

Effects of Selenium Overexposure on Glutathione Peroxidase and Selenoprotein W Gene Expression in the Jejunum

Qin Zhang, Bitao Liu, Liangyan Zheng and Zhaoxin Tang
College of Veterinary Medicine, South China Agricultural University, 510642 Guangzhou, China

Abstract: Researchers determined how oxidative stress induced by excess Se alters expression of selected selenoproteins in the jejunum from mice fed diets of different Se content. Deficient Se diet downregulated the mRNA level of GPx1, GPx4 and SEPW1; super nutrition Se dramatically increased the level of GPx1 and SEPW1 mRNA levels but had no effect on GPx2 mRNA level. The excess Se diet led to decrease GPx1 and SEPW1 mRNA levels but had little effect on the level of GPx4 mRNA. The data show that mRNA levels of the GPx family or selenoprotein W are differentially regulated by dietary Se.

Key words: Selenium, antioxidant, overexposure, mRNA, GPx2

INTRODUCTION

Selenium (Se) is an essential nutritional trace element which is best known for its antioxidant properties that are mediated by various selenoproteins. Synthesis of selenoproteins can be affected by limitations in Se supply. Both excessive and insufficient Se intake can result in adverse health effects (Bermano *et al.*, 1995; Gan *et al.*, 2002; Balogh *et al.*, 2004; Fairweather-Tait *et al.*, 2011). There are numerous reports regarding the effects of Se deficiency and excess. Gastrointestinal Glutathione Peroxidase (GPx) mRNA increases in Se-deficient mice (Brigelius-Flohe *et al.*, 2001) whereas Gpx4 mRNA decreases substantially in Se-deficient turkey liver (Sunde and Hadley, 2010). The levels of GPx1, selenoprotein W (SEPW1/SelW) are dramatically decreased in Se-deficient compared with Se-replete rats (Barnes *et al.*, 2009). Severe Se deficiency caused almost total loss of GPx1 activity and mRNA in rat liver and heart while GPx4 activity was reduced by 75% in the liver and 60% in the heart, although, mRNA levels were unchanged (Bermano *et al.*, 1995). At high concentration, selenite is known to induce apoptosis in cells by a mechanism involving free radicals (Shen *et al.*, 2001). Se and GPx levels are increased in animals treated with an excess of Se (Kaushal and Bansal, 2009). GPx mRNA level and activities were significantly increased in rats injected with 20 mg Se/kg per day but dramatically decreased upon injection of 40 or 80 mg Se/kg per day (Gan *et al.*, 2002). GPx4 mRNA level in chicken was down-regulated by an excess of Se (Zoidis *et al.*, 2010). A significant decrease in

total Superoxide Dismutase (SOD) activity was observed in animals with both excess and depleted Se (Shalimi and Bansal, 2005).

Despite this, little information is available on the effects of Se overexposure on GPx and SEPW1 gene expression in mice intestinal tract. The aim of this study was to analyze the effects of varying the level of dietary exposure to Se on antioxidant capacity as measured by GPx and SEPW1 gene expression and evaluate the dose-dependent effect of dietary supplementation containing a range of Se levels on regulation of redox status in the intestine of mice.

MATERIALS AND METHODS

Animals and diets: All experimental protocols were approved by the ethics committee of South China Agricultural University. A total of 80, 7 weeks old male BALB/c mice (Certification No. L3623, Medical Experimental Animal Center of Guangdong Province, Guangzhou) with an average Body Weight (BW) of 20 g were allotted to four groups, each of which was replicated two times with 10 mice per replicate. BALB/c mice (n = 20 per group) were fed a diet containing 0.045 (Se-marginal), 0.1 (Se-adequate), 0.4 (Se-supernutrition) or 0.8 (Se-excess) mg Se/kg. The basal diet was calculated to be adequate in protein, energy, vitamins and minerals for this class of animal except for Se content (0.045 mg kg⁻¹). The total test period was 56 days. All mice were fed with the same basal diet for 7 days to adjust the Se status and to adapt to the new environment before the experimental period.

Tissue sample preparation: Ten mice from each treatment group were selected, fasted for 12 h and then tissues and fluids harvested under general anesthesia using halothane at the end of the experiment. All blood samples were collected into sterile tubes to assay serum biochemical parameters as described. Samples of intestine were excised from individual mice, weighed immediately in an aseptic environment and then frozen in liquid nitrogen and stored at -80°C until analysis as follows. In this process, segmental samples of liver obtained after weighing were homogenized on ice using a glass-Teflon homogenizer (K5424, Glas-col; Terre Haute, IN, USA) and centrifuged at 3000×g for 10 min at 4°C. Supernatants were used immediately in further biochemical assays and protein concentrations were determined with the Bradford assay (Bradford and Dodd, 1977) using bovine serum albumin as a protein standard.

Determination of biochemical parameters: Activities of redox enzymes (GPx, SOD, Glutathione Reductase (GR), Catalase (CAT) and Lactate Dehydrogenase (LDH)) and levels of Malondialdehyde (MDA) in the liver were determined using reagent kits from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The detection range of the kits was 0.2-1000 U mL⁻¹, the detectable concentration was <0.1 U mL⁻¹ and the inter-assay and intra-assay variation coefficients were 8 and 5%, respectively. All samples were tested in duplicate.

Assay of Se content in tissues: Tissue samples were loaded and digested with reagent combination (HNO₃:H₂O₂ = 5:1) in separate series and the digestions were carried out according to the program given in Table 1. The microwave digests were cooled to room temperature which can be accelerated by immersing the segments (vial holders) into an icy water bath. Volumes of the digests and reagent blanks were then adjusted to 10.0 mL with ultrapure water. The working solution was prepared in 3.0 M HCl and Se was directly measured with hydride generation-atomic fluorescence spectrometry

(AFS-9130, Beijing Titan Instruments Co., Ltd. Beijing, China). The primary standard Se (IV) solution of 1000 mg L⁻¹ was prepared in 3.0 M HCl from SeO₂ (99.8%; Aldrich Chemical Co., Milwaukee, WI, USA) and stored in a refrigerator at 4°C. This solution is stable for at least 12 months.

Determination of selenoprotein mRNA levels in jejunum: Total RNA was isolated from tissue samples using Trizol reagent (Invitrogen; Carlsbad, CA, USA) following the manufacturer's instructions. RNA concentration was determined by measuring the absorbance at 260 nm.

Subsequently, 2 µg of RNA was treated with DNase prior to reverse transcription to cDNA using a PrimeScript RT Reagent kit (Takara; Dalian, China). The cDNA samples were stored at -20°C.

To measure the levels of GPx1, GPx2, GPx4 and SEPW1 mRNA, Real-time Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) was performed using a 7500 Real Time PCR System (Applied Biosystems; Carlsbad, CA, USA) and SYBR® Premix Ex TaqII kit (Takara; Dalian, China). The volume of each PCR reaction was 25 µL which included 2 µL cDNA, 12.5 µL SYBR® Premix Ex TaqII, 1 µL PCR forward primer, 1 µL PCR reverse primer and 8.5 µL ddH₂O. Beta-actin was used as the internal control gene and the information for all primers is listed in Table 2. All data were normalized to the internal control beta-actin and relative expression levels were calculated using the 2^{-ΔΔC_T} Method.

Statistical analysis: Results were analyzed by one-way Analysis of Variance (ANOVA), followed by a multiple comparison test (Least Square Difference; LSD).

Table 1: Low volume microwave digestion program used for digestion of biological samples

Steps	Temperature (°C)	Ramp. (min)	Hold (min)
1	20-80	10	0
2	80-140	6	0
3	140-210	4	0
4	210	0	15
5	Vent	0	20

Table 2: qRT-PCR Primers for selenoproteins used in the present study

Genes	Primer sequence	Original published sequence (Genbank accession No.)	Product (bp)
<i>Beta-actin</i>	Forward: 5'-AGCCATGTACGTAGCCATCC-3' Reverse: 5'-CTCTCAGCTGTGGTGGTGAA-3'	NM_007393	228
^a <i>GPx1</i>	Forward: 5'-GGTTCGAGCCCAATTTTACA-3' Reverse: 5'-CCCACCAGGAACCTTCTCAA-3'	NM_008160	199
^b <i>GPx2</i>	Forward: 5'-GTGCTGATTGAGAATGTGGC-3' Reverse: 5'-AGGATGCTCGTTCTGCCCA-3'	NM_030677	252
^c <i>GPx4</i>	Forward: 5'-CTCCATGCACGAATTCTCAG-3' Reverse: 5'-ACGTCAGTTTTGCCTCATTG-3'	NM_008162	117
^d <i>SEPW1</i>	Forward: 5'-CCCAAGTACCTCCAGCTCAA-3' Reverse: 5'-GCCATCACCTCTCTTCTGG-3'	NM_009156	147

^aGlutathione Peroxidase 1 (GPx1), ^bGlutathione Peroxidase 2 (GPx2); ^cGlutathione Peroxidase 4 (Gpx4) and ^dSelenoprotein W (SEPW1)

Statistical analyses were performed using SPSS Statistical Software (Version 18.0). All data are presented as means±SEM. The $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Addition of Se to the diet noticeably increased liver organ index in mice treated with 0.4, 0.8 or 0.045 mg Se kg⁻¹, compared to mice treated with 0.1 mg Se kg⁻¹ ($p < 0.05$; Table 3). A greater increase in kidney organ index was observed in mice given 0.8 mg Se kg⁻¹ in the diet compared with those receiving 0.045 or 0.1 mg Se kg⁻¹. It suggested that both liver and kidney damage occurred. Deposition of Se in the liver and kidneys increased significantly ($p < 0.05$) with increasing amounts of Se added to the diet (Table 3). This is possibly due to selenosis induced by a high dose of inorganic Se, clearly indicating that these mice were exposed to excess Se.

As shown in Table 4, the activity of GPx in the liver increased in animals fed a diet containing 0.4 mg Se kg⁻¹ but the activity was decreased when Se levels were increased further to 0.8 mg kg⁻¹ diet. Dietary Se resulted in dose-dependent increases ($p < 0.05$) in Se concentrations and GPx activities in the liver. Data from the present study also agree with previous reports (Kaushal and Bansal, 2009). In the group fed with Se at a dose of 0.4 mg kg⁻¹, an increase in the GPx activity is likely based on a free radical mechanism. The MDA levels we observed are consistent with the level of Lipid Peroxidation (LPO) found in tissues (Kang *et al.*, 2000). A highly significant increase in LPO was recorded in the liver of mice fed on both 0.4 and 0.8 mg Se kg⁻¹ diets than the Se-adequate diet-fed animals. The marked increase in LPO in the 0.8 mg Se kg⁻¹ diet group can be explained on the basis of decreased levels of GPx which would lead to peroxide accumulation and oxidative stress.

Dietary Se levels exhibited varying effects on the expression of *GPx1*, *GPx2*, *GPx4* and *SEPW1* genes in the jejunum (Fig. 1). Supernutritional Se (0.4 mg kg⁻¹) status did not change GPx1, GPx4 or SEPW1 mRNA levels in the liver (Raines and Sunde, 2011) but did affect those of the three genes in the intestine in the experiment. GPx4 mRNA level was unchanged in liver and heart of rats with severe Se deficiency diet (Bermano *et al.*, 1995). The data displayed that the level of GPx4 mRNA was downregulated with Se-deficiency diet in intestine. It indicates that there was different sensitivity for GPx4 to Se over exposure in different organs. Se deficiency or Se excess diet led to decreased GPx1 and SEPW1 mRNA level but upregulated GPx2 mRNA level, it is possibility that GPx2 is highly ranked in antioxidative selenoprotein family.

Table 3: Effects of dietary selenium at different doses on weight and Se deposition of body composition in male BALB/c mice (n = 10)

Items	0.045 mg kg ⁻¹	0.1 mg kg ⁻¹	0.4 mg kg ⁻¹	0.8 mg kg ⁻¹
Organ index				
Liver	5.70±0.08	5.32±0.28 ^a	5.82±0.45 ^b	5.74±0.23 ^b
Kidney	1.51±0.09 ^a	1.58±0.08 ^a	1.56±0.08 ^a	1.68±0.09 ^b
Se deposition				
Liver (µg/g tissue)	0.61±0.02 ^a	0.83±0.07 ^b	1.30±0.03 ^{ab}	1.56±0.03 ^c
Kidney (µg/g tissue)	0.88±0.02 ^a	0.90±0.03 ^a	1.35±0.02 ^b	1.57±0.03 ^{ab}

Within each row, mean values between columns with different superscripts (a-c) are different at $p < 0.05$ unless otherwise stated (treatment effects). Organ index = (Organ weight/body weight)×100%

Table 4: Effects of dietary selenium at different doses on antioxidant capacity in male BALB/c mice (n = 10)

Parameters	0.045 mg kg ⁻¹	0.1 mg kg ⁻¹	0.4 mg kg ⁻¹	0.8 mg kg ⁻¹
^a MDA	1.51±0.16 ^a	1.68±0.07 ^a	1.73±0.11	1.96±0.14 ^b
^d LDH	1091±33 ^a	1068±14 ^a	1186±14 ^b	1268±20 ^{ab}
^e GSH-Px	333±14 ^a	686±15 ^{ab}	751±47 ^{ab}	525±12 ^b
^f GR	34.2±9.5	46.0±5.3 ^b	40.7±1.6 ^b	24.0±2.8 ^a
^g SOD	781±23	802±23 ^b	804±26 ^b	757±18 ^a
^k CAT	32.6±0.7 ^a	45.3±4.1 ^b	30.0±3.8 ^a	26.0±0.2 ^a

^{a, b} $p < 0.05$. ^aMDA: Malondialdehyde: nmoles of MDA/mg protein; ^dLDH: Lactate Dehydrogenase: units/mg protein; ^eGSH-Px: Glutathione Peroxidase: units/mg protein; ^fGR: Glutathione Reductase: units/mg protein; ^gSOD: Superoxide Dismutase: units/mg protein; ^kCAT: Catalase: units/mg protein

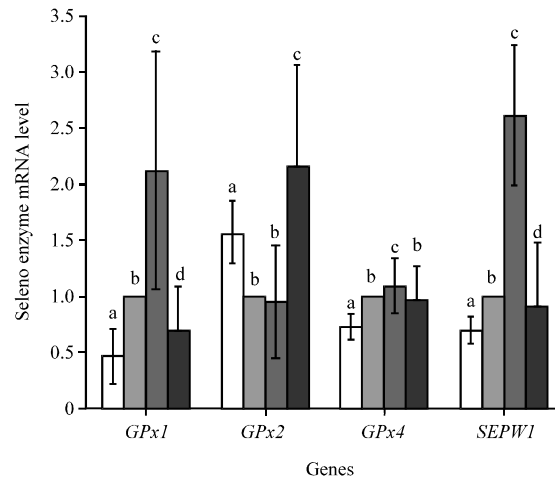


Fig. 1: Effects of different amounts of dietary selenium on mRNA levels for a subset of selenoproteins in the jejunum of male BALB/c mice (n = 6); ^{a-d}Significant difference ($p < 0.05$) by ANOVA. Values are means±SEM relative to baseline, group treated with 0.1 mg Se/kg diet. Beta-actin was used as internal control gene for normalization. White bars = Se-deficient group (0.045 mg kg⁻¹); light grey bars = Se-adequate group (0.1 mg kg⁻¹); middle grey bars = Se-supernutrition group (0.4 mg kg⁻¹); dark grey bars = Se-excess group (0.8 mg kg⁻¹). Values represent fold change in comparison with the transcription level of the group treated with 0.1 mg Se kg⁻¹ diet

CONCLUSION

Se exposure can reduce hepatic antioxidant capacity and cause liver dysfunction and mRNA levels of the GPx family or selenoprotein W are differentially regulated by dietary Se. This strongly suggests that there is a underlying mechanism in Se-dependent regulation of selenoprotein mRNA levels. For these selenoproteins with Se-regulated mRNA levels, it will also be important to determine if the protein levels of the selenoproteins are also regulated by Se status.

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