Environmental Toxicology and Pharmacology 52 (2017) 200–213

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Research Paper

Human neuronal cell based assay: A new *in vitro* model for toxicity evaluation of ciguatoxin

Teresa Coccini[a,*], Francesca Caloni[b], Uliana De Simone[a]

[a] Laboratory of Clinical and Experimental Toxicology, Poison Control Centre, Toxicology Unit, Maugeri Clinical Scientific Institutes S.p.A.-BS, IRCCS Pavia, Pavia Italy
[b] Department of Veterinary Medicine (DIMEVET), Università degli Studi di Milano, Milano, Italy

ARTICLE INFO

Keywords:
P-CTX 3C
Neurotoxicity
Marine toxins
In vitro cellular model
SH-SY5Y cells

ABSTRACT

Ciguatoxins (CTXs) are emerging marine neurotoxins representing the main cause of ciguatera fish poisoning, an intoxication syndrome which configures a health emergency and constitutes an evolving issue constantly changing due to new vectors and derivatives of CTXs, as well as their presence in new non-endemic areas.

The study applied the neuroblastoma cell model of human origin (SH-SY5Y) to evaluate species-specific mechanistic information on CTX toxicity. Metabolic functionality, cell morphology, cytosolic Ca_i^{2+} responses, neuronal cell growth and proliferation were assessed after short- (4–24 h) and long-term exposure (10days) to P-CTX-3C.

In SH-SY5Y, P-CTX-3C displayed a powerful cytotoxicity requiring the presence of both Veratridine and Ouabain. SH-SY5Y were very sensitive to Ouabain: 10 and 0.25 nM appeared the optimal concentrations, for short- and long-term toxicity studies, respectively, to be used in co-incubation with Veratridine (25 μM), simulating the physiological and pathological endogenous Ouabain levels in humans.

P-CTX-3C cytotoxic effect, on human neurons co-incubated with OV (Ouabain + Veratridine) mix, was expressed starting from 100 pM after short- and 25 pM after long-term exposure. Notably, P-CTX-3C alone at 25 nM induced cytotoxicity after 24 h and prolonged exposure.

This human brain-derived cell line appears a suitable cell-based-model to evaluate cytotoxicity of CTX present in marine food contaminated at low toxic levels and to characterize the toxicological profile of other/new congeners.

1. Introduction

Poisoning by marine algal toxins can configure a health emergency and constitutes an evolving issue constantly changing. Consumption of seafood contaminated with algal toxins results in different seafood poisoning syndromes including ciguatera fish poisoning (CFP) which is an ichthyosarcotoxism, with dramatic and clinically important neurological features, caused by the consumption of fish contaminated with toxic levels (equivalent of 0.1–5 μg/Kg P-CTX-1 of fish flesh, (EFSA, 2010)) of the voltage-sensitive sodium channel activators named ciguatoxins (CTXs).

These toxins are usually produced by the benthic dinoflagellate *Gambierdiscus toxicus* that colonises reef areas in the Pacific, Indian and Caribbean seas. Toxin accumulates in tissues of fish that eat the algae and bioaccumulates up the food chain (Tester et al., 2010), thus, humans could be intoxicated by consumption of Herbivorous and carnivorous fish containing CTXs.

Because of the "weather sealing" of the Mediterranean Sea, CTX (typically found in tropical or subtropical seas) was also identified in this new sea although so far still in some limited areas (Crete Island, Israel, the Canary Islands (Spain) and Madeira (Portugal)) (Aligizaki et al., 2008; Bentur and Spanier, 2007; Boada et al., 2010; Matute et al., 2009), as well as in new vectors (Silva et al., 2015) other than the common vectors for these phycotoxins such as finfish, some mollusks achieving higher concentrations in top predatory fish like groupers, barracuda and snapper (FDA, 2011). Recently, some Italian coastal areas have also been affected by the phenomenon of flowering of some species of benthic microalgae as *Ostreopsis ovata* which could allow harmful marine algae to become more prolific over a wider territory (Gingold et al., 2014; Tester et al., 2010).

"Emerging marine toxins", as defined by European Food Safety Authority (EFSA, 2010) are considered not only newly discovered marine toxins, or not regulated known marine toxin (Reverté et al., 2014), but also marine toxins that are currently present in the water

* Corresponding author at: Laboratory of Clinical and Experimental Toxicology, Maugeri Clinical Scientific Institutes S.p.A.-BS, IRCCS Pavia, Pavia, Italy, Via Maugeri 10, 27100 Pavia, Italy.
E-mail address: teresa.coccini@icsmaugeri.it (T. Coccini).

http://dx.doi.org/10.1016/j.etap.2017.04.003
Received 23 December 2016; Received in revised form 31 March 2017; Accepted 2 April 2017
Available online 14 April 2017
1382-6689/

Table 1
Visual observations codes.
Adapted from ICCVAM (2006).

Note Code	Note Text	Morphological characteristics by contract phase microscopy
1	Normal cell morphology	flattened-like" phenotype, cytoplasmic projections (neurites)
2	Low changes in cell morphology	shortened neurites
3	Moderate changes in cell morphology	spherical shape, shortened neurites, cell density decrease
4	High changes in cell morphology	spherical shape, shortened neurites, vacuole formation, cellular clusters, strong cell density decrease

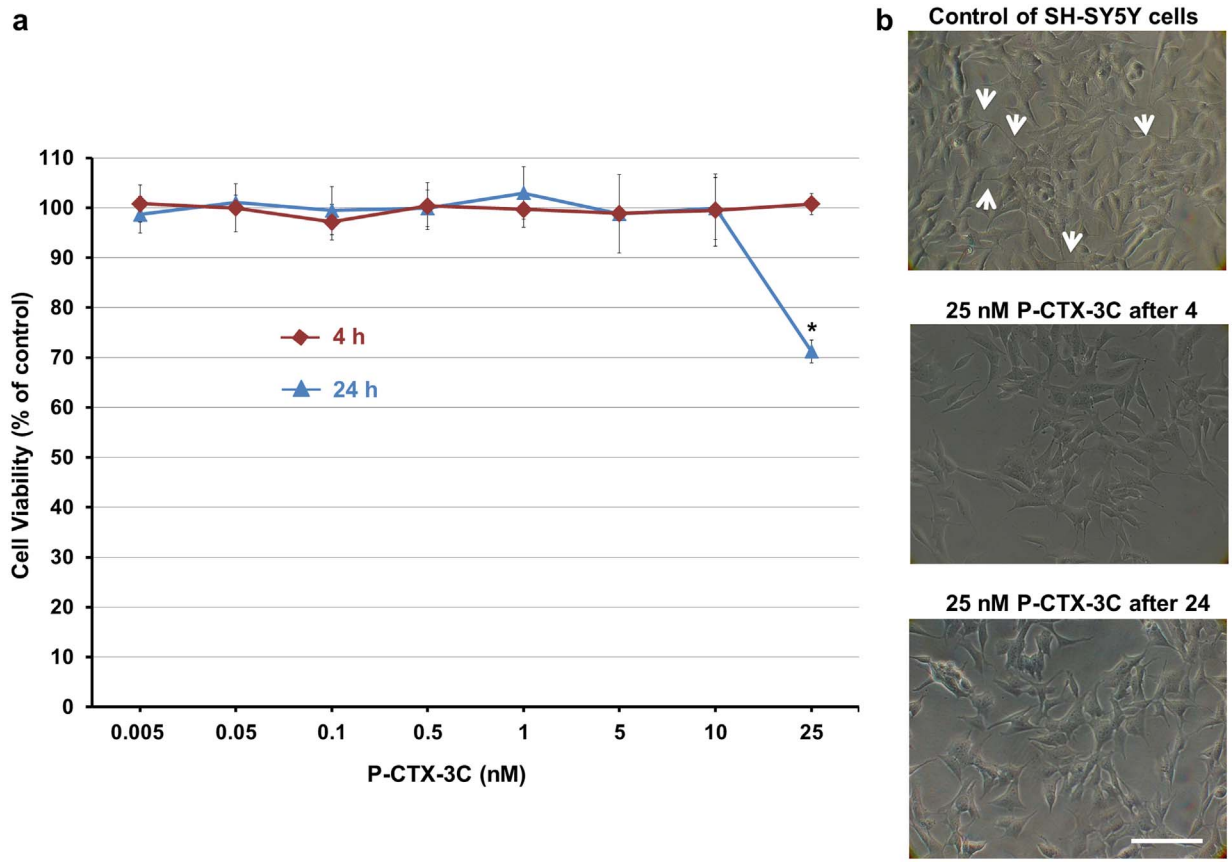

Fig. 1. Mitochondrial function measured by MTT assay in SH-SY5Y cells exposed to increasing concentration (0.005–25 nM) of P-CTX-3C after 4 and 24 h. (a): P-CTX-3C induced mortality (about 30%) at the highest concentration tested of 25 nM after 24 h, only. Data are the mean ± SD of three separate experiments each carried out in six replicates. * Different from control $P < 0.05$, statistical analysis by ANOVA followed by Tukey's test. Phase Contrast Microscopic Analysis (b): representative images of randomly selected microscopic fields by phase contrast microscopy of SH-SY5Y cells treated with 25 nM P-CTX-3C after 24 h. Ciguatoxin treatment did not induce any morphological alteration of SH-SY5Y cells (morphological code = 1). Decrease of cell density was observed at the highest concentration tested of 25 nM P-CTX-3C after 24 h only. Scale bar = 100 μm.

and seafood previously absent. The latter group includes the recently ciguatoxins appeared in the eastern Atlantic, Macaronesia (Bravo et al., 2015). The still scarce information on the mechanisms of action of CTX toxicity, the structural complexity of these marine toxins, and the limited availability of purified standards as well as the scarcity of toxin have hindered the development of methods for their detection and regulation, as well as the possibility to make precise diagnosis and treatment of intoxicated patients. Among the typical symptoms of ciguatera there are also neurological disorders such paresthesia, dysesthesia, depression, memory loss, weakness, increased nociception (Friedman et al., 2008). In severe cases the symptoms may begin as soon as 30 min after ingestion of contaminated fish, while in milder cases they may be delayed for 24–48 h. The neurological symptoms can also persist for weeks/months/years or reappear after a period of well-being. Chronic effects, including fatigue, myalgia and headaches are documented in over 20% of cases (Friedman et al., 2008).

To protect public health, monitoring programmes for marine biotoxins have been established in many countries, which often stipulate the use of animal models for detecting the presence of marine biotoxins in shellfish tissues. The mouse bioassay (MBA) has been widely used to detect CTX-group toxins in fish, but for reasons of animal welfare there is a growing concern with respect to its use. Due to its poor specificity and insufficient detection capability, the EU CONTAM Panel (EFSA, 2010) encourages research into, and the development and validation of, alternative methods. Studies for toxicity evaluation of CTX include the *in vitro* "Cell Based Assays" (CBA), most of which, with animal-derived cells (i.e., primary or immortalized cell lines, especially the N2a neurons from mouse) (Caillaud et al., 2012; Le Page et al., 2005; Pawlowiez et al., 2013; Reverté et al., 2014). These assays are based on the cellular toxicological effects (i.e., changes in the morphology or cell viability), caused by the marine toxins, which can be measured and quantified. Indeed several studies, conducted on neuroblastoma cell lines particularly of murine origin (i.e., N2a), demonstrated that CTX is a potent activator of the voltage-dependent sodium channels (VGSCs) resulting in intracellular calcium increase (Ca^{2+}), as well as a potent potassium channel inhibitor (Molgó et al., 1993; Vetter