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Selenomethionine and α-Tocopherol do not Inhibit Prostate Carcinogenesis in the Testosterone plus Estradiol-Treated NBL Rat Model

Nur Özten[1], **Lori Horton**[2], **Salamia Lasano**[2], and **Maarten C. Bosland**[1,2,3]

[1]Department of Pathology, University of Illinois at Chicago, Chicago, Illinois

[2]Department of Environmental Medicine, New York University School of Medicine, New York, New York

[3]Department of Urology, New York University School of Medicine, New York, New York

Abstract

Previous studies with selenium and/or vitamin E in prostate carcinogenesis animal models have been negative, but these models may not involve oxidative stress mechanisms. In this study, we examined the potential of selenomethionine and α-tocopherol to modulate prostate cancer development in the testosterone plus estradiol-treated NBL rat, a model that does involve sex-hormone induced oxidative stress mechanisms and prostatic inflammation. One week following implantation with hormone-filled Silastic implants, rats were fed diets containing L-selenomethionine (1. 5 or 3. 0 mg/kg), DL-α-tocopherol acetate (2,000 mg/kg or 4,000 mg/kg), or a natural ingredient control diet (NIH-07). Development of prostate carcinomas was not affected by dietary treatment with either agent. Food intake, body weight, and mortality were also not affected. The high dose of selenomethionine reduced the severity of epithelial dysplasia in the lateral prostate that was *not* associated with inflammation and α-tocopherol reduced in a dose-related fashion the incidence of marked inflammation and marked epithelial dysplasia in the lateral prostate, regardless of whether these lesions were associated with inflammation. α-Tocopherol significantly increased the incidence of adenocarcinomas of the mammary glands at both dietary concentrations. Collectively, our findings suggest that selenomethionine and α-tocopherol supplementation does not prevent prostate cancer in rats fed diets with nutritionally adequate levels of selenium and vitamin E. Importantly, the results of the current animal studies and those reported previously were fully predictive of the outcome of the SELECT trial.

Keywords

Selenium; Vitamin E; Selenomethionine; α-Tocopherol; Prostate Cancer; Hormonal Carcinogenesis; NBL Rat

Introduction

Prostate cancer is the most common non-cutaneous malignancy and the second leading cause of cancer-related deaths in men in western countries (1).Since the etiology of prostate cancer

Request for reprints: Maarten C. Bosland, Department of Pathology, University of Illinois at Chicago, 840 South Wood Street, MC 847, Room 130 CSN, Chicago, Illinois, 60612. Phone: 312-355-3724. Fax: 312-996-7586. boslandm@uic.edu.

Disclosure of Potential Conflicts of Interest

None of the authors has a potential conflict of interest to report.

is not well understood, few risk factors offer opportunities for primary prevention of this malignancy. Therefore, chemoprevention is an attractive and potentially powerful approach to prostate cancer prevention that can be mechanism-based (2,3). Selenium compounds and tocopherols are classes of chemoprevention agents that have shown promise in this respect (4,5).

A protective effect of selenium and vitamin E is biologically plausible because of their role as antioxidants (6,7) and selenium has several other properties that may confer anti-carcinogenic activity (4). Antioxidants protect cells from oxidative DNA damage, which can cause mutations and subsequent carcinogenesis (8). Inflammatory processes known to involve oxidative damage are suspected to be associated with human prostate cancer (9) and the presence of the oxidized DNA base 8-hydroxy-2'-deoxyguanosine has been observed in human prostate tissue (10).

Secondary analysis of data of the Nutritional Cancer Prevention trial with selenized yeast in men at increased risk for skin cancer yielded suggestive evidence that supplementation with this type of selenium might be protective against prostate cancer (11,12). A similar secondary analysis of the results of the ATBC trial with α-tocopherol (with or without β-carotene) in male Finnish smokers indicated a potential protective effect of vitamin E (13). These observations formed the basis for the rationale of the SELECT trial in which the potential to prevent prostate cancer was tested of selenomethionine at 200 μg/day and α-tocopherol at 400 IU/day, alone or in combination (14). However, the study was recently terminated after an interim analysis showed that neither selenium nor vitamin E prevented prostate cancer in this study and that there were possible adverse effects; a non-significant increase in type II diabetes was observed in the selenium supplemented groups (15). Interestingly, there was a statistically non-significant increased risk of prostate cancer in the vitamin E group, but not in the selenium and the selenium plus vitamin E groups.

Previous studies with selenium (as selenomethionine or selenized yeast) and/or α-tocopherol in animal models of prostate carcinogenesis have been negative, including experiments with tumor induction models (16–19). However, none of these models are known to involve oxidative stress mechanisms, which may explain these null findings. In this study, we examined the potential of selenomethionine and α-tocopherol to modulate prostate cancer development in an animal model that uniquely does involve sex-hormone induced oxidative stress mechanisms and prostatic inflammation, the testosterone plus estradiol-treated NBL rat (20–22).

Materials and Methods

Animals

Male NBL (Noble) rats (NBL/CrCrlBR) were obtained at 7 to 8 weeks of age from Charles River, Portage, MI. Rats were held in quarantine for two weeks prior to the initiation of treatment. They were housed two to a cage in solid bottom cages and were fed 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 US Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Prostate cancer induction protocol

After randomization into experimental groups (Table 1), the rats were subjected to the following treatment protocol for induction of prostate cancer (20) (Table 1). All rats received two subcutaneous Silastic tubing implants (Dow Corning, ID 0. 078 inch; OD 0. 125 inch) tightly packed over a length of 2 cm with containing crystalline testosterone (Steraloids,

Newport, RI) and one such implant packed over a length of 1 cm with crystalline 17β -estradiol (Steraloids, Wilmington, NH). The implants were sealed with medical grade silicone sealant (RTV 108, General Electric, 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 hours 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 and they were replaced once after 26 weeks. This treatment increases circulating estradiol levels by 5 to 10-fold in NBL rats while maintaining testosterone at physiologic levels (~3 ng/ml) (23).

Chemoprevention agents and diets

L-Selenomethionine (Sigma, St.Louis, MO) was mixed into the diet (NIH-07; Harlan Teklad, Madison, WI) 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, but no selenomethionine. DL-α-Tocopherol acetate (= all rac-α-tocopherol acetate) (Sigma) was mixed into the diet using a corn oil (Sigma) as vehicle at a dietary concentration of 38 g/kg diet (3. 8% w/w) to achieve final DL-α-tocopherol acetate concentrations of 2,000 or 4,000 mg/kg diet; the control diet contained the same amount for corn oil, but without tocopherol. The corn oil added 7–8 mg α-tocopherol and 35–38 mg γ-tocopherol per kg to the NIH-07 diet, which contained 22 mg/kg DL-α-tocopheryl acetate and 0.31 mg/kg selenium. The diet concentrations and form of selenium and vitamin E were selected because they were used in previous experiments with another rat model of prostate carcinogenesis (16,17) and because L-selenomethionine and DL-α-tocopheryl acetate were used in the SELECT trial (14,15). These dose levels of selenomethionine and α-tocopherol were approximately 40 and 80% of the maximally tolerated doses, as identified previously in Wistar rats, which increased mean plasma selenium levels in the selenomethionine experiment by 9–17% from 560 – 625 ng/ml in the control group to 656 – 680 ng/ml in the high selenomethionine dose group and plasma α-tocopherol levels from approximately 5 μg/ml in the control group to 15 μg/ml in either supplemented group (DL McCormick, personal communication).

Study conduct

A first group of ninety NBL rats was randomized into three treatment groups of 30 rats each, receiving no supplement (controls) or diet containing 1.5 mg/kg or 3.0 mg/kg selenium (Table 1). A second group of ninety NBL rats was also randomized into three groups of 30 rats receiving no supplement or diet containing 2,000 mg/kg or 4,000 mg/kg α-tocopherol (Table 1). Animals had *ad libitum* access to food and tap water. The animals were fed the experimental diets starting one week after implantation of the hormone-containing Silastic implants. The experimental diets were prepared every two 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 two months and monthly thereafter to assess their general health. Food intake per cage over one week was measured after the rats had been fed the experimental diets for 4, 8, 12, 24, and 48 weeks. Prostate tumor development was assessed by weekly abdominal palpation starting 26 weeks into the hormone treatment period. Rats identified as moribund were euthanized by barbiturate over-dose and exsanguination, and these rats and those found dead were necropsied immediately. At 54 weeks after the start of hormone treatment, all surviving animals were euthanized and necropsied. At necropsy, the accessory sex glands were excised *en bloc* together with the urinary bladder. Accessory sex glands, pituitaries, and all grossly observed lesions in other organs were fixed in 10% neutral buffered formalin.

Histopathologic evaluation

After fixation, the ventral prostate, dorsolateral prostate, and anterior prostate plus seminal vesicles were dissected. From the ventral prostate and grossly observed tumor masses one section was made and from all other accessory sex gland tissues 6 step sections were prepared at 250–300 micrometer intervals, which were stained with hematoxylin and eosin (24). 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 (20,23, 24). Severity and extent of dysplastic and inflammatory lesions were scored semi-quantitatively as absent, slight, moderate, or marked by a single pathologist (MCB), using previously published criteria (20,23). Mammary tumors were classified according to Russo et al. (25).

Statistical evaluation

Lesion incidence data presented reflect the presence of a particular lesion identified in a particular tissue in each animal. Differences in lesion incidence in the accessory sex glands were analyzed using Fisher's exact test (two group comparisons) and Chi Square analysis (three group comparisons and analysis for linear trend). Comparisons of animal survival were made using log-rank analysis. Analysis of body weight and water consumption data was performed using analysis of variance, with *post-hoc* comparisons using Dunnett's multiple comparison test.

Results

NBL rats in this study died on average 41–46 weeks after treatment with testosterone plus estradiol had commenced (Table 2 and Table 4). This hormonal treatment resulted in a high incidence of multifocal adenocarcinomas originating from the ducts of the dorsolateral and anterior prostate in the periurethral region, confirming previous observations (20). In the selenomethionine experiment, the prostate carcinoma incidence ranged from 93 to 97% and in the α-tocopherol experiment, it ranged from 83 to 90% (Table 2 and Table 4). In the vast majority of animals, these tumors were multifocal with 60–80% of tumor-bearing animals having more than two foci of prostate cancer (Table 2 and Table 4). Carcinomas of the anterior prostate ducts outside the periurethral area were also frequent (53–73%), but carcinomas originating in the glandular portions of the dorsolateral and anterior prostate lobes and in the seminal vesicle were very rare (Table 2 and Table 4). Lesions classified as carcinoma *in situ* (morphologically identical to carcinomas, but without clear invasive growth) were occasionally observed in the ducts and glandular portions of the anterior prostate and seminal vesicles (Table 2 and Table 4). All animals had focal dysplastic and inflammatory lesions in the lateral prostate lobes (Table 2 and Table 4) and pituitary tumors (Table 3 and Table 5). The spectrum and morphology of these neoplastic and non-neoplastic lesions (data not shown) was comparable to that observed previously in this animal model (20–23). Mammary tumors were observed in all treatment groups of both experiments and a few neoplasms were observed in sites other than the accessory sex organs or mammary glands (Table 3 and Table 5).

Effects of Selenomethionine

Feeding testosterone plus estradiol-treated NBL rats with selenomethionine mixed into the diet at 1.5 and 3.0 mg/kg did not affect prostate carcinoma incidence and multiplicity (Table 2). Selenomethionine at these doses did not affect body weight (data not shown) or mortality (Table 2). Rats in the high dose group tended to eat 3–9% more food than controls, which was statistically significant at 4 weeks, but not 8 and 24 weeks (Table 3).

The incidence of inflammation and inflammation-associated epithelial dysplasia in the lateral prostate caused by the testosterone plus estradiol treatment was also not affected by dietary

selenomethionine (Table 2). The high dose of selenomethionine significantly reduced the severity of epithelial dysplasia in the lateral prostate that was *not* associated with inflammatory lesions: the incidence of dysplastic lesions of moderate to marked severity was reduced from 100% in the control group to 59% in high dose animals, while the incidence of slightly dysplastic lesions increased to 41% (Table 2).

Selenomethionine did not affect the incidence of tumors at other sites, including pituitary tumors found in 100% of rats treated with testosterone plus estradiol, which is a known estrogen effect (Table 3).

Effect of α-Tocopherol

Feeding testosterone plus estradiol-treated NBL rats with α-tocopherol mixed into diet at 2,000 and 4,000 mg/kg did also not affect prostate cancer incidence and multiplicity (Table 4). Dietary α-tocopherol at these doses did not affect body weight (data not shown), food intake (data not shown), or mortality (Table 4).

The incidence of marked epithelial dysplasia in the lateral prostate caused by the testosterone plus estradiol treatment was significantly reduced by α-tocopherol in a dose related fashion, regardless of whether the dysplastic lesions were associated with inflammation (Table 4). α-Tocopherol also slightly, but statistically significantly, reduced the incidence of marked inflammation in the lateral prostate in a dose related fashion (Table 4).

Dietary α-tocopherol significantly increased the incidence of adenocarcinomas of the mammary glands at both dietary concentrations (Table 5). α-Tocopherol did not affect the development of pituitary tumors occurring in 100% of NBL rats treated with testosterone plus estradiol or the incidence of tumors at other sites (Table 5).

Discussion

In the present study, we demonstrated that feeding of 1.5 and 3.0 ppm selenium in the form of selenomethionine and 2,000 and 4,000 ppm α-tocopherol mixed into a natural ingredient diet did not affect prostate carcinogenesis in an animal model that involves oxidative stress and inflammation, the testosterone and estradiol treated NBL rat. Importantly, the findings of this study reproduce the result of the SELECT trial that a daily supplement of 200 μg selenium (as L-selenomethionine), 400 IU vitamin E (as DL-α-tocopherol), and their combination do not prevent prostate cancer. These findings are also consistent with our previous observations in the MNU plus testosterone-treated WU rat prostate cancer model (16,17).

The results of this study and those of the SELECT trial do not support the notion that selenium (as selenomethionine) and vitamin E (as α-tocopherol) are inhibitors of prostate carcinogenesis, as was suggested by the results of the Nutritional Cancer Prevention study that used selenized yeast and the ATBC trial with α-tocopherol (11–13). The results of many, but not all, epidemiological studies suggest a protective role of selenium against prostate cancer (26–30). Prospective studies of toenail selenium levels indicated protection (26,27), whereas no inverse relation with risk was found in most large cohorts studies of plasma selenium levels and selenium supplement use (26,28,29). Our findings are in line with those epidemiologic studies in which a lack of protective activity against prostate cancer was observed for selenium (26, 28–30) and for vitamin E, mostly in non-smokers (31–33). Protective effects of dietary and/or supplemental vitamin E have only been found in smokers in some, but not all, studies (31–33) and the possible protective effect of selenium may also be modified by smoking status as well as by vitamin E intake (28). There is even some evidence to suggest that in non-smokers, vitamin E supplements could increase prostate cancer risk (31) and plasma selenium levels were associated with increased risk in a recent study, depending on manganese superoxide

dismutase genotype (34). The present study did not assess the possible interaction between selenomethionine and α-tocopherol, but previous studies with the WU rat model did not indicate protective activity of a combination of selenomethionine and α-tocopherol (16,17). Both agents in combination may reduce the risk of fatal or advanced prostate cancer as suggested by some epidemiological studies (28,30), but the design of the present study and previous experiments with the WU rat model do not allow adequate assessment of such effects. The results of a small recent randomized clinical trial indicated that the effects of selenium supplementation on the incidence of skin cancers and internal malignancies only occurred at a selenium dose of 200 μg/day, but not at a two-fold higher dose (35). A study in dogs suggested a U-shaped dose-response relationship between selenium intake and DNA damage in prostate cells (36). These observations raise the possibility that some of the discrepancies in the literature and the lack of effects of selenium in animal studies are due to such a non-linear dose-response relationship.

One major issue not addressed in our animal experiments, SELECT, and epidemiological studies is the influence of baseline selenium and vitamin E status. In the Nutritional Cancer Prevention trial, selenium was only protective in men with blood selenium levels in the lower two tertiles (<123 ng/ml) (12). In SELECT, median baseline serum selenium levels were between 135 and 138 ng/ml with inter-quartile ranges from approx.123 to 150 ng/ml (15), well above those of the lower tertiles of the Nutritional Cancer Prevention trial (12). In our animal studies, the control diet contained 0.31 mg/kg selenium, well above the dietary requirement for rats of 0.15 mg/kg diet (37), resulting in a selenium intake of approximately 20 μg/kg body weight per day, while in the animals fed the supplemented diets, intakes were in the range of 100 and 200 μg/kg body weight per day in the low and high selenium dose groups, respectively. This probably resulted in plasma selenium concentrations in the control group of approximately 600 ng/ml and in the high selenium group of approximately 670 ng/ml (DL McCormick, personal communication) , considerably higher that those in the SELECT trial. It is conceivable that selenium is only protective against prostate cancer in men with low to marginally deficient selenium levels (36) and that this might also be the case in the rat model we used. In a recent epidemiologic study by Peters et al. (28), the mean serum selenium level was 141 ng/ml (range 51–253 ng/ml) in a US male population and similar serum selenium levels (means of 119–126 ng/ml) were found in the third NHANES study (38). Thus, even if selenium protects against prostate cancer, most US men would not have a selenium status that is sufficiently low to provide benefit from selenium supplementation.

The control diet in our animal studies contained 22 mg/kg α-tocopheryl acetate, which is the approximate dietary requirement for rats of 18 mg α-tocopherol per kg diet (37), resulting in a daily α-tocopheryl acetate intake of approximately 0.5 mg/kg body weight, while intakes in the animals fed the supplemented diets were in the range of 135 and 270 mg/kg body weight per day in the low and high α-tocopherol dose groups, respectively. This likely resulted in plasma α-tocopherol concentrations in the control group of approximately 5 μg/ml and 15 μg/ml both supplemented groups (DL McCormick, personal communication). Baseline plasma α-tocopherol levels in the SELECT trial were around 12 μg/ml and increased to approximately 18 μg/ml in the supplemented groups (15). Thus, the conditions of vitamin E animal study were comparable to those of SELECT.

The natural ingredient NIH-07 diet used in this study contains substantive amounts (approx. 400 mg/kg) of isoflavones (39) and we have shown that soy isoflavones can inhibit prostate carcinogenesis in another rat model (40) when added to a natural ingredient diet with even higher basal isoflavone levels (39). Because of this observation and the fact that the basal diet in the control and supplemented groups in the present studies was identical, it is unlikely that these dietary isoflavones modulated the effects of selenomethionine and α-tocopherol.

The form of selenium and vitamin E may also be critically important in determining the potential protective effects of these agents. For example, methylseleninic acid and methylselenocysteine, but not selenomethionine, had inhibitory activity on growth of human prostate cancer xenografts in nude mice (41) and methylseleninic acid and methylselenocysteine slowed prostate cancer development in the TRAMP model (42), whereas selenomethionine did not (19). Furthermore, it is conceivable that vitamin E may protect against prostate cancer not as all-*rac*-α-tocopherol but in its natural form of RRR-α-tocopherol or as γ-tocopherol, the predominant form of vitamin E in the US diet. There is some epidemiologic evidence to suggest that γ-tocopherol is protective against this major male malignancy (33,43).

It is possible that the NBL rat model and other currently available animal models of prostate carcinogenesis are not suitable for testing anti-prostate cancer activity of antioxidants because they lack significant oxidative stress mechanisms. However, we and others have found evidence in the NBL rat model of formation in the prostate of oxidative DNA damage (8-hydroxy-2'-deoxyguanosine), lipid peroxidation, DNA strand breaks, and reduction of activity of the antioxidant enzymes, specifically linked to the hormone treatment (22,44–46). These effects are attributed to hydroxylation of 17β-estradiol to a catechol-estrogen, which can undergo redox-cycling that results in the generation of (1) reactive quinones that can adduct to DNA and cause depurination, and (2) reactive oxygen species that can cause oxidative DNA damage and lipid peroxidation (44,47). We have found evidence to suggest that this redox-cycling mechanism indeed occurs in the testosterone plus estradiol-treated NBL rat (48).

L-Selenomethionine and all-*rac*-α-tocopherol acetate both reduced the severity of prostatic dysplasia, a lesion comparable to human PIN, and α-tocopherol acetate, but not selenomethionine, slightly reduced the severity of prostatic inflammation. However, neither agent inhibited cancer induction in the testosterone plus estradiol-treated NBL rat. The biological significance of these effects is not clear, because the dysplastic lesions in the glandular portion of the dorsal and, particularly, the lateral prostate lobes rarely if ever progress to cancer (20, unpublished data). However, these findings may suggest that the mechanism of dysplasia and inflammation induction in the NBL rat by testosterone plus estradiol involves oxidative stress and lipid peroxidation, whereas cancer induction may be specifically related to mechanisms other than oxidative DNA damage. One such alternate mechanism may be the formation of the aforementioned depurinating estrogen-quinone DNA adducts (48). Studies are ongoing in our laboratory to determine the effects of both interventions on biomarkers of oxidative stress in the prostatic target tissues of hormone-induced carcinogenesis in the NBL rat.

Interestingly, all-*rac*-α-tocopherol acetate increased the incidence of adenocarcinomas of the mammary glands from 3% to 23–27%. Historic control groups of male NBL rats treated with testosterone plus estradiol in our laboratory had a 0–8% incidence of mammary cancer (unpublished data). However, the mammary cancer incidence in the control group of the selenomethionine experiment was 27% and the incidence was 10–27% in the treatment groups of both experiments. These observations suggest that there is a wide variation in the occurrence of mammary carcinomas in NBL rats treated with testosterone plus estradiol. Thus, it is possible that the mammary cancer incidence in control animals of the α-tocopherol experiment was spuriously low resulting in an apparent increase in incidence in the rats fed the tocopherol-supplemented diets. There is no information about vitamin E and risk of male breast cancer in humans. The observation of mammary cancer in testosterone plus estradiol-treated male NBL rats may be an artifact of this model.In female NBL rats, this hormone treatment results in a high incidence of mammary cancer (49), but the effect of α-tocopherol in this model is not known. There was no evidence to indicate a protective effect of vitamin E against female breast

cancer risk in a recent randomized clinical trial (50) and there is no consistent evidence for this from epidemiologic studies (51).

The high incidence of prostate carcinomas in control animals in this study my have reduced the sensitivity of the model to inhibitory effects of chemopreventive agents. However, inhibition of prostatic dysplasia was observed in both studies. Furthermore, others have previously found inhibition of dysplasia in the testosterone plus estradiol-treated NBL rat by 4 months of dietary treatment with 9-*cis*-retinoic acid and dehydroepiandrosterone, agents that were protective in the MNU plus testosterone-treated WU rat model, but not with 4-(hydroxyphenyl)retinamide which was not inhibitory in the WU rat model (2,17,24). Thus, the NBL rat model does appear to be sensitive to inhibition of prostatic lesion development by chemopreventive agents. Our findings do not exclude the possibility that selenomethionine and α-tocopherol may have the potential to affect growth of existing metastatic prostate cancers. For example, monomethylated selenium inhibited the growth of LNCaP xenografts in nude mice (38).

Collectively, our current and previous animal model findings suggest that selenomethionine and α-tocopherol do not prevent prostate cancer. Importantly, our results are concordant with and predictive of the results of the SELECT trial and are therefore not supportive of its rationale. Much of the available epidemiological evidence about these two agents, particularly vitamin E (except in smokers), also suggests lack of protective activity when given as supplements. Alternatively, the results of our animal studies and SELECT might be interpreted to suggest that oxidative stress and resulting lipid peroxidation are not associated with increased prostate cancer risk. The findings of SELECT and the animal model studies do not exclude the possibility that other forms of vitamin E than α-tocopherol and selenium not in the form of organo-selenium compounds may provide protection against prostate cancer. It is also conceivable that these agents are protective only under conditions of selenium or vitamin E deficiency, but this was not addressed in SELECT and our animal studies.

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References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. CA Cancer J Clin. 2009 Jun 9; [Epub ahead of print].
2. Bosland MC, McCormick DL, Melamed J, Walden PD, Zeleniuch-Jacquotte A, Lumey LH. Chemoprevention strategies for prostate cancer. Eur J Cancer Prev 2002;11 Suppl 2:S18–S27. [PubMed: 12570331]
3. Klein EA. Chemoprevention of prostate cancer. Annu Rev Med 2006;57:49–63. [PubMed: 16409136]
4. Nadiminty N, Gao AC. Mechanisms of selenium chemoprevention and therapy in prostate cancer. Mol Nutr Food Res 2008;52:1247–1260. [PubMed: 18727008]
5. Ni J, Yeh S. The roles of alpha-vitamin E and its analogues in prostate cancer. Vitam Horm 2007;76:493–518. [PubMed: 17628187]
6. Jackson MI, Combs GF Jr. Selenium and anticarcinogenesis: underlying mechanisms. Curr Opin Clin Nutr Metab Care 2008 Nov;11(6):718–726. [PubMed: 18827575]
7. Mustacich DJ, Bruno RS, Traber MG, Vitamin E. Vitam Horm 2007;76:1–21. [PubMed: 17628169]
8. McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? Free Radic Biol Med 1999;26:1034–1053. [PubMed: 10232849]

9. Palapattu GS, Sutcliffe S, Bastian PJ, et al. Prostate carcinogenesis and inflammation: emerging insights. Carcinogenesis 2005;26:1170–1181. [PubMed: 15498784]
10. Chen L, Stacewicz-Sapuntzakis M, Duncan C, et al. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. J Natl Cancer Inst 2001;93:1872–1879. [PubMed: 11752012]
11. Clark LC, Dalkin B, Krongrad A, et al. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. Br J Urol 1998;81:730–734. [PubMed: 9634050]
12. Duffield-Lillico AJ, Dalkin BL, Reid ME, et al. Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. BJU Intl 2003;91:608–612.
13. Heinonen OP, Albanes D, Virtamo J, et al. Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. J Natl Cancer Inst 1998;90:440–446. [PubMed: 9521168]
14. Lippman SM, Goodman PJ, Klein EA, et al. Designing the Selenium and Vitamin E Cancer Prevention Trial (SELECT). J Natl Cancer Inst 2005;97:94–102. [PubMed: 15657339]
15. Lippman SM, Klein EA, Goodman PJ, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2009;301:39–51. [PubMed: 19066370]
16. Bosland MC, Johnson WD, Steele VE, Lubet RA, Kelloff GJ, McCormick DL. Prostate cancer chemoprevention efficacy evaluation of combined administration of L-selenomethionine + vitamin E in a rat model. Proc Am Assoc Cancer Res 2000;41:339–340.
17. McCormick DL, Rao KVN, Lubet RA, Steele VE, Kelloff GJ, Bosland MC. Differential activity of 9-*cis*-retinoic acid, α-tocopherol, and selenomethionine as chemopreventive agents in the rat prostate. Proc Am Assoc Cancer Res 1997;38:261.
18. McCormick DL, Johnson WD, Lubet RA, Steele VE, Bosland MC. Null activity of selenized yeast as a chemopreventive agent in the rat prostate. Proc Am Assoc Cancer Res (2nd ed) 2003;44:775–776.
19. Brumfield SK, Eng M, Sanda M, Pienta K. The chemopreventive activity of seleno-L-methionine in the transgenic adenocarcinoma mouse prostate model. Proc Am Assoc Cancer Res 1999;40:360.
20. Bosland MC, Ford H, Horton L. Induction of a high incidence of ductal prostate adenocarcinomas in NBL and Sprague Dawley rats treated with estradiol-17-beta or diethylstilbestrol in combination with testosterone. Carcinogenesis 1995;16:1311–1317. [PubMed: 7788848]
21. Bernoulli J, Yatkin E, Laakso A, et al. Histopathological evidence for an association of inflammation with ductal pin-like lesions but not with ductal adenocarcinoma in the prostate of the noble rat. Prostate 2008;68:728–739. [PubMed: 18302197]
22. Tam NN, Leav I, Ho SM. Sex hormones induce direct epithelial and inflammation-mediated oxidative/nitrosative stress that favors prostatic carcinogenesis in the noble rat. Am J Pathol 2007;171:1334–1341. [PubMed: 17717140]
23. Ofner P, Bosland MC, Vena RL. Differential effects of diethylstilbestrol and estradiol-17 beta in combination with testosterone on rat prostate lobes. Toxicol Appl Pharmacol 1992;112:300–309. [PubMed: 1539166]
24. McCormick DL, Rao KVN, Dooley L, et al. Influence of *N*-methyl-*N*-nitrosourea, testosterone, and *N*-(4-hydroxyphenyl)-all-*trans*-retinamide on prostate cancer induction in Wistar (WU) rats. Cancer Res 1998;58:3282–3288. [PubMed: 9699656]
25. Russo J, Gusterson BA, Rogers AE, Russo IH, Wellings SR, van Zwieten MJ. Comparative study of human and rat mammary tumorigenesis. Lab Invest 1990;62:244–278. [PubMed: 2107367]
26. Etminan M, FitzGerald JM, Gleave M, Chambers K. Intake of selenium in the prevention of prostate cancer: a systematic review and meta-analysis. Cancer Causes Control 2005;16:1125–1131. [PubMed: 16184479]
27. van den Brandt PA, Zeegers MPA, Bode P, Goldbohm RA. Toenail selenium levels and the subsequent risk of prostate cancer: A prospective cohort study. Cancer Epidemiol Biomarkers Prev 2003;12:866–871. [PubMed: 14504196]

28. Peters U, Foster CB, Chatterjee N, et al. Serum selenium and risk of prostate cancer-a nested case-control study. Am J Clin Nutr 2007;85:209–217. [PubMed: 17209198]
29. Allen NE, Appleby PN, Roddam AW, et al. Plasma selenium concentration and prostate cancer risk: results from the European Prospective Investigation into Cancer and Nutrition (EPIC). Am J Clin Nutr 2008;88:1567–1575. [PubMed: 19064517]
30. Peters U, Littman AJ, Kristal AR, Patterson RE, Potter JD, White E. Vitamin E and selenium supplementation and risk of prostate cancer in the Vitamins and lifestyle (VITAL) study cohort. Cancer Causes Control 2008;19:75–87. [PubMed: 17943452]
31. Chan JM, Stampfer MJ, Ma J, Rimm EB, Willett WC, Giovannucci EL. Supplemental vitamin E intake and prostate cancer risk in a large cohort of men in the United States. Cancer Epidemiol Biomarkers Prev 1999;8:893–899. [PubMed: 10548318]
32. Kirsh VA, Hayes RB, Mayne ST, et al. Supplemental and dietary vitamin E, beta-carotene, and vitamin C intakes and prostate cancer risk. J Natl Cancer Inst 2006;98:245–254. [PubMed: 16478743]
33. Wright ME, Weinstein SJ, Lawson KA, et al. Supplemental and dietary vitamin E intakes and risk of prostate cancer in a large prospective study. Cancer Epidemiol Biomarkers Prev 2007;16:1128–1135. [PubMed: 17548674]
34. Chan JM, Oh WK, Xie W, et al. Plasma selenium, manganese superoxide dismuthase, and intermediate- or high-risk prostate cancer. J Clin Oncol 2009;June(15) [Epub ahead of print].
35. Reid ME, Duffield-Lillico AJ, Slate E, et al. The nutritional prevention of cancer: 400 mcg per day selenium treatment. Nutr Cancer 2008;60:155–163. [PubMed: 18444146]
36. Waters DJ, Shen S, Glickman LT, et al. Prostate cancer risk and DNA damage: translational significance of selenium supplementation in canine model. Carcinogenesis 2005;26:1256–1262. [PubMed: 15817614]
37. National Research Council. Nutrient Requirements of Laboratory Animals. Washington: National Academy Press; 1995.
38. Vogt TM, Ziegler RG, Patterson BH, Graubard BI. Racial differences in serum selenium concentration: analysis of US population data from the Third National Health and Nutrition Examination Survey. Am J Epidemiol 2007;166:280–288. [PubMed: 17557900]
39. Brown NM, Setchell KD. Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones. Lab Invest 2001;81:735–747. [PubMed: 11351045]
40. McCormick DL, Johnson WD, Bosland MC, Lubet RA, Steele VE. Chemoprevention of rat prostate carcinogenesis by soy isoflavones and by Bowman-Birk inhibitor. Nutr Cancer 2007;57:184–193. [PubMed: 17571952]
41. Li GX, Lee HJ, Wang Z, et al. Superior in vivo inhibitory efficacy of methylseleninic acid against human prostate cancer over selenomethionine or selenite. Carcinogenesis 2008;29:1005–1012. [PubMed: 18310093]
42. Wang L, Bonorden MJ, Li GX, et al. Methyl-selenium compounds inhibit prostate carcinogenesis in the transgenic adenocarcinoma of mouse prostate model with survival benefit. Cancer Prev Res 2009;2:484–495.
43. Helzlsouer KJ, Huang HY, Alberg AJ, et al. Association between alpha-tocopherol, gamma-tocopherol, selenium, and subsequent prostate cancer. J Natl Cancer Inst 2000;92:2018–2023. [PubMed: 11121464]
44. Cavalieri EL, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents – DNA adducts and mutations. J Natl Cancer Inst Monogr 2000;27:75–94. [PubMed: 10963621]
45. Ho SM, Roy D. Sex hormone-induced nuclear DNA damage and lipid peroxidation in the dorsolateral prostates of Noble rats. Cancer Lett 1994;84:155–162. [PubMed: 8076372]
46. Tam NN, Ghatak S, Ho SM. Sex hormone-induced alterations in the activities of antioxidant enzymes and lipid peroxidation status in the prostate of Noble rats. Prostate 2003;55:1–8. [PubMed: 12640655]
47. Jefcoate CR, Liehr JG, Santen RJ, et al. Tissue-specific synthesis and oxidative metabolism of estrogens. J Natl Cancer Inst Monogr 2000;27:95–112. [PubMed: 10963622]
48. Cavalieri EL, Devanesan PD, Bosland MC, Badawi AF, Rogan EG. Catechol Estrogen Metabolites and Conjugates in Different Regions of the Prostate of Noble Rats Treated with 4-Hydroxyestradiol:

Implications for Estrogen-Induced Initiation of Prostate Cancer. Carcinogenesis 2002;23:329–333. [PubMed: 11872641]

49. Xie B, Tsao SW, Wong YC. Sex hormone-induced mammary carcinogenesis in female Noble rats: the role of androgens. Carcinogenesis 1999;20:1597–1606. [PubMed: 10426813]
50. Lee IM, Cook NR, Gaziano JM, et al. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. JAMA 2005;294:56–65. [PubMed: 15998891]
51. Tamimi RM, Hankinson SE, Campos H, et al. Plasma carotenoids, retinol, and tocopherols and risk of breast cancer. Am J Epidemiol 2005;161:153–160. [PubMed: 15632265]
52. Christov KT, Moon RC, Lantvit DD, et al. 9-cis-retinoic acid but not 4-(hydroxyphenyl)retinamide inhibits prostate intraepithelial neoplasia in Noble rats. Cancer Res 2002;62:5178–5182. [PubMed: 12234981]
53. Christov KT, Moon RC, Lantvit DD, et al. Prostate intraepithelial neoplasia in Noble rats, a potential intermediate endpoint for chemoprevention studies. Eur J Cancer 2004;40:1404–1411. [PubMed: 15177500]

Table 1

a. Treatment Protocol of Selenomethionine Experiment					
Group	**Number of Animals**	**From Day 1**	**From Day 7**	**After 26 Weeks**	**Terminal Sacrifice**
1	30	Testosterone plus 17β-estradiol	Control diet (NIH-07)	Testosterone plus estradiol replacement	54 wks after start
2	30	Testosterone plus 17β-estradiol	Selenomethionine mixed in diet at 1.5 mg/kg (as selenium)	Testosterone plus estradiol replacement	54 wks after start
3	30	Testosterone plus 17β-estradiol	Selenomethionine mixed in diet at 3.0 mg/kg (as selenium)	Testosterone plus estradiol replacement	54 wks after start

b. Treatment Protocol of α-Tocopherol Experiment					
Group	**Number of Animals**	**From Day 1**	**From Day 7**	**After 26 Weeks**	**Terminal Sacrifice**
1	30	Testosterone plus 17β-estradiol	Control diet (NIH-07)	Testosterone plus estradiol replacement	54 wks after start
2	30	Testosterone plus 17β-estradiol	α-Tocopherol mixed in diet at 2,000 mg/kg	Testosterone plus estradiol replacement	54 wks after start
3	30	Testosterone plus 17β-estradiol	α-Tocopherol mixed in diet at 4,000 mg/kg	Testosterone plus estradiol replacement	54 wks after start

Table 2

Effect of Selenomethionine on Prostate Carcinogenesis and Non-Neoplastic Prostate Lesions in 17β -Estradiol + Testosterone−Treated NBL Rats

Group	**1**	**2**	**3**
Selenomethionine dose (as selenium - mg/kg diet)	0	1.5	3
Effective Number of Animals	30	30	30
Mortality (wks after start hormone treatment):			
Mean week of death ± standard deviation	43 ± 6.0	41 ± 6.2	44 ± 5.7
Median week of death (95% confidence interval)	42 (40,45)	41 (39,44)	46 (41,46)
Range (minimum – maximum)	28 – 53	22 – 55	32 – 52
Lesion Incidence	**No. (%) of Rats with Lesion**:		
Adenocarcinoma:			
Total Cancer Incidence	**29 (97%)**	**28 (93%)**	**29 (97%)**
Periurethral dorsolateral/anterior prostate ducts	27 (90)	25 (83)	27 (90)
Distal anterior prostate ducts	16 (53)	19 (63)	17 (57)
Dorsolateral prostate lobe (glandular portion)	0	1 (3)	1 (3)
Anterior prostate lobe (glandular portion)	2 (7)	0	1 (3)
Seminal Vesicle (glandular portion)	1 Adenoma	0	1 Carcinoma
Tumor Multiplicity: One or two carcinomas	5 (17)	7 (23)	8 (27)
More than two carcinomas	24 (80)	18 (60)	21 (70)
Carcinoma *in situ* (but no carcinomas):			
Anterior prostate (glands & ducts)	3 (10)	0	2 (7)
Seminal vesicle (glands & ducts)	1 (3)	0	1 (3)
Focal Dysplasia in Anterior Prostate (No. animals with lesion/evaluable animals):			
Anterior prostate (glands & ducts)	16/25 (64)	23/26 (88)	15/22 (68)
Inflammation & Focal Dysplasia in Lateral Prostate:			
Inflammation (No. animals with lesion/evaluable animals):			
Total incidence	30/30 (100)	30/30 (100)	30/30 (100)
Marked inflammation	3 (10)	3 (10)	2 (7)
Slight – moderate inflammation	27 (90)	27 (90)	28 (93)
Dysplasia associated with inflammation (No. animals with lesion/evaluable animals):			
Total incidence	25/25 (100)	26/26 (100)	22/22 (100)
Moderate dysplasia	3 (12)	0	7 (32)
Slight dysplasia	22 (88)	26 (100)	15(68)
Dysplasia not associated with inflammation (No. animals with lesion/evaluable animals):			
Total incidence	25/25 (100)	26/26 (100)	22/22 (100)
Moderate – marked dysplasia	25 (100)[a]	24 (92)[b]	13 (59)[a, b]
Slight dysplasia	0[a]	2 (8)[b]	9 (41)[a, b]

Data were tested for significance using Fisher's exact test and for linear trend with dose using a 2 × 3 chi-square test.

Differences were considered statistically significant at $P \leq 0.05$ (2-sided for Fisher's exact test). Note that for lesions that can only be assessed micropscopically, the number of evaluable animals (with non-autolytic tissue) was often lower than the effective number of animals.

[a] $P = 0.0004$,

[b] $P = 0.0134$ for difference between high dose group and control or low dose group, respectively (Fisher's exact test).

Table 3

Effect of Selenomethionine on Food Intake and on Extra-Prostatic Tumor Development in 17β-Estradiol + Testosterone−Treated NBL Rats

Group	**1**	**2**	**3**
Selenomethionine dose (as selenium-mg/kg diet)	0	1.5	3
Effective Number of Animals	30	30	30
Food Intake (g/week; mean ± SD −) 4 weeks	132 ± 12.7	138 ± 6.1	143 ± 5.6*
8 weeks	140 ± 3.2	143 ± 10.0	144 ± 4.9
24 weeks	132 ± 12.7	134 ± 8.0	137 ± 3.1
Lesion Incidence	**No. (%) of Rats with Lesion**:		
Pituitary tumors:	**30 (100)**	**30 (100)**	**30 (100)**
Mammary Adenocarcinomas:			
Total Mammary Cancer Incidence	**8 (27)**	**3 (10)**	**5 (17)**
Tubulo-papillary carcinomas	5 (17)	2 (7)	4 (14)
Compact/cribriform-comedo carcinomas	3 (10)	1 (3)	1 (3)
Other tumors:			
Thymic lymphoma	3 (10)	1 (3)	3 (10)
Localized lymphoma of lymph nodes	1 (3)	1 (3)	2 (7)
Skin: Squamous papilloma	0	1 (3)	0
Adrenal pheochromocytoma	2 (7)	1 (3)	1 (3)

* $P < 0.01$ for difference with control group (ANOVA with Dunnett's multiple comparison test).

Table 4

Effect of α-Tocopherol on Prostate Carcinogenesis and Non-Neoplastic Prostate Lesions in 17β-Estradiol + Testosterone−Treated NBL Rats

Group	**1**	**2**	**3**
α-Tocopherol dose (mg/kg diet)	0	2,000	4,000
Effective Number of Animals	29	30	30
Mortality (weeks after start hormone treatment):			
Mean week of death ± standard deviation	42 ± 7.3	45 ± 6.6	42 ± 5.4
Median week of death (95% confidence interval)	42 (39,45)	43 (42,47)	41 (39,43)
Range (minimum – maximum)	27 – 54	28 – 52	27 – 54
Lesion Incidence	**No. (%) of Rats with Lesion**:		
Adenocarcinoma:			
Total Cancer Incidence	**26 (90%)**	**26 (87%)**	**25 (83%)**
Periurethral dorsolateral-anterior prostatic ducts alone	22 (76)	26 (87)	24 (80)
Distal anterior prostate ducts alone	17 (59)	22 (73)	16 (53)
Dorsolateral prostate lobe (glandular portion)	1 (3)	1 (3)	0
Tumor Multiplicity: One or two carcinomas	9 (31)	6 (20)	11 (37)
More than two carcinomas	17 (57)	20 (67)	14 (47)
Carcinoma *in situ*:			
Anterior prostate (glands & ducts)	1 (3)	0	1 (3)
Seminal vesicle (glands & ducts)	5 (17)	7 (23)	4 (13)
Dysplasia in anterior prostate/seminal vesicle (No. animals with lesion/evaluable animals):			
Anterior prostate (glands & ducts)	6/20 (30)	12/23 (52)	12/26 (46)
Seminal vesicle (glands & ducts)	3/20 (15)	2/23 (9)	3/26 (12)
Inflammation & Dysplasia in Lateral Prostate:			
Inflammation (No. animals with lesion/evaluable animals):			
Total incidence	29/29 (100)	30/30 (100)	30/30 (100)
Marked inflammation	5 (17)[a]	3 (10)[a]	0[a]
Slight – moderate inflammation	24 (83)[a]	27 (90)[a]	30 (100)[a]
Dysplasia associated with inflammation (No. animals with lesion/evaluable animals):			
Total incidence	23/23 (100)	26/26 (100)	26/26 (100)
Marked dysplasia	19 (83)[b]	15 (58)[b]	10 (38)[b]
Slight – moderate dysplasia	4 (17)[b]	11 (42)[b]	16 (62)[b]
Dysplasia not associated with inflammation (No. animals with lesion/evaluable animals):			
Total incidence	21/22 (100)	17/22 (100)	17/26 (100)
Marked dysplasia	8 (36)[c]	1 (5)[c]	1 (4)[c]
Slight – moderate dysplasia	13 (59)[c]	16 (72)[c]	16 (62)[c]
No dysplasia	1 (5)[c]	5 (23)[c]	9 (34)[c]

Data were tested for significance using Fisher's exact test and for linear trend with dose using a 2 × 3 chi-square test. Differences were considered statistically significant at $P \leq 0.05$ (2-sided for Fisher's exact test). Note that for lesions that can only be assessed micropscopically, the number of evaluable animals (with non-autolytic tissue) was often lower than the effective number of animals; the prostate of one rat in the control group was lost to cannibalism, reducing the effective number of animals.

[a] P = 0.0667; P for trend = 0.0205 (chi-square test).

[b] P = 0.0074; P for trend = 0.0018 (chi-square test).

[c] P = 0.0017; P for trend > 0.05, but 0.0017 when combining the slight-moderate and none categories (chi-square test).

Table 5

Effect of α-Tocopherol on Extra-Prostatic Tumor Development in 17β-Estradiol + Testosterone−Treated NBL Rats

Group	**1**	**2**	**3**
α-Tocopherol acetate dose (mg/kg diet)	0	2,000	4,000
Effective Number of Animals	30	30	30
Lesion Incidence	**No. (%) of Rats with Lesion**:		
Pituitary tumors:	**30(100)**	**30(100)**	**30(100)**
Mammary Adenocarcinoma:			
Total Cancer Incidence	**1 (3)**[a]	**8 (27)**[a,b]	**7 (23)**[a,c]
Tubulo-papillary carcinomas	0	5 (17)	5 (17)*
Compact tubular carcinomas	1 (3)	1 (3)	1 (3)
Cribriform carcinomas	0	2 (7)	2 (7)*
Other tumors:			
Mammary fibroadenoma	0	0	1 (3)
Thymic lymphoma	2 (7)	2 (7)	2 (7)
Localized lymphoma of lymph nodes	2 (7)	2 (7)	1 (3)
Adrenal pheochromocytoma	1 (3)	2 (7)	0
Abdominal mesothelioma	2 (7)	0	0

Data were tested for significance using Fisher's exact test and for linear trend with dose using a 2 × 3 chi-square test. Differences were considered statistically significant at $P \leq 0.05$ (2-sided for Fisher's exact test).

[a] P = 0.0381; P for trend = 0.0428 (2 × 3 chi-square test).

[b] P = 0.0257 for difference with control group (2-sided Fisher's exact test).

[c] P = 0.0523 for difference with control group (2-sided Fisher's exact test).

* One animal had both a tubulo-papillary and a cribriform carcinoma