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The cytolytic and cytotoxic activities of palytoxin

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ABSTRACT

Palytoxin (PlTX) is one of the largest compound present in nature and, with its strong ability to modify the normal function of different biological systems, is also classified as one of the most potent biotoxins. Many alterations are triggered by PlTX, directly or indirectly related to its interaction with Na^+,K^+-ATPase and the consequent conversion of this ion pump into a non-specific cation channel. The resulting perturbation of Na^+, K^+, Ca^{2+} and H^+ ion fluxes is the driving force of PlTX-induced cytotoxic events, culminating with system disruption and, finally, cell death. The modifications in the distribution of these ions across the plasma membrane play key roles in the promotion of the PlTX-induced cytolytic and cytotoxic responses. In this scenario, PlTX-specific cytolysis can be part, but might not necessarily represent a unique aspect of the cytotoxic effects of the toxin. Owing to the complex array of responses, some of them being cell-type-specific and/or affected by experimental conditions, the distinction between cytolytic and cytotoxic events becomes ill-defined, but the two responses show distinct features, whose further characterization could contribute to a better understanding of the molecular mechanism of cellular effects induced by PlTX.

Palytoxin (PlTX) is one of the largest natural substances known to date, displaying high toxicity in animals (Wiles et al., 1974). From a chemical perspective, PlTX was characterized for the first time by Moore in 1981 (Moore and Bartolini, 1981) and represents one of the largest polyether-type phycotoxin. The toxin is a non-peptide substance constituted by a long, partially unsaturated, chain of 129 carbon atoms which cooperate to the lipophilic nature of this compound. Moreover, the large number of hydroxyl, amine and amide groups as side substituents participate to the hydrophilic characteristics (Moore and Bartolini, 1981; Rossini and Hess, 2010; Uemura et al., 1981a; 1981b). These mix of hydrophilic and hydrophobic features might lead to micelles formation at high concentrations of the toxin in water, behaving in a soap-like manner, and cooperate to the high ability of PlTX to interact with biological systems (Rossini and Hess, 2010).

A study by Wiles et al. (1974) originally brought out the ability of PlTX to exert its toxic effect in a broad range of different animal species, including rats, guinea pigs, and rabbits. In particular this work highlighted the existence of a species-specific susceptibility to PlTX and an indication that its potency is strictly related to the administration route was obtained (see recent reviews by Deeds and Schwartz, 2010; Munday, 2011; Wang, 2008; Wu, 2009).

The set of observation at organismal level were later expanded by more precise and detailed investigations, to clarify the mechanistic aspects of PlTX toxicity in several systems.

The latter investigations thus showed the toxin's ability to trigger a broad variety of effects, including the contraction of vascular smooth muscle and cardiac cells (Ito et al., 1976, 1977, 1979), as well as membrane depolarization (Castle and Strichartz, 1988; Dubois and Cohen, 1977; Kudo and Shibata, 1980; Muramatsu et al., 1984; Pichon, 1982; Sheridan et al., 2005; Tosteson et al., 1991).

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Cell death is frequently recorded in isolated cells exposed to PlTX (Bignami et al., 1992; Ledreux et al., 2009; Schilling et al., 2006), where the toxin induces responses displaying uncommon characteristics. In general, cell death results in rupture of plasma membrane and the release of cellular contents *in vitro*. This phenomenon is observed also in the case of apoptosis, as the possible intervention of macrophages for the removal of membrane-enclosed cellular remnants (Orrenius et al., 2003) may not occur when systems comprise only a single cell line in culture vessels. Cytolytic and cytotoxic responses to a toxicant, therefore, often represent complementary aspects of the same process in isolated cells, as cell lysis is a consequence of collapsed cell functioning and represents the outcome of a toxic effect.

When PlTX acts on isolated cells, however, more subtle features become apparent, as cytolysis can be induced abruptly, and a description of the process might better fit the contention that cell death is the outcome of a cytolytic response.

This issue was brought to our attention by some experiments reported in the original paper by Habermann et al. (1981), when cytolysis was induced in erythrocytes that had been exposed to PlTX, after toxin removal from incubation medium and cell re-suspension in an isotonic buffer in the absence of the toxin (see, for instance, Fig. 2 in Habermann et al., 1981).

We exploited those observations in our investigations, studying the effect of PlTX on MCF-7 cells, cultured as mono-layers (Bellocci et al., 2008), and the time course of a typical cytolytic effect induced by 0.3 nM PlTX, as measured by the release of cytosolic lactate dehydrogenase (LDH) in incubation medium, is shown in Fig. 1. In the first part of cell incubation, a constant level of LDH activity was measured in our samples, independently of the presence or absence of PlTX, and it was due to the enzyme contributed by the serum added to the complete culture medium used for cell treatment. The cell exposure to 0.3 nM PlTX for such a short time in culture medium, therefore, was not sufficient, by itself, to induce cell lysis in MCF-7 cells. When the culture medium was removed, replaced by isotonic buffer devoid of PlTX, and the incubation of MCF-7 cells was continued, in turn, relevant changes in the levels of LDH were recorded in the buffer bathing the cells (Fig. 1). An abrupt increase in LDH activity, in fact, was detected in the fresh incubation buffer of cells that had been previously exposed to PlTX, and this increase progressed over time in the second part of the incubation in the absence of toxin. Background levels of LDH activity, in turn, were found in the fresh incubation buffer added to control cells in paired samples (Fig. 1).

Under these experimental conditions, therefore, cytolysis was induced by the addition of an isotonic buffer to isolated cells, and the effect depended on prior cell exposure to PlTX. Thus, cell lysis apparently resulted from some catastrophic event, and cell death could be viewed as the outcome of cytolysis.

The apparent distinction of cytolytic and cytotoxic responses induced by PlTX can be appreciated through a comparison of the behavior of different cell lines subjected to an identical experimental procedure, as reported in Fig. 2. In these experiments, the responses of human epithelial cells (MCF-7) and neuroblastoma cells (SH–SY5Y) to increasing concentrations of PlTX are compared. The analysis was carried out measuring two different parameters under our experimental conditions. The protein content recovered from cell cultures was measured as an indicator of the amount of cellular material existing in the samples, whereas the LDH activity was assayed to evaluate the extent of cytolysis occurring in those samples. In these experiments, the procedure was that described in Fig. 1, and consisted of a first incubation of cells with increasing concentrations of PlTX, that was terminated by removal of incubation medium, its replacement by an isotonic buffer devoid of toxin, and the incubation was continued for an additional hour (Fig. 2). The measurement of the protein content of our samples was carried out at the end of the first incubation, whereas the assay of LDH activity was carried out at the end of the second incubation, using aliquots of the buffer bathing the cells.

Under those conditions, increasing toxin concentrations caused a progressive decline in the cellular material remaining in culture dishes of SH–SY5Y cells at the end of the first incubation, and the levels of total protein were almost halved after treatment with 0.5 nM PlTX (Fig. 2). MCF-7 cell cultures, in turn, did not show the same effect, as the cellular material remaining in culture dishes was not decreased by increasing PlTX concentrations under these experimental conditions. The LDH activity measured in the incubation medium at the end of the second part of the incubation was found to be increased by PlTX treatment in a dose-dependent fashion in both cell lines (Fig. 2). In the case of SH–SY5Y cells, however, the response was biphasic, and LDH activity was found to decrease in the samples where the number of cells remaining in the dish after removal of culture medium had also decreased. Overall, these data showed that both cell lines could be induced to lyse by the addition of an isotonic buffer, if they had been previously exposed to effective concentrations of PlTX, but

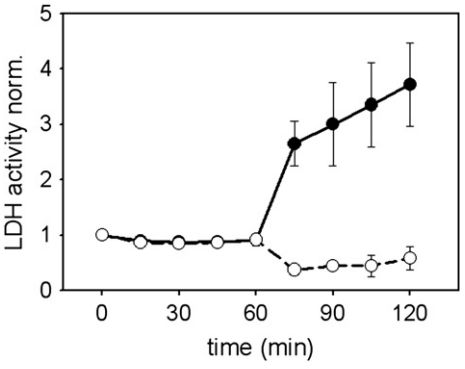

Fig. 1. Cytolytic effect triggered by PlTX in MCF-7 cells. MCF-7 cells grown as monolayer in complete culture medium received 0.3 nM PlTX (black dots), or vehicle (empty dots), and were incubated for 1 h at 37 °C. At the end of the incubation, the medium bathing the cells was removed and replaced with an isotonic buffered solution (20 mM phosphate buffer, pH 7.4, 0.15 M NaCl) devoid of toxin. The cell incubation was then continued for 1 h at 37 °C. LDH activity was measured in aliquots of incubation media which were harvested at 15 min intervals during the full incubation period. The methods used in these experiments are detailed elsewhere (Bellocci et al., 2008).