Effects of vitamin E and selenium supplementation on esophageal adenocarcinogenesis in a surgical model with rats

Xiaoxin Chen, Samer S. Mikhail, Yu Wei Ding, Guang-yu Yang, Floredeliza Bondoc and Chung S. Yang

Laboratory for Cancer Research, College of Pharmacy, Rutgers University, 164 Frelinghuysen Road, Piscataway, NJ 08854, USA

To whom correspondence should be addressed
Email: csyang@rci.rutgers.edu

Two well-known antioxidative nutrients, vitamin E and selenium, were used in this study to investigate possible inhibitory action against the formation of esophageal adenocarcinoma (EAC) in rats. In this model, carcinogenesis is believed to be driven by oxidative stress. Male Sprague–Dawley rats (8 weeks old) were divided into four groups and received esophagoduodenal anastomosis (EDA) surgery plus iron supplementation (12 mg/kg/week). Vitamin E and selenium were supplemented in the diet in the forms of α-tocopheryl acetate (750 IU/kg) and sodium selenate (1.7 mg Se/kg), which were 10 times the regular amounts in the basic AIN93M diet. At 40 weeks after surgery, all the EDA groups had lower body weights than the non-operated control group. Iron nutrition (hemoglobin, total serum iron and transferrin saturation) was normal as a result of iron supplementation after EDA. Vitamin E supplementation maintained the normal plasma level of α-tocopherol in EDA rats, but not those of γ-tocopherol and retinol. Selenium supplementation increased the serum and liver selenium contents of the EDA rats. Histopathological analysis showed that selenium supplementation increased the incidence of EAC and the tumor volume. The selenium level in the tumor is higher than that in the duodenum of the same animal. Vitamin E supplementation, however, inhibited carcinogenesis, especially in the selenium-supplemented group. We believe that vitamin E exerts its effect through its antioxidative properties, and a high dose of inorganic selenium may promote carcinogenesis by enhancing oxidative stress.

Introduction

Esophageal adenocarcinoma (EAC) has received much attention during the past 2 decades because of the rapid increase in its incidence rate, at a yearly rate of 4–10% in the USA (1,2). The 5 year survival rate is poor, at ~10%, and has not increased significantly during the past 20 years (3–5). Therefore, it is important to understand the pathogenesis and prevention of this disease.

Reflux of gastric and duodenal contents into the esophagus is known to be a risk factor for EAC. An average of ~10% of reflux esophagitis patients will develop columnar-lined esophagus (CLE, also known as Barrett’s esophagus) (4,6), a premalignant lesion in which the normal stratified keratin-producing squamous epithelium of the esophagus is replaced by mucin-secreting intestinal columnar epithelium. CLE is believed to be a tissue response to adapt to the reflux, since columnar epithelium is more resistant to chemical stress than squamous epithelium (7). According to a large autopsy study, the incidence of CLE in the general USA population was estimated to be one in 80. Moreover, the risk of CLE patients developing EAC is 30–125 times greater than in the general population (8).

In order to better understand the pathogenic process and to study possible chemopreventive strategies, we modified a surgical animal model, named esophagoduodenal anastomosis (EDA), by supplementing iron dextran after surgery (9). EDA, also known as duodenoesophagostomy, is aimed to induce a mixed reflux of gastric and duodenal contents into the esophagus via the anastomosis opening between the duodenum and the esophagus. EDA produces EAC at a low incidence (10–12). Supplementation with iron dextran i.p. prevented iron-deficiency anemia after surgery, but also produced a high rate of CLE and purely well-differentiated mucinous EAC in EDA rats (9). Since iron is known to catalyze the formation of reactive oxygen species (ROS) through the Fenton reaction (13,14), we believe that iron may promote esophageal adenocarcinogenesis through ROS-mediated oxidative damage. Several lines of evidence from studies on this model supported this hypothesis (15,16): (i) iron deposits were observed in areas of esophagitis, especially the squamocolumnar junction in which all EAC arise; (ii) overexpression of inducible nitric oxide synthase (iNOS) and nitrotyrosine were observed in rat esophagi after surgery and iron supplementation; (iii) oxidative damage to DNA, lipids and proteins was significantly higher in the esophagi of EDA rats than in those of the non-operated control rats; and (iv) columnar cells at the squamocolumnar junction, i.e. the premalignant cells for EAC, are believed to be the targets of oxidative damage since they overexpressed heme oxygenase 1 and metallothionein, which are both known to be expressed in response to oxidative stress. Therefore, we proposed that oxidative damage played an important role in the formation of EAC in the EDA model, and a similar situation might occur in humans with gastroesophageal reflux and iron over-nutrition.

Vitamin E and selenium are two well-known antioxidative nutrients. Vitamin E is the most effective chain-breaking liposoluble antioxidant in the biological membrane. Animal studies, epidemiological studies and human clinical trials have shown that vitamin E may reduce the risks of tumor development (17–19). Selenium is the essential cofactor of glutathione peroxidase, which detoxifies H2O2 and organic peroxides. Selenium supplementation has been found to inhibit cancers induced by chemical carcinogens or viruses in animals (20,21). An inverse relationship was observed between selenium levels in crops or diet and cancer mortality rate in different regions worldwide (22). Several prospective studies also showed that

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subjects with low serum selenium levels had an increased risk of developing cancer (23,24). Vitamin E and selenium are not only effective against oxidative damage alone, but also have a synergistic effect when used in combination (25,26).

In order to test our hypothesis that oxidative stress is involved in the formation of EAC, and to identify potential chemopreventive agents for human studies, we investigated herein the possible chemopreventive effects of vitamin E and selenium on the formation of EAC in this EDA model using a 2×2 factorial design.

Materials and methods

Animals and treatment

Six-week-old male Sprague–Dawley rats from Taconic Farms (Germantown, NY) were housed two per cage, separated into five groups, given respective diets and water *ad libitum*, and maintained on a 12 h light/dark cycle. They were allowed to acclimate for 2 weeks on respective diets prior to surgery. Solid food was withdrawn for 1 day before to 1 day after surgery. EDA was performed according to the procedure described previously (9), which was approved by the Animal Care and Facilities Committee at Rutgers University (protocol no. 94-017). The EDA animals were given iron dextran i.p. at 12 mg Fe/kg/week, starting 4 weeks after surgery, and continuing for the duration of the experiment (Table I). The animals were weighed once every week.

All the rats were killed by CO2 asphyxiation. The esophagus was removed, opened longitudinally and examined for gross abnormalities. If a visible tumor was observed, the length, width and height were measured and averaged for tumor diameter. Tumor volume was calculated as follows: volume = 4/3πr^3. Special care was taken to separate the esophagus from the duodenum based on the circumstances. The animals were killed at 40 weeks after EDA and iron supplementation a non-operated control rats (group V) throughout the experiment.

Table I. Effect of vitamin E and selenium on the formation of rat EAC at 40 weeks after EDA and iron supplementation

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Dietb</th>
<th>Incidence of EACc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I 28</td>
<td>AIN93M</td>
<td>67.9% (19/28)</td>
</tr>
<tr>
<td>Group II 31</td>
<td>AIN93M with 10× vitamin E</td>
<td>64.5% (20/31)</td>
</tr>
<tr>
<td>Group III 30</td>
<td>AIN93M with 10× selenium</td>
<td>90.3% (28/30)</td>
</tr>
<tr>
<td>Group IV 32</td>
<td>AIN93M with 10× vitamin E and 10× selenium</td>
<td>75% (24/32)</td>
</tr>
<tr>
<td>Group V 10</td>
<td>AIN93M</td>
<td>–</td>
</tr>
</tbody>
</table>

a All the rats received EDA and iron supplementation (12 mg Fe/kg/week) except group V served as non-operated control. The rats were placed on the respective diets starting from 2 weeks before surgery to the end of the experiment.

b AIN93M was used as the basic diet. It contains 75 IU/kg vitamin E in the form of α-tocopherol acetate and 0.17 mg/kg Se in the form of sodium selenate. Vitamin E was supplemented in the form of α-tocopherol acetate in the diet. Selenium was supplemented in the form of sodium selenate in the diet. Ten-fold supplementation increased the levels of vitamin E and Se to 750 IU/kg and 1.7 mg/kg Se, respectively.

c All the numbers are percentage of animals with EAC. The incidence of EAC of group III is significantly higher than those of groups I, II and IV (P < 0.05). The combined incidence of EAC of groups III and IV (83.9%) is significantly higher than that of groups I and II (66.1%) (P < 0.05). However, combined incidence of EAC of groups II and IV (69.8%) is not significantly different from that of groups I and III (81.0%) (P > 0.05).

Frozen on dry ice and then stored at ~80°C for the analysis of selenium and other parameters. The other half was fixed in 10% buffered formalin for 24 h and then transferred to 80% ethanol. The formalin-fixed esophagus was swiss-rolled, processed and embedded in paraffin. Five micron sections were mounted onto glass slides and used for pathological analyses. Liver was taken from the rats and processed in the same way.

Nutritional analysis

Fresh whole blood and serum were used for determination of hemoglobin, albumin, total serum iron and transferrin saturation with kits from Sigma Diagnostic (St Louis, MO). Instructions from the manufacturer were followed with slight modifications. Retinol, α-tocopherol and γ-tocopherol were measured by HPLC. In brief, fat-soluble vitamins were extracted from 150 μl of plasma with ethanol and hexane, and then dissolved in a mixture of chloroform, ethanol and acetonitrile. The HPLC system was set up using Supelco LC18 column (4.6×15 mm, 100 A; Bellefonte, PA) and a 1:1 ethanol:acetonitrile ratio as the mobile phase. A Waters 490 multiwavelength detector (Waters-Millipore, Milford, MA) was used to detect signals at 300, 325 and 450 nm.

Selenium concentration in serum and tissue was measured with inductively coupled plasma emission spectrometry/mass spectrometry (ICP ES/MS, VG Plasma Quad; Fisons Instruments, Danvers, MA). (10 μl) or frozen tissue was first digested with nitric acid, then properly diluted and loaded on the ICP ES/MS.

Histopathological analysis was carried out on the first and twentieth H&E-stained slides. EAC was diagnosed when dysplastic columnar epithelial cells invaded through the basement membrane. Dysplastic columnar cells were characterized by the partial loss of cell polarity and maturation, nuclear atypia and an increase in mitotic figures (28). Liver sections from each group were also examined for signs of toxicity, such as infiltration of inflammatory cells, vacuole formation, amyloid degeneration and necrosis.

Statistical analysis

The results on tumor incidence were analyzed by the χ2 test. The tumor volume data were analyzed by the Mann–Whitney test, and other data were analyzed by the Student’s t-test using the computer software Statview 4.2.

Results

A total of 130 rats underwent EDA, nine (7%) died before the completion of the experiment: five due to blockage of the esophagus (esophageal stricture) and four due to unknown circumstances. The animals were killed at 40 weeks after surgery. The food intake (g diet/kg body wt/day) at 20 and 40 weeks after surgery did not show any significant difference among the five groups. The body weights of all the EDA rats (groups I, II, III and IV) were significantly lower than the non-operated control rats (group V) throughout the experiment (Figure 1). No significant difference was observed among the EDA groups, which differed in dietary supplements. Selenium supplementation (groups III and IV) and vitamin E supplementation (groups II and IV) did not change the body weight significantly.

Our previous studies have shown that EDA had several nutritional problems, including decreased iron nutrition status as reflected in hemoglobin, total serum iron and transferrin saturation, decreased level of serum albumin and decreased levels of plasma fat-soluble vitamins (16). The EDA rats developed iron-deficiency anemia progressively as a result of partial loss of gastric function. In this study, supplementation of 12 mg Fe/kg/week i.p. after EDA maintained adequate iron nutrition in terms of rat hemoglobin, total serum iron and transferrin saturation (Table II). No significant difference of the iron nutrition parameters was observed among the five groups.

EDA rats had significantly lower serum albumin (data not shown) and plasma α-tocopherol, γ-tocopherol and retinol levels (group I) than non-operated control (group V) (Table I). Ten-fold supplementation of vitamin E in the form of α-tocopherol acetate (groups II and IV) significantly increased...
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the plasma level of α-tocopherol to the level comparable with, but not higher than, that of non-operated control (group V). Supplementation did not change the levels of γ-tocopherol and retinol.

Absorption of selenium was not affected by EDA, and there was no significant difference in selenium levels in serum and liver between groups I and V. Ten-fold selenium supplementation (groups III and IV) significantly increased the selenium levels in serum and in liver over the non-operated control (group V) (Table II). We also observed a significantly higher selenium level in the EDA tumors than in the duodenum of the corresponding rat (Table II).

Histopathological analysis was performed after H&E staining. The incidence of EAC in EDA animals with i.p. iron supplementation was 67.9%, which was comparable with our previous results. Under the microscope, dysplastic columnar epithelial cells invaded through the basement membrane to form a typical adenocarcinoma tumor. As expected, 10-fold vitamin E supplementation slightly decreased the EAC incidence from 67.9% (group I) to 90.3% (group III), and supplementation (groups III and IV) significantly reduced the selenium-enhanced EAC incidence from control (group V) (Table II). We also observed a significantly lower selenium in duodenum than in EAC tumor. EAC tumors of groups III and IV had higher levels of selenium than those of group I and V.

Table II. Rat nutrition after EDA and supplementation with vitamin E and selenium

<table>
<thead>
<tr>
<th></th>
<th>Group I (AIN93M)</th>
<th>Group II (AIN93M and 10× vitamin E)</th>
<th>Group III (AIN93M and 10× Se)</th>
<th>Group IV (AIN93M and 10× vitamin E and Se)</th>
<th>Group V (AIN93M)</th>
</tr>
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<tbody>
<tr>
<td>Iron nutritiona</td>
<td></td>
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<td></td>
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<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.5 ± 1.9</td>
<td>15.2 ± 1.7</td>
<td>16.1 ± 2.4</td>
<td>15.9 ± 2.1</td>
<td>13.5 ± 1.1</td>
</tr>
<tr>
<td>Total serum iron (µg/dl)</td>
<td>215.5 ± 59.9</td>
<td>198.3 ± 65.7</td>
<td>219.3 ± 119.5</td>
<td>237.7 ± 68.1</td>
<td>200.9 ± 18.1</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>32.9 ± 8.7</td>
<td>32.7 ± 12.1</td>
<td>33.6 ± 18.7</td>
<td>33.6 ± 8.9</td>
<td>38.7 ± 2.3</td>
</tr>
<tr>
<td>Fat-soluble vitamins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (µg/l)b</td>
<td>1037.4 ± 208.9</td>
<td>1541.4 ± 329.9</td>
<td>1152.7 ± 328.8</td>
<td>1622.2 ± 328.1</td>
<td>1821.6 ± 426.2</td>
</tr>
<tr>
<td>γ-Tocopherol (µg/l)b</td>
<td>13.0 ± 3.3</td>
<td>11.35 ± 1.9</td>
<td>13.2 ± 3.1</td>
<td>11.3 ± 1.9</td>
<td>16.8 ± 1.1</td>
</tr>
<tr>
<td>Retinol (µg/l)c</td>
<td>45.9 ± 6.9</td>
<td>42.6 ± 7.8</td>
<td>45.6 ± 6.7</td>
<td>43.6 ± 7.9</td>
<td>53.8 ± 6.9</td>
</tr>
<tr>
<td>Selenium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (p.p.m.)d</td>
<td>0.08 ± 0.01</td>
<td>ND</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Liver (p.p.m.)d</td>
<td>0.81 ± 0.30</td>
<td>ND</td>
<td>1.02 ± 0.03</td>
<td>1.50 ± 0.53</td>
<td>0.79 ± 0.03</td>
</tr>
<tr>
<td>Duodenum (p.p.m.)</td>
<td>0.51 ± 0.08</td>
<td>ND</td>
<td>0.58 ± 0.02</td>
<td>0.69 ± 0.08</td>
<td>0.62 ± 0.05</td>
</tr>
<tr>
<td>EAC tumor (p.p.m.)</td>
<td>0.66 ± 0.09</td>
<td>ND</td>
<td>0.86 ± 0.10</td>
<td>0.88 ± 0.07</td>
<td>ND</td>
</tr>
</tbody>
</table>

Ten samples from each group were measured. All data represent means ± SD. ND, not determined.

aNo significant difference among the five groups (P > 0.05).
bGroups II, IV and V have significantly higher levels than groups I and III (P < 0.05).
cGroups I, II, III and IV have significantly lower levels than group V (P < 0.05).
dGroups III and IV have significantly higher levels than groups I and V (P < 0.05).

Fig. 1. Rat average body weight after EDA and supplementation with vitamin E and selenium. ○, Group I: EDA with 12 mg Fe/kg/week i.p. and AIN93M; ●, group II: EDA with 12 mg Fe/kg/week i.p., AIN93M and 10-fold vitamin E; □, group III: EDA with 12 mg Fe/kg/week i.p., AIN93M and 10-fold selenium; ■, group IV: EDA with 12 mg Fe/kg/week i.p., AIN93M and 10-fold vitamin E and selenium; Δ, group V: non-operated control and AIN93M.

Fig. 2. Tumor volume of rat EAC after EDA and supplementation with vitamin E and selenium. Tumor volume of the visible EAC tumor was plotted against the group.
The combined EAC incidence of vitamin E-supplemented groups (groups II and IV, 69.8%) was also slightly lower, but not significantly different from that of non-supplemented groups (groups I and III, 81.0%) (Table I). In terms of tumor volume, vitamin E supplementation did not exert any significant effect on group II versus group I, group IV versus group III, and groups II plus IV versus groups I plus III. The combined EAC incidence of selenium-supplemented groups (groups III and IV, 83.9%) was also significantly higher than that of non-supplemented groups (groups I and II, 66.1%). A significant difference was observed between groups IV plus III and groups I plus II. No significant difference of tumor volume was observed, however, between groups III and I, and between groups IV and II (Figure 2).

Discussion

EDA partially mimics the human situation by inducing mixed reflux of gastric and duodenal contents into the esophagus, thus producing CLE, CLE with dysplasia and finally EAC. With iron supplementation (50 mg Fe/kg/week), 67.9% EAC was generated in rats, a percentage comparable with our previous results (9,16). All tumors were pure well-differentiated mucinous EAC. Oxidative damage, as a result of chronic inflammation and local iron overload, is the major factor leading to EAC in this model.

EDA rats had compromised nutrition, which does not occur in all the human EAC patients. However, there is a subgroup of EAC patients with a previous history of gastrectomy or other gastric surgery. These patients are highly susceptible to EAC as a result of reflux and direct juxtaposition of the esophagus with intestinal or ‘specialized’ CLE, according to previous studies (29,30). These patients develop malnutrition after surgery as a result of compromised function of the stomach. Nutritional supplementation of some nutrients including iron is a routine practice for such patients (31).

Vitamin E is the most effective chain-breaking lipid-soluble antioxidant in the biological membrane. It functions by stabilizing the biological membrane, protecting against oxidative stress induced by iron overload, and stimulating immune response (32). Vitamin E can also induce apoptosis in several human and rodent tumor cell lines, including human B lymphoma, glioma, breast tumor and colon cancer cell lines (33). It has been shown in both laboratory and epidemiological studies that vitamin E may reduce the risk of cancer (17–19). In a rat model of iron-induced oxidative nephrotoxicity and renal cancer, vitamin E supplementation inhibits apoptosis, oxidative DNA adduct formation and cancer incidence (34). In the present study, vitamin E supplementation slightly decreased the EAC incidence, but the result was only statistically significant in the selenium-supplemented group. Due to the partial loss of gastric function in the EDA rats, supplementation with 10-fold levels of α-tocopherol acetate in groups II and IV only increased the EDA rat plasma concentration of α-tocopherol to the normal level of the non-operated control (group V) (Table II). Supplementation at even higher levels of vitamin E may be needed to demonstrate more significant chemopreventive effects.

Selenium has long been regarded as a cancer chemopreventive agent. In a recent large clinical trial, selenium supplementation (200 μg/day of yeast selenium, selenomethionine, for an average of 4.5 years) reduced total cancer incidence by 59%, especially the incidences of lung, colorectal and prostate cancers (35). Several mechanisms have been proposed for the chemopreventive effect of selenium (36): (i) selenium-containing compounds can increase the activity of glutathione peroxidase which catalyzes the reduction of H2O2 and organic hydroperoxides (37); (ii) selenium induces apoptosis in cancer cell lines (38); (iii) selenium affects the metabolism of certain chemical carcinogens (39); (iv) selenium inhibits the formation of prostaglandin E2 in colon and the activity of thymidine kinase in mammary tumor cell lines; (v) selenium regulates the immune functions (40); and (vi) selenium may kill tumor cells through its pro-oxidant property (41,42).

However, it was out of our expectation that 10-fold selenium in the form of sodium selenate increased the EAC incidence from 67.9 (group I) to 90.3% (group III). When groups III and IV are combined, selenium supplementation increased not only the tumor incidence to 83.9 from 66.1% (groups I and II), but also the tumor volume. Although most previous studies showed some protective effect of selenium against various cancers, selenium in certain chemical forms has been found to promote cancer formation in some studies. For example, sodium selenite supplementation (4 p.p.m. selenium in drinking water) inhibited 1,2-dimethylhydrazine (DMH)-induced colon cancer in rats, but increased the incidence of DMH-induced small bowel cancer, and adenovirus type 9-induced breast fibroadenoma (43). Long-term administration of sodium selenium (2–3 p.p.m. in water) increased the incidences of spontaneous tumors and cancers from 30.8 and 16.9 to 62.5 and 41.7% in Evans rats (44). In a recent epidemiological study, Garland et al. (45) reported a moderately increased risk of melanoma, as well as of lung, colorectal and other cancers among female nurses with average or high levels of selenium in toenail samples, compared with nurses whose toenail selenium levels were low. In an epidemiological study involving an Italian population exposed to high water selenium (7–9 p.p.m., selenite), the incidence of melanoma was increased by 3.9× that of the unexposed cohort (<1 p.p.m.) (46), and the mortality for malignancies of the lymphatic-hematopoietic tissue increased in female subjects (47).

We observed significantly higher levels of selenium in rat serum and liver samples of selenium-supplemented rats (groups III and IV) than in non-supplemented rats (groups I and V). Interestingly, the selenium content in EAC tumors was significantly higher than that in the duodenum, from which the columnar cells of CLE may originate. Similarly, some human and animal cancers, such as gastric cancer (48), colon cancer (49), breast cancer (50) and lung cancer (51), contained a higher selenium level than normal tissue. In a study of human gastric cancer, high selenium levels in cancer tissue were correlated with intestinal type adenocarcinoma, a tumor quite similar to EAC (48). These results suggested that selenium might promote carcinogenesis by depositing in target tissues. Similar to our finding that vitamin E inhibits the cancer-promoting effect of selenium, dietary supplementation of 0.01–0.05% vitamin E, or other antioxidants potentiates the protective effect of 0.5% DL-methionine against 10 p.p.m. selenium (sodium selenite) as indicated by growth and liver histology (52).

Selenium of different chemical forms may have different effects on carcinogenesis. Most selenium in natural foods is in the form of organoselenium compounds, which seem to be safer and more potent than inorganic selenium for cancer chemoprevention. Dietary supplementation of an organoselen-
uum compound, 1,4-phenylenbis(methylene)-selenocyanate (p-XSC), efficiently inhibited several kinds of animal tumors (36). A recent clinical trial showing the effectiveness of selenium against several human cancers also employed organo-selenium (35). Some selenium-containing compounds have been shown to promote the development of cancers, such as bis-4-acetamino-phenyl-selenium dihydroxide for adenomatous hepatic hyperplasia and multiple thyroid adenoma, selenium diethylidithiocarbamate for hepatica, lymphoma and pulmonary tumors, and selenium sulfide for hepatocellular carcinoma (39). In the present study, sodium selenate was used because it is the chemical form used in the semi-purified AIN93 diet, and the most commonly used chemical form in commercial multivitamins.

Metallothionein overexpression is a known adaptive or protective mechanism in response to oxidative stress. In our previous study (16), metallothionein was found to be overexpressed in premalignant and EAC cells. We used metallothionein immunohistochemistry on paraffin sections to observe the difference among the EDA groups with or without chemopreventive agents (vitamin E and selenium). Nevertheless, no significant difference was observed (data not shown). It is likely that iron supplementation (12 mg/kg/week, i.p.) was so strong that the effects of vitamin E and selenium on metallothionein expression were masked.

We have proposed oxidative damage as a major causative factor leading to EAC in the EDA model. Selenium probably also promoted EAC formation by facilitating oxidative stress. According to the model proposed by Seko and Imura (41), some selenium compounds can react with glutathione and other thiols to form selenotrisulfides, which will ultimately produce superoxide and hydrogen peroxide. Selenium-promoted ROS formation is also related to the presence of iron. It has been shown that pre-treatment with an iron chelator not only reduced the acute toxicity of selenite, but also inhibited selenite-induced rat cataract (53). In the present study, iron was supplemented i.p. in all the EDA rats. It is likely that selenium may promote the formation of ROS by the Fenton reaction in the presence of excessive iron. Further investigation is warranted to determine whether organoseleno compounds, such as p-XSC, can exert a protective effect against esophageal adenocarcinogenesis.

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References


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