GENETIC IDENTIFICATION OF Na+/K+ ATPase ISOFORMS INVOLVED IN THE IN VITRO SENSITIVITY TO THE ALGAL TOXIN PALYTOXIN

1)Pelin M. 2)Brovedani V. 3)Florio C. 4)Stocco G. 5)Sosa S. 6)Tubaro A.

University of Trieste

Palytoxins (PLTXs) are highly toxic compounds identified in marine Palythoa zoanthids, Ostreopsis dinoflagellates and Thricodesmium cyanobacteria. In tropical areas, after accumulation in seafood, PLTXs induced foodborne human poisonings, characterized by severe symptoms even with lethal outcomes. In temperate areas, human poisonings ascribed to PLTXs occurred after inhalational and/or cutaneous exposure to these compounds during Ostreopsis blooms or during cleaning operations of Palythoa-containing home aquaria.

Epidemiological and molecular evidences suggest a different inter-individual sensitivity to PLTXs that could be related to genetic-dependent differences in the expression of the Na+/K+-ATPase, the molecular target for these toxins. Indeed, both lethal outcomes and absence of symptoms have been reported in humans after consumption of the same PLTX-contaminated seafood. Moreover, the binding affinity of cardioactive glycosides to Na+/K+-ATPase, drugs sharing with PLTXs the same molecular target, has been demonstrated to be isoform-dependent. Thus, this study was carried out to identify the specific Na+/K+-ATPase isoforms correlated to the in vitro sensitivity to PLTX.

To this aim, a panel of 9 cell lines (from skin, liver, breast, intestine and pancreas) were evaluated for their sensitivity to PLTX by means of cytotoxicity (EC50, concentration reducing cell viability by 50 %; MTT reduction assay) and toxin-cells binding (Kd, binding affinity, and Bmax, maximum PLTX binding; cell-based ELISA). The results were then correlated with the Na+/K+-ATPase protein expression (flow cytometry) and the gene expression for the different isoforms of the α (α1-4) and β (β1-3) subunits of the Na+/K+-ATPase (real time PCR).

Among the 9 cell lines, a significant variability of the sensitivity parameters was recorded (median EC50= 5.7x10-10 M; interquartile range = 1.5x10-10 M -1.9x10-9 M; median Kd = 8.1x10-10 M; interquartile range = 2.2x10-10 M -2.4x10-9 M; median Bmax = 0.015; interquartile range = 0.0095 – 0.02738), suggesting significant differences in the cells sensitivity towards PLTX. However, the protein expression of the Na+/K+-ATPase was not correlated with PLTX cytotoxicity (EC50 values at MTT assay; r = -0.0359, p value = 0.9270; Pearson correlation) nor with PLTX-cells maximum binding (Bmax values; r = 0.0061, p value = 0.8752; Pearson correlation) or affinity (Kd values; r = 0.5348, p value = 0.1721; Pearson correlation), suggesting that the cells sensitivity to PLTX is not related to the level of Na+/K+-ATPase protein expression. On the contrary, cells sensitivity to PLTX appears to be related with gene expression of specific α and β isoforms of the Na+/K+-ATPase. Indeed, a significant variability in gene expression for all the isoforms was found among the 9 cell lines. Intriguingly, a significant positive correlation was found between Kd values and β2 gene expression (r = 0.8052, p value = 0.0159; Pearson correlation) and between Kd values and the ratio of α1/α2 gene expressions (r = 0.7225, p value = 0.0279; Pearson correlation), suggesting a

significant role of these isoforms in PLTX binding to Na+/K+-ATPase. In addition, an inverse correlation trend was found between Kd values and the ratio of β1/β2 gene expressions (r = -0.7073, p value = 0.0728; Pearson correlation) and α2/β2 gene expressions (r = -0.7269, p value = 0.0608; Pearson correlation).

On the whole, for the first time, these data indicate a significant correlation between specific Na+/K+-ATPase isoforms and the in vitro cells sensitivity to PLTX. These results could explain the different inter-individual sensitivity of humans exposed to PLTX.