Research Communication

Homeostatic housecleaning effect of selenium: evidence that noncytotoxic oxidant-induced damage sensitizes prostate cancer cells to organic selenium-triggered apoptosis

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Abstract

The anti-cancer activity of organic selenium has been most consistently documented at supra-nutritional levels at which selenium-dependent, antioxidant enzymes are maximized in both expression and activity. Thus, there is a strong imperative to identify mechanisms other than antioxidant protection to account for selenium's anti-cancer activity. *In vivo* work in dogs showed that dietary selenium supplementation decreased DNA damage but increased apoptosis in the prostate, leading to a new hypothesis: Organic selenium exerts its cancer preventive effect by selectively increasing apoptosis in DNA-damaged cells. Here, we test whether organic selenium (methylseleninic acid; MSA) triggers more apoptosis in human and canine prostate cancer cells that have more DNA damage (strand breaks) created by hydrogen-peroxide (H_2O_2) at noncytotoxic doses prior to MSA exposure. Apoptosis triggered by MSA was significantly higher in H_2O_2-damaged cells. A supra-additive effect was observed—the extent of MSA-triggered apoptosis in H_2O_2-damaged cells exceeded the sum of apoptosis induced by MSA or H_2O_2 alone. However, neither the persistence of H_2O_2-induced DNA damage, nor the activation of mitogen-activated protein kinases was required to sensitize cells to MSA-triggered apoptosis. Our results document that selenium can exert a "homeostatic housecleaning" effect—a preferential elimination of DNA-damaged cells. This work introduces a new and potentially important perspective on the anti-cancer action of selenium in the aging prostate that is independent of its role in antioxidant protection.

Keywords: *cell fate; damage response; oxidative stress; chemoprevention*

1. Introduction

Selenium has received considerable attention as a potential cancer preventive agent because it is required for glutathione peroxidase activity, an important player in antioxidant defense [1]. For more than a decade, the importance of oxidative DNA damage and prostatic carcinogenesis has been actively investigated, culminating in the testing of supplementation with organic selenium in the largest-ever prostate cancer prevention trial, Selenium and Vitamin E Cancer Prevention Trial (SELECT). However, when SELECT was launched there was still no agreement on the most likely prostate cancer preventive mechanism of selenium. The remarkable reduction in prostate

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cancer incidence achieved by dietary selenium supplementation in the Nutritional Prevention of Cancer (NPC) Trial was seen in selenium-adequate subjects who already had maximum glutathione peroxidase activity prior to supplementation [2]. This called into serious doubt whether selenium exerts its prostate cancer preventive effect mainly through antioxidant defense [3–5].

Apoptosis induction is one of the potentially important cancer preventive mechanisms of organic selenium [6]. Supranutritional selenium supplementation in animals enhances apoptosis and reduces cancer progression in mammary gland, prostate, liver, colon, and skin [3,4,7–11]. Work from our laboratory showed that selenium-adequate, elderly dogs supplemented with organic selenium for 7 months had significantly reduced DNA damage, which was associated with no increase in glutathione peroxidase activity, yet increased apoptosis in the prostate [12]. The results of the dog study led to a new line of thinking: The lower extent of prostatic DNA damage associated with organic selenium supplementation might be the result of selenium-triggered apoptosis of prostatic cells. Moreover, to achieve a significant lowering of DNA damage level in surviving prostatic cells, organic selenium must *preferentially eliminate damaged cells,* because a nonselective triggering of apoptosis in cells would not explain the observed DNA damage reduction. Dogs with the lowest prostatic DNA damage had selenium status which paralleled the selenium status of men who had reduced prostate cancer risk in the NPC Trial and the Health Professionals Follow-up Study [13]. This provided confidence that the *in vivo* observations made in the dog study could be highly relevant to human prostate cancer protection. Hence, we reasoned that the idea that selenium might preferentially trigger apoptosis in damaged cells, a process we named homeostatic housecleaning [14], should be explored further.

To test whether organic selenium preferentially triggers apoptosis in prostatic cells with higher extent of DNA damage, we developed an *in vitro* model system in which DNA damage level could be carefully controlled. Prostate cancer cell lines were treated with hydrogen peroxide (H_2O_2) at noncytotoxic concentrations to create populations with low, medium, or high extent of DNA strand breaks. Methylseleninic acid (MSA) was used as a form of organic selenium to trigger apoptosis. In addition to apoptosis detection, clonogenic assay was employed to evaluate the influence of selenium on the proliferative potential of surviving cells. This study tested whether preferential elimination of DNA-damaged cells by selenium-triggered apoptosis is species-specific (human vs. canine cells), peculiar to H_2O_2-induced DNA damage (vs. other DNA damaging agents), or requires the persistence of DNA strand breaks.

2. Materials and Methods

2.1. Reagents

H_2O_2 solution was purchased from Mallinckrodt (Phillipsburg, NJ). Methylseleninic acid (MSA), etoposide, neocarzinostatin, and other chemicals used in the study were purchased from Sigma-Aldrich Chemical (St. Louis, MO). Annexin-V-FLUOS staining kit for apoptosis quantification was purchased from Roche Applied Science (Indianapolis, IN). Primary antibodies for immunoblot detection of cleaved poly (ADP-ribose) polymerase (Asp214) (PARP), cleaved caspase-3 (Asp175), phosphorylated-Akt (Ser473), phosphorylated-extracellular signal-regulated kinase (Erk) (Thr202/Tyr204), phosphorylated-c-jun *N*-terminal kinase (JNK) (Thr183/Tyr 185), phosphorylated-p38 (Thr180/Tyr182), survivin, and β-actin were purchased from Cell Signaling Technology (Beverly, MA). Survivin antibody from Santa Cruz Biotechnology (Santa Cruz, CA) was applied to detect survivin in TR5P cells. Chemiluminescence kit for immunobloting was purchased from Pierce (Rockford, IL).

2.2. Cell Culture

Human prostate cancer cell lines, DU-145, LNCaP, and PC-3 were purchased from ATCC (Manassas, VA). Human benign prostatic cell line, PrEC, and its complete culture medium were purchased from Lonza (Walkersville, MD). Canine prostate cancer cell lines, PC1, TR5P, and TR6LM, were established in our laboratory. PC1 and TR5P were established from primary prostate cancers individually collected from two dogs. TR6LM was derived from the lymph node metastasis of the dog from which TR5P was established. Cell culture medium, MEM (Sigma-Aldrich Chemical, St. Louis, MO), RPMI-1640 (Sigma-Aldrich Chemical, St. Louis, MO), and DMEM F-12 (GIBCO, Carlsbad, CA) supplemented with batch-matched fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA) was used in all studies. DU-145, LNCaP, and PC-3 human prostate cancer cells were maintained in DMEM F-12 supplemented with 10% (v/v) FBS, RPMI-1640 supplemented with 10% (v/v) FBS, and MEM supplemented with 7% (v/v) FBS, respectively. PrEC human benign prostatic cells were maintained and subcultured according to the manufacturer's instructions. PC1, TR5P, and TR6LM canine prostate cancer cells were maintained in RPMI-1640 supplemented with 5% (v/v) or 10% (v/v) FBS. 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 h before introducing treatment.

2.3. Treatments

To induce DNA damage, cells were exposed to H_2O_2 (1 h), etoposide (1 h), or neocarzinostatin (24 h). DMSO was used as the vehicle for etoposide and neocarzinostatin (<0.6% (v/v) final concentration). In all experiments, parental cells were exposed to equivalent amount of DMSO serving as vehicle control. Immediately after exposure to DNA damaging agents, cells were washed with PBS and cultured in fresh complete medium containing MSA for 24 h. In repair experiments, cells were cultured in fresh complete medium in the absence of H_2O_2 and MSA for 1 or 24 h to allow for DNA repair, and then exposed to MSA in complete medium for 24 h.

To inhibit JNK or p38 activation induced by H_2O_2 exposure, cells were treated with JNK activation inhibitor SP600125 (1 h) or p38 activation inhibitor PD169316 (2 h)

prior to H_2O_2 exposure. In these inhibitor studies, cells in the presence of inhibitors were exposed to H_2O_2 for 1 h and to MSA for 24 h. DMSO was used as the vehicle for SP600125 and PD169316 (<0.6% (v/v) at the final concentration). In these experiments, parental cells exposed to equivalent amount of DMSO served as vehicle control.

2.4. Assessment of DNA Damage: DNA Strand Breaks Detected by Single Cell Gel Electrophoresis (Comet Assay)

Alkaline (pH >13) or neutral (pH 8.5) Comet assay was employed to measure DNA single or double strand breaks, respectively. Ten thousand cells were suspended in 0.5% low melting point agarose and spread out on a microscope slide precoated with 1% (w/v) normal melting point agarose. Another layer of low melting point agarose was applied after the first layer solidified on ice. Slides were placed in cold lysing solution containing 2.5 M NaCl, 100 mM EDTA, 10 mM Trizma base, 1% (v/v) Triton X-100, and 10% (v/v) DMSO at pH 10.0 for 1 h. After lysing, the rest of the procedure was carried out in dim yellow light to avoid additional DNA damage. Single strand breaks were measured as described previously [13,15,16]. Briefly, slides were immersed in alkaline electrophoresis buffer (300 mM NaOH, 200 mM EDTA, pH >13) for 20 min to unwind DNA and exposed to 25 volts, 300 milliamperes for 30 min. After electrophoresis, slides were washed three times with neutralization buffer (0.4 M Trizma base, pH 7.5) and dried with 100% ethanol. For DNA double strand break measurement, slides were washed with distilled water after lysing followed by electrophoresis in tris-borate-EDTA buffer (90 mM Trizma base, 90 mM boric acid, 2 mM EDTA, pH 8.5) at 20 volts, < 10 milliamperes for 25 min and dried with ethanol [17].

DNA strand breaks were visually scored. Slides were stained with SYBR Green diluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and read using a fluorescent microscope (Nikon Instruments, Melville, NY). The extent of DNA damage was scored using the 0-4 scale scoring system of Collins [intact DNA (type 0), mild to moderate damage (type 1 or 2), and severe damage (type 3 or 4)] [12,18]. One hundred cells on each slide were randomly selected and scored. The percentage of prostatic cells bearing severe damage (type 3 and 4) was calculated.

2.5. Apoptosis Detection

Loss of phospholipid asymmetry is one of the hallmarks of the early phase of apoptosis and results in externalization of phosphatidylserine which can be detected by Annexin-V [19,20]. To quantify apoptosis, cell surface phosphatidylserine was measured by Annexin-V-fluorescein and propidium iodide double staining using a flow cytometer (Cytomics FC 500, Beckman Coulter, Miami, FL). Double staining of cells for Annexin-V-fluorescein and propidum iodide provides a reliable, high throughput method that enables discrimination of apoptotic cells from necrotic and dead cells. Studies indicate Annexin-V staining is sensitive and specific in detecting apoptosis, and strong correlation between Annexin-V and TUNEL results has been reported [21–25].

After treatments, cells were trypsinized and centrifuged at 200*g* for 5 min with spent media to collect both adherent and detached cells. Cell pellets were washed with PBS twice and suspended in annexin-V and propidium iodide labeling solution. After 20-min incubation at room temperature, cells were analyzed on a flow cytometer to detect the percentage of apoptotic and dead cells per 10,000 cells. The supra-additive effect of H_2O_2 and MSA on apoptosis was calculated as:

$$\begin{aligned}&\text{Supra-additive Effect (\%)}\\&\quad = (\text{Observed value} - \text{Predicted value})\\&\quad \div \text{Predicted value}\end{aligned}$$

The predicted value was the arithmetic sum of apoptosis observed in parental cells plus the apoptosis induced by H_2O_2 and MSA alone:

$$\begin{aligned}&\text{Predicted Apoptosis}\\&\quad = \text{Basal apoptosis in parental cells}\\&\quad + (\text{Apoptosis in } H_2O_2\text{-damaged cells}\\&\quad - \text{Basal apoptosis in parental cells})\\&\quad + (\text{Apoptosis after MSA treatment}\\&\quad - \text{Basal apoptosis in parental cells})\end{aligned}$$

For cell death, the supra-additive effect was calculated in the same manner as described for apoptosis.

The detection of cleaved PARP [26] and cleaved caspase-3 [27] by immunoblot served as an execution marker of apoptosis in DU-145 human and TR5P canine prostate cancer cells, respectively.

2.6. Western Blot Determination

Cell lysates from both adherent and detached cells were collected in 1% (v/v) Triton X-100 lysis buffer (150 mM NaCl, 50 mM Trizma base, pH 8.0) containing protease inhibitors aprotinin, leupeptin, and phenylmethanesulfonyl fluoride, and phosphatase inhibitor sodium fluoride and sodium orthovanadate. Supernatants were collected by centrifuge at 10,000*g*, 4°C for 20 min. Protein content was quantified by the Bradford dye-binding assay (Bio-Red Laboratories, Richmond, CA). Fifty to 80 μg of total protein was used for electrophoresis on 8-12% (w/v) mini SDS polyscrylamide gel with Tris-glycine buffer (25 mM Trizma base, 190 mM glycine, 0.1% (w/v) SDS). Proteins were transferred to PVDF membrane in a semi-dry condition and blocked with 5% nonfat milk in tris-buffered saline Tween-20 (TBST) buffer (20 mM Tris-HCl, 150 mM NaCl, 0.1% (v/v) Tween 20). All primary antibodies were diluted in TBST buffer and incubated with membranes overnight at 4°C. After washed three times with TBST buffer, membranes were incubated with secondary antibodies in TBST for an hour at room temperature. Membranes were developed with a

chemiluminescence kit following the manufacturer's instructions. 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).

2.7. Clonogenic Assay

To assess the proliferative potential of cells that survived after treatment, prostate cancer cells were seeded in 24-well plates at 50% confluency 18 h before DNA damage induction. After exposure to H_2O_2 or etoposide for 1 h and MSA for 24 h, a limiting dilution of 500 cells was reseeded into 6-well plates with complete culture medium not containing H_2O_2 or MSA. The medium was replenished every 3 days. Clone formation in TR5P and DU-145 cells was evaluated after 7- and 10-days culture, respectively. To fix colonies, cells were stained with 0.5% (w/v) crystal violet solution containing 6% glutaraldehyde (w/v) for 1 h. Proliferative potential was calculated as:

$$\text{Proliferative Potential}(\%) = [\text{Number of colonies composed of} > 50 \text{ cells} \div \text{Number of cells seeded}] \times \text{Plating efficiency}$$

2.8. Statistical Analysis

Data from three independent experiments were analyzed by ANOVA followed by Tukey's range test to evaluate the effect of H_2O_2 or MSA on apoptosis, cell death, and proliferative potential. Two-tailed, independent *t*-test was applied to compare differences between treated and parental cells or the reference group. A *P*-value < 0.05 was considered significant. Data are presented as mean $\pm$ SD. All statistical analyses were carried out using SAS 9.2 software (SAS Institute, Cary, NC).

3. Results

3.1. Development of *In Vitro* Model of H_2O_2-Induced Noncytotoxic DNA Damage in Human Prostate Cancer Cells

In DU-145 human prostate cancer cells, 1-h H_2O_2 exposure at concentrations of 50 and 100 μM created populations with moderate (50%) or high (90%) percentage of extensively DNA damaged cells, respectively (Table 1). Neither H_2O_2 concentration significantly increased apoptosis or cell death, *i.e.* these exposures were noncytotoxic. DU-145 parental cells not exposed to H_2O_2, which served as the low DNA damage cell population in all experiments, had 23% extensively damaged cells. The extent of H_2O_2-induced DNA strand breaks in DU-145 cells returned to preexposure level within 1 h after H_2O_2 removal (Table 1).

The sensitivity of human prostate cancer cells to MSA-triggered apoptosis was tested at selenium concentrations less than 10 μM for 24-h exposure. Selenium concentrations exceeding 10 μM were not studied, because the level is toxic to humans [28]. DU-145 human prostate cancer cells were responsive to MSA-triggered apoptosis. At a concentration of 3 μM MSA, there was increased apoptosis measured by the level of cell surface phosphatidylserine (Fig. 1A) and detection of cleaved PARP (Fig. 1B). Therefore, DU-145 cells were chosen as suitable for our *in vitro* studies. LNCaP, PC-3, and PrEC cells were resistant to MSA-induced apoptosis, and not used in further experiments.

3.2. Organic Selenium (MSA)-Triggered Apoptosis in DU-145 Human Prostate Cancer Cells: Effect of H_2O_2-Induced Noncytotoxic Damage

Compared with parental cells, DU-145 cells with H_2O_2-induced noncytotoxic DNA damage were significantly more susceptible to MSA-triggered apoptosis (Fig. 1A). Even at a concentration of 1.5 μM MSA, which did not significantly trigger apoptosis in parental cells, there was a 1.7X ($P = 0.02$) and 2.4X ($P = 0.001$) fold increase in apoptosis in cells damaged with 50 and 100 μM H_2O_2, compared with parental cells. Moreover, at both 1.5 and 3 μM concentrations of MSA, highly damaged DU-145 cells had 40% higher apoptosis than moderately damaged cells ($P = 0.04$ for 1.5 μM MSA; $P = 0.03$ for 3 μM MSA). The detection of cleaved PARP yielded results consistent with the measurement of apoptosis based upon cell surface phosphatidylserine; cells with the highest noncytotoxic DNA damage when MSA was introduced had higher levels of cleaved PARP after selenium exposure than cells with moderate DNA damage or parental cells (Fig. 1B). In addition to apoptosis, cell death was also measured. The extent of cell death after MSA exposure paralleled the extent of apoptosis, with the difference between parental cells and cells with the highest noncytotoxic DNA damage reaching statistical significance (2.1X, $P = 0.01$ for 1.5 μM MSA; 2.0X, $P = 0.02$ for 3 μM MSA) (Fig. 1C). Together these results indicated that selenium triggers higher apoptosis in H_2O_2-damaged DU-145 human prostate cancer cells.

3.3. Organic Selenium (MSA)-Triggered Apoptosis in Canine Prostate Cancer Cells: Effect of H_2O_2-Induced Damage

To test whether the ability of selenium to trigger higher apoptosis in H_2O_2-damaged prostate cancer cells was species-specific, canine prostate cancer cell lines, PC1, TR5P, and TR6LM, were studied. Before H_2O_2 exposure, 90% of these cells already had extensive DNA single strand breaks. In contrast, DNA double strand breaks were detected in a lower percentage of these cells. Therefore, double strand break induction became a better criterion to select noncytotoxic doses of H_2O_2 that would significantly increase DNA damage in canine prostate cancer cell lines. In PC1 and TR6LM cells, 1-h H_2O_2 exposure did not significantly induce extensive DNA double strand breaks even at high concentrations (Table 2). However, in TR5P cells, exposure to 400 μM H_2O_2 nearly doubled the number of extensive DNA double strand breaks; increasing from 7.8% to 13.5% ($P = 0.001$). In these cells, concentrations of H_2O_2 less than 400 μM, H_2O_2 did not significantly damage DNA, whereas concentrations exceeding

TABLE 1 *Determining the extent of DNA damage (measured as single strand breaks) in human malignant and benign prostate cell lines following exposure to noncytotoxic concentrations of hydrogen peroxide (H_2O_2)*[a]

		Extensive single strand breaks[b]			
	H_2O_2 μM	Treatment %[d]	Repair[e] %	Apoptosis[c] %	Cell death[c] %
DU-145	0	23.33 ± 5.51[§]	20.35 ± 1.51	1.28 ± 0.40	2.60 ± 0.52
	50	48.33 ± 7.23[#]	31.74 ± 7.45	1.58 ± 0.63	2.84 ± 0.41
	100	90.00 ± 2.00[*]	28.84 ± 9.87	2.06 ± 0.75	3.10 ± 0.20
LNCaP	0	31.77 ± 5.40	24.53 ± 5.40	2.30 ± 0.50	3.20 ± 0.26
	25	35.43 ± 2.15	37.03 ± 3.65	3.24 ± 0.69	3.14 ± 0.86
	50	36.46 ± 6.26	35.20 ± 4.26	2.80 ± 0.56	3.00 ± 0.35
PC-3	0	29.74 ± 4.51	26.14 ± 6.58	2.60 ± 0.30	1.60 ± 0.26
	50	29.67 ± 8.37	29.55 ± 6.61	3.20 ± 0.10	2.77 ± 0.26
	100	30.30 ± 7.94	27.54 ± 4.29	3.85 ± 0.31	2.70 ± 0.38
PrEC	0	28.73 ± 5.13[§]	22.50 ± 4.22	1.97 ± 0.75	1.55 ± 0.46
	200	50.55 ± 8.81[#]	20.43 ± 7.13	1.85 ± 0.42	1.33 ± 0.15
	400	90.10 ± 5.08[*]	35.28 ± 5.02	1.83 ± 0.78	1.60 ± 0.28

[a] *Data are expressed as mean ± SD from three independent experiments. Within each cell line, means without a common symbol differ,* $P < 0.05$.
[b] *The extent of DNA damage was scored using the 0-4 scale scoring system of Collins, and calculated as the percentage of 100 prostatic cells with severe damage (type 3 or 4).*
[c] *Cells were treated with H_2O_2 at indicated concentrations for 1 h and cultured in fresh medium in the absence of H_2O_2 for 24 h.*
[d] *Cells were treated with H_2O_2 for 1 h.*
[e] *After 1-h H_2O_2 treatment, cells were washed with PBS and cultured in complete medium in the absence of H_2O_2 for 1 h.*

500 μM H_2O_2 were cytotoxic (data not shown). Thus, a dose of 400 μM H_2O_2 was chosen for our experiments evaluating the response of TR5P canine prostate cancer cells to selenium.

MSA at a concentration of 5 μM or greater triggered apoptosis in TR5P parental cells. Similar to our observations in DU-145 human prostate cancer cells, MSA triggered more apoptosis in H_2O_2-damaged TR5P canine prostate cancer cells than in parental cells. MSA triggered higher apoptosis in TR5P cells with H_2O_2-induced DNA double strand breaks, even at 3 μM MSA, a dose that by itself did not significantly trigger apoptosis in parental cells (Fig. 2A). Compared with parental cells, MSA at 3 μM and 5 μM triggered 2.1X ($P = 0.001$) and 2.8X ($P = 0.001$) higher apoptosis in cells damaged with 400 μM H_2O_2. Apoptosis measured by the detection of cleaved caspase-3 was consistent with the level of cell surface phosphatidylserine (Fig. 2B). Also, cell death moved in parallel with apoptosis. H_2O_2-damaged cells had higher cell death triggered by selenium than parental cells (2.1X, $P = 0.01$ for 3 μM MSA; 2.6X, $P = 0.03$ for 5 μM MSA) (Fig. 2C). We concluded from these results that selenium triggers higher apoptosis in H_2O_2-damaged prostate cancer cells by a process that is species-independent.

3.4. Organic Selenium (MSA)-Triggered Apoptosis: Supra-Additive Effect in DNA-Damaged Cells

Apoptosis is a cellular response that can eliminate irreparable damaged cells. It is expected that cells with more damage are more susceptible to apoptosis. However, if the extent of selenium-triggered apoptosis in H_2O_2-damaged cells exceeds the sum of apoptosis induced by selenium or H_2O_2 alone, then it could be concluded that H_2O_2-induced damage sensitizes prostatic cells to organic selenium-triggered apoptosis. We found the extent of apoptosis in both DU-145 and TR5P prostate cancer cells exceeded the amount predicted by the arithmetic sum of apoptosis induced by H_2O_2 or MSA alone (Fig. 3, Table 3). This supra-additive effect on apoptosis induction, as well as on cell death, was significant in DU-145 human prostate cancer cells damaged with 100 μM H_2O_2 followed by treatment with 3 μM MSA (Tables 3 and 4). Under these experimental conditions, selenium-triggered apoptosis in damaged cells exceeded the predicted level of apoptosis by 71% ($P < 0.0001$). Similar to apoptosis, cell death triggered by selenium in damaged cells exceeded the predicted level by 63% ($P < 0.0001$).

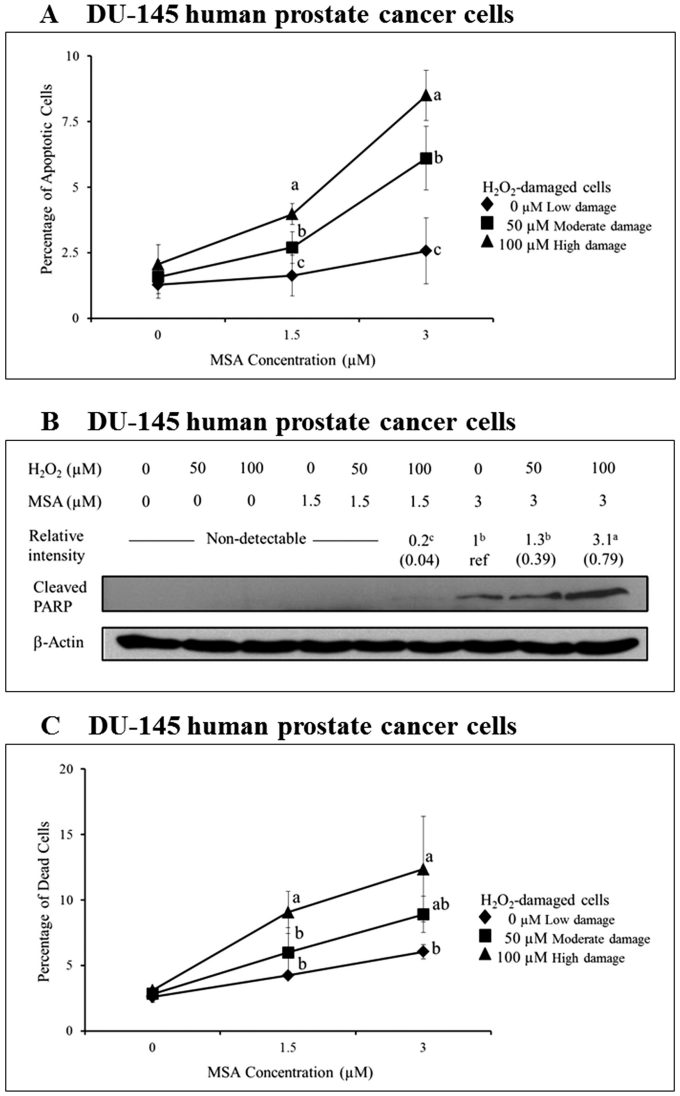

FIG 1

Organic selenium (MSA) triggers higher apoptosis in hydrogen peroxide (H_2O_2)-damaged DU-145 human prostate cancer cells. After 1-h exposure with H_2O_2 to induce noncytotoxic DNA damage, DU-145 cells were treated with MSA for 24 h. A: Percentage of apoptotic cells detected with increased level of cell surface phosphatidylserine. Significant differences in the extent of MSA-triggered apoptosis in low, moderate, and high DNA-damaged cells are seen at 1.5 μM and 3 μM MSA. B: Protein expression of cleaved PARP, an execution marker of apoptosis. The blot intensity in cells treated with 3 μM MSA alone was the reference for relative intensity ("ref" in figure). Data are expressed as mean (SD). β-actin served as the loading control. C: Percentage of dead cells. Significant differences in the extent of MSA-triggered cell death in low and high DNA damaged cells are seen at 1.5 and 3 μM MSA. Data are from three independent experiments. For (A) and (C) within each MSA exposure, means without a common letter differ, $P < 0.05$. For (B) means without a common letter differ, $P < 0.05$.

In TR5P canine prostate cancer cells, the supra-additive effect of H_2O_2 and MSA on apoptosis and cell death was even more dramatic (Fig. 3, Tables 3 and 4). Apoptosis triggered by 5 μM MSA in damaged cells exceeded predicted level of apoptosis by 184% ($P < 0.0001$). Cell death triggered by selenium in damaged cells exceeded predicted level by 176% ($P = 0.0002$). Increasing MSA concentration from 3 μM to 5 μM significantly intensified the supra-additive effect of H_2O_2 on apoptosis ($P = 0.003$). The supra-additive effect of H_2O_2 and MSA together on apoptosis and cell death shows that these two agents acting alone are less potent in killing prostatic cells. We concluded that noncytotoxic damage induced by H_2O_2 sensitizes human and canine prostate cancer cell populations to organic selenium-triggered apoptosis and cell death.

3.5. Effect of Other DNA Damaging Agents on MSA-Triggered Apoptosis

The damage caused by H_2O_2 is not limited to DNA. Other macromolecules, such as lipids and proteins, are also affected. To evaluate whether the enhanced ability of MSA to trigger apoptosis in damaged cells was peculiar to H_2O_2 treatment, two other damaging agents were studied: (1) neocarzinostatin—a DNA-specific, oxidative damaging agent; and (2) etoposide, a DNA-specific, nonoxidative damaging agent.

Neocarzinostatin was used at a concentration of 20 μM, because this concentration induced an extent of DNA single strand breaks in DU-145 cells equivalent to that induced by 50 μM H_2O_2 (Table 5). Similar to H_2O_2-induced damage, neocarzinostatin-induced damage sensitized cells to MSA-triggered apoptosis; 7.4X higher apoptosis was seen compared with nondamaged cells ($P = 0.003$) (Fig. 4A). Compared with H_2O_2-damaged cells, the 2.8X ($P = 0.001$) higher MSA-triggered apoptosis in neocarzinostatin-damaged cells suggested that DNA-specific oxidant-induced damage was more potent than nonselective oxidant-induced damage to sensitize DU-145 human prostate cancer cells to selenium-triggered apoptosis. In TR5P cells, neocarzinostatin did not induce an amount of DNA damage equivalent to that induced by 400 μM H_2O_2, so it could not be tested in canine prostate cancer cells.

Etoposide was used at concentrations of 10 and 60 μM, which induced an extent of DNA single strand breaks in DU-145 cells equivalent to that induced by 50 and 100 μM H_2O_2, respectively (Table 4). At both concentrations, etoposide-induced damage sensitized DU-145 cells to MSA-triggered apoptosis (Figs. 4A and 4B), but the magnitude was 26% lower in 60 μM etoposide-damaged cells than in 100 μM H_2O_2-damaged cells ($P = 0.01$). Etoposide failed to sensitize TR5P cells to MSA-triggered apoptosis (Fig. 4C), suggesting that oxidant-induced DNA damage was more capable of sensitizing prostate cancer cells to MSA-triggered apoptosis than nonoxidant-induced DNA damage.

3.6. Effect of Organic Selenium (MSA) on Proliferative Potential of Prostate Cancer Cells Damaged by H_2O_2

The proliferative potential of cells not eliminated by MSA-triggered apoptosis was evaluated by clonogenic assay. In DU-

TABLE 2 *Determining the extent of DNA damage (measured as double strand breaks) in canine prostate cancer cell lines following exposure to noncytotoxic concentrations of hydrogen peroxide (H_2O_2)*[a]

		Extensive double strand breaks[b]			
	H_2O_2 μM	*Treatment %*[d]	*Repair*[e] *%*	*Apoptosis*[c] *%*	*Cell death*[c] *%*
TR5P	0	7.75 ± 0.50[#]	7.67 ± 0.58	5.79 ± 0.61	3.74 ± 1.10
	400	13.50 ± 2.07[*]	6.00 ± 1.00	6.00 ± 0.79	3.20 ± 0.36
TR6LM	0	1.67 ± 0.31	1.50 ± 0.50	2.48 ± 0.51	0.70 ± 0.10
	25	1.67 ± 0.58	2.00 ± 0.60	2.73 ± 0.79	0.78 ± 0.10
	50	2.33 ± 1.53	1.75 ± 0.45	1.98 ± 0.19	0.75 ± 0.10
PC1	0	1.33 ± 0.51	1.25 ± 0.71	1.97 ± 0.75	1.55 ± 0.46
	25	1.67 ± 0.57	1.49 ± 0.28	1.85 ± 0.42	1.33 ± 0.15
	50	1.33 ± 0.58	1.33 ± 0.53	1.83 ± 0.78	1.60 ± 0.28

[a] *Data are expressed as mean ± SD from three independent experiments. Within each cell line, means without a common symbol differ,* $P < 0.05$.
[b] *The extent of DNA damage was scored using the 0-4 scale scoring system of Collins, and calculated as the percentage of 100 prostatic cells with severe damage (type 3 or 4).*
[c] *Cells were treated with H_2O_2 for 1 h and cultured in fresh medium in the absence of H_2O_2 for 24 h.*
[d] *Cells were treated with H_2O_2 for 1 h.*
[e] *After 1-h H_2O_2 treatment, cells were washed with PBS and cultured in complete medium in the absence of H_2O_2 for 1 h.*

145 parental cells, 24-h MSA exposure did not significantly affect proliferation, estimated by colony formation 10 days after treatment ($P = 0.13$) (Fig. 5A). For H_2O_2-damaged cells, interestingly, selenium influenced proliferation in a nonlinear, dose-dependent manner (Fig. 5B). DU-145 cells damaged with 50 or 100 μM H_2O_2 had significantly higher proliferative potential after 1.5 μM MSA exposure than no MSA exposure ($P = 0.01$ for moderately damaged cells; $P = 0.04$ for highly damaged cells). This proliferation-enhancing effect of selenium was not seen at 3 μM MSA. Under our experimental conditions, selenium did not affect the proliferative potential of cells damaged with etoposide ($P = 0.31$ for 10 μM; $P = 0.90$ for 60 μM) (Fig. 5C). The extent to which selenium influences the proliferative potential of neocarzinostatin-damaged DU-145 cells or H_2O_2-damaged TR5P cells could not be evaluated, because these DNA damaging agents alone completely inhibited proliferation at the doses evaluated.

3.7. Organic Selenium (MSA)-Triggered Apoptosis in H_2O_2-Damaged Prostate Cancer Cells: Effect of DNA Repair

To test whether persistence of H_2O_2-induced noncytotoxic DNA strand breaks was necessary to sensitize DU-145 human and TR5P canine prostate cancer cells to MSA-triggered apoptosis, MSA was not introduced until H_2O_2-induced DNA damage returned to pre-H_2O_2 exposure level. One hour after H_2O_2 removal, the extent of DNA strand breaks in both DU-145 and TR5P cells were not different from parental cells (Tables 1 and 2). Compared with cells immediately exposed to MSA after H_2O_2 treatment, 1-h repair did not reduce MSA-triggered apoptosis, measured by level of cleaved PARP or cleaved caspase-3 (Fig. 6). Similarly, when repair time was extended to 24 h, H_2O_2-damaged cells were still sensitized to MSA-triggered apoptosis. In fact, in DU-145 cells, sensitivity was actually enhanced after 24-h repair; exposure to 100 μM H_2O_2, 3 μM MSA triggered 57% more apoptosis after 24-h repair compared to immediate MSA exposure ($P = 0.03$). Thus, we concluded that the persistence of DNA strand breaks was not necessary for the sensitizing effect that H_2O_2-induced damage had on selenium-triggered apoptosis.

3.8. Exploration of Mechanistic Pathways

Based upon the cumulative work of other investigators [27,29,30], we reasoned that the cellular sensitivity of prostatic cells to MSA-triggered apoptosis is most likely determined by the balance between anti- and pro-apoptotic signals regulated by Akt, Erk, JNK, p38, and survivin. To test whether H_2O_2-induced DNA damage sensitizes prostate cancer cells to MSA-induced apoptosis by reducing anti-apoptotic signaling (Akt, Erk, survivin) or by enhancing pro-apoptotic signaling (JNK, p38), the activation status (phosphorylation) of kinases and the protein level of survivin were measured. After 1-h H_2O_2 exposure, neither survivin level nor kinase activation was significantly changed in DU-145 cells. In contrast, in TR5P cells, H_2O_2 exposure promoted proapoptotic signals by reducing survivin protein level and activating JNK and p38. After 1-h DNA

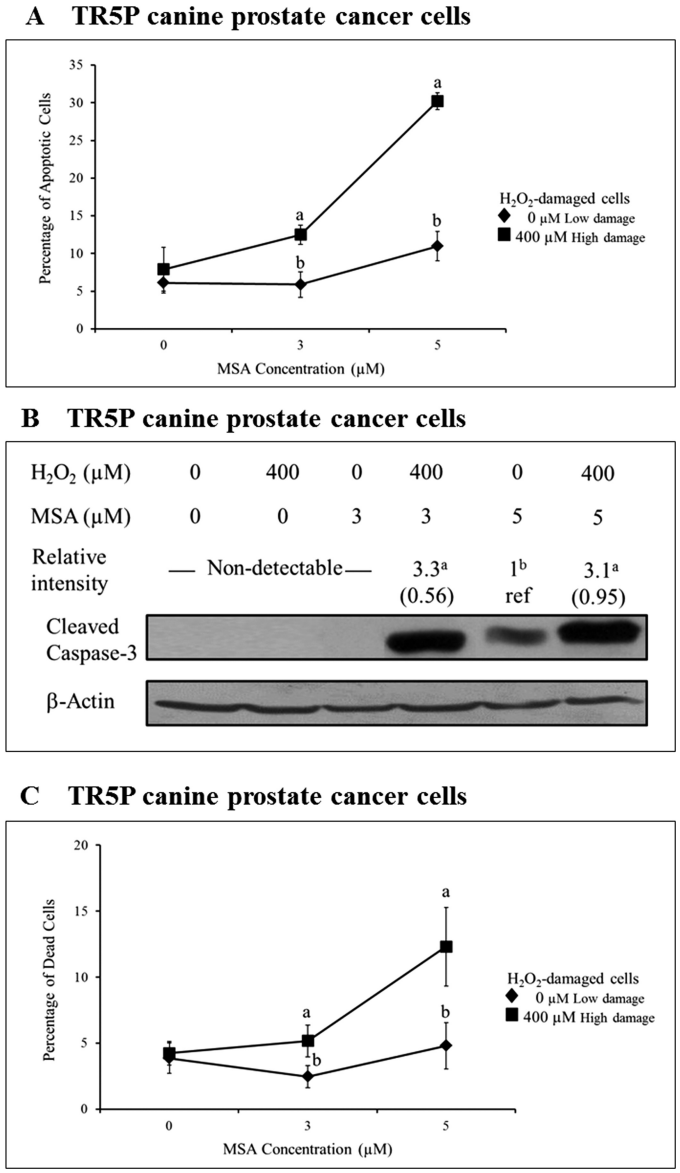

FIG 2

Organic selenium (MSA) triggers higher apoptosis in hydrogen peroxide (H_2O_2)-damaged TR5P canine prostate cancer cells. After 1-h exposure with H_2O_2 to induce noncytotoxic DNA damage, TR5P cells were treated with MSA for 24 h. A: Percentage of apoptotic cells detected with increased level of surface phosphatidylserine. Significant differences in the extent of MSA-triggered apoptosis in low and high DNA damaged cells are seen at 3 and 5 μM MSA. (B) Protein expression of cleaved-caspase-3, an execution marker of apoptosis. The blot intensity in cells treated with 5 μM MSA alone was the reference for relative intensity ("ref" in figure). Data are expressed as mean (SD). β-actin served as the loading control. C: Percentage of dead cells. Significant differences in the extent of MSA-triggered cell death in low and high DNA damaged cells are seen at 3 and 5 μM MSA. Data are from three independent experiments. For (A) and (C) within each MSA exposure, means without a common letter differ, $P < 0.05$. For (B) means without a common letter differ, $P < 0.05$.

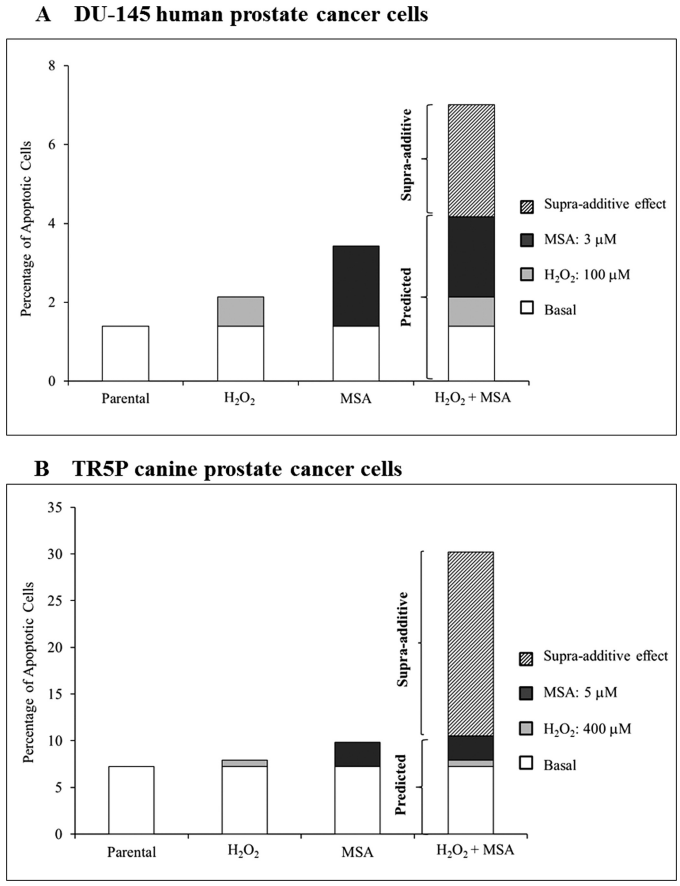

FIG 3

Supra-additive effect of organic selenium (MSA) and hydrogen peroxide (H_2O_2) on apoptosis triggered in DU-145 human and TR5P canine prostate cancer cells. After 1-h exposure with H_2O_2 to induce noncytotoxic DNA damage, DU-145 (A) and TR5P (B) cells were treated with MSA for 24 h. Apoptosis was assessed by the increased level of surface phosphatidylserine. The observed extent of MSA-triggered apoptosis in H_2O_2-damaged cells exceeded the predicted value which was the arithmetic sum of apoptosis observed in parental cells plus the apoptosis induced by H_2O_2 and MSA alone. Predicted apoptosis = Basal apoptosis in parental cells + (Apoptosis in H_2O_2-damaged cells − Basal apoptosis in parental cells) + (Apoptosis after MSA treatment − Basal apoptosis in parental cells). The difference between observed value and predicted value is supra-additive.

damage repair, reduction of survivin and activation of JNK and p38 persisted in these prostate cancer cells.

To evaluate if activation of JNK or p38, or down-regulation of survivin were necessary for sensitizing DNA-damaged TR5P cells to MSA-induced apoptosis, pathway-specific activation inhibitors were applied. SP600125 at 20 μM completely abolished JNK activation in H_2O_2-damaged TR5P canine prostate cancer cells and enhanced survivin protein level by 56% ($P = 0.04$) (Fig. 7A). However, blocking JNK activation did not prevent H_2O_2-indued DNA damage from sensitizing TR5P cells

TABLE 3 *Organic selenium (MSA)-triggered apoptosis: Supra-additive effect in hydrogen peroxide (H_2O_2)-damaged DU-145 human and TR5P canine prostate cancer cells*[a]

	Treatment		Apoptosis		
	H_2O_2 μM	Treatment %	Observed %	Predicted %	Supra-additive %
DU-145	50	1.5	2.73 ± 1.10	2.57 ± 1.00	6.62 ± 3.30[§]
	100	1.5	3.33 ± 0.83	2.67 ± 0.61	23.88 ± 4.34[#§]
	50	3	4.90 ± 1.08	3.63 ± 0.91	36.60 ± 8.12[#]
	100	3	7.00 ± 0.81	4.13 ± 0.85	70.94 ± 10.71[*]
TR5P	400	3	13.70 ± 2.26	5.80 ± 1.15	137.30 ± 8.62[#]
	400	5	30.20 ± 1.13	10.50 ± 0.71	183.61 ± 9.63[*]

[a]*Supra-additive effect is calculated as: (Observed value − Predicted value) ÷ Predicted value. The predicted value was the arithmetic sum of apoptosis observed in parental cells plus the apoptosis induced by H_2O_2 and MSA alone. Predicted apoptosis = Basal apoptosis in parental cells + (Apoptosis in H_2O_2-damaged cells − Basal apoptosis in parental cells) + (Apoptosis after MSA treatment − Basal apoptosis in parental cells). Data are expressed as mean ± SD from three independent experiments. Within each cell line, means without a common symbol differ,* $P < 0.05$.

TABLE 4 *Organic selenium (MSA)-triggered cell death: Supra-additive effect in hydrogen peroxide (H_2O_2)-damaged DU-145 human and TR5P canine prostate cancer cells*[a]

	Treatment		Cell death		
	H_2O_2 μM	Treatment %[d]	Observed %	Predicted %	Supra-additive %
DU-145	50	1.5	6.50 ± 0.96	6.03 ± 1.07	7.59 ± 1.50[§]
	100	1.5	9.47 ± 1.56	8.23 ± 0.75	15.29 ± 2.57[#]
	50	3	13.05 ± 2.61	11.22 ± 3.46	14.38 ± 2.98[#]
	100	3	20.01 ± 3.25	12.33 ± 3.98	62.91 ± 2.06[*]
TR5P	400	3	8.50 ± 2.84	3.31 ± 0.85	156.80 ± 1.53
	400	5	12.30 ± 2.11	4.45 ± 0.81	176.40 ± 0.89

[a]*Supra-additive effect is calculated as: (Observed value − Predicted value) ÷ Predicted value. The predicted value was the arithmetic sum of cell death observed in parental cells plus the cell death induced by H_2O_2 and MSA alone. Predicted cell death = Basal cell death in parental cells + (Cell death in H_2O_2-damaged cells − Basal cell death in parental cells) + (Cell death after MSA treatment − Basal cell death in parental cells). Data are expressed as mean ± SD from three independent experiments. Within each cell line, means without a common symbol differ,* $P < 0.05$.

to MSA-induced apoptosis (Fig. 7B). Similarly, the p38 activation inhibitor PD169316 (10 μM) reduced p38 activation by 65% ($P = 0.001$) in H_2O_2-damaged cells, but did not reduce the magnitude of MSA-triggered apoptosis (Fig. 7C). Taken together, our experiments exploring five molecular targets provided no clear mechanistic explanation for our observations that H_2O_2-induced damage sensitizes prostate cancer cells to organic selenium-triggered apoptosis.

4. Discussion

This study tested a hypothesis generated from our dog studies that organic selenium can preferentially eliminate DNA-damaged prostatic cells through apoptosis. Using an *in vitro* system, noncytotoxic H_2O_2 exposure was used to create prostate cancer cell populations with moderate or high extent of DNA strand breaks. Parental cells not pretreated with H_2O_2 served

TABLE 5 *Concentrations of neocarzinostatin and etoposide that induce extent of DNA damage equivalent to hydrogen peroxide (H_2O_2) exposure in DU-145 human and TR5P canine prostate cancer cells*[a]

	Agent[b]	*Concentration* μM	*Strand breaks*[c] *%*
DU-145	H_2O_2	50	53.33 ± 6.11
	Neocarzinostatin[d]	20	52.00 ± 9.54
	Etoposide	10	50.67 ± 6.03
	H_2O_2	100	77.00 ± 5.29
	Etoposide	60	71.00 ± 9.85
TR5P	H_2O_2	400	18.67 ± 1.15
	Etoposide	30	15.03 ± 1.00

[a] *Data are expressed as mean ± SD from three independent experiments.*
[b] *Cells were treated with H_2O_2 (1 h), neocarzinostatin (24 h), or etoposide (1 h).*
[c] *Single strand breaks were measured in DU-145 cells. Double strand breaks were measured in TR5P cells.*
[d] *Neocarzinostatin at higher concentrations did not further induce DNA single strand breaks in DU-145 cells or double strand breaks in TR5P cells.*

as the low DNA damage population for these experiments. We found that both human and canine prostate cancer cells were sensitized to organic selenium-triggered apoptosis by noncytotoxic DNA damage induced by H_2O_2. In studies with selective DNA damaging agents, selenium-triggered apoptosis was enhanced more by oxidant-induced DNA damage than by non-oxidant-induced DNA damage. The ability of H_2O_2-induced damage to sensitize prostate cancer cells to selenium-triggered apoptosis did not rely upon the persistence of induced DNA strand breaks.

We previously observed in selenium-adequate, elderly male dogs (physiologically equivalent to 62- to 69-year-old men) that organic selenium supplementation, without further enhancement of selenium-dependent glutathione peroxidase activity, significantly decreased the extent of DNA damage [12]. In this selenium-adequate population, the lower DNA damage level could not be attributable to increased antioxidant defense fortified by selenium supplementation. Instead, we posited that apoptosis, a cellular response to DNA damage that is promoted by supra-nutritional selenium supplementation [4,8,31,32], could drive the observed reduction in DNA damage [14]. A closer look revealed the selenium status corresponding to the lowest prostatic DNA damage in these dogs overlapped with the selenium status associated with reduced prostate cancer incidence in men reported in the NPC Trial

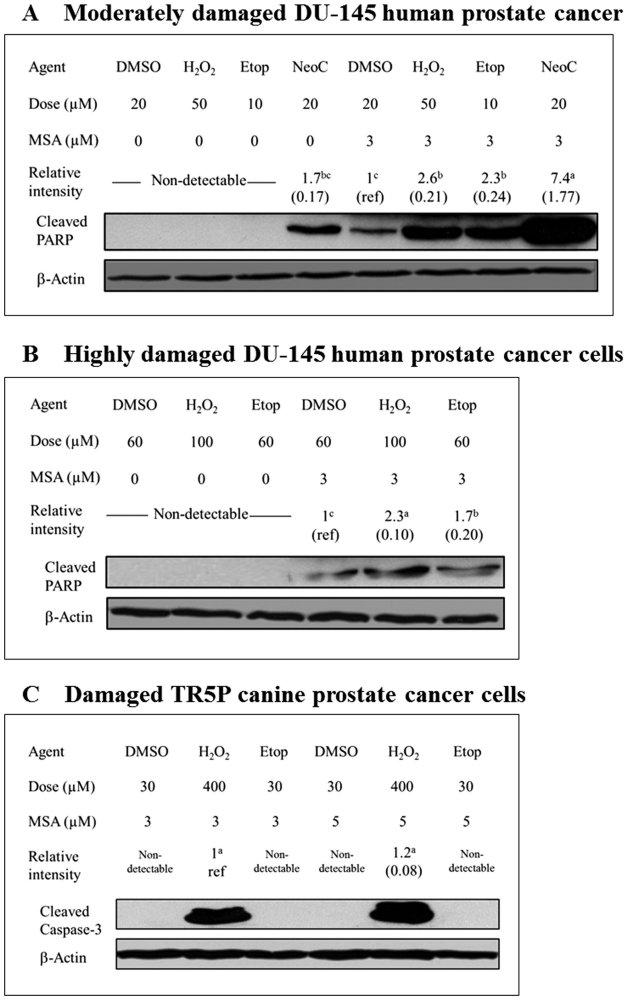

FIG 4 *Effect of the DNA damaging agents, neocarzinostatin and etoposide, on organic selenium (MSA)-triggered apoptosis in DU-145 human and TR5P canine prostate cancer cells. MSA-triggered apoptosis was compared between prostate cancer cells damaged with H_2O_2, neocarzinostatin (NeoC), or etoposide (Etop). At indicated concentrations, NeoC or Etop created equivalent amount of extensive DNA strand breaks as did H_2O_2 in the same panel. DMSO was used as the vehicle for NeoC and Etop. Cells not pretreated with DNA damaging agents were exposed to DMSO (vehicle control). A: Protein expression of cleaved PARP in moderately DNA-damaged DU-145 cells induced by H_2O_2, NeoC, or Etop. B: Protein expression of cleaved PARP in highly DNA-damaged DU-145 cells induced by H_2O_2 or Etop. The blot intensity in cells exposed to DMSO plus MSA was used as reference for relative intensity ("ref" in figure). C: Protein expression of cleaved caspase-3 in high DNA-damaged TR5P cells induced by H_2O_2 or Etop. The blot intensity in cells exposed to H_2O_2 followed by 3 μM MSA was used as the reference for relative intensity ("ref" in figure). β-actin served as the loading control. Data are expressed as mean (SD) from three independent experiments. In each panel, means without a common letter differ,* $P < 0.05$.

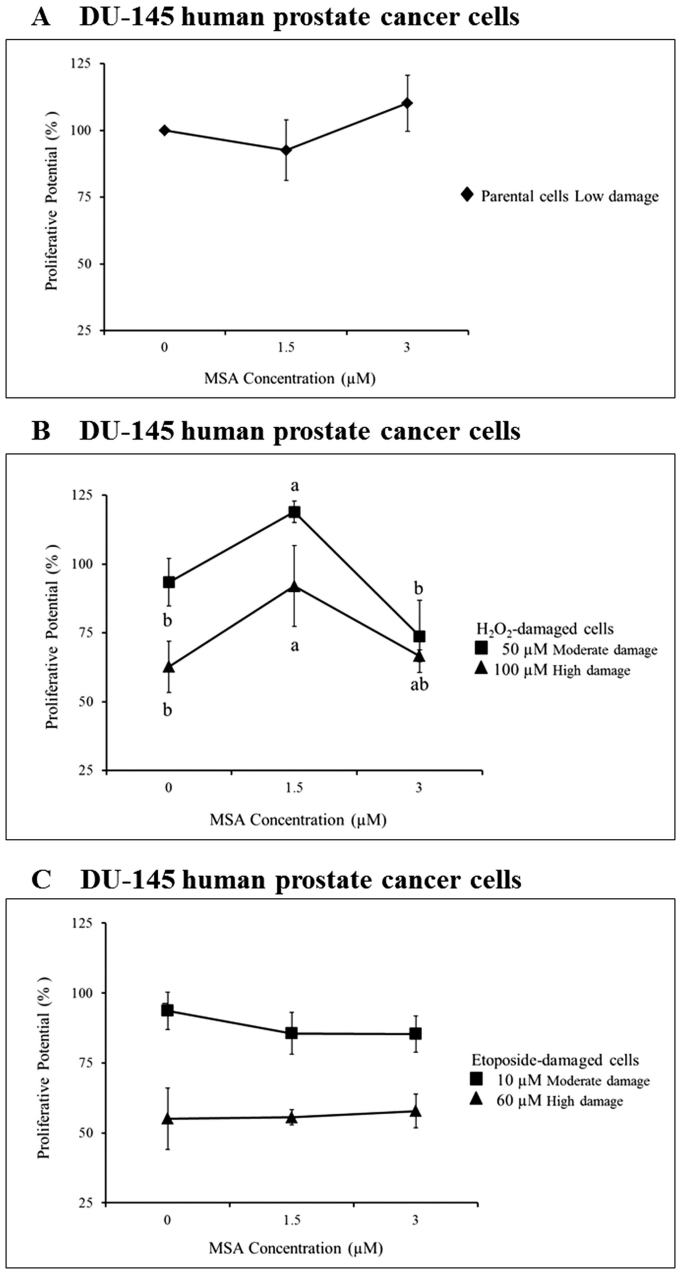

FIG 5 *Influence of organic selenium (MSA) on proliferative potential in hydrogen peroxide (H_2O_2)- or etoposide-damaged DU-145 human prostate cancer cells. DU-145 parental cells (A), cells damaged with H_2O_2 (B), or etoposide (C) were treated with MSA for 24 h. Five hundred cells from each treatment combination were reseeded to assess proliferative potential estimated by the ability to form colonies in 7 days. MSA did not affect proliferative potential of low damaged cells. Data are from three independent experiments. For (B) within each H_2O_2 exposure, means without a common letter differ, $P < 0.05$.*

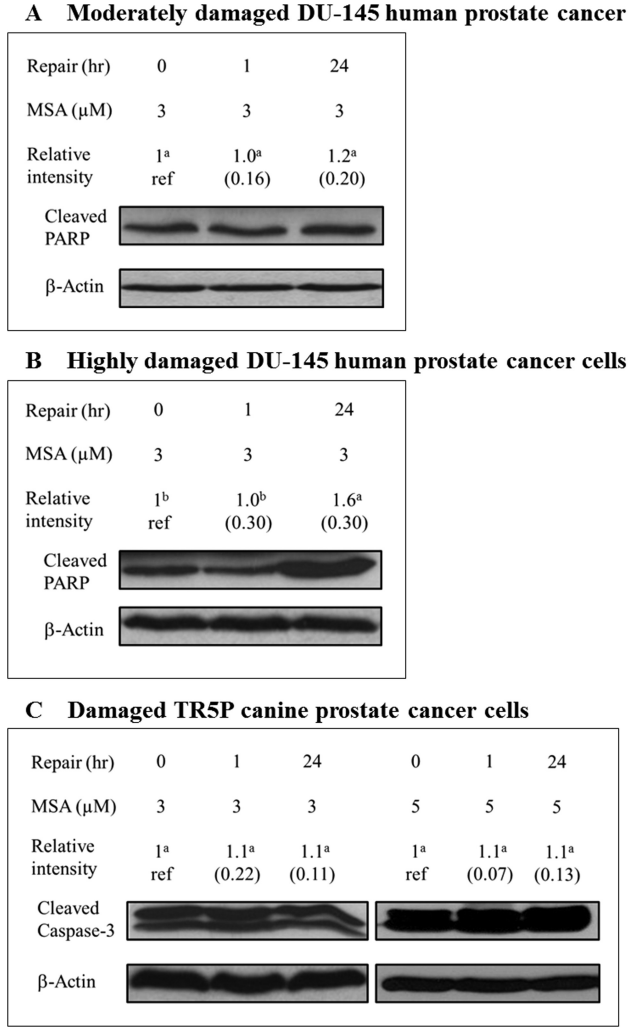

FIG 6 *Effect of DNA strand break repair on organic selenium (MSA)-triggered apoptosis in H_2O_2-damaged DU-145 human and TR5P canine prostate cancer cells. After 1-h exposure with H_2O_2 to induce noncytotoxic DNA damage, cells were immediately treated with MSA (no repair) or delayed until after DNA damage returned to basal level (1- or 24-h repair). For 1- and 24-h repair, cells were cultured in complete medium in the absence of H_2O_2 for 1 or 24 h until MSA exposure. Apoptosis was assessed after 24-h MSA treatment. A: Protein expression of cleaved PARP in DU-145 cells with moderate damage induced by 50 μM H_2O_2. B: Protein expression of cleaved PARP in DU-145 cells with the highest damage induced by 100 μM H_2O_2. C: Protein expression of cleaved caspase-3 in TR5P cells with damage induced by 400 μM H_2O_2. The blot intensity in cells immediately treated with MSA (0-h repair) was the reference for relative intensity ("ref" in figure). β-actin served as the loading control. Data are expressed as mean (SD) from three independent experiments. In each panel, means without a common letter differ, $P < 0.05$.*

and Health Professionals Follow-up Study [13]. Cells within an aging prostate gland are constantly bombarded by oxidants and other DNA damaging insults [33]. In order for any agent

to substantially reduce DNA damage through apoptosis, the agent cannot nonselectively induce apoptosis in all prostatic cells. Therefore, we put forth the hypothesis tested herein, that prostatic cells with the most DNA damage are the most vulnerable to selenium-triggered apoptosis.

Dogs and men are the only two species in which prostate cancer naturally occurs with substantial frequency, making the dog prostate a potentially important model to test the *in vivo* effects of selenium supplementation in an appropriate context [27]. However, *in vivo* studies in animal models do not allow for precise control of the extent of prostatic DNA damage, limiting the ability to determine whether or not organic selenium can trigger higher apoptosis in cells with higher extent of DNA damage. Therefore, to pursue this line of inquiry, we developed an *in vitro* model system using a well-characterized human prostate cancer cell line. Exposure of DU-145 human prostate cancer cells to H_2O_2 for 1 h at noncytotoxic concentrations created populations differing in DNA damage level. Employing this H_2O_2-damaged DU-145 model, we showed that organic selenium in the form of MSA triggered higher apoptosis in cell populations with more DNA damage, measured by strand breaks. Extending the experiment to a canine prostate cancer cell line established in our laboratory (TR5P), higher MSA-triggered apoptosis in cells damaged with H_2O_2 was also observed. From these studies, we conclude that organic selenium can preferentially trigger apoptosis in DNA damaged prostatic cells from dogs and humans.

The supra-additive effect of H_2O_2 and MSA on apoptosis and cell death indicates that cellular responses to H_2O_2 treatment sensitize prostate cancer cells to selenium-triggered apoptosis. Our experiments with etoposide, a nonoxidant DNA damaging agent, suggest the homeostatic housecleaning effect of MSA on apoptosis is not specific for oxidative DNA damage. Further, the persistence of H_2O_2-induced DNA strand breaks was not required for MSA to selectively trigger apoptosis. This "damage memory" suggests that signaling pathways activated in response to oxidant-induced damage might contribute significantly to the preferential apoptosis triggered by selenium. In prostatic cells, organic selenium initiates apoptosis through caspase-8 activation. Several pathways that may mediate MSA-triggered apoptosis in the prostate, (e.g., JNK, Akt, nuclear factor-κB, Bcl-2, and survivin) are also influenced by oxidative insults [30,31,34–47]. In cancer treatment studies, chemotherapeutic drugs that damage DNA have been shown to affect some of these pathways and synergize with selenium to kill cancer cells [30,39,48–50]. In some instances, increasing the duration of pretreatment with DNA-damaging chemotherapeutic drugs further increased the extent of apoptosis in prostate cancer cells after subsequent MSA exposure [30]. Our work extends

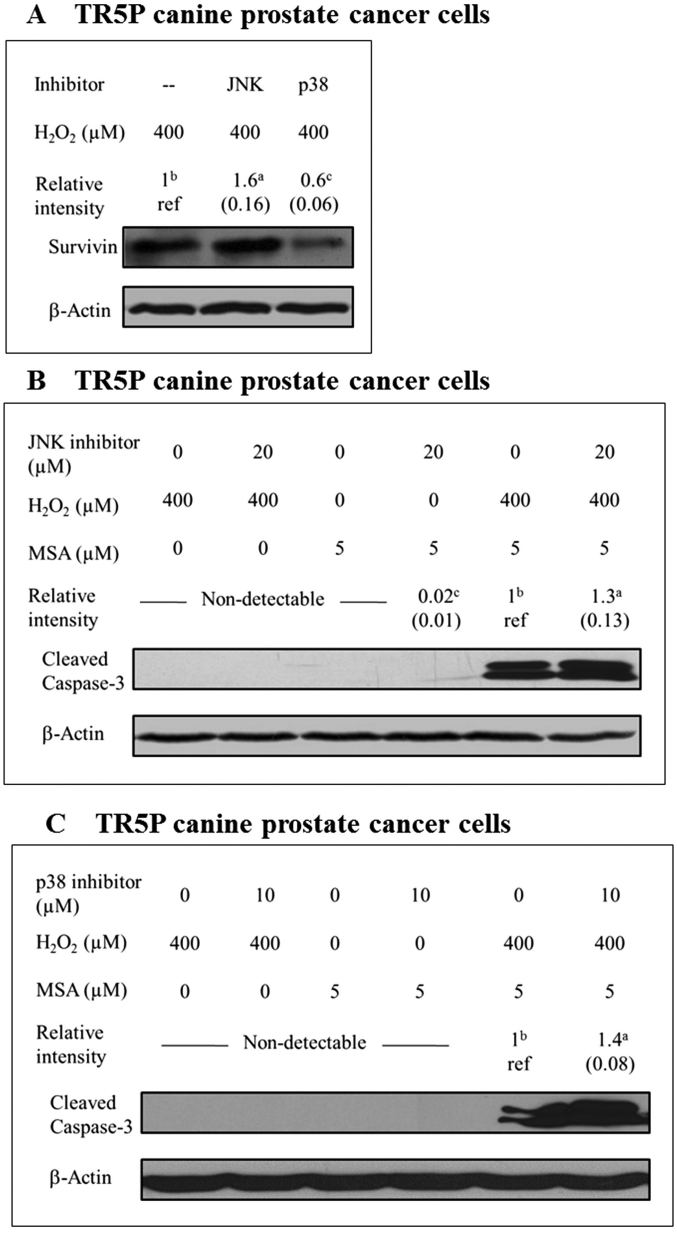

FIG 7

Inhibition of hydrogen peroxide (H_2O_2)-activated JNK or p38 with pathway-specific activation inhibitors fails to attenuate organic selenium (MSA)-triggered apoptosis in H_2O_2-damaged TR5P canine prostate cancer cells. TR5P cells were exposed to pathway-specific activation inhibitor of JNK (SP600125) or p38 (PD169316) prior to and concurrently with H_2O_2 for 1 h. A: Survivin protein level was measured after 1-h H_2O_2 exposure. In the presence of pathway-specific activation inhibitor of JNK (B) or p38 (C), cells were then treated with MSA for 24 h and protein expression of cleaved caspase-3, an execution marker of apoptosis, was measured. For (A), cells not exposed to activation inhibitors were treated with DMSO for vehicle control and served as the reference for relative intensity ("ref" in figure). For (B), (C), the blot intensity in cells treated with H_2O_2 and MSA in the absence of activation inhibitor was the reference for relative intensity ("ref" in figure). β-actin served as the loading control. Data are expressed as mean (SD) from three independent experiments. In each panel, means without a common letter differ, $P < 0.05$.

these findings that, for the valuable purpose of preventing prostate cancer, selenium may be capable of achieving selective elimination of cells with the most damage.

Not all damaged cells in the prostate will be purged through apoptosis. Therefore, we studied the influence of selenium on the proliferative potential of surviving cells. Suppressed proliferation of damaged cells could prevent prostatic cells from amplifying gene mutations and providing genetically faulty information to daughter cells, thereby reducing the likelihood of carcinogenesis [14]. Our results suggest the proliferative potential of H_2O_2-damaged prostate cancer cells is increased by some doses of selenium; at other doses, proliferation is the same or insignificantly decreased, compared with cells not treated with selenium. This suggests an added layer of complexity—that selenium might exert proproliferative and proapoptotic effects at the same time. The extent of H_2O_2-induced DNA damage might regulate these dose-dependent effects of selenium on cell proliferation, because selenium did not affect the proliferative potential of parental DU-145 cells that did not encounter H_2O_2.

Our results provoke fresh thinking on how one might apply supra-nutritional organic selenium supplementation to prostate cancer prevention. Through triggering apoptosis, organic selenium may be able to selectively eliminate DNA damaged cells, keeping genetic instability low within the prostate, and consequently attenuating carcinogenesis. This raises an unconventional possibility: For prostate cancer risk reduction, we might allow the prostate to accumulate DNA damage within a tolerable level and then intermittently supplement with organic selenium to sweep away the most damaged cells. Importantly, our data show that cellular responses to H_2O_2-induced DNA damage, rather than DNA damage *per se*, could critically regulate selenium-triggered apoptosis. We have begun to investigate several cellular pathways which are influenced by oxidative DNA damage and also regulate apoptosis—Akt, Erk, JNK, p38, and survivin. Our early results reported here indicate activation of these pathways cannot account for the higher sensitivity of H_2O_2-damaged prostatic cell populations to organic selenium-triggered apoptosis. Our future investigations, which will be complemented by the elucidation of underlying mechanisms, will aim at titrating the dose and duration of organic selenium supplementation that will achieve homeostatic housecleaning—a preferential elimination of DNA-damaged cell populations that can potentially render the prostate more resistant to cancer.

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