Insects as Biofactories to produce sphingomyelinase D for *Loxosceles* antivenom development

Mac Callum GC [1]., Smith I [1]., Birenbaum JM [1]., Rodriguez MS [2]., Targovnik AM [1]., Wolman FJ [1].,Dokmetjian JC [2]., Cascone O [1]., Roodt AR [2]., Fingermann M [2]., Miranda MV [1].

[1] *Instituto de Nanobiotecnología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.*

[2] *Administración Nacional de Laboratorios e Institutos de Salud "Dr. Carlos G. Malbrán"*

Introduction

Loxosceles species, commonly named "violin" spiders, are widely spread venomous spiders. Clinical cases of Loxosceles bites are more commonly reported in the Americas, particularly in Argentina, Brazil, Chile and Perú. Most accidents are characterized by dermonecrotic lesions, often referred to as necrotic or gangrenous arachnidism. However, in about 10% of the cases a more serious presentation occurs, characterized by a systemic evolution of the pathology that can result in a fatal outcome, mostly in children and elders. Antivenom against Loxosceles species is being produced in the Americas since the early 1960s. Its Active Pharmaceutical Ingredients are immunoglobulins or their fragments obtained from equine plasma of animals hyperimmunized with spiders' venom. Venom extraction, a very laborious task with very low yields per spider, is the most relevant production bottleneck. Herein we optimized a biotechnological process based on Spodoptera frugiperda larvae as biofactories to obtain a recombinant version from L. laeta sphingomyelinase D (rSmase). The rSmase was recovered high purity level (94.5%) in one-chromatographic step at low cost. Our version of rSmase was able to induce a neutralizing humoral response in horses' sera. The amount of venom of 17000 spiders could be replaced with rSmase from 1600 insect larvae. The use of rSmase is expected to change traditional antivenom production.

Scheme of rSph in Lepidopteran larvae and its use as an antigen

1 Equine serum is evaluated by ELISA by coating with the purified toxin.
2 Serum from animals hyperimmunized with either whole venom or recombinant protein was tested for neutralization of rSph as a way to study immunogenicity.

Conclusion

Expression was successful in both *Spodoptera frugiperda* and *Rachiplusia nu* larvae, obtaining higher yields for *Spodoptera frugiperda*. Good purification levels were achieved with cationic-exchange matrixes and higher still with IMAC. The resulting protein was not glycosylated and inoculation in rabbits demonstrated dermonecrotic lesions comparable to whole venom. Preliminary test to evaluate immunogenicity of the recombinant protein established less potency compared to whole spider venom, but further analysis are required. A high titer of equine anti-rSph antibodies was obtained. Preliminary results show that the antibodies in the hyperimmune serum have neutralizing activity against the venom. Thus, Sphyngomyelinase D represents a good example of the use of insect larvae as a small bioreactor for the production of recombinant proteins in a cost-effective and easily scalable process.

Results

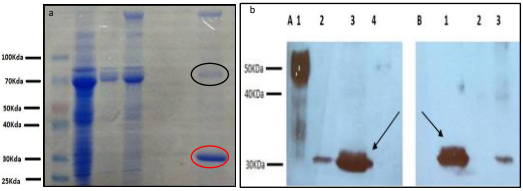

Fig 1. a. **SDS-PAGE showing IMAC purification of Smase I.** Larval homogenate from AcMNPVsph infected *S. frugiperda* at 6 dpi (1), *Pass-Through* (2), Wash steps (3-5), Eluate at 500mM imidazole (6), Eluate concentrated 7x using centrifugal filter units with 10kDa cut-off (7). Red circle indicates Smase I and black circle indicates main contaminant. IMAC purification was successful in obtaining a highly purified recombinant protein with only one major proteic contaminant in a small proportion. b. **Evaluation of Smase I expression in S. frugiperda larvae by Western blot.** A: Developed with anti-Histidine antibody. Molecular wieght Marker (1), Total homogenate from infected larvae (2), Eluate from IMAC purification (3), Positive control of a glycoprotein with His- tag (4). B: Developed with antiserum against loxosceles whole venom. IMAC eluate (1), IMAC pass-through (2), Total larval homogenate

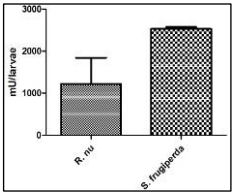

Fig 2. **Sphygomyelinase activity obtained for each larval specie after IMAC purification.** Expression was successful in both *Spodoptera frugiperda* and *Rachiplusia nu* larvae, obtaining a final yield after Ni-NTA purification of approximately 2529 ± 48 and 1223 ± 120 mU/larvae respectively.

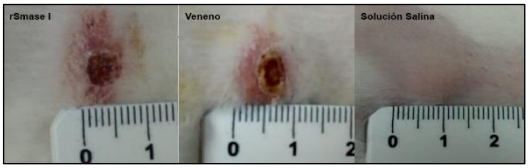

Fig 4. **Dermonecrotic activity of Smase I expressed in *S. frugiperda* assayed in mice.** A: Photograph of a mouse's upper body 7 days after inoculation of rSmase I. B: Photograph of a mouse's upper body 7 days after incoluation with venom obtained from *Loxosceles laeta* whole venom obtained by electrostimulation. C: Control mouse 7 days after inoculation. Dermonecrotic pattern seems to indicate that the recombinant protein expressed produces the same toxic skin lesions as whole spider venom whereas a control mouse inoculated with larval homogenate from non-infected larvae did not produce lesions.

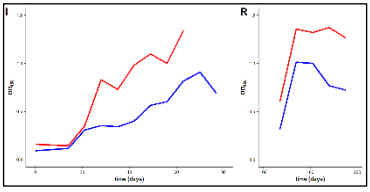

Fig 3. **Anti-sphygomyelinase recombinant antibody titer curve.** The curves are marked with different colors because two horses were immunized in parallel for 84 and 105 days, respectively.

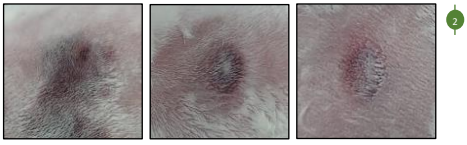

Fig 4. Neutralizing activity of hyperimmune equine serum. On the left, only *Loxosceles* venom, en el centro same dose of venom corresponding to 1 DMN as in the control together with the smallest volume of serum for 100 ul of final volume and on the right, the same as before but with the largest volume of serum for 100 ul final volume. On the left the necrotic action of the poison is visible, while on the right the equine antibodies turn out to neutralize part of the venom.