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SHORT COMMUNICATIONS

ANTIVENOM ACTIVITY OF OPOSSUM (*DIDELPHIS MARSUPIALIS*) SERUM FRACTION

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A. Rodriguez-Acosta, I. Aguilar and M. E. Giron. Antivenom activity of opossum (*Didelphis marsupialis*) serum fraction. *Toxicon* **33**, 95–98, 1995.—We have found an opossum serum fraction of approximately 97,000 mol. wt to be highly proficient in inactivating the haemorrhagic and proteolytic fractions of *Bothrops lanceolatus* venom. This antivenom substance, isolated from opossum serum or a synthetic peptide based on the aforementioned protein, would probably be useful in the medical management of *Bothrops* accidents.

Snakebite is a common medical problem in rural South America, where it is responsible for many deaths. Whole serum of opossum (*Didelphis marsupialis*) inactivates the venom of the mapanare (*Bothrops lanceolatus*) (Lacépede 1789), both *in vivo* and *in vitro*. As Vellard (1949), Perez *et al.* (1978), Moussatche *et al.* (1979) and Rodriguez-Acosta (Thesis, Universidad Central de Venezuela, 1983) reported, the whole serum of the opossum inactivates the proteolytic and haemorrhagic activity of lethal doses of *B. lanceolatus* venom in mice, pigeons and hamsters. The protective capacity of the serum was primarily associated with orosomucoid serum fraction (Moussatche *et al.*, 1979).

When opossum serum and mapanare venom were mixed, they showed no evidence of precipitation; such precipitation occurred when serum of animals hyperimmunized with mapanare venom was mixed with the venom. The mechanism of the factor in opossum serum which inactivates *B. lanceolatus* venom toxins is unknown.

Compared with commercial horse antivenin, the antivenom activity of opossum serum is potent enough to be useful in the management of snakebite accidents (see Table 2). The i.p. toxicity of opossum serum was tested in 20 g mice and no evidence of toxicity was found (data not shown).

We have found opossum serum to be highly proficient in inactivating the haemorrhagic and proteolytic fractions of *B. lanceolatus* venom. When heated serum was utilized, these effects were not observed, and the mice died (Table 1). If the serum was precipitated with ammonium sulphate, only the supernatant (immunoglobulins free) was able to protect against LD_{50} (0.5 mg/mouse) crude venom activity.

Table 1. Capacity of total serum, heated serum and different fractions of opossum (*Didelphis marsupialis*) serum obtained by DEAE-Cellulose to inactivate the haemorrhagic and proteolytic activity of mapanare (*Bothrops lanceolatus*) venom

	Total serum*	Heated serum	DEAE-Cellulose fractions 0.05 M	0.1 M†	0.15 M	0.2 M	0.3 M
Antihaemorrhagic activity	+	0	0	+	0	0	0
Anticaseinolytic activity	+	0	0	+	0	0	0

The plus sign (+) represents antihaemorrhagic or caseinolytic activity.

The zero sign (0) indicates that the serum did not inactivate venom activities.

* Sulphate ammonium precipitated supernatant without immunoglobulins: 20 mg of serum supernatant inactivated 0.5 mg (LD_{50}) of crude venom.

† Serum fraction (0.6 mg) (F-0.1 M) inactivated 0.5 mg (LD_{50}) of crude venom *in vitro*.

Venom toxicity (LD_{50}) was assayed using NIH mice (20 g).

Twenty mice were used for each experiment and the (LD_{50}) dose was calculated by probit analysis (Finney, 1962) of deaths occurring within 24 hr of venom injection. Venoms from *B. lanceolatus* were collected from specimens recently received by this laboratory from the north of Venezuela. A pool of 25 individuals was utilized. The venom was lyophilized and stored as a batch at −70°C. Inactivation capacity is the ratio of the i.m. injected LD_{50} venom in mice treated with the antivenom fraction (F-0.1) in untreated or saline-injected mice. Opossum serum was obtained from specimens in our animal-breeding facilities. Antivenin was the specific anti-bothropic equine type; Venezuelan anti-snakebite serum (FM-UCV).

The protective activity of fractions of supernatant from ammonium sulphate precipitated opossum serum followed by DEAE-Cellulose ion exchanger chromatography using linear ionic strength gradients with NaCl (0.05 M, 0.1 M, 0.15 M, 0.2 M, 0.25 M and 0.3 M) and controls is shown in Table 1.

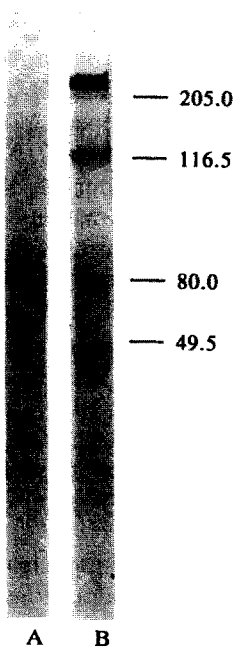

Fig. 1. (A) SDS–PAGE analysis of opossum serum fraction (97,000 mol. wt); (b) the positions of the mol. wt marker are indicated on the right.

Table 2. Effectiveness of the opossum serum fraction on mean survival times of NIH mice (20 g) injected with 2 LD_{50} *Bothrops lanceolatus* venom as compared with antivenin

Treatment	Antivenom treatment time (hr)						
	−6*	0	24	48	72	96	150
None	20 ± 4	20 ± 4	—	—	—	—	—
Human serum (8 mg)	20 ± 4	20 ± 4	20 ± 4	—	—	—	—
Antivenin (2 mg)	22 ± 4	28 ± 4	22 ± 4	—	—	—	—
Opossum serum (2 mg)	24 ± 4	A†	26 ± 4	—	—	—	—
Opossum serum (8 mg)	26 ± 4	A	A	A	A	—	—
Antivenin (4 mg)	24 ± 4	A	24 ± 4	22 ± 4	—	—	—
Opossum serum: fraction F-0.1 (1.2 mg)	26 ± 4	A	A	A	A	—	—
Antivenin (4 mg)	24 ± 4	A	24 ± 4	22 ± 4	—	—	—
Opossum serum: fraction 97,000 (2 mg)	24 ± 4	A	A	A	A	—	—
Antivenin (8 mg)	24 ± 4	A	24 ± 4	—	—	—	—

Antivenom was injected i.p. either simultaneously with the venom (i.m.), time 0, or before the injection of venom at several pretreatment times (24–150 hr).

* Injection of venom 6 hr before antivenom injection.

† A, All animals were alive during the 4 month test period.

Each experimental group comprised ten mice.

The haemorrhagic effects of mapanare venom tested *in vivo* (Kondo *et al.*, 1960) were only inactivated by DEAE-Cellulose NaCl 0.1 M–buffer PBS 0.01 M opossum serum fraction (F-0.1), when incubated together, before being injected into mice. Higher proportions of F-0.1 injected i.p. were needed to induce protection when the venom was previously injected intracutaneously into the shaved skin on the back of the mice. Five hours later, the mice were sacrificed, their skins removed and the haemorrhagic area was estimated by the diameter (cm^2) on the visceral side, representing only 2% of the positive control mice. The haemorrhage was expressed as a percentage of the haemorrhagic area produced by the venom.

Proteolytic effects of mapanare venom, tested *in vitro* (Kunitz, 1946), were only inactivated by DEAE-Cellulose NaCl–PBS 0.01 M opossum serum fraction (F-0.1).

F-0.1 serum proteins were fractionated on a gradient (5–10% acrylamide) by SDS–PAGE, in the absence of β-mercaptoethanol. After preparing the SDS–PAGE, each band was cut and pooled, and the proteins were extracted by electroelution. An exclusive relatively dense band was seen in the apparent 97,000 mol. wt region. Extracts from this and other bands were assayed for antivenom activity. This activity was found in the 97,000 band (Fig. 1). To test whether the antivenom activity of heated opossum serum, total opossum serum, F-0.1 opossum serum fraction and 97,000 band could be useful in snakebite therapy, we compared their activities with that of commercial antivenin (Table 2). The 97,000 mol. wt band was more effective than any dose of antivenin in preventing tissue damage, haemorrhagic activity and caseinolytic activity.

This antivenom substance (97,000 mol. wt), isolated from opossum serum or a synthetic peptide based on the aforementioned protein, would probably be very useful in the medical management of Crotalidae accidents.

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