LETTERS TO THE EDITOR

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EFFECT OF LYCOPENE ON IGF-I, IGF BINDING PROTEIN-3 AND IGF TYPE-I RECEPTOR IN PROSTATE CANCER CELLS

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SUMMARY

Prostate cancer is the second most common cancer that leads to death in elderly men. The risk of prostate cancer prevalence is often associated with elevated level of insulin-like growth factor I (IGF-I) and decreased level of IGF-binding protein 3 (IGFBP-3). Lycopene, a carotenoid, reduces the proliferation of cancer cells and, thus, induces apoptosis. Hence, higher intake of lycopene can be associated with a lower risk of prostate cancer. However, the mechanism of action of lycopene in prostate cancer is still unclear. The present study was carried out to find the effects of lycopene on the components of IGF system and apoptosis in an androgen-independent prostate cancer cell (PC-3). PC-3 cells were treated with various concentrations of lycopene, 20, 40 and 60 μM, each for 24, 48, 72 and 96 h. IGF-I, IGFBP-3 and IGF-I receptor (IGF-IR) levels and indication of apoptosis were evaluated. The proliferation of lycopene-treated PC-3 cells decreased significantly. At a concentration of 40 μM lycopene treatment significantly increased the level of IGFBP-3. Lycopene-induced apoptosis was indicated in annexin V and PI binding. Lycopene-induced DNA fragmentation was not detectable after 24 h after the treatment whereas the same was observed after 48 h treatment. There was a significant decrease in the IGF-IR expression after the cells were treated with lycopene and IGF-I. The data obtained suggest that the components of the IGF system may act as a positive regulator for lycopene-induced apoptosis in PC-3 cells. The results obtained are encouraging and may lead to the development of lycopene as a potential therapy for prostate cancer.

Keywords : Lycopene, IGFBP-3, IGF-I, IGF-IR, Prostate cancer

INTRODUCTION

Lycopene of tomato is the most abundant naturally occurring carotenoid, which among other major carotenoids, has the capacity to quench singlet oxygen and scavenge free radicals (1). Epidemiological data suggest that the consumption of fresh and/or processed tomato products is associated with a reduced risk of prostate cancer, and the study on the protective health benefits of tomatoes is a rewarding area of prostate cancer research (2, 3).

Insulin-like growth factor I (IGF-I) has both mitogenic and anti-apoptotic effects on the epithelial cells of prostate. A higher circulating level of IGF-I has been reported in prostate cancer patients (4, 5). IGF-I significantly increases the proliferation in PC-3 cells (5). IGF-binding protein 3 (IGFBP-3) is one of the six members of the IGFBP family and a major circulating protein in human plasma. The mitogenic effect of IGF-I is mediated *via* the IGF-I receptor (IGF-IR) and is modulated by specific binding proteins. The IGFBPs may either inhibit or potentiate IGF action (6). IGFBP-3, one of the binding proteins of IGFs, has been demonstrated to be a negative regulator of cell proliferation in prostatic and other tissues (7). In this study we investigated the effect of lycopene on IGFBP-3, IGF-IR protein expression and apoptosis in PC-3 cells.

MATERIALS AND METHODS

The cell proliferation was quantified using MTT assay, DNA synthesis was determined adopting [^{3}H] thymidine uptake. The levels of IGF-I and IGFBP-3 were determined adopting immuno-radiometric assay. IGF-IR and IGFBP-3 protein expressions were quantified adopting Western blot analysis. Apoptosis was quantified adopting annexin V and propidium iodide (PI) staining followed by flow cytometry.

RESULTS

Cell proliferation studies

Lycopene-treated PC-3 cells were observed in a microscope. The cultured PC-3 cells are shown in the Figure 1. Increased number of dead cells was observed among lycopene-treated PC-3 cells. Combined treatment of lycopene and IGF-I produced killing of cells more than with lycopene only. However, the assay depends on the functions of the mitochondria. The chemical reduction of MTT dye decreased significantly (25–30%) in 20 μM lycopene-treated cells after incubation for 72 and 96 h. In 40 μM lycopene treatment group, the cell viability was significantly ($P < 0.05$) decreased at 48 h (28%), 72 h (32%) and 96 h (33%). The maximum reduction in the viability (32–35%) was observed in 60 μM lycopene treatment group after 48, 72 and 96 h (Fig. 2). IGF-I significantly increased ($P < 0.05$) cell proliferation, whereas

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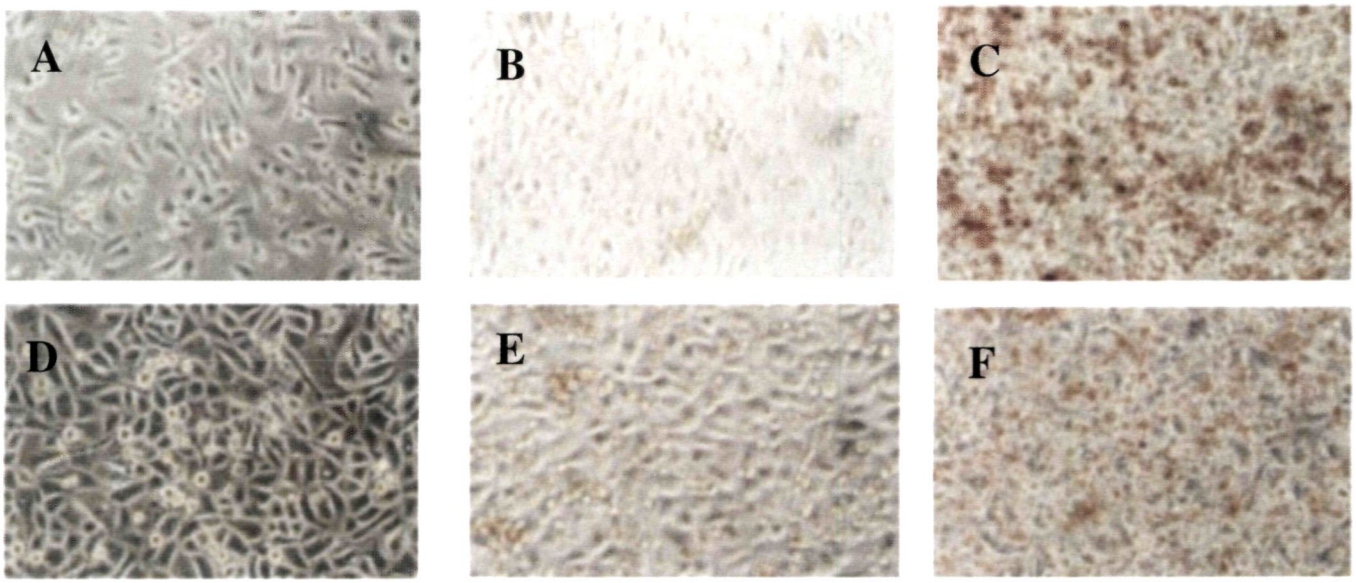


Fig. 1. Photomicrograph of PC-3 cells incubated for 48 h, visualized and photographed (magnification 10) using Nikon inverted phase contrast microscope. **(A)** Control cells treated with THF vehicle; **(B)** 40 μM lycopene **(C)** 60 μM lycopene; **(D)** 50 ng/ml of IGF-I; **(E)** 40 μM lycopene and 50 ng/ml of IGF-I; **(F)** 60 μM lycopene and 50 ng/ml of IGF-I

lycopene significantly ($P < 0.05$) brought down IGF-I- induced proliferation in PC-3 cells (Fig. 3). To further understand the effect of lycopene on IGF-I-induced cell proliferation, [^{3}H] thymidine incorporation was carried out to assess DNA synthesis. Different doses of IGF-I (10, 50 and 100 ng/ml) were administered to PC-3 cells (Fig. 4) and the effective dose was found to be 50ng/ml. It was observed that in the presence of IGF-I, lycopene inhibited the IGF-I-induced proliferation in PC-3 cells.

IGF-I and IGFBP-3

There was no difference in the levels of IGF-I in the culture media of PC-3 cells when treated with different concentrations of lycopene (Fig. 5). Lycopene treatment significantly ($P < 0.05$) increased (1.5- to 2-fold) IGFBP-3 levels both in the conditioned media (Fig. 6) and at the cellular level (Fig. 7). IGF-I (50ng/ml) treatment significantly ($P < 0.05$) decreased (2-fold) the level of IGFBP-3 in the conditioned medium. Co-treatment with lycopene and IGF-I resulted in increased levels of IGFBP-3 in the culture media.

Protein expression of IGF-IR

Lycopene (40 and 60 μM) treatment did not alter the expression of IGF-IR (Fig. 7). IGF-I (50ng/ml) treatment for 48 h significantly ($P < 0.05$) increased the expression of IGF-IR in PC-3 cells. The combined treatment of lycopene and IGF-I significantly ($P < 0.05$) decreased the level of IGF-IR in PC-3 cells when compared with the treatment of IGF-I alone.

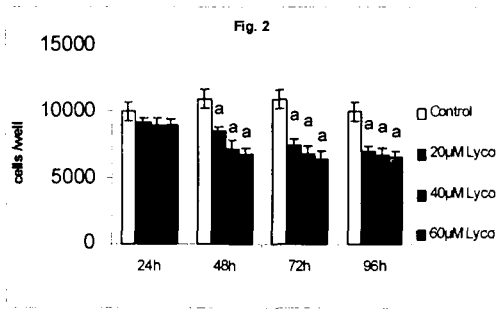
Annexin V binding and PI influx

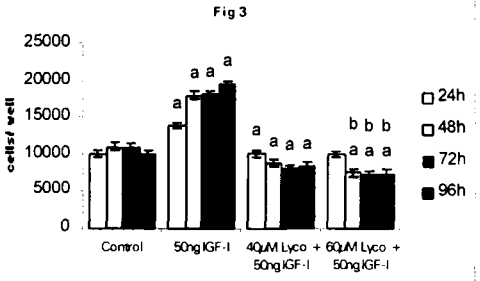
The annexin binding studies revealed that 20 μM lycopene treatment group showed 22% early apoptotic cells and 13% late apoptotic cells at 24 h (Fig. 8a). PI uptake was not altered in 20 μM lycopene-treated cells after 24 h. This indicates that on 20 μM lycopene treatment, the cells significantly increased ($P < 0.05$) early apoptosis (Fig. 8b). At 40 and 60 μM concentrations, lycopene treatment significantly increased early as well as late apoptosis in PC-3 cells (Fig. 8a and b). The time-dependent effect of lycopene in the 48 h treatment group significantly (32–35%) induced early apoptosis in 20 and 40 μM (Fig. 8c). The 60 μM lycopene-treated cells showed a significant number of early (35%) and late apoptotic cells (26%) (Fig. 8d) Annexin V staining of the cells revealed that the treatment of PC-3 cells with lycopene (20, 40 and 60 μM) for 24 and 48 h resulted in an increase in the percentage of apoptotic cells when compared with the control.

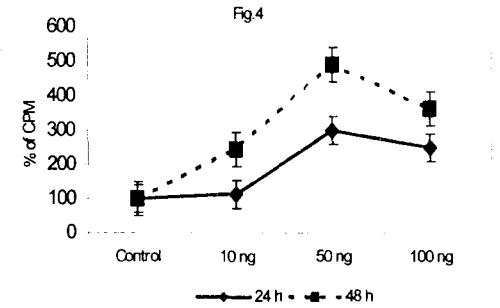
DISCUSSION

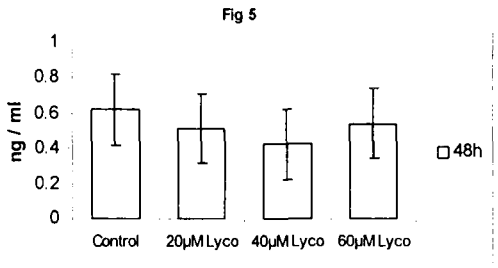
According to the previous studies, lycopene inhibits the proliferation of prostate cancer cells (8, 9). In the present study, we confirm the anti-proliferative effect of lycopene on PC-3 cells. In this study, lycopene (20, 40 and 60 μM) reduced the cell growth up to 25–35% in 48, 72 and 96 h treatment groups in a dose- and time-dependent manner. Lycopene significantly reduced the mitochondrial function through oxidative damage as evidenced (10) and it also reduced the proliferation of human hepatoma cells, which was measured by using MTT.

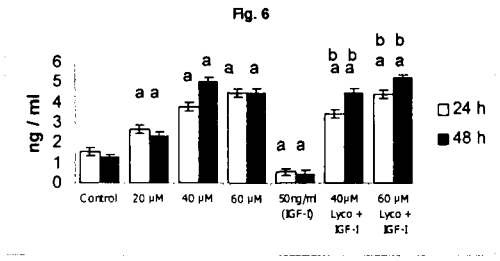
In accordance with Karas et al (11) the present findings demonstrate that lycopene is capable of inducing apoptosis after 48 h in a dose-dependent manner. Furthermore, IGF-I is a potent mitogenic growth factor and induces cell proliferation (12). Here, we have tested the role of lycopene on IGF-I-induced cell proliferation.

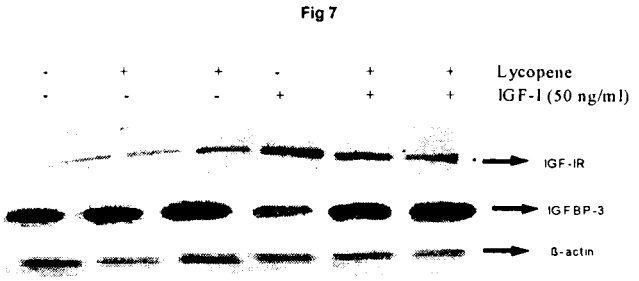












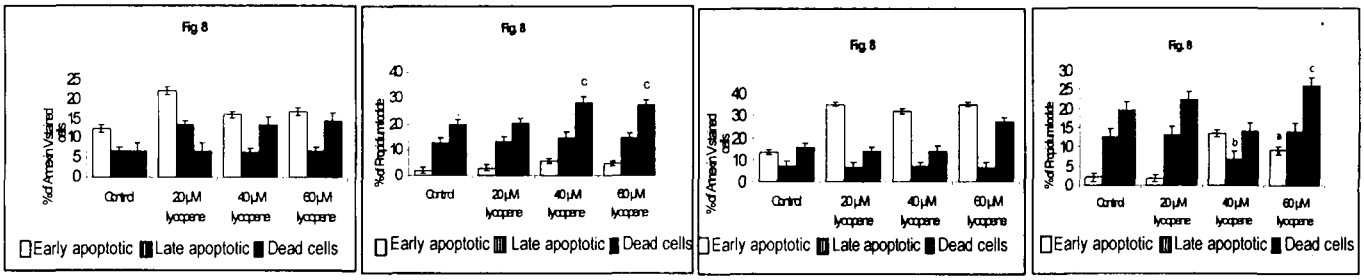


Fig. 2. Viability of PC -3 cells (MTT assay) incubated with lycopene alone for 24, 48, 72 and 96 h. Each bar represents the number of dead cells as mean ± SEM of three experiments. **Fig. 3.** MTT assay with PC-3 cells that were incubated for 24, 48, 72 and 96 h with lycopene and IGF-I (50 ng/ml). **Fig. 4.** Effect of IGF-I on the proliferation of PC-3 cells. IGF-I (10, 50 and 100 ng/ml) treated for 24 and 48 h. **Fig. 5.** Effect of lycopene on the secretory level of IGF-I in PC-3 cells incubated for 48 h with medium alone or with lycopene (20, 40 and 60 µM). **Fig. 6.** Effect of lycopene and IGF-I on the secretory level of IGFBP-3 in PC-3 cells incubated for 48 h with medium alone or with lycopene/IGF-I (50 ng/ml). Bars with different letters ('a', comparison between control and lycopene-treated cells; 'b', comparison between IGF-I and lycopene-treated cells) denote significance ($P < 0.05$). **Fig. 7.** Effect of lycopene on the expression of IGF-IR and IGFBP-3 in PC-3 cells. After 48 h exposure of lycopene (40 and 60 µM)/IGF-I (50 ng/ml), the cells were lysed for Western blot analysis of IGF-IR and IGFBP-3. Equal loading was confirmed with beta-actin. **Fig. 8.** Effect of lycopene on PC-3 cells on early or late apoptosis detected using annexin V. Graphs show the percentage of early and late apoptotic cells. PC-3 cells were incubated with 60 µM of lycopene for 48 h. **(a)** cells stained with annexin for 24 h; **(b)** cells stained with PI for 24 h; **(c)** cells stained with annexin V for 48 h; and **(d)** cells stained with PI for 48 h. Data represent mean ± SEM of three independent experiments and indicate the percentage of cells staining positively for annexin V or PI. 'a' indicates significance ($P < 0.05$) between control and lycopene-treated early apoptotic cells; 'b' indicates significance ($P < 0.05$) between control and lycopene-treated late apoptotic cells; and 'c' indicates dead cells.

The autocrine/paracrine action of IGF-I can increase cell turnover and susceptibility of malignant cells. It has been reported previously that IGF-I inhibits programmed cell death (13, 14). Lycopene treatment (20, 40 and 60 µM) for 48 h significantly increased the accumulation of IGFBP-3 protein in conditioned media of PC-3 cells. The rate of apoptosis was also observed in a duration- and dose-dependent manner. Lycopene up-regulated IGFBP-3 plasma concentration in cigarette smoke-exposed ferrets (15).

The present study demonstrates that lycopene is capable of inducing IGFBP-3 secretion, and that IGFBP-3 also reduces the amount of available ligand and IGF-I for the interaction with IGF-IR. Thus, it appears that the antiproliferative action of lycopene in androgen-independent prostate cancer cells (PC-3) results in increased IGFBP-3 production and induction of apoptosis. These results suggest that lycopene inhibits the expression of IGF-IR, increases the levels of IGFBP-3 and induces apoptosis in PC-3 cells. Treatment with lycopene showed no change in the expression of IGF-IR, but it inhibits the IGF-I-induced IGF-IR expression. More data in this context have been generated and are analyzed. The outcome will be published separately.

In conclusion, the present study demonstrates that lycopene is capable of modulating growth inhibition of prostate cancer cells *in vitro* and in inducing apoptosis. The present findings also suggest that lycopene-induced apoptosis could be mediated by the down-regulation of IGF-IR and up-regulation of IGFBP-3 in PC-3 cells. Hence, our findings have raised the possibility that IGFBP-3 may be the positive regulator for lycopene-induced apoptosis and it may have some therapeutic value for prostate cancer treatment.

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