

Effects of Lycopene Supplementation in Patients with Localized Prostate Cancer

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Epidemiological studies have shown an inverse association between dietary intake of lycopene and prostate cancer risk. We conducted a clinical trial to investigate the biological and clinical effects of lycopene supplementation in patients with localized prostate cancer. Twenty-six men with newly diagnosed prostate cancer were randomly assigned to receive a tomato oleoresin extract containing 30 mg of lycopene ($n = 15$) or no supplementation ($n = 11$) for 3 weeks before radical prostatectomy. Biomarkers of cell proliferation and apoptosis were assessed by Western blot analysis in benign and cancerous prostate tissues. Oxidative stress was assessed by measuring the peripheral blood lymphocyte DNA oxidation product 5-hydroxymethyl-deoxyuridine (5-OH-mdU). Usual dietary intake of nutrients was assessed by a food frequency questionnaire at baseline. Prostatectomy specimens were evaluated for pathologic stage, Gleason score, volume of cancer, and extent of high-grade prostatic intraepithelial neoplasia. Plasma levels of lycopene, insulin-like growth factor-1, insulin-like growth factor binding protein-3, and prostate-specific antigen were measured at baseline and after 3 weeks of supplementation or observation. After intervention, subjects in the intervention group had smaller tumors (80% vs 45%, less than 4 ml), less involvement of surgical margins and/or extra-prostatic tissues with cancer (73% vs 18%, organ-confined disease), and less diffuse involvement of the prostate by high-grade prostatic intraepithelial neoplasia (33% vs 0%, focal involvement) compared with subjects in the control group. Mean plasma prostate-specific antigen levels were lower in the intervention group compared with the control group. This pilot study suggests that lycopene may have beneficial effects in prostate cancer. Larger clinical trials are warranted to investigate the potential preventive and/or therapeutic role of lycopene in prostate cancer. *Exp Biol Med* 227:881-885, 2002

Key words: lycopene; prostate cancer; chemoprevention; treatment; connexin; tomato carotenoids

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Epidemiological studies have shown an inverse association between dietary intake of lycopene and prostate cancer risk (1). Possible mechanisms by which lycopene may prevent cancer include 1) inhibition of growth and induction of differentiation in prostate cancer cells (2-6); 2) upregulation of tumor suppressor protein Cx43 and increased gap junctional intercellular communication (7-11); and 3) prevention of oxidative DNA damage (12, 13).

Lycopene increases gap-junctional intercellular communication by increasing expression of gap junctional gene, connexin 43 (7-9). This action correlates strongly with the ability of lycopene and other carotenoids to suppress neoplastic transformation in model cell culture systems (9). This action of carotenoids has been proposed to have mechanistic significance by enabling the transfer of growth-regulatory signals between normal growth-inhibited cells and pre-neoplastic cells. Indeed, when neoplastic cells were forced into junctional communication with quiescent normal cells, the neoplastic cells became growth arrested in direct proportion to their extent of junctional communication (14). Progressive decreases with disease severity in the expression of Cx43 have been reported in the human prostate (15), and there is evidence in prostatic carcinoma cell lines that some of this loss of junctional communication may result from defects in assembly of Cx43 protein into gap junctions (16). When functional communication was restored in a human prostatic carcinoma cell line, cells had more normal differentiation, reduced proliferation, and suppressed tumorigenicity (17).

Insulin-like growth factors (IGFs) have mitogenic and antiapoptotic effects on normal and transformed prostate epithelial cells (18-20). IGF-1 is an important mitogen for prostate cells. Insulin-like growth factor binding proteins (IGFBPs) have opposing actions, in part by binding IGF-1 but also by direct inhibitory effects on target cells (18). In recent epidemiological studies, relatively high plasma

IGF-1 and low IGFBP-3 levels were independently associated with greater risk of prostate cancer (21–25). Two- to 4-fold elevated risks have been observed for prostate cancer in men in the top quartile of IGF-1 relative to those in the bottom quartile, and low levels of IGFBP-3 were associated with an approximate doubling of risk (21).

Despite the inverse association between lycopene intake and prostate cancer observed in epidemiological studies, no clinical intervention studies have previously been reported showing the effect of lycopene supplements in men with prostate cancer. We conducted a pilot study investigating the effect of lycopene supplementation on the prostate tissues and on serum levels of prostate-specific antigen (PSA), IGF-1, and IGFBP-3 in patients with localized prostate cancer. We hypothesized that lycopene supplementation would decrease growth and induce apoptosis in pre-malignant and malignant prostate cells by up-regulating Cx43, downregulating IGF-1 and decreasing the ratio of bcl-2/bax in patients with localized prostate cancer.

Clinical Trial of Lycopene in Prostate Cancer

We conducted a randomized, two-arm clinical intervention study in 35 patients with clinical stages T1 or T2 prostate cancer who were scheduled to undergo radical prostatectomy. Nine patients were excluded from analysis because they had incorrect diagnosis (one patient), or dropped out after randomization (two patients) or had previous hormone therapy (six patients). Data were collected from 26 eligible patients who were randomly assigned to the lycopene arm ($n = 15$) or the control arm ($n = 11$) of the study. Detailed description of the methodology and the results of this trial have been published elsewhere (26).

Subjects were randomly assigned to either lycopene supplementation or no intervention for 3 weeks before surgery. Blood samples were collected and food frequency questionnaires were obtained at baseline. Another blood sample was obtained for biomarker studies after 3 weeks of intervention before radical prostatectomy. Entire prostate glands were resected and specimens were evaluated for pathologic stage, Gleason score, the volume of prostate cancer as well as the extent of high-grade prostatic intraepithelial neoplasia (HGPIN). Tissue levels of Cx43, bcl-2, and bax were assessed by Western blotting in benign and malignant tissue samples. Plasma and tissue levels of carotenoids were measured by HPLC. Plasma levels of IGF-1 and IGFBP-3 were measured by enzyme-linked immunosorbent assay. Peripheral blood lymphocyte levels of 5-hydroxymethyl-deoxyuridine (5-OhmdU) were measured by gas chromatography-mass spectrometry.

Subjects randomized to the intervention arm were asked to take a tomato oleoresin extract containing 15 mg of lycopene (Lyc-O-Mato®, Lycoré Natural Products Industries, Beer-Sheva, Israel) twice daily. Subjects randomized to the control arm were asked to continue their regular diet

and were given the NCI recommendations to increase daily fruit and vegetable intake to five servings a day.

After 3 weeks of intervention or no intervention, all subjects underwent radical prostatectomy with removal of the entire prostate gland, seminal vesicals, and surrounding soft tissues. Fresh tissue samples were obtained with the exact anatomic source of each sample clearly indicated on a specimen diagram for subsequent microscopic confirmation. In cases where gross identification of the tumor was difficult to establish, a frozen section slide of the suspected area was generated and stained with hematoxylin and eosin to guide the acquisition protocols. When possible, 1 g of tissue from benign areas of the gland was kept frozen for lycopene analysis. Appropriate tissue samples were taken for Western blotting and for histologic examination. Tissue samples obtained from benign and malignant parts of the glands were stored at -70°C until biomarker studies were performed. The specimens were then entirely embedded in paraffin, step-sectioned, and microscopically examined by the study pathologists.

Changes in clinical parameters are shown in Table I. Mean plasma PSA levels decreased by 18% in the intervention group whereas they increased by 14% in the control group over the study period ($P = 0.22$). In the intervention group, 11 of 15 patients (73%) had involvement of surgical margins and/or extra-prostatic tissues with cancer, compared with 2 of 11 patients (18%) in the control group ($P = 0.02$). Twelve of 15 patients (80%) in the lycopene group had tumors that measured 4 ml or less compared with 5 of 11 (45%) in the control group ($P = 0.22$). Multifocal and/or diffuse involvement by HGPIN was observed in 10 of 15 subjects (67%) in the lycopene group compared with all 11 subjects (100%) in the control group ($P = 0.05$).

Sufficient malignant tissues were available for analysis in four subjects from the lycopene group and in four subjects from the control group. The level of Cx43 protein was 0.63 ± 0.19 optical density (OD) units in the lycopene group compared with the 0.25 ± 0.08 OD units in the control group ($P = 0.13$). The expression of cell cycle regulatory proteins, bcl-2 and bax, were not significantly different between the two groups, although bax level of the lycopene group (1.05 ± 0.29) was higher than the control group (0.68 ± 0.18).

Tissue samples from benign parts of the gland were available for biomarker analysis in eight subjects in the intervention group and six subjects in the control group. Cx43 level was 0.64 ± 0.12 in the lycopene group compared with 0.51 ± 0.10 in the control group. The expression of bcl-2 was 0.63 ± 0.04 in the intervention group and 0.58 ± 0.04 in the control group, and the expression of bax was 0.62 ± 0.10 in the intervention group and 0.79 ± 0.11 in the control group. None of the differences in the biomarkers of the two groups were statistically significant.

Plasma samples were available from 13 subjects in the intervention group and 10 subjects in the control group.

Table I. Change in Clinical Parameters in Intervention ($n = 15$) and Control ($n = 11$) Groups

| | Intervention | Control | <i>P</i> value |
|---|-----------------|-----------------|-------------------|
| PSA level (mean \pm SE, ng/ml) ^a | | | |
| Pre-intervention | 6.89 \pm 0.81 | 6.74 \pm 0.88 | |
| Post-intervention | 5.64 \pm 0.87 | 7.65 \pm 1.78 | 0.25 ^b |
| High-grade PIN (n) | | | |
| Focal | 5 | 0 | |
| Multifocal/diffuse | 10 | 11 | 0.05 |
| Gleason score | | | |
| 6 \leq | 7 | 4 | |
| >6 | 8 | 7 | 0.70 |
| Tumor volume (cm ³) | | | |
| 4 \leq | 12 | 5 | |
| >4 | 3 | 6 | 0.22 |
| Surgical stage (n) | | | |
| Confined to prostate | 11 | 2 | |
| Not confined to prostate ^c | 4 | 9 | 0.02 |

^a SE denotes standard error.^b *P* value is for comparing the change from pre- to post-intervention PSA in the two groups.^c Resection margins are positive and/or extra-prostatic invasion is present.

Mean plasma levels of IGF-1 decreased by from 233 ± 21 ng/ml to 169 ± 23 ng/ml in the lycopene group ($P = 0.0002$) and from 199 ± 20 ng/ml to 140 ± 16 ng/ml in the control group ($P = 0.0003$). Interestingly, IGFBP-3 levels also decreased in both intervention and control groups during the study period. Plasma IGFBP-3 levels of intervention group decreased from 5230 ng/ml to 3924 ng/ml and control group decreased from 5200 ng/ml to 4070 ng/ml, which were statistically significant ($P = 0.0002$ and $P = 0.0001$, respectively).

In the intervention group, plasma lycopene level increased in 5 of 11 patients, whereas only one of six subjects in the control group had an increase (Fisher's exact test, $P = 0.33$). The level of post-intervention plasma lycopene was $23.5 \mu\text{g}/\text{dl}$ in the intervention group and $17.5 \mu\text{g}/\text{dl}$ in the control group ($P = 0.15$). However, there was no significant difference between the two groups with regard to percent change of plasma lycopene level because of great variability in plasma lycopene levels and small numbers of subjects in each group. Prostatic tissue lycopene levels were 47% higher in the intervention group ($0.53 \pm 0.03 \text{ ng/g}$ of prostate tissue) compared with the control group (0.36 ± 0.06), which was a significant difference ($P = 0.02$) despite the small number of samples ($n = 8$).

Peripheral blood lymphocyte levels of 5-OHmdU were similar in both groups before and after intervention. There were no differences between the groups with respect to baseline intake of nutrients assessed by food frequency questionnaire.

Discussion and Conclusions

The results suggest that 30 mg of lycopene taken daily for 3 weeks may be sufficient to modulate prostate cancer. The microscopic extension of prostate cancer to surgical margins and/or to extra-prostatic tissues appeared to have

decreased as a result of lycopene supplementation. This finding has potential clinical implications as extension of tumor to surgical margins identifies a group of patients with poor prognosis. Patients in the lycopene group had a decrease in the plasma PSA level, which is a clinical parameter of prostate cancer burden. These results suggest that lycopene may have an antitumor effect and perhaps be useful as an adjunct to standard treatments of prostate cancer, such as surgery, radiation therapy, hormones and chemotherapy. In addition, lycopene supplementation appears to have reduced the diffuse involvement of the prostate gland with HGPIN, which is a precursor of prostate cancer (27), suggesting that lycopene may have a role in the prevention of prostate cancer.

The mechanism of the clinical effects of lycopene remains to be elucidated. Upregulation of Cx43 expression would be a potential explanation. However, although there was an increase in the expression of Cx43 in tumor tissue in the intervention group, it did not reach statistical significance, perhaps because of the small number of subjects. When the results from all 35 randomized subjects (including the nine subjects who were excluded from analysis because of protocol violation) are analyzed, Cx43 expression was significantly higher in the tumors from patients in the lycopene group ($P < 0.05$). Increased expression of Cx43 and increased junctional communication have previously been shown to occur after treatment of human and murine cells in culture with lycopene (8). Upregulated junctional communication has been linked to decreased proliferation in normal and pre-neoplastic cells (28). Therefore, our results suggest that lycopene supplementation may decrease the growth of prostate cancer, perhaps by upregulating Cx43. However, because of small sample size, no definitive conclusions can be reached. Clearly, larger clinical trials are needed to determine the efficacy as well as the appro-

Table II. Composition of Lyc-O-Mato® Capsules

| Component | Percentage (w:w) in 250 mg Lyc-O-Mato® capsule |
|------------------|--|
| Carotenoids | |
| Lycopene | 5.8–6.2 |
| Phytoene | 0.5–0.7 |
| Phytofluene | 0.5–0.6 |
| β-carotene | 0.1–0.2 |
| Total | 6.9–7.7 |
| Other components | |
| Tocopherols | 1.5–2.5 |
| Phospholipids | 14–16 |
| Phytosterols | 0.5–0.7 |
| Tomato oil | 73–76 |

priate dose and duration of lycopene supplementation in men with prostate cancer or high risk of developing prostate cancer.

It should be noted that the lycopene preparation that was used in this study was a mixture of tomato carotenoids and other tomato phytochemicals (Table II). Although lycopene was the predominant carotenoid in the capsules, there were significant amounts of phytoene and phytofluene and other bioactive compounds. It is possible that the combination of the phytochemicals present in the tomato extract was responsible for the observed clinical effects rather than lycopene alone. There are *in vitro* data suggesting synergistic effects of lycopene with phytoene, phytofluene and beta-carotene against prostate cancer cells (personal communication, Yoav Sharoni and Yossi Levy).

The differences observed in bioavailability and response to Lycomato preparation in this study are not easily explained because the preparation contains the natural tomato oleoresin present in tomato matrix in the Lyc-O-Mato® capsules used in this study. Previous studies have shown excellent bioavailability of lycopene from this preparation (29). However, it is possible that the fat and other nutrients in the diet might have influenced the bioavailability of lycopene in our study population.

Future Directions

Dose-response to lycopene should be investigated in subjects with localized and advanced prostate cancer. Clinical trials should be conducted in patients with HGPIN or elevated PSA but without a diagnosis of prostate cancer as they are at a high risk of developing prostate cancer or having occult disease. Lycopene could be compared with other promising agents such as vitamin E, selenium or soy in future clinical trials. We have found significant *in vitro* (30, 31) and clinical activity with soy isoflavones (32). We are currently conducting clinical trials investigating the effects of lycopene alone or in combination with soy isoflavones in patients with advanced prostate cancer. We plan to investigate the effects of lycopene alone and in combination with other tomato carotenoids in patients with prostate can-

cer. Lycopene should also be combined with vitamin E (33) and selenium (34) in future clinical trials.

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