Effects of Se-depletion on glutathione peroxidase and selenoprotein W gene expression in the colon

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Abstract Selenium (Se)-containing proteins have important roles in protecting cells from oxidative damage. This work investigated the effects of Se-depletion on the expression of the genes encoding selenoproteins in colonic mucosa from rats fed diets of different Se content and in human intestinal Caco-2 cells grown in Se-adequate or Se-depleted culture medium. Se-depletion produced statistically significant (\(P < 0.05\)) falls in glutathione peroxidase (GPX) 1 mRNA (60–83\%) and selenoprotein W mRNA (73\%) levels, a small but significant fall in GPX4 mRNA (17–25\%) but no significant change in GPX2. The data show that SelW expression in the colon is highly sensitive to Se-depletion.

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1. Introduction

The micronutrient selenium (Se) is essential for health\[1]. Low intake of Se has been implicated with the increased incidence of disease and Se supplementation lowered both cancer incidence and mortality from cancer\[2,3\]. Se is present in several proteins, called selenoproteins, as the amino-acid selenocysteine (Se-cys)\[4\]. Approximately 30 selenoproteins and up to 25 selenoprotein genes have been identified in mammals and they have a variety of biological roles\[4\]. They include the glutathione peroxidase family of enzymes, all of which are involved in the cell’s antioxidant systems\[5\], the thioredoxin reductases, which are involved in the redox regulation of gene expression\[6\] and a number of recently identified selenoproteins (e.g., SelX, SelN, SelW, and 15 kDa selenoprotein) that are less well characterised\[4\]. The glutathione peroxidases include cystolic glutathione peroxidase (GPX1), gastrointestinal glutathione peroxidase (GPX2), plasma glutathione peroxidase (GPX3) and phospholipid hydroperoxide glutathione peroxidase (GPX4).

The phenotype of knock-out mice lacking GPX1 and GPX2 includes colon pathology and increased susceptibility to colitis\[7\], leading to the suggestion that these enzymes are important in determining the level of anti-oxidant protection and inflammatory responses in the colon\[8\]. In addition, supplementation with Se above normal intake reduces mortality from cancer of the colon\[2,3\], as well as that of lung and prostate. These observations indicate that selenoproteins such as the GPXs are critical for colon function. However, little is known of how selenoprotein gene expression in colonic cells, particularly in vivo, is regulated by Se availability.

The aim of the present work was to investigate how Se-deficiency modulates expression of a range of selenoprotein genes in the rat colon and a human gastrointestinal cell line. Since patterns of selenoprotein activity are accompanied by altered patterns of mRNA abundances and both are regulated by Se availability\[9,10\], our approach was to use a novel ‘selenoprotein’ macroarray to screen for genes affected by Se depletion and then to extend the studies using Northern hybridisation.

2. Materials and methods

2.1. DNA probes

The GPX1, GPX4 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes were 880 bp EcoRI, 814 bp EcoRI, and 780 bp PacI/XbaI fragments, respectively, as used previously\[9\]. The human GPX2 probe (from Prof. R. Brigelius-Flohe) was a 663 bp EcoRI fragment\[11\]. The human GPX1 probe was a 300 bp BamHI/XbaI fragment\[12\] and the human GPX4 probe (from Prof. K. Yagi, Gifu) was an 874 bp HindIII/XbaI fragment\[13\]. The SelW probe (from Dr. I. Kim) was a 420 bp BamHI/XbaI fragment corresponding to the mouse brain cDNA\[14\]. The 18S rRNA probe was a 328 bp fragment corresponding to nucleotides 4852–5179 of the human ribosomal RNA sequence\[15\] cloned into the SrfI site of PCR-Script AMP vector (Stratagene) and excised using KpnI and SacI.

2.2. RNA extraction and Northern hybridisation

Total RNA was extracted by the guanidinium thiocyanate/phenol/chloroform procedure\[16\]. Northern blotting was carried out using standard procedures\[17\] and membranes pre-hybridised for 20 min at 68°C in 10 ml of QuickHyb solution (Stratagene). 25 ng of RNA probes was labelled with \(^{32}\)P-\(\omega\)-dCTP by random priming, as described previously\[9\], and hybridisation carried out for 1 h at 68°C. Non-specifically bound probe was removed from membranes by two washes in 2\(\times\) SSC (1\(\times\) SSC = 0.15 M NaCl/0.015 M sodium citrate) 0.1% SDS at room temperature for 15 min, followed by one wash in 0.1\(\times\) SSC/0.1% SDS at 60°C for 30 min. Specific hybridisation was detected using a Canberra Packard InstantImager for quantification (results for each probe were expressed per unit of hybridisation achieved with the 18S rRNA probe to allow correction for any variation between loading of RNA on the gel or transfer to
control levels by day 8 (Fig. 1). The level of GPX1 mRNA
crase in GPX1 mRNA abundance such that it returned to
Addition of selenite to the medium at day 6 led to an in-
sion remained after 4 or 6 days in the Se-depleted medium.
grown in Se-supplemented medium (Fig. 1). This low expres-
 expressins were sected ou and washed with cold sterile PBS; the colon was then cut
animals were killed by cervical dislocation and the entire colon dis-
analysis, membranes were stripped by washing in 0.1% SDS for 10 min at 95 °C before rehybridisation to other probes.

2.3. cDNA expression arrays

32P-labelled cDNA probe synthesis from 3.5 μg total RNA and
hybridisation to the Atlas Array membranes were performed according to the Atlas cDNA expression Arrays User Manual (BD Biosciences Clontech, Palo Alto, USA). Detection was performed with a phos-
phorimager (Molecular Dynamics, Phosphorimager Storm 860) using

2.4. Cell culture

Stocks of human colon adenocarcinoma cells (Caco-2) were grown at 37 °C in Dulbecco’s modified Eagle’s medium (with 4.5 g/l glucose and Glutamax) supplemented with 10% (v/v) foetal calf serum (Sigma, Poole, UK), 60 μg/ml gentamycin, 1% (v/v) non-essential amino acids and penicillin/streptomycin (100 units/ml and 100 μg/ml, respectively). Cells were transferred to serum-free medium to which was added either insulin (5 μg/ml) and transferrin (5 μg/ml) (selenium-deficient) or insulin, transferrin and selenium as sodium selenite (7 ng/ml) (selenium-replete cells) [18]. Medium was changed every 2 days.

2.5. Animals and diets

Weaning Male Hooded Lister Rats of the Rowett strain were ran-
domly allocated to groups of six and fed ad libitum on a semi-synthetic diet containing different amounts of Se for 6 weeks. The basal diet [19] contained 8 ng of Se/g (severely Se-deficient) or 111 ng of Se/g as sodium selenite. At the end of the 6 weeks, the animals were anaesthetised with isoflurane and blood samples were taken by cardiac puncture. The animals were killed by cervical dislocation and the entire colon dissected out and washed with cold sterile PBS; the colon was then cut open, the mucosa scraped off and then rapidly frozen in liquid nitrogen and stored at −80 °C.

3. Results and discussion

Caco-2 cells were grown for 2–6 days in either a Se-sup-
plemented or Se-depleted medium and RNA extracted and analysed by Northern hybridisation. Cells grown in the Se-
depleted medium showed a 60% decrease in the level of GPX1 mRNA by 2 days (P < 0.05) compared with cells grown in Se-supplemented medium (Fig. 1). This low expression remained after 4 or 6 days in the Se-depleted medium. Addition of selenite to the medium at day 6 led to an in-
crease in GPX1 mRNA abundance such that it returned to control levels by day 8 (Fig. 1). The level of GPX1 mRNA is a consistent marker of Se status in cell and animal experimen-
ts [9,10,18,20] and these changes indicate that after 2 days in Se-depleted medium the Caco-2 cells become severely Se deficient, that the effect is not increased significantly over a further 4 days and that the effect can be reversed by re-
addition of selenite; this reflects closely the changes in GPX1 mRNA in H4 hepatoma cell and FRTL-5 thyroid cells grown in similar media [18,21].

Using a custom-made macroarray and RNA extracted from
Caco-2 cells grown for 2 days in Se-deficient or Se-supple-
Table 1

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Ratio Se−/Se+</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPX1</td>
<td>0.46</td>
</tr>
<tr>
<td>GPX2</td>
<td>1.63</td>
</tr>
<tr>
<td>GPX3</td>
<td>0.67</td>
</tr>
<tr>
<td>GPX4</td>
<td>0.76</td>
</tr>
<tr>
<td>SelP</td>
<td>0.90</td>
</tr>
<tr>
<td>SBP1</td>
<td>Up</td>
</tr>
<tr>
<td>DhpB</td>
<td>0.94</td>
</tr>
<tr>
<td>SPS (SelD)</td>
<td>1.03</td>
</tr>
<tr>
<td>Phospholipase A2</td>
<td>0.95</td>
</tr>
<tr>
<td>Cu/Zn SOD</td>
<td>1.29</td>
</tr>
<tr>
<td>Mn SOD</td>
<td>1.04</td>
</tr>
<tr>
<td>SOD3 (extracellular)</td>
<td>1.58</td>
</tr>
<tr>
<td>Glutathione-S-transferase A2</td>
<td>1.50</td>
</tr>
<tr>
<td>Glutathione-S-transferase A4</td>
<td>2.76</td>
</tr>
<tr>
<td>Interleukin 1; β</td>
<td>3.89</td>
</tr>
<tr>
<td>SelN</td>
<td>0.76</td>
</tr>
<tr>
<td>SelW</td>
<td>0.27</td>
</tr>
<tr>
<td>Selß (elongation factor)</td>
<td>Down</td>
</tr>
<tr>
<td>rRNA selenocysteine associated protein</td>
<td>1.20</td>
</tr>
<tr>
<td>SelZ (l)</td>
<td>Down</td>
</tr>
<tr>
<td>TR2</td>
<td>0.73</td>
</tr>
<tr>
<td>TR3</td>
<td>1.33</td>
</tr>
<tr>
<td>MHC, class I; C</td>
<td>Up</td>
</tr>
<tr>
<td>β-Actin</td>
<td>1.08</td>
</tr>
<tr>
<td>GAPDH</td>
<td>1.11</td>
</tr>
<tr>
<td>α-Tubulin</td>
<td>1.20</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>1.09</td>
</tr>
<tr>
<td>Ribosomal protein L13a</td>
<td>0.90</td>
</tr>
<tr>
<td>Ribosomal protein S9</td>
<td>0.96</td>
</tr>
<tr>
<td>HPRT</td>
<td>1.67</td>
</tr>
<tr>
<td>Genomic DNA</td>
<td>1.19</td>
</tr>
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</table>

mRNA abundances were measured in Caco-2 cells after 2 days in Se-
deficient or Se-replete conditions. Results are the mean values obtained from two experiments each analysed using duplicate arrays. Results are expressed as a ratio of the Se-depleted cells to Se-replete cells. GPX1 and SelW mRNA abundances; showed greater than twofold change; which is considered to be significant. When expression levels were close to the limit of detection the ratio of expression is given as updown and not as a precise value. The following genes were also present on the array but expression was not detected: thioredoxin reductase 1, deiodinase type 1, II and III, 15 kDa selenoprotein, sele-
onprotein M, T, and X, mitochondrial capsule selenoprotein, SECIS-
bounding protein 2, 5-lipoxygenase, arachidonate 15-lipoxygenase types 1 and 2, arachidonate 12-lipoxygenase 5-lipoxygenase activating protein, cyclooxygenases 1 and 2, tumor necrosis factor α and β, LTβ4 receptor, thromboxane A2 receptor, prostaglandin F and D2 recep-
tors, glutathione-S-transferase theta 1 and 2, thyroid hormone recep-
tor α and β2, interferon gamma receptor and precursor, metallothionein isofrom 1fR, interleukin 1 α and β, interleukin 2, tyrosine 3-monooxygenase.

Abbreviations: Sel, selenoprotein; SOD, superoxide dismutase; TR, thioredoxin reductase; MHC, major histocompatibility complex.

mented medium, it was found that Caco-2 cells expressed ten selenoprotein mRNAs (GPX1, GPX2, GPX3, GPX4, TR2, TR3, selenoproteins N, P, W and Z) at a level that was detect-
able using this technology (Table 1). Expression of other sele-
onproteins genes on the array (see legend to Table 1) was below the level of detection. After 2 days in Se-depleted med-
ium, only two selenoproteins showed a major change in mRNA abundance compared with Se-supplemented cells (Table 1): GPX1 and Selenoprotein W (SelW), for which the ratio of relative expression was 0.46 and 0.27, respectively. In addition, Se depletion led to increased expression of the non-selenoprotein mRNAs interleukin 1β and glutathione-S-transferase A4, for which the ratio of relative expression was
3.89 and 2.76, respectively. The relative expression of GPX3, GPX4 and SelN in Se-depleted cells (0.67, 0.76, and 0.76, respectively) was less than the twofold change considered to be significant but suggested that there may be a small fall in expression. There was no evidence of changes in relative expression of GPX2, SelP, TR2 and TR3 or of non-selenoprotein antioxidant enzymes such as Mn and Cu/Zn superoxide dismutases. These array data suggest that in Caco-2 cells expression of GPX1, 2 and 4 are differentially affected by Se-depletion and that the major effect of Se-depletion on selenoprotein expression in colonic epithelial cells, as assessed by mRNA abundance, is a dramatic fall in the expression of SelW and GPX1.

To confirm these findings, two experiments were carried out. First, mRNA abundances were measured in Se-supplemented and deficient Caco-2 cells by Northern hybridisation. Second, and more importantly, since little was known about the effects of Se-depletion on the colon in vivo, selenoprotein gene expression was studied in rats that were fed diets that provided different levels of dietary Se. Quantification of Northern hybridisation data from cell culture experiments showed that there was no statistical significant difference in GPX2 mRNA levels compared to cells grown in medium supplemented with Se (Fig. 2). However, there was a small (17%) but statistically significant decrease ($P < 0.05$) in the GPX4 mRNA level in the Se-depleted cells (Fig. 2). This was in contrast with the marked decline in the level of GPX1 mRNA. These data are consistent with earlier observations that Se-supplementation of cells grown in standard medium increases GPX1 mRNA abundance but not that of GPX2 [10].

Rats were fed diets containing either an adequate supply of Se (111 ng Se/g; group D) or three levels of low Se supply ranging from a severely deficient Se supply (8 ng Se/g; group A), to deficient (33 ng Se/g; group B) or marginal supply (63 ng Se/g; group C). Previously, such diets have been found to consistently produce Se-depletion in rats [9,20]. In the present experiments measurements of liver GPX1 mRNA abundance, an accepted sensitive marker of Se-depletion, were consistent with data from earlier studies (results not shown). RNA from mucosal scrapings was subjected to Northern hybridisation with probes to GPX1, 2 and 4. Quantification of the hybridisation showed that mean GPX1 mRNA abundance was decreased by 83% ($P < 0.05$) in the colon of severely Se-deficient rats compared with tissue from the Se-adequate rats and decreased by 60% in the colon of the deficient rats (Fig. 3). In contrast, mean GPX4 mRNA abundance showed
only a small decrease (25%; \(P < 0.05\)) in the colon of the severely deficient rats and GPX4 mRNA levels showed no statistically significant change in rats fed any of the Se-deficient diets. These results confirm and extend the array data by indicating that the effect of Se in cell culture is mirrored by that in the colon in vivo and by showing that there is a differential effect of Se-depletion on GPX1, 2 and 4 in the colon.

In the liver, Se-depletion has no effect on GPX4 mRNA expression although GPX1 decreases dramatically [9]. The present data show that, in the colon, GPX4 mRNA abundance decreases as well as that of GPX1, indicating that the response of glutathione peroxidase expression to Se-depletion is different in the colon from that observed previously in liver or heart [9]. This supports the contention that regulation of selenoprotein expression by Se supply is affected differentially in different tissues [9,10].

Using a mouse SelW cDNA as probe, Northern hybridisation showed a band corresponding to SelW in RNA samples from rats fed the Se-adequate diet (Fig. 4). However, the expression of SelW mRNA was decreased in the Se-depleted colon to such an extent that SelW mRNA was barely detectable in samples from animals fed the severely Se-deficient diet (Fig. 4). Density analysis of the autoradiographs showed that the ratio of SelW/GAPDH expression ranged from 1.0 to 1.9 in the rats fed the Se-adequate diet but was zero in the rats fed the severely Se-deficient diet, since the intensity of the SelW band was below the level of detection. These data indicate that SelW expression is highly sensitive to Se-depletion. SelW is expressed in muscle [22] but has not previously been shown to be expressed in either the colon or a gastrointestinal cell line.

In summary, the present data obtained from both Caco-2 cells in culture and from rat colon tissue show that Se-depletion has differential effects on selenoprotein expression, in particular a much greater effect on the abundance of GPX1 and SelW mRNAs than that of GPX4 or GPX2 mRNA. SelW is an anti-oxidant protein [14,22,23] and therefore our hypothesis is that the large effect of Se-depletion on the expression of both SelW and GPX1, together with the smaller effect on GPX4, and despite the maintenance of GPX2 expression, lowers antioxidant protection in the colon; and in turn this would increase susceptibility to development of inflammatory responses in the gastrointestinal system [8].

References


