SYNERGISM BETWEEN FOOD TOXICANTS ACTING ON SODIUM CHANNELS

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INTRODUCTION

Ciguatoxins are emergent marine biotoxins in European Coasts that pose a risk for human health by their action on voltage-gated sodium channels (VGSC) [1]. They reach humans through contaminated fish and shellfish leading to nervous, digestive and cardiac symptoms [2]. Pyrethroids are pesticides that appear as food or water contaminants reaching humans throughout food chain. As ciguatoxins, they act by activating VGSC leading to neurological symptomatology [3].

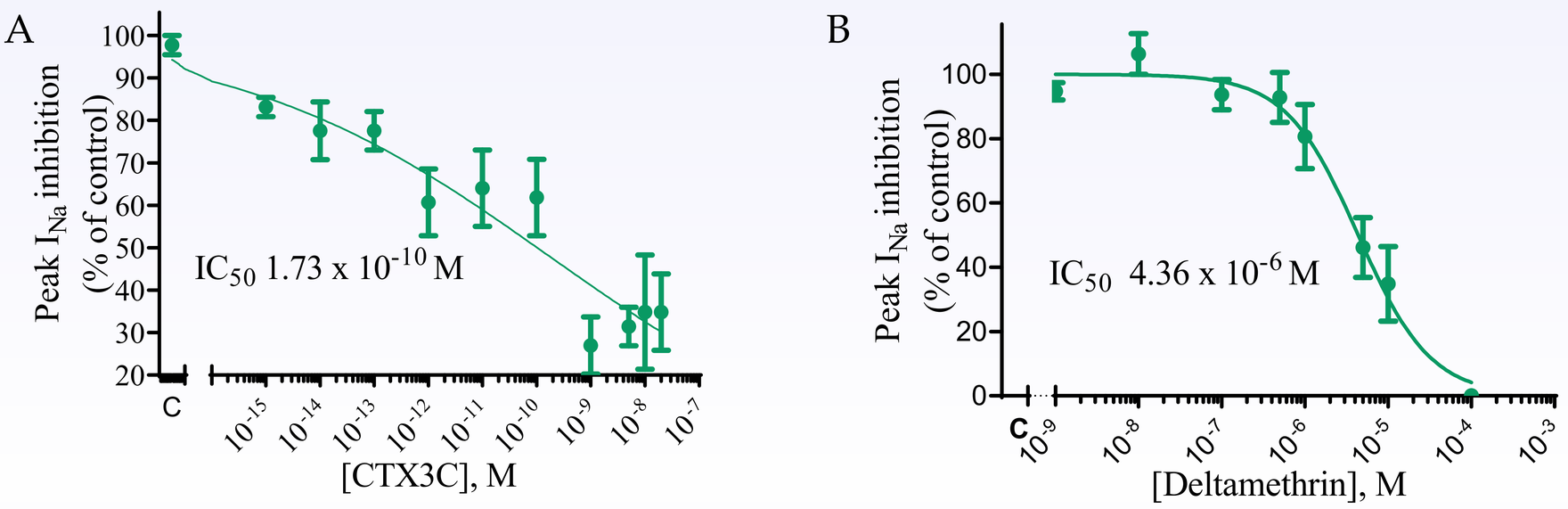
The risk for human health as well as the maximum residue levels in food components for both toxicants were assessed separately without considering their possible synergistic effects as consequence of their interaction with the same cellular target [4, 5]. There is an absolute lack of data on the possible combined cellular effects that biological and chemical pollutants may have, highlighting the importance of study the joint effects that the co-exposure to compounds with the same cell target can produce.

MATERIAL AND METHODS

Pacific ciguatoxin CTX3C was purchased from Wako (FUJIFILM Wako Chemicals Europe GmbH, Neuss, Germany). Deltamethrin was purchased from Merck (Germany). HEK293 cells transfected with the human Na_v 1.6 alpha subunit of the sodium channels were used under a MTA with Dr Andrew Powell (GlaxoSmithKline R&D, UK). Cells were cultured in DMEM/F12 medium with L-glutamine supplemented with 1% non essential aminoacids, 10% fetal bovine serum and 400 µg/ml geneticin. Cells were maintained in a humidified 5% CO_2/95 % air atmosphere at 37 °C and placed at 30 °C for 24–36 h before electrophysiological experiments.

Electrophysiological recordings of sodium currents were registered in whole-cell configuration. A protocol of voltage steps from −80 to +80 mV was applied, with 10 mV step increases and 0.15 seconds duration. Signals were recorded using a computer equipped with a Digidata 1440 data acquisition system and the pClamp10 software.

RESULTS



A. Concentration-response graph indicating the effect of CTX3C in the inhibition of the maximum peak inward sodium currents. B. Concentration-response graph indicating the effect of deltamethrin in the inhibition of the maximum peak inward sodium currents. C. Concentration-response graph for the peak inhibition of sodium currents by different deltamethrin and CTX3C concentrations relative to the CTX3C concentrations added to the recording chamber. D. Concentration-response graph for the peak inhibition of sodium currents by different deltamethrin and CTX3C concentrations relative to the deltamethrin concentrations.

Combination Index

$$CI = \frac{D1}{(DX)1} + \frac{D2}{(DX)2}$$

Where (DX)1 is the IC_{50} value of CTX3C alone, (DX)2 the IC_{50} value of deltamethrin alone and D1 and D2 are the IC_{50} values of CTX3C and deltamethrin respectively in combination. According to this method, additivity is established if CI = 1, synergism if CI < 1 and antagonism if CI > 1.

Combination Index

$$\frac{D1}{(DX)1} + \frac{D2}{(DX)2} = 0.065$$

$$0.065 < 1 \text{ synergism}$$

CONCLUSION

SYNERGISTIC EFFECT
CI < 1 between CTX3C and deltamethrin

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