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Palytoxin and other microalgal toxins belonging to different chemical classes induce cytotoxic effects involving a common set of stress response proteins

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Abstract – The complexity of effects exerted by palytoxin (PlTX) in biological systems is apparent from responses observed in different models. We have approached this complexity by proteomic analyses, to study responses induced by PlTX at a system level. The analysis of a sub-proteome of a human cell line has shown that PlTX causes changes in phosphorylation states of Hsp27 and the oxidation of DJ-1, supporting the conclusion that cell stress responses, including oxidative damage, participate to the cytotoxic effect of PlTX. A descriptive meta-analysis of proteomic data about toxic responses induced by other classes of microalgal toxins, such as okadaic acid and microcystins, reveals that several protein components participating to cell stress responses, particularly oxidative stress, are affected when the systems are exposed to effective doses of those toxins. Detected changes comprise either the total levels of individual proteins, or the relative proportions of isoforms distinguishable according to their post-translational modifications. A common set of protein effectors emerges from our analysis, suggesting that cytotoxic effects exerted by some biotoxins could share molecular processes executing cell death responses.

Toxicity pathways / toxicity networks / stress response / oxidative stress / proteomes / okadaic acid / palytoxin / microcystins

INTRODUCTION

Palytoxin (PlTX) is one of the largest marine non-proteinaceous biotoxins described so far (Ciminiello *et al.*, 2011). The toxicity of palytoxin-group toxins has been recognized long ago, and differences in toxicity according to the route of human exposure to these compounds have been also remarked, highlighting the significant complexity of health issues raised by these toxins (for recent reviews, see Deeds & Schwartz, 2010; Munday, 2011, Tubaro *et al.*, 2011). The complexity of effects exerted by PlTX in biological systems is easily appreciated by considering the mechanism of its action and the molecular bases of responses observed in animal and cellular models (Wu, 2009; Rossini & Bigiani, 2011).

PlTX has been recognized to bind to the extracellular portion of the α subunit of the Na^+,K^+-ATPase in the plasma membrane, thereby converting an energy-dependent ion transporter into a non-specific cation channel (Chhatwal *et al.*, 1983; Ito *et al.*, 1985; Castle & Strichartz, 1988). The normal functioning of

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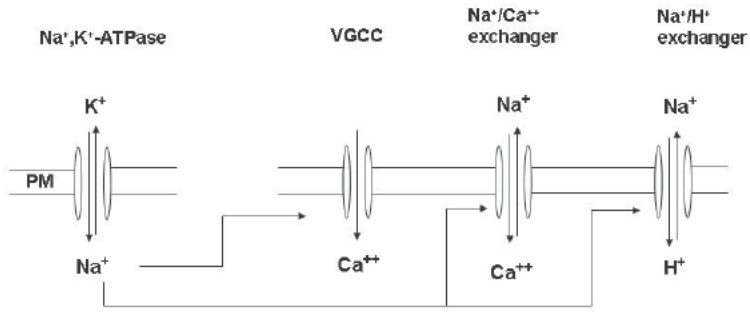

Fig. 1. Schematic representation of the molecular mechanisms of action of palytoxin. The interaction of PlTX with the Na^+, K^+-ATPase converts the pump into a cation channel, leading to sodium entrance. The depolarization of plasma membrane then determines a secondary increase of intracellular Ca^{2+} concentrations, due to the activity of the voltage-gated calcium channel and the Na^+/Ca^{2+} exchangers, acting in a reverse mode. Intracellular acidification may also ensue, as a consequence of increased activity of the Na^+/H^+ exchanger, acting in a reverse mode. The real structural organizations of ion transporters in plasma membrane have been ignored in this illustration, and different components have been represented by the same scheme. PM, plasma membrane; VGCC, voltage-gated calcium channel.

the Na^+,K^+-ATPase, involving the transfer of 2 K^+ ions inside the cell and 3 Na^+ ions outside the cell, therefore, is lost in cells exposed to PlTX (Fig. 1). The resulting cellular condition is characterized by ion imbalance, involving primarily an outflow of K^+ from the interior of the cells, and an increase in intracellular Na^+ concentrations. The raising of intracellular Na^+ ions eventually causes secondary effects, due to membrane depolarization (Fig. 1). These secondary events involve the opening of voltage-gated Ca^{2+} channels, as well as reversing of Na^+/Ca^{2+} and Na^+/H^+ antiports, determining the higher level of complexity in the alterations of cellular ion homeostasis and downstream effects of PlTX (Rossini & Bigiani, 2011).

Owing to the extreme complexity of cellular and organismal effects of PlTX, we have employed proteomic tools to study molecular responses induced by this toxin at a system level.

MATERIALS AND METHODS

The general structure of our experimental setting included exposure of the human MCF-7 cell line, used as a model system, to PlTX concentrations which could induce a toxic response, as judged by the detection of cell death within hours of toxin exposure. The details of the methodology used in our studies can be found in Sala *et al.* (2009a). Briefly, MCF-7 cells were treated with 0.03 nM PlTX for 8 h at 37°C, and the incubation was followed by cell harvesting and lysis, to yield cytosoluble extracts. The cell extracts were then used for proteomic analysis of our model system, and the protein components separated by two-dimensional electrophoresis and staining represented the sub-proteome analyzed in this study. The proteins whose cellular levels had been

altered by exposure to PlTX were detected by image analysis using appropriate softwares, and were further characterized by LC/MS/MS and immunological procedures (Sala *et al*., 2009a).

RESULTS AND DISCUSSION

The proteomic analysis of extracts from cells exposed to PlTX showed that this toxin causes changes in the phosphorylation state of Hsp27 and the oxidation state of DJ-1, which represent two stress response proteins. More precisely, PlTX treatment caused an increase in the cellular levels of Hsp27 phosphorylated in ser 82, and of an acidic form of DJ-1, representing an oxidized isoform of the protein, supporting the conclusion that cell stress responses, including oxidative damage, participate to the cytotoxic effect induced by PlTX in MCF-7 cells (Sala *et al*., 2009a).

The molecular changes induced by PlTX in the cellular pool of the two stress response proteins in MCF-7 cells attracted our attention, because increased cellular levels of phosphorylated Hsp27 isoforms and of oxidized DJ-1 have been recorded in toxic responses induced by another microalgal toxin, okadaic acid (OA), in different cellular systems (Opshal *et al*., 2010), including MCF-7 cells (Sala *et al*., 2009b). These observations were found of particular interest because the molecular mechanism of action of OA differs from that of PlTX, inasmuch as OA alters cellular functioning by binding and inhibiting the major forms of ser/thr phosphoprotein phosphatases (Bialojan & Takai, 1988), leading to a collapse of cellular functioning and cell death (Rossini, 2000).

The apparent converging of different mechanisms of action of PlTX and OA on identical cellular proteins led us to hypothesize that cross-talks might exist in the toxicity pathways of the two different chemical classes of toxins.

Based on these considerations, we approached a descriptive meta-analysis which was targeted onto proteomic data regarding the toxic responses induced by other microalgal toxins. The data gathered from literature revealed that several protein components participating to cell stress responses, particularly oxidative stress, are affected when the systems are exposed to effective doses of different toxin classes, such as OA, PlTX and microcystins. Although these toxins belong to different chemical classes and possess different molecular mechanisms of action (Bialojan & Takai, 1988; MacKintosh *et al.*, 1990; Yoshizawa *et al*., 1990; Rossini & Hess, 2010), nonetheless they share the property to induce death of cells in culture (Rossini, 2000; Gehringer, 2004; Bellocci *et al*., 2011). Major components found differentially expressed in cells exposed to those three classes of toxins included members of the Bcl-2 family of proteins involved in the control of cellular proliferation/death, proteins participating to the control of oxido-reduction states of the cells and energy metabolism, as well as the cytoskeleton (Rossini *et al.*, 2011). Detected changes comprised either the total levels of individual proteins, or the relative proportions of isoforms distinguishable according to their post-translational modifications.

The common set of protein effectors emerging from our analysis suggests that cytotoxic effects exerted by OA, PlTX and microcystins could share common molecular processes, leading to activation of cell stress responses, including oxidative stress, and resulting in cell death (Fig. 2).

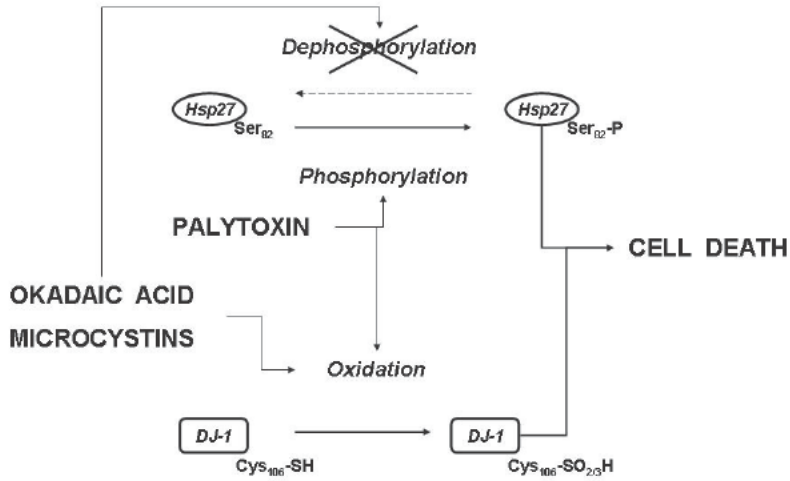

Fig. 2. Schematic representation of the cross-talks between effects of okadaic acid, microcystins and palytoxin in their mechanisms inducing cell death in biological systems (modified from Sala *et al.*, 2009a).

Further molecular analyses of cellular proteomes, including more detailed data on amino acids affected by covalent modifications of individual isoforms of cell stress proteins in cells exposed to microalgal toxins, will contribute to a better characterization of toxicity pathways and their cross-talks in biological systems.

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