UTS UNIVERSITY OF TECHNOLOGY SYDNEY

An *in vitro* study investigating the combined toxicity of the cyanotoxins β-N-methylamino-L-alanine (BMAA) and 2,4-diaminobutyric acid (2,4-DAB)

by Lisa Pu

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Masters of Science (Research)

under the supervision of
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Declaration of Original Authorship

I hereby declare the contents described in this thesis are of original research and have not been submitted to any other institute for a higher degree. The contribution to my thesis is wholly my work unless acknowledged otherwise.

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Signature removed prior to publication.

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My brother is what led me in my pursuit of understanding neurological and neurodegenerative diseases. I wish to live the life he is not able to. I hope one day, he can recover and get his life back.

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List of Publications

Steele, J. R., Italiano, C. J., Phillips, C. R., Violi, J. P., **Pu, L.**, Rodgers, K. J., & Padula, M. P. (2021) Misincorporation Proteomics Technologies: A Review. *Proteomes*. 9 (1), 2.

- Contributions involved creating several figures and diagrams to illustrate concepts.

Italiano, C. J., **Pu, L.**, Violi, J. P., Duggin, I. G., & Rodgers, K. J. (2021) Tolerance towards β-methylamino-L-alanine in *Escherichia coli* requires cysteine biosynthesis genes. *Research in Microbiology*.

- Contributions involved preparing and running samples for mass spectrometry and the analysis of glutathione levels in *Escherichia coli.*

Pu, L., Castorina, A., & Rodgers, K. J. (2022) Cyanobacterial toxins BMAA and 2,4-DAB perturb the L-serine biosynthesis pathway in SH-SY5Y neuroblastoma cells: a proteomic study. **(Submitted)**

Pu, L., Violi, J. P., Steele, J. R., Padula, M. P., & Rodgers, K. J. (2022) Changes to intracellular amino acid levels in SH-SY5Y cells exposed to the cyanotoxins BMAA and 2,4-DAB. **(Submitted)**

List of Abbreviations

2,4-DAB	L-2,4-diaminobutyric acid
3PG	3-phospho-D-glycerate
AEG	N-(2-aminoethyl) glycine
ALS/PDC	Amyotrophic lateral sclerosis/Parkinson's dementia complex
BAMA	B-amino-N-methylalanine
BMAA	β-N-methylamino-L-alanine
BMI	Body mass index
BOAA	β-N-oxalylamino-L-alanine
CSF	Cerebrospinal fluid
ER	Endoplasmic reticulum
fMND	Familial motor neurone disease
HPLC	High-performance liquid chromatography
IPA	Ingenuity pathway analysis
LOAEL	Lowest observable adverse effect level
MND	Motor neurone disease
NFT	Neurofibrillary tangle
NMDA	N-methyl-D-aspartate
NPAA	Non-protein amino acid
PHGDH	3-phosphoglycerate dehydrogenase
PPP	Pentose phosphate pathway
PSAT1	Phosphoserine aminotransferase 1
PSPH	Phosphoserine phosphatase
sMND	Sporadic motor neurone disease
SOD1	Superoxide dismutase 1
TCA	Tricarboxylic acid cycle
TDP-43	TAR DNA-binding protein 43

Abstract

Sporadic motor neurone disease is a neurodegenerative disease with poorly understood aetiology. It accounts for up to 90 to 95% of motor neurone disease cases, with the remaining 5 to 10% being familial. Development of the sporadic form of the disease may be due to a contribution of several factors such as lifestyle, genetic susceptibility, aging and environment. One of the proposed environmental factors is exposure to cyanobacterial neurotoxins. A link between exposure to cyanobacterial (blue-green algal) toxins and a high incidence of neurodegenerative diseases reported on Guam in the 1940s resulted in the discovery of the novel amino acid, β-N-methylamino-L-alanine (BMAA). BMAA is being investigated as a potential trigger for MND based on *in vitro* and *in vivo* toxicity studies as well as recent epidemiological studies that have linked exposure to cyanobacterial blooms to higher incidences of MND in several locations worldwide. In over 50 years of research, the focus has primarily been on BMAA despite there being several other isomers including L-2,4-diaminobutyric acid (2,4-DAB) which have neurotoxic effects. BMAA and 2,4-DAB are produced concurrently by cyanobacteria, and it is logical to investigate their toxicity together as well as individually. This thesis aims to investigate further the toxic mechanisms of these two isomers and how they might contribute to the development of sporadic neurodegenerative disorders.

Initially cell viability assays were performed to determine the toxicity of the neurotoxins individually, and to identify the most toxic combination. Equimolar concentrations of BMAA and 2,4-DAB resulted in the highest toxicity to the cells and was used in subsequent studies. Proteomic analysis then revealed significant enrichment in pathways involved with energy production (fatty acid β-oxidation and glycolysis) and L-serine biosynthesis. The proteomic data on the L-serine biosynthesis enzymes were then validated using RT qPCR to determine expression levels of the three enzymes involved, as well as protein levels via Western blotting. 2,4-DAB alone and in combination with BMAA significantly decreased the expression of the first enzyme involved in the L-serine biosynthesis pathway, 3-phosphoglycerate dehydrogenase (PHGDH). Supplementation with the glycolytic metabolite pyruvate before exposure to the neurotoxins was

protective and prevented the impact of the toxins on the PHGDH gene expression. These results highlight the importance of the contribution to energy dysfunction which may parallel those seen in some neurodegenerative diseases. The toxins' ability to interfere with L-serine biosynthesis enzymes may be another route by which BMAA could disrupt homeostasis in cells.

To further understand the ability of the toxins to disrupt cellular metabolism, LC-MS/MS was used to quantify the level of amino acids and antioxidant capacity of cells exposed to BMAA, 2,4-DAB and the combination. 2,4-DAB exposure showed evidence of oxidative stress which was increased when combined with BMAA. Intracellular L-alanine levels were significantly decreased following treatment with BMAA and 2,4-DAB alone. The decreases in L-alanine levels in cells might support existing studies that have demonstrated the affinity of BMAA for alanyl-tRNA synthetase. The impact of the cyanotoxins on L-serine biosynthesis could be important to the *in vivo* toxicity of BMAA since it is known that L-serine is protective, but the mechanism through which it protects against BMAA has not been identified. Since L-serine is an important amino acid in the CNS, damage to its biosynthesis by continuing exposure to these cyanotoxins could result in permanent neuronal damage.

The results of these studies contribute to the ever-growing knowledge of BMAA and its role in neurodegenerative diseases and highlight the importance of studying the toxin in combination with its isomers that are found concurrently in nature.

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