Volume 88(1) - 2015

# Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale

## 86th SIBS National Congress

Palermo, Italy, 24-25 October 2013

*Botanical Garden, Lanza Hall*

jbr

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# Effects of *Pelagia Noctiluca* crude venom on cell viability and volume regulation

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Among Cnidaria, *Pelagia noctiluca*, is one of the most dangerous jellyfish in the Mediterranean Sea, where its blooming has been very abundant for many years. Toxicology of crude venom from *P. noctiluca* stinging cells is reported in this presentation. Both *in vivo* and *in vitro* biological assays have been performed to verify and, possibly, measure the toxicity of *P. noctiluca* crude venom, whose composition is still not completely defined.

At first we tested the hemolytic activity of crude venom from single nematocysts discharged by a chemical non enzymatic method. The delivered venom induced a powerful and rapid hemolytic activity. As a second step, crude venom extracted from a population of isolated nematocysts, provoked a dose-dependent hemolysis in erythrocytes from different sources, including eel, rabbit, chicken and human. Moreover, *P. noctiluca* crude venom directly induced mitochondrial trans-membrane potential ($\Delta\Psi m$) collapse and Reactive Oxygen Species (ROS) generation in SH-SY5Y cells derived from human neuroblastoma.

In order to better characterize the biological effects of the crude venom, *in vivo* assays were also performed. Injection of crude venom into the rat paw evoked an inflammatory reaction in a dose-dependent manner. Immunohistochemical analysis showed a marked acute inflammatory response in the tissues, with accumulation of polymorphonuclear neutrophils. Treatment with melatonin as antioxidant significantly reduced the inflammatory response, thereby confirming that oxidative stress plays a major role in inducing the observed pathological changes.

In addition to hemolytic and cytolytic assays, a test on cell volume regulation capability was also chosen to describe the biological activity of *P. noctiluca* crude venom. As already demonstrated, isolated nematocytes of the sea anemone *A. mutabilis* exhibit Regulatory Volume Decrease (RVD) when stimulated with a 35% hypotonic solution. In nematocytes exposed to different concentrations of crude venom (corresponding to the amount contained in 10, 25 and 50 nematocysts/$\mu$L) RVD was partially inhibited 25 nematocysts/$\mu$L crude venom concentration and fully blocked at 50 nematocysts/$\mu$l completely recovered, therefore indicating that $K^+$ channels inhibition may account for the venom-induced RVD impairment. RVD tests were also performed on HEK293 Phoenix cells, a human embryonic kidney cell line. In control conditions, the cells stimulated by hypotonicity showed an initial swelling followed by RVD, whereas in 0.025 $\mu$g/$\mu$L crude venom-containing extracellular hypotonic solution, RVD was dramatically impaired. Furthermore, pre-incubation of cells in a crude venom-containing extracellular isotonic solution prevented RVD after hypotonic stress. Surprisingly, the presence of toxin in the extracellular isotonic solution led to cell swelling even in the absence of an osmotic gradient. This phenomenon was not observed in control conditions and the precise mechanism needs to be further elucidated.

We conclude that *P. noctiluca* crude venom extract has hemolytic activity, pro-inflammatory action, induces mitochondrial potential collapse and ROS production. In addition, crude venom inhibits RVD in both cnidarians and mammalian cells after hypotonic stress and leads to cell swelling in isotonic conditions. Our experiments add novel information to understand the mechanism of action of *P. noctiluca* venom.

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*Journal of Biological Research 2015; 88:5161*