Substrate Localisation as a Therapeutic Option for Pompe Disease

A thesis submitted to The University of Adelaide for the degree of

DOCTOR OF PHILOSOPHY

by

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October 2013

Paediatrics

Paediatrics and Reproductive Health

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Abstract

Pompe disease is a progressive form of muscular dystrophy caused by a deficiency in the lysosomal enzyme α-glucosidase (GAA). GAA catabolises glycogen and its deficiency leads to glycogen accumulation in the vesicular network of affected cells. Multiple therapies exist to treat Pompe disease but these are not completely effective (Winkel *et al.,* 2003), necessitating the development of new therapeutic strategies. A number of enzymes that reside outside of the lysosome, either in the cytoplasm (Watanabe *et al.,* 2008) or in circulation (Ugorski *et al.,* 1983), can catabolise glycogen. It was postulated that if vesicular glycogen in Pompe cells was transferred out of these compartments it could then be alternatively degraded. The ability to remove vesicular glycogen from Pompe cells may reduce the onset/progression of the disorder, providing a therapeutic option for patients.

Exocytosis is a ubiquitous cellular mechanism where intracellular vesicles fuse with the cell surface and permit vesicle content to be released from the cell. It was postulated that exocytosis may provide a mechanism to release accumulated glycogen from Pompe cells. Approximately 4% of vesicular glycogen was exocytosed from Pompe skin fibroblasts after 2 hrs in culture. Pompe cells exocytosed 2.7-fold more glycogen than unaffected cells. A cellular mechanism was therefore identified that had the capacity to release glycogen from Pompe cells.

Culture conditions can alter the amount of exocytosis in fibroblasts (Martinez *et al.,* 2000). In this study the effect of cell confluence and components of the culture media on lysosomal exocytosis was examined in Pompe skin fibroblasts. Increasing the

extracellular concentration of Ca^{2+} led to a 1.4-fold increase in glycogen release compared to cells cultured in standard media conditions. Culture confluence had a key influence on glycogen exocytosis, with sub-confluent Pompe cells releasing >80% of glycogen after 2 hrs in culture, 35-fold higher than confluent cells. Exocytic mechanisms therefore exist that allow up-regulation of glycogen exocytosis in Pompe skin fibroblasts.

A number of pharmacological compounds induce exocytosis in cultured cells (Amatore *et al.*, 2006). Pompe skin fibroblasts treated with three compounds; calcimycin, lysophosphatidylcholine and α-L-iduronidase, each demonstrated a ≥ 1.5-fold increase in glycogen exocytosis, when compared to untreated Pompe controls. Calcimycin was the most effective compound for inducing glycogen exocytosis, with 12% released after 2 hrs of treatment, but confluent Pompe cells released less than that observed from sub-confluent Pompe cells. This difference in glycogen release may have resulted from the induction of different exocytic mechanisms. Complete exocytosis, where the vesicle completely fuses with the cell surface and releases all vesicle content, is induced in sub-confluent Pompe cells. In contrast, cavicapture, involving only a partial pore opening and limited vesicle content release, is induced in response to calcimycin treatment. The identification of a compound capable of inducing complete exocytosis may therefore improve glycogen release from Pompe cells. Taken together, natural glycogen exocytosis and the ability to induce glycogen exocytosis with pharmacological compounds provided proof-of-concept for exocytic induction as a strategy to re-locate accumulated glycogen from Pompe cells, potentially providing a new therapeutic option for the disorder.

Declaration of Authenticity

I, Christopher Turner, declare this thesis contains no material which has been accepted for the award of any other degree or diploma in any University and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text. The author consents to the thesis being made available for photocopying and loan if applicable, if accepted for the award of the degree.

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Christopher Turner

Acknowledgments

I wish to thank my supervisors Peter Meikle, Doug Brooks, John Hopwood and Maria Fuller. I would also like to acknowledge the following colleagues; Sophie Lazenkis, Phil van der Ploeg, Tim Nielsen, Glen Borlace, Anthony Fidelli, Debbie Lang, Emma Parkinson-Lawrence, Jana Pacyna, Mark Proedehl and Karissa Phillis. I would like to thank Stephen Duplock, Tomas Rozek and Troy Stomski for their mass spectrometry expertise, David Stapleton for assistance with the isolation and visualization of glycogen, Greg Hodge for flow cytometry training, Lyn Waterhouse and Ruth-Ellen Williams for assistance with the TEM and confocal microscope, Alvis for assistance with preparing the TEM samples, Lou for running a number of lactate dehydrogenase assays, Kathy Nelson for providing cell lines and culturing information and Nancy Briggs for statistical analysis. I would also like to acknowledge the support of Felicity, Marilyn, Stephen, Bella and Paige Turner.

Abbreviations

^{o}C	degrees celcius
%	percentage
<	less than
>	greater than
µg	microgram
µL	microlitre
µmol	micromoles
µM	micromolar
β	beta
β-hex	beta-hexosaminidase
4-MU	4-methylumbelliferyl
AA	arachidonic acid
AMP	adenosine monophosphate
amu	atomic mass units
ANOVA	analysis of variance
BAPTA-AM	1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-
tetraacetic	acid-acetoxymethyl ester
BCA	bicinchoninic acid
BME	Basal modified Eagle's medium
BSA	bovine serum albumin
Ca^{2+}	divalent calcium ion

calcimycin	calcimycin A23187
cAMP	cyclic AMP
CRIM	cross-reactive immunological material
DAPI	4',6-diamidino-2-phenylindole
DIC	differential interference microscopy
DMEM	Dulbecco's modified Eagle's medium
EPA	eicosapentaenoic acid
EPAC	exchange proteins activated directly by cyclic AMP
ERT	enzyme replacement therapy
ESI-MS/MS	electrospray ionization tandem mass spectrometry
FBS	fetal bovine serum
g	gravitational force
GAA	acid α-glucosidase
G1P	glucose-1-phosphate
G6P	glucose-6-phosphate
HCl	hydrochloric acid
H_2O	water
HPLC	high pressure liquid chromatography
Idua	α-L-iduronidase
kDa	kilodalton
KH_2PO_4	monopotassium phosphate
LAMP	lysosomal associated membrane protein

LC/ESI-MS/MS	liquid chromatographic electrospray ionization tandem mass spectrometry
LDH	lactate dehydrogenase
LPC	lysophosphatidylcholine
LSD	lysosomal storage disorder
min	minute
mg	milligram
MLD	metachromatic leukodystrophy
mol	moles
MPR	mannose-6-phosphate receptor
MPS	mucopolysaccharidosis
MRM	multiple reaction monitoring
ms	milliseconds
MS	mass spectrometry
m/z	mass-to-charge ratio
MW	molecular weight
N_2	nitrogen
NaCl	sodium chloride
Na_2HPO_4	disodium phosphate
NaOH	sodium hydroxide
NB-NBJ	N-butyldeoxynorjirimycin
ng	nanograms
nm	nanometers

nmol	nanomoles
NRK	normal rat kidney
OD	optical density
PBS	phosphate buffered saline
PC	phosphatidylcholine
PMA	phorbol 12-myristate 13-acetate
PMP	1-phenyl-3-methyl-5-parazolone
PtdIns3K	phosphatidylinositol 3-kinase
PtdIns3P	phosphatidylinositol 3-phosphate
QC	quality control
SLP	synaptotagmin-like protein
SNAP	soluble N-ethylmaleimide-sensitive factor attachment protein
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptor
S-1-P	sphingosine-1-phosphate
Syt	synaptotagmin
TFEB	bHLH-leucine zipper transcription factor EB
TOR	target of rapamycin
VAMP	vesicle-associated membrane protein
V-ATPases	vacuolar ATPases
v/v	volume per volume
w/v	weight per volume

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