not hemorrhagic venom, but it is greater than that was reported for *C. d.terrificus* venom from Brazil (0.96). The major LNC value it would be related to the low content ratio of crotamine (8%) in *C.d.terrificus* venom from Argentina. These results provide information about *C.d.terrificus* venom composition from Argentina and they could be considered on defining the mixture of venoms for immunization to produce an effective pan-American anti-Crotalus antivenom.

BINDING OF ACIDIC PLA2 BA SPII RP4 FROM *BOTHROPS ALTERNATUS* SNAKE VENOM TO INTEGRIN αVβ3: AN IN SILICO STUDY

Emilio Angelina, Soledad Bustillo, Lucrecia Bogado, María Emilia García Denegri, Nelida Peruchena, Laura Cristina Leiva. *Laboratorio de Estructura Molecular y Propiedades (LEMyP) FaCENA, IQUIBA-NEA (UNNE-CONICET), Av. Libertad 5470, 3400, Corrientes, Argentina; Laboratorio de Investigación en Proteínas (LabInPro), FaCENA, IQUIBA-NEA (UNNE-CONICET), Av. Libertad 5470, 3400, Corrientes, Argentina*

We have previously demonstrated that BaSpIIRP4 (an acidic PLA2 from *Bothrops alternatus* venom), enhance the endothelial cells (EC) detachment effect of a snake venom metalloproteinase. Considering that the binding of cells to their ligands depends on the interaction between extracellular matrix and integral membrane proteins, this effect may be due to interactions between PLA2 and EC integrins. The integrin alpha-v beta-3 (αvβ3) is commonly expressed in endothelial cells. It has been reported that human PLA2-IIA binds to this integrin with high affinity. Moreover, it was demonstrated that arginine residues R74 and R100 are critical for this binding. In addition, the interaction svPLA2-αvβ3 integrin was also described. Considering these previous findings about the interaction of human and snake venom PLA2-IIA with integrin αvβ3, in this work we use an in silico approach to predict whether BaSpIIRP4 would also bind to the same integrin, thus supporting the hypothesis that EC detachment could be a receptor-mediated effect. Since structure of BaSpIIRP4 PLA2 has not yet been elucidated, an enzyme homology model was built with the Modeller software. PLA2 from *Bothrops jararacussu* was selected as template structure. To identify putative sites on the surface of the PLA2 model with capacity to interact with the RGD site on the headpiece of the αvβ3 integrin, the protein-protein docking server ClusPro was employed. The stability of the identified anchoring points was evaluated by Molecular Dynamic simulations with Amber16 and binding free energy calculations with MM-PBSA protocol. Three interaction sites were found, one of them showing a strong resemblance with the previously identified site on human PLA2-IIA. These results suggest that integrin αvβ3 may serve as receptor for PLA2 from *B. alternatus* venom, and this interaction could be a novel therapeutic target.

IMMUNE RESPONSE INDUCED BY NEW ADJUVANT STRATEGY TO PRODUCE CROTALIC ANTI-PLA2

Luciano Fusco, Andrea Van de Velde, David Hernández, Santiago Palma, Belkys Maleto, Gabriel Moron, Laura Leiva. *Laboratorio de investigación en proteínas (LabInPro), Universidad Nacional del Nordeste, IQUIBA-NEA, Corrientes, Argentina; Instituto de Ictiología del Nordeste, Argentina; Unidad de Investigación y Desarrollo en Tecnología Farmacéutica, Argentina; Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI), Argentina*

Snake envenomation is a serious medical problem and antivenoms are the main treatment. Antisera are produced by immunization of horses with snake venom using complete Freund's adjuvant and incomplete (booster) but it causes severe local reactions. A new adjuvant strategy is here proposed to increase efficiency in antisera production under much less morbility to immunized animals. Previous works showed that CpG-ODN formulated with a 6-O-ascorbyl palmitate nanostructure (Coa-ASC16) was more efficient as adjuvant than CpG-ODN alone using ovalbumin (OVA) as an antigen model. Here, we evaluated the immune response induced by this adjuvant strategy using crotalic PLA2 enzyme as antigen. Balb/c mice were subcutaneously immunizated on days 0, 15 and 30 with PLA2/CpG-ODN/Coa-ASC16 or PLA2/Freund's Adyuvant (complete first and incomplete-booster) (dose/mice: crotalic PLA2: 10-15μg, CpG-ODN: 30 μg). On day 50, mice were sacrificed. In both immunized mice groups, the plasma antibody titers were high (dilution1/24800), with a similar IgG1/IgG2a ratio. The IgG antibodies were then purified by affinity Sepharose-proteinG column. Indirect hemolytic activity neutralization of the PLA2 (2.5 μg) with specific antibodies (1.7-4.5 μg range; IgG anti-PLA2) were made by radial hemolysis. The evaluation did not show difference in neutralizing capacity of the antibodies produced by CpG-ODN/Coa-ASC16 or Freund's Adyuvant. Macroscopic and microscopic analysis at the site of injection of mice inoculated with Freund's adjuvant showed local damage (with non-infectious abscesses) and hypertrophy of inguinal lymph nodes, whereas mice injected with CpG-ODN/Coa-ASC16 did not. Our results shows that CpG-ODN/Coa-ASC16 produces a humoral response as strong and specific as Freund's adjuvant, with minor or null local deleterious effect. Thus, this complex emerge as a new adjuvant and a very attractive alternative for anticrotalic sera production.

AN UNUSUAL PHOSPHOLIPASE A2 FROM *PORTHIDIUM HYOPRORA* VENOM SNAKE: PURIFICATION, STRUCTURAL AND PHARMACOLOGICAL PROPERTIES

Salomón Huancahuire-Vega, Edda Evnet Newball-Noriega, Sergio Marangoni. *Departamento de Ciencias Básicas, Escuela de Medicina, Facultad de Ciencias de la Salud, Universidad Peruana Unión – Lima, Perú; Departamento de Bioquímica, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), SP, Brazil*

Snake venom PLA2 enzymes, in addition to their involvement in the digestion of prey, exhibit a wide variety of pharmacological/toxic effects by interfering in normal physiological processes of prey/victims. Often, single snake venom contains a number of PLA2 isoenzymes, and at times, different isoenzymes induce distinct pharmacological effects. However, not all PLA2 enzymes induce all the pharmacological effects; some mainly express its primary digestive function. This work describes the biochemical/pharmacological characterization of a non-toxic PLA2 designated PhTX-IV, isolated from *Porthidium hyoprora* venom by a single chromatographic step, including reverse-phase chromatography. The purification process employed C4 reverse phase high-pressure liquid chromatography. This enzyme is composed of a unique polypeptidic chain and has a molecular weight of 13.846 Da. Its complete sequence of 121 amino acids was obtained through ESI-MS/MS techniques, showing that it belongs to the Asp49 group of catalytically active enzymes and revealing a high degree of homology sequence (87-49%) with other Bothrops PLA2. PhTX-IV showed Ca2+-dependent enzymatic activity, reaching its maximal activity at pH 8 and 35-45oC. In vivo, did not show myotoxic upon muscular fibers at doses up to 100μg and neurotoxic, cytotoxic, and anti-platelet aggregation activities were absenting. Furthermore, was not lethal to mice at intravenous high doses of 100μg. Induction of local paw edema and weakly inhibited coagulation were the only toxics effects recorded. In conclusion, this enzyme, with the exception of a slight clotting time delay and a moderate edema-inducing effect, did not showed the major toxic actions reported for this type of proteins, as myotoxicity, cytotoxicity and lethality. These pharmacological characteristics suggest that the role of PhTX-IV in the pathophysiology of envenomings by *Porthidium hyoprora* might be restricted to digestive functions.

PRECLINICAL EVALUATION OF THE POLYSPECIFIC ANTIVENOM INOSERP™ PAN-AFRICA AGAINST THE VENOMS OF ELAPIDS AND VIPERIDS OF SUB-SAHARAN AFRICA REGION: NEUTRALIZATION OF TOXIC ACTIVITIES

Michel Martínez, José Almaguer, Asunción Saldivar, Raúl Soria, Henri Mathe. *Veteria Labs, S.A de C.V. Lucerna 7, Col. Juárez, Del. Cuauhtémoc, Mexico; Inosan Biopharma, S.A. Arbea Campus Empresarial, Edificio 2, Planta 2, Carretera Fuencarral a Alcobendas, Madrid, Spain*