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L-Selenomethionine Does not Protect Against Testosterone Plus 17 β -Estradiol-Induced Oxidative Stress and Pre-Neoplastic Lesions in the Prostate of NBL Rats

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Abstract

Previous animal studies examining dietary selenium effects on prostatic carcinogenesis did not show preventive benefit, including one study in a rat model involving testosterone (T) and estradiol (E2)-induced prostatic oxidative stress. Here, we examined modulation of T+E2-induced prostatic oxidative stress, dysplasia, and inflammation by L-selenomethionine at 1.5 or 3.0 mg selenium/kg in NIH-07 diet in Nbl/Crl rats treated with T+E2 for 16 weeks. Hormone treatment increased immunohistochemical staining for 8-hydroxydeoxyguanosine (8-OHdG) in the prostatic sites of T+E2-induced preneoplasia ($p < 0.05$), but selenomethionine did not attenuate 8-OHdG staining and dysplasia in the lateral prostate. Glutathione-peroxidase activity and mRNA expression were induced by T+E2 ($p < 0.05$ – $p < 0.0001$) but not changed by selenomethionine. Selenomethionine did not cause significant responses in expression and activity of glutathione-peroxidase and MnSOD, except for a reduction of MnSOD protein expression in the lateral prostate ($p < 0.01$). The absence of reduction of oxidative stress and dysplasia and the minimal effects on antioxidant enzymes caused by selenomethionine are consistent with the null effects observed in selenium supplementation animal studies and clinical trials. Significant ($p < 0.01$) opposite apoptosis/cell proliferation balance responses to selenomethionine and to T+E2 occurred in the lateral and dorsal prostate, explaining why T+E2 induces lesions selectively in the lateral lobe of NBL rats.

INTRODUCTION

Prostate cancer is the most common noncutaneous malignancy and one of the major causes of cancer-related death in men in the western world [1]. No risk factors offer opportunities for primary prevention of this malignancy [2]. Chemoprevention, i.e., the inhibition or reversal of the development or progression of cancer by chemicals, bioactive plant compounds, or dietary components, is a mechanism-based, attractive, and potentially powerful approach to prostate cancer prevention [2]. Inflammatory processes known to involve oxidative damage are suspected to be associated with human prostate cancer [3, 4] and the presence of the oxidized DNA base 8-hydroxy-2'-deoxyguanosine (8-OHdG) has been observed in human prostate tissue [5]. The use of selenium compounds has long been considered a promising approach to chemoprevention [6]. Although the mechanisms of

chemoprevention by selenium are not precisely understood, several theories have emerged. Among these, selenium metabolites have been postulated to selectively promote apoptosis and inhibit proliferation of cancer cells. It has also been proposed that selenium supplementation of individuals with low selenium status may increase the expression of antioxidant selenoproteins and thereby prevents DNA damage that could lead to mutations and subsequent carcinogenesis [6–8].

Epidemiological studies of serum/plasma or toenail selenium levels have supported a protective role of selenium against prostate cancer (9), but studies examining selenium intake or use of selenium supplements in clinical trials or cohort or case-control studies did not [9–12]. Analysis of data from the Nutritional Prevention of Cancer clinical trial with selenized yeast in men at increased risk for skin cancer [13] yielded evidence to suggest that supplementation with this type of selenium in men with low selenium status at baseline may be protective against prostate cancer as a secondary endpoint [14]. However, three subsequent large randomized clinical trials of selenium supplementation with prostate cancer as the primary endpoint did not find preventive efficacy of selenium. Selenized yeast did not prevent prostate cancer in a phase III trial with men with elevated prostate-specific antigen levels or suspect rectal exam but negative prostate biopsies [15]. Selenomethionine (200 µg as selenium) was not protective in two large randomized clinical trials, the SELECT study in average risk men (alone or with α-tocopherol at 400 IU/day) [16] and the SWOG S9917 trial with high-risk men diagnosed with high grade prostatic intraepithelial neoplasia (PIN) [17]. Of note, none of these studies included an analysis stratifying participants by baseline selenium status as was previously carried out on the results of the Nutritional Prevention of Cancer clinical trial [14].

Previous studies with selenomethionine or selenized yeast in animal models of prostate carcinogenesis have been negative, including experiments with tumor induction models [18, 19]. Selenomethionine and most other selenium compounds also do not affect the growth of xenografted human prostate cancer cells in nude mice [20]. However, methylselenocysteine decreased the growth of Dunning R3327-H prostate tumors [21] and formation of prostate tumors in TRAMP mice [22]. These null findings may be due to the lack of involvement of oxidative stress mechanisms in the induction of prostate cancer in these models.

We recently found that selenomethionine supplementation did not prevent against 17β-estradiol plus testosterone (T+E2) -induced cancer and inflammation in the prostate of NBL (Noble) rats, a finding that was fully predictive of the outcome of the aforementioned clinical trials. This animal model is unique in that involves sex-hormone-induced oxidative stress mechanisms and prostatic inflammation [23–25]. The T+E2 treatment not only induces prostate cancer and preneoplasia in these rats, but also the formation of 8-OHdG, DNA strand breaks, lipid peroxidation, and reduction of antioxidant enzyme activity [26–29]. These effects are likely due to hydroxylation of E2 to a catechol-estrogen that is capable of redox-cycling, which can result in generation of reactive quinones that can adduct to DNA and cause depurination as well as formation of reactive oxygen species that can cause oxidative DNA damage and initiate lipid peroxidation [28, 30, 31].

The present study was designed to determine whether the lack of preventive effect of supplemental selenomethionine on prostate carcinogenesis in T+E2-treated NBL rats could be explained by absence of a reduction of oxidative stress and/or protection by antioxidant mechanisms in the prostate of the selenium-adequate NBL rat.

MATERIALS AND METHODS

Animals

Male NBL (Noble) rats (NBL/CrCrIBR) were obtained at 7 to 8 weeks of age from Charles River and held in quarantine for 2 weeks before the initiation of treatment. They were housed two to a cage in solid bottom cages and were fed the NIH-07 diet (Harlan Teklad, Madison, WI) during quarantine. The protocol for these experiments was approved by the Institutional Animal Care and Use Committee in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Hormone Treatment

After randomization into six experimental groups (see Study Conduct), the rats in groups to be subjected hormone treatment received two subcutaneous silastic tubing implants (internal diameter 0.078 inch; external diameter, 0.125 inch; Dow Corning, Midland, MI) tightly packed over a length of 2 cm with crystalline testosterone (Steraloids, Newport, RI) and another implant packed over a length of 1 cm with crystalline 17 β -estradiol (Steraloids); control rats received empty implants [32]. The implants were sealed with medical grade silicone sealant (RTV 108, General Electric, Momentive Performance Materials, Waterford, NY) placed on top of small glass beads inserted in the tubing after filling with hormone. Immediately before implantation, the implants were rinsed in 70% ethanol and then soaked for 48 h in sterile saline at 37°C, which was replaced twice daily. The Silastic implants were implanted under the skin between the scapulae while the rats were anesthetized with fentanyl-droperidol. This treatment increases circulating estradiol levels by 5- to 10-fold in NBL rats while maintaining testosterone at physiologic levels (~3 ng/mL) [33].

Chemoprevention Agent and Diet

L-selenomethionine (Sigma, St. Louis, MO) was mixed into the diet (NIH-07; Harlan Teklad) using a Blend-Master Model B Lab Blender (Patterson-Kelly, East Stroudsburg, PA). A vehicle of D(+) sucrose (Sigma) was used at a dietary concentration of 10 g/kg diet (1% w/w) to achieve final selenium concentrations of 1.5 or 3.0 mg/kg diet. The control diets contained the same amount of sucrose and 0.31 mg/kg selenium, while the selenium concentration in tap water provided to the animals varied between undetectable and 6.1 ppb. The diet concentrations and form of selenium were selected based on previous experiments with rat models of prostatic carcinogenesis [18, 23]. Furthermore, L-selenomethionine was used in the SELECT trial [34] and is regarded as the dominant form of selenium in human foods. The dose levels of selenomethionine were approximately 40% and 80%, respectively, of the maximally tolerated doses identified previously in selenium-adequate Wistar rats. These levels had been reported to increase mean plasma selenium levels in the rats by 9% and 17%, respectively [18].

Study Conduct

Sixty rats were randomized into treatment groups of 10 rats each. Groups 1 and 2 received a diet with no supplement; group 1 received empty implants and group 2 received T+E2-containing implants. Groups 3 and 4 received T+E2-containing implants and were fed a diet containing 1.5 (group 3) or 3.0 mg/kg selenium (group 4). Groups 5 and 6 were fed a diet containing 1.5 and 3.0 mg/kg selenium, respectively, but were not subjected to hormone treatment. Animals had *ad libitum* access to food and tap water and were started on experimental diets 1 week after receiving the hormone-containing implants. Experimental diets were prepared every 2 weeks and stored at 4°C; fresh food was provided to the animals on Mondays, Wednesdays, and Fridays. Animals were observed daily and weighed weekly for the first 2 months and monthly thereafter to assess their general health. Food intake per cage over 1 week was measured after the rats had been fed the experimental diets for 4, 8, and 12 weeks. All rats were euthanized by barbiturate overdose and exsanguination 16 weeks after the start of hormone treatment and necropsied immediately. For studies with histopathological end-points, half of each accessory sex gland complex was fixed and stored in a 4% neutral buffered formaldehyde solution. For studies with biochemical end-points, the other half of the prostate was micro-dissected and the individual lobes, as well as the seminal vesicle, were weighed, frozen in liquid nitrogen, and stored at –80°C.

Histopathologic Evaluation

Prostate lobes were dissected after fixation, processed, and embedded in a paraffin wax. A previously described orientation was used which allowed simultaneous examination of all lobes and their ducts, including the periurethral area where carcinomas develop in this model [35]. Four µm-thick sections were made and stained with H&E. Six step sections were made from the seminal vesicle-anterior prostate (300 µm intervals) as well as the dorsal, lateral and ventral prostate (250 µm intervals). This allowed for the detection of the maximum number of microscopic-sized lesions [35]. All prostate lobes and other accessory sex glands were evaluated histopathologically and the presence, type, and size of all lesions were scored using previously published criteria [32, 35]. Lesion severity scores were assigned in a blinded fashion by a single pathologist (MCB) as follows: 0 (no lesions present) and 1, 2, or 3 to indicate, respectively, minimal, slight, or moderate severity for dysplasia and slight, moderate, or marked severity for inflammation.

Tissue Lysate Preparation

Tissue was snap frozen in liquid nitrogen and homogenized using a Mikro Dismembrator II (Braun, Melsungen, Germany). Homogenized tissue was placed on ice and lysed for 30 min using 1X Cell Lysis Buffer (Cell Signaling, Danvers, MA) containing 1mM phenylmethylsulfonyl fluoride (PMSF) and 100 nM okadaic acid. The tissue was then sonicated and insoluble cell debris was removed by centrifugation at 15,000 × g at 4°C for 15 min. Protein concentrations of the cell lysates were quantified using the BioRad Protein Dye (BioRad, Hercules, CA). Cell lysates were stored at –80°C. All assays using lysates were done in triplicate, using three independently generated lysates from each sample.

Assessment of Glutathione Peroxidase-1 (GPx-1) Activity

GPx-1 activity in tissues was measured using the standard coupled spectrophotometric method as described by Samuels *et al.* [36]. Determination of GPx-1 activity was calculated based on the consumption of reduced NADPH measured spectrophotometrically by absorbance at 339 nm in a reaction containing reduced glutathione, glutathione reductase, cell lysate, and hydrogen peroxide. GPx-1 activity was expressed as nmol NADPH oxidized/min/mg protein.

Manganese Superoxide Dismutase (SOD2) Real-time RT-PCR Analysis

cDNA was synthesized from total RNA using the Applied Biosystems High Capacity cDNA Reverse Transcription Kit. Real-time PCR was performed using Taq Man® Gene Expression Assays on an Applied Biosystems (Carlsbad, CA) StepOnePlus Real-Time PCR System. β -Actin was chosen as the housekeeping gene for normalization. Threshold cycle numbers (Ct) were used for “Ct” analysis. The primers and probes (Integrated DNA Technologies, Coralville, IA) used were as follows: 5'-GGACAAACCTGAGCCCTAAG-3' (forward primer), 5'-CAAAAGACCCAAAGTCACGC-3' (reverse primer) and 5'-TGGAGAACCCAAAGGAGAGTTGCTG-3' (TaqMan probe) for SOD2 (GenBank accession number NM_017051); 5'-GAACCCGATATAGAAGCCCTG-3' (forward primer), 5'-CCATACCAAGCCAATACCAG-3' (reverse primer) and 5'-TGTCCAAGCAGTCTAGCAACCCC-3' (TaqMan probe) for GPx1 (GenBank accession number NM_030826); and 5'-CACTTTCTACAATGAGCTGCG-3' (forward primer), 5'-CTGGATGGCTACGTACATGG-3' (reverse primer) and 5'-TCTGGGTCATCTTTTCACGGTTGGC-3' (TaqMan probe) for β -Actin (GenBank accession number NM_031144). Normalized mRNA expression values are presented in the results.

Western Blot Analysis

Tissue lysates (50 μ g) were mixed with LDS NuPAGE Sample Buffer and separated by electrophoresis through 4–12% NuPAGE Bis-Tris Gels (Invitrogen) and transferred onto PVDF membrane. Membranes were probed with the following primary antibodies: anti-cleaved caspase 3 (Cell Signaling), anti-PCNA (Cell Signaling), anti-MnSOD (Abcam), anti-GPx-1 (Santa Cruz Biotech), and as housekeeping gene/loading control anti- α -tubulin (Cell Signaling). Following primary antibody incubation overnight at 4°C, the blots were incubated with appropriate secondary horseradish peroxidase conjugated antibodies (Cell Signaling) and developed with 1X LumiGLO Reagent and 1X Peroxide. Blots were quantitated by densitometry and relative expression values normalized to α -tubulin are presented in the results.

Immunohistochemistry

Immunohistochemistry staining was performed with modifications to a previously described protocol [25]. Mouse monoclonal antibody to 8-OHdG from the Japan Institute for the Control of Aging (Fukuroi City, Shizuoka, Japan) was used. Sections were autoclaved with 0.62M ZnSO₄ at 100°C for 15 min and then treated with 2M HCl at 37°C for 30 min. Sections were then incubated with 10 % normal horse serum for 30 min at 37°C followed by

an avidin/biotin block (Vector Laboratories, Burlingame, CA). After blocking, sections were incubated overnight at 4°C with primary antibody diluted 1:40 in Dako Antibody Diluent (DakoCytomation, Glostrup, Denmark). Slides were then washed with PBS followed by incubation with peroxidase conjugated anti-mouse secondary antibody using Dako EnVision +Dual Link System-HRP (Dako). Immunoreactivity was visualized with the chromagen DAB (Dako). Human seminal vesicle was used as a positive control tissue for 8-OHdG staining [37]. Extent and intensity of staining was semi-quantitatively scored by a single pathologist (MCB) as 0 (no staining), 1 (minimal), 2 (slight), 3 (moderate), or 4 (strong).

Measurement of Tissue and Serum Selenium Levels

Following acid digestion by hydride-generation, pooled tissue samples were analyzed by electrothermal atomic absorption spectrometry using a Perkin Elmer AAnalyst 800 instrument (Perkin Elmer Corp., Wellesley, MA) equipped with automated hydride-generation and a flow injection system. Selenium was detected at 900°C by absorbance at 196.0 nm using a 2.0 nm slit. Individual serum samples were analyzed by graphite furnace atomic absorption spectrometry in the presence of 0.09% palladium chloride with the same instrument using Zeeman background correction. The minimum detection limit of these methods was 0.51 ppb. Data are expressed as ng/mg wet prostate tissue.

Statistical Evaluation

Mean values and standard errors of the mean (SEM) were calculated per group for each parameter examined. The lesion incidence data presented reflect the presence of a particular lesion identified in a specific prostate lobe. Differences in lesion incidence among the accessory sex glands were analyzed using Fisher's exact test (two group comparisons) and χ^2 analysis (three group comparisons). Serum selenium levels and differences in enzyme activity as well as gene and protein expression among different lobes were analyzed using the non-parametric Mann-Whitney (for two group comparisons) and Kruskal-Wallis tests (for three group comparisons). If data were normally distributed, they were analyzed using the parametric t-test (for two group comparisons) and ANOVA followed by a Dunnett or Tukey multiple comparison test (for three group comparisons). P values of < 0.05 were considered significant.

RESULTS

The overall results were complex and are therefore summarized in three separate tables. Table 1 shows comparisons between control rats and T+E2 treated rats. In Table 2 comparisons between T+E2 treated rats and the two selenium-supplemented T+E2-treated groups are presented. Table 3 presents comparisons between rats (not hormone-treated) fed control diet and the two diets containing selenium supplementation. We focused in this study on contrasting the lateral prostate (target of T+E2 treatment) and the dorsal prostate (not a target); results of the two other prostate lobes (ventral and anterior) which are also not targets of T+E2 are not presented here because of their lack of relevance.

Inflammation and Dysplasia

The T+E2 treatment of NBL rats selectively induced a high incidence of inflammatory lesions and dysplasia in the lateral prostate lobes (Table 1). The lesions were morphologically identical to those previously described by us and others [3, 23, 37]. Neither incidence (100%) nor severity of the inflammation and epithelial dysplasia in the lateral prostate was affected by dietary selenomethionine (Table 2).

8-OHdG Immunostaining

Oxidative DNA damage was assessed by 8-OHdG staining in various prostatic lobes, periurethral prostatic ducts, and seminal vesicle (Fig. 1). No nuclear 8-OHdG immunostaining was detected in the ventral prostate in any of the groups. Control rats not treated with T+E2 showed a low level of 8-OHdG staining in the epithelial nuclei of the lateral and dorsal prostate (Table 1), as well as the anterior prostate lobes, the periurethral prostatic ducts, and seminal vesicles (data not shown). T+E2 treatment increased immunohistochemical staining for 8-OHdG in the lateral and dorsal prostate (Table 1). The lateral prostate, but not the dorsal lobe, is the main site of T+E2-induced inflammation and dysplasia after 13–16 weeks of hormone treatment [33, 37]. There was no effect of selenomethionine supplementation on 8-OHdG staining in the prostate of rats treated with T +E2 (Table 2) and rats that did not receive T+E2 (Table 3).

Effects on Antioxidant Defense Enzymes

Activity and protein expression of GPx-1 were increased by T+E2 treatment in the lateral prostate, but not in the dorsal lobe (Table 1). Selenomethionine did not change activity and expression of GPx-1 in the lateral and dorsal prostate of T+E2-treated rats (Table 2). Selenomethionine supplementation without T+E2 did not affect mRNA expression or activity of GPx-1 in either lobe, but increased GPx-1 protein expression in the lateral, but not the dorsal prostate in a dose-related fashion (Table 3).

T+E2 treatment did not affect transcript and protein levels of the SOD2 gene in the lateral or dorsal prostate (Table 1). SOD2 transcript levels were not affected in the dorsal and lateral prostate of T+E2-treated rats fed the selenomethionine-supplemented diets compared to T +E2-treated rats on control diet (Table 2). In the dorsal prostate, low selenomethionine increased SOD2 protein expression, but in the high selenium dose group SOD2 protein expression was unchanged. In contrast, SOD2 protein expression in the lateral prostate of T +E2-treated rats on the low selenomethionine diet was not changed, but reduced to below control levels in the high selenium dose group. Selenomethionine without T+E2 treatment did not affect mRNA expression of SOD2 in either prostate lobe and protein expression in the lateral prostate (Table 3). SOD2 protein expression was reduced in the dorsal, but not the lateral prostate of both selenium-supplemented groups compared to rats on control diet.

Thus, there were minimal and lobe-specific GPx-1 and SOD2 responses to T+E2 treatment, selenomethionine alone, or their combination (Tables 1–3). T+E2 treatment increased GPx-1 expression and activity in the lateral prostate, the dysplasia and inflammation target of these hormones. Selenium supplementation had minimal effects on GPx-1 and SOD2, except for a reduction of SOD2 protein expression in the lateral prostate of supplemented animals.

Effects on Apoptosis and Cell Proliferation

Cell proliferation, as indicated by PCNA protein expression, was increased by T+E2 treatment in the lateral, but not the dorsal prostate (Table 1). Selenomethionine increased PCNA expression in the dorsal prostate of T+E2-treated rats in a dose-dependent manner ($p < 0.0001$ for linear trend), but this did not occur in the lateral lobe where high dose selenomethionine substantially reduced protein expression of PCNA to below control values (Table 2). In rats not treated with T+E2, selenomethionine reduced PCNA protein expression in the lateral, but not the dorsal prostate (Table 3).

Apoptosis, measured by cleaved caspase-3 protein expression, was significantly increased by T+E2 in the dorsal but not the lateral prostate (Table 1). Cleaved caspase-3 protein expression was increased in the lateral lobe of the high dose selenomethionine group in T +E2-treated rats, but not in the low dose group (Table 2). In the dorsal prostate, the low dose selenomethionine did not change cleaved caspase-3 expression levels, but the high selenomethionine dose reduced levels to below control values. Selenomethionine alone caused induction of cleaved caspase-3 expression in both lobes, which was dose-related ($p < 0.0001$ for linear trend) in the dorsal, but not lateral prostate (Table 3).

Selenium Levels

Serum selenium levels did not significantly change with dietary selenomethionine treatment of otherwise untreated rats and were 572 ± 39 , 683 ± 55 , and 487 ± 53 ng/ml (mean $\pm$ SEM) in the control ($n = 6$), low ($n = 8$), and high dose group ($n = 8$), respectively ($p = 0.122$). However, selenium levels in pooled dorsolateral prostate tissues were increased in a dose-dependent manner by 2-fold over the control value in the low selenium group and 2.3-fold in the high selenium group as shown in Figure 2.

DISCUSSION

The results of this study indicate that the lack of protective activity from selenomethionine against hormone-induced prostate carcinogenesis in the NBL rat model [23] is likely due to its failure to reduce oxidative stress or increase anti-oxidant mechanisms in animals fed a diet that is nutritionally adequate in selenium. This is the first study to examine the antioxidant effects of selenomethionine in the prostate in a hormonally driven prostatic carcinogenesis and oxidative stress animal model. Antioxidant enzyme expression and/or activity in target prostate tissue (the lateral but not the dorsal prostate) were only minimally affected by dietary selenomethionine in rats that were not treated with T+E2 -- GPx-1 protein expression was increased in the lateral prostate in a generally protective manner. In the lateral prostate of T+E2-treated animals, selenomethionine supplementation did not affect antioxidant enzyme expression and activity, except for a decrease in SOD2 protein expression at the high dose, which is not considered a protective effect. Oxidative DNA damage (8-OHdG staining) was not altered by selenomethionine supplementation, regardless of hormone treatment, and the induction of prostatic inflammation and dysplasia by T+E2 was not reduced. Importantly, these findings are consistent with the negative outcome of clinical trials with selenium [15–17, 38].

Previous studies have shown evidence of oxidative stress (formation of 8-OHdG) and reduction of antioxidant enzyme activity in the prostate of T+E2-treated rats, unlike we observed in this study, in addition to DNA strand breaks and induction of lipid peroxidation [26–29]. These effects may be due to the hydroxylation of E2 to a catechol-estrogen. We have previously reported evidence to suggest that catechol-estrogens are capable of redox-cycle in T+E2-treated rats [30], which may result in the generation of reactive quinones that can adduct to DNA and cause depurination [28]. Redox cycling of catechol-estrogens may also lead to the formation of reactive oxygen species that can cause oxidative DNA damage [28, 30, 31].

MnSOD and GPx-1 are thought to be essential protective enzymes against the deleterious effects of oxidative stress in the form of superoxide anions, hydroxyl radical, and hydrogen peroxide [39]. In the present study, GPx-1 activity and expression, but not SOD2 expression, were induced by the hormone treatment in the lateral prostate, the target of T+E2. This finding is not consistent with previous studies in this model [26–29] and reports of a reduction of GPx-1 expression in human prostate cancers [40] and in estrogen receptor negative breast cancer cell lines. However, GPx-1 has been reported to be upregulated by estrogens in the rat liver [41] which is thought to be the result of estradiol-mediated activation of nuclear factor kappa B (NFκB), and its consequent activation of GPx-1 [42]. Thus, the induction GPx-1 activity and expression induced by T+E2 treatment we observed in the rat lateral prostate was a protective response to increased oxidative stress (8-OHdG staining), a result of the elevated estrogen levels, or both. The absence of changes in SOD2 protein expression and GPx-1 activity and expression in T+E2-treated rats given diets containing selenomethionine and the decrease in SOD2 protein expression in the lateral prostate of high selenium dose animals, suggesting a decreased protective response, are consistent with the observed lack of reduced 8-OHdG staining or dysplasia formation in that lobe. Selenomethionine also affected SOD2 protein expression in the dorsal prostate; this effect differed in hormone-treated rats and rats not treated with T+E2 for unknown reasons.

One possible explanation for why selenomethionine was not protective in our previous NBL rat experiment is that the animals were fed a natural ingredient diet containing a nutritionally adequate level of selenium (0.31 ppm). Accordingly, the baseline selenium levels in these studies were such that selenoproteins, including GPx-1, were already maximally induced. For unknown reasons serum levels of selenium were not increased following supplementation, but prostatic selenium levels were increased in selenium-supplemented rats in a dose-related fashion. This may be related to the fact that selenomethionine is nonspecifically incorporated in all proteins as a mimic of methionine which may influence the stability of proteins and can be converted to analogues of methionine that may modulate transmethylation and polyamine biosynthesis as proposed by Jackson and Combs [43]. In previous studies using the same selenium supplementation doses, a diet containing 0.23 mg/kg selenium was used and plasma selenium levels were slightly but significantly increased after 1 or 26 weeks in the high selenium dose group but not in the low selenium group [18]. In the present study, we used a control diet containing 0.31 mg/kg selenium and measured selenium concentration in serum after 16 weeks, which may explain the difference with the previous study. Future studies with the NBL rat model should include animals that are only marginally sufficient or slightly deficient in selenium in addition to animals on a

selenium adequate diet to provide a greater spread in selenium levels and mimic low baseline conditions in humans.

We observed differential responses to T+E2 treatment and selenomethionine feeding in dorsal and lateral parts of the rat prostate regarding (a) inflammation and dysplasia, (b) the oxidative stress marker 8-OHdG, (c) expression and activity of the selenoprotein GPx-1, and (d) expression of the anti-oxidant protein manganese superoxide dismutase. The inflammation and dysplasia in this model occurred selectively in the lateral prostate and not the dorsal lobe, as we have previously observed [32, 33]. The reason for this lobe selectivity has been unclear, but the striking differences between these lobes in responses to both the T +E2 treatment and selenium supplementation suggest that their biology is fundamentally different, despite their similarities in embryology, morphology, and function. The present study provides some evidence to explain this biological difference. Oxidative stress was similar in both lobes in response to T+E2 treatment, whereas apoptosis was markedly increased selectively in the dorsal prostate, cell proliferation was selectively increased in the lateral prostate. This difference in apoptosis-proliferation balance is perhaps responsible for the differential response in lesion development of these two lobes. These prostate lobes also strikingly differed in their apoptosis and proliferation response to selenium supplementation in T+E2-treated and not hormone-treated rats. The observed opposite effect of selenomethionine on PCNA and cleaved caspase 3 expression in the lateral prostate of T +E2-treated rats may also explain the lack of inhibition by selenomethionine of development of hormone-induced dysplasia in that lobe. Of note, recently published data from a dog study indicated a lack of selenium effects on apoptosis and cell proliferation in that species [44].

In conclusion, L-selenomethionine did not enhance antioxidant protection in the T+E2-treated rat prostate or reduce prostatic oxidative stress and development of dysplasia and cancer caused by this hormone treatment of NBL rats fed a diet adequate in selenium. These findings are consistent with the negative outcome of the SELECT and SWOG S9917 trials with selenomethionine [16, 17] and a phase III trial with selenized yeast in men with elevated prostate-specific antigen levels or suspect rectal exam but negative prostate biopsies [15]. Our results and the outcomes of previous animal model studies [18, 19, 23] and these clinical trials all indicate that supplementation with selenium in the form of L-selenomethionine or selenized yeast of rats and humans consuming diets that are nutritionally sufficient in selenium is not protective against prostate cancer.

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FIGURE 1.

8-Hydroxydeoxyguanosine (8OHdG) immunostaining in the lateral prostate: representative images of sections from control (left hand panel) and T+E2-treated lateral prostate (right hand panel) stained for 8-OHdG, showing the lowest level of staining observed in tissue of control rats and the highest levels observed in tissue of T+E2 treated rats.

FIGURE 2.

Selenium levels (ng/gram wet tissue) in pooled dorsolateral prostate tissue of 4 animals per group.

TABLE 1

Summary of the effects of T+E2 treatment in the dorsal (DP) and lateral prostate (LP)

Parameter	Treatment			
	Control		T + E2	
Site	LP	DP	LP	DP
Inflammation score	0.05 ± 0.05 (10)	0.00 (9)	2.88 ± 0.13** (8)	0.00 (8)
Dysplasia score	0.00 (8)	0.00 (9)	1.25 ± 0.23** (8)	0.00 (8)
8-OHdG score	1.40 ± 0.30 (10)	0.73 ± 0.21 (9)	2.25 ± 0.25* (8)	1.38 ± 0.18* (8)
GPx-1 mRNA expression	0.87 ± 0.08 (5)	1.08 ± 0.06 (6)	2.65 ± 1.10 (5)	1.96 ± 0.60 (6)
Activity	38.9 ± 12.1 (5)	32.4 ± 9.0 (5)	89.6 ± 12.2* (5)	63.0 ± 15.6 (6)
Protein expression	1.13 ± 0.06 (3)	1.25 ± 0.05 (3)	2.99 ± 0.11** (3)	1.26 ± 0.05 (3)
SOD2 mRNA expression	0.99 ± 0.04 (4)	0.91 ± 0.04 (7)	1.04 ± 0.25 (4)	0.83 ± 0.11 (7)
Protein expression	1.79 ± 0.26 (3)	2.28 ± 0.03 (3)	2.24 ± 0.21 (3)	2.27 ± 0.04 (3)
PCNA protein expression	2.23 ± 0.06 (3)	1.24 ± 0.03 (3)	4.69 ± 0.12** (3)	1.41 ± 0.09 (3)
Cleaved caspase 3 protein expression	6.40 ± 0.20 (3)	2.41 ± 0.06 (3)	6.59 ± 0.19 (3)	4.96 ± 0.04** (3)

Mean values ± SEM (n) for scores, mRNA and protein expression, and enzyme activity are presented in units explained in Materials & Methods

* p < 0.05 for comparison with control (2-sided Mann-Whitney test or t-test as appropriate)

** p < 0.0001 for comparison with control (2-sided Mann-Whitney test or t-test as appropriate)

TABLE 2

Effects of low-dose (LSe) and high-dose (HSe) selenomethionine on T+E2-induced changes in the dorsal (DP) and lateral prostate (LP)

Parameter	Treatment					
	T + E2			T + E2 + LSe		
Site				T + E2 + HSe		
	LP	DP	LP	DP	LP	DP
Inflammation score	2.88 ± 0.13 (8)	0.00 (8)	2.50 ± 0.27 (8)	0.00 (7)	2.93 ± 0.07 (7)	0.00 (7)
Dysplasia score	1.25 ± 0.23 (8)	0.00 (8)	1.71 ± 0.18 (8)	0.00 (7)	1.43 ± 0.20 (7)	0.00 (7)
8-OHdG score	2.25 ± 0.25 (8)	1.38 ± 0.18 (8)	1.71 ± 0.29 (7)	0.93 ± 0.37 (7)	2.14 ± 0.26 (7)	2.21 ± 0.21 (7)
GPx-1 mRNA expression	2.65 ± 1.1 (5)	1.96 ± 0.60 (6)	3.61 ± 1.45 (5)	1.31 ± 0.24 (6)	2.36 ± 0.55 (5)	1.53 ± 0.36 (6)
Activity	89.6 ± 12.2 (5)	63.0 ± 15.6 (6)	107.9 ± 23.3 (5)	49.5 ± 13.3 (6)	85.7 ± 11.5 (5)	32.8 ± 7.2 (5)
Protein expression	2.99 ± 0.11 (3)	1.26 ± 0.05 (3)	2.57 ± 0.09 (3)	1.36 ± 0.07 (3)	2.68 ± 0.11 (3)	1.63 ± 0.08 (3)
SOD2 mRNA expression	1.04 ± 0.25 (4)	0.83 ± 0.11 (7)	1.37 ± 0.41 (4)	1.24 ± 0.31 (7)	1.33 ± 0.30 (4)	1.07 ± 0.22 (7)
Protein expression	2.24 ± 0.21 (3)	2.27 ± 0.04 (3)	2.37 ± 0.19 (3)	4.94 ± 0.10 [*] (3)	1.18 ± 0.10 [*] (3)	1.90 ± 0.04 (3)
PCNA protein expression	4.69 ± 0.12 (3)	1.41 ± 0.09 (3)	4.73 ± 0.11 (3)	2.67 ± 0.06 [*] (3)	1.19 ± 0.02 [*] (3)	3.16 ± 0.08 [*] (3)
Cleaved caspase 3 protein expression	6.59 ± 0.19 (3)	4.96 ± 0.04 (3)	6.55 ± 0.19 (3)	5.10 ± 0.03 (3)	13.14 ± 0.30 [*] (3)	2.37 ± 0.07 [*] (3)

Mean values ± SEM (n) for scores, mRNA and protein expression, and enzyme activity are presented in units explained in Materials & Methods

* p < 0.05 for comparison with T+E2 (ANOVA with Dunnett's test or Kruskal-Wallis with Dunn's test as appropriate)

** p < 0.01 for comparison with T+E2 (ANOVA with Dunnett's test or Kruskal-Wallis with Dunn's test as appropriate)

TABLE 3

Summary of the effects of low-dose (LSe) and high-dose (HSe) selenomethionine on the dorsal (DP) and lateral prostate (LP)

Parameter	Treatment					
	Control			HSe		
Site	LP	DP	LP	DP	LP	DP
Inflammation score	0.00 (10)	0.00 (9)	0.00 (7)	0.00 (7)	0.00 (7)	0.00 (7)
Dysplasia score	0.00 (8)	0.00 (9)	0.00 (7)	0.00 (7)	0.00 (7)	0.00 (7)
8-OHdG score	1.40 ± 0.30 (10)	0.73 ± 0.21 (9)	1.50 ± 0.32 (5)	0.90 ± 0.29 (5)	1.50 ± 0.25 (9)	0.72 ± 0.28 (9)
GPx-1 mRNA expression	0.87 ± 0.08 (5)	1.08 ± 0.06 (6)	0.79 ± 0.13 (5)	0.96 ± 0.27 (6)	0.67 ± 0.15 (5)	0.65 ± 0.14 (6)
Activity	38.9 ± 12.1 (5)	32.4 ± 9.0 (5)	127.4 ± 47.2 (5)	36.3 ± 5.9 (6)	124.0 ± 50.9 (4)	37.9 ± 8.2 (5)
Protein expression	1.13 ± 0.06 (3)	1.25 ± 0.05 (3)	2.34 ± 0.09* (3)	1.39 ± 0.08 (3)	2.69 ± 0.12* (3)	1.03 ± 0.01 (3)
SOD2 mRNA expression	0.99 ± 0.04 (4)	0.91 ± 0.04 (7)	1.04 ± 0.23 (4)	1.07 ± 0.16 (7)	0.99 ± 0.21 (4)	1.23 ± 0.17 (7)
Protein expression	1.79 ± 0.26 (3)	2.28 ± 0.03 (3)	1.23 ± 0.12 (3)	1.81 ± 0.34* (3)	1.93 ± 0.06 (3)	1.76 ± 0.07* (3)
PCNA protein expression	2.23 ± 0.06 (3)	1.24 ± 0.03 (3)	1.07 ± 0.02* (3)	1.16 ± 0.02 (3)	1.24 ± 0.02* (3)	1.25 ± 0.05 (3)
Cleaved caspase 3 protein expression	6.40 ± 0.20 (3)	2.41 ± 0.06 (3)	12.52 ± 0.02* (3)	3.17 ± 0.04* (3)	11.06 ± 0.05* (3)	5.00 ± 0.08* (3)
						p<0.001 (ANOVA + Tukey test for difference between LSe-HSe in LP and DP)
						p<0.0001 (for linear trend for DP)

Mean values ± SEM (n) for scores, mRNA and protein expression, and enzyme activity are presented in units explained in Materials & Methods

* p < 0.01 for comparison with control (ANOVA with Dunnett's test or Kruskal-Wallis with Dunn's test as appropriate)