Acute and chronic in vivo toxicity of the marine toxin palytoxin

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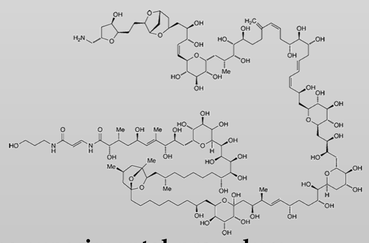
Introduction

Palytoxin (PLTX) is a marine toxin recognized amongst most poisonous substances known to date. PLTX production is associated to dinoflagellates of the genus *Ostreopsis*. Currently, this compound is considered as an emergent toxin in Europe and its prevalence in continental European waters has increased during the last years. The high toxicity of palytoxin is related with the binding to the Na^+-K^+ ATPase, converting this ubiquitously distributed enzyme in a permeant cation channel [1-3]. Several reports have shown that this toxin is responsible for human fatal intoxications, either after inhalation of toxin-containing marine aerosols or after ingestion of marine products contaminated with PLTX, such as crabs, groupers, mackerel, and parrotfish. So far, different groups have explored the acute oral toxicity of PLTX in mice however, discrepancies in the PLTX source as well as in the monitoring time for the toxic effects yielded controversial results. Although the presence of palytoxin in marine products is not yet currently regulated in Europe, the European Food Safety Authority (EFSA) expressed its opinion on PLTX toxicity and prompted the need to obtain more data regarding the *in vivo* toxicity of this compound [4]. Therefore, in this study, the acute and chronic toxicity of palytoxin was evaluated after oral administration of the toxin to mice either in a single dose and in a follow-up period of 96 hours or after chronic administration during a 28-day period.

Methods

Toxin and chemicals

PLTX (from *Palythoa tuberculosa*) was purchased from Fujifilm Wako Chemicals and dissolved at a concentration of 150 µM in DMSO



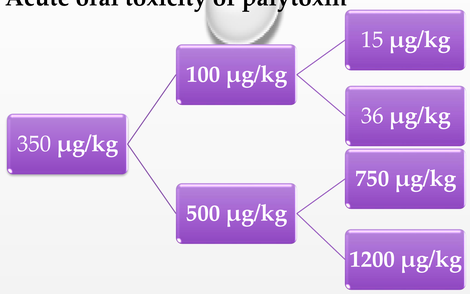
Chemical structure of PLTX

In vivo experimental procedure

In vivo studies were performed with Swiss female mice weighing 18–21 g. All animal procedures were carried out in conformity to European legislation (EU directive 2010/63/EU) and Spanish legislation (Real Decreto 53/2013, Decreto 296/2008) and to the principles approved by the Institutional Animal Care Committee of the Universidad de Santiago de Compostela. Immediately before administration, PLTX was diluted in 0.9% saline solution to achieve each dose. For acute oral toxicity, PLTX doses ranging from 15 to 1200 µg/kg body weight (bw) were administered by gavage and animals were monitored for 4 days. For chronic oral toxicity doses ranging from 0.03 to 10 µg/kg were selected. PLTX was administered by gavage every 24 h over 28 days, dissolved in a final volume of 200 µL of solution per mouse. During the experiment, moribund animals or animals obviously in pain or showing signs of severe and enduring distress were humanely sacrificed. Animals that survived the observation period were euthanized in a CO_2 chamber.

Results

Acute oral toxicity of palytoxin

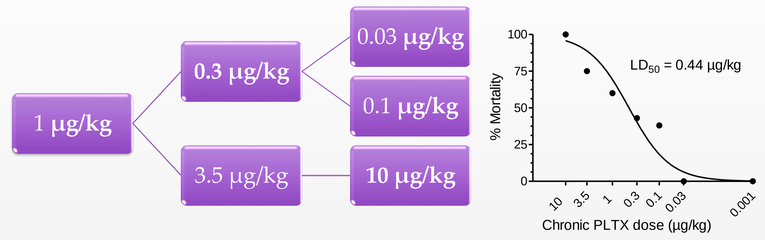


Up-and down procedure followed under OECD guidelines for PLTX single oral administration. Dose response curve with the LD_{50} for PLTX after single oral administration of 599.3 µg/kg (95% Confidence intervals (CI) from 508 to 707 µg/kg R^2 0.9712). Results are expressed as means ± SEM of the data obtained from three to nine animals.

Oral dose (µg/kg)	Total mice	Dead	Survival time (hours)
Control	7	0	0
15	3	0	96
36	6	0	96
100	6	0	96
350	4	1	3 h 25 m
500	9	3	6, 18, 4
750	5	3	4, 9, 3
1200	3	3	10 m, 15 m, 1 h 30 m

Survival times and dead rate induced by single oral administration of PLTX to mice corresponding with each treatment.

Chronic oral toxicity of palytoxin

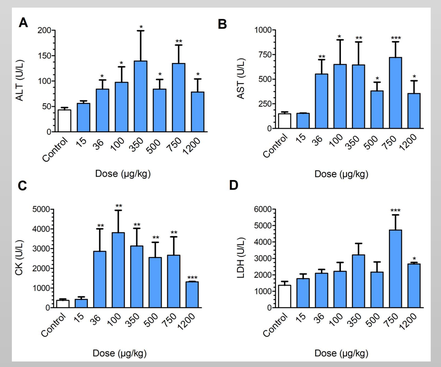


Oral doses of PLTX administered to mice for a 28-day period. Non linear fit of the mortality rate against the chronic oral toxin dosage gave an estimated LD_{50} for PLTX of 0.44 µg/kg (95% confidence interval (CI) from 0.22 to 0.9 µg/kg, R^2 = 0.9122). Values are means ± SEM of the data obtained from three to ten animals.

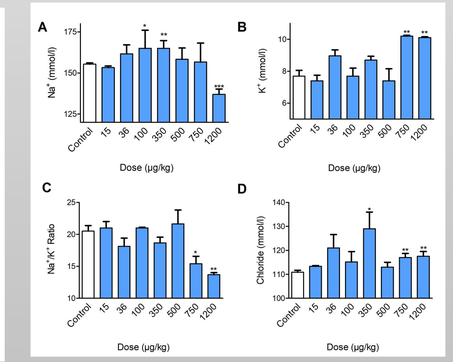
Dose (µg/kg)	Total mice	Dead	Survival time (days)
Control	6	0	28
0.03	5	0	28
0.1	8	3	15, 22, 27, 28, 28, 28, 28, 28
0.3	7	3	17, 18, 24, 28, 28, 28, 28
1	10	6	2, 2, 14, 15, 22, 25, 28, 28, 28, 28
3.5	8	6	0.3, 8, 9, 15, 15, 16, 28, 28
10	3	3	8, 10, 18

Survival times observed for each mouse fed daily with PLTX during a 28-day period. The toxin lethality increased daily feeding of mice with PLTX during 28 days, a daily oral dose of 10 µg/kg led to a mortality rate of about 100% with survival times between 8 and 18 days.

Single oral administration of PLTX by gavage to Swiss mice drastically modify blood biochemical parameters

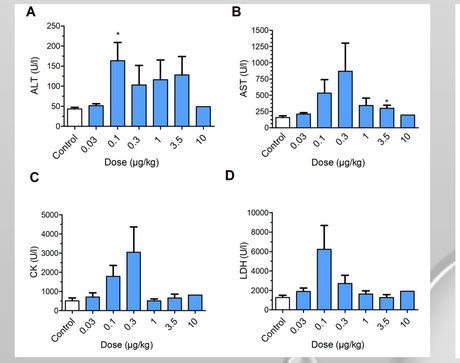


Effects of single exposure of mice to 15, 36, 100, 350, 500, 750, 1200 µg/kg of PLTX on blood levels of ALT (A), AST (B), CK (C) and LDH (D). Data are expressed as mean ± SEM from 2 to 7 determinations. *p < 0.05, **p < 0.01, ***p < 0.005 vs control values.

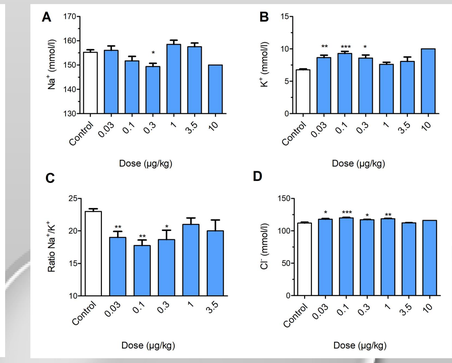


Bar graphs representing the effects of the different oral doses of PLTX on electrolyte blood levels as sodium (A), potassium (B) ratio Na^+/K^+ (C) and chloride (D). Data are expressed as mean ± SEM of 2–9 determinations. *p < 0.05, **p < 0.01, ***p< 0.005 vs control values.

Daily feeding of mice with PLTX at very low doses elicited changes in blood enzymes and in the electrolyte levels after the 28 days of the oral administration period



Effects of 28 days repeated exposure of mice to 0.03, 0.1, 0.3, 1, 3.5 and 10 µg/kg of palytoxin on blood of ALT (A), AST (B), CK (C), and LDH (D). Data are expressed as means ± SEM from 1-7 samples. * $p < 0.05$ versus control mice.



Bar graph representation of the blood levels of sodium (A), potassium (B), ratio sodium/potassium (C) and chloride (D) in control mice and in mice dosed daily by gavage with palytoxin at doses ranguing from 0.03 to 10 µg/kg. Data are expressed as means ± SEM from one to seven determinations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control mice.

Conclusions

The LD_{50} for PLTX obtained by the single oral dose was 599 µg/kg while the LD_{50} for the repeated daily exposure of PLTX was lower, with a value of 0.44 µg/kg.

The Non Observable Adverse Effect Level (NOAEL) for palytoxin was 10 µg/kg after single gavage administration to mice and 0.003 µg/kg after administration of repeated doses of PLTX.

Enzyme and electrolyte alterations on blood levels even in low doses illustrate the high toxicity of this marine compound.

Chronic studies are a valuable approach in order to prevent the human health risk associated with human fishery products and regulate the food levels of this emerging marine toxin.

References

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Acknowledgments

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