ELSEVIER

PHASE I-II PROSPECTIVE DOSE-ESCALATING TRIAL OF LYCOPENE IN PATIENTS WITH BIOCHEMICAL RELAPSE OF PROSTATE CANCER AFTER DEFINITIVE LOCAL THERAPY

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ABSTRACT

Objectives. To report a prospective trial of lycopene supplementation in biochemically relapsed prostate cancer.

Methods. A total of 36 men with biochemically relapsed prostate cancer were enrolled in a dose-escalating, Phase I-II trial of lycopene supplementation. Six consecutive cohorts of 6 patients each received daily supplementation with 15, 30, 45, 60, 90, and 120 mg/day for 1 year. The serum levels of prostate-specific antigen (PSA) and plasma levels of lycopene were measured at baseline and every 3 months. The primary endpoints were PSA response (defined as a 50% decrease in serum PSA from baseline), pharmacokinetics, and the toxicity/tolerability of this regimen.

Results. A total of 36 patients were enrolled. The median age was 74 years (range 56 to 83), with a median serum PSA at entry of 4.4 ng/mL (range 0.8 to 24.9). No serum PSA responses were observed, and 37% of patients had PSA progression. The median time to progression was not reached. Toxicity was mild, with 1 patient discontinuing therapy because of diarrhea. Significant elevations of plasma lycopene were noted at 3 months and then appeared to plateau for all six dose levels. The plasma levels for doses between 15 and 90 mg/day were similar, with additional elevation only at 120 mg/day.

Conclusions. Lycopene supplementation in men with biochemically relapsed prostate cancer is safe and well tolerated. The plasma levels of lycopene were similar for a wide dose range (15 to 90 mg/day) and plateaued by 3 months. Lycopene supplementation at the doses used in this study did not result in any discernible response in serum PSA. UROLOGY **67:** 1257–1261, 2006.

Prostate cancer is the most common noncutaneous malignancy in men in the United States.[1] In a steadily growing number of men, local therapy has failed as determined by the detection of elevated serum prostate-specific antigen (PSA) levels without evidence of clinical metastatic disease. The optimal therapy for this growing population of men is not known.[2–4] As the morbidity of long-term androgen deprivation therapy is better appreciated, the search for a less toxic therapy for this population is gaining importance.

Numerous epidemiologic studies have suggested that a diet rich in tomatoes and tomato products protects against the development of prostate cancer.[5–7] Tomatoes are the main source of dietary lycopene.[6] Lycopene is the predominant carotenoid in tomatoes and is responsible for their red color.[8] It is also the predominant carotenoid in human serum. It is a popular and commonly used supplement for prostatic disease, with no known toxicity at the commonly used doses of 5 to 15 mg/day. Studies have found a link between greater plasma lycopene levels and a lower risk of developing prostate cancer.[5,9] Several small prospective trials have suggested a potential therapeutic use of lycopene in both a neoadjuvant setting before rad-

This study was supported in part by National Institutes of Health grant P30-CA12197-2751.

Presented at the 2005 Meeting of the American Urological Association, San Antonio, Texas, May 21–26, 2005.

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Submitted: October 4, 2005, accepted (with revisions): December 15, 2005

0090-4295/06/$32.00
doi:10.1016/j.urology.2005.12.035

ical prostatectomy, as well as in advanced disease either at the initiation of hormonal ablation therapy or in the setting of hormone-refractory disease.[10–12] Very little published information is available regarding the pharmacokinetics of lycopene supplementation in humans or the optimal dose for use in clinical trials. We report a Phase I-II dose escalating trial of lycopene in patients with biochemical relapse after definitive local therapy for prostate adenocarcinoma. This is the first trial to prospectively test the clinical efficacy, pharmacokinetics, and tolerability/toxicity of lycopene in patients with biochemical relapse-only prostate cancer.

MATERIAL AND METHODS

Patients

Patients were enrolled who had biochemical relapse after definitive local therapy (either radiotherapy or surgery) for prostate adenocarcinoma. Biochemical relapse was defined using the American Society for Therapeutic Radiation Oncology criteria of three consecutive rises in PSA level separated by at least 1 month. The minimal PSA value at entry was 1 ng/mL. The Wake Forest University Health Sciences Institutional Review Board approved the protocol, and all patients signed the protocol-specific informed consent form before enrollment.

Patients were permitted to have previously undergone androgen ablation therapy but could not have had any new hormonal therapy in the 6 months before enrollment. The exclusion criteria included a history of dietary supplementation with lycopene, a history of exposure to other dietary phytotherapeutics (eg, PC-SPES, saw palmetto), and clinical evidence of metastasis (positive bone scan, computed tomography of the abdomen/pelvis, or evidence of brain metastases).

Six consecutive cohorts of 6 patients each were enrolled and received daily supplementation with an escalating dose of 15, 30, 45, 60, 90, or 120 mg/day lycopene in divided doses twice daily. Lycopene supplements (Lyc-O-Mato 6%, Natural Tomato Oleoresin, Beer-Sheva, Israel) were supplied by LycoRed Natural Products Industries. Each lycopene capsule contained 15 mg of lycopene. The PSA serum levels were measured at baseline and every 4 weeks for up to 1 year. Patients were assessed monthly for toxicity and compliance using pill counts. The PSA response was determined at 12 weeks. Responders (see next paragraph) and patients with stable disease remained in the study with regular assessments every 3 months for a total of 12 months.

Study Endpoints

The primary clinical endpoint of the study was a PSA response to lycopene supplementation, defined as a 50% reduction in PSA level from baseline maintained for at least 1 month and confirmed after at least two PSA evaluations. The secondary objectives were to determine the plasma levels of lycopene as a function of the oral dose, PSA response as a function of the lycopene dose/plasma level, duration of the PSA response, time to PSA progression, change in PSA doubling time or slope before study enrollment versus while taking lycopene, and toxicity of escalating doses of lycopene supplementation. Progression was defined as a 50% increase in the PSA level from the maximal PSA level at baseline that was confirmed by a second PSA determination at least 2 weeks later. Toxicity was graded according to the revised National Cancer Institute Common Toxicity Criteria, version 2.0.

Pharmacokinetic Studies

The blood samples for plasma lycopene levels were drawn into ethylenediaminetetraacetic acid "purple top" tubes at baseline and every 3 months for 1 year. The samples were immediately placed on ice in the dark, and then centrifuged at 2000*g* for 15 minutes. Duplicate plasma samples of 0.50 mL each were immediately mixed with 8 μL of 2.5% butylated hydroxytoluene in ethanol, vortex mixed, the air displaced with argon in subdued light, and stored at −80°C. The plasma samples (200 μL) were later mixed with 1 mL of 1 mM phosphate buffer (pH 7.4 with 0.025% ascorbic acid and 0.025% ethylenediaminetetraacetic acid) and 1 mL 30μM butylated hydroxytoluene in ethanol, and then extracted twice with 2 mL of hexane. An internal standard, tocol (Roche, Nutley, NJ), was used to correct for any differences in extraction efficiency. The upper layer was separated and dried under nitrogen and then reconstituted in a mixture of 20% tetrahydrofuran and 80% 30 μM butylated hydroxytoluene in ethanol. The lycopene concentrations were then determined using isocratic, reverse-phase, high-performance liquid chromatography using a method previously described by Hess *et al.*[13]

Statistical Analysis

A survival curve was estimated for PSA progression-free survival using Kaplan-Meier techniques. The median progression-free survival was estimated, along with the corresponding 95% confidence interval.

PSA slopes were estimated for the 24 participants who had had data measured during the year before lycopene supplementation by fitting separate linear regression models for each participant to estimate their PSA slope during that time. In each model, the outcome was the observed PSA and the predictor was the time of the measurement. Participants who were included in these analyses had at least three PSA assessments during the year before lycopene supplementation and three PSA assessments after lycopene initiation (range 3 to 12 measures available during each period). The PSA slopes were estimated for the period after lycopene supplementation for each participant using the same techniques. Doubling times and 95% confidence intervals for the doubling times were estimated using these slopes. The presupplementation and postsupplementation slopes were then compared using a paired *t* test in which each participant's change in slope (before to after) was the outcome of interest. We examined whether the before-to-after PSA slopes were different according to the lycopene dose using a general linear model with the lycopene dose as the predictor and the change in PSA slope as the outcome. We fit this model considering group as a classification variable first and then as a linear variable (to test for a possible dose-response effect). All analyses were performed using Statistical Analysis Systems, version 8 (SAS Institute, Cary, NC).

RESULTS

A total of 36 patients with biochemically recurrent prostate cancer (24 after radiotherapy and 12 after prostatectomy) were enrolled and began taking lycopene supplementation between December 2000 and January 2003. The median patient age at study entry was 74 years (range 56 to 83), and the median PSA level at baseline before lycopene supplementation was 4.4 ng/mL (range 0.8 to 24.9). Of the 36 patients, 34 were white and 2 were black. Two patients had undergone short-term neoadjuvant hormonal therapy at the time of initial ther-

apy, and no patient had undergone hormonal therapy within 4 years of enrollment.

No PSA responses occurred using a 50% decline in PSA level from baseline as the criterion. Thirteen patients (37%) had PSA progression during the study. The median time to progression was not reached.

Of the 36 patients, 24 (67%) had informative data available on serum PSA values for at least 1 year before enrolling in the trial. The calculated PSA doubling time for the entire cohort of 24 patients before lycopene supplementation was 3.7 years (95% confidence interval 1.9 to 5.4). This was not significantly different from the doubling time while the patients were taking lycopene supplements (4.1 years, 95% confidence interval 1.7 to 6.5). Similarly, the calculated PSA slope during the year before starting lycopene and during the trial was not significantly different (0.010 and 0.011, respectively, $P = 0.57$). When we examined whether there was a possible lycopene effect on the before-to-after PSA slope changes, we found no significant evidence of this ($P = 0.054$). In addition, when we tested dose as a linear trend, we again found no significant difference in PSA slopes before and during treatment ($P = 0.88$).

Lycopene supplementation was well tolerated. One patient discontinued therapy because of diarrhea (grade 2 toxicity) thought to be related to lycopene supplementation. Other toxicities were recorded but were unlikely to be secondary to lycopene supplementation. These included 1 case each of grade 1 hematuria, hematochezia, and lower extremity edema. Also, 1 patient with a long-standing history of coronary artery disease was noted to have anterior ischemia on an electrocardiogram (grade 2). Seven grade 2 transient abnormalities of either serum glucose (n = 5) or creatinine (n = 2) occurred. One patient died after 11 months on protocol of previously undiagnosed hepatocellular carcinoma.

The mean plasma lycopene level over time at the different oral doses is illustrated in Figure 1. The plasma levels increased with lycopene administration at all dose levels. The plasma levels achieved with daily doses of 15, 30, 45, 60, and 90 mg/day were similar. A significant elevation in plasma lycopene level was noted at the highest dose (120 mg) compared with the lower dose cohorts ($P < 0.0001$). A plateau in plasma lycopene level was noted after 3 months of therapy at each dose level.

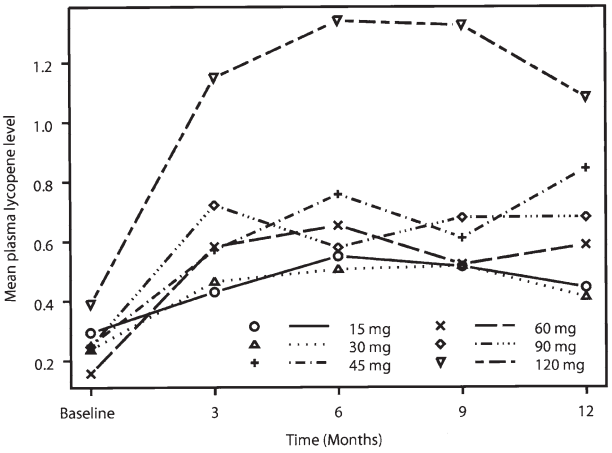

FIGURE 1. *Mean plasma lycopene level over time at six different dose levels.*

COMMENT

In this dose-escalating study, we found that lycopene supplementation for biochemically relapsed prostate cancer was well tolerated and safe. We were unable to detect any clinically significant effect of lycopene on serum PSA. This was true whether the endpoint was a 50% decline in PSA from baseline (the primary endpoint of the study) or when comparing the PSA doubling time or slope in the year before enrollment versus during therapy. The lack of effect of lycopene on PSA was not due to compliance, because the compliance rate in our trial was 100%, as measured by monthly pill counts and the rise in plasma lycopene levels across all dose levels. The latter also demonstrated that the lycopene supplement used in this trial (Lyc-O-Mato) was adequately bioavailable.

Little information is available in published reports regarding the pharmacokinetics of lycopene in humans. The half life of lycopene in human plasma has been reported to be 2 to 3 days.[14] Lycopene is transported in the blood in association with lipoproteins, particularly chylomicrons and low-density lipoprotein. A consistent finding in published studies is that food processing increases the bioavailability of lycopene.[15] Raw tomatoes and tomato juice have a lesser impact on lycopene levels than the consumption of canned tomatoes, tomato paste, or pizza sauce. The optimal dose to achieve biologically active lycopene concentrations in humans is unknown. Boileau *et al.*[16] studied tissue lycopene concentrations in male rats according to dietary lycopene supplementation. The tissue and serum lycopene concentrations increased significantly as the dietary lycopene supplementation increased from 0 to 0.5 g/kg, with little additional increase between 0.5 and 5.0 g/kg. The investigators concluded that serum and tissue lycopene reaches a plateau between 0.05 and 0.5 g/kg dietary lycopene in rats. We observed a similar plateau effect, in that similar plasma concentrations were achieved with a wide range of lycopene supplementation (15 to 90 mg/day). Furthermore, the plasma levels of lycopene reached a plateau at 3 months that remained essentially stable for the 12

months of the study. Another reason to measure serum or plasma lycopene levels in humans is the known fact that the relationship between dietary intake and serum or plasma levels is very poor.[15] We have demonstrated in this trial that substantial and sustainable increases in plasma lycopene concentrations can be achieved through daily lycopene supplementation for a wide dose range (15 to 120 mg/day). Nevertheless, it remains possible that the absence of an observed effect on PSA in this trial could have been because of the use of a lycopene supplement instead of dietary supplementation with increased consumption of processed tomatoes or tomato products.

The findings of this trial are inconsistent with two trials that tested the effect of lycopene in patients with more advanced disease.[10,11] One trial randomized 54 patients with documented metastatic prostate cancer between orchiectomy plus 6 months of lycopene supplementation (2 mg twice daily) and orchiectomy alone.[10] They found a significantly greater decrease in serum PSA level in the combined therapy arm, as well as better relief of bone pain, less lower urinary tract symptoms, and better overall survival. These impressive results using a low dose of lycopene (2 mg) and no associated toxicity prompted a second trial by the same group of 20 consecutive patients with hormone-refractory prostate cancer treated with lycopene (10 mg daily) for 3 months.[11] They reported a 35% response rate, including one complete response, associated with improved performance status in 50% and improvements in bone related pain in 10 of 16 patients and lower urinary tract symptoms in 11 (61%) of 18 patients. Our own case report, which prompted our trial, also suggested an effect against advanced disease in a patient taking lycopene and saw palmetto.[17] These excellent results in a difficult-to-treat patient population were associated with no appreciable toxicity.

The reason for the discrepancy between these two studies and our results are not immediately apparent. This is especially interesting given the low doses of lycopene used and the relatively short duration of therapy in the trials of advanced disease. Both used the same lycopene supplement formulation as was used in our study. One possible difference may be in the baseline diets between the studies. Both of the previous studies were performed in India, and the present trial was performed in the United States. Differences in the baseline diet may therefore have accounted for some of the differences. The baseline plasma levels of lycopene from the Indian trials were not reported. It is also possible that as yet poorly defined genetic polymorphisms may affect the response to lycopene supplementation so that differences in ethnicity/race of the study subjects may have affected the outcome. Another possibility is that this was due to the difference in the disease stage being treated, because both of the Indian trials focused on patients with more advanced disease than those treated in our study. However, we know of no biologic reason why advanced prostate cancer would be more responsive than at the stage of PSA relapse.

An additional concern is whether our study had sufficient power to detect a significant lycopene effect; however, the point estimates for the PSA slopes were nearly identical and showed a slightly greater PSA slope during the trial than before the trial. Thus, even with a larger sample size, which may have added precision to our estimates, the direction of the effect would not likely have changed. It remains possible that a Phase II trial with larger numbers may suggest a small effect of lycopene supplementation on serum PSA levels in men with biochemically recurrent prostate cancer that was not detected in this trial. The substantial and similar lycopene plasma level for a wide range of lycopene dose levels makes it unlikely we have missed a substantial effect on PSA in our series. Nevertheless, a larger Phase II trial at a single dose of lycopene would be required to more formally explore any potential role for lycopene supplementation in the treatment of biochemically recurrent prostate cancer.

Substantial epidemiologic data have suggested that increased consumption of tomato products and/or increased serum levels of lycopene are associated with a lower risk of prostate cancer, although this has not been found in all studies.[5–7] Our trial highlights the potential pitfalls of overinterpreting epidemiologic evidence for associations with prostate cancer risk as proof of therapeutic benefit. The results of the present trial suggest that if lycopene has an effect on prostate cancer it may be more important in protecting against carcinogenesis rather than in treating established disease, at least for biochemically relapsed disease. This is consistent with the findings of one trial that examined the effect of lycopene given neoadjuvantly before radical prostatectomy.[12] They found that lycopene supplementation was associated with a lower incidence of high-grade prostatic intraepithelial neoplasia, an entity thought to be a potential precursor lesion for invasive prostate cancer.

CONCLUSIONS

Lycopene supplementation in men with biochemically relapsed prostate cancer after failed definitive local therapy is safe and well tolerated. The plasma levels of lycopene were similar for a wide dose range (15 to 90 mg/day) and had plateaued by

3 months. Lycopene supplementation at the doses used in this study did not result in any discernible response in serum PSA.

REFERENCES

1. Jemal A, Murray T, Ward E, *et al:* Cancer statistics, 2005. CA Cancer J Clin **55:** 10–30, 2005.

2. Pound CR, Partin AW, Eisenberger MA, *et al:* Natural history of progression after PSA elevation following radical prostatectomy. JAMA **281:** 1591–1597, 1999.

3. Sartor CI, Strawderman MH, Lin XH, *et al:* Rate of PSA rise predicts metastatic versus local recurrence after definitive radiotherapy. Int J Radiat Oncol Biol Phys **38:** 941–947, 1997.

4. Lee WR, Hanks GE, and Hanlon A: Increasing prostate-specific antigen profile following definitive radiation therapy for localized prostate cancer: clinical observations. J Clin Oncol **15:** 230–238, 1997.

5. Etminan M, Takkouche B, and Caamano-Isorna F: The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. Cancer Epidemiol Biomarkers Prev **13:** 340–345, 2004.

6. Campbell JK, Canene-Adams K, Lindshield BL, *et al:* Tomato phytochemicals and prostate cancer risk. J Nutr **134:** 3486S–3492S, 2004.

7. Dagnelie PC, Schuurman AG, Goldbohm RA, *et al:* Diet, anthropometric measures and prostate cancer risk: a review of prospective cohort and intervention studies. BJU Int **93:** 1139–1150, 2004.

8. Beecher GR: Nutrient content of tomatoes and tomato products. Proc Soc Exp Biol Med **218:** 98–100, 1998.

9. Lu QY, Hung JC, Heber D, *et al:* Inverse associations between plasma lycopene and other carotenoids and prostate cancer. Cancer Epidemiol Biomarkers Prev **10:** 749–756, 2001.

10. Ansari MS, and Gupta NP: A comparison of lycopene and orchidectomy vs orchidectomy alone in the management of advanced prostate cancer. BJU Int **92:** 375–378, 2003.

11. Ansari MS, and Gupta NP: Lycopene: a novel drug therapy in hormone refractory metastatic prostate cancer. Urol Oncol **22:** 415–420, 2004.

12. Kucuk O, Sarkar FH, Sakr W, *et al:* Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. Cancer Epidemiol Biomarkers Prev **10:** 861–868, 2001.

13. Hess D, Keller HE, Oberlin B, *et al:* Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. Int J Vitam Nutr Res **61:** 232–238, 1991.

14. Stahl W, and Sies H: Lycopene: a biologically important carotenoid for humans? Arch Biochem Biophys **336:** 1–9, 1996.

15. Clinton SK: Lycopene: chemistry, biology, and implications for human health and disease. Nutr Rev **56:** 35–51, 1998.

16. Boileau TW, Clinton SK, and Erdman JW Jr: Tissue lycopene concentrations and isomer patterns are affected by androgen status and dietary lycopene concentration in male F344 rats. J Nutr **130:** 1613–1618, 2000.

17. Matlaga BR, Hall MC, Stindt D, *et al:* Response of hormone refractory prostate cancer to lycopene. J Urol **166:** 613, 2001.