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REGULATION OF GLUTATHIONE S-TRANSFERASE GENE EXPRESSION AND ACTIVITY BY DIETARY SELENIUM

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Summary: To determine selenium's effects on glutathione S-transferase gene expression and enzyme activity, weanling rats were fed a selenium-deficient diet, or the same diet supplemented with 0.1 (control) or 2.0 mg selenium/kg diet as sodium selenite, for 91 days. Consumption of either the selenium-deficient or high selenium diet increased activity of glutathione S-transferase, measured with 1-chloro-2,4-dinitrobenzene as substrate, compared to the control diet. Transcription of genes for glutathione S-transferase subunits was unaffected by selenium intake. Steady state levels of mRNA for glutathione S-transferase subunits were affected variably by changes in selenium intake, depending upon the tissue and subunit examined. These results suggest that the biological effects of selenium may be due in part to its regulation of gene expression for glutathione S-transferase family enzymes.

Chemical carcinogenesis is enhanced in experimental animals consuming diets deficient in the essential trace element selenium (1). However, the maximum protective effect of selenium in animal models of chemical carcinogenesis is achieved at levels of dietary selenium intake far in excess of those known to be nutritionally adequate (2). The mechanisms of this protective effect are not fully understood. Selenium deficiency dramatically reduces activity of selenium-dependent glutathione peroxidase (EC 1.11.1.9) (3), but high intakes of selenium do not elicit increases in enzyme activity above those achieved with a lower, but nutritionally adequate selenium intake (4). Thus, the protective effect of high selenium intakes against cancer in animal models is probably unrelated to glutathione peroxidase activity (5).

Alternative means by which selenium may inhibit chemical carcinogenesis require further consideration. Glutathione S-transferases (EC 2.5.1.18) play a significant role in xenobiotic metabolism, including the activation and deactivation of chemical carcinogens. Glutathione S-transferase activity, measured with 1-chloro-2,4-dinitrobenzene as substrate,

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increases with selenium deficiency (6). In addition, class alpha glutathione S-transferases, composed of subunits Ya and Yc, exhibit selenium-independent glutathione peroxidase activity toward organic substrates such as cumene hydroperoxide (7).

To explore additional mechanisms by which selenium may exert its cancer chemopreventive and other biological effects, these experiments were designed to measure glutathione S-transferase activity and expression of the genes for subunits Ya and Yc in tissues of rats fed diets containing deficient, adequate, or high levels of selenium. In addition, the expression of the gene for subunit Yp, a marker of neoplastic transformation in liver (8), was also determined.

Methods

Animals. Procedures related to the care and use of experimental animals were approved by the Brigham Young University Animal Care and Use Committee. Weanling male Sprague-Dawley rats were fed a basal selenium-deficient Torula yeast-based diet or the same diet supplemented with 0.1 (control) or 2.0 mg selenium/kg diet as sodium selenite. Six animals in each dietary group were fed their respective diets for 91 days. Animals were weighed weekly. At the end of the feeding period animals were killed by exsanguination via the abdominal aorta under ether anesthesia. Livers and kidneys were excised. Livers were perfused via the portal vein with cold KCl (150 mM). Portions of each tissue were aliquoted for subsequent isolation of total RNA, for enzyme assays, and - in the case of liver - for isolation of nuclei. Blood was removed from kidney samples by repeated fine mincing with surgical scissors in cold glutathione peroxidase or glutathione S-transferase assay buffer.

Enzyme assays. Glutathione peroxidase activity was assayed in 105,000 x g supernatant of liver and kidney by the method of Lawrence and Burk (3), using 2.0 mM GSH, and 0.25 mM H_2O_2 as substrate. Glutathione S-transferase activity was measured in 105,000 x g supernates by the method of Habig et al. (9), using as substrate 1-chloro-2,4-dinitrobenzene. Protein concentration was determined by the method of Lowry et al. (10).

Isolation of total RNA. Total RNA was purified from liver and kidney samples by the method of Chirgwin et al. (11) as modified by Freeman et al. (12). Concentration of RNA in the finished preparation was determined by optical density measurement at 260 nm. RNA samples were stored frozen at -70°C.

Isolation of nuclei. Liver nuclei were isolated by the method of Kasper (13) as modified by Simmons et al. (14). Nuclei suspended in storage buffer were flash frozen in liquid nitrogen and stored at -70°C until used in run-on transcription assays.

Measurement of transcription rate. The effects of different Se intakes on the relative rates of transcription of the genes for glutathione S-transferase subunits were determined in nuclear run-on transcription assays performed as described by Celano et al. (15) using [α-^{32}P]UTP to label nascent transcripts. Plasmids containing cDNA probes for glutathione S-transferase subunits were denatured by boiling for 10 minutes, then slot blotted (5 μg/slot) to nylon membranes using a slot blot apparatus. Membranes were treated by vacuum baking for 30 minutes at 80°C, followed by UV crosslinking at 120 millijoules/cm^2. Prehybridization of membranes was in Church-Gilbert buffer (16) at 65°C for 2-6 hours. Following isolation of nuclear RNA containing the labeled transcripts, equal quantities of incorporated radioactivity from each assay were added to prehybridized nylon filters containing immobilized plasmids, in fresh Church-Gilbert buffer. Hybridization proceeded overnight at 65°C. Membranes were then washed 3 times - 5 minutes, 5 minutes, then 15 minutes - in 0.2 x SSC, 0.4% SDS at 65°C (20 x SSC is 3 M NaCl, 0.3 M sodium citrate,

pH 7.0), air dried, and exposed to X-ray film using an intensifying screen at -70°C for up to 96 hours, depending on the intensity of the signal detected. Autoradiogram signal intensity was quantitated by laser densitometry.

Probes. The cDNAs for rat glutathione S-transferase subunits Ya (17), Yb1 (18) and Yc (19) were those first isolated and sequenced by Pickett and coworkers. The cDNA for subunit Yp (20) was a generous gift from Dr. Masami Muramatsu of the University of Tokyo. A cDNA for glyceraldehyde 3-phosphate dehydrogenase (21) was used as a control probe in Northern blot analysis to ensure equal loading of RNA.

Northern blot analysis. Following electrophoresis in formaldehyde-agarose gels, RNA was blotted to nylon membranes. Membranes were subsequently vacuum baked and UV-crosslinked as described above. Probes used were coding sequences from the cDNAs described above, radioactively labeled with [α-^{32}P]dCTP by random primed labeling (22). Prehybridization, hybridization, and subsequent washes were carried out according to the membrane manufacturer's directions. Autoradiography and quantitation were as described above. Signal intensities for each sample were normalized to the intensity of signal for glyceraldehyde 3-phosphate dehydrogenase from that sample.

Statistics. Student's t test was used to analyze differences between dietary groups. Differences occurring at a probability level $p < 0.05$ were accepted as statistically significant.

Results

There were no statistically significant differences in growth among rats fed the three different levels of dietary selenium.

Glutathione peroxidase activity in liver and kidney of rats fed the unsupplemented diet was less than 1% of the activity in control animals. However, increasing the dietary supplement by a factor of 20 elicited no further increases in enzyme activity.

Figure 1 shows the results of glutathione S-transferase enzyme assays performed on liver and kidney cytosols. In each tissue, increasing or decreasing selenium intake relative to the nutritionally adequate level increased enzyme activity. However, in kidney, the difference between the nutritionally adequate and high selenium intake was not statistically significant.

Figure 2 shows the results of run-on transcription assays performed with nuclei from livers of rats fed three levels of selenium. There were no discernible differences due to diet in the rates of transcription for glutathione S-transferase subunit genes.

Figure 3 shows Northern blots of total RNA from livers and kidneys of rats fed three levels of selenium. Increasing selenium intake increased steady state levels of mRNA for glutathione S-transferase subunit Ya in both tissues. In contrast, glutathione S-transferase Yc mRNA levels were decreased in kidney and affected variably in liver by increasing selenium intake. In liver, mRNA for glutathione S-transferase subunit Yp was undetectable, while in kidney, Yp mRNA levels were reduced by either dietary selenium supplement.

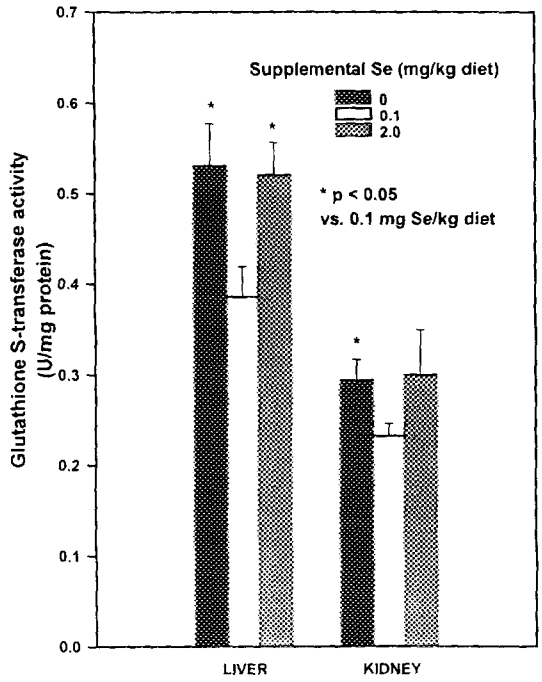

Figure 1. Activity of glutathione S-transferase, measured with 1-chloro-2,4-dinitrobenzene as substrate, in livers and kidneys of rats fed three levels of dietary selenium. Means + SEM are shown for each tissue and dietary group. Asterisks indicate statistically significant differences within a tissue from diet supplemented with 0.1 mg selenium/kg diet.

Discussion

The observation that deficient and high dietary selenium intakes both increased glutathione S-transferase activity, compared to that observed in rats fed a nutritionally adequate level of selenium, suggests that such changes occurred by different mechanisms. In selenium deficiency, selenium-dependent glutathione peroxidase activity in liver and kidney is virtually abolished. To compensate, activity of glutathione S-transferase class alpha enzymes, which exhibit selenium-independent glutathione peroxidase activity, may increase. This compensation would also increase total glutathione S-transferase activity, measured with 1-chloro-2,4-dinitrobenzene.

An additional explanation is that selenium deficiency increases concentrations of reduced glutathione (23). Reduced glutathione is required in the metabolism of inorganic selenium (24) and is the reducing agent in the glutathione peroxidase reaction. Lacking selenium in the diet, and with a markedly reduced activity of selenium-dependent glutathione

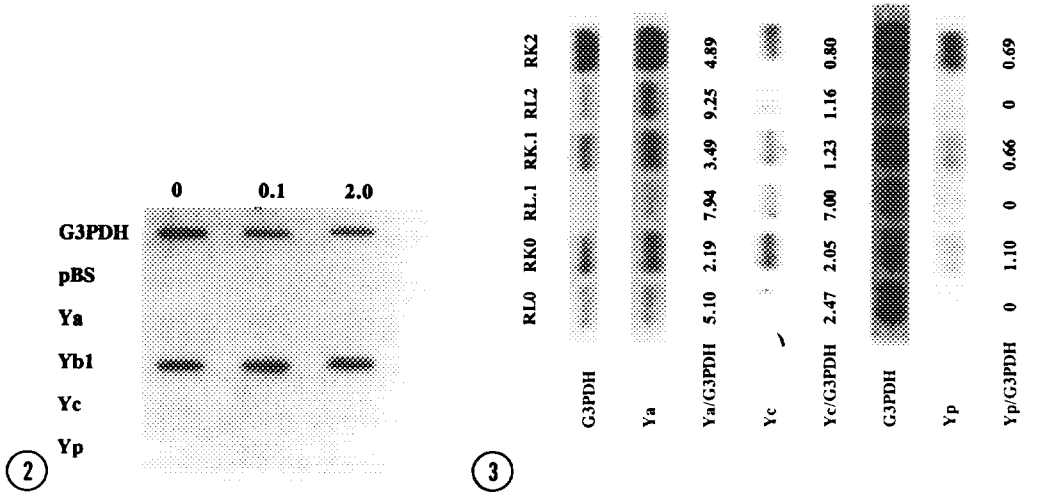

Figure 2. Results of run-on transcription assays of glutathione S-transferase subunit gene transcription. Nuclei were isolated from livers of rats fed Torula yeast diets supplemented with 0, 0.1 or 2.0 mg selenium/kg diet. Positive controls were glutathione S-transferase subunit Yb1 and glyceraldehyde 3-phosphate dehydrogenase (G3PDH). The plasmid pBlueScript (pBS) (Stratagene, La Jolla, CA) was used as a negative control.

Figure 3. Northern blots of liver and kidney RNA from rats fed Torula yeast diets supplemented with 0, 0.1 or 2.0 mg selenium/kg diet. Blots were probed with coding sequences from the cDNAs for glutathione S-transferase subunits Ya, Yc, and Yp. Loading of RNA was quantitated by probing with glyceraldehyde 3-phosphate dehydrogenase (G3PDH). Signal intensity produced by glutathione S-transferase subunit probes was normalized to that of glyceraldehyde 3-phosphate dehydrogenase.

peroxidase, additional glutathione would be available for other processes, such as conjugation by glutathione S-transferases.

Consistent with this explanation, a high intake of selenium would be expected to reduce availability of glutathione for use in other reactions. Therefore, an increase in glutathione S-transferase activity due to high selenium intake is somewhat paradoxical. The simplest explanation for this observation is that 1-chloro-2,4-dinitrobenzene, the substrate used to assay glutathione S-transferase activity, is metabolized by almost all subclasses of glutathione S-transferase enzymes. Hence, activity of one subclass of enzymes may be enhanced by one dietary treatment, while a different dietary intervention increases activity of another subclass of enzymes. The net effect in each case would be an increase in total activity, measured with 1-chloro-2,4-dinitrobenzene.

It is possible, although less likely, that increased glutathione S-transferase activity represents a pathologic response to toxic levels of selenium. The toxicity of high selenium exposure is well documented (25). However, intakes required to produce toxicity symptoms

are usually higher than 2.0 mg Se/kg diet. Furthermore, the high selenium supplement had no inhibitory effect on growth in this study. If toxicity was occurring in these animals, its manifestations were much more subtle.

Selenium's effects on transcription of genes for glutathione S-transferase subunits were minimal. Subunit Yb1, although not a class alpha subunit with glutathione peroxidase activity, was included as an additional positive control probe in the run-on assay due to its previously demonstrated high transcription level (26).

In contrast to its lack of effect on transcription, selenium intake markedly alters steady state levels of mRNA for subunits Ya, Yc, and Yp. This suggests that mRNA turnover is the pretranslational step at which selenium exerts its greatest effect on expression of these genes. The effects of selenium on mRNA for glutathione S-transferase subunits are specific to the subunit and tissue studied. The same pattern - no effect on transcription, but altered steady state mRNA levels - is seen in the case of selenoprotein genes (27,28).

There was little correlation between expression of genes for class alpha subunits and activity of glutathione S-transferase measured with 1-chloro-2,4-dinitrobenzene. The most likely explanation for this observation, as noted above, is that this substrate is well utilized by almost all glutathione S-transferase enzymes, not just those of the alpha class whose gene expression was examined in this study.

In summary, transcription of genes for glutathione S-transferase subunits is unaffected in rat liver by selenium intake. However, steady state levels of mRNA for glutathione S-transferase subunits are affected variably in liver and kidney by increasing or decreasing dietary selenium relative to the nutritionally adequate level. Activity of glutathione S-transferase, measured with 1-chloro-2,4-dinitrobenzene, is increased by both inadequate and high selenium intakes, compared to that characteristic of a nutritionally adequate intake. These findings suggest that regulation of gene expression for enzymes of the glutathione S-transferase family may be an additional mechanism by which dietary selenium exerts its biological effects.

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