

Monomethylated Selenium Inhibits Growth of LNCaP Human Prostate Cancer Xenograft Accompanied by a Decrease in the Expression of Androgen Receptor and Prostate-Specific Antigen (PSA)

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OBJECTIVES. Epidemiological studies and prevention trials suggest selenium is a promising preventive agent for prostate cancer. Selenium-containing compounds inhibited the growth of prostate cancer cell lines including androgen sensitive LNCaP and androgen insensitive DU145 and PC3 cells in vitro. Previous study revealed a novel mechanism of selenium action in which selenium (methylseleninic acid (MSA)) markedly reduced androgen receptor (AR) signaling in prostate cancer cells, suggesting that selenium might act as an antiandrogen, which could serve as a therapeutic agent for prostate cancer. In this study, we tested whether selenium (methylselenocysteine (MSC)) affects tumor growth of human prostate cancer cells by targeting AR signaling in vivo.

METHODS. Prostate tumor xenografts were established in nude mice by co-inoculating LNCaP cells with Matrigel. The mice-bearing tumors were treated with or without MSC (100 µg/mouse/day) via intraperitoneal injection for 2 weeks. The effect of MSC on tumor growth, AR, and prostate-specific antigen (PSA) expression was examined.

RESULTS. Methylselenocysteine (MSC) significantly inhibited LNCaP tumor growth ($P < 0.05$). AR expression in tumor tissues and serum PSA levels were considerably decreased in MSC-treated mice compared to the vehicle controls.

CONCLUSIONS. Pharmacological dose of MSC inhibits the growth of LNCaP human prostate cancer in vivo accompanied by a decrease in the expression of AR and PSA. These findings suggest that selenium (MSC) can serve as a therapeutic agent aimed at disruption of AR signaling for prostate cancer. *Prostate* 66: 1070–1075, 2006. © 2006 Wiley-Liss, Inc.

KEY WORDS: selenium; androgen receptor; PSA; prostate cancer

INTRODUCTION

Accumulating epidemiological and molecular evidence suggest that selenium protects against the development of variety cancers including prostate, colon, esophagus, lung, and gastric cardia [1–4]. Numerous case-control studies have demonstrated an inverse relationship between selenium status and prostate cancer risk [5–9]. A landmark study of selenium as a chemopreventive agent initiated by Larry Clark demonstrated that supplementation of people with selenized yeast (200 µg of selenium) is capable of reducing the overall cancer morbidity by nearly 50% [1]. Patients

Abbreviations: AR, androgen receptor; PSA, prostate-specific antigen; MSC, methylselenocysteine; MSA, methylseleninic acid.

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receiving the supplement showed a significantly lower incidence of developing lung (RR = 0.54), colon (RR = 0.42), and prostate cancer (RR = 0.37). Extending the analysis [10] reaffirmed the significant reduction in prostate cancer incidence by selenium (RR = 0.48, 95% CI = 0.28–0.80). The current Selenium and Vitamin E Chemoprevention Trial (SELECT) is based on the promising epidemiological and prevention studies on selenium in prostate cancer. The goal is to investigate the efficacy of selenium, either alone or in combination of Vitamin E, in reducing prostate cancer incidence among average risk of 32,400 healthy men who are 55 years of age or older [11].

The anticancer activity of selenium is dependent on its chemical form. In general, inorganic selenium compounds, such as selenate or selenite, are known to produce genotoxic effects, and are therefore not preferred for medicinal use especially at high doses. Organic selenium-containing compounds are better tolerated, but are different in their anticancer activity depending on their pharmacokinetic and pharmacodynamic properties. The metabolism of selenium to a monomethylated intermediate, methylselenol, is presumed necessary for the expression of anticancer activity [12–14]. Methylselenol is highly reactive and difficult to formulate. Stable precursors, such as methylselenocysteine (MSC) or methylseleninic acid (MSA), or selenomethionine, which can be converted endogenously to methylselenol, are good selenium precursors for generating methylselenol, the active selenium metabolite that exhibit anticarcinogenic activity.

Methylselenocysteine (MSC) has a naturally occurring selenoamino acid, which is synthesized by plants including garlic, onions, broccoli, and species of the genus *Astragalus*. Extracts of selenium-enriched garlic have shown that MSC is the most active ingredient in the chemoprevention of mammary cancer. MSC metabolism has been studied in animals and the chemopreventive effect of MSC is believed to occur due to the generation of monomethylated selenium (MSA) species by endogenous enzymes. It undergoes a facile one-step cleavage by β -lyase to provide a steady stream of the active selenium metabolite, methylselenol. MSA species have been shown to generate superoxide, induce apoptosis and arrest cancer cells in culture. MSC was found very effective in reducing colon cancer in rats. Thus selenomethionine and MSC have emerged as the dietary supplements of choice based upon experimental efficacy in animals, and MSC clearly becomes the most potent carcinostatic selenium compound. MSC is at least twice as active as L-selenomethionine in suppressing mammary premalignant lesions and mammary tumorigenesis in rodents [15,16]. Unlike L-selenomethionine, which is incorporated into

proteins in place of methionine, MSC is not incorporated into any proteins thereby being fully bioavailable for chemoprevention and the synthesis of selenium-containing enzymes such as glutathione peroxidase. Preliminary animal data indicates that MSC is less toxic than L-selenomethionine when included in diets which may be related to the accumulation of L-selenomethionine in tissues. Due to its proved anticancer activity and low toxicity *in vivo*, MSC was used in this *in vivo* study.

Cell culture studies demonstrated that selenium inhibited the growth of prostate cancer cell lines including androgen sensitive LNCaP, and androgen insensitive DU145 and PC3 cells [17–20]. We recently reported that MSA is able to decrease markedly AR transcript and protein levels [19]. The expression of prostate-specific antigen (PSA), a well-known androgen-regulated gene, is also inhibited by MSA [17,19,21]. Recent study demonstrated that MSA but not selenite inhibited AR signaling in LNCaP cells [21]. This unique antiandrogen activity suggests that selenium may serve as a therapeutic agent for prostate cancer. In this study, the effect of selenium (MSC) on human prostate cancer cell tumor growth was examined.

MATERIALS AND METHODS

Cell Culture

The LNCaP cells were maintained in RPMI 1640 supplemented with 10% of FBS, 100 U/ml of penicillin, and 100 μ g/ml of streptomycin at 37°C in 5% CO₂ incubator.

Animal

Male athymic nude mice (NCR-NU, body weight, 20–25 g), 5–6 weeks of age, were obtained from NCI (NCI, Frederick, MD). The mice were kept four mice/cage and maintained according to Institutional Animal Care and Use Committee approval.

Tumors

LNCaP cells were cultured in RPMI1640 supplemented with 10% FBS. The cells were harvested when 90%–95% confluent and 4×10^6 cells were co-inoculated with 100 μ l of Matrigel (BD Biosciences, Palo Alto, CA) into the right flank of the animal. Animals were examined twice weekly for the development of palpable tumors at the site of injection.

Treatments

When tumor volumes reached approximately 150–200 mm³, tumor-bearing mice were randomly assigned to treatment groups receiving either MSC (Sigma, St. Louis, MO) at a dose of 100 μ g/mouse/day or vehicle

(PBS) via intraperitoneal injection for 14 consecutive days. Body weights and tumor volumes were monitored twice weekly. Tumor length, width, and height were measured using calipers twice weekly. The tumor volume was calculated using the formula $0.5236 \times L \times W \times H$. Mice were sacrificed after 2 weeks of treatment. At the termination of treatment, mice were anesthetized with Ketamine, and blood was collected by orbital sinus method for serum PSA determination. Tumors were removed, weighed, and immediately frozen for RNA and protein extraction.

Determination of PSA Secretion

The serum was collected at the end of experiments. Twenty-five micro liters of serum was used to determine PSA secretion. Levels of PSA in the serum of tumor-bearing mice were determined by ELISA with the use of antiPSA as primary antibody as described by the manufacturer's protocol (Bechman Coulter, Fullerton, CA).

Western Blot Analysis

Details of the procedure were described previously [22]. Forty micrograms of protein were resolved in 8% SDS-PAGE depending on the molecular weight of the protein to be detected. After blocking overnight at 4°C in 5% milk in PBS-0.1% Tween 20, membranes were incubated overnight with antibodies against either AR (Santa Cruz Biotechnology, Santa Cruz, CA) or α -actin (Sigma). Immunoreactive bands were quantitated using volume densitometry and normalized against α -actin. Following secondary antibody incubation, immunoreactive proteins were visualized with an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Buckinghamshire, England).

Statistical Analysis

Values were expressed as the mean \pm SE. Student's *t*-test (two-tailed) was used to determine the significance between treatments and untreated controls, and $P < 0.05$ was considered significant.

RESULTS

Selenium Inhibits LNCaP Prostate Cancer Proliferation In Vivo

We have demonstrated that MSA inhibited prostate cancer cell growth in vitro [19]. To test whether selenium affect prostate cancer tumor growth in vivo, LNCaP tumor xenografts were established by co-inoculation of LNCaP cells with Matrigel subcutaneously into male nude mice. Selenium treatment started when tumors reached about 150–200 mm³ in

size. The mice were randomly divided into 2 groups of 12 mice each. One group was received MSC only, and another received PBS as controls. MSC was injected intraperitoneally into the nude mice at concentration of 100 μ g/mouse/day for 14 days. The dose of MSC was chosen based on the report that the maximum tolerated dose (MTD) of MSC is 200 μ g/mouse/day \times 28 days [23]. MSC at 200 μ g/mouse/day for 28 days is demonstrated to be a safe and nontoxic dose and schedule [23]. As controls, the mice were injected with PBS. The tumor volumes were measured twice a week. At the end of experiments, the mice were sacrificed and body weight was recorded. In addition, the tumors were weighed and the blood was collected for measurement of serum PSA levels. There was no significant change in body weight between MSC treatment group and control group. Treatment of MSC significantly inhibited tumor growth ($P < 0.05$) compared to the control (Fig. 1).

Selenium Reduced Androgen Receptor and Androgen-Regulated Gene Expression

We previously demonstrated that selenium significantly suppressed AR expression and AR-regulated gene PSA expression in LNCaP cells in vitro [19]. To determine whether selenium affects AR and PSA expression in vivo, we determined AR protein expression in the tumor tissues using Western blot. The levels of AR protein expression were considerably reduced in

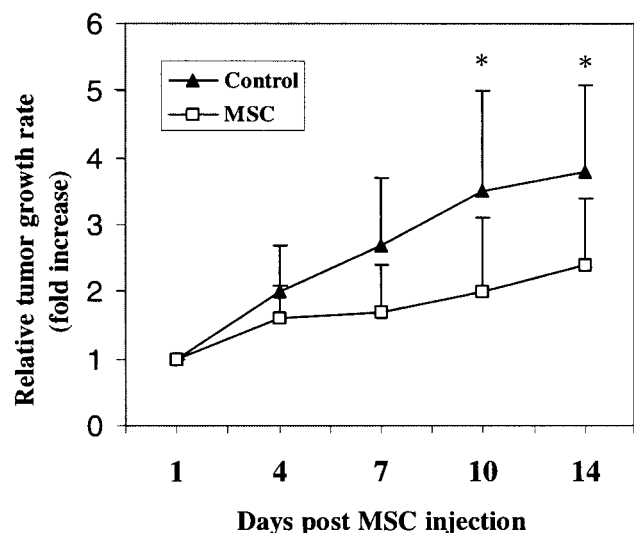


Fig. 1. Effect of methylselenocysteine (MSC) on LNCaP xenografts in vivo. LNCaP tumor xenografts were established by co-inoculation of LNCaP cells with Matrigel s.c. into male nude mice. The mice were randomly divided into 2 groups of 12 mice each. One group was received MSC only, and another received PBS as controls. MSC was injected intraperitoneally into the nude mice at concentration of 100 μ g/mouse/day for 14 days. As controls, the mice were injected with saline. Bars, \pm SE. *, represent statistically significance.

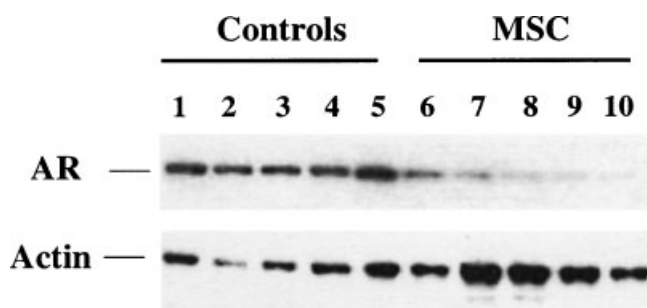


Fig. 2. Effect of MSC on AR expression in representative samples bearing LNCaP tumors analyzed by Western blot using antibody against AR. Lanes 1–5 were controls from LNCaP tumors treated with saline only; lane 6–10 were samples from LNCaP tumors treated with MSC. The same membrane was reprobbed with antibody against α -actin as an internal control.

MSC-treated group compared to the controls (Fig. 2). The serum PSA levels were determined by ELISA as described previously [24] and found that serum PSA levels were significantly lower ($P < 0.05$) in the MSC-treated group than in the controls (Fig. 3). These results demonstrated that selenium (MSC) inhibited LNCaP tumor growth in nude mice model, accompanied by a decrease in AR protein and AR-regulated gene PSA expression.

DISCUSSION

We previously demonstrated a novel mechanism of selenium action in prostate cancer in which selenium disrupts AR signaling through reducing the levels of AR expression in LNCaP cells in vitro [19]. In this study, we extended these findings by demonstrating that selenium (MSC) inhibits the tumor growth of experimental xenograft of human LNCaP prostate cancer accompanied by a reduction of AR and AR-regulated gene PSA expression in vivo.

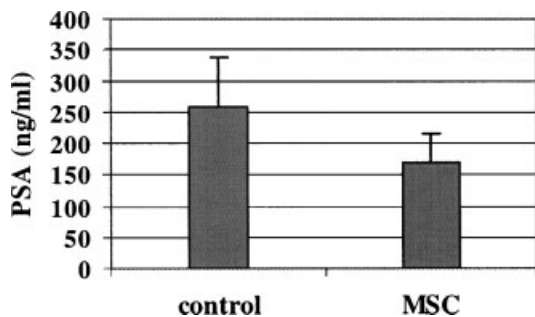


Fig. 3. Effect of MSC on serum PSA levels in mice-bearing LNCaP tumors. Serum PSA levels were measured using ELISA as described in the Materials and Methods, and normalized to tumor weight. The serum PSA levels were expressed as ng/ml of serum/gram of tumor. Each group contains 12 mice; bars, \pm SE.

Accumulating evidence suggests that organic selenium-containing compounds have better anticancer activity and are less toxic compared to inorganic selenium compounds. For these reasons, selenomethionine, presumably the major form of selenium in selenized yeast, is selected for the supplement used in SELECT trial. Despite the above advantage of organic selenium, inorganic selenium was also shown to have anticancer activity in prostate cancer. Dietary supplement of selenium (sodium selenate but not MSC) at dose of 3 ppm in drinking water resulted in a reduction of tumor growth in hormone refractory human PC3 prostate tumors in mice [25]. It would be interesting to compare the effect of organic selenium and inorganic selenium in the xenograft model of LNCaP human prostate cancer. The dose of MSC used in this study is 100 μ g/mouse/day for 14 days, which is within the MTD of 200 μ g/mouse/day \times 28 days reported [23]. Although the dose of MSC is justified, it is important to realize that the equivalent dose in a human would be well into toxic ranges. In humans, epidemiologic data suggest that 750–850 μ g are the upper limit of safe daily exposure [26]. It is therefore interesting to further investigate using multiple doses of MSC to be able to show a dose response curve.

A common characteristic of all selenium compounds that express significant experimental carcinostatic activity in vitro and in vivo is their interaction with thiols and the generation of free radical species. Cell culture studies demonstrated that selenium inhibited the growth of prostate cancer cell lines including androgen sensitive LNCaP and androgen insensitive DU145 and PC3 cells [17–20]. Most recently, we demonstrated that AR signaling intensity is a key factor in determining the sensitivity of prostate cancer cells to selenium inhibition of cell growth [36]. There are a number of potential mechanisms proposed for the antiproliferative effects of selenium, including antioxidant effects, enhancement of immune function, stimulation of apoptosis, and induction of cell-cycle arrest [18]. We recently reported that MSA is able to dramatically decrease AR signaling in prostate cancer cells [19]. The downregulation of AR signaling by selenium could be through multiple processes. We recently demonstrated that selenium decreases AR transcriptional initiation, posttranscriptional, and post-translational modifications [37]. The expression of PSA, a well-known androgen-regulated gene, is also inhibited by MSA [19]. The fact that selenium disrupts AR signaling suggests that selenium may serve as an antiandrogen for prostate cancer chemoprevention and therapy.

Androgen signaling through androgen receptor (AR) plays an important role not only in maintaining the function of the prostate, but also in promoting the

development of androgen-independent prostate cancer. A common treatment modality for prostate cancer is androgen deprivation, which can be achieved by surgical castration, chemical castration, or a combination of surgical and chemical castrations. Chemical castration may involve LH-RH analogs (e.g., leuprolide acetate or goserelin acetate), steroid antiandrogens (e.g., cyproterone acetate), nonsteroid antiandrogens (e.g., flutamide and bicalutamide), or a combination of these agents. The goal of these androgen deprivation treatments is either blocking androgen-AR binding or reducing the levels of androgen. Since AR plays essential role in androgen-independent prostate cancer, almost all of androgen deprivation therapies are either blocking ligand-AR binding (flutamide or bicalutamide) or reducing the levels of ligand (LH-RH analogs). Although antiandrogen treatment is effective, the antitumor effects can be temporary. Virtually every patient will relapse due to the growth of androgen-independent prostate cancer cells. The major reason is that AR signaling remains intact and is often hyperactive in androgen-independent prostate cancer. Several possible mechanisms have been suggested for this androgen-independent cancer growth including: (1) AR mutation or amplification [27–29]; (2) activation of AR in a ligand-independent manner by growth factors and cytokines [30–32]; (3) activation of co-regulators [33,34]; and (4) AR activation by tissue androgen of uncertain origin [35]. There are urgently needed for testing new therapies based on novel mechanisms to target AR signaling for androgen-independent prostate cancer. Reducing AR expression may represent an attractive approach to target androgen signaling in prostate cancer. Our data demonstrated that selenium reduces AR expression, a completely different mechanism from the conventional androgen deprivation therapies, to disrupt androgen signaling in prostate cancer [19]. This unique antiandrogen activity suggests that selenium may serve as a therapeutic agent for prostate cancer. This concept was validated in this study in which selenium inhibits LNCaP tumor growth accompanied by a reduction of AR and PSA expression *in vivo*.

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