Toxins 2021

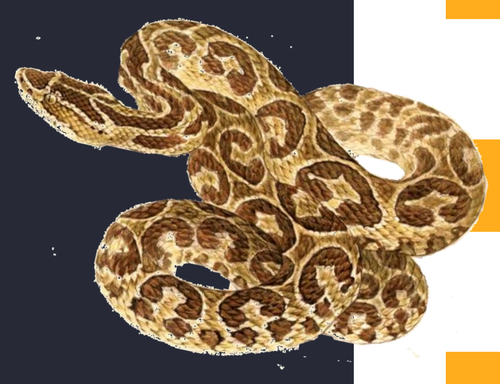
# Evaluation of *in vitro* muscle regeneration after myonecrosis induced by *Bothrops alternatus* and *Bothrops diporus* venoms from Northeastern Argentina

Luciano S. Fusco, Andrea C. Van de Velde, Laura C. Leiva, Soledad Bustillo*

*solebustillo@yahoo.es

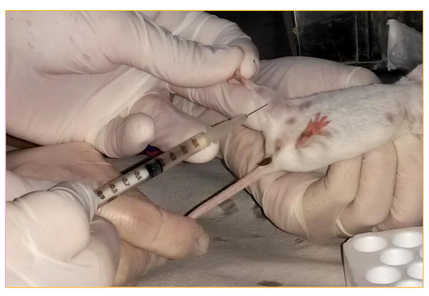
## INTRODUCTION

The majority of snakebites in northeastern Argentina are caused by *Bothrops alternatus* (yarará grande) and *Bothrops diporus* (yarará chica), reptiles that belong to the Viperidae family. The specific treatment of these ophidian envenomations is serotherapy with antivenoms that ensures a rapid distribution of antibodies and controls the systemic alterations but not always the local damage in the bite site where traces of venom are capable to preclude a successful regenerative response.

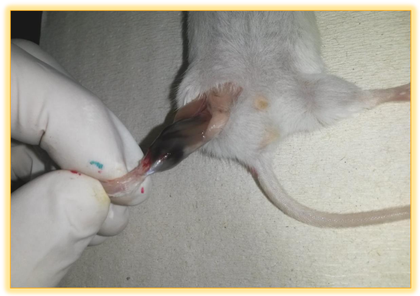


In this work, we explore the characteristics of muscle tissue during the critical period after *Bothrops alternatus* or *Bothrops diporus* venom injection and their potential inhibitory effect on muscle differentiation using an *in vitro* study model.

## Methods



Groups of CF-1 mice were injected intramuscularly in the right gastrocnemius with 50 μg of *B. alternatus* or *B. diporus* venoms. Control mice: PBS



Sacrifice and muscle disection

0-1-3-24-168 h

Liquid Nitrogen

Pulverization and filtration

Muscle Homogenates

- Quantification and analysis of proteins
  - ELISA
  - Western Blotting
- Myogenesis evaluation
  - Cytotoxicity C2C12 cell line
  - Myoblast differentation C2C12 cell line

## Results

The amount of both venoms in muscle homogenates decreased over time, with even traces of venom (5-13 μg/mL) being observed 168h after inoculations. No significant differences were detected between *B. alternatus* and *B. diporus* venom treatments.

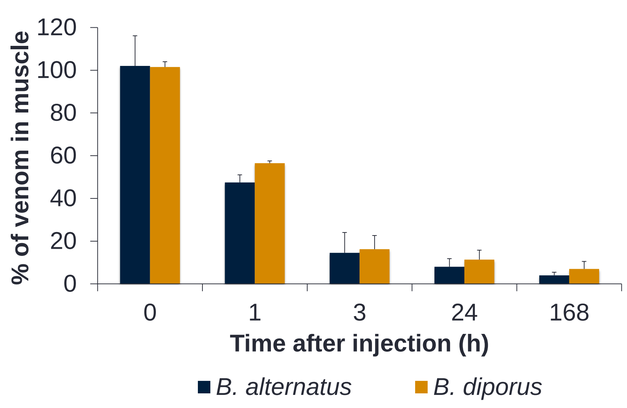



Figure 1. Quantification of venom by ELISA

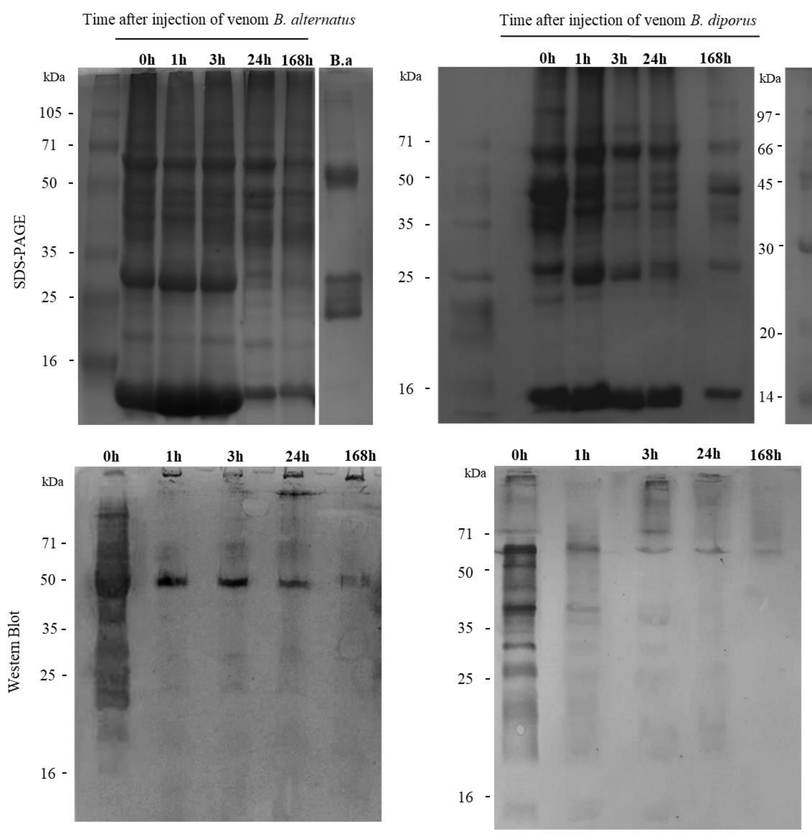



Figure 2. Immunodetection of venom proteins by Western Blotting

Identification by immunoblotting showed typical venom protein bands with molecular masses between 20 and 100 kDa for *B. alternatus* and 14 and 100 kDa for *B. diporus* whose intensities gradually decreased with time. An intense band of ~60 kDa, characteristic of metalloproteases, was mainly visualized even after 7 days of both treatments.

Muscle homogenates obtained from mice after 24h and 168h were considered non or minimally cytotoxic (85-100% cell viability) and used for myogenic assays (Data not shown).
Control homogenates (injected only with PBS) and myogenic control cultures (incubated only with DMEM-SFB 1%), showed a similar large proportion of mature myotubes (Figure 3, A and B). In contrast, a complete lack of myoblast fusion was evidenced when myogenic cells were incubated with muscle homogenates from mice injected with either of the two botropic venoms (Figure 3 C, D, E and F). Once more, the effect was more pronounced in the case of *B. diporus* venom treatments, where even some rounded or detached cells could be observed (Figure 3, D and F).

.

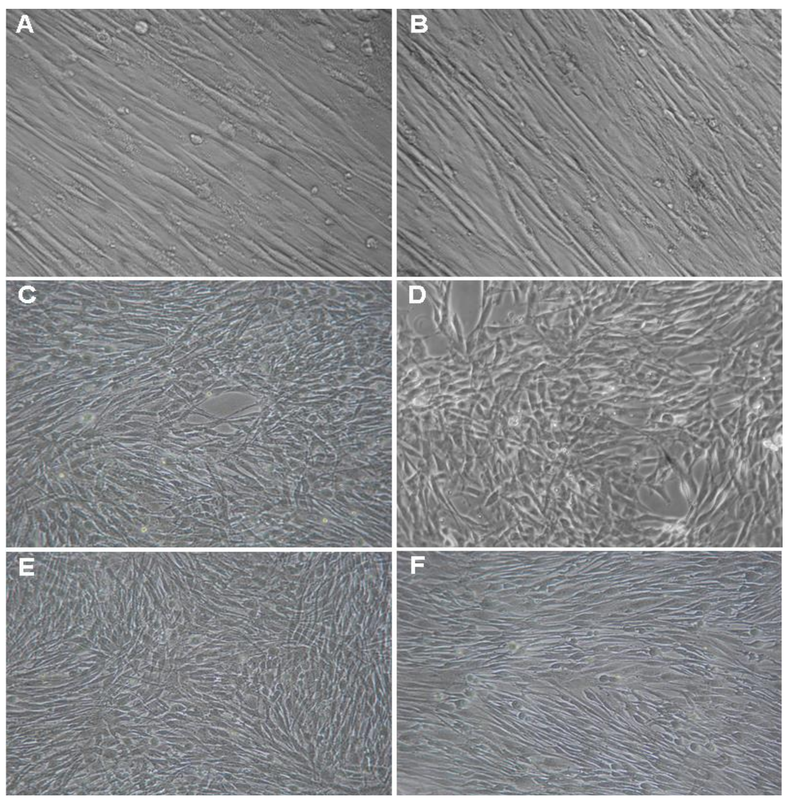



Figure 3. Myogenesis of myoblasts of the C2C12 line treated with muscle homogenates. A. Myogenic control. B. Control Muscle homogenate (PBS). C and E. *B. alternatus* treatments (24 and 168h). D and F. *B. diporus* treatments (24 and 168h)

## Conclusion

These preliminary findings showed that traces of venom present in the muscle tissue after several hours of intoxication, would prevent a successful regenerative response. Future studies will show if local administration of antivenom or specific antibodies, complementary to serotherapy, could improve the prognosis of snakebite poisonings by accelerating muscle regeneration processes.

## Contact Information

Grupo de Investigación Biológicas y Moleculares (GIByM). IQUIBA-NEA CONICET. Universidad Nacional del Nordeste (UNNE). Email: solebustillo@yahoo.es