Toxicology Letters 229 (2014) 440–450

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

The marine toxin palytoxin induces necrotic death in HaCaT cells through a rapid mitochondrial damage

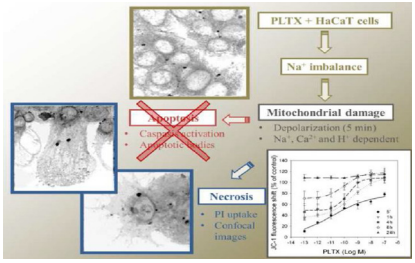
Marco Pelin, Silvio Sosa, Sabrina Pacor, Aurelia Tubaro *, Chiara Florio

Department of Life Science, University of Trieste, Via Valerio 6, 34127 Trieste, Italy

HIGHLIGHTS

- PLTX induces an early and irreversible necrotic cell death.
- Apoptosis occurrence was excluded.
- Necrosis is related to an early mitochondrial depolarization already after 5 min.
- Mitochondrial damage is strictly dependent on ionic imbalance.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form 21 July 2014
Accepted 22 July 2014
Available online 24 July 2014

Keywords:
Palytoxin
HaCaT cells
Mitochondria
Necrosis
Apoptosis

ABSTRACT

Palytoxin (PLTX) is one of the most toxic algal biotoxin known so far. It transforms the Na^+/K^+-ATPase into a cationic channel inducing a massive intracellular Na^+ influx. However, from a mechanistic point of view, the features and the intracellular pathways leading to PLTX-induced cell death are still not completely characterized. This study on skin HaCaT keratinocytes demonstrates that PLTX induces necrosis since propidium iodide uptake was observed already after 1 h toxin exposure, an effect that was not lowered by toxin removal. Furthermore, necrotic-like morphological alterations were evidenced by confocal microscopy. Apoptosis occurrence was excluded since no caspases 3/7, caspase 8, and caspase 9 activation as well as no apoptotic bodies formation were recorded. Necrosis was preceded by a very early mitochondrial damage as indicated by JC-1 fluorescence shift, recorded already after 5 min toxin exposure. This shift was totally abolished when Na^+ and Ca^{2+} ions were withdrawn from culture medium, whereas cyclosporine-A was ineffective, excluding the occurrence of a controlled biochemical response. These results clearly establish necrosis as the primary mechanism for PLTX-induced cell death in HaCaT cells. The rapidity of mitochondrial damage and the consequent irreversible necrosis rise serious concerns about the very fast onset of PLTX toxic effects.

1. Introduction

Palytoxin (PLTX), a marine toxin identified in *Palythoa* zoanthid corals, *Ostreopsis* dinoflagellates and *Trichodesmium* cyanobacteria (Kerbrat et al., 2011; Moore and Scheuer, 1971; Tubaro et al., 2011a), represents an increasing hazard for human health. It is considered one of the most complex and large non-proteinaceous and not polymeric molecules found in nature (Fig. 1). Depending on the natural source, there are several structural analogs of PLTX. Four of them are the most studied and characterized under a chemical and/or biological point of view: (i) 42-OH-PLTX, the main

Abbreviations: BNIP3, Bcl-2/adenovirus E1B 19-kilodalton interacting protein; LDH, lactic dehydrogenase; DAPI, 4′,6-diamidino-2-phenylindole; DIL, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DMA, 5-(*N*,*N*-dimethyl) amiloride; ENaC, epithelial Na^+ channel; MFI, mean fluorescence intensity; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MPTPs, mitochondrial permeability transition pores; PI, propidium iodide; PBS, phosphate buffer saline; PLTX, palytoxin; PFA, paraformaldehyde; NHE, Na^+/H^+ exchanger; ROS, reactive oxygen species; TTBS, tween/tris-buffered salt solution.

* Corresponding author. Tel.: +39 40 5588835; fax: +39 40 5583215.
E-mail addresses: marco.pelin@hotmail.it (M. Pelin), silvio.sosa@econ.units.it (S. Sosa), pacorsab@units.it (S. Pacor), tubaro@units.it (A. Tubaro), florioc@units.it (C. Florio).

http://dx.doi.org/10.1016/j.toxlet.2014.07.022
0378-4274/

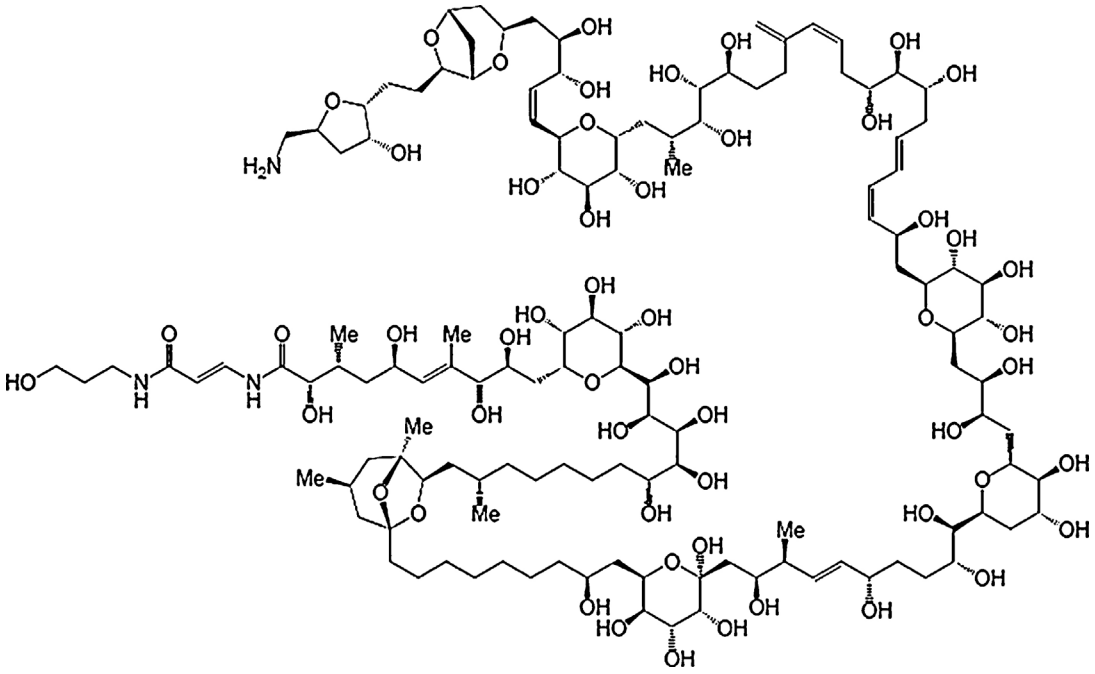


Fig. 1. Molecular structure of PLTX.

compound of *Palythoa toxica* (Ciminiello et al., 2009), whose acute toxicity in mice after oral exposure is comparable to that of PLTX (Tubaro et al., 2011b); (ii) a stereoisomer of 42-OH-PLTX extracted from *Palythoa tuberculosa*, whose cytotoxicity is 100 times lower than that of PLTX (Ciminiello et al., 2014); (iii) ostreocin-D, produced by *Ostreopsis siamensis*, which toxicity appears to be lower than that of PLTX (Ito and Yasumoto, 2009); and (iv) ovatoxin-a, the major toxin produced by *Ostreopsis* cf. *ovata* in the Mediterranean Sea (Ciminiello et al., 2012), which is still under investigation for its biological effects.

Human poisonings ascribed to PLTXs exposure are usually associated to the ingestion of contaminated seafood that, in some cases, resulted in fatal outcomes in tropical areas. In the last two decades, PLTXs have become a relevant hazard also in temperate areas, such as the Mediterranean Sea due to the increasing presence of toxic dinoflagellates of the genus *Ostreopsis*. In this area, episodes of human intoxications characterized by respiratory distress were associated to inhalational exposure to marine aerosol during *Ostreopsis* cf. *ovata* blooms. Moreover, dermatological problems have been frequently ascribed to PLTX after skin contact with seawater during *Ostreopsis* blooms (Durando et al., 2007; Tichadou et al., 2010; Tubaro et al., 2011a). Similarly, severe respiratory reactions, dermatitis and/or other systemic symptoms were reported in people handling *Palythoa*-containing home aquaria, where PLTXs were detected (Deeds and Schwartz, 2010; Hoffmann et al., 2008; Nordt et al., 2011).

Recently, to investigate the effects of PLTX at the skin level, we have studied its activity on the human skin HaCaT cell line, that turned out to be one of the most sensitive cell models to the cytotoxic effect of the toxin (Pelin et al., 2011). At the molecular level, PLTX cytotoxicity is mediated by the interaction with the Na^+/K^+ ATPase pump (Habermann, 1989), which is converted into an open uncontrolled cationic channel. At the cellular level, PLTX toxic effects have been tentatively ascribed to an early mitochondrial dysfunction, as well as to a mitochondrial-mediated oxidative stress (Gabrielson et al., 1992; Kano et al., 1987; Pelin et al., 2013a) leading to a rapid cell death. However, the main features and mechanisms of PLTX-induced cell death have not yet been completely elucidated.

In general, cell death can occur at least by two different well-known mechanisms: apoptosis and necrosis. Even though they can be often interconnected, apoptosis and necrosis are distinct mechanisms of cell death with defined morphological and biochemical features. The first one is an ATP-dependent mechanism characterized by cytoplasmic shrinkage and vacuolisation, chromatin condensation at the nuclear level notwithstanding the intact structure of the cytoplasmic compartments, DNA fragmentation and apoptotic bodies formation (Kanduc et al., 2002). Conversely, necrosis displays early signs of mitochondrial dysfunction (i.e., mitochondrial swelling) that result in production of reactive oxygen species (ROS). Cell membranes become early permeable during the process, with a consequent cell and organelles swelling and, ultimately, plasma membrane rupture with leakage of cell contents (Ziegler and Groscurth, 2004).

Despite the continuously increasing data regarding the characteristics of PLTX interaction with its molecular target, the detailed mechanisms and the intracellular pathways leading to cell death after this first molecular event are still not completely characterized. Indeed, several studies report evidence for PLTX-induced both apoptotic- (Valverde et al., 2008a) and necrotic-like damages (Sagara et al., 2013). Hence, in the present study the main features of PLTX-dependent cell death and the relevant possible intracellular pathways were investigated in skin HaCaT keratinocytes. The study clearly demonstrates that PLTX induces a very rapid mitochondrial damage leading to a necrotic-like cell death.

2. Materials and methods

2.1. Chemicals

HaCaT cell line was purchased from Cell Line Service (DKFZ, Eppelheim, Germany) and all cell culture reagents were from Euroclone (Milan, Italy). Palytoxin, isolated from *P. tuberculosa*, was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan; lot number WKL7151, purity >90%) and aliquots stocked in EtOH 50% at −20 °C. Mitochondrial staining kit JC-1, propidium iodide (PI), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide