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INNOVATIVE IN VITRO STRATEGIES FOR NEUROTOXICITY TESTING,

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Background

Alternative approaches such as in vitro and non-mammalian models as part of an integrated test strategy could accelerate the process of neurotoxicity evaluation of a wide range of chemicals and reduce and refine animal use. Screening of marine neurotoxins requires the use of the mouse bioassay where thousands of mice are sacrificed every year. Therefore, there is an urgent need for in vitro alternatives in this field.

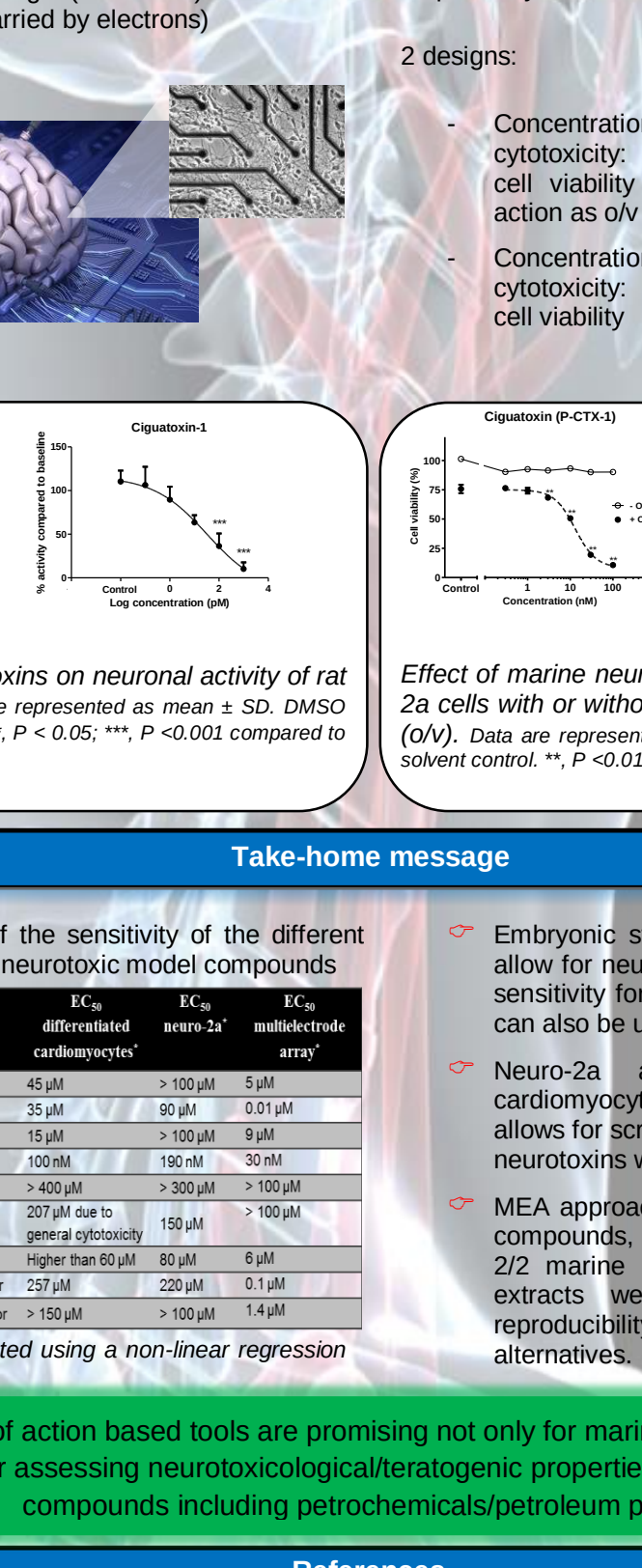
Strategy

Because a wide range of marine neurotoxins targets ion channels/pumps and neuronal receptors[1], we developed three mode of action based models: embryonic stem cell-derived cardiomyocytes with beating as a final readout, mouse neuroblastoma cells with cytotoxicity as a measure of toxicity and rat cortical neurons with changes in neuronal activity as a functional endpoint.

Cell-based assays for neurotoxicity screening

Embryonic stem cell-based model

Neurons and cardiac cells share common ion channels[2]. Therefore, embryonic stem cells were differentiated into cardiomyocytes prior to exposure to neurotoxins.



Multielectrode array (MEA)

Rat cortical neurons comprising a wide range of ion channels/pumps and neurotransmitter receptors targeted by marine neurotoxins[3].

- Neuronal activity recording
- Changes in voltage (ion fluxes) transduced into currents (carried by electrons)

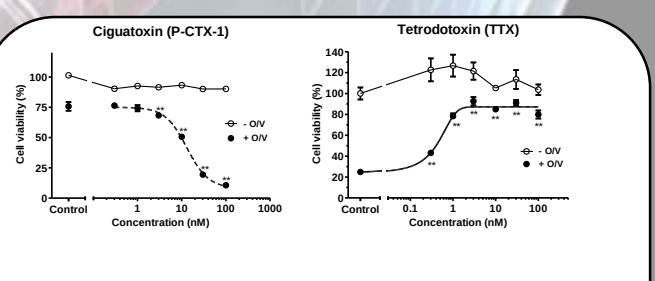
Neuroblastoma neuro-2a assay

Marine neurotoxins alone do not have any effect on neuro-2a cells viability at real-life concentrations. Cells become responsive to marine neurotoxins only when first incubated with ouabain and veratridine (o/v), Na^+/K^+-ATPase blocker and Na^+ channel opener respectively.

2 designs:

- Concentrations of o/v inducing 80% cytotoxicity: Na^+ channel blockers increase cell viability through an opposite mode of action as o/v
- Concentrations of o/v inducing 20% cytotoxicity: Na^+ channel openers decrease cell viability

Effect of marine neurotoxins on neuronal activity of rat cortical neurons. *Data are represented as mean ± SD. DMSO was used as solvent control. *, P < 0.05; ***, P <0.001 compared to solvent controls.*



Effect of marine neurotoxins on cell viability of neuro-2a cells with or without addition of ouabain/veratridine (o/v). *Data are represented as mean ± SD. DMSO was used as solvent control. **, P <0.01 compared to solvent control.*

Take-home message

Table 1. Comparison of the sensitivity of the different models for screening of neurotoxic model compounds

Compound	Mode of action	EC_{50} differentiated cardiomyocytes*	EC_{50} neuro-2a+	EC_{50} multielectrode array*
Diphenhydramine	Na^+ channel blocker	45 µM	> 100 µM	5 µM
Veratridine	Na^+ channel opener	35 µM	90 µM	0.01 µM
Isradipine	Ca^{2+} channel blocker	15 µM	> 100 µM	9 µM
Verapamil	Ca^{2+} channel blocker	100 nM	190 nM	30 nM
Sematilide	K^+ channel blocker	> 400 µM	> 300 µM	> 100 µM
Clofilium	K^+ channel blocker	207 µM due to general cytotoxicity	150 µM	> 100 µM
Amiodarone	K^+ channel blocker	Higher than 60 µM	80 µM	6 µM
Ouabain	Na^+/K^+ ATPase blocker	257 µM	220 µM	0.1 µM
Digoxin	Na^+/K^+ ATPase inhibitor	> 150 µM	> 100 µM	1.4 µM

EC_{50} values were calculated using a non-linear regression model

- Embryonic stem cell-derived cardiomyocytes allow for neurotoxins detection but with a low sensitivity for marine neurotoxins. This assay can also be used for developmental toxicity
- Neuro-2a assay: less sensitive than cardiomyocytes for model compounds but allows for screening of a wide range of marine neurotoxins with high sensitivity
- MEA approach: sensitivity of 88% (7/9 model compounds, 6/6 pure marine neurotoxins and 2/2 marine neurotoxins present in seafood extracts were correctly identified), good reproducibility compared to existing in vitro alternatives.

These mode of action based tools are promising not only for marine neurotoxin screening but also for assessing neurotoxicological/teratogenic properties of a wide variety of compounds including petrochemicals/petroleum products.

References

[1]Nicolas, J., Hendriksen, P. J. M., Gerssen, A., Bovee, T. F. H. and Rietjens, I. M. C. M. (2014), Marine neurotoxins: State of the art, bottlenecks, and perspectives for mode of action based methods of detection in seafood. Mol. Nutr. Food Res., 58: 87–100

[2]Nicolas, J., Hendriksen, P. J. M., de Haan, L. H., Koning, R., Rietjens, I. M. C. M. and Bovee, T. F. H. (2014), In vitro detection of cardiotoxins or neurotoxins affecting ion channels or pumps using beating cardiomyocytes as alternative for animal testing. Toxicol. In Vitro., 29(2): 281-288

[3]Nicolas, J., Hendriksen, P. J. M., van Kleef, R. G. D. M., de Groot, A., Bovee, T. F. H., Rietjens, I. M. C. M. and Westerink, R. H. S. (2014), Detection of marine neurotoxins in food safety testing using a multielectrode array. Mol. Nutr. Food Res. 58(12) (2014) 2369-78

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