Investigating the Toxicity and Mechanisms of Non-Protein Amino Acids

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DECLARATION

I, Kate Samardzic, certify that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by an Australian Government Research Training Program.

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LIST OF PUBLICATIONS

Rodgers, K.J., **Samardzic, K.**, and Main, B.J. (2015) Toxic Nonprotein Amino Acids, in *Plant Toxins*. Springer. p. 1-20.

Samardzic, K., and Rodgers, K.J. (2017). Oxidised protein metabolism: recent insights. Biol Chem, 398: p. 1165-1175.

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Samardzic, K. and K.J. Rodgers. (2019). Cytotoxicity and mitochondrial dysfunction caused by the dietary supplement L-norvaline. Toxicol in Vitro, 56: p. 163-171.

Samardzic, K. and K. J. Rodgers (2019), Cell death and mitochondrial dysfunction induced by the dietary non-proteinogenic amino acid L-azetidine-2-carboxylic acid (Aze). Amino Acids, 8: p. 1221-1232.

Giannopoulos, S., **Samardzic, K.**, Raymond, B.A., Djordjevic, S.P., and Rodgers, K.J. (2019) L-DOPA causes mitochondrial dysfunction in vitro: a novel mechanism of L-DOPA toxicity uncovered. Int J Biochem Cell Biol, 117.

CONFERENCE PROCEEDINGS

Algal Research Symposium, Sydney, NSW, Australia 2015

Oral Presentation

"Phytotoxic Activity of Non-Protein Amino Acids Synthesised by Cyanobacteria"

Society of Environmental Toxicology and Chemistry, Hobart, TAS, Australia 2016

Oral Presentation

"Phytotoxic Activity of Non-Protein Amino Acids Synthesised by Cyanobacteria"

FameLab, Sydney, NSW, Australia 2018

Oral presentation

"Mitochondria: Cellular Superheroes and their Kryptonite"

Multifaceted Mitochondria, San Diego, CA, USA 2018

Poster Presentation

"Mitochondrial Dysfunction caused by Non-Protein Amino Acid Dietary Supplements"

AMP Amplify PhD Pitch Competition, Sydney, NSW, Australia 2018

Oral Presentation

"Supplement Dos and Don'ts"

AussieMit, Melbourne, VIC, Australia 2018

Poster Presentation

"Mitochondrial Dysfunction caused by Non-Protein Amino Acid Dietary Supplements"

ABSTRACT

Non-protein amino acids (NPAAs) are amino acids not normally used in protein synthesis. However, a small subset of this type of amino acid, known as amino acid analogues, can be mistakenly utilised in protein synthesis due to their structural similarity to a canonical protein amino acid. This process has been implicated in the development of neurodegenerative disease. This research set out to investigate the toxicity and mechanisms of two lesser studied NPAAs which enter the human food chain and have the potential to contribute to the development of disease. These were L-norvaline (Nva), an analogue of the branched chain amino acids that is usually found in dietary supplements consumed by bodybuilders, and L-azetidine-2-carboxylic acid (Aze), an analogue of L-proline that is produced by sugar beets and enters the human food as a dietary supplement and as fodder for livestock.

Initial experiments sought to identify whether either Nva or Aze induced cell death using metabolic and imaging assays. Both NPAAs were cytotoxic to human neuroblastoma cells, confirming the rationale behind the present investigation and providing the impetus for further research. Cell death pathways were also investigated, and death was determined to be necrotic following Nva exposure, and both necrotic and apoptotic following treatment with Aze. The toxicity of these NPAAs to mitochondria was then characterised using immunofluorescence microscopy and bioenergetic flux assays. These revealed, for the first time, that Nva and Aze cause mitochondrial dysfunction. Distinct morphological changes and bioenergetic failure were common to both conditions, however, for Nva bioenergetic failure was only observed in the presence of a nitric oxide synthase inhibitor due to Nva's secondary action as an arginase inhibitor.

Finally, a proteomic characterisation of cells exposed to both NPAAs was performed to further elucidate the molecular mechanisms involved in their toxicity. This was the first study to perform this analysis in human cells treated with either NPAA and revealed disruptions to processes that precede protein translation. Changes to proteins involved in the processing of DNA and RNA during cell cycle progression indicate, for the first

time, that NPAA exposure exerts toxic effects upstream of protein translation. These results identify Nva and Aze as NPAAs with significant potential for toxicity that should, therefore, be consumed with caution. Furthermore, these results add to the existing knowledge of the mechanisms of both these individual NPAAs, and amino acid analogues in general.

CONTENTS

LIST OF TABLES AND FIGURES

***Please note that the Tables and Figures listed above include those in chapters one, two and five only.**

LIST OF ABBREVIATIONS

ATP	adenosine triphosphate
AD	Alzheimer’s disease
ALS	Amyotrophic Lateral Sclerosis
ALS-PDC	Amyotrophic Lateral Sclerosis-Parkinsonism-Dementia Complex
aaRS	aminoacyl transfer ribonucleic acid synthetase
BCA	bicinchoninic acid
BP	Biological Process
CC	Cellular Compartment
DNA	deoxyribonucleic acid
DTT	dithiothreitol
DMEM	Dulbecco's Modified Eagle's Medium
EMEM	Eagle’s Minimum Essential Medium
EF-Tu	elongation factor Tu
ER	endoplasmic reticulum
E. coli	*Escherichia coli*
FDR	false discovery rate
fALS	familial Amyotrophic Lateral Sclerosis
FBS	foetal bovine serum
GO	Gene Ontology
HSP	heat shock protein
HD	Huntington’s disease
IRES	internal ribosome entry site

DAB	L-2, 4-diaminobutanoic acid
L-DOPA	L-3,4- dihydroxyphenylalanine
Aze	L-azetidine-2-carboxylic acid
Nva	L-norvaline
BMAA	L-β-*N*-methylaminoalanine
BOAA	L-β-*N*-oxalylamino-L-alanine
LFQ	label free quantification
LeuRS	leucyl-transfer ribonucleic acid synthetase
LC-MS	liquid chromatography-mass spectrometry
mRNA	messenger ribonucleic acid
MS	Multiple Sclerosis
NO	nitric oxide
NPAA	non-protein amino acid
OXPHOS	oxidative phosphorylation
PD	Parkinson's disease
PBS	phosphate buffered saline
PI	Propidium Iodide
ROS	reactive oxygen species
RFC	replication factor C
RNA	ribonucleic acid
sALS	sporadic Amyotrophic Lateral Sclerosis
SOD1	superoxide dismutase 1
SLE	Systemic Lupus Erythematosus

THOC	THO complex
tRNA	transfer ribonucleic acid
TCEP	tris(2-carboxyethyl)phosphine
UPR	unfolded protein response
VCP	valosin containing protein
ZnF	zinc finger

***Please note that the abbreviations listed above include those in chapters one, two, five and six only**