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Effect of Lycopene on Prostate LNCaP Cancer Cells in Culture

LINDA KIM, M.Sc., A. VENKET RAO, Ph.D., and LETICIA G. RAO, Ph.D.

ABSTRACT

Epidemiological studies have shown an inverse relationship between serum lycopene levels and the risk of prostate cancer. The objective of this study was to measure the effect of lycopene on the proliferation of LNCaP human prostate cancer cells in culture. A new, water-dispersible lycopene in an appropriate vehicle was used. The stock solution was diluted in the medium to obtain lycopene concentrations of 10^{-6}, 10^{-5}, and 10^{-4} M; their corresponding vehicles were similarly diluted to be used as controls. Cells were grown for 48 hours in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics. Lycopene was then added at different concentrations, and the cells were allowed to grow for 24, 48, 72, and 96 hours. Lycopene at concentrations of 10^{-6} and 10^{-5} M significantly reduced the growth of LNCaP cells after 48, 72, and 96 hours of incubation, by 24.4% to 42.8% ($P < .05$). The inhibitory effect of lycopene was significantly higher than that of the corresponding vehicle controls. In a follow-up experiment, a lower range of lycopene concentrations (10^{-9} to 10^{-7} M) was used to determine whether there was a dose-response effect. Lycopene significantly decreased the growth of cells in a dose-dependent manner when cells were incubated for 24, 48, 72, or 96 hours (F = 3.150, 11.27, 54.51, and 297.5, respectively; $P < .05$). The growth inhibitory effect of lycopene on human prostate cancer cells observed in this study suggests a possibly important role for lycopene as an antioxidant in human prostate cancer; however, investigations of other mechanisms are warranted.

INTRODUCTION

PROSTATE CANCER IS a commonly diagnosed cancer[1] and is the second most common cause of cancer deaths among North American men.[2] Although genetic factors and age are important determinants of risk, there is increasing evidence from epidemiological, experimental, and metabolic studies suggesting that environmental exposures, including diet, play an important role in the progression of prostate cancer. Nutritional intervention studies have focused on possible agents with the potential to prevent prostate cancer. The role of highly reactive oxygen species (ROS) and oxidative damage to biomolecules is one of the main foci of recent research related to cancer and cardiovascular diseases. Oxidative stress has been widely postulated to be involved in the causation and progression of several chronic diseases including prostate cancer.[3–8]

Dietary antioxidants, which inactivate ROS and provide protection from oxidative damage,[3–8] are being considered as important preventive strategic molecules for cancer and other chronic diseases. These antioxidants possess

Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada.

the ability to quench singlet oxygen free radicals, among other ROS, and thus protect against cellular oxidative damage.[9]

More specifically, lycopene, a predominant carotenoid naturally present in tomatoes and other fruits, is a potent antioxidant and the most significant free radical scavenger.[19] The antioxidant properties of lycopene have raised interest in the tomato as a food with potential anticancer properties.[20] To date, although the effects of lycopene on the growth of several cancer cell lines have been studied,[27] very little evidence has been reported with prostate cancer cells of human origin.

The objective of the present study was to measure the effect of lycopene on the growth and proliferation on human prostate cancer LNCaP cells.

MATERIALS AND METHODS

Cell culture

The human prostate cancer cell line, LNCaP, was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were maintained in a 75 cm^2 flask in RPMI-1640 medium containing 2 mM glutamine, 10 μg/ml streptomycin, and supplemented with 10% fetal bovine serum and antibiotics and subcultured weekly. For experimental use, the cells were plated into 12-well culture dishes with a density of 5×10^5 cells/ml. At 48 hours after subculture, the unattached cells were washed and fresh medium containing different concentrations of lycopene was added. The cells were further cultured for 24, 48, 72, or 96 hours under standard cell culture conditions. Respective vehicle controls of similar dilution for each concentration of lycopene were also evaluated. All cell cultures were maintained in a carbon dioxide incubator at 37°C with a 5% CO_2 atmosphere.

Lycopene sample preparation

A new, micro-emulsion with 0.258% lycopene (LycoRed Natural Products Industries Ltd., Beer Sheva, Israel) in an appropriate vehicle was used for this study. A 1×10^{-3} M stock solution of lycopene was prepared fresh and diluted before use in the RPMI-1640 medium; lycopene was added to the cultures at final concentrations ranging from 10^{-9} to 10^{-4} M. The corresponding vehicle cultures received the carrier solvent in the same dilution as the lycopene.

Cell proliferation

The cells were cultured for 48 hours before treatment with lycopene. After incubation periods of 24, 48, 72, and 96 hours, any floating cells were washed off, and the remaining cells were detached from the wells with trypsin-EDTA and collected for cell counting. Cell proliferation was measured by direct cell counts with a hemacytometer.

Statistical analysis

For *in vitro* growth assays, replicates of two experiments each in triplicate were performed. Results are expressed as mean ± SEM. Statistical differences were analyzed by a one-way analysis of variance (ANOVA) followed by the Dunnett multiple comparison test and Student's *t* tests (Instat, v. 2.02, GraphPad Software, San Diego, CA); a probability value lower than .05 was considered statistically significant.

RESULTS

The figures illustrate the dose-dependent effect of lycopene at concentrations of 10^{-9} to 10^{-5} M on the growth of LNCaP cells after various times of incubation. Lycopene was observed to be stable under the standard cell culture conditions and incubation periods used. After 24 hours of incubation, lycopene inhibited the proliferation of LNCaP cells in a dose-dependent fashion (n = 6, F = 3.150, $P < .05$; Fig. 1). At 10^{-7} M, the decrease in LNCaP cell number was 20.4% that of the vehicle at the same dilution ($P < .05$). After 48 hours of treatment, lycopene inhibited the proliferation of LNCaP cells in a dose-dependent fashion (n = 6, F = 11.27, $P < .001$; Fig. 2), and at lycopene concentrations of 10^{-8}, 10^{-6}, and 10^{-5} M, cell numbers were significantly reduced, by 3.8%, 24.4%, and 25.6%, respectively, compared with the vehicles at the

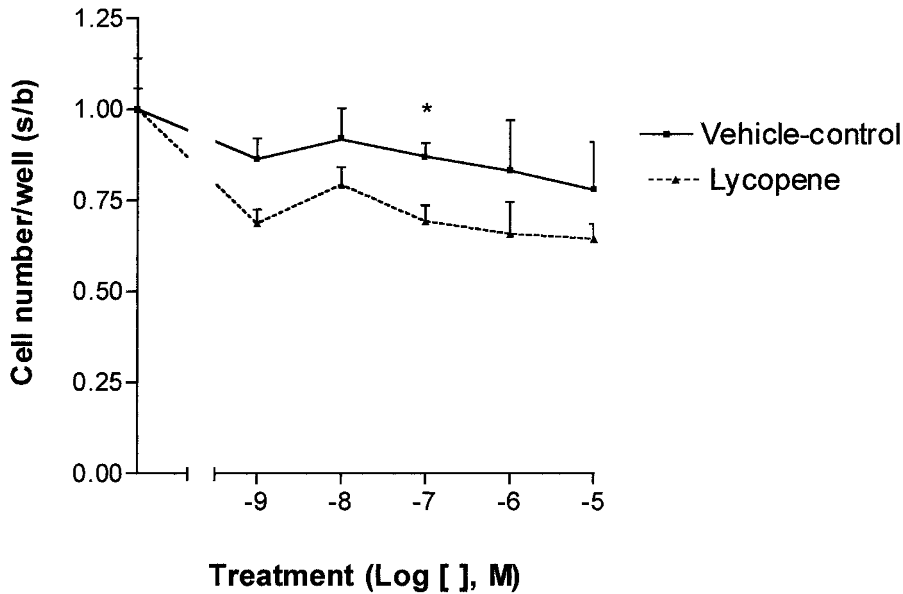

FIG. 1. Dose-response effect of lycopene on LNCaP cell number after 24 hours of incubation. After allowing LNCaP cells to stabilize for 48 hours, cells were treated with lycopene concentrations of 10^{-9} to 10^{-5} M and incubated for 24 hours. Cells were then collected and counted using a hemacytometer. Values are means ± SEM, n = 6. *, $P < .05$, significantly different from vehicle-control.

same dilution ($P < .05$). Similarly, after 72 hours of incubation, lycopene caused a dose-dependent inhibition of LNCaP cell growth (n = 6, F = 54.51, $P < .0001$; Fig. 3), and lycopene concentrations of 10^{-8}, 10^{-6}, and 10^{-5} M significantly reduced the growth of LNCaP cells compared with their vehicles at the same dilution (by 15.2%, 31.9%, and 25.5%, respectively; $P < .05$). After an incubation of 96 hours, lycopene was observed to significantly

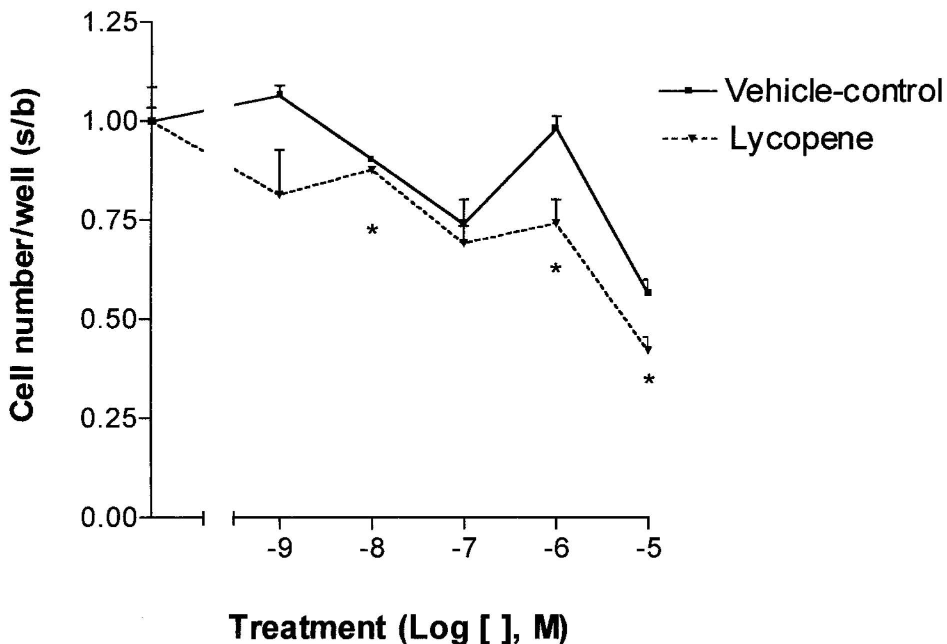

FIG. 2. Dose-response effect of lycopene on LNCaP cell number after 48 hours of incubation. After allowing LNCaP cells to stabilize for 48 hours, cells were treated with lycopene concentrations of 10^{-9} to 10^{-5} M and incubated for 48 hours. Cells were then collected and counted using a hemacytometer. Values are means ± SEM, n = 6. *, $P < .05$, significantly different from vehicle-control.

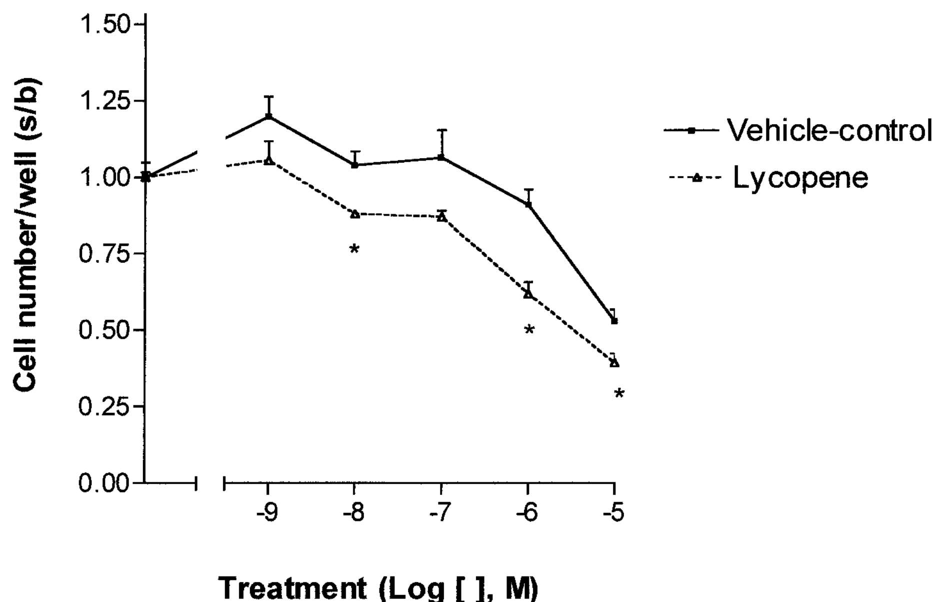

FIG. 3. Dose-response effect of lycopene on LNCaP cell number after 72 hours of incubation. After allowing LNCaP cells to stabilize for 48 hours, cells were treated with lycopene concentrations of 10^{-9} to 10^{-5} M and incubated for 72 hours. Cells were then collected and counted using a hemacytometer. Values are means ± SEM, n = 6. *, $P < .05$, significantly different from vehicle-control.

decrease cell numbers by 42.8% at 10^{-6} M and 30.8% at 10^{-5} M ($P < .05$), and lycopene also dose-dependently inhibited LNCaP cell proliferation (n = 6, F = 297.5, $P < .0001$; Fig. 4). The inhibitory effect of lycopene at each of these concentrations was significantly greater than that of the respective vehicle control ($P < .05$). Cell growth was completely inhibited with 10^{-4} M lycopene concentrations at all incubation periods (data not shown).

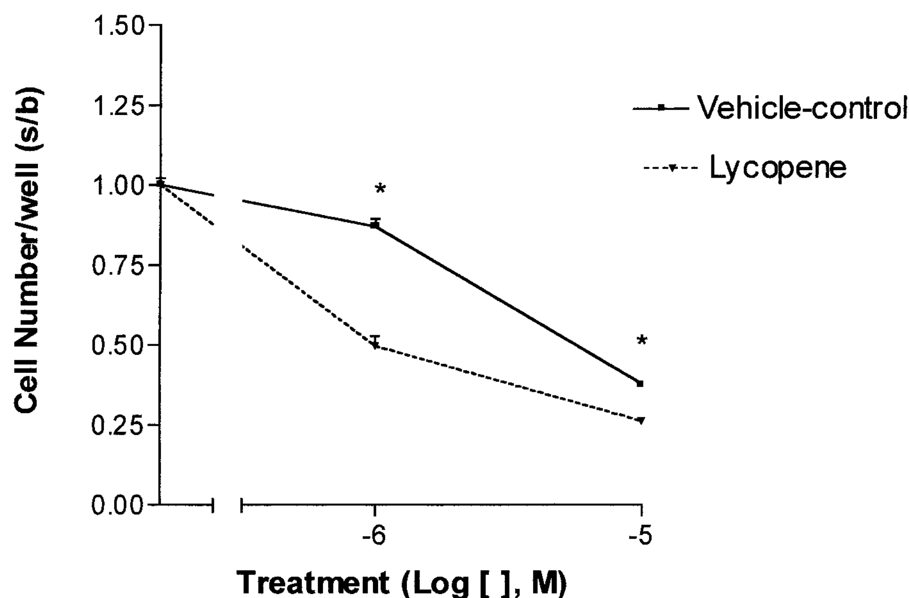

FIG. 4. Dose-response effect of lycopene on LNCaP cell number after 96 hours of incubation. After allowing LNCaP cells to stabilize for 48 hours, cells were treated with lycopene concentrations of 10^{-6} and 10^{-5} M and incubated for 96 hours. Cells were then collected and counted using a hemacytometer. Values are means ± SEM, n = 6. *, $P < .05$, significantly different from vehicle-control.

DISCUSSION

Prostate cancer has become an important public health problem in the Western world. Recent interest in lycopene has focused on its antioxidant properties and its association with decreased risk of chronic diseases such as cancer and cardiovascular diseases. Lycopene has been found to be concentrated in the prostate and other body tissues such as liver, adrenals, and adipose tissue.[24,28] The presence of lycopene in the prostate[24] suggests the hypothesis that lycopene may have direct effects within the prostate and may contribute to the reduced prostate cancer risk observed in individuals who consume high amounts of tomato-based, lycopene-rich foods.[24,26]

The results from this experiment provide evidence that lycopene can inhibit the growth of human prostate cancer cells and that the inhibition depends on the concentration as well as the duration of exposure to lycopene. In this study, when cells were exposed to lycopene for extended periods, cell growth and viability were significantly decreased. The effect of lycopene was dose-dependent and was greater at the higher concentrations of 10^{-5} and 10^{-6} M. With lycopene concentrations of 10^{-4} M, LNCaP cell proliferation was completely stopped at all incubation periods (data not shown). However, this effect was also seen when cells were treated with the vehicle-control at the same lycopene concentration. It is possible that at this concentration the effects of lycopene and those of certain components present in the vehicle are indistinguishable.

Although the exact mechanism by which lycopene reduces the growth of human prostate cancer cells is not well understood, the decrease in LNCaP cell proliferation by lycopene may in part be due to direct effects of lycopene on cellular processes controlling cell growth and protection against oxidative damage caused by ROS. ROS are thought to be involved in stimulating the activity of cell-cycling genes and, in particular, in enhancing cancer cell proliferation. In turn, there is also evidence that cancer cells produce more ROS, therefore providing for a greater continuous proliferation of cancer cells.[29] In addition, because ROS react with the cell membrane, they are likely to cause damage to normal, healthy cells, resulting in cell death. This leads to an environment in which cancer cells proliferate without competition, as they may acquire resistance to the effect of ROS. Lycopene is able to capture the free radicals produced by ROS and thereby reduce the effects caused by them. However, hypothesized mechanisms, specifically related to the reduction of prostate cancer cell growth, are limited and remain speculative. Therefore, additional studies are needed to determine the mechanism of action of lycopene on LNCaP cells.

Lycopene, which is present in high amount in tomatoes and tomato-derived products, has been shown in several *in vitro* culture studies to have a growth inhibitory effect on mammary, lung, and endometrial cancer cell proliferation.[27] Additional roles of lycopene, such as the induction of gap-junction communication between cells through the increased synthesis of connexin 43, were found.[31] This effect was associated with the ability of lycopene to restore the loss of gap junctions that occurs in malignant processes. To date, much of the tissue culture research involving the anticarcinogenic effects of lycopene has investigated various forms of cancer other than prostate. One *in vitro* study demonstrated a strong inhibitory effect on prostate carcinoma PC-3 cell proliferation resulting from the simultaneous addition of lycopene and α-tocopherol at physiological concentrations.[32] A more recent study by Xu et al.[33] reported that lycopene contained in micelles was taken up by LNCaP cells and was stable for up to 96 hours under standard cell culture conditions. Neither the micelles themselves nor lycopene solubilized in micelles at concentrations up to 10 μg/ml in the cell culture medium produced inhibition of cell proliferation.

Due to its extreme hydrophobicity, lycopene is insoluble in tissue culture media. As well, lycopene is highly unstable and degrades rapidly when dissolved in organic solvents or aqueous systems containing organic cosolvents and when exposed to light or air. This study was the first to use a water-dispersible form of lycopene as a convenient method for delivering lycopene to the cells.

In conclusion, the observed results suggest that the effect of lycopene could be due to its

antioxidant properties, which are thought to be responsible for the inhibition of human prostate LNCaP cancer cell growth. These results propose possible implications with respect to the use of lycopene in the chemoprevention of human prostate cancer.

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Address reprint requests to:
A. Venket Rao
Department of Nutritional Sciences
Faculty of Medicine
150 College Street
Toronto, Ontario
Canada M5S 3E2

E-mail: v.rao@utoronto.ca