

Hormetic Dose Response of NaAsO₂ on Cell Proliferation of Prostate Cells in Vitro: Implications for Prostate Cancer Initiation and Therapy

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Abstract

Sodium meta-arsenite (NaAsO₂) has been suggested to play a role both in initiation/progression of prostate cancer and as a future antiprostate cancer drug. We have studied the effects of NaAsO₂ on cell proliferation of prostate cancer and noncancer cells, breast cancer cells, and adrenocortical carcinoma cells in vitro. Further, we have investigated the effect of NaAsO₂ on the androgen receptor. We report that NaAsO₂ alters the cell proliferation of prostate cells, in a hormetic manner, by increasing cell proliferation at low concentrations and decreasing the cell proliferation at high concentrations. No activation of the androgen receptor was detected. We conclude that NaAsO₂ is able to increase cell proliferation of prostate cells in vitro at low concentrations, while it decreases cell viability at high concentrations. This novel finding has to be further addressed if NaAsO₂ should be developed into an antiprostate cancer drug.

Keywords

androgen receptor activation, cell viability, hormetic effects, prostate cancer

Introduction

Prostate cancer is among the most common forms of cancer with 1.3 million new cases worldwide during the year of 2018, accounting for approximately 14% of all cancers diagnosed in men. Prostate cancer is expected to cause more than 350 000 deaths year 2018.¹ Growth of prostate cancer is dependent on androgens acting on the androgen receptor. The main treatment of prostate cancer is androgen deprivation therapy either by chemical or surgical castration.

The association between inorganic arsenic exposure from drinking water and prostate cancer is well documented from epidemiological studies.²⁻⁵ The role of inorganic arsenic in the development of prostate cancer and possible mechanisms behind the effect has been reviewed by Benbrahim-Tallaa and Waalkes.⁶ Due to the high incidence and mortality in prostate cancer, a number of new treatment strategies and drugs are under development. Sodium meta-arsenite (NaAsO₂) has been proposed as a new antiprostate cancer drug.⁷⁻¹⁰ Sodium meta-arsenite has successfully been used in the treatment of acute promyelocytic leukemia (APL) and has also been used in clinical trials for treatment of solid tumors of various origin,¹¹ including patients with

metastatic prostate cancer (European Clinical Trials Database number 2006-005607-33). Zhang et al¹⁰ have reported that combinations of NaAsO₂ and taxane drugs have synergistic antiproliferative effects on prostate cancer cells in vitro. Phatak et al⁹ found that NaAsO₂ exerted antiproliferative effects on a wide range of cancer cells in vitro, including prostate cancer cells. Further, they concluded that NaAsO₂ is a telomere-targeting agent and that this could be the mechanism for the antiproliferative effect. Kim et al⁷ reported that NaAsO₂ reduced cell viability of both androgen-sensitive and androgen-insensitive prostate cancer cells in vitro. The authors also reported that NaAsO₂

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induces apoptosis, necrosis, and autophagy in the studied prostate cancer cell lines and suggest that this effect could be via a reactive oxygen species (ROS)-dependent mechanism. Recently, Kim et al⁸ demonstrated reduced levels and stability as well as translocation to the nucleus of androgen receptors and reduced levels of prostate-specific antigens (PSAs) after treatment of prostate cancer cells with NaAsO₂.

In the present study, we have investigated the effects of NaAsO₂ on cell proliferation of cancer and normal cells derived from the prostate, adrenal cortex, and breast. Adrenocortical and breast cells were included in the study to enable a comparison between cell types and to investigate whether observed effects were specific to prostate cells. We report that NaAsO₂ exerts a hormetic effect on cell proliferation of both normal and cancer prostate cells, with a stimulated proliferation at low concentrations of NaAsO₂.

Materials and Methods

Chemicals and Cell Culture

Sodium meta-arsenite was purchased from Sigma-Aldrich (Stockholm, Sweden). Human breast cancer MCF-7 cells were cultured in Dulbecco Modified Eagles Medium (DMEM)/F:12 medium (Sigma-Aldrich, Stockholm, Sweden) supplemented with 10% fetal bovine serum (Gibco, Stockholm, Sweden), 2 mM L-glutamine (Gibco, Stockholm, Sweden), 6 ng/mL insulin (Gibco, Stockholm, Sweden), and 100 U/mL penicillin/100 µg/mL streptomycin (Gibco, Stockholm, Sweden). Human adrenocortical NCI-H295R cells were cultured in DMEM/F:12 medium (Sigma-Aldrich, Stockholm, Sweden) supplemented with 1% ITS Plus premix (BD Biosciences, Gothenburg, Sweden), 2.5% NuSerum (VWR, Stockholm, Sweden), 2 mM L-glutamine (Gibco, Stockholm, Sweden), and 100 U/mL penicillin/100 µg/mL streptomycin (Gibco, Stockholm, Sweden). Human prostate RWPE cells were cultured in Keratinocyte Serum Free Medium (Gibco, Stockholm, Sweden) supplemented with 0.05 mg/mL bovine pituitary extract and 5 ng/mL human recombinant epidermal growth factor. Human prostate LNCaP cells were cultured in RPMI 1640 medium (Gibco, Stockholm, Sweden) supplemented with 2 mM L-glutamine (Gibco, Stockholm, Sweden), 10% fetal bovine serum (Gibco, Stockholm, Sweden), 1 mM sodium pyruvate (Gibco, Stockholm, Sweden), and 100 U/mL penicillin/100 µg/mL streptomycin (Gibco, Stockholm, Sweden). Human prostate VCaP cells were cultured in DMEM/F:12 medium (Sigma-Aldrich, Stockholm, Sweden) supplemented with 2 mM L-glutamine (Gibco, Stockholm, Sweden), 10% fetal bovine serum (Gibco, Stockholm, Sweden), and 100 U/mL penicillin/100 µg/mL streptomycin (Gibco, Stockholm, Sweden). All cells were cultured as monolayers in a humidified environment at 37°C with 5% CO₂ and subcultured weekly.

Cell Proliferation Assay

To assay the effect of NaAsO₂ on the cell proliferation in LNCaP, RWPE, VCaP, MCF-7, and NCI-H295R cells, the

capacity of the cells to reduce a tetrazolium compound (MTS) to the colored product formazan was analyzed. The reaction is dependent on mitochondrial function, and the amount of formazan formed is directly proportional to the number of living cells in culture. Cells were seeded in 96-well plates with a density of 1×10^4 cells per well and incubated for 48 hours. The cells were then treated with NaAsO₂ dissolved in water. Cells were treated with NaAsO₂ in concentrations ranging from 0.01 to 300 µM. Cell proliferation was analyzed after 24 hours of exposure, using the CellTiter 96 AQueos One Solution Proliferation Assay System (Promega, Stockholm, Sweden) in accordance with the manufacturer's recommendations. The absorbance was measured using a Wallac Victor2 1420 microplate reader (PerkinElmer, Massachusetts, USA). Relative effects on cell proliferation were compared to the vehicle treated control.

Androgen Receptor Activation Assay

To investigate whether NaAsO₂ could activate the androgen receptor, LNCaP, VCaP, and RWPE cells were transfected with a luciferase reporter plasmid, where the expression of luciferase is under the control of a promoter containing androgen receptor responsive elements.¹² The cells were seeded in 96-well plates with a density of 1×10^4 cells per well and incubated for 48 hours. The cells were then transfected with 0.4 µg luciferase plasmid per well. To standardize for transfection efficiency, the cells were cotransfected with a renilla luciferase plasmid. The transfection was performed with 0.3 µL Lipofectamine 2000 per well in accordance with the manufacturer's recommendations. Following transfection, the cells were incubated for 24 hours and then treated with various concentrations of NaAsO₂ for 24 hours. The luciferase activity was assayed using the Dual-Luciferase Reporter Assay System (Promega, Stockholm, Sweden). The luciferase activity is expressed as percentage compared to the vehicle-treated control group. Testosterone was used as a positive control.

Statistical analysis

Statistical analysis was performed by Kruskal-Wallis test, followed by a Mann-Whitney *U* test for individual comparisons against the control. The statistical analysis was performed using Minitab 16. *P* < .05 was considered statistically significant.

Results

Sodium Meta-Arsenite Alters the Cell Proliferation of Prostate Cells in a Hormetic Manner

We found (Figure 1A-C) that NaAsO₂ treatment altered the proliferation of prostate LNCaP, VCaP, and RWPE cells in a hormetic manner, resulting in stimulated cell proliferation with up to approximately 40% in the concentration range 0.1 to 40 µM (depending on the cell line) and reduced cell viability at higher concentrations.

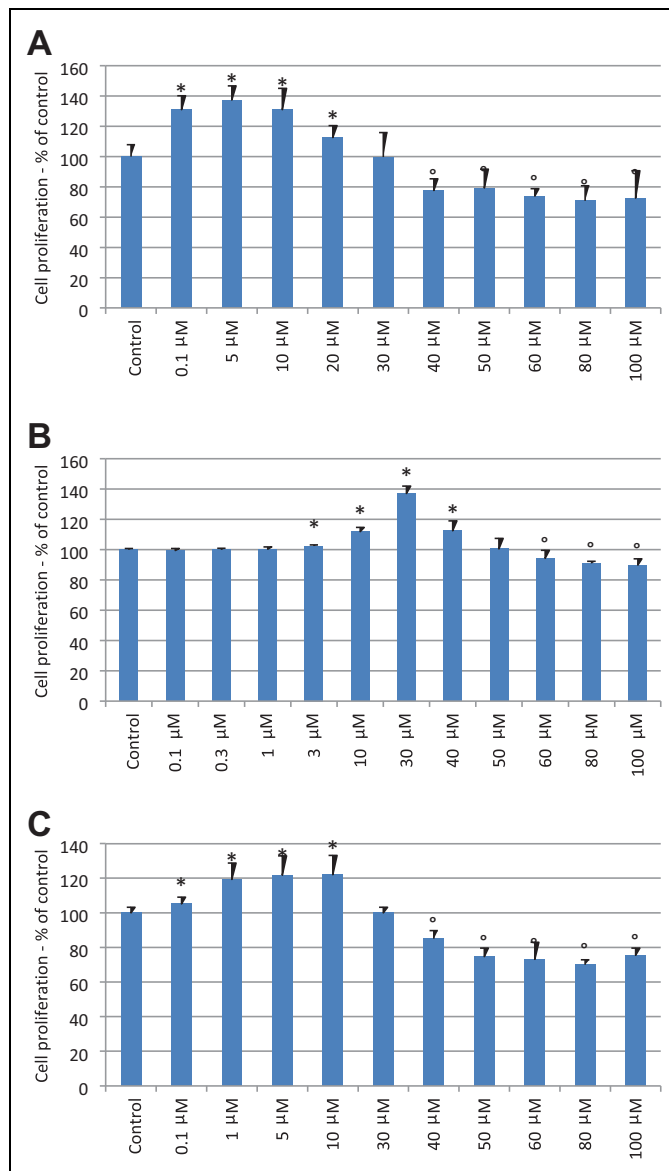


Figure 1. Cell proliferation of prostate LNCaP (A), VCaP (B), and RWPE (C) cells cultured and treated as described in the Materials and Methods section. The cell proliferation is expressed as percentage of the vehicle-treated control. Each bar represents the mean value, and the error bars represent the standard deviation ($n = 24$ for the control group and $n = 8$ for the treated groups). P values $< .05$ were considered statistically significant. *Statistically significant increase in cell proliferation; °Statistically significant decrease in cell proliferation.

Effects of NaAsO₂ on the Cell Proliferation of Cancer Cell Lines Derived From Breast and Adrenal Cortex

The proliferation of breast cancer MCF-7 cells was increased by treatment with NaAsO₂ in the concentration 3 and 10 μM, although the stimulatory effect was only 5% to 7% (Figure 2A). At the concentration 30 μM and above, NaAsO₂ was found to significantly decrease the cell viability. The proliferation of

adrenocortical carcinoma NCI-H295R cells was found to be altered by NaAsO₂ treatment in a monotonic manner, where increased levels of NaAsO₂ resulted in decreased cell proliferation (Figure 2B).

Sodium Meta-Arsenite Does Not Activate the Androgen Receptor

The effect of NaAsO₂ on androgen receptor activation was assayed at nontoxic concentrations in LNCaP, VCaP, and RWPE cells. We found that NaAsO₂ was unable to activate the androgen receptor in LNCaP cells (Figure 3A) and VCaP cells (Figure 3B). The transfected RWPE cells were not responsive to the positive control testosterone (data not shown) and excluded from further analysis of receptor activation.

Discussion

Arsenic exposure via drinking water affects millions of people worldwide, and an association with increased incidence of prostate cancer has been reported.²⁻⁵ Prostate cancer is a disease with high incidence and mortality. The possibility that arsenite may play a role in the initiation and progression of prostate cancer as well as being a promising candidate compound as an antiprostate cancer drug is intriguing. The molecular events by which arsenite initiates cancer in normal prostate cells at low concentrations are probably different from the molecular events causing cell deterioration in prostate cancer cells at high concentrations. NaAsO₂, a water soluble and orally bioavailable arsenic compound, has been reported to significantly decrease the proliferation of prostate cancer cells⁷⁻¹⁰ with suggested mechanisms at multiple levels, including that NaAsO₂ could act as a telomere-targeting agent, that it could induce apoptosis and necrosis via a ROS-dependent mechanism, or that it could reduce androgen receptor levels, stability, and translocation into the nucleus.

In this article, we report that the effect of NaAsO₂ on the proliferation of both cancer and normal prostate cells is in fact hormetic with stimulated cell proliferation in the low- to medium-concentration range and reduced cell proliferation at high concentrations. We have only observed this clear hormetic effect in prostate cells. In cells derived from other organs, NaAsO₂ exhibited either a clear monotonic response on the cell proliferation with increasing concentrations of NaAsO₂ causing a decrease in cell proliferation or a more unclear dose-response relationship. The hormetic response of NaAsO₂ on the proliferation of prostate cells is a novel finding and should be of high relevance to address further whether NaAsO₂ is to be developed into an antiprostate cancer drug, as the induced proliferation of prostate cancer cells following low-dose exposure to NaAsO₂ could counteract the desired result of the drug therapy (ie, decreased proliferation of prostate cancer cells). We have also investigated whether the stimulated cell proliferation caused by low doses of NaAsO₂ could be via an

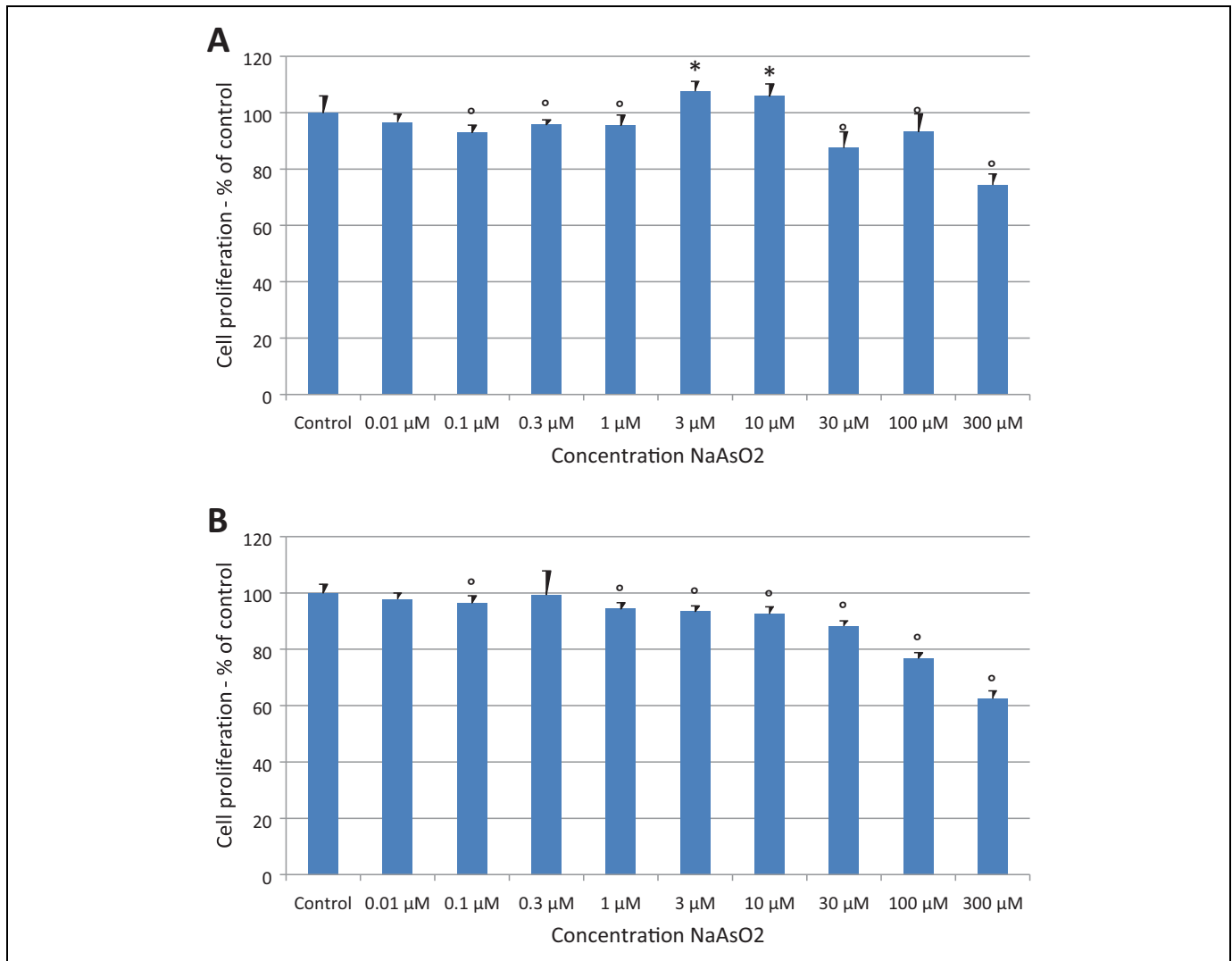


Figure 2. Cell proliferation of breast cancer MCF-7 (A) cells and adrenocortical carcinoma NCI-H295R (B) cells cultured and treated as described in the Materials and Methods section. The cell proliferation is expressed as percentage of the vehicle-treated control. Each bar represents the mean value, and the error bars represent the standard deviation ($n = 24$ for the control group and $n = 8$ for the treated groups). P values $< .05$ were considered statistically significant. *Statistically significant increase in cell proliferation; ^oStatistically significant decrease in cell proliferation.

androgen receptor-dependent mechanism but found that NaAsO₂ is unable to activate the androgen receptor. While androgen receptor signaling is an important regulator of proliferation of prostate cells in many cases, this signaling pathway is not the only mechanism by which proliferation is regulated. Our finding that NaAsO₂ is affecting the proliferation of prostate cells, but is unable to activate the androgen receptor, indicates that NaAsO₂ acts via one of the other proliferation-regulating pathways in prostate cells. The human ether-a-go-go-related gene potassium channel (hERG) has been identified as a novel regulator of growth and death in tumor cells.¹³ Zhang et al¹⁴ recently reported dual effects of arsenic trioxide on tumor cells. They reported stimulated proliferation at low doses, associated with increased expression of hERG and accelerated hERG channel activation, and inhibited proliferation

at high doses, associated with reduced expression of hERG. Further research is needed to clarify the mechanism for the effect of NaAsO₂ on prostate cell proliferation.

The effects of arsenite on prostate cells can be compared with the effects of isoflavons, especially genistein and daidzein, on mammary cells. In estrogen-dependent human breast cancer MCF-7 cells, genistein enhances cell proliferation at low concentrations (0.01 to 10 μM), while higher cell concentrations inhibit cell growth.^{15,16} A dose-dependent stimulation of tumor growth has been reported in experimental animals after treatment with isoflavons via the feed.¹⁷

Dose-response relationships between arsenic exposure and health effects are debated, and whether there is a dose threshold below which exposure is not harmful.¹⁸ Exposure to arsenic in drinking water is associated with increased mortality in various

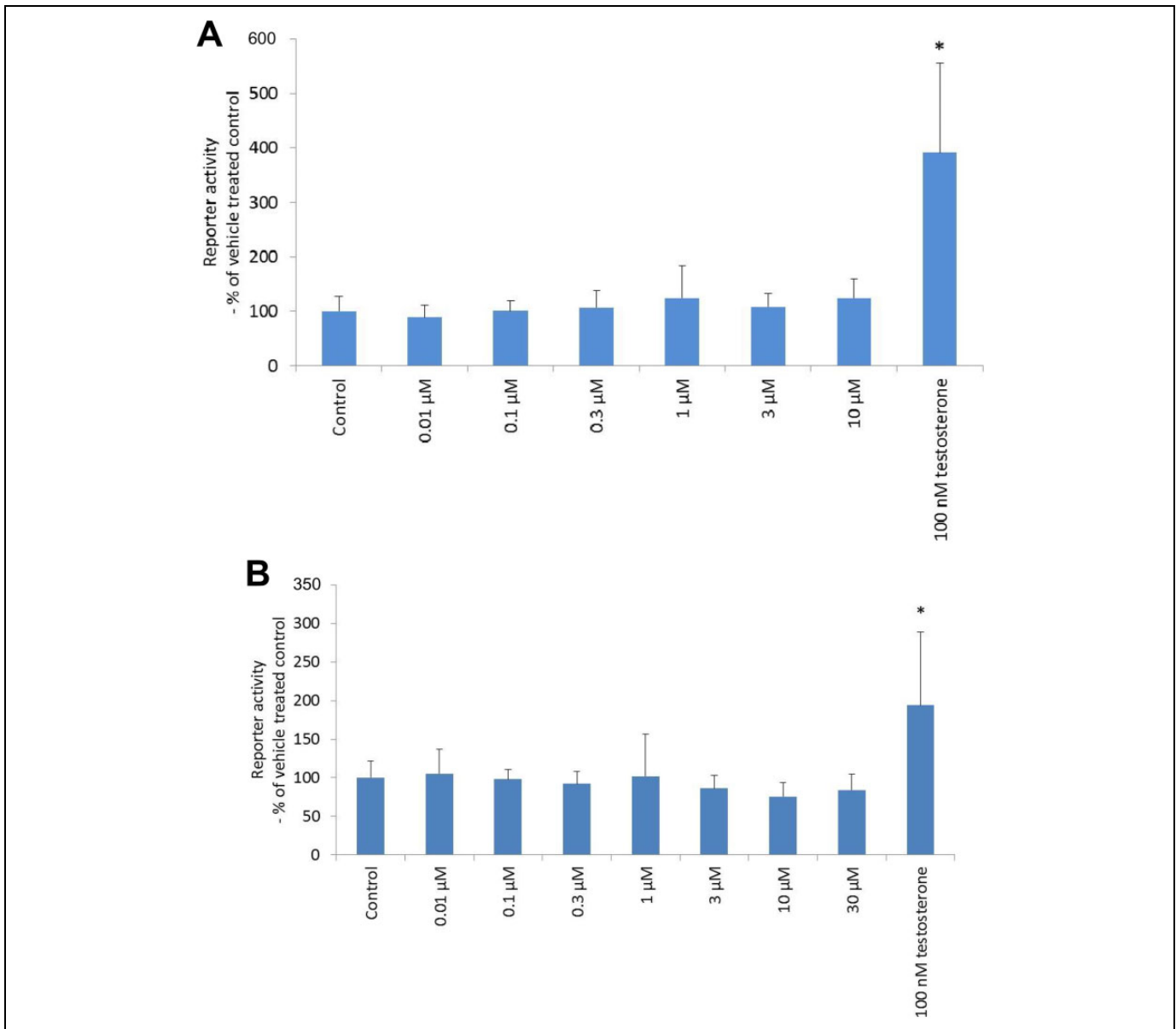


Figure 3. Androgen receptor activity in prostate LNCaP (A) and VCaP (B) cells cultured, transfected, and treated as described in the Materials and Methods section. The reporter activity is expressed as percentage of the reporter activity in the vehicle-treated control. Each bar represents the mean value, and the error bars represent the standard deviation ($n = 16$ for the control group and $n = 8$ for the treated groups). P values $<.05$ were considered statistically significant. *Statistically significant increase in androgen receptor activity.

forms of cancer, including prostate cancer.^{2,3,6} Epidemiological studies with low exposures are sparse. However, a US prospective cohort study reported an association between low to moderate exposure to arsenic (measured as urinary levels) and increased mortality in prostate cancer (hazard ratio 3.30; 95% confidence interval 1.28-8.48).⁴ A dose-dependent association between arsenic in drinking water and prostate cancer was also reported in an ecologic study from Iowa, United states.⁵

In conclusion, we report that the suggested antiprostata cancer drug NaAsO₂ in fact stimulates the proliferation of prostate cancer cells in vitro at low concentrations and that this is an androgen receptor-independent effect. This novel finding needs

to be further addressed if NaAsO₂ should be developed as an antiprostata cancer drug.

Declaration of Conflicting Interests

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