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Selenium Form-Dependent Anti-Carcinogenesis: Preferential Elimination of Oxidant-Damaged Prostate Cancer Cell Populations by Methylseleninic Acid is Not Shared by Selenite

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Abstract

Selenium has received considerable attention as a cancer preventive agent. But the puzzling, disquieting results of the Selenium and Vitamin E Cancer Prevention Trial (SELECT) have called into question how much is really understood about the biology behind selenium and cancer risk. This predicament should provide researchers with a renewed stimulus for exploring mechanisms of selenium anti-carcinogenesis. One such line of inquiry is homeostatic housecleaning — that selenium can preferentially eliminate DNA-damaged cell populations through apoptosis, consistent with the decreased DNA damage and increased apoptosis observed in the prostate of selenium-replete dogs after receiving additional dietary selenium supplementation. Because growing experimental evidence suggests the anti-carcinogenic effects of selenium on prostatic cells are form-dependent and apoptosis is a DNA damage response, the aim of this research was to determine whether selenite, a form of selenium that induces DNA damage, possesses potent homeostatic housecleaning activity. To test this hypothesis, we exposed human and canine prostate cancer cells to non-cytotoxic concentrations of hydrogen peroxide (H_2O_2) to create cell populations with higher levels of oxidant-induced DNA damage, and then evaluated the extent to which oxidant damage sensitizes prostate cancer cell populations to selenite-triggered apoptosis compared to apoptosis triggered by methylseleninic acid (MSA), a non-DNA damaging methylselenol precursor we previously showed to have strong homeostatic housecleaning activity. In this brief communication, we report that non-cytotoxic oxidant-induced damage does not sensitize prostate cancer cell populations to selenite-triggered apoptosis. Intensity of apoptosis triggered by MSA in H_2O_2-damaged prostate cancer cells was 3 times higher than undamaged cell populations not exposed to H_2O_2 ($P \leq 0.01$). In contrast, neither human nor canine prostate cancer cells with oxidant-induced damage had a significant increase in intensity of selenite-triggered apoptosis compared to undamaged cells. The divergent results between MSA and selenite in our experiments contribute to a growing catalogue of observations that suggest there are important form-dependent differences in the extent to which selenium can impact the emergence of prostate cancer. By carefully documenting the form-dependent biological effects of selenium and other nutrients, we commit ourselves to more precisely qualifying the implications of laboratory results and to more carefully designing and interpreting the results of large-scale human trials.

Keywords: Cancer prevention; DNA damage response; Apoptosis; Carcinogenesis; Nutrition; SELECT; Trace mineral

Abbreviations: FBS: Fetal Bovine Serum; H_2O_2: Hydrogen Peroxide; MSA: Methylseleninic Acid; PARP: Poly (ADP-ribose) Polymerase; SELECT: Selenium and Vitamin E Cancer Prevention Trial

Introduction

Personalizing nutrition for disease prevention — minimizing the risk for diseases such as prostate cancer by optimizing nutrient intake — remains one of the major challenges that scientists and health professionals face today. Emerging data on the relationship between selenium intake and human health point to a U-shaped dose response, suggesting that more selenium is not necessarily better [1-4]. Moreover, careful evaluation of the relationship between selenium status and cancer risk suggests that the anticancer benefit of selenium supplementation in humans and animals cannot be explained simply by antioxidant protection, because it occurs at selenium levels at which selenium-dependent antioxidant enzymes are already maximized [3,5-8]. More than ever before, investigators are motivated to explore new mechanisms of selenium anti-carcinogenesis [9-11].

Our work is pointing to a new idea of how selenium might diminish prostate cancer risk — by sweeping away damaged prostatic cells, rather than by protecting cells from damage. This line of reasoning was set in motion by the results of a randomized feeding trial in dogs, the only non-human species to frequently develop prostate cancer during aging [12]. In that study [8], dietary selenium supplementation lowered prostatic DNA damage but increased apoptosis, leading to the hypothesis that selenium might exert its cancer-preventive effect by selectively increasing apoptosis in the most highly DNA-damaged cells. In order to achieve a significant lowering of DNA damage level in the prostate, selenium would have to preferentially eliminate populations of damaged cells, because a non-selective triggering of apoptosis would not explain the overall reduction in DNA damage observed in the prostate of selenium-supplemented dogs.

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Pursuing this line of reasoning, in a recent paper [13] we documented the ability of selenium to preferentially sweep away damaged cells — a process we call homeostatic housecleaning — using an *in vitro* assay so that DNA damage level could be more carefully controlled in human and canine prostate cancer cell populations. We showed that non-cytotoxic oxidant-induced damage sensitizes prostate cancer cell populations to apoptosis triggered by methylseleninic acid (MSA), a proximal precursor of methylselenol [14]. Methylselenol production is thought to play an important role in the anti-tumorigenic activity of dietary selenium [10,15]. Taken together, our results point to a new way in which selenium might render an aging prostate under oxidative attack more resistant to cancer.

At the same time, work by us and others in the field of selenium and cancer has emphasized that the anticancer effects of selenium may be form-dependent [16-19]. It is plausible that a selenium compound that induces DNA damage might have superior homeostatic housecleaning activity than compounds like MSA that do not induce DNA damage. Here, in this brief research communication, we report our experience testing the hypothesis that selenite, an inorganic form of selenium that induces DNA damage [20], might have stronger apoptosis-triggering activity in oxidant-damaged cell populations. Our results comparing MSA and selenite add meaningfully to a growing body of experimental evidence that reveal mainly divergent effects of these two forms of selenium on prostate cancer cells.

Materials and Methods

Cell lines and assay conditions

The human prostate cancer cell line DU-145 was purchased from ATCC (Manassas, VA). The canine prostate cancer cell line TR5P was established in our laboratory from a dog with spontaneous prostatic carcinoma [21]. DU-145 human and TR5P canine prostate cancer cells were maintained in DMEM F-12 (GIBCO, Carlsbad, CA) supplemented with 10% batch-matched fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA) and RPMI-1640 supplemented with 5% FBS, respectively. The cell culture condition was 37°C with a humidified atmosphere of 5% CO_2 in air. For all experiments, cells were seeded at 50% confluency 18 hours before introducing treatment.

To create prostate cancer cell populations with non-cytotoxic oxidant damage, cells were exposed to hydrogen peroxide (H_2O_2) (Malinckrodt, Phillipsburg, NJ) for one hour and then DNA strand breaks were measured by Comet assay [22,23]. Dose-finding studies were used to determine the concentration of H_2O_2 that induced non-cytotoxic oxidant damage, as previously reported [13]. In DU-145 cells without H_2O_2 exposure, 23% of cells had extensive DNA damage (single strand breaks). One-hour exposure of DU-145 cells to 100 µM H_2O_2 yielded a cell population in which 90% of cells had extensive DNA damage ($P<0.0001$) but was non-cytotoxic — no significant increase in apoptosis or cell death quantified by Annexin-V-fluorescein and propidium iodide [24,25]. Thus, a dose of 100 µM H_2O_2 was chosen for experiments with DU-145 cells. In TR5P canine prostate cancer cells, 90% of cells already had extensive DNA single strand breaks before H_2O_2 exposure, whereas only 8% of cells had extensive DNA double strand breakage. Therefore, double strand break induction was a preferred criterion to select doses of H_2O_2 that would significantly increase DNA damage in TR5P cells. Exposure to 400 µM H_2O_2 for one hour increased the number of cells with extensive DNA damage to 13.5% ($P=0.001$). Doses of H_2O_2 exceeding 400 µM were cytotoxic to TR5P cells. Thus, a dose of 400 µM H_2O_2 was chosen for experiments with TR5P cells.

To assay the ability of MSA or selenite to preferentially trigger apoptosis in oxidant-damaged cell populations, cells were washed with PBS immediately after H_2O_2 exposure and incubated in fresh complete medium containing methylseleninic acid (MSA) or sodium selenite for 24 hours. Methylseleninic acid was chosen for these experiments because the metabolism of this selenium compound to the anti-tumorigenic metabolite methylselenol does not require lyases, which are absent in many cell lines [14,15]. Dose-finding experiments for MSA and selenite identified concentrations of each selenium compound that enabled useful comparison of apoptosis intensity between oxidant-damaged versus undamaged cell populations. Selenite concentrations exceeding 10 µM were not studied, because the level is toxic to humans [26]. Comet assay confirmed that at the concentrations used, exposure to selenite, but not exposure to MSA, induced DNA strand breaks as previously reported [20]. After 24 hours incubation, cells were harvested to determine intensity of apoptosis.

To detect apoptosis in cells, cleaved PARP [27,28] or cleaved caspase-3 [29,30] was measured by immunoblot. Cell lysates from both adherent and detached cells were collected in 1% Triton X-100 lysis buffer containing protease inhibitors. Protein content was quantified by the Bradford dye-binding assay (Bio-Red Laboratories, Richmond, CA). Fifty µg of total protein was used for electrophoresis on 8-12% mini SDS polyacrylamide gel with Tris-glycine buffer (25 mM Trizma base, 190 mM glycine, 0.1% SDS). Proteins were transferred in a semi-dry condition. Membranes were developed with a chemiluminescence kit (Pierce, Rockford, IL) following the manufacturer's instructions. Primary antibodies for immunoblot detection were purchased from Cell Signaling Technology (Beverly, MA). The detection of cleaved PARP served as an execution marker of apoptosis in DU-145 human prostate cancer cells. For TR5P canine prostate cancer cells, since primary antibody for dog cleaved PARP was not commercially available, cleaved caspase-3 was chosen as the apoptosis marker. Beta-actin was used as the loading control. Blot intensity was quantitated by Kodak Image Station 440CF with 1D Image Analysis Software (Kodak, Rochester, NY) and expressed in arbitrary units.

Data analysis

Blot intensity of cleaved PARP and cleaved caspase-3 was used to determine the homeostatic housecleaning activity of MSA and selenite. To test each selenium compound for homeostatic housecleaning activity (i.e., the preferential elimination of oxidant-damaged cell populations), prostate cancer cells were exposed to non-cytotoxic concentrations of H_2O_2 to create cell populations with increased oxidant-induced DNA damage. Then we evaluated the extent to which oxidant damage sensitized prostate cancer cell populations to MSA-triggered or selenite-triggered apoptosis. For DU-145 human and TR5P canine prostate cancer cells, this was determined by comparing the extent to which the observed intensity of selenium-triggered apoptosis in oxidant-damaged cells exceeded an expected value, which was equal to the sum of intensities of basal apoptosis in untreated cells, plus apoptosis induced by H_2O_2 alone, and apoptosis induced by selenium alone. Data from three independent experiments were analyzed by two-tailed, independent t-test to compare differences between the effect of selenium treatment on blot intensity of apoptosis markers in oxidant-damaged versus undamaged cells. In this manuscript, the term "undamaged cells" refers to cell populations that were not exposed to H_2O_2. A p-value<0.05 was considered significant. Data are presented as mean and standard deviation (SD). All statistical analyses were carried out using SAS 9.2 software (SAS Institute, Cary, NC).

Results

In DU-145 human prostate cancer cells, we evaluated whether the intensity of apoptosis triggered by MSA in H_2O_2-damaged prostate cells exceeded the sum of the basal apoptosis in untreated cells plus apoptosis induced by MSA alone and H_2O_2 alone. Figure 1a shows that MSA at 3 μM triggered significantly greater apoptosis (3X increase, P=0.01) in H_2O_2-damaged cells than expected. A similar threefold increased intensity of apoptosis triggered by MSA (5 μM) was seen in H_2O_2-damaged TR5P canine prostate cancer cells compared to undamaged cells not exposed to H_2O_2 (P=0.004; Figure 1b), evidence of preferential elimination of oxidant-damaged cell populations by MSA-triggered apoptosis.

In contrast, apoptosis triggered by selenite was not higher in H_2O_2-damaged cells compared with undamaged cells. Non-cytotoxic oxidant damage did not sensitize DU-145 cells to selenite-triggered apoptosis; an increase in apoptosis was not detected by PARP cleavage in either undamaged or H_2O_2-damaged DU-145 cells exposed to 10 μM selenite (Figure 1a). In TR5P canine prostate cancer cells, selenite at 5 μM induced apoptosis in undamaged cells. However, the intensity of selenite-triggered apoptosis was not significantly higher in cell populations with oxidant-induced damage (P=0.18; Figure 1b). We concluded that, unlike for MSA-triggered apoptosis, non-cytotoxic oxidant-induced damage does not sensitize prostate cancer cells to selenite-triggered apoptosis.

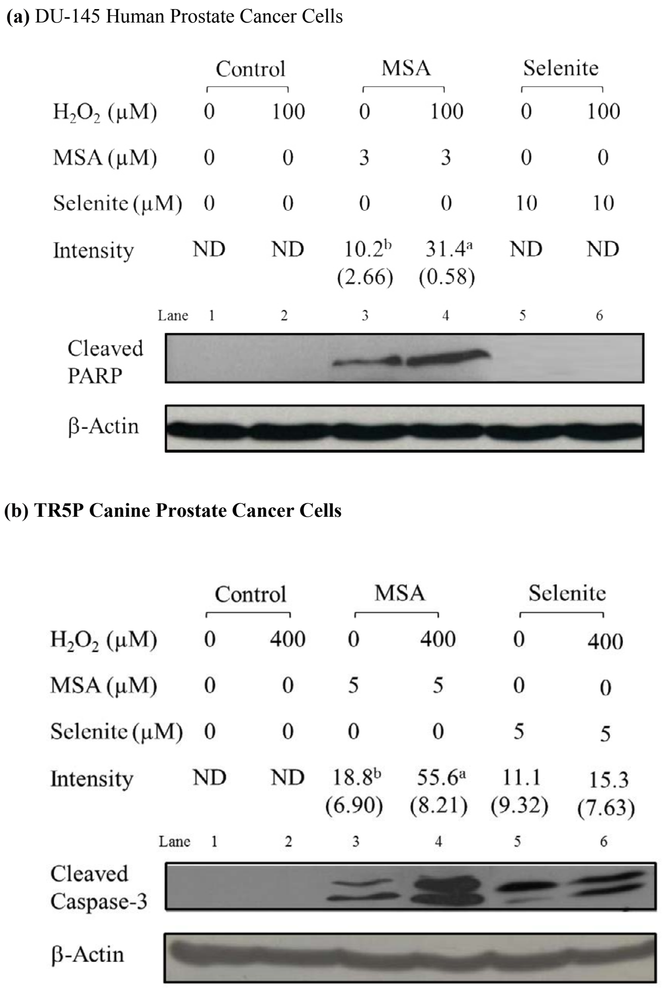

Figure 1: Apoptosis induced by methylseleninic acid (MSA) or selenite in hydrogen peroxide (H_2O_2)-damaged DU-145 human and TR5P canine prostate cancer cells. After 1-hour exposure with H_2O_2 to induce non-cytotoxic DNA damage, cells were washed with PBS and treated with MSA or selenite in complete medium for 24 hours, and then intensity of apoptosis was determined using cleaved PARP and cleaved caspase-3. Cells exposed to assay conditions without H_2O_2 or selenium served as comparisons. (**a**) Protein expression of cleaved PARP in DU-145 human prostate cancer cells exposed to 3 μM MSA or 10 μM selenite. (**b**) Protein expression of cleaved caspase-3 in TR5P canine prostate cancer cells exposed to 5 μM MSA or 5 μM selenite. Blot intensities are expressed as mean (SD) in arbitrary units from three independent experiments. β-actin, which served as the loading control, shows there was equivalent loading in all lanes (using intensity of untreated cells in Lane 1 as reference, the relative intensity for Lane 2-6 in panel a was 0.99, 0.98, 0.98, 0.95, 0.98, respectively; in panel b the relative intensity for Lane 2-6 was 0.97, 0.99, 0.97, 0.98, 1.01). For MSA, homeostatic housecleaning activity is determined by comparing the intensity of apoptosis marker in Lane 4 versus the sum of Lanes 1, 2, and 3. For selenite, homeostatic housecleaning activity is determined by comparing the intensity of apoptotic marker in Lane 6 versus the sum of Lanes 1, 2, and 5. Since under the conditions of these experiments there was no apoptosis signal in Lane 1 (there was no significant basal apoptosis in untreated cells) or Lane 2 (there was no apoptosis signal for H_2O_2 treatment alone because the H_2O_2 dose used was intended to be DNA-damaging, but not cytotoxic), the comparison of interest is simplified to Lane 4 vs. Lane 3 for MSA and Lane 6 vs. 5 for selenite. For each cell line within the same selenium treatment group, mean values without a common superscript differ at P<0.05. ND: non-detectable.

Discussion

Prostate cancer is the ultimate product of dysregulated homeostasis within the aging prostate. Through triggering apoptosis, selenium may selectively eliminate DNA-damaged cells, keeping genetic instability low within the prostate, and consequently attenuating carcinogenesis. If this process of preferential elimination of DNA-damaged cell populations — which we have called homeostatic housecleaning [13] — can potentially render the prostate more resistant to cancer, it is important to identify the forms of selenium with the most potent homeostatic housecleaning activity. In this communication, we show that in contrast to methylseleninic acid (MSA), selenite does not preferentially trigger the apoptosis of oxidant-damaged prostate cancer cell populations. The magnitude of apoptosis triggered by selenite was not different between undamaged prostate cancer cell lines and cells after non-cytotoxic damage induced by H_2O_2 exposure.

These results add to a growing body of experimental evidence and clinical suspicion that the anti-carcinogenic effects of selenium are form-dependent. For example, supplementation with selenium in the form of selenium yeast significantly decreased prostate cancer incidence in the men of the Nutritional Prevention of Cancer Trial [5]. Yet, in the Selenium and Vitamin E Cancer Prevention Trial (SELECT), no such cancer-protective advantage of selenomethionine was observed; in fact, supplementation of men with the highest baseline selenium status was associated with an increased risk for prostate cancer [4]. These discordant results raise questions whether particular forms of selenium might elicit superior prostatic responses. These highly publicized results have motivated researchers to begin to report their experience with dietary supplementation studies directly comparing selenomethionine and selenium yeast [16,31]. Clearly, this is only a starting point. The interrogation of a broader range of prostatic responses and a more extensive array of selenium compounds are needed to better understand critical differences in the selenium form-dependent effects that may impact cancer risk.

Our initial work on selenium-triggered apoptosis and prostate cancer showed that non-cytotoxic oxidant damage sensitizes cells to MSA-triggered apoptosis [13]. We reasoned that selenite might have even more potent homeostatic housecleaning activity because selenite has been shown to induce DNA strand breaks [32], which might render selenite better equipped than MSA (which does not damage DNA) to trigger apoptosis in prostate cell populations after non-cytotoxic H_2O_2 exposure. Here, we show this is not so. These discordant results — positive homeostatic housecleaning activity with MSA, negative homeostatic housecleaning activity with selenite — are consistent with previous studies showing these two compounds exert divergent effects on an array of mechanisms of anti-carcinogenesis in prostatic cells as diverse as reactive oxygen species production [33], p53 activation [30,34], cell cycle arrest [35,36], androgen receptor signaling [37,38], matrix metalloproteinase production [39,40], and apoptosis [28,30,33,35,41]. Of particular relevance to the results reported here are the findings of Hu and colleagues [28] that MSA can potentiate apoptosis induced by chemotherapeutic drugs, whereas selenite cannot. Their results from evaluating MSA and selenite in a cancer treatment setting are complemented by our experiments, which evaluate the ability of MSA and selenite to trigger apoptosis following non-cytotoxic oxidant damage, suggesting form-dependent effects of selenium may indeed impact the emergence of cancer in a cancer-preventive context — regulating the elimination of damaged epithelial cell populations in the aging prostate exposed to pro-oxidant pressures. Future studies might be directed toward defining the extent to which the expression of specific selenoproteins modulates selenium-induced elimination of oxidant-damaged prostatic cells.

Finally, as scientists and health professionals continue to collectively re-think the role of selenium and other nutrients in cancer prevention, investigators must work to carefully document the form-dependent effects of nutrients. By avoiding a mindset of *naïve substitution* — seeing one form of nutrient as equivalent to another — we make surer progress toward understanding the implications of our laboratory findings and side-stepping errant assumptions. Moreover, this disciplined approach might help us to avoid potential pitfalls in the design and interpretation of large-scale human trials.

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