Antiviral activity observed in the hemolymph of *Podalia sp* (*Lepidoptera: Megalopygidae*)

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Introduction. The control of human viruses is of high interest in human and animal health. Several works have demonstrated the presence of bioactive peptides and their potential use as therapeutic agents in insect hemolymph. However, relatively little data are available on molecules from insects with antiviral activities. So, the objective of this study, is to identify the potential antiviral of a protein isolated from hemolymph of larvae of *Podalia sp* (*Lepidoptera: Megalopygidae*)

Methods. In this study, the effects of supplementation of infected culture with hemolymph from larvae of *Podalia sp* (*Lepidoptera: Megalopygidae*) (1%v/v) were investigated. The effect of hemolymph on virus growth was measured on confluent monolayers of infected cells with measles, influenza (H1N1), herpes, and rubella virus (enveloped virus). Picornavirus, a non enveloped virus, also was used. The cultures were observed daily for evidence of cytopathic effect. The analyses of the viral replication also was performed by qPCR analysis at 96 hs after infection. The antiviral protein responsible for this activity was isolated and purified by gel filtration chromatography using a gel filtration column system (Superdex 75) and further fractionated using a Resource-Q ion exchange column system.

Results. Cytotoxicity and genotoxicity of Podalia hemolymph was evaluated and no adverse effect was observed. Experiments with the purified protein led to a 243-fold reduction in influenza virus production, 729-fold reduction in measles virus production and a 2.187-fold reduction in picornavirus production. Assays using qPCR to determine viral mRNA present in the treated and infected cells also was performed. The replication of these virus was compared between treated and untreated infected cells. The purified antiviral protein was able to reduce at least 10^3 times the replication of herpes and rubella virus. Heating and freezing seem to have no influence over its antiviral activity. Also, the protein does not display virucidal activity and does not act on receptors on the cell membrane. The observations suggest an intracellular mechanism of action and that the protein may act as a constitutive agent that affects the innate antiviral immune response.

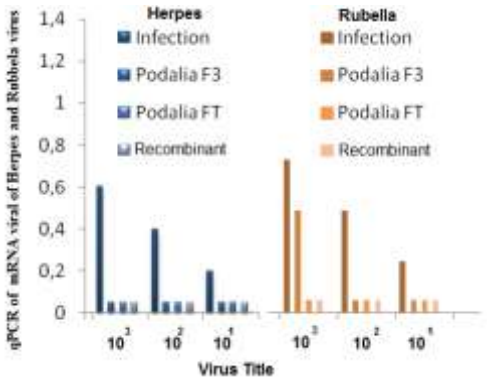

Figure 1 - qPCR of herpes simplex viral DNA and rubella mRNA. VERO and SIRC cells were infected with herpes or rubella virus with 10^1, 10^2 and 10^3 TCID50 and treated with total hemolymph, purified fraction 3 or an antiviral recombinant protein. Total mRNA was determined at 96 hs after infection. After reverse transcription, the levels of intracellular herpes or rubella mRNA were measured by qPCR. At photo below, the protective effect of hemolymph in L929 cells infected with EMC virus

Conclusions. The results obtained showed that the purified antiviral protein, obtained from podalia hemolymph, was unable to inhibit virus replication of all kind of virus. At qPCR, a significantly reduction in the virus replication also was observed. This antiviral protein does not display virucidal activity and does not act on receptors on the cell membrane. The observations suggest an intracellular mechanism of action.

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References.

Greco et al. Antiviral research, 84 (2009) 84–902009. **doi:10.1016/j.antiviral.2009.07.018**
Carmo ET al. Antiviral research, 94, 126-130 (2012), doi:10.1016/J.antiviral.2011.12.010;