Selenium prevents tumor development in a rat model for chemical carcinogenesis

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Previous studies in animals and humans have shown that selenium compounds can prevent cancer development. In this work we studied the tumor preventive effect of selenium supplementation, administrated as selenite, in the initiation, promotion and progression phases in a synchronized rat model for chemically induced hepatocarcinogenesis, the resistant hepatocyte model. Selenite in supra-nutritional but subtoxic doses (1 and 5 p.p.m.) was administrated to the animals through the drinking water. Such supplementation during the initiation phase did not have a tumor preventive effect. However, selenite treatment during the promotion phase decreased the volume fraction of pre-neoplastic liver nodules from 38% in control animals to 25% (1 p.p.m.) and 14% (5 p.p.m.) in the selenite-supplemented groups. In addition the cell proliferation within the nodules decreased from 42% in the control to 22% (1 p.p.m.) and 17% (5 p.p.m.). Immunoistochemical staining for the selenoenzyme thioredoxin reductase 1 revealed an increased expression of the enzyme in liver nodules compared with the surrounding tissue. The activity was reduced to 50% in liver homogenates from selenite-treated animals but the activity of the selenoenzyme glutathione peroxidase was essentially unaltered. Selenite treatment (5 p.p.m.) during the progression phase resulted in a significantly lower volume fraction of liver tumors (14 compared with 26%) along with a decrease in cell proliferation within the tumors (34 compared with 63%). Taken together our data indicate that the carcinogenetic process may be prevented by selenium supplementation both during the promotion and the progression phase.

Introduction

An anticarcinogenic effect of selenium compounds has been demonstrated in several animal models designed for studies of different forms of cancers (1,2). In animal models using 2-acetylaminofluorene (2-AAF) to induce liver tumor development, selenium compounds have been shown to reduce the growth of enzyme-altered liver foci (3–6). Although selenium does not seem to interfere with intracellular carcinogen activation or interaction with cellular macromolecules (7) selenium compounds do have an effect on 2-AAF hepatotoxicity (6). The tumor preventive effect of selenium has, however, not yet been studied in the sequential initiation–promotion model permitting differentiation of initiation, promotion and progression.

The tumor preventive effect of selenium compounds has also been shown in human cancers (8). The mechanisms of the selenium effect are still not fully understood. Data support the activation of the tumor suppressor protein p53 (9,10), increased resistance against oxidative stress due to induction of selenoproteins as well as interactions with cell-cycle signal transduction (1,2,11,12). It is important to further investigate the molecular mechanisms of this potential tumor preventor before it can be used as a tool for preventive therapy in patients with a high risk of cancer development.

We showed recently that the cytosolic selenoenzyme thioredoxin reductase (TrxR1), was significantly increased in 2-AAF-induced liver nodules and that this induction was specific for the focal lesions (13). The difference in activity between liver nodules and the surrounding parenchyma was 3.5-fold, indicating a differential and tumor-specific effect. TrxR1 is a selenoenzyme that is preferentially induced by selenium supplementation at sub-toxic levels over a transient period of several weeks (14). The thioredoxin system, comprising TrxR, thioredoxin and NADPH, is an efficient antioxidant system in the cell acting directly on radicals (15), but importantly also, the enzyme is responsible for regeneration of other antioxidants like ubiquinol and indirectly also vitamin E (16).

In this work we have studied the effect of selenium in a sequential initiation–promotion model for experimental hepatocarcinogenesis. The model is described by Solt and Farber (17) and is called the resistant hepatocyte model, implementing the appearance and selection of resistant hepatocytes with up-regulated cellular defence as determinants for tumor cell growth advantage during promotion and progression (18). In this work the effect of selenium on tumor initiation, promotion and progression has been followed. The appearance of focal lesions and their development into advanced lesions/hepatomas have been quantified as have the rate of cell proliferation in the lesions and surrounding liver tissue. In addition the expression of the selenoenzymes TrxR and glutathione peroxidase (GPx) have been studied.

Materials and methods

Chemicals

The basal rat diet R36 was purchased from Lactamin (Sweden). 2-AAF (Fluca, Switzerland) was mixed with the modified Altromine1314 diet (Altromine, Germany), which resembles the basal diet R36. Anti-GST-Yp/Yf subunit

Abbreviations: 2-AAF, 2-acetylaminofluorene; BrdU, 5-bromo-2′-deoxyuridine; DEN, diethylnitrosamine; GPx, glutathione peroxidase; GST, glutathione S-transferase; LI, labeling index; PH, partial hepatectomy; TrxR, thioredoxin reductase.
7 antibodies were from Biotrine International, Dublin, Ireland. Trx from Escherichia coli was purchased from Promega. Bovine TrxR for the activity assay standard curves was obtained from IMCO. Sodium selenite and all chemicals employed for enzyme assays were procured from Sigma Chemical.

**Animal experiments**

Fisher-344 rats, weighing 140–160 g, were purchased from Charles River Sverige AB, Uppsala, Sweden. Animals were maintained in a 12-h light and dark cycle and fed a standard chow diet (Altromin modified 1314, Altromin International, Lage, Germany) until the start of the experiment. The animals were allowed to acclimatize in the animal room for 4 days before use. Temperature, humidity and ventilation in the animal room were controlled in accordance with international standards.

The animals were divided into eight groups with eight rats in each group. For groups I-IV and VII-VIII the process was initiated with diethylnitrosamine (DEN) dissolved in saline (50 mg/ml), and injected intraperitoneally in a necrogenic dose of 200 mg/kg rat body wt. Promotion was performed using 0.02% 2-AAF-diet for 4 days followed by a 2/3 partial hepatectomy (PH). After PH, two intra-gastric injections of 2-AAF (20 mg/ml emulsified in agar) were performed on days 2 and 4. In protocol I-VI, rats were killed at 7 and 21 days post-PH. In experiments VII-VIII, rats were killed at 21 days and 3, 6, 9 and 12 months post-PH. Selenium was administrated in the drinking water at a concentration of 1 and 5 mg/l according to the protocol described in Figure 1. In experiment II, selenium was administrated 1 week before and 1 week after injection of DEN. In experiments III, IV and VI selenium was given 1 week before 2-AAF feeding and during the following 5 weeks. In experiment VIII selenium was continuously given from the time-point 3 weeks after PH and through the entire progression phase until the rats were harvested. The rats were drinking 10–12 ml water/100 g body wt corresponding to a dose of 10 μg selenite/100 g body wt and 50 μg selenite/100 g body wt for the 1 and 5 p.p.m. dosages, respectively. These dosages are the same or lower than the dosages used previously in tumor preventive studies made in rats (3–7). In this way the effect of selenium on initiation, promotion and progression could be investigated sequentially.

**Determination of selenium in serum samples from rat**

The total selenium concentration in serum samples from rats was determined by using an ICP-MS instrument (ELAN 6100 DRC, Perkin-Elmer SCIEX, Concord, ON, Canada) equipped with a dynamic reaction cell (DRC). Briefly, selenium as 78Se was recorded, blank subtracted and quantified against an external calibration curve. Further details regarding the analyses and instrumental settings have been described elsewhere (19,20).

**Determination of nodule and tumor density**

Rats were killed after exsanguination via the abdominal aorta under ether anaesthesia. The livers were taken out, weighed and sliced in 4-mm-thick slices. For experiments I-VI representative slices were harvested and fixed in 4% buffered formaldehyde and processed for histological investigation. In experiments VII-VIII slices with visible lesions were harvested and processed. Paraffin sections were incubated with a monoclonal antibody towards intracellular glutathione S-transferase 7-7 (anti-GST-Yp/Yf subunit 7) to visualize initiated hepatocytes, liver foci and nodules as described earlier (21). Liver foci and nodules were quantified by number and size using morphometric densitometry, expressing numbers and volumes in relation to surface area of the section and volume fraction, respectively. The relative volumes of the lesions were calculated as described earlier (21).

**Labeling index**

Three days before killing, the rats were administered 5-bromo-2-deoxyuridine (BrdU) by Aztec micro-osmotic pumps (1 μl/h, 3 days) implanted subcutaneously on the back of the animals. Incorporated BrdU was detected by BrdU-immunohistochemistry. BrdU-labeled and -unlabeled nuclei were counted in six image fields (×250) corresponding to 600–800 cells, which was sufficient according to cumulative mean. The labeling index (LI) was counted in the surrounding parenchyma as well as in liver nodules. In experiments VII-VIII advanced liver nodules were easily detectable, while remodeling nodules and nodules with low growth potential were harder to demarcate. The LI was calculated under guidance from GST-stained consecutive sections with computerized morphometry.

**Immunohistochemical staining**

For immunostaining of TrxR1, 4-μm liver tissue sections were deparaffinized in xylol and re-hydrated in decreasing concentrations of ethanol. The tissue sections were heated in a microwave oven for 10 min in 0.01 M citrate buffer, pH 6.0, rinsed in 0.05 M Tris-saline buffer (TBS), pH 7.6. The automatic DAKO TechMate 500 was used for staining the slides with anti-TrxR polyclonal IgG (Upstate, USA, Cat. # 07-078) at a 1:200 dilution in DAKO ChemMate antibody diluent (DAKO A/S, Denmark) for 25 min in room temperature, humidity and ventilation in the animal room were controlled in accordance with international standards.

Fig. 1. Schematic presentation of selenium treatment during the initiation phase (experiment II), promotion phase (experiments III-VI) and during the progression phase (VII-VIII). Block arrow indicates initiation with diethylnitrosamine 200 mg/kg body wt in saline. White box indicates 4 days feeding with 0.02% 2-AAF in the diet, line arrow directed upwards represents PH; line arrow pointing down indicates 2-AAF injections (20 mg/kg body wt). Open triangles indicate time for harvest.
Temperature. For visualization Dako ChemMate Detection Kit Peroxidase/ DAB (#K5001, Dako A/S, Denmark) was used. The sections were counterstained with hematoxylin.

The specificity of the primary antibody reaction was confirmed in separate experiments by adsorption of the anti-TrxR antibody with excess amounts of the recombinant rat TrxR1, kindly provided by Dr Elias Amør, Karolinska Institutet, Sweden.

**Enzyme assays**

Liver tissue was homogenized at 4 °C in a solution containing 50 mM Tris and 1 mM EDTA in a final concentration of 2 g tissue/10 ml. The homogenates were centrifuged at 25,000 g for 10 min, the supernatant was collected and the protein concentration was determined by the Biuret procedure using bovine serum albumin as the standard (22). The activities of TrxR in the supernatants were determined using insulin disulfides as substrate, essentially as described by Holingren and Björnstedt (15). The activities of GPx were determined by using the method described by Lawrence and Burk (23).

**Ethical approval**

This study was approved by the Stockholm South Local Community for Ethical Review of animal experiments, no. S128-03.

**Results**

The total selenium concentration in serum samples from the rats treated with 5 p.p.m. selenite in the drinking water was slightly higher compared with the levels in animals drinking only water (Table I). The selenium concentration in serum did not increase with duration of treatment and showed no tendency to accumulate with time compared with the control.

**Effects of selenite during initiation**

After 7 days post-PH the relative nodule density was 1 ± 0.3% in the non-selenite-treated group and 4 ± 1.5% in the selenite-treated group, a difference that was statistically significant (P = 0.005). Twenty-one days post-PH the relative nodule density was 38 ± 8% in the non-selenite-treated group (Figure 2A) and 44 ± 15% in the selenite-treated group (Figure 2B), a difference that was not statistically significant (P = 0.22).

There was no difference between the number of nodules in the selenite-treated and non-treated group.

**Effects of selenite during promotion**

**Density of nodules and cell proliferation.** After 21 days post-PH the relative nodule density was 38% in the non-selenite-treated group. In the livers of animals receiving 1 p.p.m. selenite in the drinking water during the promotion period the density of the liver nodules was 25%, and the density decreased even further, to 14%, in animals receiving 5 p.p.m. selenite (P = 0.05 and P < 0.001, respectively) (Figure 2).

**Table I.** Selenium concentration in serum (ng/ml) from rats obtained (A) selenite treatment during the promotion phase, experiments I–V and (B) selenite treatment during the progression phase, experiments VI–VIII

<table>
<thead>
<tr>
<th>Time post-PH</th>
<th>0 p.p.m. Se</th>
<th>5 p.p.m. Se</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 7 days</td>
<td>506 ± 51</td>
<td>578 ± 48</td>
<td>P = 0.006</td>
</tr>
<tr>
<td>21 days</td>
<td>540 ± 10</td>
<td>647 ± 27</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>B 21 days</td>
<td>514 ± 16</td>
<td>655 ± 25</td>
<td>P = 0.05</td>
</tr>
<tr>
<td>3 months</td>
<td>664 ± 12</td>
<td>723 ± 21</td>
<td>P = 0.18</td>
</tr>
<tr>
<td>6 months</td>
<td>720 ± 19</td>
<td>754 ± 8</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>9 months</td>
<td>659 ± 54</td>
<td>746 ± 27</td>
<td>P = 0.01</td>
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<tr>
<td>12 months</td>
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**Fig. 2.** Liver sections, stained immunohistochemically with anti-GST antiserum after 21 days post-PH in animals treated with (A) 0 p.p.m. selenite, (B) 5 p.p.m. during the initiation, (C) 1 p.p.m. selenite during the promotion and (D) 5 p.p.m. selenite during the promotion. Nodule density (volume fraction) in (A) is 38 ± 8%, (B) 44 ± 15%, (C) 25 ± 12%, (D) 14 ± 5%. Statistical analysis using Student’s t-test gives P = 0.05 in (C) and P < 0.001 in (D) compared with the nodule density in (A). The difference between (A) and (B) was not statistical significant.
compared with the non-treated rats and parenchyma gives value in the nodules in the non-treated rats. The LI value in the surrounding 5 p.p.m. selenite treatment.

non-DEN-treated rats compared with the DEN-treated rats with or without 5 p.p.m. selenite treatment.

The LI value in the surrounding parenchyma gives $P = 0.04$ in the 5 p.p.m. selenite-treated rats compared with the non-treated rats and $P = 0.08$ and $P < 0.001$ in the non-DEN-treated rats compared with the DEN-treated rats with or without 5 p.p.m. selenite treatment.

$\frac{\text{Relative liver mass}}{100 \text{ g rat}}$ 7 and 21 days post-PH after treatment with 0, 1 and 5 p.p.m. selenite during the promotion and as a control, 0 and 5 p.p.m. in the absence of DEN. Statistical analysis using Student’s $t$-test gives $P = 0.02$ (5 p.p.m. selenite) compared with the non-treated rats and $P = 0.03$ in the non-DEN-treated rats compared with the DEN-treated rats.

Three months treatment with selenite in the progression phase did not have any effect on the volume fraction of tumor tissue within the rat livers. After 6 months, however, there was a statistically significant effect on the volume fraction of tumor tissue, $10 \pm 6\%$ in the non-treated animals compared with only $1.5 \pm 1.2\%$ in the selenite-treated rats ($P = 0.02$). The tumor density values after 9 months were unfortunately not conclusive because of too few animals in this group.

Seven days post-PH. The BrdU LI and volume fraction of focal liver lesions at 7 days post-PH clearly showed an almost complete mitoinhibition of the surrounding hepatocytes in all 2-AAF-treated groups (data not shown). In the presence of the drug, selenium seemed to have only a minor effect on liver toxicity generating a growth pressure on the foci and nodules that resulted in almost 100% of the cells passing S-phase during the 3 days of BrdU exposure. The results at 21 days showed an increased ability of the hepatocytes to recover from the mitoinhibitory effect of 2-AAF with selenium in a dose-dependent fashion.

Relative liver mass. The liver cell proliferation after PH is dependent on the relative functional liver mass, which, in this model, relates to liver weight per body weight and 2-AAF toxicity. The cell proliferation in experiments V and VI at 21 days after PH reflects the liver regeneration after recovery from 2-AAF mitoinhibition. The selenium-dependent increase in proliferation rate of surrounding hepatocytes seen in experiments III and IV (Figure 3) is interesting as it could be a result of selenium decreasing the toxicity of 2-AAF.

In Figure 4 the relative liver weights at 7 and 21 days post-PH are depicted. It is obvious that at 1 week no significant effect of selenium on liver regeneration is seen. At 21 days, though, the selenium-treated rats in experiments III and IV are approaching the normal expected relative liver weight faster than the rats drinking water. It is noteworthy that the weight of the surrounding, non-nodular liver parenchyma in all groups amount to around the same weight, 3 g/100 g of rat body wt.

**Selenoenzyme activities.** We know from recently published data (13) and from immunohistochemical staining (Figure 8A) that the activity of TrxR1 is significantly higher in the liver nodules than in the surrounding tissue during conditions where 2-AAF have been taken out. The enzyme activity data from rats killed at 21 days after PH showed a marked increase in activity in the rats carrying liver nodules (Figure 5). In the selenium-treated groups the activity was lower than in the control group (Figure 5). This decrease can be explained by the lower volume fraction of liver nodules in these livers, but an effect of the activity of the enzyme in the lesions after selenium treatment could not be excluded. It is noteworthy that the TrxR1 activity levels were the same in rats treated with 1 and 5 p.p.m. selenite, although the volume fraction of liver nodules were significantly lower in the livers from rats drinking water with 5 p.p.m. selenite.

In contrast to TrxR1, there were no significant alterations in the activities of GPx (Figure 5).

Effects of selenite during progression

Density of nodules and cell proliferation. Three months treatment with selenite in the progression phase did not have any effect on the volume fraction of tumor tissue within the rat livers. After 6 months, however, there was a statistically significant effect on the volume fraction of tumor tissue, $10 \pm 6\%$ in the non-treated animals compared with only $1.5 \pm 1.2\%$ in the selenite-treated rats ($P = 0.02$). The tumor density values after 9 months were unfortunately not conclusive because of too few animals in this group.
One year after PH, animals from both the selenite-treated group and the non-treated group had macroscopically visible nodular liver lesions some of them with morphology of hepatoma. However, the selenite-treated rats had a significantly lower volume fraction of these lesions within their livers than the non-treated rats, 14 compared with 26% \( (P = 0.01) \) (Figure 6) and the cell proliferation in these tumors were significantly lower compared with the non-treated animals; LI was 34 compared with 63% \( (P = 0.04) \) (Figure 7). In both groups GST-positive liver nodules were seen. Most of them were irregular and not surrounded by a pseudocapsule like the more proliferative lesions. There was a non-significant increase in volume fraction of liver nodules in the selenite-treated animals (Figure 6). The lesions defined as liver nodules (i.e. GST-positive and no morphological basis for hepatocellular carcinoma) had a lower LI in the selenite-treated rats, indicating a lower proliferation rate in these lesions (Figure 7).

The proliferation in the surrounding tissue was lower in the selenite-treated group compared with the control group, 4 compared with 8% \( (P = 0.05) \) reflecting a lower regenerative driving force in these livers.

*Selenoenzyme activity.* After 1 year, lower TrxR1 levels in the liver cell extracts from the selenium-treated animals were recorded compared with the levels in non-treated rats (data not shown)—probably due to a lower volume fraction of tumor tissue in the selenium-treated group. However, because of great variance between the rat livers after 1 year due to the heterogeneous phenotype of the tumors, no conclusions can be drawn from these measurements.

**TrxR immunohistochemistry**

Immunohistochemical staining using anti-TrxR antibodies showed that the liver nodules at 21 days post-PH stained strongly positive for the enzyme in comparison with the surrounding liver parenchyma. The non-nodular hepatocytes stained positive only weakly. The staining was specific for the hepatocytes, leaving the bile duct cells, endothelial cells and Kupffer cells negative (Figure 8A). The staining reaction...
was almost completely abolished if the antibodies were pre-mixed with excess amount of TrxR1 protein, confirming a high specificity of the primary antibody (Figure 8B).

The staining of the parenchyma surrounding the lesions was not visibly affected by selenium treatment (Figure 9B). Interestingly, in the progression phase the GST-positive liver nodules did not stain positively compared with the surrounding (data not shown). Only the rapidly proliferating lesions showed a significant positive staining (Figure 9C) and the staining intensity was not affected by selenium treatment (Figure 9D).

Discussion

In the resistant hepatocyte model selenium treatment covering the promotion phase significantly decreased the volume in the livers occupied by liver nodules. The effect was dose-dependent and significant at both doses of 1 and 5 p.p.m. sodium selenite in drinking water. It is well established from earlier work that the size of the pre-neoplastic cell population at risk for further progression reflects the risk of liver cancer development (24). The results therefore suggest that selenium is able to reduce the risk for liver cancer even when it is used only during a short period of time covering the promotion phase of the carcinogenic process.

It has been shown in in vitro studies of cell lines and also in vivo after PH that selenium inhibits regenerative cell proliferation at doses corresponding to 6 p.p.m. in the diet (4). At the dose of 5 p.p.m. in the drinking water selenium allowed an earlier recovery from 2-AAF mitoinhibition. Selenium contributes to reduce the toxicity of 2-AAF during promotion and thereby reduce the time of selection of the resistant initiated hepatocytes. In the presence of a toxic promoter selenium obviously contributes to the cellular defence.

Selenium treatment starting after initiation and promotion and covering only the progression phase of the process also significantly reduced the risk of development of liver cancer, although without the ability to eliminate the risk. During progression no toxic treatments are given and the effect of selenium is harder to explain. One important finding in the work is that the background cell proliferation in the surrounding hepatocytes is lower in the presence of added selenium than in the group requiring pure water.

The selenoenzyme GPx (cytosolic) was not altered by selenium treatment. Interestingly TrxR1, another selenoenzyme, is increased in liver nodules 3-4-fold over background activity (13). Selenite treatment reduced this enzyme over-expression by 50% in 25 200 g supernatants from total liver homogenates at both selenium doses used. As immunohistochemical analysis revealed that there was a clear over-expression of TrxR1 also in these nodules compared with the control tissue, the decrease in enzyme activities may at least in part reflect the lower volume fraction of nodules after selenium treatment.

Immunohistochemical investigation clearly shows a high and exclusive expression of TrxR1 in liver nodules under the influence of the promoter and during the period of rapid cell proliferation. During the progression phase the TrxR1 expression in the nodules corresponded to the level of the surrounding tissue. However, rapidly growing lesions, that is persistent liver nodules and hepatomas, did express high levels of TrxR1. The results support the idea that TrxR1 is expressed as a component of the resistant phenotype in growing cells under toxic selective pressure. It is intriguing that TrxR1 also seems to be a marker for neoplastic lesions with a high risk of malignant transformation during the progression phase or a marker for hepatocellular carcinoma. Future studies will show the value of this marker in clinical practice as a predictor of neoplastic transformation in chronic liver disease.

Doxorubicin-resistant neoplastic lung cancer cells have recently been shown to exhibit an increased sensitivity to toxic doses of selenium (25,26). In these cells selenium was not able to further induce TrxR1, which resulted in cell death upon exposure of selenium. If this is true also for the resistant cells in the liver tumor model an interesting new possibility for successful treatment of resistant neoplasia appears.

Previously published data suggesting that selenoproteins do not play a major role in the preventive effects of selenium support our data concerning the low effects of selenium on selenoprotein levels (1,11). Therefore, other mechanisms must also be considered. Of special interest is the ability of low-molecular-weight selenium compounds to redox-regulate proteins and transcription factors important for cell growth and proliferation. This can be exemplified by the inhibition of AP-1 DNA binding (27), inhibition of human Trx and ribonucleotide reductase (28,29).

Selenium-mediated redox regulation of p53 and activation of the DNA-repair branch of the p53 pathway has been reported (10). As selenite treatment during the initiation phase, when the first critical DNA-damage occurs, did not have any effect, this suggested mechanism does not seem to be of major importance in our model studied here. Other, more possible mechanisms include specific induction of apoptosis in pre-neoplastic/neoplastic lesions thereby preventing the development of manifest tumors (1,11). Both caspase-dependent and -independent selenite-mediated apoptosis have been reported (26,30) and seems to be associated with the inhibition of several important intracellular signalling pathways (31).

In the work of Mukherjee et al. it is shown that selenomethionine does reduce lipid peroxidation in rat liver from rats fed 2-AAF (4). Their data also indicate an effect on glutathione...
metabolism and maintenance of high intracellular glutathione levels in both nodular lesions and surrounding liver parenchyma upon selenium administration during long-term 2-AAF feeding. GPx was the only selenium-dependent enzyme investigated in these studies and this enzyme was unaffected or slightly increased by 2-AAF as well as by selenomethionine treatment. No significant differences were shown between the focal lesions and the surrounding parenchyma. Interestingly, glutathione reductase was decreased by selenium supplementation of carcinogen-treated animals compared with those treated with only carcinogen. This alteration was seen in focal lesions as well as in the surrounding parenchyma and was opposed to the alterations in glutathione levels. Superoxide dismutase activity was decreased and catalase activity was increased in 2-AAF-induced liver nodules and surrounding parenchyma compared with normal liver. Selenium treatment restored the superoxide dismutase activity to normal levels, but did not affect catalase. The results support the general hypothesis of the importance of free radical formation and oxidative stress in carcinogenesis and that selenium could act by increasing the cellular defence against free radicals.

In conclusion, we have collected data from a sequential animal model of experimental hepatocarcinogenesis showing that sodium selenite is able to reduce the risk for cancer development in the liver. This potential is clear in promotion as well as in progression phases of the carcinogenic process, but selenium treatment during the initiation phase did not have any effect. The tumor preventive effects of selenium in the different steps of carcinogenesis have not been reported previously. The selenium effect differs in the pre-neoplastic or neoplastic lesions compared with the surrounding parenchyma.

Our data support previous results from other systems, that selenium is a potent cancer preventive agent. Future studies will focus on underlying molecular mechanisms.

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