

Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial

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Objective To test if supplemental dietary selenium is associated with changes in the incidence of prostate cancer.

Patients and method A total of 974 men with a history of either a basal cell or squamous cell carcinoma were randomized to either a daily supplement of 200 µg of selenium or a placebo. Patients were treated for a mean of 4.5 years and followed for a mean of 6.5 years.

Results Selenium treatment was associated with a significant (63%) reduction in the secondary endpoint of prostate cancer incidence during 1983-93. There were 13 prostate cancer cases in the selenium-treated group and 35 cases in the placebo group (relative risk, RR=0.37, P=0.002). Restricting the analysis to the 843 patients with initially normal levels of prostate-specific antigen (≤ 4 ng/mL), only four cases were diagnosed in the selenium-treated group and 16 cases were diagnosed in the placebo group after a 2 year

treatment lag. (RR=0.26 P=0.009). There were significant health benefits also for the other secondary endpoints of total cancer mortality, and the incidence of total, lung and colorectal cancer. There was no significant change in incidence for the primary endpoints of basal and squamous cell carcinoma of the skin. In light of these results, the 'blinded' phase of this trial was stopped early.

Conclusions Although selenium shows no protective effects against the primary endpoint of squamous and basal cell carcinomas of the skin, the selenium-treated group had substantial reductions in the incidence of prostate cancer, and total cancer incidence and mortality that demand further evaluation in well-controlled prevention trials.

Keywords Prostate cancer, selenium, chemoprevention, cancer screening, diet, trace minerals, nutrition, epidemiology, clinical trials

Introduction

Prostate cancer is the most common cancer and the second leading cause of cancer death in American men [1]. It is estimated that in 1997, there will be 209 000 new prostate cancer cases and 41 800 deaths from prostate cancer. The epidemiology of prostate cancer is complex, with few established risk factors; those most established are family history, age, country, race and testosterone deficiency [2]. Interest in the role of diet in the aetiology of prostate cancer is increasing with several proposed associations [3]. Dietary factors which have been investigated include dietary fat [4,5], lycopene, soy

products [6], protease inhibitors [7], retinol [8], vitamin E [9] and the essential trace element selenium (Se) [10,11].

The introduction of PSA screening for prostate cancer coincided with a dramatic increase in incidence in the late 1980s and early 1990s, presumably because of the earlier diagnosis of prevalent cases of cancer. The incidence doubled from 1984 to 163/100 000 in 1993 in the USA. This dramatic increase in incidence in the past decade underscores the importance of enhancing primary and secondary prevention efforts, although the benefits of PSA screening are still controversial.

The largest current phase III prostate cancer prevention trial [12] uses a prevention strategy based on antiandrogen therapy with a 5 α -reductase inhibitor which prevents the formation of 5 α -dihydrotestosterone,

an active derivative of testosterone. Preclinical trials have shown some preventive efficacy [13].

The association of prostate cancer with the intake of certain dietary nutrients suggests that they may be useful preventive agents. To our knowledge, there are no current phase III prostate cancer prevention trials testing the efficacy of dietary ingredients, although there are some small pilot studies to establish feasibility. A recent lung cancer prevention trial (ATBC Trial) that used β -carotene and α -tocopherol in a factorial design in Finnish smokers observed a reduced incidence of prostate cancer in patients who received 50 IU of α -tocopherol acetate [9].

Dietary Se has been inversely associated with the risk of cancer since the 1960s [14], with substantial preclinical evidence that dietary Se can significantly reduce the incidence of many cancers. Selenium supplements have been used in over 100 carcinogenesis experiments, with two-thirds of the studies showing a significant reduction in cancer incidence. Several ecological epidemiological studies have suggested that low blood Se levels increase the risk of cancer [14]. Only two analytical epidemiological studies have investigated the association of serum Se and prostate cancer. The first study, within the Lipids Research Clinics trial, found a borderline increase in the incidence of prostate cancer in patients with low Se levels [10]. However, this study lacked sufficient power to detect modest differences in risk. The second study in Sweden [11], a low Se region, found a significantly increased risk of prostate cancer and BPH in patients with lower Se levels.

Selenium is involved in the biosynthesis of testosterone [15] and can stimulate the production of pituitary and adrenal hormones [16]; therefore, Se can be theoretically linked to the function of the pituitary-adrenal-gonadal axis, prostate pathophysiology and to prostate carcinogenesis. Recently, a new prostate selenoprotein has been identified which preferentially incorporates Se when compared with glutathione peroxidase [17]; the significance of this discovery for prostate carcinogenesis remains to be determined.

The male study patients from the Nutritional Prevention of Cancer Trial, a double-blind trial of dietary Se supplementation, provide the opportunity to test whether Se supplementation reduces the incidence of prostate cancer in a population not recruited or selected for a urological study.

Patients and methods

The study was a double-blind, placebo-controlled, randomized cancer prevention trial using a nutritional dose of Se in patients with a history of non-melanoma skin cancer. The randomization procedure was blocked by

time and stratified by clinic. Patients were eligible for randomization if they had: (i) a history of two or more basal cell carcinomas (BCCs) or one squamous cell carcinoma (SCC) of the skin, with one of these carcinomas occurring within the previous year; (ii) a 5-year life expectancy; and (iii) no reported internal malignancies treated within the previous 5 years. The intervention agent was 200 μ g of Se supplied as a 0.5 g high-Se yeast tablet (Seleno Precise) by Cypress Systems, Inc., Fresno, CA, USA for Nutrition 21, La Jolla, CA, USA. The placebo of brewer's yeast was identical in smell and appearance to the intervention agent.

The first patients were randomized to the trial in 1983. The primary endpoints for the trial were the incidence of BCCs and SCCs of the skin. In 1990, after the initiation of the trial, several secondary endpoints were added to the trial, i.e. mortality from all causes and cancer, and the incidence of lung, colon and prostate cancers.

At each 6 monthly clinic visit, the study participants were interviewed to identify the incidence of illness and the use of prescribed and proprietary drugs. In addition, patients provided a sample of blood for the analysis of Se levels as one method of monitoring for toxicity. Additional aliquots of blood from the patients were stored at -80°C and were used for the retrospective determination of plasma PSA levels using the Abbott Diagnostics IMX PSA assay (Abbott Park, IL, USA), a microparticle enzyme immunoassay. The first available blood after randomization was used for the determination of the initial PSA levels.

The selenium content of each batch of tablets were determined in the laboratories of Dr G.F. Combs (Cornell University, Ithaca, NY, USA) and of Dr I.S. Palmer (South Dakota State University, Brookings, SD, USA) [18]. The Se concentration was determined by atomic absorption spectrophotometry (Perkin-Elmer 3030, Perkin-Elmer Corp, Norwalk, CT, USA) equipped with an electrodeless discharge lamp and automatic Zeeman-effect background correction. Quality control included multiple aliquots of exhaustively analysed human plasma as external-control samples. A coefficient of variation of $<7\%$ (for duplicate analyses) was the criterion for acceptance [19].

Patients who discontinued treatment were followed annually to identify new illness and confirm their vital status. All identified cancers were confirmed by medical documentation and reviewed by an appropriate medical specialist. Each cancer was staged using the TNM classification system. Prostate cancers were classified as local for TNM Stage 0, 1 and 2; advanced prostate cancers were defined as TNM Stage 3 and 4.

Incidence data were evaluated using standard survival analysis techniques, including log-rank statistics and

Cox proportional hazards models; all *P* values were two-tailed. The intention-to-treat model was used for all analyses.

Results

There were no significant differences between the treatment and placebo groups for the baseline risk factors (Table 1). Plasma Se concentration of the Se-treated group increased by $\approx 67\%$ to a mean of 190 ng/mL within the first year of randomization. At the end of 1993, the trial had 6464 person-years of follow-up and only three patients were lost to follow-up. Patients in the Se-treated group remained on treatment for a mean of 4.5 years, compared with 4.4 years in the placebo group. They also had a slightly longer follow-up (6.2 vs 6.1 years).

There were no dermatological signs of Se toxicity during the trial. There were no statistically significant differences in the incidence of new BCCs or SCCs between the groups, although slightly more cases of both types of skin cancer occurred in the Se group [20].

Forty-eight patients were diagnosed with a new prostate cancer; patients randomized to the Se group had a statistically significant lower incidence (63%) of prostate cancer (relative risk [RR]=0.37, *P*=0.002) than the placebo group (Table 2). Patients in the lowest tertile of baseline plasma Se had a statistically significant treatment effect (RR=0.08), as did patients in the middle tertile (RR=0.30); patients in the highest tertile had a non-significant reduction in risk (RR=0.85).

Table 3 presents the occurrence of prostate cancer by

Table 1 Baseline characteristics of the male study population

Characteristic	Se	Placebo
Patients randomized*	479	495
Age (years) (mean[sd])	64.4 (8.8)	63.2(9.5)
Plasma Se (ng/mL) (mean[sd])	115.0 (22.0)	115.0 (21.6)
Initial PSA > 4 ng/mL (% of subjects)	10.4	9.0

*Excludes six patients with a history of prostate cancer before randomization.

Table 2 Tertile of baseline selenium

Tertile Se (ng/mL)	Se cases	Placebo cases	RR	<i>P</i>
<106.4	1	13	0.08	0.002
106.4–121.2	4	13	0.30	0.03
>121.2	8	9	0.85	0.75
Total	13	35	0.37	0.002

Table 3 The incidence of prostate cancer by initial PSA level, 1983–93

	Person years	Se cases	Placebo cases	RR	<i>P</i>
All patients	6464	13	35	0.37	0.002
With PSA*	6186	12	32	0.38	0.003
Diagnosis > 2 years	4319	7	28	0.25	<0.001
First PSA (ng/mL)					
≤4	3984	4	16	0.26	0.009
>4	336	3	12	0.19	0.005
4–10	281	1	6	0.11	0.02
>10	56	2	6	0.48	0.39

*44 of 48 prostate cancer cases had a valid prediagnosis PSA estimate.

both time since randomization and initial PSA level. The RR for all patients (0.37) and those with an initial PSA (0.38) are similar. Excluding cases that were diagnosed in the first 2 years of the study (six Se, seven placebo) eliminated those cases with a short duration of treatment. The use of this 2-year treatment lag enhanced the treatment effect (RR=0.25). Stratification on initial PSA level did not substantially affect the estimate of the RR for either normal (RR=0.26) or elevated PSA values (RR=0.19). There is a suggestion that patients with a modest PSA increase (4–10 ng/mL) had a stronger treatment effect (RR=0.11) than patients with PSA values of >10 ng/mL (RR=0.48); this difference in treatment effect was not statistically significant.

The Se-treated group had a significantly lower incidence of prostate cancer (RR=0.42, *P*=0.02) for local disease (TNM stage 0–2) and advanced disease (TNM stage 3, 4) (RR=0.27, *P*=0.03). The patient age at diagnosis was similar for both treatment groups, at 71.9 years for placebo and 72.4 years for the Se-treated group. However, the younger patients, (<65 years) had a stronger treatment effect (RR=0.09) than older patients (RR=0.49), although statistically these treatment effects based on age are no different (*P*=0.10).

When considering only events that occurred after the definition of additional endpoints in 1990, there were significantly more cases (*n*=27) in the placebo group than in the Se group (nine cases, RR=0.33, *P*=0.002). For each calendar year in which a case of prostate cancer occurred, the incidence was lower in the Se group.

Discussion

Selenium is an essential nutrient with a recommended daily allowance of 70 µg in men. The variable distribution of Se in soil and foods produce geographical

regions and populations with varying Se status. This study was conducted in a lower Se region of the USA, the eastern coastal plains, where the dietary Se intake has been estimated to be 90 µg per day. The dietary Se intake in the UK of 30–40 µg per day would correspond to the lowest tertile of trial patients [21]. The 200 µg supplement of Se used in this trial increased the plasma levels of study patients to those that would be found in the regions of the USA with adequate Se.

Patients in the Se-treated group had a statistically significant reduction (63%) in the incidence of prostate cancer during the first decade of the trial and during the last 4 years of the trial after the definition of prostate cancer as a trial endpoint. These results cannot be explained by a favourable distribution of prostate cancer risk factors in the Se group, as these patients were older and had a higher proportion of initially high PSA levels at entry to the trial.

The blinded phase of the trial ended ahead of schedule on January 31, 1996. This decision to open the trial was made by the trial safety monitoring committee primarily because of a statistically significant reduction in total cancer mortality and other secondary endpoints in the Se group for the 1983–93 dataset. Overall, there was no significant difference in the incidence of the primary study endpoints of non-melanoma skin cancer. However, there were significant reductions in the additional endpoints of total cancer incidence and mortality, and the incidence of lung, colon, and prostate cancers. There was also an insignificant 17% reduction in all-cause mortality [20].

One of the principal strengths of the present study is that patients were recruited independently of their urological symptoms, thus the results should be more generalizable than if the study patients had been recruited from urology clinics. Conversely, this means that patients had to seek their own urological care from community physicians.

The availability of frozen plasma samples from early in the trial is another strength of the study, as this allowed PSA levels to be determined in most patients. This permitted stratification by the initial PSA level, eliminating the possibility of a favourable distribution of patients with elevated PSA levels (i.e. prevalent prostate cancer cases) for the treatment group. There was also no apparent difference in the treatment effect between patients with normal and elevated initial PSA values.

The inverse association between baseline plasma Se levels and the magnitude of the treatment effect is consistent with both the epidemiology of Se and cancer, and how the protective effect of a nutritional supplement would manifest itself, if a nutritional deficiency increased the risk of disease. As the epidemiology suggests that individuals with low Se levels are at higher risk of

cancer, it would be expected that these individuals would benefit most from a nutritional supplement. The enhancement of the treatment effect with a 2-year treatment lag model and a weaker treatment effect of patients with PSA levels > 10 ng/mL are also consistent with a biological model of cancer prevention.

In summary, it appears that Se supplementation decreases the incidence of prostate cancer in men with a history of non-melanoma skin cancer. The results are consistent both over time and by baseline Se levels. Adjusting for initial PSA levels does not materially alter the protective effect, except for patients with PSA levels > 10 ng/mL. Similarly, the TNM stage of the patients with prostate cancer is consistent with what would be expected from an effective cancer prevention agent, as is the strong protective effect for patients who were randomized at less than 65 years of age.

Prostate cancer was included as an additional endpoint in 1990 because of its high incidence and the suggestion of a possible treatment effect in the early data obtained before 1990. Limiting the data to events occurring after the definition of the endpoints allows testing of the *a priori* hypothesis that Se supplementation reduces the risk of prostate cancer. Although based on fewer cases, this treatment effect was highly statistically significant.

Supplementation with a nutritional dose of the essential trace element Se significantly reduced the incidence of prostate cancer in a population of patients with non-melanoma skin cancer. To our knowledge, this is the first completed double-blind randomized controlled trial to specifically test if a dietary supplement can prevent prostate cancer. These results require confirmation in independent trials, but suggest that Se supplementation may be important for both the primary and secondary prevention of prostate cancer.

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References

- 1 Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1996. *CA Cancer J Clin* 1996; 46: 5–7.
- 2 Whittemore AS, Kolonel LN, Wu AH *et al*. Prostate cancer in blacks, whites, and Asians in the United States and Canada. *J Nat Cancer Inst* 1995; 87: 652–61.
- 3 Kolonel LN. Nutrition and prostate cancer. *Cancer Cause Control* 1996; 7: 83–94.
- 4 Wynder EL, Rose DP, Cohen LA. Nutrition and prostate

- cancer, a proposal for dietary intervention. *Nutr Cancer* 1994; 22: 1
- 5 Giovannucci E, Rimm EB, Colditz GA *et al.* A prospective study of dietary fat and risk of prostate cancer. *J Nat Cancer Inst* 1993; 85: 1571
 - 6 Adlercreutz H, Honjo H, Higashi A *et al.* Urinary excretion of ligands and isoflavonoid phytoestrogens in Japanese men and women. *Am J Clin Nutr* 1991; 54: 1093-5
 - 7 Wynder EL. Editorial: prostate cancer — nutrition adjunct therapy. *J Urol* 1996; 56: 1364-6
 - 8 Rohan TE, Howe GR, Burch JD, Jain M. Dietary factors and risk of prostate cancer. A case-control study in Ontario, Canada. *Cancer Causes Control* 1995; 6: 145-54
 - 9 The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *New Engl J Med* 1994; 330: 1029
 - 10 Criqui MH. Selenium, retinol, retinol-binding protein and uric acid associations with cancer mortality in a population based prospective case-control study. *Ann Epidemiol* 1991; 1: 385-93
 - 11 Hardell L, Degerman A, Tomic R, Marklund SL, Bergfors M. Levels of selenium in plasma and glutathione peroxidase in erythrocytes in patients with prostate cancer or benign hyperplasia. *Eur J Cancer Prev* 1995; 4: 91-5
 - 12 Brawley OW, Thompson IM. Chemoprevention of prostate cancer. *Urology* 1994; 43: 594-9
 - 13 Tsukamoto S, Akaza H, Imada S *et al.* Chemoprevention of rat prostate carcinogenesis by use of finasteride or casodex. *J Nat Cancer Inst* 1995; 87: 842-3
 - 14 Clark LC. The epidemiology of selenium and cancer. *Fed Proc* 1985; 44: 2584-9
 - 15 Bedwal RS, Nair N, Sharma MP, Mathur RS. Selenium — its biological perspectives. *Med Hypoth* 1993; 41: 150-9
 - 16 Potmis A, Nonavinakere VK, Rasek HR, Early JL. Effect of selenium (Se) on plasma ACTH, beta-androphin, corticosterone and glucose in rat: Influence of adrenal enucleation and metyrapone pretreatment. *Toxicol* 1993; 79: 1-9
 - 17 Kalckloech M, Kyriakopoulos A, Hammel C, Behne D. A new selenoprotein found in the glandular epithelial cells of the rat prostate. *Biochem Biophys Res Comm* 1995; 217: 162-70
 - 18 Olson OE, Palmer IS, Cary EE. Modification of the official fluorometric method for selenium in plants. *J Assoc Off Anal Chem* 1975; 58: 117-26
 - 19 McShane LM, Clark LC, Combs GF Jr, Turnbull BW. Reporting the accuracy of biochemical measurements for epidemiologic and nutrition studies. *Am J Clin Nutr* 1991; 53: 1354-60
 - 20 Clark LC, Combs GF Jr, Turnbull BW *et al.* For The Nutritional Prevention of Cancer Study Group. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: a randomized clinical trial. *JAMA* 1996; 76: 1957-6
 - 21 Rayman MP. Dietary selenium. Time to act. *Br Med J* 1997; 114: 387-8

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