Regulation of Growth and Polyamine Metabolism in MCF-7 Human Breast Adenocarcinoma Cells by Selenium

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Selenium is a known cancer chemopreventive agent owing to its antitoxic nature and antitumorogenic effect. In this study the cytocidal response of sodium selenite on MCF-7 human breast adenocarcinoma cells was examined. After 48 h the number of cells in culture containing high concentration of selenium i.e. 10 and 25 μM decreased by 35% and 70% respectively, but at low concentrations i.e. 1 μM and 5 μM, their number increased by 10% and 15%, respectively. Growth stimulatory concentrations of selenium resulted in significant stimulation of polyamine levels. Significant increase in glutathione levels was also observed with 5 μM of selenium. Growth inhibitory concentrations of selenium resulted in inhibition of polyamine levels. Increase in glutathione content was much less with 10 μM selenium when compared with 5 μM of selenium. Regulation of polyamine biosynthesis by selenium could be due to the action of selenium on thiol groups which in turn are required for the activity of ornithine decarboxylase. Selenium resulted in inhibition of DNA synthesis as observed by cell cycle analysis and higher concentration of selenium resulted in DNA fragmentation.

Cancer chemoprevention involves the use of naturally occurring or synthetic agents against clinically detectable cancers. There are several reports in literature demonstrating that selenium supplementation to diet or water can inhibit chemical-carcinogen-induced tumorigenesis in skin1, liver2, colon3 and mouse mammary glands4.

It has been reported earlier that selenium at lower concentration favours cell growth but at higher concentration inhibits growth5,6. Excess selenium in the form of sodium selenate is inhibitory to cell proliferation7. A possible correlation between selenium and regulation of polyamines in liver and lymphoid organs has been reported earlier8. Polyamines are essential for growth and differentiation. Selenite concentration known to inhibit cell proliferation, inhibited both ornithine decarboxylase (ODC) and spermidine acetyl transferase (SAT) activities in vitro8.

Earlier reports show that selenium plays a major role in polyamine metabolism8 and ODC which catalyses the rate limiting step in polyamine biosynthetic pathway, requires SH groups for its activity9 and can be inhibited in vitro by compounds that react with thiol groups10. Therefore, it is possible that selenium may be involved in the interference of the polyamine synthesis. This work reports the effect of sodium selenite on MCF-7 human breast adenocarcinoma cells and the possible role of selenium on growth and polyamine metabolism in MCF-7 cells has been worked out.

MATERIALS AND METHODS

All of the tissue culture chemicals such as RPMI-1640 and antibiotics were purchased from Sigma Chemical Co. St. Louis, MO, U.S.A. Dansylchloride, propidium
Table I - Effect of selenium on polyamine content

<table>
<thead>
<tr>
<th>Group</th>
<th>Putrescine (nmol/10⁶ Cells)</th>
<th>Spermidine (nmol/10⁶ Cells)</th>
<th>Spermine (nmol/10⁶ Cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.07 ± 0.32</td>
<td>11.16 ± 0.48</td>
<td>4.73 ± 0.30</td>
</tr>
<tr>
<td>1 µM selenium</td>
<td>4.5 ± 0.41*</td>
<td>13.04 ± 1.08*</td>
<td>6.50 ± 0.33*</td>
</tr>
<tr>
<td>5 µM selenium</td>
<td>4.96 ± 0.06*</td>
<td>18.31 ± 0.29*</td>
<td>7.82 ± 0.16*</td>
</tr>
<tr>
<td>10 µM selenium</td>
<td>2.96 ± 0.31*</td>
<td>9.89 ± 0.24*</td>
<td>4.12 ± 0.26*</td>
</tr>
<tr>
<td>25 µM selenium</td>
<td>3.3 ± 0.67*</td>
<td>5.66 ± 0.94*</td>
<td>1.65 ± 0.38*</td>
</tr>
</tbody>
</table>

Effect of different concentrations of selenium on putrescine, spermidine and spermine levels in MCF-7 cells. 5x10⁶ cells were treated with indicated concentrations of selenium at 48 h after seeding. Cells were harvested 24 h later for polyamine estimation as mentioned in Materials and Methods section. Results are mean ± SD of 3-4 replicates.

* represents significant increase over the control P ≤ 0.05.

iodide, RNaseA, 4,6-diamidino-2-phenyl-2-phenyl-indole (DAPI), 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB), sodium selenite, putrescine, spermine and spermidine were also obtained from Sigma Chemical Co. Thymidine [³H] was obtained from the Bhabha Atomic Research Centre, Mumbai, India. Fetal calf serum (FCS) was obtained from Biological Industries, Kibbutz Beit Haemek, Israel. All other chemicals used were of analytical grade.

Cell culture: MCF-7, a human breast adenocarcinoma cell line, was obtained from the National Centre for Cell Sciences (NCCS), Pune, India. The cell line was regularly maintained in RPMI-1640 medium supplemented with 0.2% sodium bicarbonate, 10% FCS and antibiotics (50 µg/ml of penicillin and 100 µg/ml of streptomycin) under a humidified atmosphere of 95% air and 5% CO₂ at 37°C. For drug studies and for other experiments, a number of 60 mm petri dishes were plated with approximately 10⁶ cells in medium for 48 h. Drugs were added 48 h after plating. The time of treatment of drugs varied with each experiment set up as indicated in the figure legends. Following exposure to drugs, the cells were rinsed and trypsinized. Cell growth was monitored by the counting of viable cells after trypsin blue dye exclusion test using a hemocytometer. Each experiment was repeated at least 3-4 times and each treatment was in triplicate. The percentage of trypsin blue excluded cells (live cells) was more than 95% in untreated group.

Polyamine measurements: For polyamine assay, cells (Approximately 10⁶ cells) were resuspended in 250 ml of 2% perchloric acid and kept for 24 h at 4°C. Cells were pelleted from perchloric acid. The supernatants were used for polyamine estimation. Dansyl derivatives were prepared according to Seller [11] and polyamines were separated by thin layer chromatography (TLC) on 0.2 mm thick silica gel G plates, using ethylacetate:cyclohexane (2:3, v/v) as the solvent. Quantification of polyamines was accomplished using a Camag TLC Scanner with the TLC II software programme Cats 3. The concentration of unknown samples was determined against standard polyamines.

Glutathione content: Cells were suspended in 200 µl of 5 M sodium chloride, 5 mM EDTA and 250 mM glacial acetic acid. The homogenate was centrifuged at 2000 x g for 10 min. The supernatant (0.1 ml) was mixed with 0.9 ml of 0.1 M phosphate buffer (pH 8.0) and 2.0 ml of 0.6 mM 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB). The absorbance was measured at 412 nm. The concentration of unknown samples was determined against standard glutathione.

Cell cycle analysis: Cells were grown and treated with drug(s) for 48 h, harvested by trypsinization, washed with cold PBS and pelleted by centrifugation at 2000 x g for 10 min at 4°C. The pelleted cells were processed for cell cycle analyses as described earlier [13]. Samples were examined using EPIC®XL-software (Coulter Corporation, Miami, Florida, USA) and then analysed using MULTICYCLE software (Phoenix Flow Systems Inc., San Diego CA, USA) for cell cycle analysis.

Quantitative assay of DNA fragmentation: Assays were conducted in triplicate in a flat bottom, 96-well microtiter
Fig. 1: Dose dependent effect effect of selenium

Effect of different concentration of selenium on MCF-7 cells. Cells were plated at a density of 5 x 10^5 cells/well in triplicate in 96 well growth plates. Two day after plating, the medium was replaced with the same medium but containing different concentrations of selenium. Following selenium treatment 2.5 μCi/ml of [3H] thymidine was added per well. Triplicate wells were counted for cell number after 48 h of drug treatment by harvesting and radioactivity incorporated was measured using scintillation spectrometer. Results are mean ± SD of triplicate samples. SD<5% of average is not shown.

plate in RPMI 1640 medium with 10% FCS in a total volume of 0.1 ml. Approximately 5 x 10^4 cells were seeded in each well. Wells for total counts received 0.1 ml of medium, whereas, experimental wells received appropriate concentrations of drugs in medium. DNA fragmentations were measured by the release of [3H] thymidine-labeled DNA fragments after a 24 h incubation as described earlier 13,14.

Cell morphology. MCF-7 cells were exposed to 5 μM or 10μm selenium for 24 h. Cells were washed with PBS, fixed in 70% ethanol and then stained with 10 μg/ml DAPI. Alterations in cell nuclei were photographed under a fluorescence microscope 13,14 (Ernest Leitz, GMBH, Wetzlar, Germany). All the above experiments were conducted in triplicate and repeated at least 3-4 times. Results are mean ± SD of triplicate values. SD<5% of the average value are not shown.

Fig. 2: Glutathione content of MCF-7 cells treated with selenium

Glutathione (GSH) content in selenium treated MCF-7 cells. The content was measured after 24 h of selenium treatment. Bars represent mean±SD of 3-4 replicates. The fig depicts that b and c are significantly higher than a (P<0.001) whereas c is significantly lower than b (P<0.05)

RESULTS

The cytocidal response of sodium selenite on MCF-7 human breast adenocarcinoma cells showed that after 48 h, the number of cells in culture containing 25 μM of selenium decreases by 70% (P<0.05) when compared with control values. A 10 μM concentration of selenium led to 35% (P<0.05) decrease in the number of cells but lower concentration led to increase by 15% (P<0.05) (Fig.1).

Different concentrations of selenium which are stimulatory (1 μM, 5 μM) and others which are inhibitory (10 μM and 25 μM) to growth were used on MCF-7 cells to study their relative effects on polyamine levels. Concentration of selenium that resulted in stimulation of growth (1 μM and 5μM) also resulted in increased levels of putrescine, spermidine and spermine (Table 1), whereas, the concentrations of selenium that were inhibitory to cell growth (10 μM and 25 μM) resulted in the inhibition of putrescine, spermidine and spermine (Table 1). A 1 μM concentration of selenium led to increase of putrescine, spermidine and spermine by 10%, 17% and 37% respectively, whereas, 5 μM concentration of selenium resulted
Fig. 3: DNA histogram as analysed by flow cytometry of MCF-7 cells.

Cells (5 x 10^6) were treated for 48 h with different concentrations of selenium, harvested and fixed in 70% ethanol followed by staining with propidium iodide. Samples were examined using EPIC® XL: software. A control B: 5 μM selenium, C: 10 μM selenium. Percentage distribution of cells in each phase of the cell cycle are shown.

Fig. 4: Fragmentation of DNA produced by selenium in MCF-7 cells was measured as described in Materials and methods section after a 24 h incubation. Each point represents mean ± SD of 3-4 replicates.

in 22%, 64% and 65% increase of putrescine, spermidine and spermine respectively over the control values (P<0.05 for all the values). Higher concentrations of selenium i.e. 10 and 25 μM resulted in inhibition of putrescine by 27% (P<0.05), 20% (P<0.05) respectively over the control whereas inhibition of spermidine with the above concentrations was 11% (P<0.05) and 50% (P<0.05) respectively and that of spermine was 11% (P<0.05) and (P<0.05) respectively.

Selenium resulted in marked increase of glutathione concentration in MCF-7 cells. Glutathione level of the MCF-7 cells was 10.4±0.2 μM/10^6 cells. 5 μM of selenium resulted in higher glutathione content (20.5 ±1.4 μM/10^6 cells) as compared to 10 μM of selenium (16.5±0.9 μM/10^6 cells (Fig. 2).

The effect of selenium on cell cycle of MCF-7 cells was examined by using a flow cytometer. The cells were collected 48 h after exposure to two different concentrations of selenium 5 μM and 10 μM. The results are shown in Fig. 3. At 5 μM and 10 μM of selenium there was significant inhibition of S-phase cells, whereas, a significant increase in G1 phase cells was observed. However, percentage of cells in G2/M was significantly lower with 10 μM of selenite as compared to 5 μM concentration.

The significant influence of two different effects of selenium led to the investigation whether this effect was
Fig. 5: Morphological alteration of MCF-7 cells nuclei after incubation with 5 µM and 10 µM of selenium. Cells were incubated in the presence of 5 µM and 10 µM of selenium for 24 h. Cells were washed with PBS, fixed in ethanol and stained with DAPI as described in the materials and methods section. The morphological changes in cell nuclei were observed under fluorescence microscope. A: untreated control, B: cells treated with 5 µM of selenium, C: cells treated with 10 µM of selenium. Arrows indicate MCF-7 cells with apoptotic nucleus (condensed or fragmented)

A result in triggering programmed cell death. Fig. 4 demonstrates that 5.0 µM of selenium when given for 24 h did not result in DNA fragmentation of MCF-7 cells, whereas, 10 µM of selenium resulted in DNA fragmentation as measured by [3H] thymidine incorporation.

Typical morphological changes of MCF-7 nuclei after 24 h incubation with these two concentrations of selenium are shown in Fig. 5. Only the cells treated with 10 µM of selenium showed DNA fragmentation as observed by the presence condensed fragmented nuclei (Fig. 5C). No such fragmentation was observed in cells treated with 5 µM selenium treated or (Fig. 5B) or untreated cells (Fig. 5A).

DISCUSSION

Several experiments have demonstrated that selenium supplementation to the diet or water can inhibit chemical carcinogen-induced tumorigenesis in skin, liver, colon, and mammary glands. Selenium chemopreventive action is mostly in the promotional stage of carcinogenesis, a step that does not involve chemical carcinogen metabolism. Even in in vitro cultures selenium influences either normal or transformed cells. While low concentrations of selenium stimulate the growth of mouse mammary epithelial cells, higher concentrations inhibit cell growth and the presence of even higher amounts of this element are cytotoxic.

Selenium is known to inhibit the proliferation of responsive cells. Its mechanism of action in vivo, however, is still not understood. Selenium acts through an increase of glutathione peroxidase activity which may prevent the formation of carcinogenic oxygen radicals and thereby defend the organism against the initiation of carcinogenic process. It is also known to inhibit protein synthesis and cell proliferation possibly due to the stimulation by GSSG of a protein kinase able to phosphorylate eukaryotic initiation factor 2 (eIF-2); thereby inactivating it.

Earlier investigation by Colombatto et al. have shown that selenium plays a major role in polyamine metabolism in liver as well as in lymphoid organs. In order to better understand the mechanism of action of selenium, its effects on the regulation of growth and polyamine metabolism in MCF-7 human breast adenocarcinoma cells was investigated.

When MCF-7 cells were subjected to different concentrations of selenium for 48 h, the number of cells in culture containing low concentrations of selenium that is 1 µM and 5µM, increased by 10% and 15% respectively. Higher concentrations of selenium i.e 10 µM and 25 µM inhibited the cell growth by 35% and 70% respectively as compared with controls in culture without selenium.

Concentrations of selenium that were stimulatory to growth of MCF-7 cells resulted in increase in polyamine levels where as the concentrations of selenium that were inhibitory to cell growth resulted in inhibition of polyamines.
Since it is well established that polyamine biosynthetic pathway requires SH group for its activity and can be regulated in vitro by compounds that can react with thiol groups, it is therefore conceivable that action of selenium may involve interference with polyamine synthesis. These results are further supported by the recent reports that growth inhibitory concentrations of selenomethionine and synthetic selenium derivative p-xSC reduce the cellular polyamine content.

The analysis of the cell cycle by flow cytometry showed that both concentrations inhibited the number of cells in S-phase and increased the percentage of cells in G1-phase. However, 10 μM of selenium that inhibited cell proliferation, resulted in remarkable inhibition of percentage of cells in G2/M phase when compared to 5μM selenium. Earlier reports on the cell cycle analysis of the TM6 mouse mammary tumor cell line showed that selenite arrests cells in S/G2-M phase and in contrast, methylselenocysteine arrested cells in G1 phase. The variable effect of selenite observed in the present study could be due to differences in the two cell lines. Thus, it is concluded that the growth inhibitory effects of selenium could be due to inhibition of DNA synthesis as observed by cell cycle analysis and higher concentration of selenium results in fragmentation of DNA.

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REFERENCES