhagic effect, according to Gutiérrez *et al.*, 1981 method [6].

Neutralization of the edema–forming activity. Previous experiences have established the minimum edema–forming dose (MED) from venom of *B. alternatus* offsprings in 0.38 μg [12]. This data was used for the determination of the neutralization capacity of the studied antivenom on this effect. Mice from 18 to 20 g were administered intraperitoneally with 0.5 ml of antivenom, and 2.28 μg venom in 0.05 ml PBS in the left plantar pad. Groups of 5 individuals received the antivenom 30 minutes before, simultaneously and 30 minutes after the injection of the venom. Animals were anesthetized and euthanized 60 minutes after the administration of the antivenom and both limbs were separated from the rest of the body to determine the increment of weight due to edema.

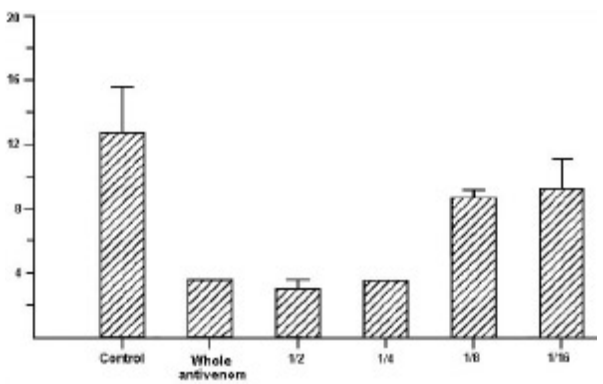

Figure 1. *Neutralization of the hemorrhagic activity from venom of* ***Bothrops alternatus*** *offsprings. Graphic shows the neutralizing capacity of the whole antivenom and different dilutions of it (X axis). Y axis represents mean diameter values (mm) of the hemorrhagic halo.*

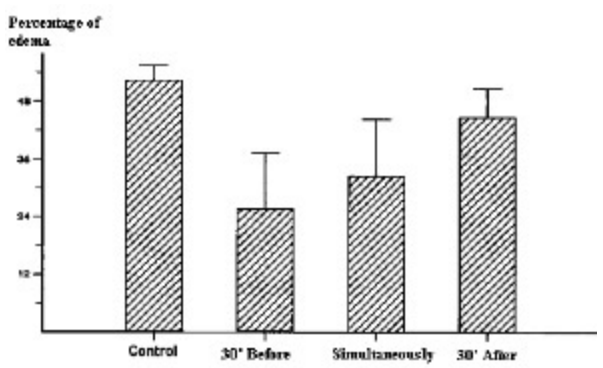

Figure 2. *Neutralization of the edema–forming activity from venom of* ***Bothrops alternatus*** *offsprings. Mice were injected with 2.28 μg of the venom (challenge dose) and 500 μl of the antivenom 30 minutes before, simultaneously and after it.*

RESULTS

The antivenom satisfactorily neutralized the hemorrhagic and edema–forming activities induced by venom from *Bothrops alternatus* offsprings, in spite of the fact that for the preparation of the antigenic booster for horses only venom from adult snakes was used.

ED_{50} was calculated by linear regression. The dose of injected venom was constant (9 μg), and the consequent hemorrhagic halo showed an inverse proportion between μg of the venom and the different dilutions of the antivenom. These haloes were similar when whole antivenom and dilutions up to 1/4 were used, while 1/8 and 1/16 dilutions had a lower neutralizing activity (Figure 1). Statistical analysis showed $r^2 = 0.860$; the calculated ED_{50} was 172 μl antivenom/venom.

When the antivenom was injected prior to venom, the edema–forming activity was best neutralized, while in the simultaneous injection a lower neutralizing activity was evident. Late administration of the antivenom (30 minutes after venom injection) did not show significant differences with those animals which received the venom only (Figure 2).

DISCUSSION

Bothrops alternatus venom was barely studied in the past. Our study showed that the hemorrhagic activity from venom of offsprings was four times more powerful than in adults (MHD 3.6 μg). This effect could also be observed with the edema–forming activity as it showed 10 times the power of the adult venom (4.4 μg) [12]. This is remarkable as both offspring and adult snakes inhabit the same geographical area.

Despite the significant differences in the intensity of the hemorrhagic and edema–forming activities from venoms of adults and offsprings, the neutralizing capacity of the antivenom was efficient for both of them [4].

Compared to adults, offsprings venom had a greater vasculotoxic and edema–forming activity. Nevertheless, the antivenom was capable to neutralize both effects when it was administered in time. This antivenom is the same used in previous experiences to neutralize the same toxic activities of adult venom, but from a different lot.

It is important to study the neutralizing activity of the bivalent antivenom in the neutralization of the toxic activities of offsprings venom, considering that it is not used in the preparation of the antigenic booster for the immunization of horses. Paradoxically, compared to adults a lower antivenom dose is needed to neutralize the hemorrhagic activity of offsprings venom (172 vs. 282.95 μl).

Results may confirm the existence of a total immunologic identity between offspringss and adults. Differences might be attributable to variations in the

concentration of some enzymes in comparison to others, being this related to the ontogeny of the animals.

Resumen

Zeinsteger, P.A.; Maruñak, S.L.; Acosta de Pérez, O.: ***Neutralización de las actividades hemorrágica y edematizante de veneno de viboreznos de Bothrops alternatus.*** Se efectuaron pruebas de neutralización de las actividades hemorrágica y edematizante causadas por veneno de viboreznos de *Bothrops alternatus* (víbora de la cruz) de un mes de vida, utilizando un antiveneno bivalente de origen equino, producido en el Laboratorio Central de Salud Pública de La Plata, Provincia de Buenos Aires, Argentina. Se determinó que 172 µl de antiveneno neutralizan el 50% de la actividad hemorrágica inducida por 9 µg de veneno (dosis de reto). La neutralización de la actividad edematizante fue más efectiva cuando se administró el antiveneno previo al veneno. Se concluye que el antiveneno utilizado neutraliza satisfactoriamente las actividades tóxicas estudiadas, a pesar que para la obtención del mismo, se inmunizan equinos con veneno de ejemplares adultos.

Palabras clave: *Bothrops alternatus*, neutralización, actividad hemorrágica, actividad edematizante.

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