18ª Reunião Cientifica Anual 2016

18 RCA Instituto Butantan, São Paulo, Brasil de 31/11 a 02/12, 2016

Characterization of inorganic elements, proteins and hemostatic activity present in coxal fluid of *Ornithodoros brasiliensis* (Acari: Arrgasidae)

Esther Tanigushi, Fernanda Faria, Daniel Pimenta, Marcela Fanhani, Cibele B. Zamboni, Ronaldo Zucatelli Mendonça, Simone M. Simons.

Introduction: Ornithodoros brasiliensis, has distribution restricted to the Rio Grande do Sul. Due to its quick engorgement, hydrostatic balance is maintained through the secretion of excess body fluids and ions in the coxal liquid (CL), by coxal gland during and after feeding. The CL has ionic composition, protein and blood coagulation inhibitory substances almost unknown; however, these molecules can be antihemostatic strategies for this tick. **Objectives:** In this study was screening biological activity, elemental composition and doing proteomic studies in the coxal liquid from tick O. brasiliensis. Methods: The colony of O. brasiliensis is kept in a controlled environment chamber. The ticks feed in rabbits (Oryctolagus cuniculus) (CEUAIB 1281/14). CL was collected during and after tick feeding. Protein concentrations were determined by Bradford (1976). CL samples were submitted to affinity chromatography in resin (Heparina Sepharose – GE) in FPLC system (ÄKTA-GE). Separated protein samples were put together in fractionated pools (P1 to P7), dialyzed against NaCl 3mM and concentrated in a freeze-dryer. Later, we analyzed samples with gel electrophoresis (SDS-PAGE 12,5%), and inhibitory activity tests over factor Xa with or without phospholipids and ClCa₂. Preliminary proteomic studies with mass spectometry MALDI/TOF and researches in ESTs database and cDNA library from salivary gland of O. brasiliensis, showed proteins expressed during and after the hematophagy. Neutron Activation Analysis (NAA) determined ionic concentration of CL, through irradiation on nuclear reactor IEA-R1 of IPEN/SP (3-4.5MW, pool type). Results: The revealed SDS-PAGE 12.5% bands of separated samples P1 to P5 of chromatography had molecular mass between 67 and 15 kDa. It was not possible to see bands P6 and P7. The crude CL, P3 and P6 showed inhibition around 30% over FXa, but on P1 and P7 the inhibition was absent. The phospholipids and ClCa₂ addition to reaction increased the inhibitory activity around 50%. P5 had the better inhibition percentage on activity of the enzyme. NAA revealed the presence of Br, Mg, K, P, Ca, besides high concentrations of Na, Cl and S, suggest the association with osmotic pressure regulation mechanisms during tick feeding. Proteomic analysis showed vitellogenin and hemelipoglycoprotein, proteins involved in vitellin (reserve substance in eggs) synthesis and transport of blood cells heme group, respectively. Discussion: Even though results are still preliminary, they represent important discoveries regarding biological activity, elemental and protein composition of coxal liquid from tick O. brasiliensis. The CL has shown great inhibitory potential over FXa, therefore, the next steps will be crucial for appropriate purification and characterization of the relevant protein in this secretion.

Financial support: CNPq and FAPESP