Inhibitory Effect of Selenium on N-Nitrosodiethylamine and Phenobarbital-Promoted Rat Liver Carcinogenesis

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Summary  The chemoprotective efficacy of selenium, a dietary micro-nutrient, against chemically-induced hepatocarcinogenesis in rats was investigated. Carcinogenesis was performed by a single intraperitoneal injection of N-nitrosodiethylamine (DEN, 200 mg/kg body weight) with phenobarbital (0.05%) as a promoter in rats. When selenium-supplemented (4 ppm) drinking water was provided ad libitum throughout the experimental period, before the initiation, or during the promotion period it was found to be effective in elevating hepatic glutathione peroxidase activity to a statistically significant level measured either in hyperplastic nodules or in the non-nodular surrounding tissue compared with the level of the carcinogen control. Selenium treatment also increased the activity of this enzyme in kidney, lung, and erythrocytes to a significant level compared with the tissue or cell levels of the carcinogen control rats. A decrease in the number and size of hyperplastic nodules was observed in the selenium-treated groups. Hence, a direct correlation may be made between the hepatic selenium content with the formation of hyperplastic nodules and non-nodular surrounding liver tissues. Our results further confirm the fact that selenium is particularly protective in limiting the action of DEN during the initiation phase of hepatocarcinogenesis.

Key Words: selenium, N-nitrosodiethylamine, glutathione peroxidase

Hepatocarcinogenesis induced by various carcinogens such as N-nitrosodiethylamine (DEN), 2-acetylaminofluorene (2-AAF), dimethylnitrosoamine (DMNA), 3'-methyl-4-dimethylaminoazobenzene (3'-met-DAB), p-dimethylaminoazoben-
zene (p-DAB), aflatoxin B₁ (AFB₁), etc. is a multistep complex process [1]. Rat serves as a favorite model that facilitates the study of the mechanism of chemical carcinogenesis starting from a normal cell to malignant transformation. Interference of any one recognizable stage during this complex and intricate phenomenon could disrupt the entire process as a whole.

Four decades ago, selenium (Se) was recognized as an essential nutrient on the basis of its ability to serve interchangeably with vitamin E in the prevention of vascular or muscular signs in experimental animals [2]. The metabolic basis of this nutrient remained unclear, however, until it was discovered that the enzyme glutathione peroxidase contained selenium as an essential component at its catalytic site [3].

There is increasing epidemiological evidence to show the chemoprotective effect of selenium [4, 5]. Negative associations have also been found between serum selenium levels and liver cancer [6]. Although inorganic selenium at levels above the dietary requirement has been shown to inhibit carcinogenesis [7], chronic feeding of inorganic selenium at levels greater than 5 ppm proved to be toxic in rodents [8].

In experimental animals, dietary selenium has been found to inhibit carcinogenesis in the mammary gland [9], skin [10], colon [11], pancreas [12], and liver [12].

During the last 30 years, changes in activities of enzymes or isoenzymes in experimental hepatocarcinogenesis and in transplantable tumors have been extensively studied by various workers [13, 14]. Hepatic preneoplastic foci induced by various carcinogens in different models were extensively reviewed by Farber [1, 15]; and a large number of marker enzymes useful for their identification, i.e., foci of altered enzyme activity in hepatomas, were reviewed by Sato [13]. However, very meager information has emerged so far regarding the anti-neoplastic efficacy of dietary selenium on DEN-induced hepatocarcinoma in rats.

In the present study, we exerted extensive effort to ascertain the basic mechanism of selenium-mediated prevention of DEN-induced hepatocarcinogenesis in rats, with special attention to marker enzymes, and also to know at which stage of the process selenium acts as an effective chemopreventive agent. Furthermore, attempts were also made to correlate the hepatic concentration of selenium and the activities of marker enzymes during different phases of selenium administration.

MATERIALS AND METHODS

Animals and diet. Male, albino rats of the Wistar strain, at the age of 6 weeks, were used in these experiments. The rats were procured from Tamil Nadu Veterinary College, Chennai, India. They were fed normal rat chow marketed by M/s. Hindustan Lever Ltd., Mumbai, India, and were provided with clean drinking water ad libitum. Analysis showed that the rat chow used to feed our experimental animals contained 0.1 ppm of selenium, which is believed to satisfy the
normal requirement of rats [16].

Experimental design. To determine the chemopreventive efficacy of selenium and to identify the stage(s) at which selenium could be an effective anti-neoplastic agent during DEN-induced hepatocarcinogenesis, we divided the rats into eight different groups, as depicted in Fig. 1.

Liver tumors were induced in groups A, B, C, and D with a single intraperitoneal injection of DEN (Sigma, St. Louis, MO, USA) at a dose of 200 mg/kg body weight in saline at the age of 10 weeks. Two weeks after the DEN administration, the carcinogenic effect was promoted by phenobarbital (PB, Sigma) given in the rat chow (0.05% w/w) up to 14 successive weeks. The method to achieve DEN-induced liver tumor formation and promotion with PB was carried out according to the method of Yoshiji et al. [17].

Control animals (group a) were given the normal rat chow without additional

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**Fig. 1.** Schematic representation of experimental regimen. ↓ DEN (200 mg/kg i.p.), ■ basal diet and selenium supplementation in drinking water (4 ppm), □ basal diet and normal drinking water, ■ basal diet with PB (0.05%) and normal drinking water, □ basal diet with PB (0.05%) and selenium supplementation in drinking water (4 ppm), \$ time of sacrifice.
selenium supplementation, but the group B, b, C, c, D, and d animals were supplemented with 4 ppm of selenium (as sodium selenite) in the drinking water for various periods of time as indicated below. Fresh drinking water supplemented with selenium was replaced every 2 days. Daily food and water intake were noted, and weights of the animals from each group were recorded every second day. The time of DEN administration was taken as 0 (zero); minus (−) and plus (+) signs represent the time in weeks before and after DEN administration, respectively. The schedule of selenium treatment in groups B, b, C, c, D, and d was as follows: groups B and b, −4 to +16; groups C and c, −4 to 0; groups D and d, +2 to +16. The experiments were terminated 16 weeks after DEN administration.

All the experimental animals were killed by cervical decapitation 20 weeks after the initiation of the experiment. Blood was collected in tubes containing EDTA and centrifuged at 3,000 rpm for 15 min. The buffy coat was removed, and the packed cells were washed three times with physiological saline. The washed cells were lysed by suspension in hypotonic buffer [18] and centrifuged at 15,000 × g for 30 min [19]. The resulting pellet contained the erythrocyte membrane, and the supernatant represented the hemolysate. Livers were perfused in situ with cold 0.15 M NaCl at 37°C. Animals were fasted overnight before sacrifice.

Hyperplastic nodules and surrounding non-nodular liver tissues were obtained from all the groups treated with DEN. The greyish-white hyperplastic nodules were easily identified from the surrounding reddish-brown liver tissue. The nodules were eventually divided into three categories in accordance with their respective size and total area of the liver parenchyma occupied (< 1 mm, > 1-3 mm, and > 3 mm), as described previously by Moreno et al. [20] for different carcinogen-fed rats.

Biochemical assays. The liver, kidney, and lung samples were homogenized with a motor-driven Teflon-coated homogenizer in ice-cold 0.1 M Tris-HCl buffer (pH 7.4) to give a 10% homogenate. Hemoglobin in washed erythrocytes was estimated according to the method of Drabkin and Austin [21]. Glutathione peroxidase was determined according to the method of Rotruck et al. [3], and protein was estimated by the method of Lowry et al. [22]. Selenium concentrations in plasma, liver, kidney, and lungs samples were determined by the fluorometric method of Olson et al. [23].

Glutathione peroxidase assay and the biochemical estimations were performed on hyperplastic nodules and surrounding non-nodular liver tissue and/or whole liver of both treated and control groups.

Statistical analysis. Statistical significance of differences between groups was examined by Student's t-test. The level of significance was evaluated with p-values.
RESULTS

Food and water intake
During the entire period of our study no difference in food or water consumption was observed among the various groups of animals. Food and water intakes were 8.9–11.1 g/100 g/day and 16.8–19.2 ml/100 g/day, respectively, for all rat groups.

Mortality
A total of three rats died before the end of the study (i.e., 20 weeks), two from group A (16.6%) and one from group D (8.3%). None of the rats from any other group died during the experimental period.

Body and liver weights
Table 1 shows the body, and liver weights, relative liver weight, and weights of spleen, lung, and kidney of the different groups of rats that were killed at the end of the study. The final body weight of the carcinogen (DEN) control group A was lower \((p<0.01)\), and the liver weight was higher, than that of untreated normal control (group a). Treatment with selenium (4 ppm) increased the final body weight of animals in groups B, C, and D as compared with that of group A and maintained the normal body weight of animals in groups b, c, and d. Thus, selenium supplementation had practically no adverse effect on the growth response of the rats. The liver weight of carcinogen (DEN)-fed rats was slightly higher \((p<0.05)\) than that of the control (group a) rats. On the other hand, the relative liver weight in the rats of group A was found to be significantly higher \((p<0.01)\) than that of group a; although selenium supplementation reduced the relative liver

<table>
<thead>
<tr>
<th>Groups</th>
<th>Effective no. of rats</th>
<th>Final body weight (g)</th>
<th>Liver weight (g)</th>
<th>Relative liver weight (g liver weight/100 g body)</th>
<th>Spleen (g)</th>
<th>Lungs (g)</th>
<th>Kidney (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10/12</td>
<td>270±20.3(^{a})</td>
<td>13.26±2.24(^{a})</td>
<td>5.03±0.84(^{a})</td>
<td>0.48±0.2</td>
<td>1.92±0.2</td>
<td>3.08±0.2</td>
</tr>
<tr>
<td>a</td>
<td>8/8</td>
<td>312±21.5</td>
<td>10.05±2.56</td>
<td>3.18±0.66</td>
<td>0.51±0.1</td>
<td>1.84±0.2</td>
<td>3.21±0.2</td>
</tr>
<tr>
<td>B</td>
<td>12/12</td>
<td>295±20.6</td>
<td>11.10±2.33</td>
<td>3.77±0.65(^{a})</td>
<td>0.53±0.3</td>
<td>2.02±0.3</td>
<td>3.33±0.3</td>
</tr>
<tr>
<td>b</td>
<td>8/8</td>
<td>307±24.3</td>
<td>10.56±1.19</td>
<td>3.44±0.58</td>
<td>0.71±0.4</td>
<td>2.39±0.3</td>
<td>3.29±0.02</td>
</tr>
<tr>
<td>C</td>
<td>12/12</td>
<td>285±29.2</td>
<td>11.36±2.30</td>
<td>3.99±0.76(^{a})</td>
<td>0.56±0.3</td>
<td>2.12±0.4</td>
<td>3.18±0.4</td>
</tr>
<tr>
<td>c</td>
<td>8/8</td>
<td>304±30.9</td>
<td>10.21±2.06</td>
<td>3.36±0.73</td>
<td>0.69±0.3</td>
<td>2.28±0.4</td>
<td>3.26±0.4</td>
</tr>
<tr>
<td>D</td>
<td>11/12</td>
<td>283±27.6</td>
<td>11.92±2.43</td>
<td>4.19±0.61(^{a})</td>
<td>0.51±0.2</td>
<td>1.93±0.2</td>
<td>3.02±0.4</td>
</tr>
<tr>
<td>d</td>
<td>8/8</td>
<td>313±32.3</td>
<td>10.45±1.88</td>
<td>3.36±0.65</td>
<td>0.70±0.4</td>
<td>2.26±0.5</td>
<td>3.31±0.5</td>
</tr>
</tbody>
</table>

Each value represents the mean±SD. \(^{a}\)Significant difference as compared with group A; \(^{b}\)as compared with group a; \(^{c}\)as compared with group b; \(^{d}\)as compared with group c; \(^{e}\)as compared with group d; \(^{*}\)p<0.05, \(^{\circ}\)p<0.01, \(^{\circ\circ}\)p<0.001.
weights in groups B, C, and D, compared with group A. No significant change was observed in spleen, lung, and kidney weights of the different experimental groups.

Effect of selenium on nodule growth

The appearance of visible hepatocyte nodules, nodule incidence with total number of nodules, and relative size of nodules in different selenium-treated groups are depicted in Table 2. All the selenium-fed groups (B, C, and D) showed marked reductions in all of the above parameters. However, the maximum beneficial effect was observed in group B animals where administration of selenium was started 4 weeks before DEN administration and continued for 20 weeks. Relative decreases in total number of nodules, nodule incidence, and relative size of nodules were also observed in group C and D animals, where selenium was

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats with nodules/total no. of rats</th>
<th>Nodule incidence</th>
<th>Total no. of nodules</th>
<th>Relative size (mm) of nodules (% of total no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;1 mm</td>
</tr>
<tr>
<td>A</td>
<td>10/10</td>
<td>100%</td>
<td>513</td>
<td>26.5</td>
</tr>
<tr>
<td>B</td>
<td>4/12</td>
<td>33.3%</td>
<td>63</td>
<td>61.4</td>
</tr>
<tr>
<td>C</td>
<td>7/12</td>
<td>58.3%</td>
<td>142</td>
<td>41.8</td>
</tr>
<tr>
<td>D</td>
<td>7/11</td>
<td>63.6%</td>
<td>204</td>
<td>41.6</td>
</tr>
</tbody>
</table>

Table 2. Effect of selenium supplementation (4 ppm) on the development of hepatocyte nodules, their number, incidence, and size in different experimental groups (see Materials and Methods).

Table 3. Change in the total hepatic selenium (Se) concentration and glutathione peroxidase (GPx) activity in different experimental groups (see Materials and Methods).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Se concentration (μg/g tissue)</th>
<th>GPx activity (μg of glutathione utilized/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoma</td>
<td>0.33±0.02 NS(*)</td>
<td>38.9±2.49 NS(*)</td>
</tr>
<tr>
<td>Surrounding</td>
<td>0.35±0.02 NS(*)</td>
<td>45.8±3.05 NS(*)</td>
</tr>
<tr>
<td>a Whole liver tissues</td>
<td>0.55±0.08</td>
<td>87.6±8.06</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoma</td>
<td>0.41±0.02 NS(*)</td>
<td>71.9±8.5 NS(*)</td>
</tr>
<tr>
<td>Surrounding</td>
<td>0.47±0.03 NS(*)</td>
<td>76.7±7.7 NS(*)</td>
</tr>
<tr>
<td>b Whole liver tissues</td>
<td>0.73±0.12</td>
<td>93.8±8.7</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoma</td>
<td>0.36±0.03 NS(*)</td>
<td>41.5±2.9 NS(*)</td>
</tr>
<tr>
<td>Surrounding</td>
<td>0.38±0.02 NS(*)</td>
<td>58.1±4.8 NS(*)</td>
</tr>
<tr>
<td>c Whole liver tissues</td>
<td>0.56±0.09</td>
<td>88.8±5.3</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoma</td>
<td>0.39±0.03 NS(*)</td>
<td>49.9±4.6 NS(*)</td>
</tr>
<tr>
<td>Surrounding</td>
<td>0.40±0.03 NS(*)</td>
<td>62.8±5.9 NS(*)</td>
</tr>
<tr>
<td>d Whole liver tissues</td>
<td>0.67±0.11</td>
<td>90.6±8.4</td>
</tr>
</tbody>
</table>

Each value represents the mean±SD. * Significant difference as compared with group A; a as compared with group a; c as compared with group b; d as compared with group c; e as compared with group d; *p<0.05, @ p<0.01, † p<0.001; NS not statistically significant.

Table 4. Glutathione peroxidase activity and selenium concentration in kidney, lung, erythrocytes, and serum of rats of different experimental groups (n=6) (see Materials and Methods).

<table>
<thead>
<tr>
<th>Particulars</th>
<th>a</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>c</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>GPx</td>
<td>40.28±3.21</td>
<td>33.8±3.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.21±4.1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>43.21±3.33&lt;sup&gt;h&lt;/sup&gt;</td>
<td>36.33±3.91&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>0.42±0.02</td>
<td>0.39±0.03&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.39±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44±0.04&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.37±0.03&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lungs</td>
<td>GPx</td>
<td>22.38±2.18</td>
<td>17.88±2.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.64±2.16&lt;sup&gt;h&lt;/sup&gt;</td>
<td>25.12±1.89&lt;sup&gt;h&lt;/sup&gt;</td>
<td>20.88±1.86&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>0.39±0.02</td>
<td>0.35±0.04&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.40±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43±0.03&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.36±0.02&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>GPx</td>
<td>7.08±0.66</td>
<td>4.12±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.01±0.62&lt;sup&gt;h&lt;/sup&gt;</td>
<td>8.42±0.56&lt;sup&gt;h&lt;/sup&gt;</td>
<td>4.93±0.51&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Se</td>
<td>0.68±0.04</td>
<td>0.48±0.03&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.72±0.06&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.84±0.07&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.51±0.04&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum</td>
<td>GPx</td>
<td>0.52±0.03</td>
<td>0.24±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58±0.06&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.66±0.06&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.45±0.04&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as μg of glutathione utilized/min/mg protein for GPx in kidney and lung, μg of glutathione utilized/min/mg of hemoglobin for GPx in erythrocytes, μg/g of tissue for Se in kidney and lung, and μg/ml for Se in erythrocytes and serum. Each value represents the mean±SD. *Significant difference as compared with group A; <sup>b</sup>as compared with group a; <sup>c</sup>as compared with group b; <sup>d</sup>as compared with group c; <sup>e</sup>as compared with group d; <sup>*</sup>P <0.05, <sup>##</sup>P <0.001; NS not statistically significant.
administered in the initiation and promotion phase respectively, of carcinogenesis.

Table 3 summarizes the hepatic concentration of selenium and glutathione peroxidase activity. The values of both selenium concentration and glutathione peroxidase activity were found to be directly proportional to the total intake of selenium in each of the groups (B, C, and D). A significant difference in both selenium concentration and glutathione peroxidase activity was observed in both hepatoma and surrounding liver tissues as compared with the values for the carcinogen (group A) control rats and also with their respective controls, viz., groups b, c, and d.

Glutathione peroxidase activity and selenium concentration in kidney, lung, erythrocytes, and serum of rats of different experimental groups are shown in Table 4. The glutathione peroxidase activity and selenium concentration were found to be lower in carcinogen (group A) control rats than in untreated control rats (group a). However, there was no significant change in the selenium concentration in the lungs. The above parameters inclined towards normal on selenium supplementation.

DISCUSSION

The results of our present investigation clearly demonstrate that in this particular two-stage model of hepatocarcinogenesis in rats, the supplementation with 4 ppm selenium during the entire experimental period, before initiation, or during promotion greatly reduced the incidence, multiplicity, and size of visible persistent nodules with a concurrent decrease in the glutathione peroxidase activity and selenium concentration. In the promotional event, however, these changes did not have any statistical significance. Our data thus reveal the unique protective role of selenium against chemically induced liver tumorigenesis in rats and corroborate our previous findings [24].

The anticarcinogenic potential of selenium is primarily manifested in the initiation phase and secondarily in the promotion stage. In this regard, it is interesting to note that continuous long-term exposure to 4 ppm of selenium would elicit a greater protection in terms of the magnitude of preneoplasia than exposure at either the initiation or promotion phase alone.

Results observed from our study indicate that supplementation with 4 ppm of selenium in the drinking water, especially during the entire period of the study, resulted in fewer rats developing visible persistent nodules and in a smaller number of nodules than those observed in DEN control animals. Another striking observation of the study was that the selenium mediated inhibition of the appearance of persistent nodules more than 3 mm in size with a concurrent attenuation of nodular volume. Although it is evident that not all the hepatocyte nodules become cancerous during the lifespan of the animals, numerous observations support the concept that the nodules are the precursors of hepatic cancer [1, 25]. Moreover, there is a large body of evidence to show the correlation between the
number and size of hyperplastic nodules and hepatocarcinoma in both experimental and human disease [26, 27]. In view of this, inhibition of nodule growth and enhancement of their regression by selenium, as observed in our study, may be important for cancer prevention, especially if one considers that the persistent nodules are easily recognizable and have a low tendency to regress spontaneously. We observed that the food and fluid intakes and changes in body weights among different experimental groups were found to be statistically similar. This feature is of paramount importance because nutritional depreciation causing body weight loss may parallel a decrease in tumor volume [28]. Thus, the observed inhibitory effect of selenium on nodule appearance and growth is likely to be mediated through the impairment of the nutritional status of the experimental animals.

Results of this study suggest that selenium inhibits both the initiation and promotion phases of carcinogenesis. This finding confirms the study by Ip and Ganther [29] in which they used 7, 12-dimethylbenzanthracene-induced mammary tumor model although a slightly different dosage of selenium was used (5 ppm). However, Marshall et al. [30] cited that 4 ppm of selenium can inhibit 50% of liver tumors induced by 2-AAF. In addition, Kobayashi et al. [31] also suggested that supplementation with 4 ppm can reduce 56% of glandular stomach cancers induced by N-methyl-N'-nitro-N-nitrosoguanidine.

It was reported that the cytotoxic effect of a high level of selenium may not be the direct cause of the antitumor action and that the accumulation of selenium in tissues to a very high level would not be beneficial [10]. The antitumor activity of selenium has been demonstrated in animal models, and methylated selenides or selenobetaine has been proposed to have a more powerful activity than selenium [32]; and selenodiglutathione, a metabolite of selenite with reduced glutathione, has been shown to be a more potent agent for cancer prevention than any other methylated metabolite of selenium [31].

Antitumor activity of selenium has been demonstrated in animal models and methylated selenides or selenobetaine have been implicated to have a more powerful activity [9, 32], and selenodiglutathione, a metabolite of selenite with reduced glutathione has been shown to be a more potent agent for cancer prevention than any other methylated metabolites of selenium [29, 33]. Although the detailed mechanism of the selenium-mediated antitumor action is unknown, seleno proteins with the known biological functions may be possibly involved in the antitumor action. It is reasoned from the fact that selenides, the immediate precursors for the selenium moieties of seleno proteins [34], have more powerful antitumor action than selenite or selenate [35].

DEN is a procarcinogen [36]. Metabolic activation [37] of chemical carcinogens to ultimate carcinogens requires specific enzymes and cofactors, and it is possible that selenoenzymes or selenium metabolites can affect initiation of carcinogenesis [10]. The above possibility agrees with the fact that selenium is more active in initiation than in promotion phases. Inhibitory effect of selenium on 7, 12-dimethylbenzanthracene, a strong tumor initiator, binding to DNA in
vivo supports the idea that selenium may be involved in the initiation phase of carcinogenesis [38].

Immunopotentiation by selenium treatment has been demonstrated in animal models [39], and it should be considered that the antitumor action of selenium may be mediated through the potentiation of the immune system.

Lanfear et al. [40] reported the antitumor action of selenium in the promotional phase of carcinogenesis, in that selenium might be involved in cellular proliferation process. Their study agrees with our findings. However, the actual mechanism by which selenium would affect the control of the cell cycle is not known [6].

Antioxidants have the capacity to scavenge free radicals directly or to interfere with the generation of free radical events, which results in the inhibition of neoplastic processes [41]. It has been reported that free radicals play an important role in the complex course of multistep carcinogenesis [42]. Selenium has been showed to possess several activities including antioxidant potency [6]. Numerous studies indicate that dietary supplementation with selenium (Na2SO3) cause an increase in selenium-dependent glutathione peroxidase level in various tissues including liver [8]. This enzyme is responsible for inhibiting DNA damage due to oxidative stress [3, 43]. Oxidative damage has been implicated in the initiation and/or post-initiation of the carcinogenic process [37]. Thus, it is possible that the inhibition of liver carcinogenesis by selenium may be due to an increase in glutathione peroxidase activity. This is a reasonable hypothesis, in view of the fact that DEN is also capable of inducing oxidative DNA damage [44].

Tissue selenium concentrations in rats supplemented with selenium were elevated in proportion to the length of the supplementation; and all rats in the selenium-supplemented groups showed no pathological changes, indicating that 4 ppm of selenium is not actually toxic to rats.

We reasoned from the results of this experiment that the antitumor activity exerted from selenium supplementation is not dependent on the non-specific selenium toxicity but rather on specific biomolecules associated with selenium in the body.

Considering selenium as a prophylactic antitumor agent, a moderately high level (4 ppm) of selenium supplementation should be supplied for the replenishment of the metabolic pool of selenium so that this agent can be useful in the prevention of carcinogenesis.

REFERENCES


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