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Cellular effects of Coenzyme Q10 and Triton X on primary chicken embryo heart and muscle cell cultures

By

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Abstract

Coenzyme Q10 is a lipid-soluble coenzyme, synthesized in mammalian tissue to support energy production, and also act as an antioxidant. Certain medication, stress and age may deplete the body's endogenous Coenzyme Q10 store. Numerous disease conditions have been shown to benefit from Coenzyme Q10 supplementation. It is a lipid-soluble component of virtually all cell membranes, and is located in the hydrophobic domain of the phospholipid bilayer of cellular membranes. It is also the only known lipid-soluble antioxidant that animal cells can synthesize *de novo*, and for which there exist enzymatic mechanisms which can regenerate it from its oxidized product formed in the course of its antioxidant function. The aim of this study was to investigate the cellular effects of Coenzyme Q10 and Triton X-100 on primary chicken embryo heart and muscle cell cultures. Triton X-100, a well known membrane disrupter, extensively used by cell biologists for that purpose, was used to investigate whether Coenzyme Q10 might offer protection to cell membranes exposed to disruption. Due to the correlation found between the chemical structures of nonylphenol and Triton X-100, it was decided to determine whether Triton X-100 possess estrogenic properties. Using the Recombinant Yeast Screen Assay for estrogenic activity, it was found that Triton X-100 induced weak estrogenic activity.

The primary heart and skeletal muscle cell cultures were established by harvesting skeletal muscle tissue and hearts from 13 day old chicken embryos. After establishment of the cell

cultures, the concentrations of Coenzyme Q10 and Triton X-100 were tested for cytotoxicity using the MTT, NR, and CV assays, in the form of a combined colorimetric cytotoxicity assay. The MTT assay revealed an increase in cell viability in both cell cultures upon exposure to Triton X-100 and Coenzyme Q10, alone, and in combination. Triton X-100 and Coenzyme Q10, alone, and in combination, caused a decrease in lysosomal membrane integrity, as measured by the NR assay, and both substances, alone, and in combination, had no effect on cellular proteins, as measured by the CV assay.

Scanning electron microscopy (SEM) was done to determine the cellular effect of heart and skeletal muscle cell cultures on the external surface, more specifically the membranes, of cells in culture. Triton X-100 in the concentrations used in the study, caused membrane disruption, ranging from complete membrane lyses at the highest concentrations to membrane ruptures and apoptotic blebbing in lower concentrations. SEM revealed that no adverse effects were caused by Coenzyme Q10 on the membrane structure, in dissimilarity, cell differentiation and proliferation, including myoblast formation were seen in the presence of all the concentrations of Coenzyme Q10. Numerous ion channels were observed on cellular surfaces exposed to Coenzyme Q10. Upon exposure to 0.005% Triton X-100, after pre-treatment with Coenzyme Q10, SEM revealed a “membrane patch” formation on membranes disrupted by Triton X-100. Damage to cell membranes in the presence of Triton X-100, were less severe when cells were pre-treated with Coenzyme Q10. Confocal microscopy was utilized to investigate intracellular occurrences in the presence of Triton X-100 and Coenzyme Q10. Using Mito Tracker Red to stain active respiring mitochondria and DAPI to stain nuclei, confocal microscopy confirmed the observations made by SEM, that Coenzyme Q10 enhance cell proliferation and differentiation, and that the adverse effects to cells exposed to Triton X-100 are less severe after pre-treatment with Coenzyme Q10. ROS generation was detected, using dichlorodihydrofluorescein diacetate, in cultures exposed to Triton X-100, and none in the presence of Coenzyme Q10. In the presence of Triton X-100, after pre-treatment with Coenzyme Q10, ROS generation was remarkably lower.

The study provided apparent evidence that Coenzyme Q10 offer protection to cardiac and skeletal muscle cells in culture after exposure to relatively low concentrations of the membrane disrupter Triton X-100. Coenzyme Q10 also promotes the process of proliferation and differentiation in primary chicken embryonic cultures of heart and skeletal muscle cells.

Declaration

I, Marnie Potgieter, hereby declare that this research dissertation is my own work and has not been presented for any degree at another University;

Signed: …….....

Date: …….....

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"Except the Lord build the house, they labour in vain that build it: except the Lord keep the city, the watchman waketh but in vain. It is in vain for you to rise up early, to sit up late, to eat the bread of sorrows: for so He giveth his beloved sleep." (Psalm 127:1-2)

List of Abbreviations, Symbols and Chemical Formulae

%	Percentage
˙QH	Ubisemiquinone or univalently reduced state of Coenzyme Q10
°C	Degrees centigrade
β-gal	β-galactosidase
µg	Microgram
µg/µl	Microgram per microlitre
µl	Microlitres
µm	Micrometer
3D	Three dimensional
4-HB	4-hydroxy benzoic acid
8-OH-dG	8-hydroxy-deoxyguanosine
AB	Alamar Blue
abs	Absorbance
acetyl-CoA	Acetyl-coenzyme A
ADP	Adenosine 5'-diphosphate
AFM	Atomic force microscopy
AIDS	Acquired immunodeficiency syndrome
AIF	Apoptosis inducing factor
ALS	Amyotrophic lateral sclerosis
ANOVA	Analysis of variance
AOA1	Ataxia-oculomotor-aprataxia type 1
APTX	Gene that codes for aprataxin
asc˙	Ascorbyl radical
ATP	Adenosine triphosphate
Bax	BCL2-associated X protein

c fos	Protooncogene
Ca^{2+}	Calcium ion
CCCP	Carbonyl cyanide m-chloro phenylhydrazone
CDK	Cyclin-dependent kinase
CHF	Congestive heart failure
cm^2	Centimetres squared
CMC	Critical micelle concentration
c-myc	Proto-oncogene with sequence homology to viral avian myelocytomatosis viral oncogene (v-Myc)
CO_2	Carbon dioxide
CoA	Coenzyme A
COPD	Chronic obstructive pulmonary disease
Coq 1-9	The Nine Coq proteins
Coq	Coenzyme Q10 genes
CoQ	Fully oxidized ubiquinone form
CoQ/CoQ10	Coenzyme Q10
COQ1-8	Biosynthetic enzymes
COQ1-9	Q-deficient yeast mutants
CoQ9	Coenzyme Q9
$CoQH^-$	Radical semiquinone intermediate
CoQH2	Fully reduced ubiquinol form
COX	Human complex IV
CPRG	Chlorophenol red-β-d-galactopyranoside
CV	Crystal violet
Da	Dalton
DAPI	4´,6-diamidino-2-phenylindole dihydrochloride
DCF	Dichlorofluorescein
DCFH	Dichlorodihydrofluorescein

DCH_2FDA	Dichlorodihydrofluorescein diacetate
ddH_2O	Double distilled water
DMAPP	Dimethylallyl diphosphate
DMEM	Dulbecco's Modified Eagle's Medium
$DMQH_2$	5-demethoxyubiquinol
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DPBS	Dulbecco's Phosphate Buffered Saline
dsDNA	Double stranded DNA
e^-	Electron
E13	Embryonic day 13
E2	17β-estradiol
E6	Embryonic day 6
EC50	half maximal effective concentration
EDC	Endocrine disrupting chemical/s
EDTA	Ethylene diamine tetra acetate
ER-α	Human estrogen receptor-α
etc.	et cetera
ETFDH	Electron-transferring-flavoprotein dehydrogenases gene
$FADH_2$	Flavin adenine dinucleotide
FBS	Foetal bovine serum
$Fe^3O_2^{\cdot -}$	Perferryl radical
FRDA	Friedrich's ataxia
g	Gram
G1	A period in the cell cycle during interphase
G2	The third, final, and usually the shortest subphase during interphase within the cell cycle
GAII	Glutaric aciduria type II
GI	Gastro-intestinal

H^+	Hydrogen ion
H_2O	Water
H_2O_2	Hydrogen peroxide
HBSS	Hanks Balanced Salt Solution
HCl	Hydrochloric acid
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
hr	Hour/hours
i.e.	That is
IC50	half maximal (50%) inhibitory concentration (IC) of a substance
Inc	Incorporated
IPP	Isopentenyl-PP
KCl	Potassium chloride
kDa	Kilodalton
KH_2PO_4	Potassium dihydrogen phosphate
$L^{\cdot}$	Carbon-centered radical
LH	Polyunsaturated fatty acid
$LOO^{\cdot}$	Lipid peroxyl radicals
LOOH	Lipid hydroperoxide
M	Mitosis
M	Molar
mg	Milligram
mg/ml	Milligrams per millilitre
Mg^{2+}	Magnesium ion
ml	Millilitre
mm	Millimetres
mM	Millimolar
MRFs	Myogenic regulatory factors
MTT	1-(4,5-Dimethylthiazol-2-yl)–3,5 diphenylformazan

mV	Millivolt
Myf5	Myogenic factor 5
MyoD	Myogenic factor D
Na^{2+}	Sodium ion
Na_2HPO_4	Disodium hydrogen phosphate
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
$NaHCO_3$	Sodium hydrogen carbonate
NF_KB	nuclear factor-kappa B
ng/L	Nanogram per liter
nm	Nanometre
NOX	NADH oxidase
NP	Nonylphenol
NPE	Nonylphenol ethoxylate/s
NR	Neutral red
N-SMase	Neutral sphingomyelinase
O_2^-	Superoxide
OH^-	Hydroxyl ion
OsO_4	Osmium tetraoxide
OXPHOS	Oxidative phosphorylation
Pax7	Transcription factor
PBS	Phosphate Buffered Saline Solution
PDSS1	Prenyldiphosphate synthase, subunit 1
PDSS2	Decaprenyl diphosphate synthase, subunit 2
PHB	Polyprenyl-4-hydroxybenzoate
P_i	Phosphate
PI	Propidium iodide

-PP	Diphosphate
PSF	Penicillin, streptomycin, fungizone
PTP	Permeability transition pore
p-Value	Probability value
Q 1 – 5	Five different concentrations of Coenzyme Q10, used in the study
Q	The fully oxidized state of Coenzyme Q10 or ubiqunone
RCBA	Recombinant Yeast Screen Assay
RIE	Relative induction efficiency
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RSV-BH	Rous sarcoma virus (high titre strain)
RSV-BH-Ta	Mutant form of the Rous sarcoma virus
RuO_4	Ruthenium oxide
SAM	S-adenosylmethionine
SD	Standard deviation
SDH	Succinate dehydrogenase
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning Electron Microscopy
SMP	Submitochondrial particles
TEM	Transmission Electron Microscopy
TX 1 – 5	Five different concentrations of Triton X-100, used in the study
ug/ml	Microgram per millilitre
VitE-$O^{\cdot}$	α-tocopheroxyl radical
VLDL	Very low density lipoproteins

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