Herbal/Hormonal Dietary Supplement Possibly Associated with Prostate Cancer Progression

Shahrokh F. Shariat,1,2 Dolores J. Lamb,2,3 Radha G. Iyengar,4 Claus G. Roehrborn,3 and Kevin M. Slawin2

Abstract

Background: Patients seek herbal/hormonal dietary supplements (HHDS) to prevent and/or solve health and aging issues. After two men developed an unusual course of clinically aggressive prostate cancer within months of starting daily consumption of the same HHDS product, we investigated the effect of this product on prostate cancer progression.

Methods: We evaluated serum levels of total testosterone, luteinizing hormone, and follicle-stimulating hormone and screened prostate biopsy and metastatic specimens for androgen receptor protein expression and mutations. We did hormone analyses and capillary electrophoresis. We tested the effect of the HHDS product on androgen receptor-negative (DU-145 and PC-3) and androgen receptor-positive (LNCaP) human prostate cancer cell lines.

Results: Both patients had low hormone levels. The androgen receptor was expressed in all primary and metastatic prostate cancer tissues and no mutations were identified. Hormone analysis revealed that the HHDS contained testosterone and estradiol. The HHDS product was a more potent dose-dependent stimulator of cancer cell growth than testosterone both in androgen receptor-negative and receptor-positive cell lines. Blocking experiments with increasing concentrations of bicalutamide did not prevent the HHDS product–stimulated growth. We filed an adverse event report with the Food and Drug Administration who issued a warning letter. The manufacturer responded by removing this HHDS product from the market.

Conclusions: The HHDS product contained one or more endocrinologically active tumor-promoting components that had cellular androgen receptor status–independent activity. The HHDS product exhibited potent prostate cancer growth stimulatory activity that was more powerful than that of testosterone, independent of the androgen-receptor status of prostate cancer cells, and resistant to antiandrogen blockade.

Patients seek herbal/hormonal dietary supplements (HHDS) to prevent and/or solve health and aging issues. The prevalence of HHDS use by adults is estimated to be 42% to 69% in the United States with an estimated out-of-pocket expenditure of about 34.4 billion dollars (1–4). Individuals use HHDS for self-improvement, failure or distrust of conventional medicine, and because they believe that these natural products are safe and drug free. Among the HHDS inundating the marketplace, the sale of androgenic steroids preparations is exponentially increasing. In 2004, U.S. expenditures on testosterone supplements were estimated to be $425,000,000 (1–4). A search using the Google internet engine for the terms “testosteron" in August 2006, yielded >3,230,000 hits. Eighty-two percent of the first 100 sites endorsed over-the-counter supplements that guarantee a “fountain of youth”, maintenance of a “youthful” heart, relief of stress, and improvements in mood disturbances, stamina, energy, strength, and virility. Despite these glowing promises, there may be cause for concern. Two of our patients developed aggressive prostate cancer several months after starting the same HHDS (brand name not mentioned due to legal reasons), leading us to investigate its effect on prostate cancer growth.

Patients and Methods

Case 1. A 67-year-old White male with well-controlled hypertension and hyperlipidemia presented with a two-month history of nonproductive cough, frequent urination, and loss of appetite accompanied by an involuntary weight loss of >40 pounds. A chest radiograph showed multiple bilateral lung nodules (Fig. 1A). A bone scan revealed the existence of metastatic disease involving the right rib cage, upper lumbar spine, right sacrum, and both pubic rami (Fig. 1B). Total serum prostate-specific antigen (T-PSA) was markedly elevated at 74 ng/mL. A digital rectal examination revealed a very large, asymmetric, bilaterally nodular prostate. He had two prior prostate cancer screenings that were normal (normal digital rectal examination and T-PSA of 1.3 ng/mL 2 years prior and T-PSA of 2.0 ng/mL 1 year before diagnosis). An ultrasound-guided systematic 12-core prostate biopsy showed the presence of prostate adenocarcinoma with Gleason score 6 (3 + 3) in all cores (Fig. 1C). A computed tomography–guided lung biopsy revealed metastatic prostate adenocarcinoma (Fig. 1D).
The patient initiated a regimen of combined androgen blockade involving an luteinizing hormone–releasing hormone agonist plus a p.o. antiandrogen (i.e., Lupron and Casodex, respectively). After 2 months of androgen ablation–hormone therapy, the number of lung nodules on chest radiography decreased and his T-PSA reached a nadir of 3.6 ng/mL. Two months later, however, a brain magnetic resonance imaging revealed the presence of an intrasellar pituitary mass. Trans-sphenoidal hypophysectomy revealed metastatic prostate adenocarcinoma (Fig. 1E). The patient subsequently received palliative radiation therapy and salvage chemotherapy. He was alive at last follow-up but suffered from widely metastatic disease.

The patient's history was unusual. The patient mentioned that he began taking a HHDS product (brand name not mentioned due to legal reasons) purchased via the Internet 10 months before the onset of his symptoms. He sought to develop stronger muscles and enhanced sexual performance. He took two capsules daily for 6 months, as recommended by the manufacturer. Initially, he gained 10 pounds in muscle mass and attained a higher than average energy level.

Case 2. A 51-year-old African-American male with hypertension had a serum T-PSA of 21.7 ng/mL during his yearly prostate cancer screening. Digital rectal examination showed a bilaterally hard and enlarged prostate. His previous screening results were normal based on a digital rectal examination and serum T-PSA levels of 1.5 ng/mL 4 years prior, 2.3 ng/mL 2 years prior, and 2.1 ng/mL 1 year prior. Systematic 12-core transrectal ultrasound-guided prostate biopsy showed prostate adenocarcinoma with Gleason score 7 (4 + 3) in 10 cores. A chest radiograph revealed multiple bilateral small nodules. Magnetic resonance imaging of the abdomen and pelvis suggested extracapsular extension, seminal vesicle involvement, and pelvic lymph node enlargement. The bone scan revealed an increased uptake in L4 and L3 vertebrae.

Combined androgen blockade (i.e., Zoladex and Casodex, respectively) resulted in a decrease in his T-PSA to a nadir of 1.3 ng/mL 3 months after start of hormone therapy. However, his T-PSA subsequently increased to 4.6 ng/mL at 10 months, 7.0 ng/mL at 12 months, and 23.7 ng/mL at 15 months. His bony symptoms...
worsened, and he underwent palliative radiotherapy. The patient was alive with widely metastatic disease at last follow-up.

The patient mentioned that he began taking the same HHDS product as the first patient described above, ~ 11 months before his diagnostic visit. He purchased the HHDS product over the internet after reading an advertisement in a fitness journal. Initially, he gained ~ 20 pounds in muscle mass but started rapidly losing weight after 3 months. He stopped taking the HHDS product after his diagnosis.

Male reproductive hormone status. At time of diagnosis, serum levels of total testosterone were 182 and 154 ng/dL (reference range: 350-720); serum levels of luteinizing hormone were 3.1 and 6.3 IU/L (reference range for males, 7-24); and serum levels of follicle-stimulating hormone were 0.9 and 1.3 IU/L (reference range for adult males, 1.5-12.4), respectively, for cases 1 and 2. These low hormone levels are thought to reflect the administration of exogenous testosterone found to be present in the HHDS product, as this leads to a marked decrease in gonadotropin-releasing hormone pulse frequency (5, 6). This in turn inhibits luteinizing hormone and follicle-stimulating hormone release from the pituitary (7).

Ingredients of the HHDS. The ingredients listed on the label of the HHDS product were as follows: 60 mg of a proprietary blend of six testosterone precursors (androstenediones and androstenediols), 100 mg chrysin, and 100 mg elk velvet antler per serving. Hormone analysis for total testosterone (Chiron Corp. Diagnostic) revealed that the HHDS contained testosterone and estradiol. Dehydroepiandrostosterone levels were below the assay detection limits.

Capillary electrophoresis confirmed the presence of chrysin and elk velvet antler. Chrysin is a flavonoid found in some plants, including the geranium-like plants called Pelargonium. Chrysin is a popular nutritional supplement among male body builders and other athletes because of its aromatase-inhibitory action blocking metabolism of androstenedione and testosterone to estrogens (8, 9). Elk antler velvet is marketed as an alternative medicine remedy for disorders such as impotence in men and infertility in women. Despite its popularity, a recent placebo-controlled, randomized clinical trial revealed that elk velvet antler supplementation did not enhance men’s athletic performance or change hormonal response during stress (10).

Androgen receptor immunohistochemistry and mutation analysis. The androgen receptor signaling pathway mediates the actions of androgens in target tissues. In the prostate, it plays an important role in the development of organs and cellular differentiation. Androgens also play a critical role in the initiation, proliferation, and progression of prostate cancer to ligand-independent disease (11, 12). We screened the prostate biopsy specimens from both patients, as well as the lung and pituitary specimens from the first patient for expression of the androgen receptor protein. The staining protocol has been described elsewhere (1:50 dilution of mouse monoclonal antibody G122-25; BD PharMingen; ref. 13). The androgen receptor was expressed in all primary and metastatic prostate cancer tissues.

Androgen receptor gene mutations can be associated with aggressive prostate cancer behavior, disease progression, and metastasis (reviewed in ref. 14). Accordingly, we investigated whether androgen receptor mutations were present in the laser microdissected prostate cancer biopsy specimens from both patients using PCR amplification of exons 2 to 8 and direct sequencing (15). No mutations were identified.

The HHDS product stimulates the growth of prostate cancer cell lines. Hormone-refractory (DU-145 and PC-3) and hormone-sensitive (LNCaP) human prostate cancer cell lines were obtained from American Type Culture Collection. All cell lines were maintained in the respective American Type Culture Collection—recommended complete growth medium. Before treatment, the cells were allowed to adhere to the plate for 24 h. Experiments were done in triplicate and repeated in three independent experiments.

We tested the hypothesis that the HHDS product stimulates the growth of prostate cancer cells. Cells were plated in 6-well culture plates at 2 × 10^4 cells per well and treated with the HHDS product (100 μg/mL per well), testosterone (24 pg/mL per well), or no drug (control). Medium was changed every 2 days, and the cells harvested and counted on days 2, 4, and 6. Cell growth is expressed as the relative increase in number of cells compared with controls (medium vehicle only without the HHDS product or testosterone). The LNCaP and DU-145 cell lines were treated with increasing doses of the HHDS product (100 μg/mL), testosterone (24 pg/mL), or no drug (vehicle only).

![Graphs showing the growth of prostate cancer cell lines with and without the HHDS product.](https://example.com/graphs.png)
product(100, 200, 400, 600, 800, and 1,000 A

A LNCaP, PC-3, and DU-145 treated with serially increasing doses of the HHDS product in a dose-dependent manner. Cell growth curves with 95% confidence intervals for LNCaP, PC-3, and DU-145 are shown. Cell growth is expressed as the relative increase in number of cells compared with medium without HHDS product. These assays were done in triplicate and repeated in three independent experiments. Differences were assessed using ANOVA (SPSS, version 13.0). A P value of ≤0.05 were considered significant.

DU-145 cell lines (Fig. 2) grew significantly faster when treated with the HHDS product compared with testosterone. The PC-3 cell line grew only when treated with the HHDS product. These findings suggest that the HHDS product is a more potent stimulator of prostate cancer cell growth than testosterone, and that it stimulates growth in vitro regardless of the androgen-responsiveness of prostate cancer cells.

We then assessed the effect of increasing doses of the HHDS product on prostate cancer growth. Prostate cancer cells were plated and treated with serially increasing doses of the HHDS product (100, 200, 400, 600, 800, and 1,000 µg/mL) or no drug (control) are shown. Cell growth is expressed as the relative increase in number of cells compared with control. Medium was changed on day 2, and the cells were harvested and counted on day 4. Cell growth is expressed as the relative increase in number of cells compared with control. The proliferation of all three cell lines was enhanced in a HHDS product dose-dependent manner (Fig. 3).

Finally, we used increasing concentrations of the potent nonsteroidal antiandrogen (bicalutamide) to define the androgen specificity on HHDS product–induced growth of LNCaP cells. Bicalutamide is a competitive antagonist of androgen receptor widely used for growth inhibition of androgen-responsive prostate cancer growth. Prostate cancer cells were plated and treated with the HHDS product (100 µg/mL) and increasing doses of the bicalutamide (0.01, 0.01, or 1 µmol/L) or bicalutamide alone (0.01 µmol/L). Cell growth is expressed as the relative increase in number of cells compared with controls (HHDS product alone, 100 µg/mL). Bicalutamide inhibited LNCaP cell growth in a dose-dependent fashion. However, the growth-inhibitory effect of bicalutamide was only transient when tested in combination with the HHDS product (Fig. 4). These findings suggest that the HHDS product stimulates prostate cancer cell growth effectively, bypassing the androgen receptor pathway in prostate cancer cells, while also rendering the cancer cells resistant to standard antiandrogen therapy.

Discussion

Unlike prescription and over-the-counter drugs, the law does not require nutritional supplements to undergo premarket approval for safety and efficacy. The Dietary Supplement Health and Education Act in 1994 allows HHDS manufacturers to assume the sole responsibility for ensuring the effectiveness and safety of their products. Thus, the current Food Drug Administration regulatory system provides little oversight or assurance that HHDS will have predictable pharmacologic effects or even that product labels provide accurate information to consumers. In the present study, the HHDS product evaluated listed ingredients that were not present, misrepresented the concentrations of the ingredients present, and failed to list all the steroids.

The HHDS product contained one or more endocrinologically active tumor-promoting components that had cellular androgen receptor status–independent activity. It is impossible to draw firm conclusions regarding the causative role of this HHDS product on the development and progression of prostate cancer. However, based on the history of the two patients and the in vitro experiments, the product exhibits potent prostate cancer growth stimulatory activity. This growth-promoting effect was more powerful than that of testosterone, independent of the androgen-receptor status of prostate cancer cells, and resistant to antiandrogen blockade. However, the predominant effect of the HHDS product is proandrogenic because the stimulation of cell growth is most profound in the androgen-responsive cells, and that bicalutamide blocks most, but not all, of the proliferative effects of the HHDS product. The data in Fig. 4 suggest a second nonandrogenic growth stimulatory effect of the HHDS, resulting in the activation of an alternative growth pathway that effectively bypasses the androgen receptor, causing resistance to the prostate cancer growth inhibitory actions of bicalutamide. To our knowledge, this is a new type of adverse effect from HHDS. Based on clinical data and the cell

Fig. 3. The HHDS product enhances prostate cancer cell proliferation in a dose-dependent manner. Cell growth curves with 95% confidence intervals for LNCaP, PC-3, and DU-145 treated with serially increasing doses of the HHDS product (100, 200, 400, 600, 800, and 1,000 µg/mL) or no drug (control) are shown. Cell growth is expressed as the relative increase in number of cells compared with medium without HHDS product. These assays were done in triplicate and repeated in three independent experiments. Differences were assessed using ANOVA (SPSS, version 13.0). A P value of ≤0.05 were considered significant.

Fig. 4. The HHDS product activates an alternative growth pathway that effectively bypasses the androgen receptor, causing resistance to the prostate cancer growth inhibitory actions of bicalutamide. Cell growth curves with 95% confidence intervals for LNCaP treated with either the antiandrogen bicalutamide alone (0.01 µmol/L) or the HHDS product (100 µg/mL) with increasing doses of the antiandrogen bicalutamide (0.01 to 1 µmol/L). The cell growth is expressed as the relative increase in number of cells compared with HHDS product alone (100 µg/mL). These assays were done in triplicate and repeated in three independent experiments as described above.
culture experiments, we filed an adverse event report with the Food Drug Administration. After acquiring an affidavit from both patients, a Food Drug Administration field officer collected their medical records as well as the HHDS. The Food Drug Administration issued a warning letter “Labeling/Promotional Claims False and Misleading/New Drug/Misbranded” leading to the removal of this HHDS from the market by the manufacturer. This not the first instance in which HHDS have caused potentially serious harm. In June 2000, for example, Nortier and colleagues (16) reported an outbreak of urinary tract cancers in Belgium among users of a Chinese herbal product that contained aristolochic acid, a known carcinogen found in a herb called Aristolochia fangchi. Moreover, germander has been shown to be associated with acute hepatitis, comfrey has been associated with hepatic veno-occlusive disease, kava kava with liver toxicity, yohimbine with seizures and renal failure, PC-SPES with endocrinological toxicity, and ephedra with cardiovascular death. The potential for harmful effect of HHDS on patient health (i.e., side effects, decreased compliance, and drug-HHDS interaction) indicates a need for improved physician-patient communication and patient education on alternative therapies. Documentation of HHDS use should become part of routine assessment for all patients, particularly cancer patients. If physicians are aware that patients are using or combining these agents with conventional treatment, they can assist them in making more informed choices and monitor them for possible interactions and side effects. For example, currently, the diagnosis of prostate cancer is a contraindication to testosterone replacement therapy. Moreover, all hypogonadal men have to be screened for prostate cancer before initiation of testosterone replacement therapy and need to be followed with frequent digital rectal and T-PSA examinations during testosterone treatment (17–19). Given that testosterone supplements are in high demand, there is significant concern that HHDS other than the one evaluated in the current study may pose an urgent human health risk. Expanded research initiatives are required to define the mechanism, safety, and efficacy of common HHDS. Finally, evidence-based information regarding side effects, contraindications, and drug interactions should be made easily accessible to patients through the internet.

References
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