In vivo and in vitro influence of selenium on DNA/RNA synthesis in spleen and lymphocytes in culture—Possible mediation of changes in GSH/GSSG ratio

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Received 5 June 2000; revised 29 September 2000

Effect of superanutritional levels of selenium (Se) as sodium selenite (0.5 and 1.5 ppm) given orally to Balb/c mice for one and two weeks was observed on the rate of DNA/RNA synthesis, levels of reduced as well as oxidized glutathione (GSH and GSSG) and glutathione peroxidase (GSH-Px)/glutathione-S-transferase (GSH-S-transferase) activities in spleen. Similar effect of three different concentrations of Se (10⁻⁷, 10⁻⁵ and 10⁻³ M) in culture media was also observed on the rate of DNA/RNA synthesis in proliferating lymphocytes taken from mice spleen. The results of the present study indicated that with increasing concentration and duration of Se treatment in vivo and in vitro, a marked inhibition of the rate of DNA/RNA synthesis was observed. Levels of total glutathione and GSSG in spleen were elevated significantly only after two weeks in 1.5 ppm treatments. Glutathione peroxidase activities in spleen decreased (p<0.05) in 1.5 ppm group at one week and in 0.5 ppm group at two week treatment. At higher Se treatment, the activity recovered towards control. However, GSH-S-transferase in spleen remained unchanged at all treatment intervals. The results indicated that changes in glutathione system by increasing Se concentration might account for inhibition of rate of DNA/RNA synthesis.

Interest in selenium (Se) aroused by establishment of its role as possible anticarcinogenic agent as well as an antioxidant and its relation to vitamin E metabolism. There is extensive documentation over past few years regarding the inhibitory role of Se on carcinogenesis in the range of tissues in both mouse and rat. Majority of the reports show that Se supplementation inhibits tumorigenesis in a number of mammalian species and the protective effect extends to chemical, radiation and oncogenic virus induced transformations while Se deficiency enhances tumor development.

The mechanism underlying the chemopreventive effect of Se is still not much clear. However, it may be attributed to growth inhibitory effects which are linked to inhibition of DNA/RNA synthesis. Selenium exerts biphasic effect on cell growth/proliferation and DNA synthesis. At low dose (10⁻⁷ M), Se enhances cell proliferation in vitro but at higher dose (10⁻⁵ M), Se shows both reversible and irreversible tumor suppression.

Selenium have also been reported to effect glutathione concentration in short term animal feeding experiments. It was hypothesized that perturbations in glutathione metabolism induced by Se may account for its cancer chemopreventive activity. Following an initially non-enzymatic reaction with glutathione to form selenotrisulfides (GS-Se-SG), the reduction of selenite to hydrogen selenide is catalyzed by glutathione reductase via the intermediary selenopersulfide step. This relationship between glutathione metabolism and selenium raises the possibility that Se may be a glutathione perturbing agent. In some studies it has been shown that Se supplementation causes an increase in oxidized glutathione (GSSG) as well as the ratio of GSSG to GSH (ref. 10).

To evaluate the possible mechanism of inhibition of DNA/RNA synthesis by Se in the present studies, the levels of GSH-Px/GSH-S-transferase and ratio of GSH/GSSG were also determined at the similar concentration of Se.

Materials and Methods
Female Balb/c mice weighing approximately 20g were obtained from the Central Animal House of the Panjab University, Chandigarh (India) and were maintained on pellet diet and water ad libitum. For the present study, the animals were divided into two sets of three groups each. In one set, the treatment was given for one week whereas in the other for two weeks. Each group contained five animals.

The animals in each set of three groups were treated as—Group I served as a control and each animal was given 0.2 ml distilled water orally daily.
Animals in Group II and III were administered orally 2.5 and 7.5 μg Se (equivalent to 0.5 and 1.5 ppm Se) as sodium selenite in 0.2 ml of distilled water respectively. After the completion of treatments in each group, the animals were sacrificed by cervical dislocation under mild anesthesia and removed spleens.

DNA synthesis rate—For measuring the rate of DNA synthesis, 15 μCi ³H-thymidine (Sp. Act. 18000 mCi/ml) was injected ip to the animals four hours before sacrifice. DNA content in the extract was estimated by the standard biochemical method. One ml of DNA extract was added to the 8 ml scintillation fluid, counts were taken to represent the rate of DNA synthesis as DPM/mg DNA.

RNA synthesis rate—For measurement of RNA synthesis rate, 15 μCi of ³H-uridine (Sp. Act. 16000 mCi/ml) was used in place of ³H-thymidine. Rest of the procedure is the same as for DNA synthesis rate. RNA content in the extract was estimated by the standard biochemical method.

Total glutathione and oxidized glutathione—Total and oxidized glutathione levels in the spleen at the end of Se treatment were estimated by the enzymatic method.

Glutathione peroxidase and glutathione-S-transferases—Activity of GSH-Px and GSH-S-transferases in spleen were determined by the method given by Paglia and Valentine and Habig et al. respectively. Protein estimation was done by the standard biochemical method of Lowry et al.

Selenium estimation—Selenium content in spleen was estimated by the fluorimetric method.

Se influence on DNA/RNA synthesis in lymphocytes in culture—Lymphocytes collected from the spleen of mice were incubated in 96-well plates as follows—100 μl of lymphocytes suspension (6×10⁶ cells/ml) in medium was mixed with 100 μl of Con A (final concentration of Con A as 5 μg/well). To evaluate the DNA/RNA synthesis rate in the cells in culture, 1 μCi ³H-thymidine/³H-uridine per well were added after 66 hr of incubation. After 6 hr of further incubation, the cells were harvested in a semiautomatic harvester. Cell on filter sheet were dried, filters removed from sheet and added to the scintillation vials for liquid scintillation counting and DPM/well was calculated for all the wells as a measure of the uptake of both the ³H-labelled compounds.

To see the influence of Se on the lymphocytes in culture Se as sodium selenite was added separately at a concentration of 10⁻⁷, 10⁻⁵, and 10⁻³ M to the media and lymphocytes were cultured as described above.

Results

Significant increase in the Se levels were observed in the spleen in different treatment groups at different intervals (Table 1). Further, there was a significant inhibition of both DNA and RNA synthesis with increasing concentration of Se in both sets of mice treated for one and two weeks (Table 1).

In vitro studies on the influence of Se on the lymphocytes in culture, there was a significant inhibition in the uptake of ³H-thymidine and ³H-uridine with increasing Se concentration in the culture which directly indicated the DNA/RNA synthetic rate respectively (Table 2). Total and oxidized glutathione levels in the spleen after Se supplementation at 0.5 and 1 ppm levels for 1 and 2 weeks were investigated. Not much change was observed in the levels of the total glutathione both in one and two weeks of treatments at 0.5 ppm Se level. However at 1.5 ppm Se treatments, a significant increase in total glutathione level was observed at two weeks treatment interval (Table 3). Similar increase obtained in the levels of

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Selenium content (ng/g tissue)</th>
<th>DNA synthetic rate (dpm/mg DNA)</th>
<th>RNA synthetic rate (dpm/mg RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>511.9±24.79</td>
<td>46872±7940</td>
<td>36683±6250</td>
</tr>
<tr>
<td>0.5 ppm</td>
<td>653.7±23.73**</td>
<td>32142±414**</td>
<td>29866±4704*</td>
</tr>
<tr>
<td>1.5 ppm</td>
<td>607.5±42.73***</td>
<td>32013±8167**</td>
<td>30053±4766*</td>
</tr>
<tr>
<td>Two weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>511.9±24.79</td>
<td>33177±3563</td>
<td>12124±4700</td>
</tr>
<tr>
<td>0.5 ppm</td>
<td>678.4±55.25**</td>
<td>22285±3826**</td>
<td>9761±935***</td>
</tr>
<tr>
<td>1.5 ppm</td>
<td>754.2±40.58***</td>
<td>17104±530***</td>
<td>7963±734***</td>
</tr>
</tbody>
</table>

P values—* < 0.05; ** < 0.01; *** < 0.001
GSSG was, however, more significant which resulted in the significant reduction in the ratio of GSH/GSSG at 1.5 ppm Se both at one and two weeks of treatments (Table 3).

In the present study, no significant change in the activity of the GSH-S-transferase was found as a result of the Se treatment. No change in GSH-Px activity in spleen was also observed. On further treatment, of Se, a significant decrease in the activity of this enzyme was observed. However 1.5 ppm Se treatment group for two weeks showed recovery in the GSH-Px activity towards control levels (Table 4).

**Discussion**

In the present study, a significant increase in the Se level and a progressive decrease in the rate of synthesis of DNA and RNA in spleen was observed with increase in dose and time of Se treatment. This study showed that inhibition of rate of DNA/RNA synthesis occurred both in vivo and in vitro. There have been several studies in the earlier years to find out the correlation between in vivo and in vitro inhibition. However, the mechanism underlying this inhibition in the rate of DNA/RNA synthesis is not known completely.

Selenium is rapidly taken up by the cells and metabolized to hydrogen selenium and some other metabolic products which are intermediates in the synthesis of selenoproteins and their interaction with cellular macromolecules. As a result of this, Se indirectly modulates a series of intercellular events which may result in the rapid synthesis or degradation of certain selenoproteins and some of these have been reported to be involved in DNA synthesis inhibition by Se in the interaction of the selenoproteins in nucleic acid synthetic pathways.

Levels of GSSG increased significantly at higher Se doses at two week interval and hence a decrease in the ratio of GSH/GSSG was observed in the present study. Similar results have been obtained by some workers earlier. Activity of GSH-Px and GSH-S-transferases were not altered in the present study at higher doses of Se which caused nucleic acid synthesis inhibition. Therefore, it was concluded that despite having most of Se in it, GSH-Px was unresponsive to high Se levels and hence the activity of this enzyme was not involved in the inhibition of DNA/RNA synthesis. There is abundant evidence in the literature that changes in GSH-Px function can not explain the chemopreventive effects of Se in vivo and on cells growth in vitro as GSH-Px levels in tissues are not enhanced at levels of Se which are growth inhibitory. Some studies also show that GSH-S-transferases is not much responsive to dietary Se. However this enzyme shows reduction of a wide variety of disulfides by glutathione and enhances the conjugation of glutathione with a wide range of compounds such as carcinogen metabolites and hence may indirectly prevent carcinogen-DNA interaction. But at growth inhibitory levels of Se, the levels of GSH-S-transferase are unaltered. Therefore the inhib-

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**Table 2**—Influence of different concentrations of sodium selenite on lymphocyte proliferation and uptake of $^3$H-thymidine/$^3$H-uridine

<table>
<thead>
<tr>
<th>Treatments</th>
<th>$^3$H-thymidine uptake (dpm/well)</th>
<th>$^3$H-uridine uptake (dpm/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54401±4976</td>
<td>16392±58755</td>
</tr>
<tr>
<td>$10^3$ M Se</td>
<td>32216±5070***</td>
<td>82957±16627***</td>
</tr>
<tr>
<td>$10^4$ M Se</td>
<td>27316±8723***</td>
<td>63499±5079***</td>
</tr>
<tr>
<td>$10^5$ M Se</td>
<td>667±433***</td>
<td>3461±1653***</td>
</tr>
</tbody>
</table>

$P$ values: * $< 0.05$; ** $< 0.01$; *** $< 0.001$

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**Table 3**—Influence of selenium on levels of total glutathione, reduced and oxidized glutathione (GSSG) and GSH/GSSG ratio in spleen

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total glutathione</th>
<th>GSH (ng/mg protein)</th>
<th>GSSG</th>
<th>GSH/GSSG ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>One week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2716.8±277.0</td>
<td>2472.7±278.8</td>
<td>202.76±48.68</td>
<td>12.64±2.95</td>
</tr>
<tr>
<td>0.5 ppm</td>
<td>2496.9±284.6</td>
<td>2160.2±173.4</td>
<td>211.12±49.51</td>
<td>10.92±3.89</td>
</tr>
<tr>
<td>1.5 ppm</td>
<td>2778.1±325.9</td>
<td>2519.5±279.6</td>
<td>249.75±47.32</td>
<td>10.12±0.97*</td>
</tr>
<tr>
<td>Two weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2716.8±277.0</td>
<td>2472.7±278.8</td>
<td>202.76±48.68</td>
<td>12.64±2.95</td>
</tr>
<tr>
<td>0.5 ppm</td>
<td>2367.3±240.7</td>
<td>2189.3±125.4*</td>
<td>175.04±24.91</td>
<td>12.76±2.09</td>
</tr>
<tr>
<td>1.5 ppm</td>
<td>3114.7±184.0*</td>
<td>2726.2±55.9*</td>
<td>423.82±97.22*</td>
<td>7.22±2.91**</td>
</tr>
</tbody>
</table>

$P$ values: * $< 0.05$; ** $< 0.01$; *** $< 0.001$
bition dose not seem to be mediated also by the changes in GSH-S-transferase activity.

Recent studies have shown that GSH on reacting with sodium selenide produces a compound called selenodiglutathione which have been shown to specifically inactivate the elongation factor as a result of which biosynthesis of several essential specific selenoproteins is altered which may lead to reduced cell proliferation. Also selenium has been shown to react with thiols and corresponding selenotrisulfides are formed which have been established as the inhibitors of DNA and RNA polymerases.

In summary, the present study showed the inhibition of DNA/RNA synthesis in vivo and in vitro selenium exposure, and it was hypothesized that the inhibition was mediated by changes in GSH and GSSG levels.

References