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Improvements in the use of neuroblastoma x glioma hybrid cells (NG108-15) for the toxic effect quantification of marine toxins

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ABSTRACT

New advances in the use of NG108-15 cells for marine toxins detection and quantification are presented. We have established modifications on ouabain and veratridine proportion to obtain good toxins dose-response curves on this cell line for neurotoxins acting on voltage gated sodium channel (VGSC). Interesting, differences in the toxic response were observed between two VGSC activating toxins, brevetoxin-3 and pacific ciguatoxin-1. For non-VGSC acting lipophilic toxins, several factors that may influence toxin detection and quantification were analyzed. One hour cultures and forty-eight hours of exposure time, compared with 24 h of culture and 24 h of exposure, would increase NG108-15 cell maximal yessotoxin (YTX) and azaspiracid-1 (AZA-1) toxic response whereas no change was observed for okadaic acid (OA), dinophysistoxin-1 and pectenotoxin-2. Dose-response curves obtained for YTX or AZA-1 showed variability according to the day of the experiment while good reproducibility was obtained for OA. Evaporation time of toxin solutions before cell exposure could be an important source of variability in AZA-1 toxic response evaluation.

1. Introduction

Marine toxins from Harmful Algal Blooms (HAB) can have impact on coastal resources or implicate human health risk through food-borne intoxications (Hallegraeff, 2004; Sobel and Painter, 2005). Cell-based assays for toxicity evaluation contribute to the understanding of the potency of marine toxins, to the characterization of their mechanism of action (Botana, 2000) and, as other biological models, to identify and follow new toxic compounds through purification steps to contribute to develop analytical procedures for their identification. Additionally, cell-based assays could favour the reduction of animal testing in food safety monitoring programs which currently use for some marine toxins mouse bioassays.

Previous reviews regarding the specific use of cell-based assays for the evaluation of marine toxins illustrate their potency as toxicological models (Garthwaite, 2000; Rossini, 2005; Cañete and Diogène, 2008).

Regarding previous comparative studies on the use of two established neuronal cell lines; neuroblastoma × glioma hybrid cells (NG108-15) and Neuro-2a neuroblastoma cells (Cañete and Diogène, 2008) for the identification and quantification of marine toxins toxic effect, we have selected in the present study NG108-15 cells to evaluate the capacity of this cell line to be used in a toxicological cell-based assay for the evaluation of the toxic potential of neurotoxins and other types of toxins. In our previous studies, NG108-15 cells were more sensitive to pectenotoxin-2 (PTX-2) than Neuro-2a cells and a preliminary study with domoic acid (DA) seems to show

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a better capacity of NG108-15 than Neuro-2a cells to detect DA toxicity. NG108-15 used for cytoxicity evaluation of PTX-2 would represent an important reduction of the use of expensive standard toxin, and could be used in a routinely marine toxin detection laboratory. For okadaic acid (OA), dinophysistoxin-1 (DTX-1) and palytoxin dose-response curves obtained for NG108-15 cells supports this model as a biological, suitable and sensitive tool to their toxicity detection and quantification.

First studies on NG108-15 cells to evaluate their capacity to detect toxicity of neurotoxins acting on VGSCs, saxitoxin (STX) and brevetoxin-3 (PbTx-3), were carried adapting the method used to evaluate cytotoxic response on Neuro-2a cells (Manger et al., 1993; Dickey et al., 1999) and ouabain and veratridine (O/V) concentrations were established based on Neuro-2a experience (with a fixed proportional factor of 10:1 respectively). Under these experimental conditions NG108-15 showed a narrow range of effect (quantitatively), with smaller differences in viability between the control and the concentration of toxin having the maximum effect. Preliminary results on O/V proportions in NG108-15 cells showed important improvement in offsetting O/V effect by VGSC inhibiting toxin or potentiate VGSC acting toxins effect by incrementing veratridine concentration in regard to ouabain.

Non-VGSC acting lipophilic toxins such as yessotoxin (YTX) and azaspiracid-1 (AZA-1) have been demonstrated to increase strongly their maximum toxic effect on mammalian cell cultures in 48 h exposure time compared with 24 h (Twiner et al., 2005; Pérez-Gómez et al., 2006). In these studies, dose-response curves obtained after 48 h exposure allow to estimate, in all cases, the 50% effect concentration (EC50) which is around 20 nM for YTX (Pérez-Gómez et al., 2006) and around 1.1–7.9 for AZA-1 (Twiner et al., 2005).

In the present study a better O/V proportion obtained for NG108-15 cells to evaluate neurotoxins acting on VGSCs is presented. Dose-response curves of STX, PbTx-3 under O/V optimal conditions on NG108-15 are presented and compared to dose-response curves obtained under first O/V concentrations selected. Pacific ciguatoxin-1 (P-CTX-1), another VGSC activating toxin, was exposed under both O/V conditions (proportion 10/1 and 1/1). Results are presented and compared with those obtained for STX and PbTx-3.

For non-VGSC acting lipophilic toxins OA, DTX-1, PTX-2, YTX and AZA-1, several assays are proposed in order to optimize the experimental protocol including incubation time and exposure time. Variability in toxic response estimation in different days as well as the importance of evaporation time of toxin solutions was evaluated for OA, YTX and AZA-1.

2. Material & methods

In the present study, different methods used to evaluate cytotoxic response of marine toxins on NG108-15 cells have been developed according to previous work on Neuro-2a cells (Manger et al., 1993; Dickey et al., 1999) and NG108-15 cells (Cañete and Diogène, 2008). Suitability of this cellular model to evaluate the toxic effect of toxins studied is determined by the possibility to generate dose-response curves. Sensitivity of the model to different marine toxins was evaluated by the EC50s values obtained and maximal toxic effects observed.

2.1. NG108-15 cell culture maintenance

NG108-15 cells (ATCC, HB12317), were cultured in 10% FBS/Dulbecco's Modified Eagle's Medium (DMEM) at 37 °C and 5.0% CO_2 in an incubator (Binder, Tuttlingen, Germany). Culture medium DMEM was supplemented with 0.2% Pyridoxine-HCL solution (2 g/L), 2% L-glutamine solution (200 mM), 0.5% antibiotic solution (10 mg/mL streptomycin and 1000 μ/mL penicillin), 0.1 mM hypoxantine, 400 nM aminopterin, and 0.016 mM thymidine. Cells subcultures were made three times per week (dilution 1/4), in 75 cm^2 flasks. An approximate confluence of 90% was obtained with 30 mL of 10% FBS/DMEM in two days.

2.2. Seeding of cells into 96 well plates for cytotoxicity assays

For cell viability assays, 96 well plates (flat bottom) were prepared with cells obtained from a 90 to 100% confluence flask. Inocula of 200 μL cell suspension were added to each well. Cell densities were approximately in the range of 25 000–50 000 cells/well for NG108-15. For the whole study all conditions (controls included) were tested at least in duplicate.

2.3. Toxin preparation

Previous to toxin exposure of cells, defined aliquots of toxin solutions were dispensed on vials and evaporated under gentle N_2 flux at 40 °C using a Turbovap (Zymark corp., Hopkinton, Massachusetts). Evaporated extracts were dissolved in 5% FBS culture medium (concentrated dose) and were added to the corresponding wells. Dilutions from the concentrated dose were prepared, and added to a minimum of two wells (duplicates). Volume in each well was adjusted with 5% FBS culture medium (as complementary medium) to a final volume of 230 μL.

2.4. Toxin exposure of cells and response evaluation

Toxins studied in this work included neurotoxins acting on VGSCs (STX, PbTx-3 and P-CTX-1) and other toxins which mechanism of action does not involve VGSCs (OA, DTX-1, PTX-2, YTX and AZA-1). For neurotoxins acting on VGSCs cells with or without O/V were exposed to increased concentrations of commercial toxins. Ouabain and veratridine concentrations were selected to produce mortality in approximately 20% or 80% of the cell population depending on whether the toxins activate (e.g., PbTx-3 and P-CTX) or inhibit (e.g., STX) sodium channels. Two different proportional factors of O/V concentration were studied. A 10:1 (O/V) proportion was chosen based on experience on Neuro-2a cells. A 1:1 O/V proportion was also used in this study after a series of assays with NG108-15 to determine the optimal O/V proportion causing the highest increment of response for toxins acting on VGSCs. These different proportional factors of O/V (10:1 and 1:1) were obtained using concentrations of O/V at 0.75/0.075 mM or 0.35/0.35 mM in VGSC inhibiting