Cytotoxicity and detection of new analogues of palytoxin

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Palytoxin (PLTX) is the parent compound of a family of marine toxins, detected in corals of the genus *Palythoa*, in benthic dinoflagellates of the genus *Ostreopsis* and in cyanobacteria of the genus *Trichodesmium*. Recently, a new PLTX analogue, named ovatoxin-a (OVA-a), has been identified as the major toxin produced by *O. ovata* in the Mediterranean Sea, while a diastereoisomer of 42-hydroxy-palytoxin (42-OH-PLTX), with a configurational inversion at C50, was isolated from *Palythoa tuberculosa* corals. Due to the structural difference from the parent compound PLTX, their toxicological potential can be different.

In spite of the small amount of pure analogues available, preliminary *in vitro* studies were carried out to evaluate their cytotoxicity. Considering the increasing human cases of dermotoxicity ascribed to palytoxins after marine aerosol exposure during *Ostreopsis* blooms and/or handling zoanthid corals in home aquaria, their effect on human skin keratinocytes was evaluated by the MTT assay. After a 4-h exposure, the cytotoxicity of OVA-a as well as that of the 42-OH-PLTX isomer was lower than PLTX's. In more details, OVA-a and the 42-OH-PLTX isomer reduced cell viability with an EC_{50} of $1.3x10^{-9}$ M (95% confidence limits, CL: 0.6-$2.7x10^{-9}$ M) and $8.3x10^{-9}$ M (95% CL: 6.3-10.7 x 10^{-9} M), respectively, being almost two and three orders of magnitude less toxic than PLTX (EC_{50} of $3.1x10^{-11}$ M; 95% CL: 0.9-$9.8x10^{-11}$ M). Moreover, the new 42-OH-PLTX isomer was about two orders of magnitude less toxic than 42-OH-PLTX (EC_{50} of $1.0x10^{-10}$ M; 95% CL: 0.5 - 2.5 x 10^{-10} M). Even if lower than that of PLTX, the cytotoxicity of OVA-a, 42-OH-PLTX and its isomer was displayed at nanomolar concentrations after a short exposure (4 h).

Considering that OVA-a is the major PLTX analogue in Mediterranean Sea and the possible presence of 42-OH-PLTX and/or its isomer in corals, their detection is crucial. Thus, an indirect sandwich ELISA, based on murine monoclonal anti-PLTX antibody and rabbit polyclonal antibodies, was developed. It proved able to detect PLTX, 42-OH-PLTX and OVA-a in the same manner, and to a less extent also the 42-OH-PLTX isomer.