The Effect of the Cyanobacterial Toxin Saxitoxin on Neurodevelopment

By

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Table of Contents

List of Figures

List of Tables

Abstract

The potent neurotoxin saxitoxin (STX) belongs to a group of structurally related analogues, collectively known as the paralytic shellfish toxins, produced by both marine and freshwater phytoplankton. This group of toxins act by blocking the voltage-gated sodium channels, halting the inflow of sodium ions and the subsequent generation of action potentials. While acute exposure has been well researched, with safety guidelines applied, chronic low dose exposure from neither marine or freshwater sources has been investigated. Given the role of cellular electrical activity in neurodevelopment this latter pattern of exposure may be of significant public health concern. This background has been addressed in chapter 1; “Low dose extended exposure to saxitoxin and its potential neurodevelopmental effects: a review”, and the published manuscript can be found in Appendix 1.

Given this lack of investigation we aimed to determine if STX had an adverse effect on neurodevelopment following low dose extended exposure using two models of neuronal development. Further, we aimed to establish an assay which could be used to determine if any adverse neurodevelopmental effects recorded were due to direct STX toxicity.

Firstly, using model neuronal cell lines it was shown that STX at or below the current drinking water guideline (0.25-3μg/L) caused a significant concentration dependent decrease in the development of neuronal morphology following an extended exposure period. This research is presented in chapter 2; “Extended low-dose exposure to saxitoxin inhibits

neurite outgrowth in model neuronal cells" and the published manuscript can be found in Appendix 2.

In addition to investigating the neurodevelopmental effects of STX, an assay measuring viability indirectly through cellular metabolism was established to be used with STX. The assay was used to eliminate the possibility of non-specific cell toxicity as a cause of the effects on neurodevelopment. The assay was successfully optimised in two cell lines and tested with STX (0.25-10μg/L) and $ZnSO_4$ (10^{-4}-10^{-1}M), a known cytotoxic compound. The assay showed that STX is not toxic in our cell line under the conditions used for chapter 2. These results are reported in Chapter 3; "Optimisation of a real time resazurin based assay for use in OVCAR-3 and SH-SY5Y cells".

Moving to a model which more accurately models mammalian neuronal differentiation, the effect of STX at the drinking water guideline (3μg/L) and a predicted algal bloom concentration (10μg/L) was investigated using embryonic stem cells. Cells were differentiated using a previously described method of neuronal differentiation and assessed by examination of morphological development of neuronal features and expression of gene markers. A concentration dependent decrease in morphological neuronal index scores was recorded, confirming the results of chapter 1, in addition the expression of neuronal markers *nestin* and *MAP2* were increased following exposure to STX (3μg/L) while *β_{III}-Tubulin* was delayed by 3 days in both STX treatment groups. This research is presented in chapter 4; "Low dose exposure to saxitoxin affects neuronal differentiation of D3 embryonic stem cells".

These results suggest that STX, and potentially its analogues, interfere with proper neuronal development at environmentally relevant concentrations. Whilst further work is required to investigate the mechanisms causing the adverse effects seen, the work presented here raises awareness that this pattern of exposure could be of significant public health concern and deserves further investigation.

Student declaration

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Abbreviations

%CV	co-efficient of variability
AB	Alamar Blue
b.w	body weight
BBB	blood brain barrier
C1-4	C-toxins
CNS	central nervous system
d.p.c	day(s) post coitum
dcSTX	decarbamoylated
DMEM	Dulbecco;s Modified Eagle Medium
EB #	EB day
EBs	embryoid body
EFSA	European Food Safety Authority
EROD	ethoxyresorufin-*0*-deethylase
FBS	foetal bovine serum
GPx	glutathione peroxidase
GTXs	gonyuatoxins
HAB	harmful algal blooms
hERG	human ether-a-go-go
IF	intermediate filament
LDH	lactate dehydrogenase
LIF	leukaemia inhibitory factor
LPS	lipopolysaccharide
MAP2	microtubule-associated protein 2
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

$NA_v1.1$-1.9	α-subunits
neoSTX	neosaxitoxin
P-loop	pore loop
PAC	powdered activated carbon
PLL	poly-L-lysine
PNS	peripheral nervous system
PROD	penthoxyresorufin-*O*-deethylase
PSP	paralytic shell fish poisoning
PSTs	paralytic shellfish toxins
RA	retinoic acid
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
S1-S6	transmembrane segments
SOD	superoxide dismutase
ß1-4	ß-subunits
STX	saxitoxin
STXeq	saxitoxin equivalents
TTX	tetrodotoxin
VGCC	voltage-gated calcium channel
VGSC	voltage-gated sodium channel