Characterization of palytoxin induced inflammation at the skin level: an *in vitro* study on keratinocytes and immunity cells co-cultures

M. Pelin[1], S. Sosa[1], C. Ponti[1], D. Gibellini[2], C. Florio[1], A. Tubaro[1]

[1] Dept. of Life Science, University of Trieste, Via L. Giorgieri 7/9, 34127 Trieste, Italy
[2] Dept. of Haematology and Oncological Sciences, University of Bologna, Via Massarenti 9, 40138 Bologna, Italy

Palytoxin (PLTX) is a non-proteinaceous marine toxin identified in corals of the genus *Palythoa* and subsequently detected in *Ostreopsis* microalgae and *Trichodesmium* cyanobacteria. These organisms are common in tropical or subtropical areas, but in recent years *Ostreopsis* has also spread in the Mediterranean area, where its blooms are often associated to conjuntivities, rhinorrhea, fever and dermatitis in people after recreational and occupational exposure (Tubaro et al., 2011). After cutaneous contact to PLTX and/or analogues, skin irritation and inflammatory effects were observed, suggesting that the skin could be one of the main target organs of the toxin. Indeed, PLTX's dermotoxicity is still a rather underestimated, but increasingly expanding, sanitary problem. With this study we aimed to investigate the inflammatory effects of PLTX after cutaneous exposure. To this end, we used the skin keratinocytes HaCaT cell line, a predictive model for the evaluation of *in vitro* toxicity at skin level. The effect on the inflammatory cytokines IL-6, IL-8, TNF-α and IL-1α was initially investigated. At sub-cytotoxic concentration (10^{-11} M), PLTX caused a rapid increase of IL-6, IL-8 and TNF-α mRNA expressions, followed by IL-6 and IL-8 release after 24 h exposure. These data are in line with a previous study showing accumulation of IL-8 and TNF-α mRNAs in human macrophages (Crinelli et al., 2012). Furthermore, other two inflammatory mediators, histamine and prostaglandin E_2, were released in a time-dependent manner, already after 1 h exposure.

With the aim to evaluate the involvement of these inflammatory mediators in the progression of PLTX skin inflammation, co-cultures of immunity cells with HaCaT cells were set up. To this aim, undifferentiated (monocytes) and PMA-differentiated (macrophages) THP-1 cells, and primary cultures of human lymphocytes were chosen. The replicative and migratory responses of monocytes, macrophages and lymphocytes were studied after exposure to PLTX-treated HaCaT conditioned media. As well as PMA, conditioned media induced a time-dependent differentiation of THP-1 cells, that reached a maximum after 72h of treatment. Similarly, a significant increase in cell proliferation was observed in lymphocytes exposed to conditioned media for 48 and 72 h.

Finally, the chemotactic potential of the IL-6, IL-8 and histamine-containing conditioned media on monocytes, macrophages and lymphocytes was investigated. PLTX-treated HaCaT cells conditioned media stimulated a migratory response in all three cell models with an order of potency of monocytes>lymphocytes>macrophages. Moreover, lymphocytes and macrophages chemotaxis entity was not statistically different from that induced by the positive control MIP-3α, suggesting that for lymphocytes and macrophages the maximum migration was attained. On the whole, this data suggest an involvement of macrophages, but especially of lymphocytes, in the modulation of inflammation at the skin level by PLTX. However, further experiments are required to elucidate the role of other cells, such as dendritic cells.

Crinelli et al., 2012, Plos ONE 7(6):e38139
Tubaro et al., 2011, Toxicon 57(3):478-95