The Effects of Selenomethionine and Wheat Biofortified with Selenium on DNA Damage and Cell Death in Human Lymphocytes

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Abstract

Selenium (Se) is an essential micronutrient, being a component of more than twenty seleno-proteins in humans. Previous studies suggested that increased intake of Se may reduce the risk of degenerative diseases including cancer; however, excessive intake can be toxic. Wheat is one of the major dietary sources of Se in humans, mainly in the form of L-selenomethionine (Se-met) but the impact of this source of Se on human health at the genome level was previously unexplored.

This PhD project aimed to (a) determine the safe dose-range and bio-efficacy of Se-met *in vitro*; (b) identify the optimal concentration of Se-met for reduction of genome damage *in vitro*; (c) investigate the optimal concentration of Se-met for improving resistance to gamma radiation or hydrogen peroxide induced genome damage *in vitro*; d) determine the bioavailability and bioefficacy of Se *in vivo*, in the form of either Se-met or wheat biofortified with Se; e) identify the nutrients and food groups that are correlated with Se intake/status and f) identify the nutrients, food groups and plasma mineral concentrations that are correlated to baseline lymphocyte DNA damage.

The *in vitro* study was performed on the peripheral blood lymphocytes isolated from six males and cultured with media supplemented with Se-met in a series of Se concentrations from 3 to 3850 µg Se/l while keeping the total methionine (i.e. Se-met + L-methionine) concentration constant. Baseline genome stability of lymphocytes and the extent of DNA damage induced by 1.5 Gy γ-ray or 7.5 µM hydrogen peroxide (H_2O_2) were investigated using the Cytokinesis-block Micronucleus Cytome (CBMN-Cyt) assay and the alkaline Comet assay with and without glycosylase (Fpg or Endo III) treatment after 9 days of culture. Results showed that high Se concentrations (≥1880 µg Se/l) caused strong inhibition of cell division, extensive DNA damage and increased cell death indicating cytotoxicity and genotoxicity. Baseline frequency of nucleoplasmic bridges (NPBs) and nuclear buds (NBud) declined significantly as Se concentration increased from 3 µg Se/l to 430 µg Se/l (P trend = 0.03 and 0.008, respectively); however, a significant trend of increase in Comet DNA damage was also observed (P trend <0.05) in lymphocytes. Selenium concentration (≤ 430 µg Se/l) had no significant effect on baseline frequency of micronuclei (MN) or DNA oxidation and had no protective effect against γ-ray-induced or H_2O_2-induced genome damage in lymphocytes.

A randomised double-blind placebo-controlled intervention trial was conducted on healthy South Australian males (n = 62, age (mean ± SD) 56 ± 7.0 years) with Se

dosage increased every 8 weeks for a total duration of 24 weeks. This study compared the bioavailability, by using plasma Se concentration as the biomarker, and bioefficacy of Se, by using platelet glutathione peroxidase (GPx) activity and lymphocyte DNA damage as biomarkers, from wheat process-fortified with Se-met (PROFORT) and high-Se wheat biofortified with Se (BIOFORT) compared to non-fortified normal (CONTROL) wheat. It was found that increased Se intake from BIOFORT wheat increased plasma Se concentration effectively in a dose-response manner from a baseline of 122 µg/l up to 190 µg/l ($P<0.001$). Increased Se intake from PROFORT wheat also increased plasma Se with a plateau at 140 µg/l, being therefore less effective than BIOFORT wheat ($P<0.001$). There was no significant change in Se status in the CONTROL group. Improved plasma Se concentrations had no effect on platelet GPx activity or lymphocyte DNA damage in either of the intervention groups.

Results from the food frequency questionnaire (FFQ) survey (n = 173) and plasma Se concentration survey (n = 179) suggested that the study population screened for participation in the *in vivo* trial described above had a mean plasma Se concentration (± SD) of 102 (± 12) µg/l and a mean (± SD) estimated Se intake of 165 (± 68) µg/d. This is a higher estimated Se intake than found in previous Australian studies. The major dietary sources of Se were found to be bread/cereals, fish/seafood and meat. However, increased intake of nuts/seeds, which are rich in Se, may have undesirable effects on lymphocyte DNA oxidation in this Se-replete population.

In conclusion, the *in vitro* studies suggest that (1) Se-met at higher concentrations at greater or equal to 1880 µg Se/l is cytotoxic; (2) Se-met may improve specific genome stability biomarkers such as nucleoplasmic bridge and nuclear bud at concentrations up to 430 µg Se/l, but further studies are needed to verify this effect. The *in vivo* studies in older men showed that Se from BIOFORT wheat is more effective in raising plasma Se concentration than Se from wheat process-fortified by the addition of Se-met, when both wheat products were subjected to strong heat. However, the platelet GPx activity and lymphocyte DNA damage appeared not to be modified by improved Se status.

This work contains two publications:

1) "The effect of selenium, as selenomethionine, on genome stability and cytotoxicity in human lymphocytes as measured by the cytokinesis-block micronucleus cytome assay". ***Mutagenesis*** 2009 May;24(3):225-32. 2) "Increased consumption of wheat biofortified with selenium does not modify biomarkers of cancer risk, oxidative stress or immune function in Australian males" ***Environmental Molecular Mutagenesis.*** 2009 July; 50 (6):489-501

The latter one was not able to be published in a journal of higher impact factor due to part of the data had been published elsewhere. Both articles are attached in Appendix.

Declaration

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 to Jing Wu 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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* List of publications:

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"Increased consumption of wheat biofortified with selenium does not modify biomarkers of cancer risk, oxidative stress or immune function in Australian males" Epub ahead of print in ***Environmental Molecular Mutagenesis.*** DOI: 10.1002/em

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List of abbreviations

ACCV	Anti-Cancer Council of Victoria
AIDS	Acquired immunodeficiency syndrome
ALS	Alkali labile site
ANOVA	Analysis of variance
ATM	Ataxia telangiectasia mutated gene
ATP	Adenosine tri-phosphate
ATR	Ataxia telangiectasia mutated and Rad3-related gene
AU	Arbitrary unit
BIOFORT	Wheat biofortified with selenium
BNed	Binucleated
BRCA	Breast cancer gene
Ca	Calcium
CBMN Cyt assay	Cytokenesis-block micronucleus cytome assay
CHK2	Background checkpoint kinase 2 gene
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Cu	Copper
$CuSO_4$	Copper sulphate
CV	Coefficient of variation
Cyto-B	Cytochalasin B
DAN	Diaminonaphthalene
DI	Deiodinase
DMABP	3,2'-dimethyl-4-aminobiphenyl
DMSO	Dimethyl sulfoxide
DSB	Double strand break
EDTA	Ethylenediaminetetraacetic acid
Endo III	Endoneclease III
FBS	Foetal bovine serum
Fe	Iron
FFQ	Food frequency questionnaire
Fpg	Formanidopyrimidine-DNA glycosylase

*Gadd*45	Growth arrest and DNA damage gene
GPx	Glutathione peroxidase
GSH	Reducing glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
HBSS	Hanks balanced salt solution
$HClO_4$	Perchloric acid
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HNO_3	Nitric acid
hTERT	Human telomerase reverse transcriptase
ICP-MS	Inductive coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
IDI	Iodothyronine deiodinase
IFN-γ	Interferon gamma
IL-2	Interleukin-2
IMVS	Institute of Medical and Veterinary Science
K	Potassium
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
LDL	Low-density lipoprotein
Mg	Magnesium
MN	Micronuclei
MNed	Micronucleated
MT	Metallothionein
Mtase	Cytosine-5-methyltransferase
MTHFR	Methylenetetrahydrofolate reductase
MUFA	Monounsaturated fatty acid

Na	Sodium
NaCl	Sodium chloride
Na_2CO_3	Sodium carbonate
Na_2HPO_4	Sodium phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NBud	Nuclear bud
NCEFF	National Centre of Excellence in Functional Foods
NDI	Nuclear division index
NHMRC	National Health and Medical Research Council
NOAEL	No observable adverse effect level
NPB	Nucleoplasmic bridge
8-OHdG	8-hydroxy-2-deoxyguanosine
P	Phosphorus
PBS	Phosphate buffered saline
PHA	Phytohaemagglutinin
PHGPx	Phospholipid hydroperoxide glutathione peroxidase
PROFORT	Wheat process-fortified with selenomethionine
PUFA	Polyunsaturated fatty acid
p-XSC	1,4-phenylenebis(methylene)selenocyanate
RDA	Recommended daily allowance
RDI	Recommended daily intake
ROS	Reactive oxygen species
S	Sulphur
SAM	S-adenosylmethionine
SARDI	South Australia Research and Development Institute
SCGE	Single-cell gel electrophoresis
SDG	Selenodiglutathione
SE	Standard error
Se	Selenium
SeAM	Se-adenosylmethionine
SECIS	Selenocysteine insertion sequence

SE-EMP	Selenium exchangeable metabolic pool
Sel	Selenoprotein
Se-met	Selenomethionine
SD	Standard deviation
SNP	Single nucleotide polymorphism
SPS2	Selenophosphate synthetase-2
SSB	Single strand break
Top II	Topoisomerase II
TrxR	Thioredoxin reductase
UTR	Untranslated region
WAS	Waite Analytical Services
XPA	Xeroderma pigmentosum group A protein
Zn	Zinc