Effects of synthetic ciguatoxin CTX3C and 44-methylgambierone (MTX3) on voltage-gated sodium channels and their in vivo toxicity

Andrea Boente-Juncal, Sandra Raposo-García, Celia Costas, M Carmen Louzao, Carmen Vale*, Luis Botana

Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain.
*Correspondence: mdelcarmen.vale@usc.es; Tel.: +34-982-822-223.

Introduction

Gambierdiscus species are marine dinoflagellates producers of toxins causative of a widespread human illness known as Ciguatera Fish Poisoning (CFP) which comprises gastrointestinal, neurological, and cardiovascular symptoms. Blooms of these dinoflagellates have expanded worldwide reaching even European coasts. In fact, the presence of *Gambierdiscus* species and the related toxins and CFP intoxications have been repeatedly identified in Europe during the last decades, especially in the Canary Islands [1,2] and Madeira [3]. Besides ciguatoxins, which can cause long term neurological complications in humans as a consequence of their permanent activation voltage-gated sodium channels [4-6], the structure of an additional ciguatoxin-related toxin named 44-methylgambierone (MTX3) has been recently elucidated [7]. Initial studies on the biological activity of 44-methylgambierone described an effect similar to that of the synthetic ciguatoxin CTX3C although of much lower potency [7].

Objectives

- Explore the relative *in vivo* and *in vitro* toxicities and activities of these compounds.
- Evaluate neurotoxic effect of CTX3C and MTX3 together with ouabain and veratridine
- Compare their effects on voltage-gated ion channels.
- Determine oral chronic *in vivo* toxicity after an administration period of 28 days to assess behavioral and biochemical alterations.

Methods

Neuroblastoma cell line. Human SH-SY5Y were used for determination of cellular viability. Cells were exposed for 4 h to CTX3C (Wako) or MTX3 (Cifga) in the presence or absence of Ouabain (O) and Veratridine (V).

Determination of cellular viability. Treated cells were rinsed and incubated for 1 h with a solution of MTT (methylthiazolyldiphenyl-tetrazolium bromide) salt dissolved in Locke's solution. Absorbance was measured at 595 nm in a spectrophotometer plate reader Synergy 2.

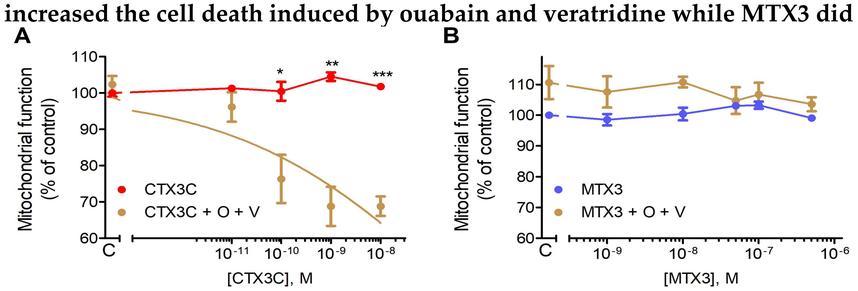
hNa$_v$ 1.6 HEK cell line. HEK293 cells transfected with hNa$_v$ 1.6 were used under an MTA with Dr Andrew Powell (GlaxoSmithKline R&D, UK). Before electrophysiological experiment cells were incubated at 30 °C for 24–36 h.

Electrophysiology. Whole cell configuration recordings were conducted to register the activation of voltage-gated sodium channels. Voltage steps from −80 to +80 mV were applied. A computer-controlled current and voltage clamp amplifier was used. Signals were recorded and analyzed using a computer equipped with a Digidata 1440 data acquisition system and the pClamp10 software.

***In vivo* experimental procedure.** *In vivo* studies were performed with Swiss female mice weighing 23–26 g. 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 USC. CTX3C at 10, 32 and 100 ng/kg bw (body weight) or MTX3 at 550 or 1760 ng/kg bw were administered by gavage to mice for 28 days.

Results

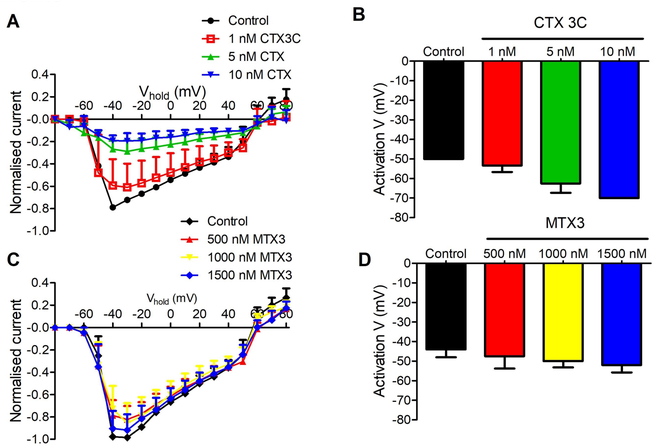
CTX3C increased the cell death induced by ouabain and veratridine while MTX3 did not



Effect of CTX3C and MTX 3 on cell viability. Four hours exposure of SH-SY5Y cells to different concentrations of CTX (0.01 to 10 nM) or MTX3 (1 to 300 nM) alone or in co-incubation with 10 nM ouabain and 150 µM veratridine. A) CTX3C in co-incubation with O+V induced cell death in a concentration dependent manner. B) MTX3 alone or in combination with O+V did not affect cellular viability. Results are expressed as mean ± sem.

Results

CTX3C decreased the sodium current amplitude and hyperpolarized the activation potential of voltage-gated sodium channels but MTX3 had no effect on sodium channel currents



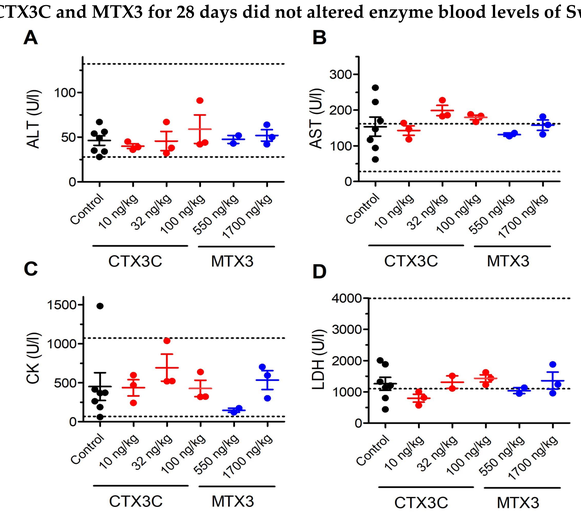
Concentration-dependent effects of CTX3C and MTX3 on the Na$_v$1.6 voltage-gated sodium. (A) I–V relationship for the effect of 1, 5 and 10 nM CTX3C. (B) Pooled results for the concentration dependent effects of CTX3C on the voltage activation of sodium channels. (C) MTX3 at concentrations ranging from 500 to 1500 nM, did not affect the current through the Na$_v$1.6 channel. (D) Effect of MTX3 on the voltage activation of Na$_v$1.6 currents. Results are expressed as mean ± sem.

Chronic toxicity elicited by daily oral CTX3C and MTX3 administration

	Total mice	Dead	Survival time (days)	Mortality %
Control	7	0	28	0
10 ng/kg CTX3C	3	0	28	0
32 ng/kg CTX3C	3	0	28	0
102 ng/kg CTX3C	3	0	28	0
550 ng/kg MTX3	2	0	28	0
1760 ng/kg MTX3	3	0	28	0

Oral doses of CTX3C and MTX3 and survival times observed after daily toxin administration for 28-day period.

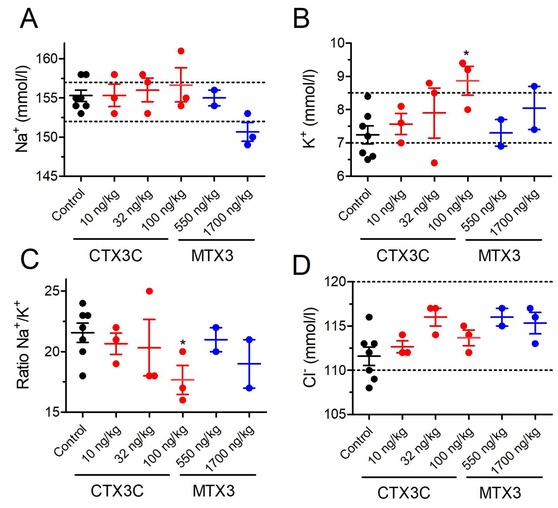
Daily oral doses of CTX3C and MTX3 for 28 days did not altered enzyme blood levels of Swiss mice



Scatter plot graphs showing representing blood levels of alanine transaminase (ALT) , aspartate transaminase (AST), creatin kinase (CK), and lactate dehydrogenase (LDH) in control Swiss female mice and in mice dosed daily by gavage with CTX3C and MTX3. The respective minimum and maximum reference blood values for each parameter are marked by the pointed lines.

Results

CTX3C and MTX3C induced subtle changes in electrolyte blood levels



*Blood levels of sodium, potassium, ratio sodium/potassium and chloride in control Swiss female mice and in mice dosed daily by gavage, over a 28-day period, with CTX3C or MTX3, * $p < 0.05$ versus control mice. The respective minimum and maximum reference values of blood electrolyte levels are indicated by the pointed lines.*

Conclusions

1. Neither CTX3C nor MTX3 alone affected neuronal cells viability or animal death after chronic oral treatment.
2. CTX3C exacerbated the cell death induced by veratridine and ouabain while MTX3 did not modify it.
3. Synthetic CTX3C inhibited voltage-gated sodium currents in a concentration-dependent manner and caused the activation of the channels at more hyperpolarizing potentials but MTX3 did not affect neither the sodium current amplitude nor the voltage activation of sodium channels.
4. Daily administration of CTX3C and MTX3 did not alter blood biochemical parameters of Swiss mice.
5. The results presented here confirm previous findings indicating that MTX3 exhibited lower potency than CTX3C.

Acknowledgments

The research leading to these results has received funding from the following FEDER cofunded-grants. From Conselleria de Cultura, Educacion e Ordenación Universitaria, Xunta de Galicia, 2017 GRC GI-1682 (ED431C 2017/01). From Ministerio de Ciencia e Innovación IISCIII/PI19/001248. From European Union Interreg AlertoxNet EAPA-317-2016, Interreg Agritox EAPA-998-2018, and H2020 778069-EMERTOX. Andrea Boente-Juncal (FPU16/07129) and Celia Costas (FPU18/05681) are recipients of a fellowship from Ministerio de Educación, Cultura y Deporte, Spain.

References

1. Estevez, P.; Sibat, M.; Leão-Martins, J.M.; Tudó, A.; Rambla-Alegre, M.; Aligizaki, K.; Diogène, J.; Gago-Martinez, A.; Hess, P. Use of Mass Spectrometry to Determine the Diversity of Toxins Produced by *Gambierdiscus* and *Fukuyoa* Species from Balearic Islands and Crete (Mediterranean Sea) and the Canary Islands (Northeast Atlantic). *Toxins* **2020**, *12*.
2. Perez-Arellano, J.L.; Luzardo, O.P.; Perez Brito, A.; Hernandez Cabrera, M.; Zumbado, M.; Carranza, C.; Angel-Moreno, A.; Dickey, R.W.; Boada, L.D. Ciguatera fish poisoning, Canary Islands. *Emerg Infect Dis* **2005**, *11*, 1981-1982.
3. Otero, P.; Pérez, S.; Alfonso, A.; Vale, C.; Rodríguez, P.; Gouveia, N.N.; Gouveia, N.; Delgado, J.; Vale, P.; Hirama, M., et al. First toxin profile of ciguateric fish in Madeira Arquipelago (Europe). *Analytical chemistry* **2010**, *82*, 6032-6039.
4. Martín, V.; Vale, C.; Hirama, M.; Yamashita, S.; Rubiolo, J.A.; Vieytes, M.R.; Botana, L.M. Synthetic ciguatoxin CTX 3C induces a rapid imbalance in neuronal excitability. *Chemical research in toxicology* **2015**, *28*, 1095-1108.
5. Martín, V.; Vale, C.; Rubiolo, J.A.; Roel, M.; Hirama, M.; Yamashita, S.; Vieytes, M.R.; Botana, L.M. Chronic ciguatoxin treatment induces synaptic scaling through voltage gated sodium channels in cortical neurons. *Chemical research in toxicology* **2015**, *28*, 1109-1119.
6. Vale, C.; Antero, A.; Martín, V. Pharmacology of ciguatoxins. *Phycotoxins, Chemistry and Biochemistry; Alfonso, LBAA, Ed.; Wiley Blackwell: Hoboken, NJ, USA* **2015**, 23-48.
7. Boente-Juncal, A.; Álvarez, M.; Antelo, Á.; Rodríguez, I.; Calabro, K.; Vale, C.; Thomas, O.P.; Botana, L.M. Structure Elucidation and Biological Evaluation of Maitotoxin-3, a Homologue of Gambierone, from *Gambierdiscus belizeanus*. *Toxins* **2019**, *11*.