Inhibition of androgen receptor signaling by selenite and methylseleninic acid in prostate cancer cells: two distinct mechanisms of action

Bryan Husbeck,[1] Rumi S. Bhattacharyya,[2] David Feldman,[2] and Susan J. Knox[1]

Departments of [1]Radiation Oncology and [2]Endocrinology, Stanford University, Stanford, California

Abstract

The development of prostate cancer and its progression to a hormone-refractory state is highly dependent on androgen receptor (AR) expression. Recent studies have shown that the selenium-based compound methylseleninic acid (MSeA) can disrupt AR signaling in prostate cancer cells. We have found that selenite can inhibit AR expression and activity in LAPC-4 and LNCaP prostate cancer cells as well but through a different mechanism. On entering the cell, selenite consumes reduced glutathione (GSH) and generates superoxide radicals. Pretreatment with *N*-acetylcysteine, a GSH precursor, blocked the down-regulation of AR mRNA and protein expression by selenite and restored AR ligand binding and prostate-specific antigen expression to control levels. MSeA reacts with reduced GSH within the cell; however, *N*-acetylcysteine did not effect MSeA-induced down-regulation of AR and prostate-specific antigen. The superoxide dismutase mimetic MnTMPyP was also found to prevent the decrease in AR expression caused by selenite but not by MSeA. A Sp1-binding site in the AR promoter is a key regulatory component for its expression. Selenite decreased Sp1 expression and activity, whereas MSeA did not. The inhibition of Sp1 by selenite was reversed in the presence of *N*-acetylcysteine. In conclusion, we have found that selenite and MSeA disrupt AR signaling by distinct mechanisms. The inhibition of AR expression and activity by selenite occurs via a redox mechanism involving GSH, superoxide, and Sp1. [Mol Cancer Ther 2006;5(8):2078–85]

Introduction

The androgen receptor (AR) not only plays an important role in the development of androgen-dependent prostate cancer but also is present and active in hormone-refractory disease (1, 2). Androgen binding stimulates AR translocation to the nucleus, where it interacts with specific androgen-responsive elements on the promoters of target genes involved in the proliferation and differentiation of prostate cells (3, 4). Androgen deprivation continues to be the standard therapy for advanced and metastatic prostate cancer. Although prostate cancer initially responds to androgen withdrawal, most of these cancers eventually progress to a hormone-refractory state with a potentially fatal outcome (5–7). Several AR-related mechanisms influence the development of hormone-refractory prostate cancer. Increased AR expression, mutations in the AR ligand-binding domain, and ligand-independent activation of the AR may allow prostate cancer to progress in an androgen-deprived environment (8–11). Therefore, novel therapies that target the AR and its regulatory pathways have significant implications for prostate cancer prevention and the treatment of neoplastic disease.

Selenium is a key component of several functional selenoproteins required for normal heath. Epidemiologic studies suggest that an inverse relationship exists between serum selenium levels and cancer risk (12, 13). The secondary clinical findings of Clark et al. in the National Prevention of Cancer trial showed that selenium supplementation may potentially reduce the incidence of prostate cancer by 50% (14). In an attempt to confirm this finding, the Selenium and Vitamin E Chemoprevention Trial has enrolled >35,000 men to determine if selenium alone or in combination with vitamin E can prevent prostate cancer (15). A ''watchful waiting'' trial is also being conducted to study the effect of selenium on the progression of clinical prostate cancer using time to disease progression and prostate-specific antigen (PSA) velocity as the primary end points (16). Although the mechanisms for potential prostate cancer prevention and therapy by selenium compounds in humans have not been fully elucidated, it is conceivable that effects on the AR may play a role at least in part.

Selenium compounds have been shown to inhibit cell growth and induce apoptosis in a variety of human cancer cells *in vitro*, including androgen-dependent and androgen-independent prostate cancer cells (17–19). The antitumor activities of selenium compounds are dependent on the dose and chemical form. The inorganic form of selenium, selenite, undergoes thiol-dependent reduction to hydrogen selenide (H_2Se; ref. 20). H_2Se can supply selenium for the synthesis of selenoproteins or undergo

Received 1/30/06; revised 5/9/06; accepted 6/7/06.

Grant support: Department of Defense grants W81XWH-04-1-0160 and T32 DK007217 and NIH grant DK 42482.

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Requests for reprints: Susan J. Knox, Department of Radiation Oncology, Stanford University Medical Center, 300 Pasteur Drive, Stanford, CA 94305. Phone: 650-725-2720; E-mail: sknox@stanford.edu

doi:10.1158/1535-7163.MCT-06-0056

sequential enzymatic methylation to yield monomethylated, dimethylated, and trimethylated metabolites (21). Studies using animal carcinogenesis models have implicated the monomethyl selenium metabolite, methylselenol (CH_3SeH), as an active anticancer selenium metabolite (22). The further oxidative metabolism of H_2Se can also produce superoxide anions and induce oxidative stress (20, 23, 24).

Reactive oxygen species and the intracellular redox state have emerged as important mediators of cell signaling (25, 26). Glutathione (GSH) is the main intracellular thiol-based antioxidant and high doses of selenite not only consume total GSH but also generate superoxide radicals (20, 23, 24). Alterations in the intracellular redox state can affect the activity of redox-sensitive proteins via the oxidation of critical cysteine residues, which may in turn have downstream effects on signal transduction and gene transcription. Furthermore, excessive production of reactive oxygen species can overwhelm the buffering capacity of a cell and induce apoptosis. Several studies have shown that decreased GSH and superoxide formation are causally related to the induction of apoptosis in selenite-treated cancer cells (18, 27–29).

Recently, methylseleninic acid (MSeA; CH_3SeO_2H) has been reported to down-regulate PSA expression via disruption of AR signaling in several prostate cancer cell lines (30–32). The metabolism of MSeA is different from selenite in that it bypasses the H_2Se metabolite pool. On entering the cell, MSeA reacts directly with reduced GSH to produce CH_3SeH, the putative active selenium metabolite for cancer prevention (22). Here, we show that selenite, in equivalent doses to MSeA, can also decrease AR expression and signaling in LAPC-4 and LNCaP prostate cancer cells. Because both selenite and MSeA react with reduced GSH, we examined how modulation of the intracellular redox state in prostate cancer cells could influence their response to selenite and MSeA. Our data suggest that the inhibition of AR signaling by selenite occurs by a redox-dependent mechanism that is distinct from MSeA.

Materials and Methods

Cell Culture and Treatments

LAPC-4 prostate cancer cells (provided by Dr. Charles Sawyers, University of California at Los Angeles) were cultured in phenol red–free RPMI 1640 (Life Technologies, Rockville, MD) supplemented with 10% fetal bovine serum (Gemini Bio-Products, Woodland, CA) at 37°C in a humidified atmosphere with 5% CO_2. LNCaP prostate cancer cells and OVCAR-3 ovarian cancer cells (American Type Culture Collection, Rockville, MD) were cultured in RPMI 1640 (with phenol red) supplemented with 10% fetal bovine serum. Selenite and *N*-acetylcysteine were supplied by Sigma (St. Louis, MO), MSeA was obtained from Selenium Technologies (Lubbock, TX), and the superoxide dismutase mimetic MnTMPyP was provided by Alexis Biochemicals (San Diego, CA). For androgen-defined experiments, charcoal-stripped fetal bovine serum (Omega Scientific, Tarzana, CA) and the synthetic androgen R1881 (NEN Life Science Products, Boston, MA) were used. All other chemicals and reagents were supplied by Sigma unless otherwise noted.

Cell Proliferation Assay

Cells were seeded in 96-well plates (10,000 per well), allowed to attach overnight, and on the following day treated with selenite for 24, 48, and 72 hours. Cell survival was assayed using the CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay (Promega, Madison, WI). The MTS solution was added directly to the wells and incubated at 37°C for 3 hours and the absorbance was read at 490 nm with a 96-well plate reader (V_{max} Kinetic Microplate Reader, Molecular Devices, Sunnyvale, CA).

Transient Transfection Assay

LAPC-4 cells were seeded at 10^5 per well in 24-well tissue culture plates. The AR promoter (−5,400/+580)-luciferase construct in the pGL3 basic vector was provided by Donald J. Tindall (Mayo Clinic, Rochester, MN). The TransLucent Sp1 reporter vector was purchase from Panomics (Redwood City, CA). Transfections were done using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). Each transfection contained 0.1 μg pAR-luc or pSp1-luc DNA and 0.01 ng pSV40-*Renilla* DNA. The control plasmid pSV40-*Renilla* was for normalization. Luciferase activity was measured using the Promega dual luciferase assay system on the luminometer TD-20 (Turner Design, Sunnyvale, CA).

Western Blot Analysis

Cells pellets were resuspended in lysis buffer [50 mmol/L HEPES (pH 7.5), 0.5% NP40, 0.5% sodium deoxycholate, 50 mmol/L NaCl, 1 mmol/L EDTA, 0.1 mmol/L sodium orthovanadate], incubated on ice for 20 minutes, and spun at 14,000 × *g* to collect whole-cell lysates. Nuclear extracts were prepared using Nuclear and Cytoplasmic Extraction Reagents (Pierce, Rockford, IL). The lysates were run on NuPAGE 10% Bis-Tris gels (Invitrogen). Proteins were transferred to polyvinylidene difluoride membranes and blocked with 5% milk/TBS [100 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl/0.1% Tween 20]. Primary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and included rabbit polyclonal anti-AR (N-20), goat polyclonal anti-PSA (C-19), goat polyclonal anti-actin (C-11), and mouse monoclonal anti-Sp1 (E-3). The secondary antibodies were conjugated to horseradish peroxidase and detected with enhanced chemiluminescence Western blotting detection reagents (Amersham Biosciences, Piscataway, NJ).

Real-time Reverse Transcription-PCR

Total RNA was isolated from LAPC-4 cells using TRIzol reagent (Invitrogen) and cDNA was synthesized using SuperScript RT II (Invitrogen) and an oligo(dT) primer. An aliquot of the reverse transcription product was amplified by real-time PCR using gene specific primers and the DyNAmo SYBR Green PCR kit (New England Biolabs, Beverly, MA) using the OptiCon2 real-time PCR detection

system (Bio-Rad Laboratories, Waltham, MA). Expression levels of mRNA for AR, PSA, estrogen receptor-β, glucocorticoid receptor, progesterone receptor, and vitamin D receptor were measured using specific primers for each gene. The mRNA expression of TATA box-binding protein was used as a control. Changes in gene expression were determined using the comparative $C_T(\Delta\Delta C_T)$ method as described (33).

PSA Assay

Conditioned medium samples from control or treated LAPC-4 cells were collected and centrifuged at low speeds to remove cell debris. PSA concentrations were determined in conditioned medium using an ELISA kit (Diagnostic Systems Laboratories, Webster, TX) according to the manufacturer's instructions. The PSA values were normalized to total protein per sample.

[^{3}H]-Dihydrotestosterone-Binding Assay

Radioligand binding assays were done as described previously (34) using [^{3}H]-dihydrotestosterone as the ligand to assess functional AR expression. Cells were grown to 70% confluence before treatment. High-salt extracts were prepared from harvested cells and aliquots were incubated overnight at 4°C with [^{3}H]-dihydrotestosterone (Amersham Biosciences) at a single saturating concentration. Nonspecific binding was assessed in parallel assays containing 250-fold excess radioinert 5-dihydrotestosterone (Steraloids, Inc., Wilton, NH) and subtracted from total binding to yield specific binding. Specific binding is a quantitative assessment of functional AR. Receptor concentrations were expressed as fmol ligand bound/mg protein.

Results

Selenite Inhibits LAPC-4 Growth and AR and PSA Expression

LAPC-4 human prostate cancer cells express a wild-type AR and respond to androgen with increased proliferation and increased expression and secretion of PSA (35). The effect of selenite on the proliferation of LAPC-4 cells was measured using the MTS assay. Figure 1A shows the dose response of LAPC-4 cells to increasing concentrations of selenite for 24, 48, and 72 hours. LAPC-4 cells treated with 2.5 μmol/L selenite showed no change relative to control; however, cell proliferation was inhibited with time in a dose-dependent fashion after treatment with 5 or 10 μmol/L selenite. We next tested whether the inhibition of cell growth by selenite was associated with decreased AR expression. First, we assessed the effects of selenite on the transcriptional activity of the AR promoter. LAPC-4 cells were transfected with an AR promoter-luciferase construct and then treated with selenite for 24 hours. Figure 1B shows the dose-dependent inhibition of AR promoter activity by selenite. Importantly, decreased AR promoter activity was observed after treatment with 2.5 μmol/L selenite, suggesting that the inhibition of AR transcription occurs before any decrease in cell number. The decrease in AR promoter-driven luciferase activity after exposure to selenite for 24 hours was coupled to decreased AR protein levels as determined by Western blot analysis (Fig. 1C).

The AR is the most important regulatory factor for PSA gene transcription. To test whether the modulation of AR expression by selenite was associated with decreased PSA expression at the transcriptional level, we measured AR

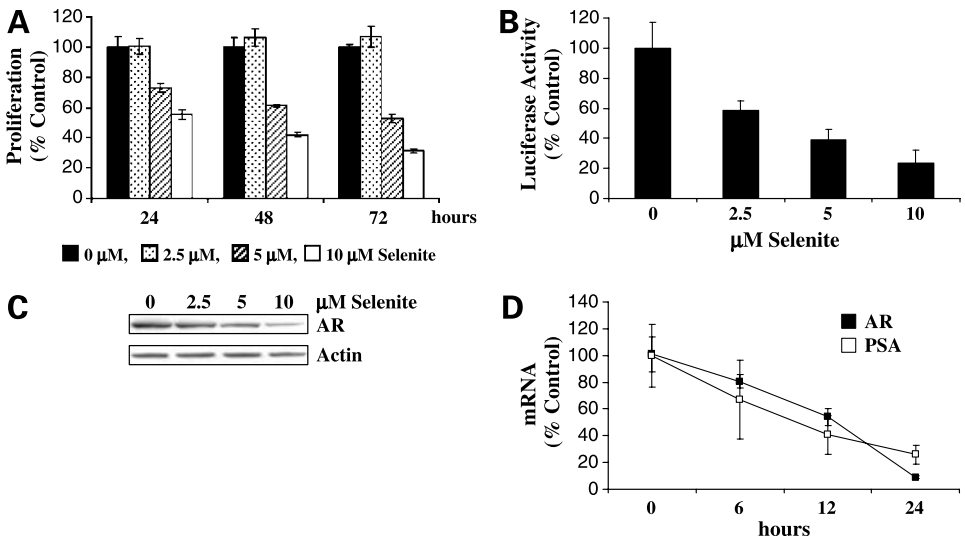

Figure 1. Effect of selenite on LAPC-4 cell proliferation and AR and PSA expression. **A,** LAPC-4 cells were treated with selenite at the indicated concentrations for 24, 48, and 72 h and cell proliferation was measured by MTS assay. **B,** LAPC-4 cells were cotransfected with the AR promoter-luciferase construct, pAR-luc, and pSV40-ren and then treated with selenite for 24 h. Luciferase activity was normalized to *Renilla* and expressed as percentage of control. **C,** AR protein expression in LAPC-4 cells after exposure to selenite for 24 h as detected by Western blot analysis. Actin protein expression was used to normalize for loading. **D,** LAPC-4 cells were treated with 10 μmol/L selenite for 6, 12, and 24 h and AR and PSA mRNA was measured by real-time reverse transcription-PCR. The expression of TATA box-binding protein was used for normalization. *Points,* mean of three experiments; *bars,* SD.

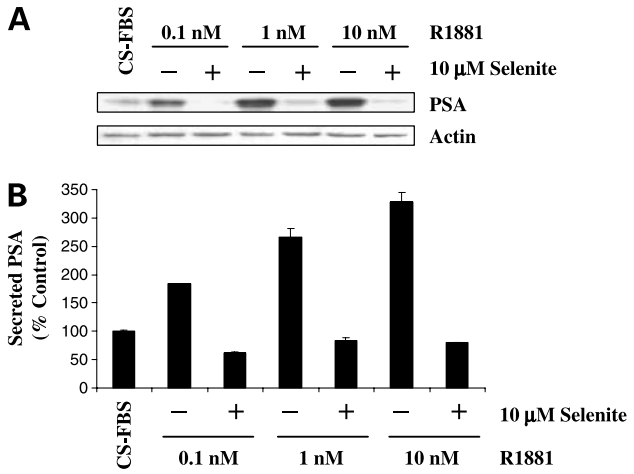

Figure 2. Selenite inhibits R1881-induced PSA expression. **A,** increasing amounts of R1881 were added to LAPC-4 cells growing in hormone-depleted medium and cellular PSA was detected by Western blot analysis 24 h later. Actin protein expression was used to normalize for loading. **B,** ELISA detection of secreted PSA in the conditioned medium from the same cells. PSA values were normalized to total protein per sample. *Columns,* mean of three experiments; *bars,* SD.

and PSA mRNA quantitatively by real-time reverse transcription-PCR. LAPC-4 cells were treated with 10 μmol/L selenite for various lengths of time and the results are shown in Fig. 1D. The expression of AR and PSA mRNA followed a similar time response pattern after dosing with selenite. Transcript levels decreased as early as 6 hours after exposure to selenite and the inhibition was approaching 100% after 24 hours.

Selenite Interferes with R1881-Induced PSA Expression in LAPC-4 Cells

The experiments described in Fig. 1 were done in cells cultured in 10% fetal bovine serum. We also tested the effects of selenite on PSA protein expression in LAPC-4 cells cultured in charcoal-stripped fetal bovine serum with increasing concentrations of R1881, a potent synthetic androgen. Treatment of LAPC-4 cells with R1881 for 24 hours led to a dose-dependent increase in cellular PSA protein levels (Fig. 2A). Simultaneous treatment with 10 μmol/L selenite inhibited the induction of PSA by R1881. An ELISA was done to measure the amount of secreted PSA into the conditioned medium from the same cells. Figure 2B shows that selenite was also able to completely suppress R1881-induced PSA secretion.

N-Acetylcysteine Attenuates Selenite-Induced Down-regulation of AR and PSA

The proapoptotic activity of selenite is mainly dependent on its ability to deplete GSH and induce oxidative stress (18, 27–29). We examined how modulation of intracellular GSH could influence selenite-induced down-regulation of AR expression and activity. LAPC-4 cells were pretreated with 10 mmol/L *N*-acetylcysteine for 24 hours and exposed to selenite for an additional 24 hours. Pretreatment with *N*-acetylcysteine increased intracellular GSH levels in LAPC-4 as much as 80% (data not shown). As shown in Fig. 3A, the addition of *N*-acetylcysteine blocked the decrease in AR protein levels following treatment with selenite. A radioligand binding assay was done with [^{3}H]-dihydrotestosterone to assess functional AR expression after treatment with selenite. Figure 3B shows that pretreatment with *N*-acetylcysteine restored functional AR levels to control after exposure to selenite. The effect of *N*-acetylcysteine on AR expression occurred at the transcriptional level. Using real-time reverse transcription-PCR, we found that *N*-acetylcysteine was able to inhibit the down-regulation of AR mRNA by selenite (Fig. 3C). Consequently, *N*-acetylcysteine supplementation also maintained normal levels of PSA mRNA and secreted PSA in LAPC-4 cells treated with selenite (Fig. 3C and D).

N-Acetylcysteine Does Not Affect MSeA-Induced Suppression of AR and PSA Expression

MSeA has been shown previously to inhibit AR expression and signaling in LAPC-4 and LNCaP prostate cancer cells (30–32). Because MSeA reacts with reduced GSH within the cell, we tested whether altering the intracellular GSH content with *N*-acetylcysteine could also modulate the effect of MSeA on the AR. LAPC-4 cells were treated with 10 mmol/L *N*-acetylcysteine for 24 hours and then exposed to 10 μmol/L MSeA for another 24 hours. Figure 4A shows that pretreatment with *N*-acetylcysteine did not inhibit MSeA-induced down-regulation of AR protein levels. Similarly, *N*-acetylcysteine did not prevent the decrease in PSA secretion caused by MSeA in the same cells (Fig. 4B). We next tested the effect of *N*-acetylcysteine on selenite- and MSeA-induced inhibition of AR and PSA expression in LNCaP prostate cancer cells, which express a mutant but functional AR (35), to determine the universality of this response. LNCaP cells were treated with 10 mmol/L *N*-acetylcysteine for 24 hours and then dosed with 5 μmol/L selenite or MSeA for 24 hours. Selenite and MSeA decreased AR protein expression and PSA secretion in LNCaP cells, and *N*-acetylcysteine pretreatment was again found to only block the inhibition caused by selenite but not by MSeA (Fig. 4C–E). We also tested the effects of selenite on the AR-positive OVCAR-3 ovarian cancer cell line. Inhibition of OVCAR-3 cell growth by selenite was associated with decreased AR expression, which was inhibited by the addition of *N*-acetylcysteine (see Supplementary Fig. S1).[3] The results show that selenite treatment can elicit similar molecular effects in different cell backgrounds.

Differential Involvement of Superoxide in Selenite- versus MSeA-Mediated Down-regulation of the AR

Superoxide produced as a result of selenite metabolism is an important mediator of selenite-induced apoptosis (27–29). We tested whether the inhibition of AR expression by selenite or MSeA in prostate cancer cells was mediated

[3] Supplementary material for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

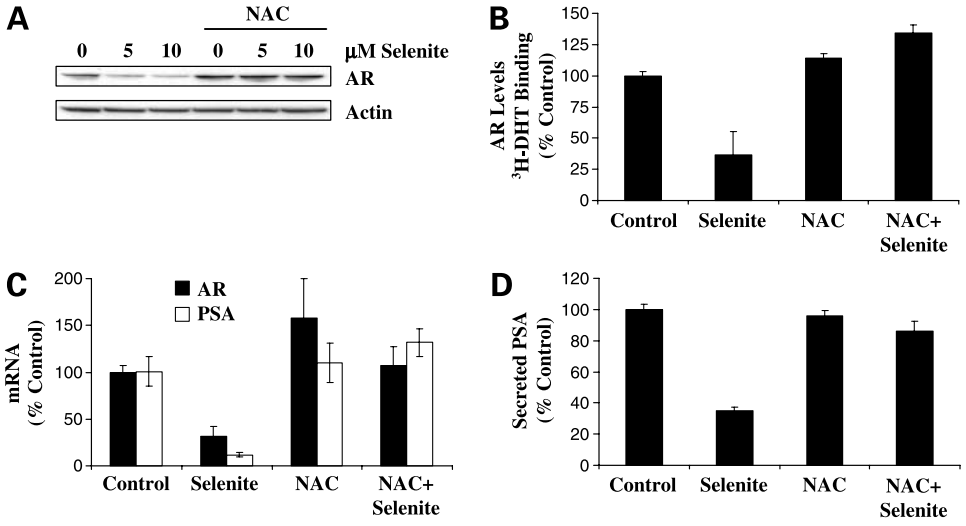

Figure 3. *N*-acetylcysteine inhibits selenite-induced down-regulation of the AR and PSA. LAPC-4 cells were pretreated with 10 mmol/L *N*-acetylcysteine (*NAC*) for 24 h and then treated with selenite for another 24 h. **A,** AR protein expression determined by Western blot analysis after exposure to 5 or 10 μmol/L. Actin protein expression was used to normalize for loading. **B,** functional AR levels measured by [^{3}H]-dihydrotestosterone (*^{3}H-DHT*) binding. **C,** AR and PSA mRNA measured by real-time reverse transcription-PCR. **D,** ELISA detection of secreted PSA after exposure to 10 μmol/L selenite with or without *N*-acetylcysteine pretreatment. *Columns,* mean of three experiments; *bars,* SD.

at least in part by superoxide. LAPC-4 and LNCaP cells were exposed to selenite or MSeA in the presence or absence of 5 μmol/L MnTMPyP for 24 hours and AR protein expression was measured by Western blot analysis. MnTMPyP is a stable manganese-porphyrin complex that has been shown to dismutate superoxide radicals to hydrogen peroxide (36). Figure 5 shows that MnTMPyP was able to prevent the decrease in AR protein caused by selenite but not by MSeA in both LAPC-4 and LNCaP cells. The data implicate a role for superoxide in the down-regulation of the AR by selenite but not MSeA.

Effects of Selenite and MSeA on Sp1

The Sp1 transcription factor and its DNA-binding motif play an important role in regulating the transcriptional activities of the AR promoter (37). Sp1 activity is redox sensitive (38). Therefore, we tested whether selenite had an effect on Sp1 activity. Using a Sp1-luciferase reporter vector, we observed decreased Sp1 activity in LAPC-4 cells after 8 hours of exposure to selenite (Fig. 6A). Both LAPC-4 and LNCaP cells were then exposed to selenite or MSeA for 8 hours and Sp1 was measured in the nuclear extracts by Western blot analysis. As shown in Fig. 6B, selenite decreased Sp1 expression in both LAPC-4 and LNCaP cells, whereas MSeA did not. In addition, pretreatment with *N*-acetylcysteine blocked the effect of selenite on nuclear Sp1 expression. The results indicate that reduced nuclear Sp1 expression leading to decreased Sp1 activity may be the mechanism by which selenite inhibits AR expression.

Effects of Selenite and MSeA on the Expression of Other Steroid Receptors

To determine whether the effects of selenite or MSeA are specific for the AR and not a generalized response, we

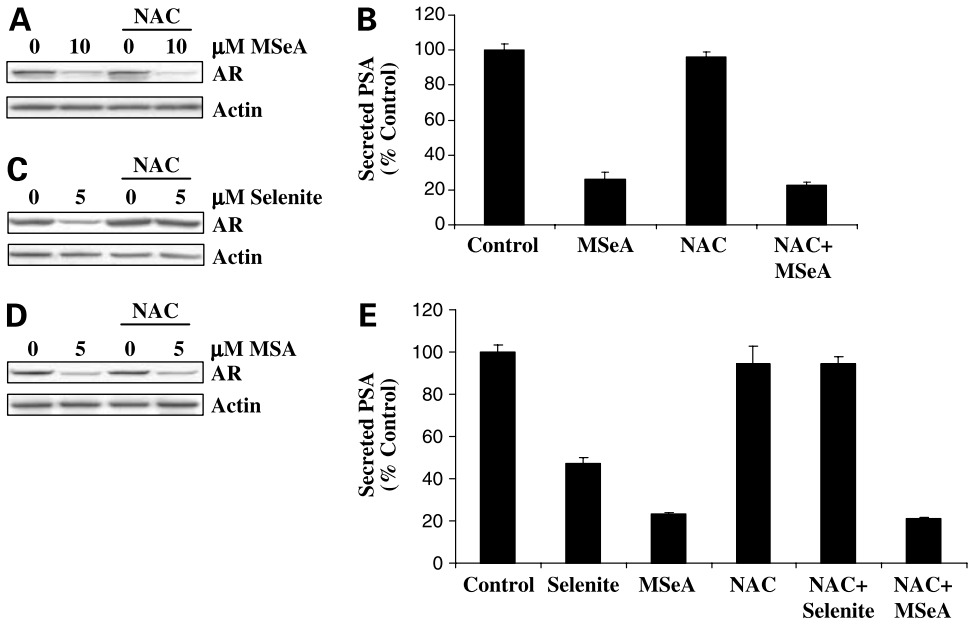

Figure 4. *N*-acetylcysteine does not inhibit MSeA-induced down-regulation of the AR and PSA. **A,** LAPC-4 cells were pretreated with 10 mmol/L *N*-acetylcysteine for 24 h and then treated with 10 μmol/L MSeA for another 24 h and AR protein expression was detected by Western blot analysis. **B,** ELISA detection of secreted PSA from the same cells. LNCaP cells were pretreated with 10 mmol/L *N*-acetylcysteine for 24 h and then treated with 5 μmol/L selenite (**C**) or MSeA (**D**) for 24 h and AR protein expression was detected by Western blot analysis. **E,** ELISA detection of secreted PSA from the same cells. Actin protein expression was used to normalize for loading. *Columns,* mean of three experiments; *bars,* SD.

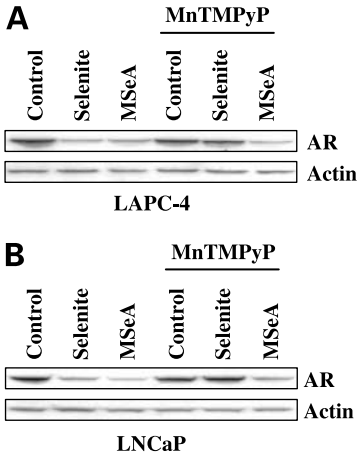

Figure 5. Role of superoxide in selenite- and MSeA-induced inhibition of AR expression. LAPC-4 and LNCaP cells were treated with selenite or MSeA in the presence or absence of 5 μmol/L MnTMPyP. **A,** Western blot analysis of AR protein expression in LAPC-4 cells 24 h after treatment with 10 μmol/L selenite or MSeA. **B,** LNCaP cells 24 h after treatment with 5 μmol/L selenite or MSeA. Actin protein expression was used to normalize for loading.

measured the expression of other steroid receptors to identify additional signaling pathways that may be altered by selenium. LAPC-4 cells were treated with 10 μmol/L selenite or MSeA for 6 and 24 hours and estrogen receptor-β, glucocorticoid receptor, progesterone receptor, and vitamin D receptor mRNA was measured quantitatively by real-time reverse transcription-PCR (Table 1). There was a trend toward most genes showing decreased RNA expression by 6 hours and increased RNA expression after 24 hours of treatment with selenite or MSeA. Only the vitamin D receptor showed decreased expression after 6 and 24 hours of selenite exposure. Whether the vitamin D receptor is inhibited by selenite in a redox-dependent fashion similar to the AR is not known.

Discussion

There have been numerous studies showing that selenium compounds have potential chemopreventive and chemotherapeutic effects on prostate cancer. Selenium can be dosed in a variety of chemical forms, each of which are uniquely metabolized, and may work through different anticancer mechanisms. Most recently, the selenium metabolite CH_3SeH and its precursor MSeA have been shown to disrupt AR expression and signaling in prostate cancer cells (30–32). MSeA was specifically developed because selenomethionine, the form of selenium currently being given to men in the ongoing Selenium and Vitamin E Chemoprevention Trial, is poorly metabolized to CH_3SeH in cell culture and therefore is not suitable for mechanistic studies (22). Whereas MSeA reacts with GSH to deliver CH_3SeH directly to cells (22), selenite undergoes thiol-dependent reduction via reduced GSH to H_2Se, which is then methylated to produce CH_3SeH (20, 21). H_2Se can also be incorporated into selenoproteins as selenocysteine or react with molecular oxygen to generate superoxide radicals (20, 23, 24). Our goal was to characterize the effects of selenite on AR signaling in prostate cancer cells and to determine whether selenite and MSeA shared similar molecular mechanisms of action.

We found that selenite, in equivalent doses to MSeA, also decreased AR expression and activity in LAPC-4 and LNCaP prostate cancer cells. LAPC-4 cells express a wild-type AR as opposed to LNCaP cells, which have a mutant but functional AR (35). Thus, the inhibition of AR signaling by selenite seems to occur regardless of the AR genotype. Selenite suppressed AR promoter activity in a dose-dependent fashion and inhibitory effects were observed at a dose that did not effect cell proliferation, suggesting that the decrease in AR transcription caused by selenite precedes any change in cell number. AR and PSA mRNA expression was reduced in LAPC-4 cells as early as 6 hours after exposure to selenite. Previous studies in our laboratory have shown that selenite can deplete total GSH and shift the redox balance of LAPC-4 cells toward an oxidative state within the same period under identical treatment conditions (19). Therefore, we examined how modulation of the intracellular redox state in prostate cancer cells could influence their response to selenite and MSeA. Increasing the GSH concentration with *N*-acetylcysteine blocked the down-regulation of the AR by selenite at the transcriptional level and restored AR signaling. In contrast, *N*-acetylcysteine had no effect on the inhibition of AR expression caused by MSeA. These results show that the down-regulation of the AR by selenite occurs by a redox-dependent mechanism that is distinct from MSeA.

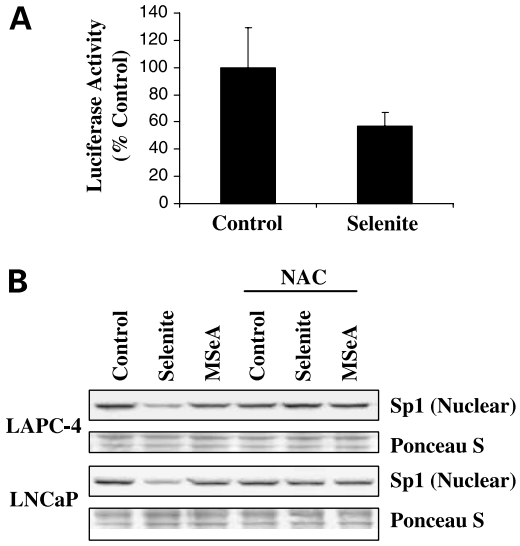

Figure 6. Effects of selenite and MSeA on Sp1. **A,** LAPC-4 cells were cotransfected with the Sp1 reporter vector, pSp1-luc, and pSV40-ren and then treated with 10 μmol/L selenite for 8 h. Luciferase activity was normalized to *Renilla* and expressed as percentage of control. *Columns,* mean of three experiments; *bars,* SD. **B,** Western blot analysis of Sp1 protein expression in the nuclear extracts of LAPC-4 and LNCaP cells exposed to selenite or MSeA for 8 h with or without *N*-acetylcysteine pretreatment. Ponceau S–stained bands were used to show equal loading of samples.

Table 1. Changes in steroid receptor gene expression induced by selenite and MSeA in LAPC-4 cells

Gene	Treatment	Fold change in RNA expression relative to control	
		6 h	24 h
Estrogen receptor-β	Selenite	0.99	2.45
Glucocorticoid receptor	Selenite	0.73	5.44
Progesterone receptor	Selenite	0.43	3.26
Vitamin D receptor	Selenite	0.65	0.11
Estrogen receptor-β	MSeA	0.73	2.29
Glucocorticoid receptor	MSeA	0.83	2.07
Progesterone receptor	MSeA	0.90	2.49
Vitamin D receptor	MSeA	0.22	2.69

Previous studies showing that the apoptotic activity of selenite in prostate cancer cells can be inhibited by the addition of superoxide dismutase or MnTMPyP support the existence of a causal role for superoxide in selenite-induced cell death (27–29). Although the induction of apoptosis by MSeA in prostate cancer cells is not inhibited by superoxide dismutase (28), studies have shown that MSeA is also capable of generating superoxide radicals *in vitro* (39). We used MnTMPyP to examine the role played by superoxide in selenite- or MSeA-induced down-regulation of the AR. Our finding that MnTMPyP was able to prevent the decrease in AR expression caused by selenite but not by MSeA implicates a role for superoxide in the disruption of AR signaling by selenite in prostate cancer cells. Using patient-matched pairs of normal and malignant prostate cells, we have shown previously that normal cells are more resistant to selenite-induced apoptosis than the cancer-derived cells (40). The normal cells from the same study also showed higher levels of manganese superoxide dismutase compared with the cancer-derived cells. This increased manganese superoxide dismutase expression may play an important role in eliminating superoxide radicals produced as a result of selenite metabolism. Based on our current results, it would be of interest to determine if selenite also preferentially decreased AR expression in prostate cancer cells that are deficient in manganese superoxide dismutase.

Sp1 is a ubiquitously expressed transcription factor that recognizes GC-rich regions in the promoters of target genes (41). The Sp1-binding sequence is the major positive regulatory element in the AR promoter (37). Because Sp1 transactivation is redox sensitive (38), we did experiments to determine if selenite could inhibit Sp1-mediated transcriptional activity. Using a Sp1 reporter construct, we were able to show reduced Sp1 activity in prostate cancer cells exposed to selenite. This inhibition of Sp1 activity by selenite was associated with decreased Sp1 expression in the nucleus. The effect of selenite on Sp1 expression was shown to be redox dependent, as *N*-acetylcysteine prevented this decrease. Given that Sp1 regulates the activation of many genes involved in tumor growth and survival (42, 43), it is likely that other anticancer properties of selenite may also be mediated through the inhibition of Sp1. Although MSeA has been shown to inhibit AR gene transcription, our present findings suggest that it does not seem to occur via a mechanism involving decreased Sp1 expression. A scheme summarizing the possible differential effects of selenite and MSeA on AR expression is shown in Fig. 7.

The development of prostate cancer and its progression to a hormone-refractory state is highly dependent on AR expression (1, 2). The finding that hormone-refractory prostate cancer is not associated with a loss of AR expression but is instead characterized by the presence of a functionally intact, although frequently overexpressed or mutated AR, has heightened interest in the AR as a therapeutic target (2). Current hormone therapy for prostate cancer only reduces circulating androgen levels or blocks agonist binding to the AR without decreasing AR levels. Strategic targeting of the AR with ribozymes, antisense oligomers, and small interfering RNAs has been shown to significantly inhibit prostate cancer growth both *in vitro* and *in vivo* (44–47). Thus, the ability of different selenium compounds to reduce AR levels in prostate cancer has many therapeutic indications and needs to be explored further. However, it is not yet known whether the concentrations of selenium necessary for inhibition of AR signaling can be safely achieved in human tissues. The average plasma selenium concentration in U.S. adults is 1.5 μmol/L and supplementation with selenium in the trial by Clark et al. elevated the concentration to 2.4 μmol/L (14). Data from Longnecker et al. revealed that serum concentrations of 3 to 5 μmol/L selenium can be achieved by people taking high doses of selenium without any apparent adverse effects (48). Hopefully, ongoing animal studies and future human trials will address questions regarding optimal dosage regimens and enable the full potential clinical utility of selenium compounds in prostate cancer to be realized.

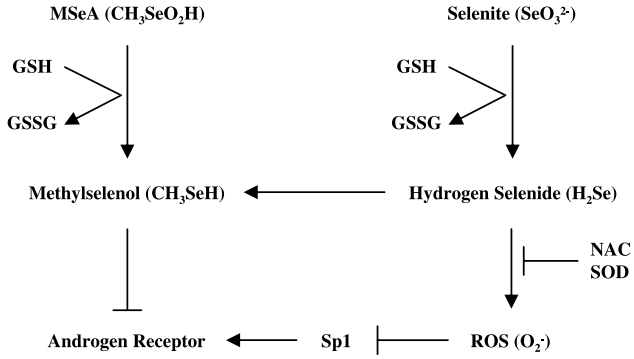

Figure 7. Schematic illustration showing the inhibition of AR expression by selenite and MSeA in prostate cancer.

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