Oral chronic toxicity of the marine toxin tetrodotoxin

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INTRODUCTION

Tetrodotoxin (TTX) is a very toxic compound responsible for human intoxications after ingestion of contaminated fishery products. TTX was initially associated mainly with human fatalities occurring in Asiatic countries [1], but it has expanded to other regions including European countries. In Europe, the first non-fatal human intoxications by TTX was reported more than 10 years ago after the ingestion of a *Charonia lampas* trumpet shell captured in the Portuguese coast and commercialized in Spain [2]. Since then, during the last decade, the presence of the TTX-containing pufferfish *Lagocephalus sceleratus* has been reported in European coasts, mainly in the Mediterranean Sea [3,4] with some fish tissues containing TTXs amounts as high as 2 mg/kg [5]. Moreover, an increasing concern regarding food safety has been raised after the detection of TTX in mussels, oysters and clams harvested in UK, Greece, the Netherlands [6-8] and Spain. The current European legislation in marine toxins has not yet regulated the levels of TTX in fishery products. Considering initial data on the acute oral toxicity of TTX [9] and in view of the EFSA opinion remarking the need for additional chronic toxicity studies to further reduce the uncertainty of the likely future toxin regulation.

The main objective of this work was to evaluate the oral chronic toxicity of TTX over a period of 28 days using protocols internationally validated to test the toxicity of this compound.

METHODS

All animal procedures were carried out in conformity to European and Spanish legislation and to the principles approved by the Institutional Animal Care Committee of the Universidad de Santiago de Compostela. TTX was purchased from Tocris and quantified against a certified reference material (CRM TTX) acquired from CIFGA. Swiss female mice were fed with different TTX doses by gavage every 24 h over 28 days. First dose of TTX evaluated to test its chronic toxicity was 20 µg/kg/day followed by 25, 44, 75 and 125 µg/kg. During experiments, control and TTX-treated mice were weighted weekly at day 0, 7, 14, 21 and day 28, and food consumption was also registered at the same intervals. Euthanasia was performed on day 28. Before the last TTX-dosage, on day 27, control and TTX-treated mice were placed in metabolic cages for 24 h to monitor urine and feces production and obtain samples. Finally, animals were euthanized in a CO_2 chamber.

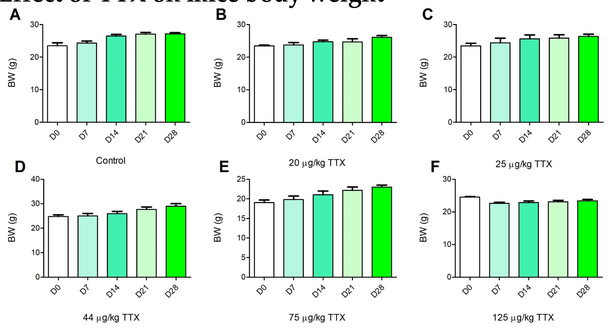
RESULTS

Percent of mortality and survival times observed for each mouse fed daily with TTX during a 28 day period

Dose (µg/kg)	Total mice	Dead	Survival time (days)	Mortality %
Control	10	0	28	0
20	8	0	28	0
25	3	0	28	0
44	10	0	28	0
75	4	2	1, 5, 28, 28	50
125	5	2	3, 7, 28, 28, 28	40

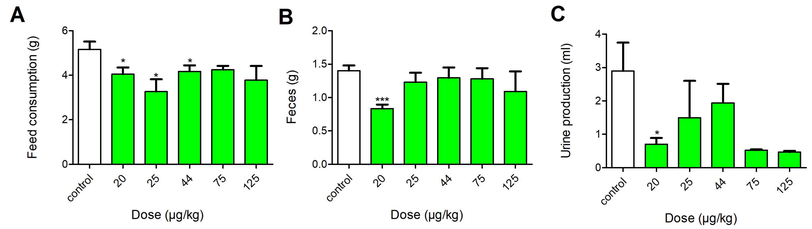
Mortality rate evoked in the repeated 28 day oral toxicity study in mice after gavage administration of tetrodotoxin at doses of 20, 25, 44, 75, 125 µg/kg and survival times corresponding to each treatment.

Effect of TTX on mice body weight



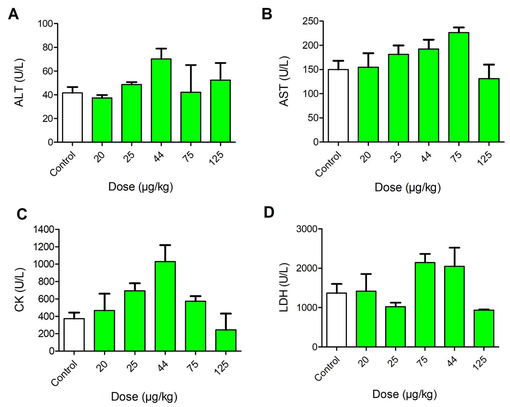
Effect of repeated oral exposure of mice to different doses of TTX on body weight (BW) during the treatment period. During experiments, control and TTX-treated mice were weighted weekly at day (D0), day 7 (D7), day 14 (D14), day 21 (D21) and day 28 (D28).Values are expressed as mean ± SEM. All animals increase their BW except those treated with the highest doses of TTX (125 µg/kg).

Results food intake, feces and urine production during 24 h after the last TTX dose

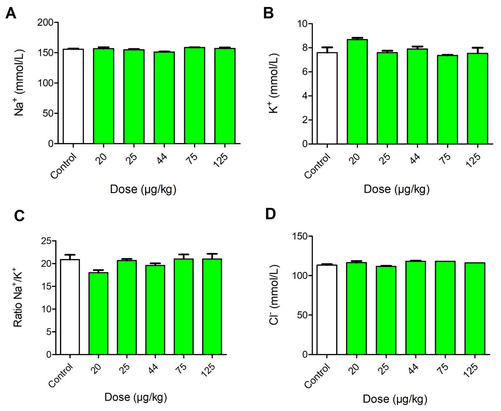


Food intake (A), feces (B) and urine production (C) over a 24 h period in control mice and in mice treated with TTX. Feed consumption slightly decrease in treated mice. Urine production was decreased in mice treated with TTX at doses of 20 µg/kg, but no significant changes were found in higher doses mainly due to the limited number of animals.

Results of 28-day repeated exposure of mice to TTX on blood biochemical parameters



Bar graphs showing no effects of daily TTX administration on either blood alanine aminotransferase (ALT) , aspartate aminotransferase (AST), creatin kinase (CK) and lactate dehydrogenase (LDH). TTX was administered daily by gavage and after sacrifice blood levels of ALT (A), AST (B), CK (C), and LDH (D) were measured. Data are expressed as means ± SEM.



Bar graphs representation of the blood levels of sodium (A), potassium (B), ratio sodium/potassium (C) and chloride (D) in control Swiss female mice and in mice dosed daily by gavage with TTX at 20, 25, 44, 75 and 125 µg/kg. No electrolyte dysregulation were present after administration of repeated low doses of TTX.

Urine parameters in control mice and in mice treated daily with TTX

	Control (n=10)	20 µg/kg TTX (n=4)	25 µg/kg TTX (n = 3)	44 µg/kg TTX (n=10)	75 µg/kg TTX (n = 2)	125 µg/kg TTX (n = 3)
Urine protein (g/l)	0.9 ± 0.7	0.3 ± 0.2	0.4 ± 0.3	0.3 ± 0.0	3 ± 2	1
Glucose (mmol/l)	0.6 ± 0.4	3 ± 0.0	1.1 ± 0.9	3.3 ± 3.0	3	7.7 ± 4.7
Ketones (mmol/l)	0.15 ± 0.15	0.8 ± 0.4	Negative	Negative	1.5	1 ± 0.5
Hemoglobin (Ery/µl)	Negative	2.5 ± 2.5	Negative	4 ± 1.6 *	Negative	Negative
Bilirubin (µmol/l)	6.7 ± 5.1	8.5 ± 4.9	Negative	35.2 ± 8.7 *	Negative	50
Urobilinogen (µmol/l)	1.7 ± 1.7	8.5 ± 4.9	Negative	3.4 ± 2.3	Negative	52.3 ± 17.7

Analytical results of urine in control animals and in animals treated daily with TTX at doses of 20, 25, 44, 75 and 125 µg/kg for 28 days. Values are expressed as mean ± SEM and the number of animals used to evaluate urine parameters is shown in parenthesis for each dose. For some mice-group, ketones, hemoglobin, bilirubin and urobilinogen were not present in urine samples and are expressed in the table as negative. * $p < 0.05$ versus control animals. For urine protein, glucose, ketones, blood hemoglobin, and bilirubin values are represented by mean ± SEM.

CONCLUSIONS

- Animals treated with TTX at doses of 125 µg/kg showed a decrease in urine production compared with control animals. Additionally, high doses of TTX provoked proteinuria bilirubinuria and presence of urobilinogen in urine, symptoms indicative of nephrotoxicity.
- The safety level of the TTX dose established by EFSA did not cause alterations in mice after 28 daily oral treatment.
- Combinations of different toxins should be considered for public health purposes specially if they share a common cellular target.
- Further studies are needed to evaluate the potential harmful effects of chronic exposure to low oral doses of TTX.

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