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

By

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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.
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 Ugedoen het vervul my met verwondering.
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<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>%</td>
<td>percentage</td>
</tr>
<tr>
<td>®</td>
<td>registered sign</td>
</tr>
<tr>
<td>°C</td>
<td>degrees celcius</td>
</tr>
<tr>
<td>µl</td>
<td>microliter</td>
</tr>
<tr>
<td>µm</td>
<td>micrometer</td>
</tr>
<tr>
<td>10q24</td>
<td>the gene location for MYOF</td>
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<tr>
<td>11q12-13</td>
<td>gene location for AHNAK</td>
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<td>14q32</td>
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</tr>
<tr>
<td>2p13</td>
<td>gene location for DYSF</td>
</tr>
<tr>
<td>8-OH-dG</td>
<td>8-hydroxy-deoxyguanosine</td>
</tr>
<tr>
<td>A/J</td>
<td>Albino mouse strain with spontaneous progressive muscular dystrophy due to dysferlin mutation</td>
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<tr>
<td>aa</td>
<td>amino acids</td>
</tr>
<tr>
<td>A-band</td>
<td>anisotropic band</td>
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<tr>
<td>ABTS⁺</td>
<td>2,2'-azinobis(3-ethylbenzothiazoline sulphonate)</td>
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<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ADP-Fe³⁺</td>
<td>Adenosine diposphate iron tri-oxide</td>
</tr>
<tr>
<td>AED</td>
<td>animal equivalent dose</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AU</td>
<td>arbitrary units</td>
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<tr>
<td>Balb/c</td>
<td>albino, laboratory-bred strain of the house mouse</td>
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<tr>
<td>BAR</td>
<td>family of genes</td>
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<tr>
<td>BHP</td>
<td>tert-butylhydroperoxide</td>
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<tr>
<td>BHT</td>
<td>butylated hydroxytoluene</td>
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<tr>
<td>Bin-1</td>
<td>conserved member of the BAR family of genes implicated in myoblast differentiation and membrane deformation</td>
</tr>
<tr>
<td>BMD</td>
<td>Becker muscular dystrophy</td>
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<tr>
<td>bp</td>
<td>base pair</td>
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<tr>
<td>BSA</td>
<td>Body surface area</td>
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<tr>
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<td>Caenorhabditis elegans</td>
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<tr>
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<td>myoblast mouse cell line</td>
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<tr>
<td>Ca²⁺</td>
<td>Calcium</td>
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<tr>
<td>CAT</td>
<td>catalase</td>
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<tr>
<td>CAV3</td>
<td>caveolin 3 gene</td>
</tr>
<tr>
<td>CD4⁺</td>
<td>A glycoprotein expressed on the surface of T helper cells (cluster of differentiation)</td>
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</table>
cDNA complementary deoxyribonucleic acid
CH calponin homology
CK Creatine kinase
CMD/MDC Congenital muscular dystrophies
Co Company
CO₂ Carbon dioxide
CoQ Coenzyme Q
CoQ10 Coenzyme Q10
COQ2 OH-benzoate prenyl-transferase gene
CoQH₂ reduced form of CoQ10/ubiquinol
COX cyclooxygenase
CPK creatine phosphokinase
CT computed tomography
C-terminal carboxy terminal
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 dehydroepiandrosterone
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
dysferlin gene
DYSF Dysferlin
Dysf" 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
H2O2 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
IkBα nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
K conversion factor
K2PO4 potassium phosphate
Kb kilobyte
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
O2−  superoxide
O3  singlet oxygen
OGHD  oxoglutarate dehydrogenase
OH−  hydroxyl radical
OH  hydroxide
ONOO−  peroxynitrite
OPMD  Oculopharyngeal muscular dystrophy
OSI  oxidative stress index
OsO4  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
r²-value  coefficient of determination
RNA  Ribonucleic acid
ROS  reactive oxygen species
Rpm  revolutions per minute
RuO4  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 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/J Olahsd.

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 Zwischenscheibe disc
ZNF9 Zinc finger protein 9.
When you love what you’re doing, it’s hard not to.

Michael S Pepper