ZINC AND GENOMIC STABILITY

A thesis submitted to the University of Adelaide
for the degree of Doctor of Philosophy

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7.3 Zinc and genomic stability: *in vivo* (Genome health effect of Zinc supplementation in an elderly South Australian population with low Zinc status)

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Abstract

Zinc (Zn) is an essential trace element required for both optimal human health and maintaining genomic stability. The main aim of this thesis was to address important knowledge gaps regarding the possible impact of Zn status on genomic stability events in both lymphocytes and epithelial cells using both *in vitro* and *in vivo* models. The project also aimed to study the differential impact of Zn Carnosine (ZnC) and Zn Sulphate (ZnSO₄) on genome stability as the former is a newly emerging commercially available supplement renown for its antioxidant capacity. The *in vitro* studies investigated the effects of ZnSO₄ and ZnC on cell proliferation via MTT assay and DNA damage rates and was measured using both the comet assay and the Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay in the WIL2-NS human lymphoblastoid cell line and HOK cell line. This study also investigated the impact of Zn status on both telomere length and telomere base damage *in vitro*. An *in vivo* study was designed to further investigate the effect of Zn supplementation in minimising genome instability events in lymphocytes. An increased intake of Zn may reduce the risk of degenerative diseases but may be toxic if taken in excess. This study aimed to investigate whether taking daily supplements of 20 mg of Zn as Zn Carnosine can improve Zn status, genome stability events and Zn transporter genes in an elderly South Australian cohort characterised by having low plasma Zn levels. In conclusion, the *in vitro* studies suggest that 1) Zn deficiency (0 µM) and high Zn concentrations increase DNA damage; 2) Zn at 4-16 µM is optimal in maintaining genome stability events; 3) Zn at 16-32 µM is optimal in protecting the cell against DNA damage induced by irradiation and hydrogen peroxide challenges; and 4) Zn may play an important role in telomere maintenances. The *in vivo* study suggests that Zn supplementation may be beneficial in an elderly population with marginal lowered Zn status by raising plasma Zn levels, lowering DNA damage events and modifies Zn transporter gene expression.
Declarations

I, Razinah Sharif certify that 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 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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Signature: ........................................ Date: ......................................
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Presentations and Publications arising from the thesis

Abstract/Poster Presentations


**Oral Presentations**

1. Zinc and Genomic Stability. Wednesday Wrap. School of Medicine, University of Adelaide. 16th September 2009.

2. Zinc and Genomic Stability. Wednesday Wrap. School of Medicine, University of Adelaide. 14th December 2011.


Publications


List of Abbreviations

ACCV   Anti Cancer Council of Victoria
AOA   Antioxidant Activity
ANOVA   Analysis of Variance
AP1   Activator Protein 1
APE   Apyrimidinic Endonuclease
ATCC   American Type Culture Collection
aTL   Absolute Telomere Length
ATM   Ataxia Telangiectasia Mutated
ATR   Ataxia Telangiectasia and Rad3 Related
ATRIP   Ataxia Telangiectasia and Rad3 Related Interacting Protein
AU   Arbitrary Unit

BCA   Bicinchoninic Acid
BER   Base Excision Repair
BN   Binucleate
BNed   Binucleated
BHMT   Betaine-homocysteine-S-methyltranferase
BSA   Bovine Serum Albumin

Ca   Calcium
CBMN Cyt assay   Cytokinesis Block Micronucleus Cytome assay
cDNA   Complementary Deoxyribonucleic Acid
CRP   C-Reactive Protein
CSIRO   Commonwealth Scientific and Industrial Research Organisation
CT   Cycle Threshold
Cu   Copper
CuSO4   Copper Sulphate
Cu/ZnSOD   Copper Zinc Superoxide Dismutase
CV   Coefficient of Variation
Cyto-B   Cytochalasin B

DCF   2′7′-dichlorofluorescein
DCFH   2′7′-dichlorofluorescein hydrochloride
dH2O   Distilled Water
DMSO   Dimethyl Sulfoxide
DNA   Deoxyribonucleic Acid
DNMT   Deoxyribonucleic Acid Methyltransferase
DTT   Dithiothreitol
EDTA | Ethylenediaminetetraacetic Acid
--- | ---
ELISA | Enzyme-linked Immunosorbent Assay
eSOD | Erythrocyte Superoxide Dismutase
FapyGua | 2,6-diamino-4-hydroxy-5-formamidopyrimidine
FapyAde | 4,6-diamino-5-formamidopyrimidine
FBS | Foetal Bovine Serum
Fe | Iron
FeCl$_3$.6H$_2$O | Iron Chloride
FFQ | Food Frequency Questionnaire
Fpg | Formanidopyrimidine-DNA Glycosylase
FRAP | Ferric reducing Ability of Plasma
GAPDH | Glyceraldehyde 3-Phosphate Dehydrogenase
gDNA | Genomic Deoxyribonucleic Acid

H$_2$O$_2$ | Hydrogen Peroxide
HBSS | Hanks Balanced Salt Solution
Hcy | Homocysteine
HCl | Hydrochloric Acid
HOK | Human Oral Keratinocyte
HUMN | HUman MicroNucleus/ The International Collaborative Project on Micronucleus Frequency in Human Populations
H$_2$O | Water
ICPOES | Inductively Coupled Plasma Optical Emission Spectrometry
IL-6 | Interleukin-6
IMVS | Institute of Medical and Veterinary Science
IR | Irradiated
K | Potassium
Kb | Kilobases
MDA | Malondialdehyde
Mg | Magnesium
MgCl$_2$ | Magnesium Chloride
MNi | Micronuclei
MNed | Micronucleated
MnSOD | Manganese Superoxide Dismutase
mRNA | Messenger Ribonucleic Acid
MT | Metallothionein
MT1A | Metallothionein-1A
MTR | Methionine Synthase
MTT | 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
M2nD | Marginal Zinc Deficiency
Na  
Sodium
NaCl  
Sodium Chloride
NaF  
Sodium Fluoride
NaOH  
Sodium Hydroxide
NBud  
Nuclear Bud
NDI  
Nuclear Division Index
NFκB  
Nuclear Factor kappa-light-chain-enhancer of activated B cells
NI  
Non Irradiated
NK  
Natural Killer
NPD  
Nucleoplasmic Bridge
NO  
Nitric Oxide
Na₄P₂O₇.10H₂O  
Sodium Pyrophosphate
Na₃VO₄  
Sodium Orthovanadate

8-OHdG  
8-Hydroxy-2-deoxyguanosine
8-oxoG  
8-Oxoguanine
8-oxodG  
8-Oxo-2'-deoxyguanosine
OGG1  
8-Oxoguanine DNA glycosylase
OKM  
Oral Keratinocyte Medium
OKGS  
Oral Keratinocyte Growth Supplement

P  
Phosphorus
p53  
p53 Tumor Suppressor genes
PARP  
Poly (ADP-ribose) Polymerase
PBL  
Peripheral Blood Lymphocyte
PBS  
Phosphate Buffered Saline
PCR  
Polymerase Chain Reaction
PHA  
Phytohemagglutinin
PMSF  
Phenylmethanesulfonylfluoride

Q-FISH  
Quantitative Fluorescent In Situ Hybridization

RDA  
Recommended Daily Allowance
RDI  
Recommended Daily Intake
Ref1  
Redox Factor-1
RPMI  
Roswell Park Memorial Institute
ROS  
Reactive Oxygen Species
RT  
Real Time
RT  
Room Temperature
RTPCR  
Real Time Polymerase Chain Reaction

S  
Sulphur
SAM  
S-adenosyl Methionine
SE  
Standard Error
SD  
Standard Deviation
SDS  
Sodium Dodecyl Sulfate
<table>
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<tr>
<td>SDS-PAGE</td>
<td>Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis</td>
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<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>SOD</td>
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<td>$\gamma$-H2AX</td>
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