The Prostate 70:1127–1133 (2010)

Combined Inhibitory Effects of Soy Isoflavones and Curcumin on the Production of Prostate-Specific Antigen

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BACKGROUND. Sustained chronic inflammation in the prostate promotes prostate carcinogenesis. Since an elevated level of prostate-specific antigen (PSA) per se reflects the presence of inflammation in the prostate, intervention to improve the PSA value might potentially have beneficial effects for the prevention of the development of prostate cancer. Isoflavones and curcumin have anti-inflammatory and anti-oxidant properties. We examined the biological effects of soy isoflavones and curcumin on LNCaP cells. After that, we conducted a clinical trial for men who received prostate biopsies, but were not found to have prostate cancer, to evaluate the effects of soy isoflavones and curcumin on serum PSA levels.

METHODS. The expression of androgen receptor and PSA were examined in LNCaP cells before and after treatment of isoflavones and/or curcumin. Eighty-five participants were randomized to take a supplement containing isoflavones and curcumin or placebo daily in a double-blind study. Subjects were subdivided by the cut-off of their baseline PSA value at 10 μg/ml. We evaluated values of PSA before and 6 months after treatment.

RESULTS. The production of PSA were markedly decreased by the combined treatment of isoflavones and curcumin in prostate cancer cell line, LNCaP. The expression of the androgen receptor was also suppressed by the treatment. In clinical trials, PSA levels decreased in the patients group with PSA ≥ 10 treated with supplement containing isoflavones and curcumin ($P = 0.01$).

CONCLUSIONS. Our results indicated that isoflavones and curcumin could modulate serum PSA levels. Curcumin presumably synergizes with isoflavones to suppress PSA production in prostate cells through the anti-androgen effects. *Prostate 70: 1127–1133, 2010.*

KEY WORDS: **prostate cancer; isoflavones; curcumin; PSA**

INTRODUCTION

Prostate cancer is the most common neoplasm in Caucasian men, while the incidence in Asians has been relatively low. Observational studies have suggested that diet is one of the most contributing factors for the lower observed incidence and mortality of prostate cancers in Asia [1]. Environmental factors such as dietary habits may play a major role in the causation of prostate cancer. Besides genetic factors, changes of lifestyle, especially dietary changes, should modify the risk of carcinogenesis. For example, Japanese men who migrated to the U.S. have experienced increasing rates of prostate cancer over consecutive generations [2].

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Received 26 December 2009; Accepted 20 January 2010
DOI 10.1002/pros.21147
Published online 26 March 2010 in Wiley InterScience (www.interscience.wiley.com).

Westernization of dietary habits among Japanese and Koreans may contribute significantly to the increased morbidity and mortality of prostate cancer in those country [3]. One of the reasons why Asians have a low risk of prostate cancer may be the ample intake of soy. Soy foods have been postulated to reduce the risk of a number of chronic diseases, including coronary heart disease, osteoporosis, and cancers [4–6]. The amount of dietary soy intake is also associated with a decreasing risk of prostate cancer [4–6].

Soy isoflavones are nonsteroidal diphenolic compounds that inhibit prostate cancer cell growth in vitro as well as tumor growth in mice [7–12]. The isoflavone aglycones are genistein, daidzen, and glycitein. Genistein, the predominant isoflavone in soy, has been shown to have anti-oxidant activity and to inhibit tumor growth through anti-proliferative and anti-angiogenic mechanisms [13]. In addition to estrogenic activities, genistein can induce apoptosis and inhibit the activation of the anti-apoptotic protection factor, nuclear factor-κB (NF-κB) in prostate cancer cells [14]. Intestinal bacteria convert daidzein into equol. Among Japanese, those who are able to convert daidzein to equol are less prone to develop prostate cancer [15]. Thus the ability to produce equol from daidzein or equol itself is closely related to a lower incidence of prostate cancer. The serum level of active isoflavones is found to be markedly lower in Americans as compared to Japanese and Koreans. The proportion of equol producers is also lower in former than in the latter [16]. Several clinical trials investigating the efficacy of soy isoflavones on prostate-specific antigen (PSA) have been previously undertaken, although the results are not consistent.

Epidemiological studies suggest that natural dietary ingredients used in Asian countries have anti-carcinogenic potential against prostate cancer [17]. Curcumin is a major yellow pigment in turmeric, which is widely used as a spice and coloring agent in several foods such as curry. Extensive studies have revealed that curcumin regulates the expression of inflammatory enzymes, cytokines, adhesion molecules, and cell survival proteins [18]. Curcumin exerts strong anti-oxidant and anti-inflammatory activities by suppressing both constitutive and inducible NF-κB and activator protein-1 activation [19]. In addition, curcumin has chemosensitive and radiosensitive effects by down-regulating the MDM2 oncogene through the PI3K/mTOR/ETS2 pathway [20]. Thus the potential therapeutic role of curcumin in prostate cancer is worthy of further evaluation and clinical trials.

In this study, we examined the effects of isoflavones and curcumin on the expression of PSA and androgen receptor in prostate cancer cells. Furthermore, we conducted a double-blind placebo-controlled clinical trial to evaluate the effects of soy isoflavone and curcumin on serum PSA levels in men who did not have detectable prostate cancer by biopsy.

MATERIALS AND METHODS

Cells and Culture Conditions

The human prostate cancer cell line LNCaP was obtained from the American Type Culture Collection (Rockville, MD). The cells were routinely maintained in RPMI 1640 supplemented with 10% FCS, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured at 37°C in a humidified incubator with 5% CO_2. Curcumin (Medi Herb, Inc., Bangalore, India) was dissolved in ethanol at a concentration of 10 mM and stored at −20°C. Isoflavones (NICHIMO Co., Ltd, Tokyo, Japan) were dissolved in DMSO at a concentration of 20 mg/ml and stored at −20°C in the dark.

Measurement of PSA in Conditioned Medium

LNCaP cells were cultured in culture medium for 24 hr and then treated with curcumin, isoflavones, or a combination of the two agents. After a 48 hr treatment, the conditioned media were collected, centrifuged to remove residual cells, and stored at −20°C. For quantitative analysis of the amount of PSA secreted by LNCaP cells, an immunoassay procedure was performed using a commercial PSA assay kit (R&D Systems, Inc., Minneapolis, MN). MTS assay was performed at the same time, and results were used for normalization of PSA levels.

Immunoblotting

Subconfluent LNCaP cells were treated with curcumin, isoflavones, or a combination of the two agents for 24 hr. Cells were washed twice with cold PBS and then lysed in RIPA buffer on ice for 30 min. The cell lysate was centrifuged at 15,000 rpm for 30 min at 4°C, and the supernatant was collected. Protein concentrations were measured by a BCA protein assay kit (Pierce, Inc., Rockford, IL). Protein samples were separated by SDS–PAGE and transferred onto a PVDF membrane (Millipore, Inc., Tokyo, Japan). Immunoblotting was performed using rabbit anti-androgen receptor antibody (Santa Cruz Biotechnology, Inc., CA; 1:2,000), mouse anti-PSA antibody (DakoCytomation, Kyoto, Japan; 1:1,000), or mouse anti-human β-actin antibody (Sigma-Aldrich Co., St. Louis, MO; 1:10,000 dilution) as an internal loading control. Immunoreactive proteins were visualized with ECL detection reagents (GE Healthcare Biosciences, Tokyo, Japan).

Randomized Placebo-Controlled Double-Blind Study for the Effect of Isoflavones and Curcumin on the Serum PSA Levels

One hundred men were recruited who underwent systematic prostate biopsy (14 cores) at the Teikyo University Hospital consecutively because of elevated levels of PSA and were not found to have either cancer or prostatic intraepithelial neoplasia (PIN). Serum levels of PSA were measured by Chemiluminescence Enzyme Immunoassay with a LUMIPULSE kit (FUJIREBIO, Tokyo, Japan). Patients who had allergies against either soy or turmeric; who had already taken supplements containing either isoflavones or curcumin; or who had been treated for cardiovascular disease, liver disease, renal failure, or asthma were excluded from the study. Patients who had a history of any malignancy and who took anti-androgen drugs such as finasteride for their lower urinary tract syndrome were excluded.

Patients were randomized to receive either isoflavones + curcumin (supplement) or placebo in a double-blind study. Participants were asked to take either isoflavones (40 mg) and curcumin (100 mg) or placebo for 6 months. Isoflavones contained 66% daidzen, 24% glycitin, and 10% genistin. Tablets of supplements and placebo were designed and manufactured by Angfa, Inc. (Tokyo, Japan) and SECOM Medical System Co., Ltd (Tokyo, Japan). Each subject gave informed written consent to participate in this study, which was approved by the local ethics committee. In 100 subjects, 42 out of 50 who were assigned to take placebo and 43 out of 50 to take supplements completed the trial. There were no significant adverse effects either in the placebo or supplement groups except one subject on placebo who experienced severe diarrhea during the trial and dropped out subsequently. Fourteen other subjects dropped out of the trial either because of the inability to visit the ambulatory clinic due to the deterioration of other medical conditions. We compared PSA levels at 6 months between the supplement and placebo groups.

Statistical Analysis

Statistical analysis was performed using the SAS statistical software package version 9.13 (SAS Institute, Inc., Cary, NC). *T*-test was used to compare the differences in 6-month PSA levels between the supplement and placebo groups. A *P*-value of <0.05 was considered to be statistically significant. Since PSA levels at the baseline affected ones at 6 months, we performed analysis of covariance (ANCOVA) using baseline PSA levels, which was changed binary variable. We divided subjects into subgroups by a cut-off of 10 ng/ml of baseline PSA level; Subgroup 1: PSA <10; Subgroup 2: PSA ≥ 10. We compared the change of PSA levels between the baseline and the end of the study. We examined the statistical interactions of the intake of supplement and PSA levels at the baseline by ANCOVA.

RESULTS

Combined Inhibitory Effects of Isoflavones and Curcumin on PSA Production and Expression of Androgen Receptor

To determine the effects of isoflavones, curcumin, and their combination on PSA production, immunoassay was performed on LNCaP cells at various concentrations. Treatment of the cells with 10 μg/ml isoflavones alone caused 40% inhibition of PSA secretion to the supernatant compared to control, whereas treatment of the cells with 20 μM curcumin caused 20% inhibition. A combination treatment with 10 μg/ml isoflavones and 20 μM curcumin caused almost complete inhibition of PSA production in LNCaP cells (Fig. 1). Analysis by immunoblotting was also performed on LNCaP cells before and after treatment of isoflavones and/or curcumin. These results showed that the amounts of PSA production by LNCaP cells were significantly reduced when cells were treated with 25 μM curcumin. A further decrease in PSA level was observed when cells were treated with a combination of 10 μg/ml isoflavones and 25 μM curcumin (Fig. 2). We also examined the effects of these two compounds on the expression of androgen receptors. After 24 hr incubation, the expression of androgen receptor was inhibited by treatment with 10 μg/ml isoflavones or 25 μM curcumin (Fig. 2). The combination of isoflavones and curcumin inhibited the expression of androgen receptors additively.

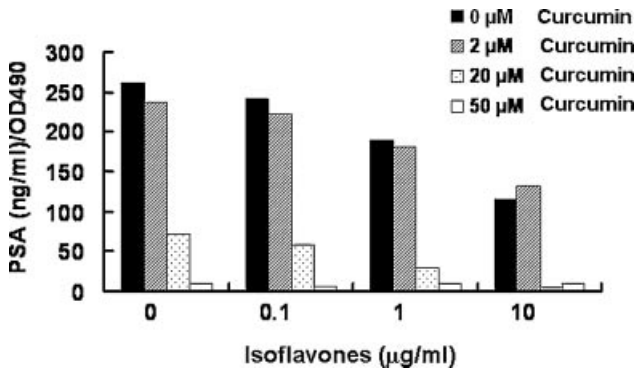

Fig. 1. PSA secretion in LNCaP cells after treatment with curcumin and/or isoflavones. Cells were treated with various doses of curcumin and/or isoflavones for 48 hr.

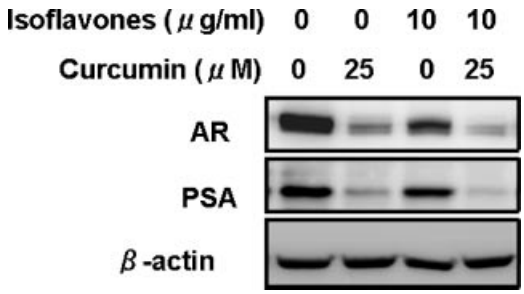

Fig. 2. Androgen receptor and PSA expression in LNCaP cells after treatment with curcumin and/or isoflavones. Immunoblot analysis demonstrated synergistic effects of isoflavones and curcumin after 24 hr. Immunoblots were probed for mouse anti-human β-actin antibody as an internal control.

Effects of Supplements on PSA Levels in Men Without Prostate Cancer

The 85 men who completed the clinical trial were all Japanese. At baseline, the median age of the population was 73 years (range: 50–86). Serum PSA levels at the baseline and at the end of the study were not different between supplement and placebo groups (Table I). The number of subjects in the individual subgroups were: Subgroup 1 (PSA at the baseline <10): Placebo: 32 (37.6%), Supplement: 28 (32.9%); Subgroup 2 (PSA at the baseline ≥ 10): Placebo: 10 (11.8%), Supplement: 15 (17.7%) (Table II). In two subgroups divided by the cut-off of PSA ≥10 ng/ml, serum PSA levels at the baseline and the end of the study between supplement and placebo groups were not different (Table II; Fig. 3). Considering the interaction of initial PSA levels and the intake of supplements, analysis by ANCOVA showed that supplement group had significantly larger decrease in PSA levels in Subgroup 2 (initial PSA ≥ 10 ng/ml) (Table III).

DISCUSSION

Several lines of evidence indicate that oxidative stress may play an important role in carcinogenesis. In the prostate, the aging process shifts this redox balance towards a more oxidative state which may be due in part to a decline of anti-oxidative enzymes like super-oxide dismutase and catalase [21]. Proposed prostate cancer preventive agents like isoflavones, selenium, lycopene, or green tea have well-known anti-oxidant activities. In addition to these agents, curcumin is also a promising candidate [17,22]. Curcumin has been shown to cause apoptosis and cell-cycle arrest with inhibited cell growth, activation of signal transduction, and transforming activities in both androgen-dependent and independent prostate cancer cells [18,22,23]. The present study was undertaken to evaluate the potential efficacy of isoflavones, curcumin, and their combination as prostate cancer preventive agents. The central findings of this study are that a combined treatment of curcumin and isoflavones enhance the inhibition of PSA production and the expression of androgen receptor in LNCaP cells.

PSA is the leading marker for prostate cancer, although it has a low sensitivity, and does not have a clear cut-off level to produce a dichotomous result. In the Prostate Cancer Prevention Trial, even within the 0–4.0 ng/ml interval, the PSA level was a continuously increasing marker of prostate cancer risk including high-grade tumors, with no boundary below which no prostate cancer was found [24,25]. Thus lowering the PSA threshold has been proposed to increase the detection of cancers, although the unavoidable tradeoff is an increased number of subjects who are false positives [26]. Currently there is no consensus for the management of biopsy-negative subjects. Repeated biopsies are recommended based on the fact that a second biopsy can detect cancer in around 30% of subjects [27]. However, patients may not agree with the immediate re-biopsy. Furthermore, subjects who do not have detectable cancer after a second biopsy may be

TABLE I. Patient Characteristics and PSA Changes

	Supplement (n = 42)	Placebo (n = 43)
Age[a]	72.4 (59–86)	71.3 (50–84)
PSA at the baseline[b]	10.5 ± 9.5	8.0 ± 6.7
PSA at 6 months[b]	7.4 ± 4.6	7.1 ± 5.6

[a]Mean (min–max).
[b]Mean ± SD.

TABLE II. Patient Characteristics and PSA Level of Subgroup

Subgroup	Supplement	Age	PSA at the baseline	PSA at 6 months
1: PSA < 10	Placebo (n = 32)	72 (50–82)	5.2 ± 2.2	4.8 ± 2.2
	Supplement (n = 28)	74 (61–86)	6.1 ± 1.9	5.9 ± 2.6
2: PSA ≥ 10	Placebo (n = 10)	74 (71–84)	17.0 ± 8.4	14.2 ± 7.2
	Supplement (n = 15)	73 (59–82)	18.8 ± 12.4	10.2 ± 6.2

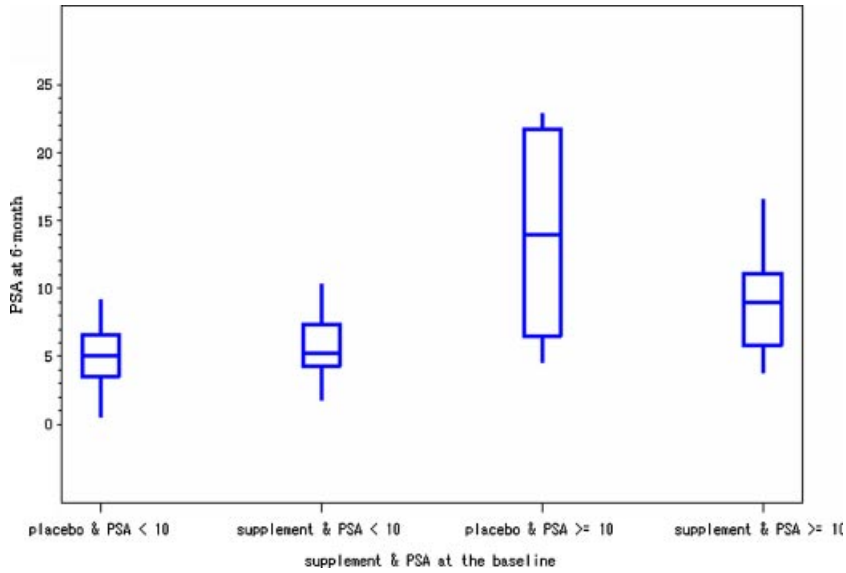

Fig. 3. Serum PSA levels at 6 months between subgroups: Subgroup I: PSA $<$ I0; Subgroup 2: PSA $\geq$ I0. PSA levels were compared among subgroups by *t*-test, and ANCOVA was used to compare the differences in PSA levels at 6 months. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

left for observation to see if there will be a further rise of PSA.

Elevated serum levels of PSA reflect not only the existence of cancer but also inflammation in the prostate. Sustained chronic inflammation in the prostate may promote prostate carcinogenesis [28]. In addition, PSA, a serine protease, plays a significant role in prostate tumor growth by regulating various proangiogenic and anti-angiogenic growth factors [29]. Thus intervention to improve the PSA value might have beneficial effects for the prevention of the development of prostate cancer. Several trials to date have examined the effects of isoflavones on serum PSA levels, although the results are not consistent [17,30–32]. The strengths of this study are the randomized, controlled, parallel-arm design; the blinding of investigators and participants; the relatively long (6 months) duration of the intervention; and the larger sample size than in previous studies. In our study, PSA levels significantly decreased in the supplement-treated subgroup compared with that of the placebo group only in those subjects whose baseline PSA levels were over 10 ng/ml ($P = 0.02$). Currently, we do not have a solid explanation for our findings that the effects of soy isoflavones and curcumin were seen only in those subjects whose PSA is over 10. One speculation is that isoflavones and curcumin might improve the asymptomatic inflammation in prostates with high serum PSA levels.

The limitation of this preliminary clinical study was that cohort of patients accrued likely had already been placed on diets rich in soy isoflavones or curcumin and they were not controlled for the content of diets. We did not measure the plasma levels of soy isoflavones and curcumin to see whether their basal levels might affect the observed changes in serum PSA levels, or to show supplementation achieved a biologically relevant increase in serum levels for each of these agents. To further identify the role of derivatives of isoflavones, we need to evaluate the serum levels of equol and daidzen influenced by the intake of soy isoflavones.

TABLE III. ANCOVA with Interaction

Variable	Estimate	SE	95% CI	*P*-value
Supplement 1[a]	1.05	1.05	(−1.03, 3.13)	0.32
Supplement 2[b]	−4.00	1.65	(−7.28, −0.71)	0.02
PSA at the baseline (≥10/<10)	9.36	1.46	(6.45, 12.27)	<0.0001

[a]Supplement use in PSA $<$ 10.
[b]Supplement use in PSA $\geq$ 10.

CONCLUSIONS

A combined treatment of soy isoflavones and curcumin inhibited the production of PSA and the expression of androgen receptor in cultured prostate cancer cells in vitro. In a randomized, placebo-controlled clinical trial, a combined treatment of soy isoflavones and curcumin decreased serum PSA in those subjects whose baseline PSA was more than 10 ng/ml. Our data show that the effects of isoflavones and curcumin on PSA production in prostate cells, particularly in combination, may have therapeutic advantages in the patients with high PSA level who has negative prostate biopsies.

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