In vitro effects of palytoxin and 42-hydroxy-palytoxin on skeletal muscle cells

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The marine polyethers palytoxins (PLTXs) are considered among the most toxic natural compounds, although their actual toxic potential for humans is not completely characterized. PLTXs have been detected in different species of marine organisms, and occasionally, they have been associated with cases of human food-born poisonings. After oral and cutaneous exposure, common signs and symptoms ascribed to PLTXs include myalgia and elevated serum levels of creatine phosphokinase, which suggest skeletal muscle as one of the main targets of these toxins (Tubaro et al., 2011a).
42-hydroxy-palytoxin (42-OH-PLTX) is a congener of PLTX isolated in the last years from the coral *Palythoa toxica* (Ciminiello et al., 2009) and identified also in marine cyanobacteria of the genus *Trichodesmium* (Kerbrat et al., 2011), suggesting a possible wide distribution of this analogue in marine environment as well as in home aquaria. Acute oral toxicity studies in mice on both PLTX and 42-OH-PLTX evidenced symptoms and hematoclinical alterations supporting epidemiological data indicating skeletal muscle tissue as a sensitive target for these compounds (Sosa et al., 2009; Tubaro et al., 2011b). Thus, 42-OH-PLTX was investigated for its *in vitro* effects on mouse skeletal muscle cells, in comparison to the parent compound PLTX. After 24 h exposure, 42-OH-PLTX and PLTX exerted a concentration-dependent cytotoxic effect with an EC_{50} of 0.36 ± 0.06 nM, comparable to that of PLTX (IC_{50} = 0.54 ± 0.07 nM). Each toxin induced an increase of cell volume within 10 min, with a toxic insult developing within 1 h. Moreover, Ca^{2+} imaging experiments revealed that both PLTX and 42-OH-PLTX induced a transitory intracellular Ca^{2+} increase (transient phase) followed by a slower and more sustained Ca^{2+} increase (long-lasting phase). The long-lasting phase, sustained by the massive and prolonged Ca^{2+} influx from the extracellular compartment, is also responsible for the cytotoxic effect of these toxins in myocytes.
42-OH-PLTX and PLTX impair also the functional properties of the muscle cells: after 24 h incubation with 0.1nM 42-OH-PLTX or PLTX, the cells maintained the responsiveness to the neurotransmitter acetylcholine (Ach) but the amplitude of ACh-induced intracellular Ca^{2+} variation was significantly reduced in comparison to control cells.
In conclusion, 42-OH-PLTX, similarly to PLTX, provokes a potent cytotoxic effect on skeletal muscle cells, inducing morphological and functional alterations by the impairment of intracellular Ca^{2+} homeostasis. These data indicate that not only PLTX, but also 42-OH-PLTX, could be involved in the muscular effects in human poisonings ascribed to palytoxins.

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