BRIEF COMMUNICATION

Effects of Dietary Selenium Supplementation on DNA Damage and Apoptosis in Canine Prostate

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The trace mineral selenium inhibits cancer development in a variety of experimental animal models. We used an *in vivo* canine model to evaluate the effects of dietary selenium supplementation on DNA damage in prostate tissue and on apoptosis in prostate epithelial cells. Sexually intact elderly male beagle dogs were randomly assigned to receive an unsupplemented diet (control group) or diets that were supplemented with selenium (treatment group), either as selenomethionine or as high-selenium yeast at 3 µg/kg or 6 µg/kg body weight per day for 7 months. The extent of DNA damage in prostate cells and in peripheral blood lymphocytes, as determined by the alkaline comet assay, was lower among the selenium-supplemented dogs than among the control dogs (prostate *P*<.001; peripheral blood lymphocytes *P* = .003; analysis of variance) but was not associated with the activity of the antioxidant enzyme glutathione peroxidase in plasma. The median number of terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling-positive (i.e., apoptotic) prostate epithelial cells was 3.7 (interquartile range = 1.1–7.6) for the selenium-supplemented dogs and 1.7 (interquartile range = 0.2–2.8) for the control dogs (*P* = .04, Mann–Whitney *U* test). These data suggest that dietary selenium supplementation decreases DNA damage and increases epithelial cell apoptosis within the aging canine prostate. **[J Natl Cancer Inst 2003;95: 237–41]**

Prostate cancer is the second leading cause of cancer-related mortality among men in the United States *(1)*. Selenium, an essential nutrient required for the activities of a number of metabolically important enzymes, including the antioxidant glutathione peroxidase, inhibits cancer development in a variety of experimental animal models *(2–4)*. In 2001, the National Cancer Institute initiated the Selenium and Vitamin E Prostate Cancer Prevention Trial (SELECT) to evaluate whether daily dietary supplementation with selenium and/or vitamin E decreases the incidence of prostate cancer. However, it is not known what dietary form or dose of selenium might offer the most potent cancer-protective effects.

Selenium-dependent glutathione peroxidase and thioredoxin reductase protect the body from the endogenous products of cellular metabolism that have been implicated in DNA damage, mutagenesis, and carcinogenesis *(5–7)*. A shift in the pro-oxidant–antioxidant balance within the prostate has been proposed as a factor that contributes to prostate carcinogenesis *(8–11)*. We hypothesized that selenium supplementation exerts its anticarcinogenic effect by reducing the naturally occurring genotoxic stress within the aging prostate. Because the influence of aging on prostate cancer development is similar in dogs and humans, the only two species in which prostate cancer occurs spontaneously with appreciable frequency *(12,13)*, we examined the effects of dietary selenium supplementation on DNA damage and apoptosis in elderly beagle dogs that were physiologically equivalent to 62- to 69-year-old men and free of prostate cancer.

Forty-nine elderly (i.e., 8.5- to 10.5-year-old) sexually intact male, retired breeder dogs weighing 9–18 kg were purchased from a local supplier. After 4 weeks of acclimation, the dogs were randomly assigned to the control group (n = 10 dogs), which was fed a maintenance diet that contained 0.3 ppm selenium (Science Diet® Canine Maintenance; Hills Pet Nutrition, Inc., Topeka, KS), or to one of the four daily treatment groups, which received either the maintenance diet plus 3 µg/kg/day selenomethionine (Solgar Vitamin and Herb, Leonia, NJ) (n = 10 dogs), 6 µg/kg/day selenomethionine (n = 10 dogs), 3 µg/kg/day high-selenium yeast (SelenoExcell®; Cypress Systems, Fresno, CA) (n = 10 dogs), or 6 µg/kg/day high-selenium yeast (n = 9 dogs). The daily selenium intake for the dogs in the control group was approximately 6 µg/kg body weight. All dogs had nutritionally adequate selenium status prior to the start of the experiment [mean pretreatment plasma selenium concentration *(14)* was 275 ng/mL (range = 228–339 ng/mL)]. The dogs were fed their respective diets for 7 months. At the end of that period, peripheral blood lymphocytes were harvested from whole blood *(15–17)* that was obtained from each dog, and the dogs were then euthanized in accordance with guidelines set forth by the American Veterinary Medical Association Panel on Euthanasia *(18)*. The prostate was collected *in toto* from each dog within 15 minutes after euthanasia. Prostate tissue (50–80 mg) was harvested fresh to prepare prostate cell suspensions for alkaline comet assay. The remaining prostate was fixed in formalin, embedded in paraffin, and step-sectioned at 4-mm intervals.

The extent of DNA damage in prostate cells and in peripheral blood lymphocytes was measured by single-cell gel electrophoresis (alkaline comet assay) *(19)*. The extent of DNA damage was visually scored in 100 randomly selected cells from each sample using previously described criteria *(20,21)* (Fig. 1, A). The ApopTag™ peroxidase *in situ* apoptosis detection kit (Intergen, Inc., Purchase, NY) and a modification of the terminal deoxynucleotidyl trans-

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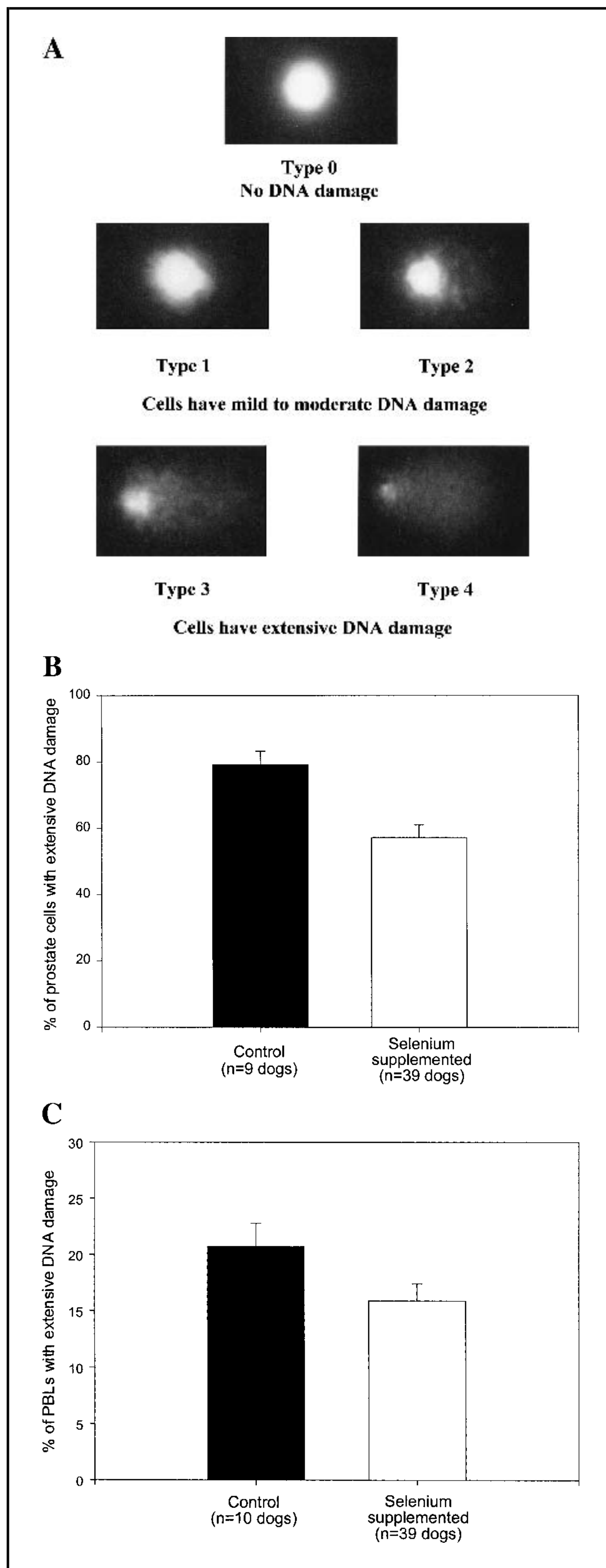

Fig. 1. DNA damage in prostate cells and peripheral blood lymphocytes (PBLs) from control dogs and dogs that received daily selenium supplementation. **A**) Extent of DNA damage in prostate cells and PBLs was measured by single-cell gel electrophoresis (alkaline comet assay) as described by Singh et al. *(19)*. Under the assay conditions used in this experiment, comet tails reflect the electrophoretic migration of DNA fragments that result from strand breaks, alkali-labile sites, crosslinks, or base excision repair sites *(19)*. Extent of DNA damage was scored in 100 randomly selected cells from each sample (50 cells from several different fields from each of two replicate slides) by an examiner who was blinded to treatment group. Each cell was visually scored as previously described *(20, 21)* according to the following criteria: no damage (type 0), mild to moderate damage (type 1 and type 2), and extensive DNA damage (type 3 and type 4). Extent of DNA damage within prostate cells or PBLs was expressed as the percentage of cells with extensive DNA damage (the total number of cells that displayed type 3 or type 4 DNA damage). **B**) DNA damage in prostate cells. Within 15 minutes of euthanasia, the prostate was collected from each dog at necropsy, and 50–80 mg of prostate tissue was placed in 1 mL of cold Hanks' balanced salt solution containing 20 m*M* EDTA and 10% dimethyl sulfoxide (DMSO) *(24)*. One dog in the control group had a tissue sample that was insufficient for further analysis. Tissue was then minced with fine scissors, and 50 μL of the resulting cell suspension was mixed with 1 mL of RPMI-1640 medium containing 10% fetal bovine serum for subsequent electrophoresis. Cytospin preparations of the cell suspensions indicated that greater than 90% of the cells had an epithelial morphology; the mean percentage of viable cells, as estimated by the trypan blue exclusion assay, was 80%. **Bars** = mean percentage (and the upper 95% confidence interval) of prostate cells that displayed type 3 or type 4 DNA damage. **C**) DNA damage in PBLs. PBLs were freshly harvested from whole blood *(15–17)* that was obtained from each dog after 7 months of treatment and prior to euthanasia. Cytospin preparations confirmed that more than 90% of the cells in this enriched cell population were lymphocytes; mean percentage of viable cells, as estimated by the trypan blue exclusion assay, was 91%.

ferase–mediated dUTP nick end-labeling (TUNEL) method *(22)* were used to determine the frequency of apoptosis within sections of dog prostatic tissue. Histopathologic evaluation of formalin-fixed, step-sectioned prostate tissue sections stained with hematoxylin and eosin revealed no foci of carcinoma in any of the dogs. The activity of selenium-dependent glutathione peroxidase in plasma collected immediately prior to euthanasia was assayed by the method of Lawrence and Burk *(23)* using 0.25 m*M* H_2O_2 as the acceptor substrate. All aspects of this experimental protocol were approved by the Purdue University Animal Care and Use Committee.

Analysis of variance was used to determine the statistical significance of differences between the control dogs and the selenium-supplemented dogs in the extent of DNA damage in prostate cells or peripheral blood lymphocytes after 7 months on the respective diets. Because no consistent differences in effects were observed with respect to the different forms or doses of selenium the dogs received, in all analyses control dogs were compared with all selenium-supplemented dogs. The median number of apoptotic epithelial cells within prostate tissue sections from control and selenium-supplemented dogs per ×200 microscope field were compared with the use of the Mann–Whitney *U* test. Fisher's exact test was used to compare the percentage of dogs in each treatment group that had more than 30 apoptotic cells per ×200 microscope field. This cutoff point represented a level of apoptosis that exceeded the mean number plus three standard deviations of apoptotic cells in prostate samples from dogs fed the control diet. A *P* value of less than .05 was considered statistically significant, and all tests of statistical significance were two-sided.

After 7 months of treatment, the percentage of prostate epithelial cells and peripheral blood lymphocytes with extensive (i.e., types 3 and 4; Fig. 1) DNA damage was statistically significantly lower in the selenium-supplemented dogs than in the control dogs (mean percentage of prostate cells with extensive DNA damage was 79.1% for the control group and 57.2% for the selenium-treated group [difference = 21.9%, 95% confidence interval [CI] = 13.6% to 30.1%, *P*<.001]; mean percentage of peripheral blood lymphocytes with ex-

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tensive DNA damage was 20.7% for the control group and 15.9% for the selenium-treated group [difference = 4.8%, 95% CI = 1.7% to 7.9%, P = .003]) (Fig. 1, B and C). The mean percentage of prostate cells with extensive DNA damage in dogs in each of the four selenium treatment groups was statistically significantly lower than it was in dogs in the control group (mean percentage of prostate cells with extensive DNA damage was 79.1% for control dogs and 49.1% for dogs receiving 6 μg/kg/day high-selenium yeast [difference = 30.0%, 95% CI = 23.8% to 36.2%, P<.001]; 56.9% for dogs receiving 3 μg/kg/day high-selenium yeast [difference = 22.2%, 95% CI = 13.5% to 30.9%, P = .003]; 63.9% for dogs receiving 6 μg/kg/day selenomethionine [difference = 15.2%, 95% CI = 4.0% to 26.4%, P = .01]; and 58.1% for dogs receiving 3 μg/kg/day selenomethionine [difference = 21.0%, 95% CI = 13.5% to 28.5%, P<.001]). After 7 months of treatment, the mean (± standard deviation) glutathione peroxidase activity in plasma of control dogs that received a selenium-adequate diet was 25.5 ± 6.1 nm/mg protein, which was not statistically significantly different from the mean glutathione peroxidase activity in plasma of selenium-treated dogs (P>.05).

A very low level of apoptosis was observed within prostate cells from the

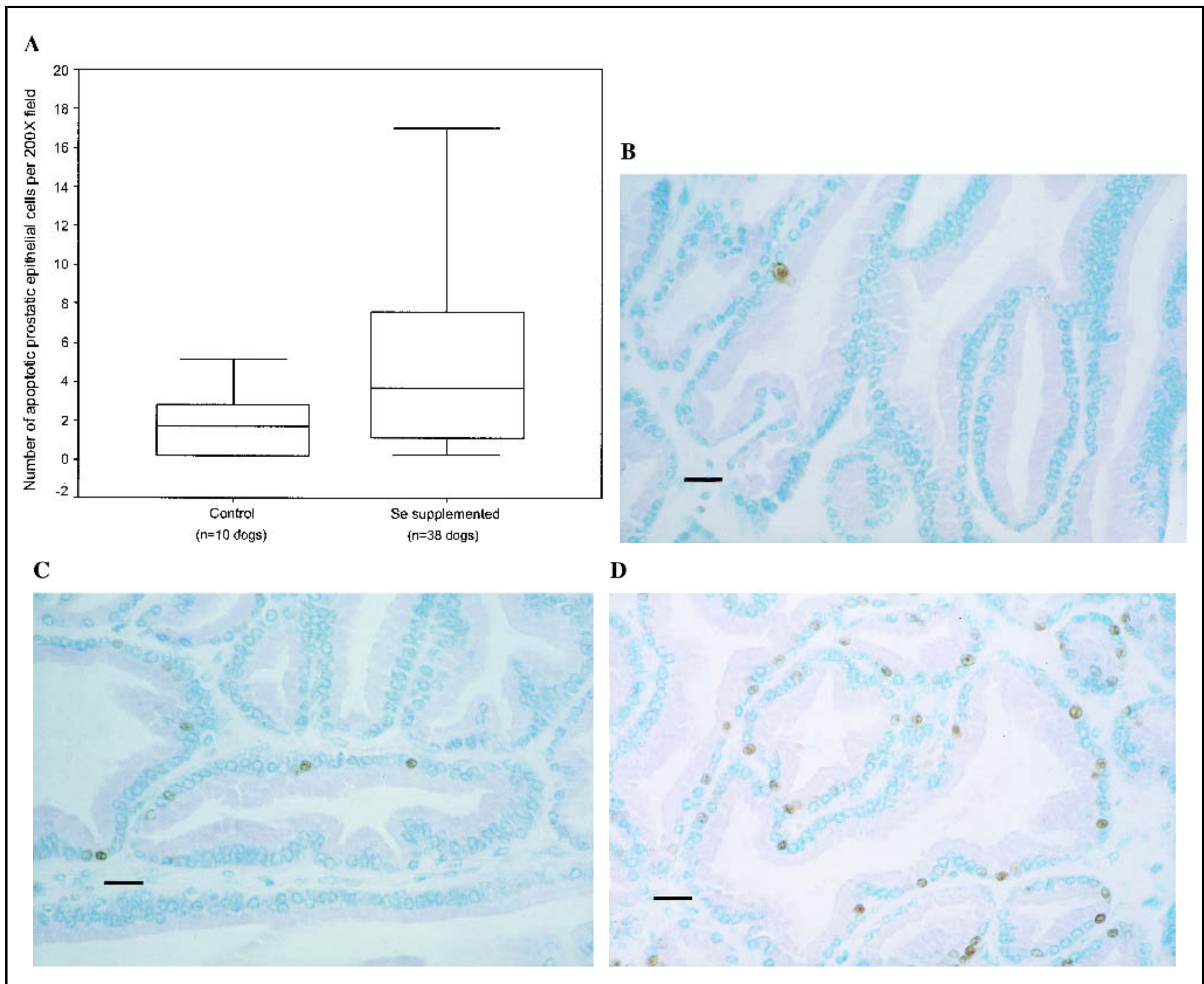

Fig. 2. Prostatic epithelial cell apoptosis in control dogs and dogs receiving daily selenium supplementation. A modified terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling (TUNEL) method was used to measure prostatic epithelial cell apoptosis *in situ* in formalin-fixed tissue specimens *(22)*. For each dog, the number of prostate epithelial cells with positive nuclear staining was counted in randomly selected, noncontiguous, ×200 microscopic fields. An average of 23 fields in one tissue section was evaluated for each dog. Immunopositive stromal cells, inflammatory cells, or epithelial cells that were shed into the acinar lumen were not counted. Microscopic fields that contained areas that displayed intense inflammation were not scored. **A**) Data are displayed in a box and whisker plot (prostate tissue from one selenium-supplemented dog did not react to staining). The **center horizontal line** indicates the median value for each group. The length of each box (interquartile range) indicates the range of the central 50% of values, with the box edges placed at the first and third quartiles. **Whiskers** (the lines extending beyond the box) show the range of observed values that are within 1.5 times the interquartile range. **Panels B, C,** and **D**) Representative photomicrographs of TUNEL-stained prostate tissue from a control dog (**B**) and a selenium-treated dog (**C**) demonstrate the increased number of epithelial cells with TUNEL-positive nuclear staining (brown) associated with selenium treatment. **Panel D** shows a region of markedly increased apoptosis ("hot spot") within the prostate of a selenium-treated dog. In each of these ×200 photomicrographs, the scale bar = 50 μm.

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10 control dogs (median number of TUNEL-positive epithelial cells/×200 field = 1.7 cells; interquartile range = 0.2–2.8 cells) (Fig. 2, B). By contrast, 38 dogs treated with selenium for 7 months had an approximately twofold increase in the median number of apoptotic cells per field compared with control dogs (median = 3.7 cells; range = 1.1–7.6 cells) (P = .04) (Fig. 2, A and C). Foci of increased apoptosis (i.e., apoptotic hot spots), which were defined as those microscopic fields in which there were more than 30 apoptotic cells, were present in prostate tissue sections from 16 (42%) of 38 selenium-supplemented dogs (Fig. 2, D) but in prostate tissue sections from only one (10%) of 10 control dogs (P = .07). There were also no statistically significant differences between the two groups of dogs when the cutoff point for apoptotic hotspots was 20, 40, or 50 apoptotic cells per ×200 microscope field (P = .07 for each cutoff point).

Our results show that daily supplementation with nontoxic doses of selenium is associated with a decrease in the steady-state level of DNA damage and an increase in epithelial cell apoptosis within the aging canine prostate. Importantly, these effects of selenium supplementation were observed in dogs that had no histologic evidence of prostate cancer and that were of a comparable physiologic age to that of men enrolled in SELECT. We used the alkaline comet assay as a simple, robust method to assess DNA integrity in prostate cells to measure the effect of nutritional intervention on the level of genotoxic stress within the prostate. Two different forms and doses of selenium were consistently associated with a reduction in the steady-state level of DNA damage within the prostate of elderly dogs to levels lower than those measured in the prostate of young adult dogs (data not shown). These biologic responses within the canine prostate were accompanied by statistically significant increases in plasma and toenail selenium concentrations over the treatment period (data not shown). At the end of the study, mean concentration of selenium in toenails collected from selenium-treated dogs was roughly equivalent to the average selenium level found in toenails of men in the Health Professionals Study (data not shown) *(25).*

The specific mechanism by which selenium supplementation exerts its anticarcinogenic effect on the prostate is unknown *(26,27).* A reduction in the steady-state level of DNA damage within prostatic epithelial cells could result from a decrease in the rate of DNA damage formation, an increase in the rate or efficiency of DNA damage repair *(28),* or the preferential elimination of epithelial cells that have the most extensive DNA damage. With regard to the latter possibility, selenium has been shown to induce apoptosis in several *in vitro* models of cancer *(27,29–32).* Our data support the hypothesis that selenium sensitizes prostatic epithelial cells with extensive DNA damage to apoptosis *in vivo.* Our data also suggest that the effects of selenium on the level of DNA damage are independent of the effects of selenium supplementation on glutathione peroxidase activity. This observation in dogs is consistent with data from a randomized clinical trial of selenium supplementation in humans *(14),* in which a 63% reduction in prostate cancer incidence was observed in selenium-supplemented men who already had maximal expression of plasma glutathione peroxidase prior to intervention (Combs GF Jr, Clark LC: unpublished data).

In summary, daily supplementation with nontoxic doses of selenomethionine or high-selenium yeast given prior to the development of carcinoma is associated with a reduction in the accumulation of genotoxic damage within the aging canine prostate. Therefore, selenium may benefit the aging prostate by decreasing the accumulation of DNA damage in epithelial cells even before these cells show cytologic changes suggestive of malignancy. We believe that DNA damage and apoptosis are selenium-responsive events that may be important regulatory points in multistep prostatic carcinogenesis. Further study of the process of carcinogenesis within the prostate of animal species vulnerable to spontaneous cancer development may provide important insights into the putative anticancer mechanisms of selenium and identify biomarkers that predict the prostate's response to selenium.

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Notes

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