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Sodium Selenite, Dietary Micronutrient, Prevents the Lymphocyte DNA Damage Induced by *N*-Nitrosodiethylamine and Phenobarbital Promoted Experimental Hepatocarcinogenesis

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Abstract Selenium (Se), a micronutrient, has a long history in chemoprevention of mammary and colon cancers in rodent models. Se is a current clinical trial, having shown promise in prevention of prostate and other human cancers. The mechanisms involved in the in vivo anti-carcinogenic activity of Se remain to be elucidated. In the present study, we examined the effect of sodium selenite supplementation in lymphocytes, obtained from hepatoma bearing rats on DNA damage in correlation with oxidative stress. In addition, this study examined the supplementation of Se at 4-ppm levels in the form of sodium selenite either before initiation or during initiation and/or promotion phase's increases lymphocyte Se concentrations. This in turn improves lymphocyte resistance to oxidative stress and protection against the lymphocytes DNA damage. Supplementation of Se increased lymphocyte Se concentration and reduced lymphocytes DNA damage as determined by single cell gel electrophoresis. The enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase, and catalase were found to be decreased while the thiobarbituric acid reactive substances level was increased in the lymphocytes of hepatoma bearing rats. Furthermore, the reactive oxygen species such as superoxide radicals and hydroxyl radicals were also found to be high in lymphocytes. Our present results explain the understanding of unique association between anti-peroxidative effect of Se and ultimately the capability of Se to prevent cancer. J. Cell. Biochem. 88: 578–588, 2003.

Key words: *N*-nitrosodiethylamine; comet; DNA damage; selenite; oxidative stress; hepatoma

There has been an increasing concern in recent years about the role of dietary supplements in relation to human health. Although research has focused mainly on the action of single compounds, there is still a lack of definitive evidence on their mechanisms of action.

Selenium is an important dietary anti-carcinogen. Se intake and plasma levels have been reported to correlate inversely with cancer mortality [Clark et al., 1996]. Laboratory investigations also reveal that Se supplementation can substantially reduce cancer risk in animals treated with various chemical carcinogens [Jacobs, 1983; Combs and Williams, 1998; Thirunavukkarasu et al., 2001]. The anti-carcinogenic effects of Se are not limited to a specific carcinogen, or to a specific tissue, suggesting a general metabolic phenomenon [Combs and Williams, 1998]. Although the exact mechanisms for its anti-carcinogenic activity remain to be elucidated, it is widely believed that multiple pathways are involved [Sunde, 1990; El-Bayoumy, 2001]. These include the role of Se on carcinogen metabolism, its effect on the immune system [Jacobs, 1983; Cor, 1984; Ip et al., 1991; Taiq and Preiss, 1992; El-Bayoumy, 2001], as well as induction of programmed cell death in cancer cells [Behne et al., 1996]. However, the in vivo anticarcinogenic activity in cancer bearing animals remains unclear.

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One of the most important mechanisms related to the anti-carcinogenic effects of Se is believed to be its potent cytotoxicity, as observed in various types of malignant cells [Lanfear et al., 1994; Shen et al., 2000]. On the other hand in vivo studies clearly indicate that the anti-cancer property of Se is not directly related to the cytotoxic effect of Se but specific biomolecules associated with them [Combs and Williams, 1998; Ip, 1998; El-Bayoumy, 2001; Thirunavukkarasu et al., 2001]. Therefore, it is essential to determine which types of Se compounds provide optimal protection against cancer with the least toxicity. Inorganic Se compounds appear to cause distinctly different cellular effects from those elicited by organic forms of Se in vitro and in vivo in pre-clinical and clinical investigations [Thompson et al., 1994]. Se exerts many of its biological actions through the expression of specific selenoproteins in which Se is present as specific selenocysteine residues encoded by a TGA triplet [Rafferty et al., 1998]. Insertion of Se at these TGA-directed sites requires Se to be present in a chemically active form similar to selenide, and current evidence indicates that selenite is a more potent precursor of selenide than selenomethionine [Patterson et al., 1989; Ganther, 1999; El-Bayoumy, 2001]. Further evidence for the increased bioactivity of selenite over selenomethionine comes from the observation that selenite, but not selenomethionine, can be converted within the cell into selenodiglutathione, a form of Se, which appears to regulate the apoptosis [Lanfear et al., 1994; Wu et al., 1995]. So, in the present study we used Se in the form of sodium selenite.

Hepatocarcinogenesis can be induced by various procarcinogens, for example, *N*-nitrosodiethylamine (DEN), 2-acetylaminofluorene, and aflatoxin B_1, is an intricate phenomenon and a complex process involving multiple stages [Coker et al., 1991]. Chemically induced rat liver carcinogenesis has been considered as one of the best-known experimental models of carcinogenesis, allowing screening of potential compounds on different phases (e.g., initiation and promotion) of neoplastic development. In the present study, we selected DEN to induce the liver cancer because of the simple metabolic pathways involved and its potent carcinogenic activity [Yoshiji et al., 1991].

DNA damage has been recognized as the onset of many diseases, including cancer and could be a useful biomarker of the oxidative status and antioxidant defense system of the organism [Sun, 1990]. Several methods exist to study DNA damage. A relatively simple technique called single cell gel electrophoresis (EP) (comet assay) has been developed to evaluate DNA damage (specifically single strand breaks and alkaline-labile sites) in any eukaryotic cell population. This method has been mainly applied to quantify DNA damage and DNA repair capacity subsequent to UV ionizing radiation and oxidative damage in vitro models [Dizdaroglu, 1991; Hegler et al., 1993; Dhawan et al., 2001].

As part of larger project, we already reported that supplementation Se decreased thiobarbituric acid reactive substances (TBARS) and increased antioxidants enzymes in erythrocytes of hepatoma bearing rats [Thirunavukkarasu and Sakthisekaran, 2001]. In the present study, we examined the effect of Se exposing on DNA damage in lymphocytes obtained from hepatoma bearing rats. The interest in lymphocytes is due, not only to the fact that they are considered to be a good model to reflect actual state of the body but also serve as a reliable model for studying the effect of the addition of specific antioxidants to the diet [Riso et al., 1999].

MATERIALS AND METHODS

Animals and Diet

Male, Wistar strain albino rats, of age 6 weeks were used in these experiments. The rats were procured from Tamil Nadu Veterinary College, Chennai, India. They were fed with normal rat chow marketed by M/s. Hindustan Lever Limited, Mumbai, India and were provided with clean drinking water ad libitum. The rat chow used to feed our experimental animals contains 0.1 ppm Se, which is believed to satisfy the normal requirement of rats [Newberne et al., 1978].

Chemicals and Their Sources

The following were purchased from the indicated sources: DEN, phenobarbital (PB), bovine serum albumin, and sodium selenite (Sigma Chemical Co., St. Louis, MO). All other chemicals, including solvent, used were of high purity and of analytical grade marketed by SD Fine Chemicals, Mumbai and Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Experimental Design

Animals were divided into eight groups. Each group consists of six animals. Liver tumors were induced in groups 2, 3, 5, and 7 with a single intraperitoneal injection of DEN at a dose of 200-mg/kg-body weight, at the age of 10 weeks. Two weeks after DEN administration, the carcinogenic effect was promoted by PB (0.05%). The promoter was supplemented to the experimental animals through rat chow up to 14 successive weeks [Yoshiji et al., 1991; Thirunavukkarasu et al., 2001].

Group 1: Normal control animals received normal rat chow.
Group 2: Hepatoma induced animals [single intraperitoneal injection of DEN, (200 mg/kg body wt.) in saline at ten weeks of age. Two weeks after the administration DEN, PB (0.05%) was administrated as the promoter of carcinogenesis. The promoter was incorporated into the rat chow upto 14 successive weeks].
Group 3: Hepatoma induced animals (as in group 2) treated with sodium selenite (4 ppm through drinking water) throughout the study. Sodium selenite was supplemented 20 weeks, i.e., 4 weeks before the administration of DEN and 16 weeks after the administration of DEN.
Group 4: Control animals treated with sodium selenite alone (as in group 3, throughout the study).
Group 5: Hepatoma induced animals (as in group 2) pretreated with sodium selenite for 4 weeks before administration of DEN.
Group 6: Control animals treated with sodium selenite alone (for 4 weeks as in group 5).
Group 7: Hepatoma induced animals (as in group 2) post treated with sodium selenite (sodium selenite was supplemented 14 weeks in the promotion phase).
Group 8: Control animals treated with sodium selenite alone (for 14 weeks as in group 7) [details, Thirunavukkarasu et al., 2001].

The experiments were terminated 16 weeks after DEN administration. All the experimental animals fasted overnight and were killed by cervical decapitation, 20 weeks after the initiation of the experiment. Blood was collected in tubes; tubes containing EDTA as the anticoagulant, lymphocytes were separated out.

Lymphocytes Separation

Lymphocytes were recovered from whole blood by means of a density gradient separation with histopaque 1077. In detail, 10 ml (70 μl) of whole blood (kept on ice after the drawing and processed within 15 min) were gently mixed with 110 ml (900 μl) of cold RPM1 1640 medium supplemented with 10% fetal calf serum in micro tubes. Ten milliliters (100 μl) of histopaque 1077 were carefully under layered. The samples were then centrifuged at 200*g* for 4 min at 4°C and about 10 ml (100 μl) of the middle/top histopaque layer drawn and added to 100 ml (1 ml) of PBS (Ca^{2+}, Mg^{2+} free) to wash the cells. After being washed, the lymphocytes were recovered, resuspended in PBS, and counted by hemocytometer. Adding 1% triton and quickly freezing and defrosting the samples, cell membrane lysis was performed.

Comet Assays

Comet assay was performed by the method of Dhawan et al. [2001] with slight modifications [Singh et al., 1988]. Lysis solution (without sodium sarcosinate and with 10% DMSO—freshly prepared), Tris-HCl neutralization (0.4 M, pH 7.5) buffer and EP buffer (300 mM NaOH, 1 mM EDTA) were prepared as described by Singh et al. [1988]. In brief, the cells were suspended in 110 μl of low melting point agarose (0.65% LMPA-w/v in PBS, pH 7.4) and pipetted onto a frosted glass microscope slide pre-coated with 140 μl of 1% normal melting point agarose (NMPA) (in PBS, pH 7.4). The agarose was allowed to set for 10 min at 4°C and thereafter, the cover slip was removed and the slides were exposed for 24 h to lysis solution. Finally, the slides were rinsed with distilled water and EP buffer to remove salts. These slides were exposed to alkaline EP buffer (pH 13.0) for 40 min, and subjected to EP for 20 min (300 mA, 25 mV). Then the alkali was neutralized with Tris-HCl buffer; the slides rinsed with distilled water and methanol, and were stained with ethidium bromide.

Slide Scoring

Slides were scored using nebug, an image analysis system attached to a fluorescence microscope equipped with appropriate filters. The microscope was connected to a computer through a charge coupled device (CCD) camera to transport images to software for analysis. The final magnification was $\times 400$, the parameters taken for the lymphocytes were: tail DNA (%), tail length (migration of the DNA away from the nucleus, μm), and tail moment (arbitrary units). Images from 100 cells (50 each replicate slide/10 randomly selected different field) were analyzed.

Biochemical Investigations

Se concentration in lymphocytes was determined by the fluorometric method of Olson et al. [1975]. TBARS were measured by the method of Hogberg et al. [1974]. The enzymatic antioxidants, glutathione peroxidase (GPx) was determined according to the method of Rotruck et al. [1973], superoxide dismutase (SOD) by Marklund and Marklund [1974], and catalase (CAT) by the method of Sinha [1972]. Superoxides were estimated by the method of Nishikimi et al. [1972] in lymphocytes in which SOD was inhibited with diethyldithio carbamide. Hydroxyl radicals were estimated by the method of Gutteridge [1981].

Statistical Analysis

Statistically significant ($P < 0.05$) differences between different groups were done using ANOVA and Student's t-test. Each value in the results section represents two-way significance tests, i.e., b, represents significance against group 2 DEN-control and a, c, d, and e represent the same against their respective controls (groups 1, 4, 6, and 8).

RESULTS

In our previous findings we observed that supplementation of Se in the form of sodium selenite significantly reduced the tumor incidence 63.3, 56.8, and 33.3% in group 3, group 5, and group 7 respectively. This effect was consistent in terms of nodular volume, etc., we also observed, no change in food and water intake between the experimental groups during the experimental period. We did not observe any toxic symptoms between the experimental groups during the experimental period. The non-toxic symptoms of Se were also confirmed by histological studies [Thirunavukkarasu et al., 2000, 2001].

The observed tail DNA, tail length, and tail moment in hepatoma bearing and Se treated animals were compared with their respective control animals. Initiation of hepatoma with DEN and promotion with PB resulted in considerable DNA damage in lymphocytes. However, control animals treated with Se alone (group 4, group 6, and group 8), did not show any statistically significant DNA damage as evident from Table I and Figure 1. Se supplementation before initiation and/or during initiation and during promotion stages of hepatoma bearing animals results in decrease of DNA damage in lymphocytes. However, the highly significant level was observed in group 3 where Se was supplemented for 20 weeks, i.e., before initiation to end of the experiment. When Se was supplemented only during initiation or promotion, a significant reduction in the DNA damage was observed in hepatoma bearing animals. In contrast, the DNA damage provoked in the

TABLE I. Tail DNA, Tail Length, and Tail Moment in Control and Experimental Groups (for Details See Materials and Methods)

Particulars	Tail DNA (%)	Tail length (mm)	Tail moment (mm)
Group 1	$7.65 + 0.64$	$13.53 + 0.98$	$0.84 + 0.06$
Group 2	$24.98 + 3.01a^{\#}$	$44.65 + 6.48a^{\#}$	$6.46 + 0.69a^{\#}$
Group 3	$10.68 + 1.42b^{\#}c^{*}$	$1.39 + 1.96b^{\#}c^{*}$	$1.84 + 0.09b^{\#}c^{NS}$
Group 4	$7.84 + 0.98$	$12.96 + 1.21$	$0.88 + 0.06$
Group 5	$16.41 + 2.01b^{@}d^{@}$	$29.34 + 3.54b^{\#}d^{@}$	$2.63 + 0.14b^{\#}d^{@}$
Group 6	$6.98 + 0.56$	$12.64 + 0.91$	$0.90 + 0.07$
Group 7	$20.01 + 1.18b^{*}e^{\#}$	$33.08 + 1.84b^{*}e^{\#}$	$0.90 + 0.07b^{*}e^{\#}$
Group 8	$7.48 + 0.83$	$12.68 + 0.84$	$0.90 + 0.06$

Groups were treated as in Materials and Methods; each value represents mean ± SD; a as compared with group 1; b as compared with group 2; c as compared with group 4; d as compared with group 6; e as compared with group 8 ($^{*}P < 0.05$, $^{@}P < 0.01$, $^{\#}P < 0.001$, NS—not statistically significant).

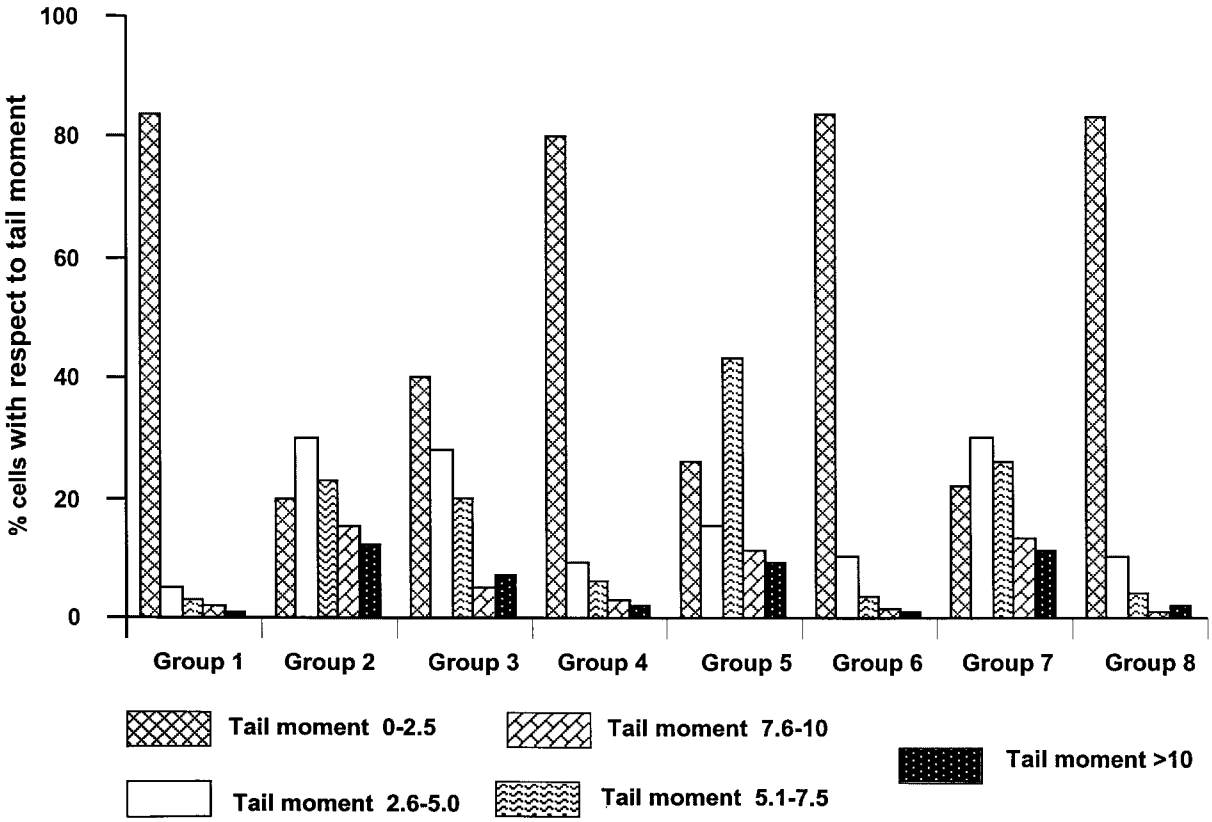

Fig. 1. Percent distribution of cells with respect to tail moment (μm) in control and experimental groups (for details see Materials and Methods).

hepatoma bearing animals was prevented, in a time of Se supplementation rather than duration-dependent fashion.

Figure 2 shows the Se concentration in lymphocytes of control and experimental groups. Se concentration was found to be significantly lowered ($P < 0.001$) in hepatoma bearing animals (group 2) when compared to control (group 1) animals. Upon Se supplementation, its concentration was found to be increased in group 3 ($P < 0.001$), group 5 ($P < 0.05$), and group 7 ($P < 0.001$) animals in duration dependent manner compared to group 2 animals. However, when group 3, group 5, and group 7 animals were compared with their respective control groups Se concentration was found to be lowered in group 5 ($P < 0.01$) and group 7 ($P < 0.05$) animals, in contrast there was no statistical significance in group 3 animals.

Figures 3 and 4 represent the level of TBARS, hydroxyl radical, superoxide radical, GPx, SOD, and CAT in lymphocytes of control and experimental animals. In hepatoma bearing (group 2) animals, the status of TBARS, hydroxyl radical, superoxide radical were significantly ($P < 0.001$) increased whereas the activities of GPx, SOD, and CAT were significantly ($P < 0.001$) decreased compared with group 1 control animals. Se supplementation throughout the study (group 3 animals) shows significant ($P < 0.001$) decrease in the status of TBARS, hydroxyl radical, and superoxide radical, on the other hand activities of GPx, SOD, and CAT were found to be increased ($P < 0.001$) compared with group 2 animals. Group 3 animals when compared with their pair-fed (group 4) animals, the levels of TBARS ($P < 0.05$), hydroxyl radical ($P < 0.001$), superoxide radical ($P < 0.01$) were increased whereas the activities of GPx, SOD, and CAT did not show any statistical difference.

Significantly lowered levels of TBARS ($P < 0.01$), hydroxyl radical, and superoxide radical ($P < 0.001$) and increased ($P < 0.01$) activities of GPx, SOD, & CAT were observed in group 5 as compared with group 2 rats. On the other hand, group 5 animals when compared with their pair fed (group 6) animals, the levels of TBARS, ($P < 0.01$) hydroxyl radical, and superoxide

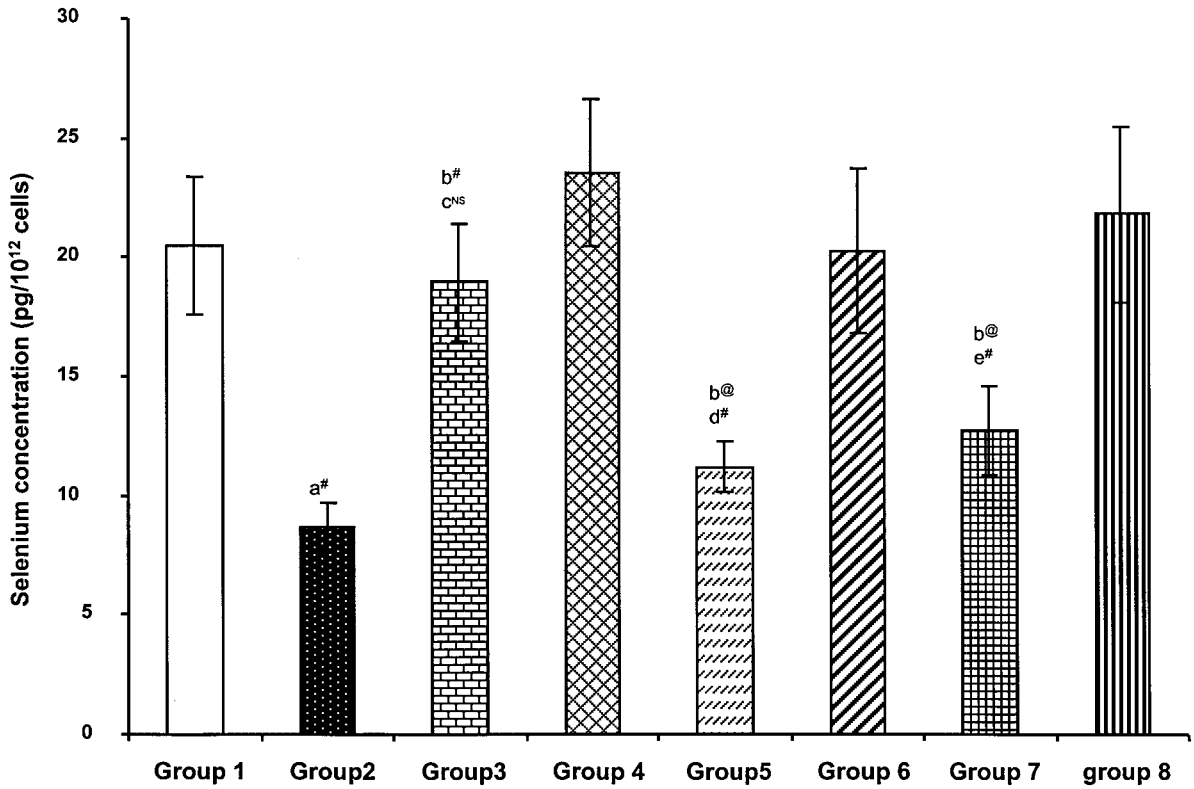

Fig. 2. Selenium concentration in control and experimental groups (for details see Materials and Methods). Groups were treated as in Materials and Methods; each value represents mean ± SD; a as compared with group 1; b as compared with group 2; c as compared with group 4; d as compared with group 6; e as compared with group 8 ($^{*}P < 0.05$, $^{@}P < 0.01$, $^{\#}P < 0.001$, NS—not statistically significant).

radical ($P < 0.001$) were increased and the activities of GPx ($P < 0.05$), SOD ($P < 0.01$), & CAT ($P < 0.05$) were found to be lowered. Se post-treated group 7 animals also showed a decreased levels of TBARS ($P < 0.05$), hydroxyl radical ($P < 0.001$), superoxide radical ($P < 0.01$), and increased activities of GPx ($P < 0.01$), SOD ($P < 0.05$), & CAT ($P < 0.05$) when compared with group 2 animals. Moreover, group 7 animals when compared with their pair-fed (group 8) animals the levels of TBARS ($P < 0.01$), hydroxyl radical, and super oxide radical ($P < 0.001$) were found to be higher and the activities of GPx ($P < 0.01$), SOD ($P < 0.001$), and CAT ($P < 0.01$) were found to be deceased.

DISCUSSION

The role of oxidative stress in the development of some chronic diseases including cancer has been widely reported in literature [Diplock, 1991; Ames et al., 1993; Davies, 1995]. Furthermore, it was demonstrated that antioxidant enzymes, the main scavengers of free radicals, are altered during carcinogenesis or after tumor formation [Ames et al., 1993; Thirunavukkarasu and Sakthisekaran, 2001]. The mechanism and implication of this abnormality, however is still unclear [Scholz et al., 1990; Nakae et al., 1997]. These studies clearly indicate that cancer cells/mutant cells are responsible for oxidative stress in cancer bearing host. However, many different variables, such as, the type of cell and its state, the general condition of the organism and numerous physiological, environmental, and dietary factors affect the oxidative stress [Anderson et al., 1994]. So, researchers are viewing more attention using the antioxidant substances in cancer chemotherapy or adjuvant chemotherapy. Those compounds present in food substances; easily available, acting as anticancer and also antioxidant were given more attention. One such compound is Se; Se in the form of sodium selenite is known to play a vital role.

A marked increase in the DNA damage and elevated levels of hydroxyl radical, superoxide radical, and TBARS were observed in the

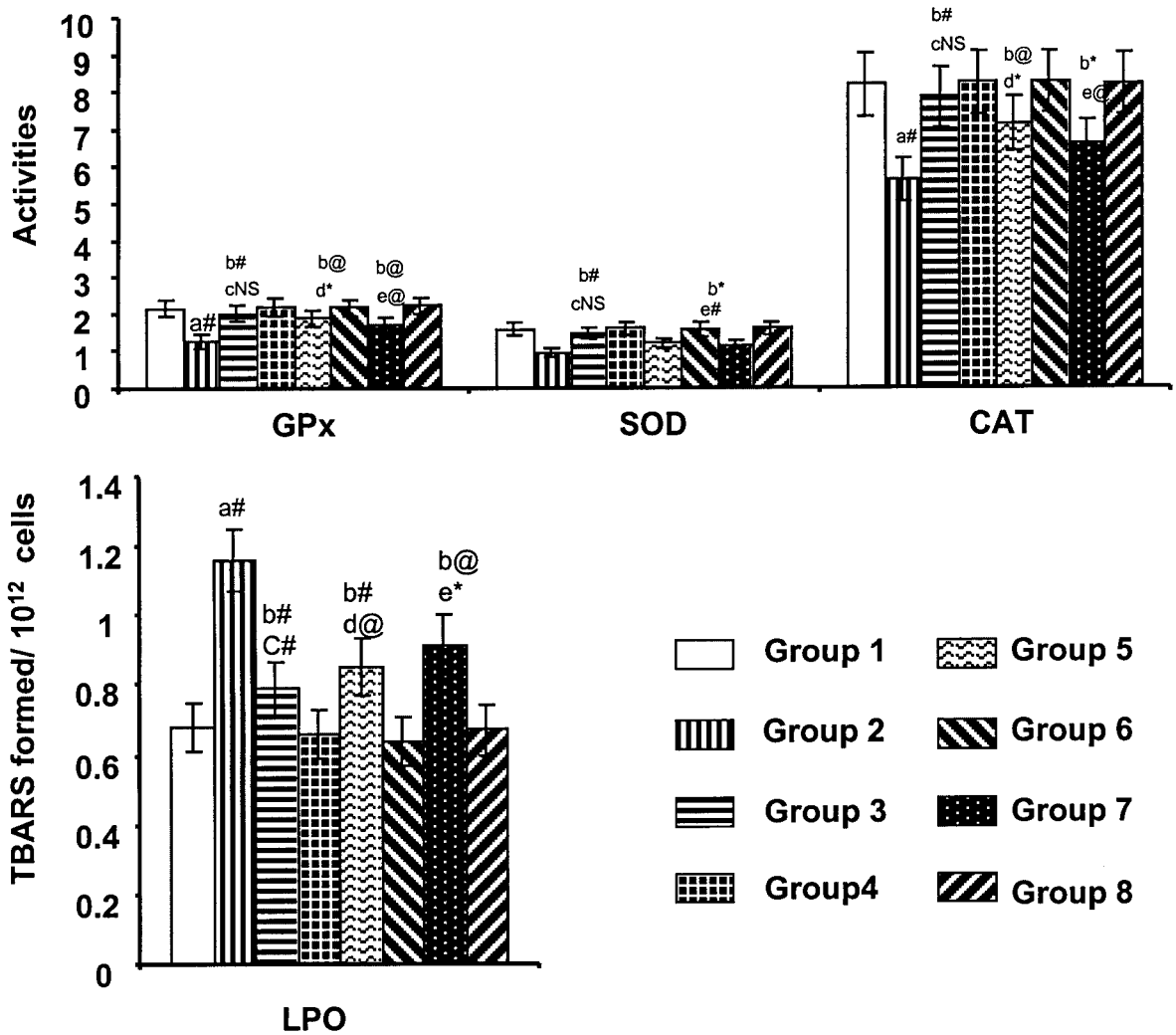

Fig. 3. Levels of lipid peroxidation, and activities of some enzymic antioxidants in lymphocytes of control and experimental groups. Groups were treated as in Materials and Methods; each value represents mean ± SD; values are expressed as follows; GPx, ng of GSH utilized/10^{12} cells; SOD, units/10^{12} cells; CAT, nmoles of H_2O_2 utilized/10^{12} cells; a as compared with group 1; b as compared with group 2; c as compared with group 4; d as compared with group 6; e as compared with group 8 ($^{*}P < 0.05$, $^{@}P < 0.01$, $^{\#}P < 0.001$, NS—not statistically significant).

lymphocytes of carcinoma bearing rats. The increased level of TBARS in lymphocytes of carcinoma bearing animals may be due to uncompromised production of reactive oxygen species in tumor site [Salim, 1993; Diplock et al., 1994]. We also observed increased levels of TBARS and decreased antioxidant enzyme activities in various organs of hepatoma-bearing rats [Thirunavukkarasu and Sakthisekaran, 2001]. The observed increase susceptibility of the lymphocytes to DNA damage in carcinoma animals may be due to that effect. In the present study, we also observed increased levels of superoxide and hydroxyl radicals in lymphocytes of hepatoma bearing animals.

Reduced Se levels in various organs of tumor bearing animals and serum were reported by various authors [Werner, 1999; Yu et al., 1999; Brown and Arthur, 2001] and also by us [Thirunavukkarasu et al., 2000]. In the present study, we observed the decreased levels of lymphocyte Se concentration in cancer bearing animals. Around 65% of dietary Se in the rats is thought to be associated with liver [Ryszka et al., 1994] and the cause of hepatic Se deficiency in the hepatoma animals. Se is incorporated into polypeptide in the form of amino acid selenocysteine [Stadtman, 1996] and it is possible that a disruption in selenocysteine synthesis or an inhibition into proteins, would lead

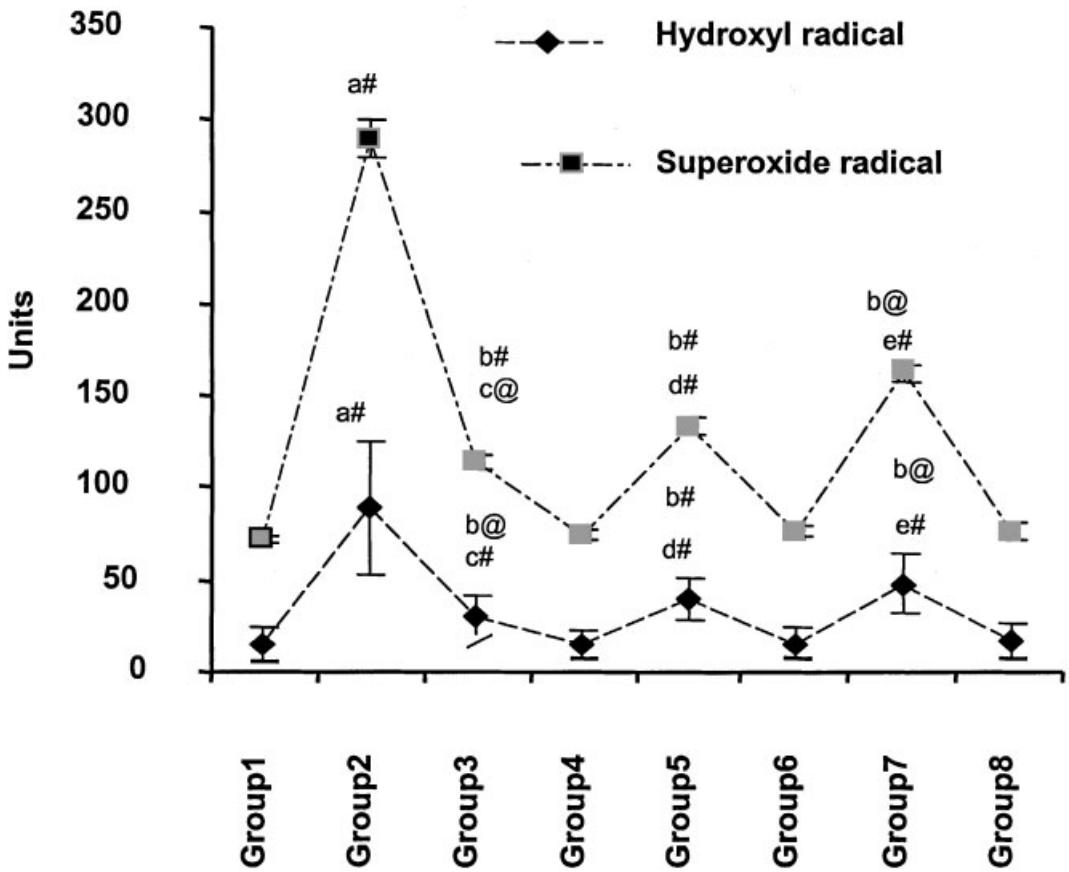

Fig. 4. Levels of superoxide radical, hydroxyl radical in lymphocytes of control and experimental groups. Groups were treated as in Materials and Methods; each value represents mean ± SD; values are expressed as follows: hydroxyl radical, nmoles of MDA/10^{12} cells; superoxide radical, nmoles of NBT reduced/10^{12} cells; a as compared with group 1; b as compared with group 2; c as compared with group 4; d as compared with group 6; e as compared with group 8 ($^{*}P < 0.05$, $^{@}P < 0.01$, $^{\#}P < 0.001$, NS—not statistically significant).

to a deficiency in cellular Se. Decreased Se concentration directly correlates with increased DNA damage observed in the present study.

Se deficiency in animals is associated with reduced weight gain and alterations in P450 dependent drug metabolism, which are the common symptoms in cancer patients and also cancer bearing animals. In previous studies we observed these symptoms in hepatoma bearing animals [Thirunavukkarasu et al., 2000]. Moreover, Se deficiency has been shown to impair the conversion of the pro-hormone thyroxin (T4) to 3,3,5′-tri-iodothyronine (T3), by disabling the selenoenzymes growth and developments [Arthur et al., 1990]. It is noteworthy that growth hormone deficiency and hypoparathyroidism have been reported in cancer patients [Grieve et al., 1983]. These phenomena have been attributed to the secondary effect of the disturbed Se metabolism. The results suggest that the demand for the Se is increased, in cancer condition by at least certain levels.

We have further shown reduced levels of antioxidant enzymes in lymphocytes of hepatoma bearing animals. Se status does not influence transcription of GPx, but it has been shown that Se deficiency in rats can reduce the steady state level of GPx RNA, suggesting that Se stabilizes GPx RNA prior to translation [Hill et al., 1992]. GPx has been demonstrated to be absolutely necessary for cellular survival under normoxic conditions in vitro, with a 19% inhibition of GPx activity effecting a 50% reduction in cell survival [Michiels et al., 1994]. In the presence of glutathione, the enzyme catalyses the reduction of lipid and non-lipid peroxides through the oxidation of two molecules of GSH and has been found to be 2,200 times more effective than Cu, Zn-SOD, and 14 times more effective than CAT; unlike those able to inhibit

the peroxidation of cellular membranes [Michiels et al., 1994]. GPx deficiencies have been reported in liver cancer animals and humans [Sung et al., 1999]. It has been noted that hepatoma-bearing rats develop reactive oxygen species, and it is known that the elevation of ROS can be the consequence of sustained oxidative damage. As such, the Se deficiency, and the resulting impairment in GPx activity could contribute to the production of these ROS. It should be noted in this study that function of GPx in blood cell is to reduce hydrogen peroxide and organic hydroperoxide, not phospholipid hydroperoxide. So observed decreased activity of GPx can directly correlate with increased levels of superoxide and hydroxyl radical observed in the present study.

DNA damage observed in lymphocytes (group 2) might be a secondary effect of hepatoma bearing host. Supplementation of Se throughout the study (20 weeks) and pretreatment (4 weeks) has shown to be more effective than post treatment (16 weeks). This may be possible that Se supplementation above nutritional level can increase various selenoproteins [Thompson et al., 1994; Behne et al., 1996; Thirunavukkarasu et al., 2002], which in turn may reduce carcinogen–DNA interaction [Swenberg et al., 1991]. This is possible in the present study because DEN is a procarcinogen [Ames et al., 1993]. Metabolic activation of this chemical carcinogen to ultimate carcinogens requires specific enzymes and cofactors, and it is possible that selenoenzymes (or) Se metabolites can affect initiation of carcinogenesis [Cor, 1984; Ip et al., 1991; Lanfear et al., 1994]. Increased levels of GSH, which in turn may reduce the carcinogen and DNA interaction by providing large nucleophilic pool for electrophilic carcinogen [Swenberg et al., 1991]. DEN is also one of the electrophilic carcinogens [Bartsch et al., 1989]. The positive effect of Se on post-treatment also cannot be ruled out.

The DNA damage observed in untreated lymphocytes (group 2) seems to indicate the strand breaks of endogenous oxidative damage produced from carcinogen exposure. We also observed some damage in normal drug control animals and this could depend on the fact that a certain DNA damage is always present in the cell and that strand breaks occur as intermediates in the preparation process in which specialized enzymes recognize abnormalities and remove them by excision, re-synthesis, and rejoining of DNA strands [Riso et al., 1999]. When the animals were treated with carcinogens like DEN, the damage is worsened. In this condition it is possible to evaluate the potentiality of a cell to protect itself against an oxidative stress. This potential depends on the total antioxidant capacity of the cell, which could be sensitive to the antioxidant substances when introduced with diet.

In summary, lymphocytes Se depletion may result in significant impairment of cellular antioxidative defense and increases the susceptibility of lymphocytes to oxidative stress. This unique association between oxidative stress induced in cancer bearing host and Se supplementation may help us to understand the chemopreventive ability of selenite against cancer. Considering Se as a prophylactic antitumor agent, a moderately high level (4 ppm) of Se should be supplied for the replenishment of the metabolic pool of Se.

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