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Null Activity of Selenium and Vitamin E as Cancer Chemopreventive Agents in the Rat Prostate[1]

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Abstract

To evaluate the potential efficacy of selenium and vitamin E as inhibitors of prostate carcinogenesis, four chemoprevention studies using a common protocol were performed in a rat model of androgen-dependent prostate cancer. After stimulation of prostate epithelial cell proliferation by a sequential regimen of cyproterone acetate followed by testosterone propionate, male Wistar-Unilever rats received a single intravenous injection of *N*-methyl-*N*-nitrosourea (MNU) followed by chronic androgen stimulation via subcutaneous implantation of testosterone pellets. At one week post-MNU, groups of carcinogen-treated rats (39–44/group) were fed either basal diet or basal diet supplemented with L-selenomethionine (3 or 1.5 mg/kg diet; Study 1); DL-α-tocopherol (Vitamin E; 4000 or 2000 mg/kg diet; Study 2); L-selenomethionine + Vitamin E (3 + 2000 mg/kg diet or 3 + 500 mg/kg diet; Study 3), or selenized yeast (target selenium levels of 9 or 3 mg/kg diet; Study 4). Each chemoprevention study was terminated at 13 months post-MNU, and prostate cancer incidence was determined by histopathologic evaluation. No statistically significant reductions in prostate cancer incidence were identified in any group receiving dietary supplementation with selenium and/or vitamin E. These data do not support the hypotheses that selenium and vitamin E are potent cancer chemopreventive agents in the prostate, and when considered with the recent clinical data reported in the SELECT trial, demonstrate the predictive nature of this animal model for human prostate cancer chemoprevention.

Keywords

Prostate cancer; chemoprevention; selenium; vitamin E; selenomethionine

INTRODUCTION

The prostate presents perhaps the ideal target for human cancer chemoprevention: Prostate cancer occurs in high incidence in Western male populations (1,2), the incidence of both putative preneoplastic prostate lesions and prostate cancers increases with age (3,4); and precancerous and early cancerous lesions may remain at a subclinical stage for many years,

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thus offering an extended period for interventions directed at the prevention of clinically significant disease (5,6).

Because carcinoma of the prostate occurs primarily in elderly men, any delay in its development that may be achieved through pharmacologic, hormonal, or nutritional interventions could result in substantial reductions in cancer morbidity and mortality. Furthermore, even a modest reduction in the slope of the cancer latency curve could delay the onset of clinically significant disease until far later in life. As such, the often decades-long latent period for prostate cancer development suggests that strategies designed to inhibit tumor progression could be effective when initiated in middle-aged or elderly men, with the goal of stabilizing and/or reversing preneoplastic or incipient neoplastic lesions. Stabilizing or reversing preneoplastic lesions or early neoplasms may not only reduce prostate cancer incidence and associated morbidity, but could also result in a significant decrease in prostate cancer mortality.

The potential activity of selenium (Se) as a cancer preventive agent has been of interest since its identification in the 1970's as a component of glutathione peroxidase (7). Se is present at the active site of the enzyme, and mediates glutathione peroxidase-catalyzed reduction of hydrogen peroxide and lipid hydroperoxides (8,9). Human exposure to Se is extensive, and results primarily from consumption of foodstuffs containing selenoamino acids such as selenomethionine (SeMet; 10, 11). Regional variations in Se levels in foods and drinking water have led to the hypothesis that at least some geographic differences in cancer incidence patterns may reflect differences in population Se status (12).

Experimental data from studies conducted in animal models and epidemiologic data from studies of human populations suggest a possible inverse relationship between Se intake and cancer risk in several organs. Dietary supplementation with Se inhibits cancer induction in a number of *in vivo* carcinogenesis models, including animal models for neoplasms of the skin, mammary gland, liver, and colon (reviewed in 13). However, Se compounds are not universally active as chemopreventive agents: negative results and/or enhancement of carcinogenesis have been reported in animal models for cancer of the pancreas, liver, and skin (14–16).

Epidemiologic investigations of Se status and cancer risk provide a similar picture. Recent studies have noted an inverse relationship between Se status and cancer risk in several tissues, including the esophagus, stomach, lung, and prostate (17). In the prostate, early studies by Willett *et al*. (18) and Criqui *et al*. (19) suggested higher prostate cancer risk in men with low serum Se; the results of two more recent investigations also suggest that human prostate cancer risk may be inversely related to Se status (20,21). By contrast, the European Prospective Investigation into Cancer and Nutrition (EPIC) trial found no relationship between plasma Se levels and prostate cancer risk (22); a similar null relationship was reported in the Carotene and Retinol Efficacy (CARET) Trial (23). Interestingly, although the results a study using samples from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening (PLCO) Trial failed to demonstrate a relationship between serum Se and prostate cancer risk in the overall study population, the results of this trial data did suggest a possible protective effect of high serum Se in individuals with a high intake of vitamin E (24).

Of particular relevance to the present studies is the Nutritional Prevention of Cancer (NPC) Study, an intervention trial in which a significant reduction in prostate cancer incidence was reported in men who received Se supplements (as selenized yeast) for periods averaging 4.5 years (25–27). While the NPC trial represents a potentially landmark finding, it should be noted that the study was not conducted as a prostate cancer prevention trial, but was designed to study the effect of Se administration on skin cancer. As such, evaluation of the effects of Se supplementation on prostate cancer incidence and the observation of Se protection against prostate cancer were *post-hoc* processes (secondary endpoints), and were outside of the original

hypothesis that was investigated. Interestingly, the original hypothesis studied by Clark *et al.* (25,26), that skin cancer incidence would be reduced by Se supplementation, was not substantiated by this work. More recently, the Vitamins and Lifestyle (VITAL) study found no association between use of Se supplements and prostate cancer risk (28).

The results of the NPC trial reported by Clark and colleagues provided the primary rationale supporting the design and conduct of the SELECT trial, a prospective randomized Phase III intervention trial for prostate cancer prevention in which more than 35,000 men received SeMet (200 μg/day), α-tocopherol (vitamin E; 400 IU/day), SeMet + vitamin E, or placebo (29,30). This study was recently terminated after an interim analysis showed no prostate cancer risk reduction in groups receiving SeMet and/or vitamin E, and possible adverse effects of the interventions being tested (31). Notably, the SELECT trial investigators identified a marginally significant ($p = 0.06$) increase in prostate cancer risk in the vitamin E group, but not in groups exposed to either selenium alone or selenium plus vitamin E.

The present report summarizes the results of four *in vivo* studies that were performed to evaluate the efficacy of SeMet, vitamin E, SeMet + vitamin E, and selenized yeast as inhibitors of androgen-dependent carcinogenesis in the rat prostate. These studies were designed in consideration of the results reported by Clark and colleagues (25), and provide an experimental correlate to the NPC, PLCO, and SELECT trials. The Wistar-Unilever rat model employed in these studies has been used extensively to identify agents with cancer preventive activity in the prostate; we have previously reported that prostate carcinogenesis in this model can be inhibited by 9-*cis*-retinoic acid (32); Bowman-Birk Inhibitor (33); a soy isoflavone mixture (33); dehydroepiandrosterone (34); and 16α-fluoro-5-androsten-17-one (fluasterone; 35). Most prostate tumors in the Wistar-Unilever rat model are adenocarcinomas originating in the dorsolateral prostate (36). The morphology of these cancers has been described in detail (36), and is to a large extent comparable to human prostate cancer (37,38). Prostate cancers induced in the model grow progressively (39), and eventually develop into large pelvic masses that kill the host by obstructing urinary flow. Gross metastatic lesions have been identified in approximately 60% of animals in which prostate cancers have been allowed to progress until death occurs (36).

Importantly, studies in this model with chemopreventive agents for which human data exist suggest that the model is predictive of human responses, as shown with N-(4-hydroxyphenyl) retinamide (4-HPR; fenretinide; 37, 40), anti-androgens (41,42), and the results of the present studies. Preliminary reports of individual studies comprising portions of the present data have been presented at U.S.-based and international scientific meetings (43–45).

MATERIALS AND METHODS

Four separate 13-month carcinogenesis studies were conducted using a common protocol to determine the efficacy of (a) SeMet; (b) vitamin E; (c) SeMet + vitamin E; and (d) selenized yeast as inhibitors of prostate cancer induction in Wistar-Unilever (W/U) rats by *N*-methyl-*N*-nitrosourea (MNU) + testosterone.

Animals and animal husbandry

For each study, approximately 140 male Wistar-Unilever rats (HsdCpb/WU; 7 to 8 weeks of age at the time of receipt) were purchased from virus-free barrier colonies at Harlan/Sprague-Dawley, Limburg, Netherlands. All rats were held in quarantine for a minimum of one week prior to the initiation of hormone pretreatment. Throughout all studies, rats were housed in pairs on hardwood bedding in suspended polycarbonate cages. All animals were housed in windowless rooms that were illuminated for 12 hours each day and maintained at 22 ± 1°C and within the range of 30% to 70% relative humidity. Throughout all studies, animals had

free access to chow diet (Teklad 4% Fat Rat/Mouse diet [Harlan/Teklad, Madison, WI] in the SeMet, vitamin E, and SeMet + vitamin E studies; Purina 5001 Laboratory Chow [PMI Nutrition, Brentwood, MO] in the Se Yeast study) and City of Chicago drinking water (supplied by automatic watering system).

Pretreatment

After release from quarantine, rats received daily oral (gavage) doses of 50 mg cyproterone acetate (in sesame oil [5 ml/kg]; Berlex Laboratories, Wayne, NJ, or Sigma Chemical, St. Louis, MO) per kg body weight for 21 consecutive days. One day after the final dose of cyproterone acetate, rats received daily subcutaneous injections of 100 mg testosterone propionate (in sesame oil [2 ml/kg]; Sigma) per kg body weight for three days. This sequence of anti-androgen (cyproterone acetate) followed by androgen (testosterone propionate) results in maximal stimulation of prostatic epithelial proliferation at approximately 60 hours after the first dose of androgen. Administration of carcinogen at the time of maximum proliferation of the prostate epithelium maximizes the neoplastic response in this tissue.

Carcinogen administration and hormone post-treatment

Dosing solutions containing MNU (Ash-Stevens, Inc., Detroit MI) were prepared immediately prior to use in a vehicle of sterile saline (pH 5.0), and were protected from light during all manipulations. At 60 hours after the first dose of testosterone propionate, all rats received a single intravenous injection of 30 mg MNU per kg body weight. One week after MNU exposure, carcinogen-treated rats received subcutaneous implants of two silastic capsules, each containing 40 mg crystalline testosterone (Sigma). Silastic capsule implants were replaced at intervals of 26 weeks throughout the study.

Administration of chemopreventive agents

L-Selenomethione (≥ 98% purity) and DL-α-tocopherol acetate (Vitamin E; ≥ 96% purity) were purchased from Sigma. Se yeast (SelenoExcell, Cypress Systems, Fresno, CA) was supplied by the Division of Cancer Prevention, National Cancer Institute. Dose levels of each agent that were used in prostate cancer chemoprevention studies were selected to prevent suppression of body weight gain or other clinical evidence of systemic toxicity, as determined in a preliminary six-week toxicity/diet tolerance study that was conducted for each agent or agent combination.

One week after MNU administration, rats were assigned to experimental groups (39 to 44 rats per group, depending on study) using a computer-generated randomization process that blocks for body weight. Dietary administration of chemopreventive agents was initiated at this time, and was continued until the termination of each study at 13 months. To optimize diet uniformity, SeMet and vitamin E were mixed into experimental diets using a sucrose carrier (total level of agent + sucrose carrier = 10 g/kg); control diets in those studies were supplemented with sucrose carrier only. Fresh batches of each experimental diet were prepared weekly, and were stored at −20 °C prior to use; batches of experimental diets were analyzed monthly throughout each study to confirm the concentration of chemopreventive agents. All food and bedding materials in animal cages were changed twice weekly.

In-life observations

In all studies, rats were observed at least once daily to assess their general health, and were weighed weekly. In selected studies, blood samples for quantitation of plasma levels of chemopreventive agents were collected after 1 and 26 weeks of agent administration. Beginning at 6 months post-MNU, each rat was palpated weekly to monitor the development of accessory sex gland masses.

Plasma drug level analyses

Plasma Se levels were analyzed using a using a fluorometric procedure (46,47), and National Bureau of Standards oyster standard for quality control. After sample digestion with nitric, perchloric, and hydrochloric acids, the selenite in the digestate was treated with 2,3-diaminonaphthalene (DAN) and extracted with cyclohexane. The fluorescence of the Se-DAN complex in the extract was measured using an excitation wavelength of 366 nm and an emission wavelength of 606 nm.

Plasma tocopherol levels were quantitated by HPLC, using a minor modification of the method of Driskell *et al.* (48). Plasma samples were mixed with ethanol, vortex mixed, extracted with hexane, and centrifuged. The hexane layer was removed and evaporated, and the residue was dissolved in ethanol for analysis using a solid phase consisting of a Whatman Partisil C18 ODS-3 (10 μm) column and an isocratic mobile phase consisting of 100% methanol. Detection was via UV absorbance at 285 nm.

Post-mortem procedures

Rats identified as moribund were euthanized and necropsied immediately. At 13 months post-carcinogen, surviving animals in each study were euthanized by CO_2 inhalation and necropsied. At necropsy, the accessory sex glands were excised *en bloc* with the urinary bladder. The urinary bladder was then removed from the tissue block, and a total weight was obtained for the accessory sex glands. Accessory sex glands were fixed in 10% neutral buffered formalin for histologic processing.

The approach used for histologic preparation of accessory sex glands has been described in detail (37). Histopathologic evaluations were performed on all gross lesions in the accessory sex glands, on step sections taken at intervals of 250 μm from the dorsolateral prostate, anterior prostate, and seminal vesicle (six step sections per tissue), and on one section from the ventral prostate. Microscopic lesions in the accessory sex glands were classified using previously described criteria (37,38).

Statistical evaluations

Lesion incidence values were calculated as the number of rats in an experimental group that demonstrated a specific lesion, divided by the "effective number of animals" in that group. The "effective number of animals" in each group includes all animals that survived for longer than 9 months (and were therefore considered at risk for prostate carcinogenesis), but excludes any animals whose tissues were lost to follow-up as a result of either cannibalism or autolysis.

Statistical comparisons of inter-group differences in lesion incidence were limited to histologically confirmed tumors only. Evidence of anticarcinogenic activity was defined as a statistically significant ($p < 0.05$) reduction in the incidence of cancer in a group receiving a chemopreventive agent in comparison to its dietary control group. For each study, separate evaluations are presented for (a) the total incidence of cancers in all accessory sex glands combined and (b) cancers that were clearly confined to the dorsolateral + anterior prostate. Comparisons of prostate cancer incidence and animal survival at study termination were performed using chi-squared analysis and Fisher's exact test. Comparisons of continuous data (animal body weights and plasma levels of chemopreventive agents) were performed using analysis of variance, with *post-hoc* comparisons made using Dunnett's test.

RESULTS

Chemoprevention efficacy evaluation of L-selenomethionine in the rat prostate

The first study was performed to evaluate the chemopreventive efficacy of SeMet administered as a single agent. In this study, groups of 39 or 40 MNU-treated rats received either basal diet only (control), or basal diet supplemented with SeMet at doses of 3.0 or 1.5 mg/kg diet. The high dose (3.0 mg SeMet per kg diet) was selected on the basis of body weight data generated in a preliminary six-week dose tolerance study in which SeMet was administered at doses ranging from 0.75 mg/kg diet to 12 mg/kg diet (data not shown).

In the chemoprevention study, dietary supplementation with SeMet had no effect on prostate cancer induction by MNU + testosterone (Table 1). The total incidence of accessory sex gland cancers in the dietary control group was 79% (30/38); by comparison, incidences of accessory gland cancer in groups fed the low and high doses of SeMet were 77% (30/39) and 68% (25/37), respectively ($p > 0.10$ for both comparisons). A similar pattern was seen when comparisons were limited to cancers that were clearly confined to the dorsolateral + anterior prostate: in comparison to a prostate cancer incidence of 53% (20/38) in the dietary control group, groups fed the low and high doses of SeMet demonstrated cancer incidences of 44% (17/39) and 51% (19/37), respectively ($p > 0.10$ for both comparisons).

The SeMet dose levels used in this study induced no evidence of toxicity in any treated animal. Survival curves for both groups treated with SeMet were comparable to that of the dietary control group at all times throughout the exposure period (data not shown). At study termination, survival was 77% (30/39) and 85% (34/40) in groups exposed to the low and high doses of SeMet, respectively, versus a survival of 82% (32/39) in the dietary control group (Table 1). Body weights were also comparable in all groups at all times in the study; at study termination, mean body weights in groups receiving dietary supplementation with SeMet were 100.5% and 102.6% of dietary controls.

Administration of the high dose (3 mg/kg diet) of SeMet resulted in a modest (11 to 16%) but statistically significant increase in plasma Se levels after both 1 and 26 weeks of exposure (Table 2). Plasma Se levels in rats receiving the low dose (1.5 mg/kg diet) of SeMet were similar to dietary controls at both time points. The apparent temporal variation in plasma Se levels in the study is worthy of note, as higher plasma Se levels were measured in samples collected from all groups (including dietary controls) at study week 26 (July) versus those collected during study week 1 (January). Although the reasons underlying this variation are not clear, seasonal changes in the composition of the chow diets used in the study may be responsible for these temporal differences in plasma Se levels within all experimental groups.

Chemoprevention efficacy evaluation of DL-α-tocopherol (vitamin E) in the rat prostate

The second study was performed to evaluate the chemopreventive efficacy of DL-α-tocopherol acetate (vitamin E, administered as a single agent) in the rat prostate cancer model. In this study, groups of 40 MNU-treated rats received either basal diet only (control), or basal diet supplemented with vitamin E at 4000 or 2000 mg/kg diet. The dose of 4000 mg vitamin E/kg diet was selected as the high dose for the chemoprevention trial on the basis of the results of a six-week toxicity/diet tolerance study, in which a suppression of body weight gain was identified in rats fed vitamin E at 5000 mg/kg diet (data not shown).

As was the case with SeMet alone, comparisons of (a) total cancer incidence in all accessory sex glands and (b) incidences of cancers that were clearly limited to the dorsolateral + anterior prostate failed to identify any chemopreventive activity of vitamin E in the rat prostate (Table 3). In comparison to a 63% incidence (25/40) of accessory sex gland cancers in the dietary control group, groups fed the low and high doses of vitamin E demonstrated accessory sex

gland cancer incidences of 53% (21/40) and 68% (27/40), respectively ($p > 0.10$ for both comparisons). The high dose of vitamin E did appear to protect against cancer induction in the seminal vesicle (5% incidence [2/40] versus 20% incidence [8/40] in dietary controls; $p < 0.05$). However, this decrease was more than offset by an increase in the incidence of cancers confined to the dorsolateral + anterior prostate in the group receiving high dose vitamin E (55% [22/40] in the high dose vitamin E group versus 35% [14/40] in the dietary control group). Although only marginally significant ($0.05 < p < 0.10$), the increased prostate cancer incidence in rats receiving the high dose of vitamin E is of interest in consideration of the results of the SELECT trial, in which a marginally significant ($p = 0.06$) increase in prostate cancer risk was seen in the group exposed to vitamin E only (31).

Of concern was a statistically significant decrease in survival in rats receiving the high dose of vitamin E (Table 3). At study termination, survival in the group receiving the high dose of vitamin E was 75% (30/40). This survival is significantly ($p < 0.05$) poorer than both the 93% survival (37/40) observed in dietary controls and the 95% survival (38/40) in rats receiving the low dose of vitamin E.

Neither dose level of vitamin E had any effect on animal body weight at any time in the study. Vitamin E also induced no evidence of gross toxicity identifiable through clinical observations, gross pathology, or other evidence of organ-specific toxicity identified at the terminal necropsy.

Dietary supplementation with vitamin E resulted in a 3- to 4-fold elevation of plasma α-tocopherol levels in comparison to levels measured in the dietary control group ($p < 0.01$ versus dietary control for both dose groups; Table 4). Although mean plasma α-tocopherol levels were higher in rats fed the high dose of vitamin E than in rats fed the low dose, differences in plasma α-tocopherol levels in the two groups fed supplemental vitamin E did not differ significantly from one another.

Chemoprevention efficacy evaluation of combined administration of L-selenomethionine+ DL-α-tocopherol (vitamin E) in the rat prostate

Because the SELECT trial was designed to evaluate the possible interaction between Se and vitamin E in prostate cancer chemoprevention, a third study was performed in our rodent model to determine the chemopreventive efficacy of combined administration of SeMet + vitamin E. In this study, SeMet was administered at 3.0 mg/kg diet in combination with vitamin E at either 500 or 2000 mg/kg diet.

Consistent with the results of studies in which SeMet or vitamin E were administered as single agents, comparisons of cancer incidence in all accessory sex glands and in the dorsolateral + anterior prostate failed to identify any chemopreventive activity of the agent combination (Table 5). In comparison to a 60% incidence (25/42) of accessory sex gland cancers in the dietary control group, a cancer incidence of 67% (28/42) was seen in both groups fed the SeMet + vitamin E ($p > 0.10$ for both comparisons). Comparisons of cancer incidence in the dorsolateral + anterior prostate also failed to identify evidence of anticarcinogenic efficacy for the agent combination: prostate cancer incidences (40% [17/42] and 38% [16/42]) in groups fed SeMet + the low and high doses of vitamin E were both greater than was the cancer incidence in dietary controls (26% [11/42]; $p > 0.10$ for both comparisons).

As was seen in the studies with SeMet alone and with vitamin E alone, administration of SeMet + vitamin E in combination induced no clinical evidence of toxicity in any treated animal. Survival at study termination was comparable in all groups (Table 5). Mean terminal body weights in groups treated with SeMet + vitamin E were 98.8% and 102.3% of dietary controls.

Chemoprevention efficacy evaluation of selenized yeast in the rat prostate

In the NPC trial, Clark *et al*. (25) used selenized yeast as a means for Se administration. To provide an experimental correlate for this intervention trial, a study was performed to investigate the efficacy of selenized yeast as an inhibitor of cancer induction in the rat prostate model.

The source of selenized yeast used in our rat prostate cancer chemoprevention study was identical to that used by Clark and colleagues; the lot of yeast used in our studies contained 1225 mg Se/kg diet. Prior to the chemoprevention study, a preliminary toxicity/diet tolerance study was performed in which rats were fed selenized yeast at levels ranging from 1.64 to 19.6 g per kg diet; these dietary levels of selenized yeast provided target Se levels ranging from 2 to 24 mg/kg diet. The results of this preliminary study (not shown) demonstrated significant body weight loss within two weeks in rats fed selenized yeast at levels that provided dietary Se supplements of ≥ 12 mg/kg diet. By contrast, dietary administration of selenized yeast at doses that provided supplemental Se at levels ≤ 9 mg/kg diet had no effect on body weight gain, and induced no other clinical evidence of toxicity during a six-week exposure period. On the basis of these results, selenized yeast levels of 7.35 and 2.45 g/kg diet were selected for use in the prostate cancer chemoprevention bioassay; these doses of selenized yeast provided target dietary Se supplements of 9 mg/kg diet and 3 mg/kg diet, respectively.

The data presented in Table 6 demonstrate that selenized yeast conferred at most very modest protection against prostate cancer induction by MNU + testosterone. Selenized yeast had no statistically significant effect on the total incidence of accessory sex gland cancers: whereas the dietary control group demonstrated an 81% incidence (35/43) of cancer in all accessory sex glands combined, the total incidences of accessory sex gland cancers in groups fed the low and high doses of selenized yeast were 74% (31/42) and 68% (30/44), respectively ($p > 0.10$ for both comparisons). When the analysis is limited to lesions that were clearly confined to the dorsolateral + anterior prostate, selenized yeast did demonstrate possible dose-related chemopreventive activity: in comparison to a 53% incidence (23/43) of prostate cancer in dietary controls, rats fed the high dose of selenized yeast demonstrated a cancer incidence of 36% (16/44; $0.05 < p < 0.10$). By contrast, the 45% incidence (19/42) of cancers that were clearly confined to the dorsolateral + anterior prostate in the group receiving the low dose of selenized yeast did not differ from control ($p > 0.10$).

Survival in both groups receiving chronic dietary exposure to selenized yeast was reduced from that in dietary controls; the 65% survival (28/43) in rats fed the low dose of selenized yeast was significantly reduced ($p < 0.05$) in comparison to the dietary control group (86% [38/44]; Table 6). Body weights were comparable in all study groups throughout the study; at study termination at 13 months, mean body weights in groups fed the low and high doses of selenized yeast were 103.7% and 101.8% of control body weights, respectively. No evidence of Se toxicity was identified in any animal on the basis of clinical observation.

Dietary administration of selenized yeast resulted in dose-related increases in plasma Se (Table 7). At the high dose of selenized yeast (target Se level of 9 mg/kg diet) plasma Se levels were increased by 29% and 22% from control after 1 week and 26 weeks of exposure. At the low dose of selenized yeast (3 mg/kg diet), direct comparisons can be made between Se administered as selenized yeast and as SeMet. At this dose level, the percentage increase in plasma Se (versus the parallel control group) in rats fed selenized yeast were greater than those seen in rats fed SeMet: after 1 and 26 weeks of exposure, plasma Se levels in the group exposed to selenized yeast at a target Se concentration of 3 mg/kg diet were increased by 23% and 15%, respectively (Table 7), whereas plasma Se levels were increased by 17% and 12% in groups fed SeMet at 3 mg/kg diet (Table 2).

DISCUSSION

The results of the present series of rodent chemoprevention studies do not support the hypotheses that Se and vitamin E are effective agents for prostate cancer chemoprevention. Notably, the lack of activity of SeMet in our rat model is consistent with the results of the SELECT trial, in which SeMet was found to be inactive in prostate cancer chemoprevention in humans (31). When administered as a single agent at doses of 1.5 mg/kg diet or 3 mg/kg diet, SeMet was non-toxic, but was also completely inactive in prostate cancer chemoprevention in the Wistar-Unilever rat model. The lack of activity of SeMet in this study was not a function of dose; similar doses of SeMet and other Se compounds confer protection against carcinogenesis in animal models for cancer in several other organ sites (reviewed in 13).

Vitamin E also failed to demonstrate significant evidence of efficacy in prostate cancer prevention in the Wistar-Unilever rat model. Neither dose level of vitamin E had any significant effect on the total incidence of accessory sex gland cancers. A small but significant reduction in the incidence of seminal vesicle cancers was seen in rats receiving the high dose of vitamin E; however, the group receiving the high dose of vitamin E also demonstrated a quantitatively larger (but only marginally significant; $0.05 < p < 0.10$) increase in the incidence of cancers that were confined to the dorsolateral + anterior prostate. In comparison to the very high incidence of prostate cancer in humans, primary carcinomas of the seminal vesicles are very rare; a recent case study identified only approximately 50 case reports of primary cancers of the seminal vesicles in the published literature (49). In consideration of both the questionable anatomic relevance of this malignancy to human prostate cancer, and the fact that rats receiving the high dose of vitamin E demonstrated a quantitatively larger increase in the number of cancers that were clearly limited to the dorsolateral + anterior prostate, the protection against seminal vesicle cancer seen in the present study is considered to be of little or no significance to the prevention of human prostate cancer.

The results of our vitamin E study in the Wistar-Unilever rat are consistent with the results of the two largest randomized intervention trials in which vitamin E supplementation was evaluated for efficacy in prostate cancer chemoprevention. The marginally significant ($0.05 < p < 0.10$) increase in prostate cancer risk seen in rats fed the high dose of vitamin E in our study is closely correlated to the marginally significant ($p = 0.06$) increase in prostate cancer risk identified in the SELECT trial cohort that was exposed to vitamin E (31). Our data are also consistent with the results of the Physician's Health Study II, in which it was recently reported that supplementation with vitamin E had no significant effect on prostate cancer risk (50).

Additional negative data were obtained in the chemoprevention efficacy evaluation of the combination regimen of SeMet + vitamin E. In this study, groups exposed to SeMet + vitamin E demonstrated small (and non-significant) increases in the total incidence of cancer in the accessory sex glands, and in the incidence of cancers that were clearly confined to the dorsolateral + anterior prostate; no evidence of anticarcinogenic activity was identified. Again, these data correlate well with the results of the SELECT trial, in which a non-significant increase in prostate cancer risk was seen in the group receiving SeMet + vitamin E.

The efficacy evaluation of selenized yeast provided the only potential evidence of chemopreventive activity against prostate cancer that was found in the four studies. In this study, rats receiving the high dose of selenized yeast (target Se level of 9 mg/kg diet) demonstrated a modest reduction in the incidence of cancers that were clearly confined to the dorsolateral + anterior prostate. However, this decrease was not significant at the 5% level of confidence ($0.05 < p < 0.10$), and selenized yeast had no significant effect on the total incidence of accessory sex gland cancers. The results of a preliminary toxicity/dose selection study

demonstrated that the high dose of selenized yeast used in this study (target Se dose of 9 mg/kg diet) is very close to the maximum tolerated dose for this agent; although no adverse effects of administration of selenized yeast at this dose were seen in either the preliminary toxicity study or the chemoprevention study, administration of selenized yeast at a target Se level of 12 mg/kg induced significant systemic toxicity in the preliminary study, as indicated by rapid body weight loss. On this basis, it is considered highly unlikely that greater chemopreventive efficacy can be achieved through administration of selenized yeast at doses higher than those used in the present investigation.

The results of the present prostate cancer chemoprevention efficacy evaluation of selenized yeast in rats differ from those of the NPC trial reported by Clark and colleagues (25,26). In that trial, selenized yeast was administered to 974 male skin cancer patients in the attempt to prevent the development of additional skin cancers. Although administration of selenized yeast failed to reduce skin cancer incidence in that population, *post-hoc* evaluations of cancer incidence identified a reduction in prostate cancer risk in individuals exposed to selenized yeast. This finding was a major factor underlying the rationale for the design of the SELECT trial (30,51); in that trial, however, the apparent chemopreventive activity of Se that was found in the NPC trial was not confirmed (31). The results of the present studies in a rodent model system, in which no prostate cancer chemopreventive activity was identified for SeMet, vitamin E, or SeMet + vitamin E (originally presented in abstract form in 43, 44) provided what appear to be accurate predictions of the results of the SELECT trial.

The lack of activity of Se compounds and vitamin E as chemopreventive agents in the rat prostate cancer model cannot be ascribed to lack of oral bioavailability. In all studies, chemopreventive agents were administered at a high dose that approximates the maximum tolerated dose (MTD) for that agent; dose levels of each agent or agent combination were selected on the basis of preliminary toxicity/diet tolerance studies. Furthermore, administration of the high doses of each agent studied resulted in statistically significant increases in plasma levels of Se or vitamin E. It is also important to note that both SeMet and selenized yeast were found to be inactive in prostate cancer chemoprevention when administered at Se equivalent doses at which significant chemopreventive activity has been reported in other tissues (reviewed in 13).

The lack of chemopreventive activity of Se and vitamin E also cannot be ascribed to the lack of sensitivity of the model system used for efficacy evaluations. We have previously reported that prostate carcinogenesis in the Wistar-Unilever rat model can be inhibited by a broad range of agents with apparently diverse mechanisms of action. These agents include the RAR/RXR pan-agonist, 9-*cis*-retinoic acid (32); PTI G-2535, a characterized mixture of soy isoflavones (33); the soy-derived protease inhibitor, Bowman-Birk Inhibitor (33); the adrenal 17-ketosteroid, dehydroepiandrosterone (DHEA; 34), and the minimally androgenic DHEA analog, fluasterone (35). It should be noted, however, that several chemopreventive agents (*e.g.*, difluoromethylornithine [DFMO] and oltipraz) that demonstrate a broad range of anticarcinogenic activity in other organ sites are inactive in our rat prostate cancer model (43).

In conclusion, the results of four studies using a well-studied rat model for prostate carcinogenesis failed to identify any statistically significant chemopreventive activity of SeMet, vitamin E, SeMet + vitamin E, or selenized yeast. Comparisons of the present experimental data with the results of the SELECT trial (SeMet, vitamin E, and SeMet + vitamin E) and the Physicians Health Study II (vitamin E) suggest that the results of prostate cancer chemoprevention studies in the Wistar-Unilever rat model may serve as useful predictors of the results of large-scale, randomized clinical intervention trials for prostate cancer prevention.

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Table 1

Survival and prostate cancer incidence in rats receiving L-selenomethionine

Group	1	2	3
Chemopreventive agent	Control	SeMet	SeMet
Chemopreventive agent dose level (mg/kg diet)	-	1.5	3.0
Survival at study termination	32/39 (82)	30/39 (77)	34/40 (85)
Effective number of animals for histopathology	38	39	37
	No. (%) of rats with lesion:		
All accessory sex glands combined (dorsolateral and anterior prostate plus seminal vesicle):			
Adenocarcinoma or carcinosarcoma, all	30 (79)	30 (77)	25 (68)
Macroscopic size	11 (29)	10 (26)	6 (16)
Microscopic size only	19 (50)	20 (51)	19 (51)
Carcinoma *in situ* only	3 (8)	5 (13)	8 (22)
Dorsolateral plus anterior prostate (clearly confined to these glands):			
Adenocarcinoma, all	20 (53)	17 (44)	19 (51)
Macroscopic size	0	0	0
Microscopic size only	20 (53)	17 (44)	19 (51)
Carcinoma *in situ* only	3 (8)	4 (10)	6 (16)
Dorsolateral prostate region (originating from dorsolateral or anterior prostate or seminal vesicle):			
Adenocarcinoma or carcinosarcoma, all	8 (21)	9 (23)	6 (16)
Macroscopic size	7 (18)	7 (18)	5 (14)
Microscopic size only	1 (3)	2 (5)	1 (3)
Anterior prostate/seminal vesicle region (originating from anterior prostate or seminal vesicle):			
Adenocarcinoma, all	5 (13)	3 (8)	1 (3)
Macroscopic size	4 (11)	3 (8)	1 (3)
Microscopic size only	1 (3)	0	0
Seminal vesicle only (clearly confined to this gland):			
Adenocarcinoma, all	1 (3)	4 (10)	1 (3)
Macroscopic size	0	0	0
Microscopic size	1 (3)	4 (10)	1 (3)
Carcinoma *in situ* only	0	1 (3)	2 (5)
Sarcoma	1 (3)	0	0

Table 2

Plasma Se levels in rats receiving L-selenomethionine

		Plasma Se levels (ng/ml)	
Group	**SeMet dose (mg/kg diet)**	**1 week**	**26 weeks**
1	0	537 ± 52	803 ± 21
2	1.5	549 ± 42	803 ± 35
3	3.0	627 ± 65*	897 ± 11†

*$p < 0.05$ versus dietary control.

†$p < 0.01$ versus dietary control.

Table 3

Survival and prostate cancer incidence in rats receiving DL-α-tocopherol (vitamin E)

Group	1	2	3
Chemopreventive agent	Control	Vitamin E	Vitamin E
Chemopreventive agent dose level (mg/kg diet)	-	2000	4000
Survival at study termination	37/40 (93)	38/40 (95)	30/40 (75)†
Effective number of animals for histopathology	40	40	40
	No. (%) of rats with lesion:		
All accessory sex glands combined (dorsolateral and anterior prostate plus seminal vesicle):			
Adenocarcinoma or carcinosarcoma, all	25 (63)	21 (53)	27 (68)
Macroscopic size	3 (8)	4 (10)	3 (8)
Microscopic size only	22 (55)	17 (43)	24 (60)
Carcinoma *in situ* only	7 (18)	9 (23)	4 (10)
Dorsolateral plus anterior prostate (clearly confined to these glands):			
Adenocarcinoma, all	14 (35)	15 (38)	22 (55)*
Macroscopic size	0	0	0
Microscopic size only	14 (35)	15 (38)	22 (55)*
Carcinoma *in situ* only	7 (18)	7 (18)	4 (10)
Dorsolateral prostate region (originating from dorsolateral or anterior prostate or seminal vesicle):			
Adenocarcinoma or carcinosarcoma, all	2 (5)	3 (8)	3 (8)
Macroscopic size	2 (5)	2 (5)	3 (8)
Microscopic size only	0	1 (3)	0
Sarcoma	0	1 (3)	1 (3)
Anterior prostate/seminal vesicle region (originating from anterior prostate or seminal vesicle):			
Adenocarcinoma, all	1 (3)	1 (3)	1 (3)
Macroscopic size	1 (3)	1 (3)	1 (3)
Microscopic size only	0	0	0
Seminal vesicle only (clearly confined to this gland):			
Adenocarcinoma, all	8 (20)	4 (10)	2 (5)†
Macroscopic size	0	0	0
Microscopic size	8 (20)	4 (10)	2 (5)
Carcinoma *in situ* only	0	4 (10)	0
Sarcoma	0	2 (5)	0

* $0.05 < p < 0.10$ versus dietary control.

† $p < 0.05$ versus dietary control.

Table 4

Plasma α-tocopherol levels in rats receiving DL-α-Tocopherol (vitamin E)

		Plasma α-Tocopherol levels (μg/ml)	
Group	**DL-α-Tocopherol dose (mg/kg diet)**	**1 week**	**26 weeks**
1	0	4.9 ± 0.6	4.7 ± 2.0
2	2000	15.7 ± 1.8*	14.0 ± 3.7*
3	4000	16.0 ± 2.7*	15.8 ± 1.9*

* $p < 0.01$ versus dietary control.

Table 5

Survival and prostate cancer incidence in rats receiving combined administration of L-Selenomethionine + DL-α-tocopherol (vitamin E)

Group	1	2	3
Chemopreventive agents	Control	SeMet + E	SeMet + E
SeMet + vitamin E doses (mg/kg diet)	None	3 + 500	3 + 2000
Survival at study termination	33/42 (79)	34/42 (81)	35/42 (83)
Effective number of animals for histopathology	42	42	42
	No. (%) of rats with lesion:		
All accessory sex glands combined (dorsolateral and anterior prostate plus seminal vesicle):			
Adenocarcinoma or carcinosarcoma, all	25 (60)	28 (67)	28 (67)
Macroscopic size	14 (33)	10 (24)	11 (26)
Microscopic size only	11 (26)	17 (40)	17 (40)
Carcinoma *in situ* only	7 (17)	4 (10)	5 (12)
Sarcoma	1 (2)	0	0
Dorsolateral plus anterior prostate (clearly confined to these glands):			
Adenocarcinoma, all	11 (26)	17 (40)	16 (38)
Macroscopic size	0	0	0
Microscopic size only	11 (26)	17 (40)	16 (38)
Carcinoma *in situ* only	9 (21)	7 (17)	8 (19)
Dorsolateral prostate region (originating from dorsolateral or anterior prostate or seminal vesicle):			
Adenocarcinoma or carcinosarcoma, all	7 (17)	3 (7)	7 (17)
Macroscopic size	7 (17)	3 (7)	7 (17)
Microscopic size only	0	0	0
Anterior prostate/seminal vesicle region (originating from anterior prostate or seminal vesicle):			
Adenocarcinoma, all	6 (14)	5 (12)	3 (7)
Macroscopic size	6 (14)	5 (12)	3 (7)
Microscopic size only	0	0	0
Seminal vesicle only (clearly confined to this gland):			
Adenocarcinoma, all	4 (10)	5 (12)	5 (12)
Macroscopic size	0	1 (2)	1 (2)
Microscopic size only	4 (10)	4 (10)	4 (10)
Carcinoma *in situ* only	0	0	0

Table 6

Survival and prostate cancer incidence in rats receiving selenized yeast

Group	1	2	3
Chemopreventive agents	Control	Se yeast	Se yeast
Selenized yeast dose level (mg/kg diet)	None	2450	7350
Target selenium dose level (mg/kg diet)	0	3.0	9.0
Survival at study termination	38/44 (86)	28/43 (65)†	33/44 (75)
Effective number of animals for histopathology	43	42	44
	No. (%) of rats with lesion:		
All accessory sex glands combined (dorsolateral and anterior prostate plus seminal vesicle):			
Adenocarcinoma or carcinosarcoma, all	35 (81)	31 (74)	30 (68)
Macroscopic size	18 (42)	16 (38)	15 (34)
Microscopic size only	17 (40)	15 (36)	15 (34)
Carcinoma *in situ* only	5 (12)	3 (7)	5 (11)
Neurofibrosarcoma	0	0	1 (2)
Dorsolateral plus anterior prostate (clearly confined to these glands):			
Adenocarcinoma or carcinosarcoma, all	23 (53)	19 (45)	16 (36)*
Macroscopic size	1 (2)	0	0
Microscopic size only	22 (51)	19 (45)	16 (36)
Carcinoma *in situ* only	6 (14)	2 (5)	8 (18)
Dorsolateral prostate region (originating from dorsolateral or anterior prostate or seminal vesicle):			
Adenocarcinoma or carcinosarcoma, all	10 (23)	10 (24)	8 (18)
Macroscopic size	9 (21)	10 (24)	8 (18)
Microscopic size only	1 (2)	0	0
Neurofibrosarcoma	0	0	1 (2)
Anterior prostate/seminal vesicle region (originating from anterior prostate or seminal vesicle):			
Adenocarcinoma, all	6 (14)	5 (12)	6 (14)
Macroscopic size	6 (14)	5 (12)	6 (14)
Microscopic size only	0	0	0
Seminal vesicle only (clearly confined to this gland):			
Adenocarcinoma or carcinosarcoma, all	4 (9)	1 (2)	3 (7)
Macroscopic size	2 (5)	0	1 (2)
Microscopic size only	2 (5)	1 (2)	2 (5)
Carcinoma *in situ* only	2 (5)	1 (2)	1 (2)

* $0.05 < p < 0.10$ versus dietary control.

† $p < 0.05$ versus dietary control.

Table 7

Plasma Se levels in rats receiving dietary supplementation with selenized yeast

			Plasma Se levels (ng/ml)	
Group	**Selenized yeast dose (mg/kg diet)**	**Target selenium dose (mg/kg diet)**	**1 week**	**26 weeks**
1	0	0	560 ± 64	625 ± 69
2	2450	3.0	691 ± 54†	719 ± 89*
3	7350	9.0	725 ± 76†	764 ± 46†

* $p < 0.05$ versus dietary control.

† $p < 0.01$ versus dietary control.