ZINC AND GENOMIC STABILITY

A thesis submitted to the University of Adelaide

for the degree of Doctor of Philosophy

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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 ($ZnSO_4$) 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 $ZnSO_4$ 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

1. **Sharif, R**., Thomas, P., Zalewski, P., Graham, R. & Fenech, M. The effect of Zinc Sulphate and Zinc Carnosine on cytotoxicity and genotoxicity in the WIL2-NS lymphoblastoid cell line. 19th International Conference on Nutrition. 4-9th October 2009, Bangkok, Thailand.

2. **Sharif, R**., Thomas, P., Zalewski, P., Graham, R. & Fenech, M. The effect of Zinc Sulphate and Zinc Carnosine on cytotoxicity and genotoxicity in the WIL2-NS lymphoblastoid cell line. Australian Science Medical Research 2010. 9-10th June 2010, Adelaide, Australia.

3. **Sharif, R**., Thomas, P., Zalewski, P., Graham, R. & Fenech, M. The effect of Zinc Sulphate and Zinc Carnosine on cytotoxicity and genotoxicity in the WIL2-NS lymphoblastoid cell line. Nutrigenomics Symposium, CSIRO. 30th July 2010, Adelaide, Australia.

4. **Sharif, R.** Thomas, P. Zalewski, P. and Fenech, M. Zinc deficiency increases genome instability in Human Oral Keratinocytes (HOK). Nutrition in Medicine Conference. 13–15th May 2011, Bondi, Australia.

5. **Sharif, R.** Thomas, P. Zalewski, P. and Fenech, M. Zinc deficiency increases genome instability in Human Oral Keratinocytes (HOK). Australian Science Medical Research 2011. 9-10th June 2011, Adelaide, Australia.

6. **Sharif, R.** Thomas, P. Zalewski, P. and Fenech, M. Zinc deficiency increases genome instability in Human Oral Keratinocytes (HOK). XI Asian Congress on Nutrition 2011. 13-16th July 2011, Singapore.

7. **Sharif, R.** Thomas, P. Zalewski, P. and Fenech, M. Zinc deficiency increases genome instability in Human Oral Keratinocytes (HOK). Postgraduate Research Conference, Faculty of Health Science, University of Adelaide. 25th August 2011, Adelaide, Australia.

8. **Sharif, R.** Thomas, P. Zalewski, P. and Fenech, M. The effect of Zinc Sulphate and Zinc Carnosine on cytotoxicity and genotoxicity in the WIL2-NS lymphoblastoid cell line. NSNZ & *NSA* Joint Annual Scientific Meeting. 30th November-2nd December 2011, Queenstown, New Zealand.

9. **Sharif, R.** Thomas, P. Zalewski, P. and Fenech, M. Zinc supplementation influences genomic instability biomarkers, antioxidant activity and Zn transporter genes in an elderly Australian population with low Zn status. International Society for Zinc Biology 2012 Conference. 15-19th January 2012, Melbourne, Australia.

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.

3. Zinc and Genomic Stability. Special Seminar. Genome Stability Laboratory. Yong Loo Lin School of Medicine. National University of Singapore. 11th July 2011.

4. The effect of Zinc Sulphate and Zinc Carnosine on genome stability and cytotoxicity in the WIL2-NS lymphoblastoid cell line. International Society for Zinc Biology 2012 Conference. 15-19th January 2012. Melbourne, Australia.

Publications

1. **Sharif, R**., Thomas, P., Zalewski, P., Graham, R. & Fenech, M. (2011) The effect of Zinc Sulphate and Zinc Carnosine on cytotoxicity and genotoxicity in the WIL2-NS lymphoblastoid cell line. *Mutation Research*. **720(1-2)**: 22-33.

2. **Sharif, R**., Thomas, P., Zalewski, P. & Fenech, M. (2011) Zinc deficiency or excess within the physiological range increases genome instability and cytotoxicity, respectively, in human oral keratinocyte cells. Genes and Nutrition. In press.

3. **Sharif, R**., Thomas, P., Zalewski, P. & Fenech, M. (2011) The role of zinc in genomic stability. Mutation Research. In press.

4. O'Callaghan, N., Baack, N., **Sharif, R**., and Fenech, M. (2011) A qPCR-based assay to quantify oxidized guanine and other FPG-sensitive base lesions within telomeric DNA. Biotechniques. Vol. 51 (6): 403–412.

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
C_T	Cycle Threshold
Cu	Copper
$CuSO_4$	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
dH_2O	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
$FeCl_3.6H_2O$	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_2O_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_2O	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
MZnD	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
NPB	Nucleoplasmic Bridge
NO	Nitric Oxide
$Na_4P_2O_7.10H_2O$	Sodium Pyrophosphate
Na_3VO_4	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

SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SNP	Single Nucleotide Polymorphism
SOD	Superoxide Dismutase
TANK1	Human Tankyrase 1
TBAR	Thiobarbituric Acid Reaction
TBD	Telomere Base Damage
TI	Tail Intensity
TL	Telomere Length
TM	Tail Moment
TPEN	N,N,N'N'-tetrakis(-)[2-pyridylmethyl]-ethylenediamine
TPTZ	Tripyridyl Triazine
WAS	Waite Analytical Service
WHO	World Health Organization
WIL2-NS	WIL2-NS Lymphoblastoid Cell Line
WST-1	2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt
Zn	Zinc
ZnC	Zinc Carnosine
ZnD	Zinc Deficiency
ZnAD	Zinc Adequate
$ZnSO_4$	Zinc Sulphate
ZIP1	ZIP1 human Zinc transporter gene
γ-H2AX	genes coding for Histone 2A (phosphorylated)