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Cellular effects of Coenzyme Q10 and Resveratrol in the SJL/J dysferlinopathy mouse model

By

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Submitted in partial fulfillment of the requirements for the degree

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Department of Anatomy

University of Pretoria

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Supervisor: **Prof E Pretorius**

Co-supervisor: **Dr M Beukes**

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Abstract

The muscular dystrophies (MDs) are genetic disorders of muscle degeneration due to mutations in genes that encode a wide variety of proteins. Dysferlinopathy encompasses a large variety of neuromuscular diseases characterized by the absence of dysferlin in skeletal muscle and an autosomal recessive mode of inheritance. Dysferlinopathy can manifest as limb girdle muscular dystrophy type 2B (LGMD 2B), Miyoshi myopathy (MM) or distal myopathy with anterior tibial onset (DMAT). The first symptoms usually appear during the second or third decade of life as clumsiness when running, fatigue when walking long distances and difficulty in climbing stairs. Progression of the disease eventually leads to a loss of ambulation.

A deficit in membrane-repair machinery in dysferlinopathy suggested a direct role for dysferlin in the Ca^{2+}-dependent membrane-repair process. Recently, dysferlin has also been implicated in the process of chemotaxis. Evidence exists that free radical mediated injury contributes to the pathogenesis of muscle necrosis in the muscular dystrophies. The imbalance of free radical synthesis and antioxidant capacity has been suggested to contribute to the necrotic process.

It is therefore imperative to explore the effect of antioxidant supplementation in the MDs. The present study followed a novel approach in investigating the cellular effects afforded by the supplementation of the SJL/J mouse model for dysferlinopathy with the antioxidants, Coenzyme

Q10 (CoQ10) and resveratrol. The study aimed to determine, at cellular level, the histopathology and ultrastructural changes in the SJL/J mouse model following a 90 day trial with antioxidant supplementation. In addition to studying the morphology, the study paid attention to non-specific parameters. The study mainly focused on the histopathology and ultrastructural alterations in the SJLL/J mouse. In addition the oxidative stress index of the affected quadriceps muscle was determined.

The outcome provides evidence that increased oxidative stress levels are present in the SJL/J mouse. Antioxidant supplementation with CoQ10 at 120mg/kg/day or a resveratrol/CoQ10 combination supplementation at 40 and 60mg/kg/day, decreased the levels of oxidative stress and dystrophic markers at a cellular level. In addition, increased physical strength was observed. This thesis provides evidence to create a new platform for combination therapeutic strategies.

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Declaration

I, Marnie Potgieter, hereby declare that this thesis entitled:

Cellular effects of Coenzyme Q10 and Resveratrol in the SJL/J dysferlinopathy mouse model

which I herewith submit to the University of Pretoria for the Degree of Doctor of Philosophy in Anatomy, is my own original work and has never been submitted for any academic award to any other tertiary institution for any degree.

30 November 2009

Date

Marnie Potgieter

I will praise thee; for I am fearfully and wonderfully made: marvellous are thy works; and that my soul knoweth right well.

Psalm 139:14

Ek wil U loof, want U het my op 'n wonderbaarlike wyse geskep. Wat U gedoen het vervul my met verwondering.

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To all my friends and family, thank you for your love and support.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	percentage
®	registered sign
°C	degrees celcius
µl	microliter
µm	micrometer
10q24	the gene location for *MYOF*
11q12-13	gene location for AHNAK
14q32	gene location for AHNAK nucleoprotein 2
2p13	gene location for *DYSF*
8-OH-dG	8-hydroxy-deoxyguanosine
A/J	Albino mouse strain with spontaneous progressive muscular dystrophy due to dysferlin mutation
aa	amino acids
A-band	anisotropic band
$ABTS^{+}$	2,2'-azinobis(3-ethylbenzothiazoline sulphonate)
ADP	Adenosine diphosphate
$ADP\text{-}Fe^{3+}$	Adenosine diposphate iron tri-oxide
AED	animal equivalent dose
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AU	arbitrary units
Balb/c	albino, laboratory-bred strain of the house mouse
BAR	family of genes
BHP	tert-butylhydroperoxide
BHT	butylated hydroxytoluene
Bin-1	conserved member of the BAR family of genes implicated in myoblast differentiation and membrane deformation
BMD	Becker muscular dystrophy
bp	base pair
BSA	Body surface area
BW755c	
c.elegans	*Caenorhabditis elegans*
C2C12	myoblast mouse cell line
Ca^{2+}	Calcium
CAT	catalase
CAV3	caveolin 3 gene
$CD4^{+}$	A glycoprotein expressed on the surface of T helper cells (cluster of differentiation)

cDNA	complementary deoxyribonucleic acid
CH	calponin homology
CK	Creatine kinase
CMD/MDC	Congenital muscular dystrophies
Co	Company
CO_2	Carbon dioxide
CoQ	Coenzyme Q
CoQ10	Coenzyme Q10
COQ2	OH-benzoate prenyl-transferase gene
$CoQH_2$	reduced form of CoQ10/ubiquinol
COX	cyclooxygenase
CPK	creatine phosphokinase
CT	computed tomography
C-terminal	carboxy terinal
Cu,Zn SOD	Copper/Zinc superoxide dismutase
DACM	distal anterior compartment myopathy
DAPC	dystrophin associated protein complex
DFBN9	a specific type of autosomal recessive deafness in humans
DGC	Dystrophin-glycoprotein complex
DHEA	dehydroepiandosterone
DHPR	dihydropyridine receptor
DM	Myotonic dystrophy
DMAT	Distal myopathy with anterior tibial onset
DMD	Duchenne muscular dystrophy
DNA	deoxyribonucleic aced
DPC	dystrophin protein complex
DTT	1,4-Dithiothreitol
dy/dy	homozygous dystrophic mouse strain with dy mutation, suggested to be a mutation in the M-chain gene; animals display a more severe phenotype than the mdx mouse
DYSF	dysferlin gene
Dysf^{im}	Allele responsible for decreased levels of dysferlin in SJL/J mice; inflammatory myopathy allele
E	Expect value. The E-value is a parameter that describes the number of hits on can 'expect' to see by chance when searching a database of a particular sized.
EAE	experimental autoimmune encephalitis
EAM	Autoimmune myositis
EBD	extensor digitorum brevis
ECM	extra cellular matrix
EDL	extensor digitorum longus
EDMD	Emery-Dreifuss muscular dystrophy
EDTA	ethylenediaminetetraacetic acid

EHL	extensor hallicus longus
EM	electron microscopy
F28+	28th generation
F4/80	an antibody used to identify mouse macrophages
FA	focal adhesion
FDA	Food and Drug Administration
FER-1	*C. elegans* ferlin-1 gene
FER-1	nematode protein ferlin-1
FER1L1	dysferlin
FER1L2	otoferlin
FER1L3	myoferlin
FER1L4-6	proteins that are predicted form the human and mouse genomic sequences but have not yet been characterized
FKRP	Fukutin-related protein
FSHD	Facioscapulohumeral dystrophy
g	gauge
g	gram
Glocuse-6-P	glucose-6-phosphate
Gluconate-6-P	Gluconate-6-phosphate
GM-CSF	monocyte-colony stimulating factor
GPx	glutathione peroxidase
GRMD	golden retriever muscular dystrophy
GSH	glutathione
H	Hydrogen
H_2O_2	hydrogen peroxide
HED	human equivalent dose
HEPA	high efficiency particulate air
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
H-zone	*Heller* zone
I-band	isotropic band
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IFN-γ	interferon-γ
IgE	immunoglobulin E
IL	interleukin
ILK	integrin-linked kinase
IU	international units
IVC	individually ventilated microisolator-cages
IκBα	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
K	conversion factor
K_2PO_4	potassium phosphate
Kb	kilobyte

KCl	potassium chloride
kDa	kilodalton
kg	kilogram
Km	The K_m factor, body weight (kg) divided by BSA (m^2), is used to convert the mg/kg dose used in a study to an mg/m^2 dose
kV	kilo volt
$L^{\bullet}$	Carbon-centered radical
LARGE	The LARGE gene was so named because it covers over 660 kb of genomic DNA; the protein it encodes is a putative glycosyltransferase.
LDH	Lactate dehydrogenase
LGMD	Limb girdle muscular dystrophy
LGMD 2B	Limb girdle muscular dystrophy type 2B
$LOO^{\bullet}$	lipid peroxyl radicals
LOOH	lipid hyperoxide
M	molar
MAC	membrane attack complex
MCK	myosin creatinine phosphokinase
MD(s)	Muscular dystrophy/dystrophies
MDA	Malondialdehyde
mdx	Dystrophin-deficient mouse model for Duchenne muscular dystrophy
mg/kg	milligram per kilogram
mg/kg/day	milligram per kilogram per day
mg/m^2	milligram per square meter
MHC-1	myosin heavy chain class I
min	minutes
ml	millilitre
M-line	*mittel* line
mm	millimetre
mM	millimolar
MM	Miyoshi myopathy
mmol/g	millimoles per gram
Mn-SOD	Manganese superoxide dismutase
MRC	Medical Research Council
MRC-5	human lung cell line
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MYOF	the gene for myoferlin
n	sample size
Na_2CO_3	sodium carbonate
NAD^+	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate

NCL-Hamlet	Mouse monoclonal antibody against dysferlin
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer
nm	nanometer
nmol/g	nanomoles per gram
NO•	nitric oxide
NOS	nitric oxide synthase
N-terminal	Nuclear terminal
O_2^-	superoxide
O_3	singlet oxygen
OGHD	oxoglutarate dehydrogenase
OH^-	hydroxyl radical
OH	hydroxide
$ONOO^-$	peroxynitrite
OPMD	Oculopharyngeal muscular dystrophy
OSI	oxidative stress index
OsO_4	osmium tetroxide
PA	Pennsylvania
PBS	Phosphate buffered saline
PD	proximodistal phenotype
pH	measure of the acidity or basicity / potential of Hydrogen
POMT1	Protein O-linked mannose β-1,2-N-acetylglucosaminyltransferase.
PT	posterior tibial
PUFA	polyunsaturated fatty acids
P-value	level of significance / probability value
r^2-value	coefficient of determination
RNA	Ribonucleic acid
ROS	reactive oxygen species
Rpm	revolutions per minute
RuO_4	ruthenium tetroxide
S100A10	a protein encoded by the *S100A10* human gene
S100A11	a protein encoded by the *S100A11* human gene
SD	standard deviation
SE	standard error
sec	seconds
SEM	scanning electron microscopy
SH3	domain in myoferlin that may mediate interactions with other proteins
SJL/J	Swiss Jim Lambert; Dysferlin-deficient strain of Swiss mice; animal model for dysferlinopathy

SJL/Olac	SJL strain obtained by the Clinical Research Centre, Harrow from the Jackson Laboratory, Bar Harbor in 1975, to OLAC, now Harlan Laboratories in 1977. This strain is now known as SJL/JOlaHsd
SOD	superoxide dismutase
SR	sarcoplasmic reticulum
STIR	short-time-inversion-recovery
SWR/J	Swiss mice used widely in research as general purpose strain
TA	anterior tibial/tibialis anterior
TAS	Total antioxidant status
TBA	thiobarbituric acid
TBARS	TBA reactive substances
TCA	trichloroacetic acid
TCAP	Telethonin, a protein that interacts with, or “caps”, another protein in muscle called titin.
TEM	transmission electron microscopy
TNF	tumor necrosis factor
TNFα	tumor necrosis factor-α
TNFα(-/-)	TNFα null mice
TRIM 32	One of 37 TRIM proteins containing a tripartite motif (TRIM).
T-tubule	transverse tubule
U/l	unit per liter
UPBRC	University of Pretoria’s Biomedical Research Centre
USA	United States of America
UV	ultra violet
WW	a protein-binding domain on the dystrophin protein that include two conserved moieties of tryptophan, with W representing the letter code of tryptophan
Z-disc	*Zwishenscheibe* disc
ZNF9	Zinc finger protein 9.

When you love what you're doing, it's hard not to.

Michael S Pepper