Sodium selenite inhibits interleukin-6-mediated androgen receptor activation in prostate cancer cells via upregulation of c-Jun

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Abstract

Background— It has been suggested that interleukin-6 (IL-6) may modulate androgen receptor (AR) action to accelerate prostate cancer (PCa) progression. Selenium compounds are highly recommended as a promising chemopreventive agent for PCa. This study was to determine if selenium can repress IL-6 mediated AR action in PCa progression.

Methods— Cell proliferation, prostate-specific antigen, gene transfer, and western blot assays were used to study the effects of sodium selenite and methylseleninic acid on IL-6 mediated AR action on an AR expressing human prostate cancer cell line, LNCaP.

Results— We found that sodium selenite, but not methylseleninic acid, significantly ($p < 0.05$) inhibited IL-6-induced trans-activating activity of AR and cell proliferation in LNCaP cells. Interestingly, although sodium selenite did not show effect on activation of both STAT3 and ERK1/2 in the presence of IL-6, an increased expression of c-Jun was detected in cells after treatment with sodium selenite. Indeed, we showed overexpression of c-Jun blocked IL-6-induced AR activation.

Conclusions— Taken together, our results suggest that sodium selenite not methylseleninic acid can inhibit IL-6-mediated AR activation by increased c-Jun in LNCaP cells. Sodium selenite may be a proper selenium form for further testing its potency on intervening IL-6-mediated PCa progression.

Keywords

Selenium; IL-6; prostate cancer; c-Jun; androgen independent growth

1. Introduction

Prostate cancer is the most commonly diagnosed solid tumor and the second leading cause of cancer mortality in men in the Western world [1]. Clinical data demonstrate that serum interleukin-6 (IL-6) levels in patients with hormone-refractory prostate cancer are elevated and usually accompanied by high levels of prostate specific antigen (PSA) [2,3]. Recent studies implicate that IL-6 may function as an autocrine or paracrine growth factor since it stimulates proliferation of various cancer cells, including prostate cancer cells [3–8]. IL-6 has been shown

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to activate androgen receptor (AR) -dependent gene expression in prostate cancer cells in the absence of androgens [4,9,10]. Intracellular signaling pathways, such as those mediated by mitogen-activated protein kinases (MAPK), and signal transducer and activators of transcription-3 (STAT3), are involved in the signaling cross-talking with the AR. Activation of STAT3 has been implicated in the activation of AR induced by IL-6 in an androgen independent manner in LNCaP cells, an androgen responsive human prostate cancer cell line [9,10].

Selenium is a chemopreventive agent, for which there are considerable epidemiological and clinical studies indicating a protective role against several different types of cancer, including that of the prostate. An inverse association between selenium levels in the serum or toenails and the subsequent risk of developing prostate cancer has recently been reported [11,12], which stimulates a great deal of interests in understanding the mechanism of selenium-mediated chemoprevention. Selenium is an essential trace element and exists in both organic and inorganic forms. Sodium selenite and methylseleninic acid (MSeA) are the two most studied selenium compounds for prostate cancer prevention. MSeA is a synthetic organic compound of selenium, relatively stable in solution, and presumably produces methylselenol as an anti-cancer effector [13]. Selenomethionine (Se-Met) can be metabolized and converted into methylselenol in the liver and is currently undergoing a prostate cancer intervention clinical trial SELECT [14]. Although numerous studies have suggested the inhibitory role of selenium in the growth of prostate cancer cells *in vitro*, a comprehensive understanding of the mechanism underlying selenium anticancer effects on IL-6 mediated prostate cancer cell growth, is currently lacking. Selenium compounds have been shown to inhibit cell proliferation, induce apoptosis and prevent tumor progression [15–17].

2. Materials and methods

2.1. Cell culture and reagents

Human prostate cancer LNCaP cells (American Type Culture Collection, Manassas, VA) was maintained in RPMI 1640 (Mediatech, Hercules, CA) containing 5% fetal bovine serum (FBS, Biofluids, Rockville, MD) at 37°C and 5% CO_2. For experiments, cells were cultured for 24 h in serum-free RPMI 1640 in order to avoid potential interference of existing steroids in FBS. Cells were treated in RPMI 1640 with 5% charcoal-stripped FBS supplemented with different chemicals as indicated. Mibolerone (Mib, New England Nuclear, Boston, MA), a nonmetabolizable synthetic androgen, was used at 1 nmol/l concentration. Recombinant human IL-6 (Leinco Technologies, Inc, St. Louis, MO) was used at 25 ng/ml in all experiments.

2.2. Growth responses and PSA Levels

Cells were plated in 24-well plates at 2×10^4 cells/well. Forty-eight to 72 h after plating, cells were treated with MSeA or sodium selenite (Sigma) at different doses in the presence or absence of Mib or IL-6 as indicated. MTS assay, a non-radioactive cell proliferation assay, composed of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (Promega, Madison, WI) was used to determine cell proliferation 72 h after treatments. To measure secreted PSA levels, 400 μl of spent medium from cells treated for 72 h were collected. PSA protein levels were determined using specific immunoassays (Mayo Immunochemical Core Facility) as previously reported [18].

2.3. Western blot analysis

Cells were seeded at 1×10^6 cells/plate in 100-mm dishes. Cells grown in log phase were co-treated with IL-6 and various concentrations of MSeA or sodium selenite for different time points. The cells were collected by centrifugation and washed with cold PBS. Cell lysates were prepared in a lysis buffer (PBS containing 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS

plus freshly added protease inhibitors, 100 μg/ml phenylmethylsulfonyl fluoride, 30 μl/ml aprotinin, and 1 mmol/l sodium orthovanadate) and used for Western blot analysis. The sample filters were immunoblotted with specific primary antibody to c-Jun, STAT3, phospho-STAT3 (Cell Signaling, Beverly, MA), ERK1/2, phospho-ERK1/2 (Santa Cruz Biotechnology, Santa Cruz, CA) and horseradish peroxidase-conjugated secondary antibodies and visualized by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ).

2.4. Transcriptional reporter assays

LNCaP cells were plated into 12-wells plate. Cells at 50–70% confluence were transfected with the appropriate constructs (6-kb PSA promoter-pGL3, hK2-3 x ARE-SV40 minimal promoter pGL3 or empty pGL3 vector) tagged with luciferase gene by using the method described previously [19]. Twenty-four hours after transfection, cells were treated with MSeA or sodium selenite in combination with Mib or IL-6. Whole cell lysate was prepared for luciferase assay according to the manufacturer's instructions (Promega). CMV-β-galactosidase (β-gal), 0.2 μg/well, expression vector was also co-transfected for normalization of transfection efficiency. The experiments were done in triplicate and repeated three times, and the standard deviations (SDs) were calculated.

2.5. Statistical analysis

All values are given as means plus SDs. Means of groups were compared with the Student's t test and $p < 0.05$ was used as the level of significance.

3. Results

3.1. Inhibition of IL-6-mediated cell growth and down-regulation of IL-6-induced PSA protein expression by sodium selenite

When LNCaP cells were exposed to sodium selenite or MSeA in the presence of IL-6 for 72 h, a significant reduction in cell growth was observed only with selenite exposure but not with MSeA (Fig. 1A). As expected, PSA production was stimulated by IL-6. A significant inhibition of PSA upregulation induced by IL-6 was detected in cells treated with sodium selenite (Fig. 1B). A dose-dependent inhibition of PSA protein expression by sodium selenite was also detected in LNCaP cells treated with mibolerone (Fig. 1C). These data suggest that sodium selenite, but not MSeA, can block IL-6 mediated cell growth and PSA upregulation in LNCaP cells.

3.2. Inhibition of IL-6 induced androgen independent AR trans-activation by sodium selenite

PSA is mainly regulated by the AR signals at the transcriptional level. Thus, we used the PSA gene as a model to investigate effects of selenium on AR transactivation activity stimulated by androgens or IL-6. Cells were transfected with the PSA-6kb promoter that contains androgen responsive elements (ARE). AR binds directly to the ARE of target genes for androgen action. Following transfection, cells were treated with either sodium selenite or MSeA at various concentrations in the presence of androgen. Effects of both sodium selenite and MSeA in androgen-mediated AR transactivation activity were then measured by a luciferase reporter assay. We found that both sodium selenite and MSeA significantly inhibited the AR transactivation activity in a dose-dependent manner (Fig. 2A). These results, are consistent with data from previous studies by others [20–25], and suggest that both the compounds were equally effective in inhibiting androgen-mediated AR action. We next tested the effect of these compounds on IL-6-mediated AR transactivation. Interestingly, we found that only sodium selenite, but not MSeA, significantly inhibited IL-6-induced AR transactivation activity in LNCaP cells (Fig. 2B). Taken together, these results suggest that sodium selenite, but not MSeA, is the potent inhibitor of IL-6-mediated AR transactivating function.

3.3. Both sodium selenite and MSeA had no effects on IL-6-induced activation of STAT3 and ERK1/2

IL-6 can mediate primarily two signal transduction pathways, Janus kinases/STAT3 and ERK 1/2 in prostate cells [26,27]. To examine if these 2 pathways can be affected by the selenium compounds, LNCaP cells were exposed to either compound in the presence of IL-6 for 30 min. Expression and phosphorylation activation of STAT3 and ERK1/2 were analyzed by Western blot. Consistent with previous studies [27], we detected an increased activation of STAT3 and ERK1/2 via phosphorylation but not the total STAT3 and ERK1/2 protein levels in LNCaP cells after stimulation with IL-6. Neither sodium selenite nor MSeA had any effect on IL-6-induced activation of STAT3 and ERK1/2 (Fig. 3). No alteration of total AR protein was observed following a longer-term exposure (up to 2 h, data not shown).

3.4. Sodium selenite increased c-Jun expression and overexpression of c-Jun inhibited the AR transactivation activity

To dissect the molecular mechanisms underlying selenite-mediated inhibition of AR activation induced by IL-6, we analyzed the effects of both sodium selenite and MSeA on expression of c-Jun in LNCaP cells. As shown in Figure 4A by Western blot analysis, a significant increase of c-Jun protein expression was detected in cells after 24 h treated with sodium selenite, but not in cells treated with MSeA. To further examine the functional contribution of c-Jun to the regulation of AR transactivation, we used a c-Jun expression vector and co-transfected cells with hK2-3ARE, a vector linked with the luciferase gene [28]. As shown in Figure 4B, AR transactivation activity stimulated by IL-6 was significantly inhibited by c-Jun in LNCaP cells in a dose dependent manner. The results strongly suggest that sodium selenite, but not MSeA, has strong inhibitory effect on IL-6-mediated AR transactivation in LNCaP cells and this inhibitory effect is mediated by overexpression of c-Jun.

4. Discussion

Our studies suggested that sodium selenium, but not MSeA, inhibits IL-6-mediated androgen receptor activation in prostate cancer cells. Using LNCaP cells, we showed that sodium selenite, but not MseA, down-regulates the expression of PSA and AR transactivation induced by IL-6. In addition, sodium selenite shows an inhibitory effect on IL-6-induced proliferation of LNCaP cells. Moreover, sodium selenite induces c-Jun protein expression which in turn interferes with the AR transactivating activity. Thus, sodium selenite inhibits IL-6-mediated AR activation in LNCaP cells via upregulation of c-Jun, supporting a potential intervention role of sodium selenite in controlling prostate cancer progression.

Immunohistochemical staining of tumor biopsies showed that AR was expressed in most prostate cancer cells. More importantly, expression of AR usually correlates to the clinical development of the patients with prostate cancer. For example, a higher level of AR expression is found in patients with relapsed androgen refractory prostate tumors than those with the primary tumors [29,30] and up to 30% of the patients with recurrent tumors show expression of AR protein [31–33]. The AR in those androgen refractory tumors is still functional and can respond to a number of non-androgen factors including IL-6 [34,35]. The potential role of IL-6 in the development and progression of prostate cancer has been suggested by numerous studies [3–8]. IL-6 has been shown to activate AR -dependent gene expression in prostate cancer cells independent of androgens [34,35]. Barton BE et al. have recently shown that IL-6 signaling causes a change from hyperplasia to neoplasia in rat prostate epithelial cells [36]. Activation of intracellular kinases MERK1/2 and a latent transcription factor, STAT3, has been implicated in the activation of AR-dependent gene expression induced by IL-6 in prostate cancer cells [9,34,35]. In addition, phosphatidylinositol 3-kinase (PI 3-K)/AKT has also been considered as one of early down-stream mediators for activation of the AR by IL-6 [4,10]. However,

conflicting results are shown in the literature regarding the influence of the PI 3-K/AKT pathway on the AR [27]. It has been suggested that activated STAT3 by IL-6 binds AR to enhance AR's transactivation function [27]. Consistent with previous studies [27], we detected an increased cell proliferation and activation of STAT3 and ERK1/2 via phosphorylation in LNCaP cells after stimulation with a recombinant human IL-6. Here we identified that although both sodium selenite and MSeA equally effective inhibit androgen mediated AR transactivation, only sodium selenite showed inhibitory effects on IL-6-mediated transactivation of AR. Moreover, Wu Y et al., [37] showed that selenium could deactivate AKT in prostate cancer cells. In consistence with this finding we also found selenium compounds used could reduce phosphorylated AKT (data not shown). Yet, we demonstrated MSeA did not inhibit IL-6 mediated AR activation. We concluded that AKT did not play a role in selenite mediated repression of AR activation by IL-6.

Dietary supplement of selenium may be an attractive approach to intervene prostate cancer development and progression. The efficacy was suggested Clark et al. [38] who showed 63% reduction of prostate cancer incidence with supplementation of selenite-enriched yeast. Higher serum selenium levels were found to be associated with a reduced (up to 50%) risk of metastasis in prostate cancer [12]. Sodium selenite and MSeA, are comprehensively studied selenium compounds, have been reported to be effective in inhibiting growth of most of the prostate cancer cell lines including LNCaP. Previous studies indicate that MSeA inhibits the expression of PSA protein by androgens in LNCaP cells [20,21,23]. However, in the present study, we provide evidence suggesting that sodium selenite, but not MSeA, down-regulates the expression of PSA and AR transactivation induced by IL-6 in LNCaP cells. Interestingly, both sodium selenite and MSeA showed no inhibitory effects on IL-6-induced activation of STAT3 and ERK1/2, important early events in IL-6-mediated signaling pathways in prostate cancer cells as reported in previous studies [9,34,35]. Instead, it appears that upregulation of c-Jun may play a critical role in sodium selenite induced inhibition of AR activation in response to IL-6.

c-Jun, a member of the basic leucine zipper family, is a main component of AP-1 protein complex which can directly activate or induce transcription of many genes containing AP-1 DNA binding sequences. It was reported that antioxidants such as quercetin can either inhibit or induce the activation and expression of c-Jun depending on cell types [39,40]. For example, angiotensin-II-induced c-Jun N-terminal kinase (JNK) mediated c-Jun activation was inhibited by quercetin in rat aortic smooth muscle cells [41], resulting in a decrease in both phosphorylation and activation of c-Jun. On the other hand, quercetin, resveratrol [42] and perillyl alcohol [43] can enhance the expression of c-Jun which in turn inhibited AR function in human prostate cancer cells. In this study, we showed that sodium selenite, but not MSeA, induces the over-expression of c-Jun in prostate cancer cells. It has been shown that the DNA binding domain of nuclear receptors may interact with the leucine zipper of c-Jun and result in mutual repression [41,44]. Sato et al. [44] demonstrated that there was a direct protein–protein interaction between the DNA- and ligand-binding domains of the AR and the leucine zipper region of c-Jun. Thus, the leucine zipper region of c-Jun by interacting with the DNA binding domain might affect the function of the AR. Meanwhile, this interaction may repel the binding of the coactivator, cyclic AMP response element-binding protein binding protein (CBP), to AR [45]. It is possible that increased c-Jun binding to AR-STAT3 complex might disassociate p300/CBP and interfere with the function of AR-STAT3. We will further address this potential question in the future studies.

In conclusion, we showed that sodium selenite, but not MSeA, significantly inhibited IL-6-induced cell proliferation and *trans*-activating activity of AR in LNCaP cells, presumably via up-regulation of c-Jun. Thus, sodium selenite may have strong effects in inhibiting progression of prostate cancer. A sodium selenite intervention strategy aimed at dampening the amplitude

of AR signaling in androgen dependent and independent pathways could be helpful for controlling prostate cancer in high risk men. Combined with androgen ablation, selenite intervention might also be useful for the prevention of relapse after endocrine therapy. Further studies will focus on the molecular mechanisms of sodium selenite-associated up-regulation of c-Jun and determine if the differential effects of the selenite compound on IL-6-induced AR activation can also be observed in any other dietary selenium compounds.

Acknowledgements

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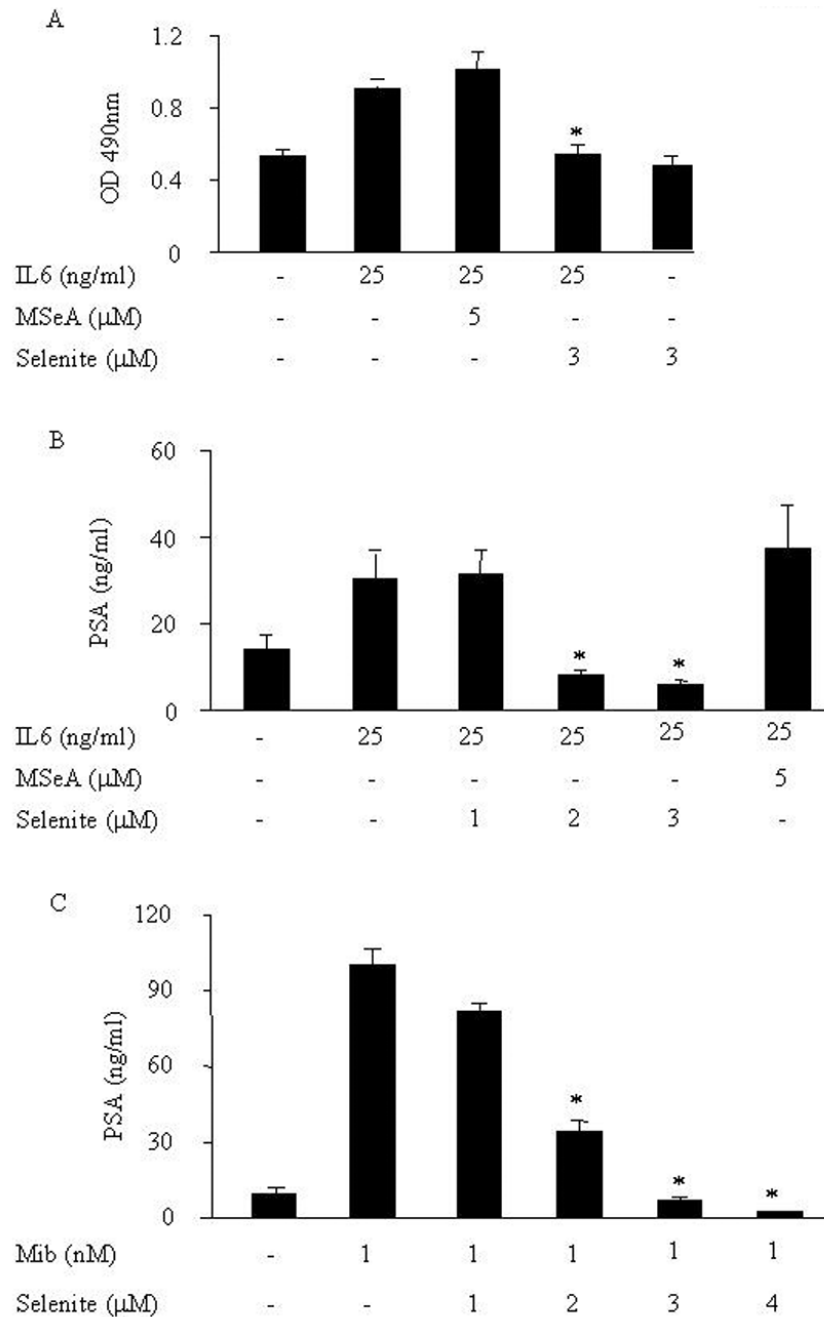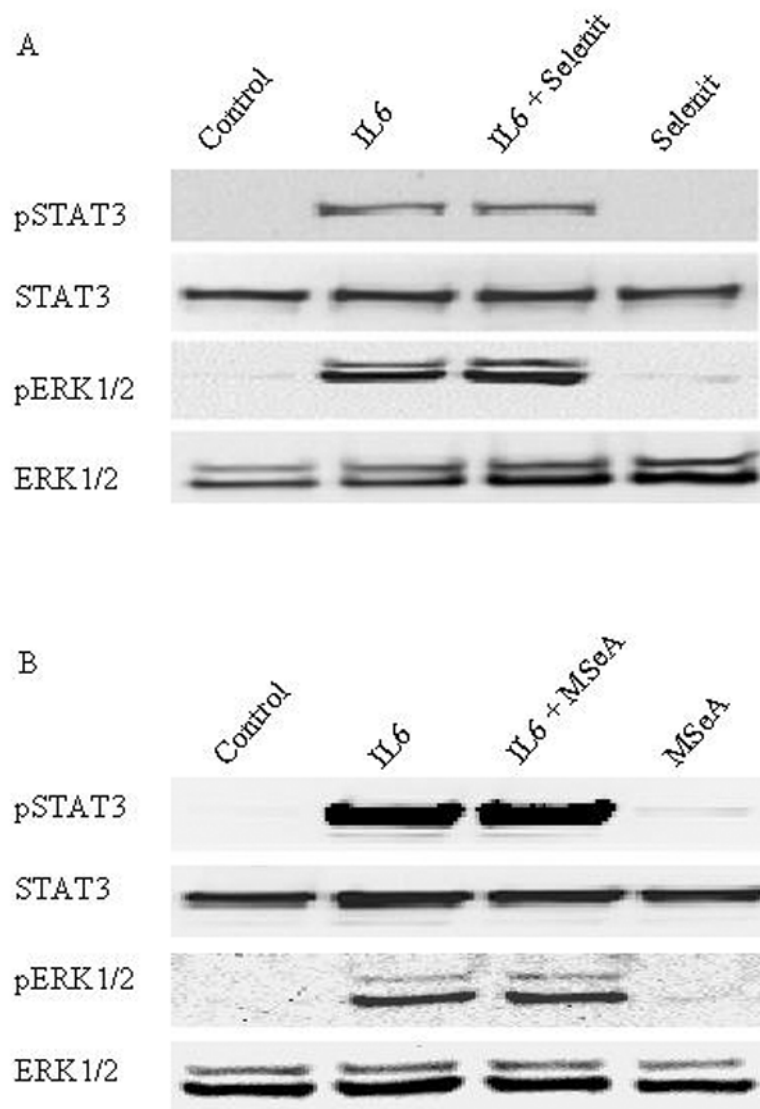
Fig 1.
Effects of sodium selenite or MSeA on IL-6-mediated cell growth (A) and IL-6 (B) or Mib (C) induced PSA protein expression in LNCaP cells. (A) After treated with or without IL-6 in the presence or absence of 5 μmol/l MSeA or 3 μmol/l selenite for 72 h, cell growth was measured by the MTS assay. (B) After treated with or without IL-6 in the presence or absence of 1, 2, 3 μmol/l selenite or 5 μmol/l MSeA for 72 h, 400 μl of spent medium was collected and PSA protein expression was determined by immunoassay. (C) After treated with or without Mib in the presence or absence of 1, 2, 3, 4 μmol/l selenite for 72 h, 400 ul of spent medium were collected and PSA protein expression was determined by immunoassay.
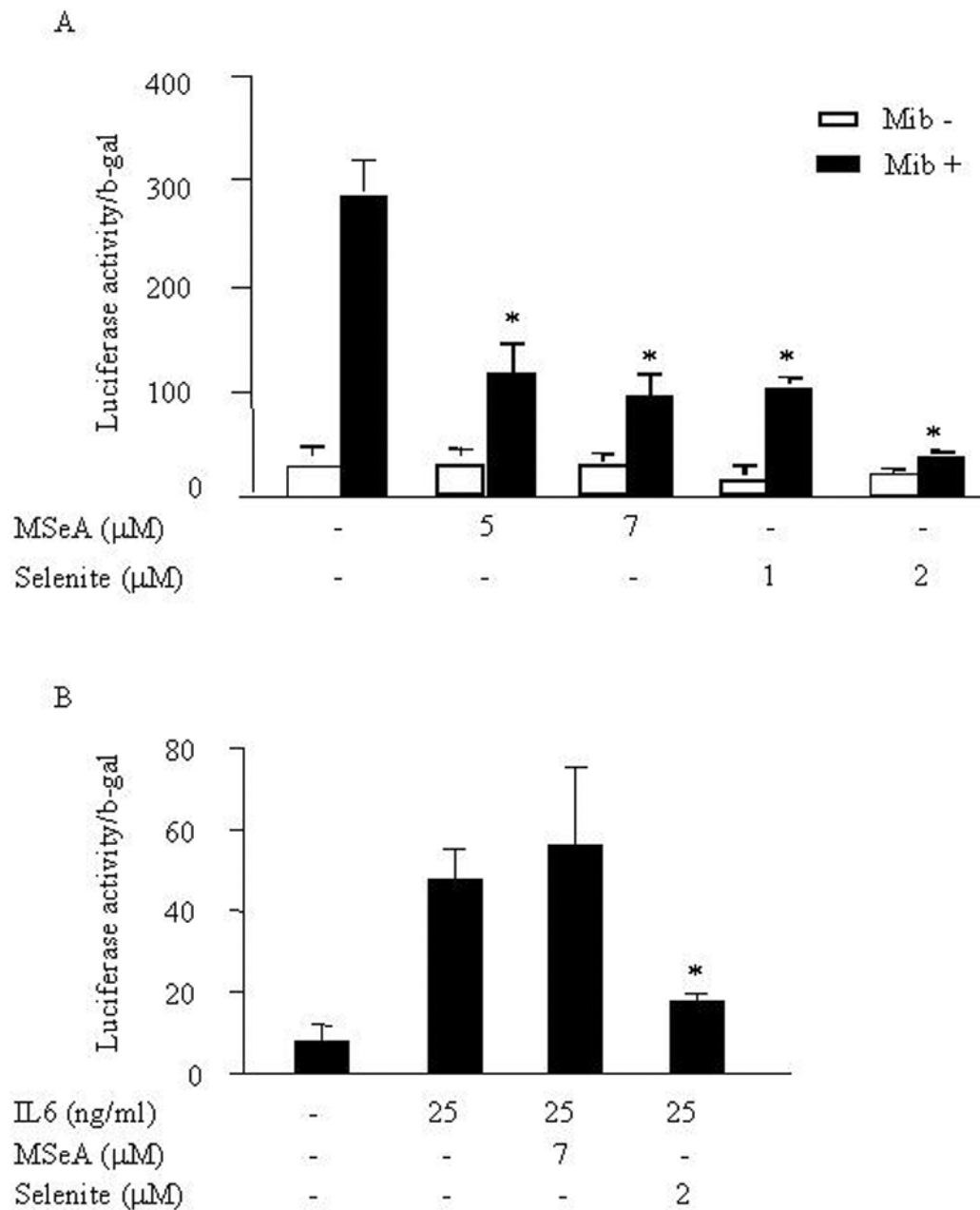
Fig 2.
Effects of sodium selenite or MSeA on Mib or IL-6-stimulated AR transactivation activity in LNCaP cells. (A) After co-transfected with PSA-6kb promoter-luciferase reporter (1 μg/well) and CMV-β-gal vectors (0.2 μg/well) for 24 h, LNCaP cells were treated with Mib or without and plus or minus MSeA or selenite as indicated. Twenty-four hours later, cell lysate was collected and transcriptional activity of PSA-6kb and expression of β-gal were determined by luciferase and β-gal assay, respectively. The relative luciferase activities were normalized by β-gals. (B) Effects of sodium selenite and MSeA on IL-6 stimulated AR transactivation activity in LNCaP cells were determined by luciferase and β-gal assays.

Fig 3.
Effects of sodium selenite or MSeA on IL-6-induced expression and activation of STAT3 and ERK1/2 in LNCaP cells. (A) After treated with or without IL-6 in the presence or absence of 3 μmol/l selenite for 30 min, whole cell lysates were prepared and expression of total and phosporylated STAT3 and ERK1/2 were determined by Western blot analysis using specific antibodies. (B) Effects of MSeA on IL-6-induced expression of total and phosphorylated STAT3 and ERK1/2 in LNCaP cells were shown by Western blot analysis.
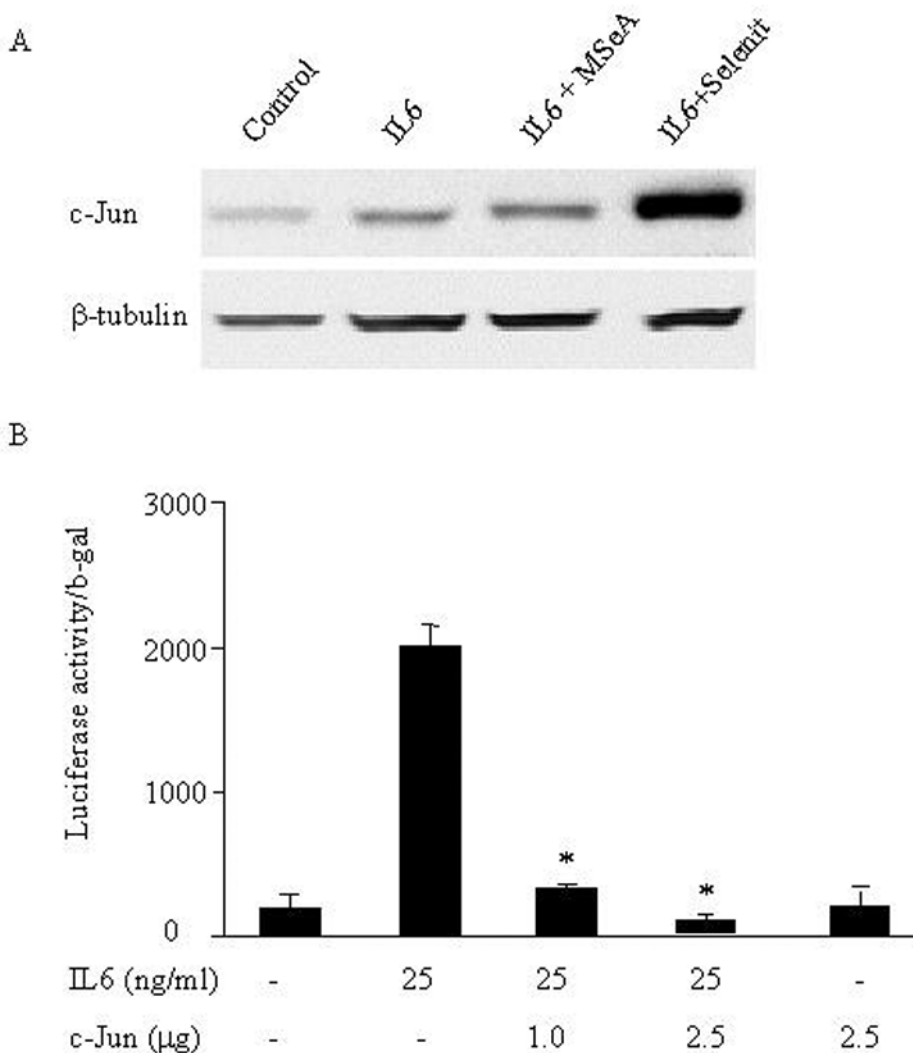
Fig 4.
Effects of sodium selenite or MSeA on IL-6-induced c-Jun expression and overexpression of c-Jun on IL-6-mediated AR transactivation in LNCaP cells. (A) Effects of sodium selenite or MSeA on IL-6-induced c-Jun expression. After LNCaP cells treated with or without IL-6 and plus or minus sodium selenite (3 μmol/l) or MSeA (5 μmol/l) for 24 h, the expression of c-Jun in whole cell lysates was analyzed by western blot analysis. β-tubulin was used as an internal control for protein loading and transfer efficiency. (B) Effects of overexpression of c-Jun on IL-6-mediated AR transactivation in LNCaP cells. After cotransfected with hK2-3ARE promoter-luciferase reporter (1 μg/well), CMV-β-gal vectors (0.2 μg/well) and plus or minus c-Jun expression vector (1 or 2.5 μg/well) for 24 h, cells were treated with or without IL-6. Cell lysate was collected 24 h later and transcriptional activity of hK2-3ARE and expression of β-gal were determined by luciferase and β-gal assays, respectively. The relative luciferase activities were normalized by β-gal.