

**DEVELOPMENT OF NEUROPATHOLOGY
IN MURINE MPS IIIA AND MPS VII AND
THE EFFECT OF *N*-
BUTYLDEOXYNOJIRIMYCIN TREATMENT
ON MPS IIIA MICE**

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**Thesis submitted for the degree of
Doctor of Philosophy**

in

Discipline of Genetics

**School of Molecular and Biomedical Sciences
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The University of Adelaide**

February 2014

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Abstract

The mucopolysaccharidoses (MPSs) are a family of heritable diseases caused by deficiencies in glycosaminoglycan (GAG) degrading lysosomal enzymes. GAGs accumulate in a range of tissues, resulting in diverse pathology that includes brain degeneration. The secondary accumulation of glycosphingolipids, specifically G_{M2} and G_{M3} gangliosides, occurs in the MPS brain. In MPS IIIA and MPS VII mouse models GAGs and gangliosides began to accumulate prior to the onset of behavioural changes. G_{M2} levels began to rise early, following the trend of GAG accumulation, and increased to 548% and 219% of normal levels in MPS IIIA and MPS VII respectively. G_{M3} levels began to rise later, reaching a peak of 484% and 313% of normal in MPS IIIA and MPS VII respectively.

Given that brain G_{M2} and G_{M3} accumulation precedes behavioural deficits, it is possible that these gangliosides contribute to brain degeneration. Thus, gangliosides may be a target for the treatment of MPS brain disease. *N*-butyldeoxynojirimycin (*NB*-DNJ) is an iminosugar capable of crossing the blood brain barrier and reducing brain ganglioside synthesis, consequently decreasing overall brain G_{M2} and G_{M3} levels. *NB*-DNJ treatment of MPS IIIA mice decreased brain G_{M2} and G_{M3} levels in the short but not in the long term. Despite this, the innate fear response was restored and learning ability was equivalent to normal with both lengths of treatment.

MPS IIIA mice treated with *NB*-DNJ also had a reduction in cytokine gene expression, astroglial activation and oxidation of inflammatory lipids. Whether MPS IIIA behavioural improvements were due to a delay in ganglioside accumulation with *NB*-DNJ treatment, or due to an anti-inflammatory function of *NB*-DNJ is not known. However, this thesis

demonstrates that *NB-DNJ* can improve MPS brain dysfunction in the MPS IIIA mouse model and may be a potential therapy for CNS disease for children with MPS.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Xenia Kaidonis and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Xenia Kaidonis

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Acknowledgements

Over the course of my PhD, many people have shared their support and expertise. First and foremost, I'd like to thank my supervisors Dr Sharon Byers and Dr Janice Fletcher for their guidance, support and patience. Their knowledge in the field of mucopolysaccharidosis research is extraordinary and I was fortunate to have had the opportunity to learn from them.

Thank you to the members of the Matrix Biology Unit, past and present: Ainslie, Matilda, Nathan, Elle, Carmen, Wan Chin, Krystyna, Kavita, Mardiah, Chun Hong, Xiao Dan, Zarpana, Marleesa, Chun Hao, Wesley and Sharvin. Their friendship, support and good humour made the lab a truly enjoyable place to come to every day. I would especially like to thank Ainslie for her help over the years, particularly with the behaviour testing of MPS IIIA mice and *NB-DNJ* injections.

Special thanks must go to Enzo Ranieri, who taught me mass spectrometry, and to Peter Sharp, who passed on some of his vast knowledge of lipid biochemistry. Both were always happy to lend their equipment and to give their time for me and for this I am very grateful. Thank you also to Dr Craig Freeman for helping me with methods for the hydrolysis of heparan sulphate and the separation of glucuronic acid and iduronic acid residues.

Thank you to Dr Tomas Rozek for allowing me to use the mass spectrometer at the University of South Australia when our machine at the Women's and Children's hospital was finally decommissioned. Thank you to the Women's and Children's hospital animal house staff for their excellent care of the mice used in this study. Thank you also to the SA Pathology histology department (WCH site) for use of their cryostat.

To the University of Adelaide department of genetics and post-graduate coordinators Dr Michael Lardelli and Dr Frank Grutzner, thank you for your support throughout my candidature and for providing such a well organised programme.

I would like to acknowledge funding for this work by the NH&MRC and the kind donation of NB-DNJ used for the oral treatment of mice by Actelion.

Finally, I would like to thank those most important to me: Tom, my parents and sisters, for their constant love, encouragement and support throughout this journey.

Abbreviations

12(s)-HETE	12(s)-hydroxyeicosatetraenoic acid
13(s)-HODE	13(s)-hydroxyoctadecadienoic acid
15(s)-HETE	15(s)-hydroxyeicosatetraenoic acid
6-keto-PGF1 α	6-keto-prostogmandin F1 alpha
α <i>Syn</i>	alpha-synuclein
AA	arachidonic acid
AAV	adeno-associated virus
BBB	blood brain barrier
BLAST	basic local alignment search tool
BMT	bone marrow transplant
CB	cerebellum
<i>Ccl3</i>	chemokine (C-C motif) ligand 3
Cer	ceramide
CH	cerebral hemisphere
CL	cardiolipin
CNS	central nervous system
CS	chondroitin sulphate
CSF	cerebrospinal fluid
<i>Ctsb</i>	cathepsin B
<i>Cypa</i>	cyclophilin A
d ₃ -G _{M1}	N-octadecanoyl-d ₃ -monosialoganglioside 1
DAPI	4',6-diamidino-2-phenylindole
DHA	docosahexaenoic acid
DEAE	diethylaminoethyl

DS	dermatan sulphate
EDTA	sodium ethylenediaminetetraacetic acid
EPA	eicosapentaenoic acid
ERT	enzyme replacement therapy
FITC	fluorescein isothiocyanate
GAG	glycosaminoglycan
Gal	galactose
GalNAc	<i>N</i> -acetylgalactosamine
GalNAc T	<i>N</i> -acetylgalactosamine transferase
GalT I	galactosyltransferase I
GalT II	galactosyltransferase II
GFAP/ <i>Gfap</i>	glial fibrillary acidic protein
Glc	glucose
GlcA	glucuronic acid
GlcT	glucosyltransferase
G _M	monosialoganglioside
G _{M2} -AP	monosialoganglioside 2 activator protein
GSL	glycosphingolipid
GT	gene therapy
GUSB	β -glucuronidase
HA	hyaluronan
Hex A/B	hexosaminidase A/B
HIV	human immunodeficiency virus
HS	heparan sulphate
HSD	highly significant difference
IdoA	iduronic acid
<i>Ifn</i> γ	interferon gamma

<i>Il1β</i>	interleukin 1 beta
iso-PGF2α	iso-prostaglandin F2 alpha
KS	keratan sulphate
LC-ESI MS/MS	liquid chromatography- electrospray ionisation tandem mass spectrometry
LSD	lysosomal storage disorder
MCB	membranous cytoplasmic bodies
<i>Mip1a</i>	chemokine (C-C motif) ligand 3
MPA/B	mobile phases A/B
MPS	mucopolysaccharidosis
MRM	multiple reaction monitoring
MS/MS	tandem mass spectrometry
NB-DGJ	<i>N</i> -butyldeoxygalactonojirimycin
NB-DNJ	<i>N</i> -butyldeoxynojirimycin
NEB	New England Biolabs
NPC	Niemann Pick C
NSAID	non-steroidal anti-inflammatory drug
OCT	optimal cutting temperature
PA	phosphatidic acid
PBS	phosphate buffered saline
PC	phosphatidylcholine
PCR	polymerase chain reaction
PE	phosphatidylethanolamine
PGD2	prostaglandin D2
PGE2	prostaglandin E2
PGF2α	prostaglandin F2 alpha
PI	phosphatidylinositol

PUFA	polyunsaturated fatty acid
RAPC	repeated acquisition and performance chamber
ROS	reactive oxygen species
RVD1 α	resolvin D1 alpha
SA	sialic acid
SAT I	sialotransferase I
SDS	sodium dodecyl sulphate
SDT	substrate deprivation therapy
SHIRPA	SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment
SM	sphingomyelin
SOD	superoxide dismutase
TAE	tris-acetate/ sodium ethylenediaminetetraacetic acid
TFA	trifluoroacetic acid
<i>Tgfβ1</i>	transforming growth factor beta 1
TLC	thin layer chromatography
<i>Tnfα</i>	tumor necrosis factor alpha
<i>Tnfrs1α</i>	tumor necrosis factor family receptor superfamily member 1a
TXB2	thromboxane B2
UA	uronic acid
UDP	uridine diphosphate