EFFECTS OF A MUSHROOM MYCELIUM EXTRACT ON THE TREATMENT OF PROSTATE CANCER

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ABSTRACT

Objectives. To determine whether supplemental amounts of a polysaccharide/oligosaccharide complex obtained from a shiitake mushroom extract (SME) would lower the prostate-specific antigen (PSA) level in patients with prostate cancer.

Methods. A total of 62 men (mean age 73.2 years, range 53.6 to 85.5) with histologically proven prostate cancer who had two consecutive elevated PSA readings were accrued to the study during a 3-month period. This was an open-label study in which the patients received oral administration of capsules containing SME given three times daily for 6 months. The endpoint for the trial was the lowering of the PSA levels.

Results. Of the 62 men enrolled in the study, 61 were assessable. At 4 months, 1 patient withdrew because of unrelated surgery and 7 withdrew because of disease progression; none had responded with a decrease of greater than 50% in the PSA level. By 6 months, a total of 23 patients had progression and none had responded. Thirty-eight patients had stable PSA levels after 6 months. Although not the primary endpoint of the study, in other studies these patients could have been included as responders. When the patients’ rates of PSA rise before study entry were analyzed, 4 (7%) had stabilized disease while taking SME. Thus, the final results for our study patients were 0 with a complete response, 0 with a partial response, 4 (7%) with stable disease, and 23 of 61 with progression while taking SME.


The number of people using complementary and alternative medicine (CAM) to augment their health and cancer care needs is growing,1 but research into the use patterns and efficacy of these therapies has been minimal. CAM is defined as “therapies used along with conventional medicine that are noninvasive, pleasant, stress-reducing, and can be used in states of sickness or health.”2

However, often lacking is objective information to assess whether some or any of these therapies are efficacious. Although the publication rate of clinical trials assessing CAM therapies has increased, it still is a relatively small database from which to draw conclusions about CAM efficacy.3 Patients are spending considerable time and money on remedies that are often unproven. In 1990, an estimated 425 million visits to CAM providers in the United States were reported, exceeding the number of annual visits to all primary care physicians (388 million).4 Out-of-pocket expenditures for CAM therapies in 1990 were an estimated $10.3 billion. The prevalence of CAM use among adult patients with cancer is estimated to be approximately 31%, on the basis of a recent systematic review of 26 surveys from 13 countries.5 Among men with, or at a high risk of, prostate cancer (CaP), CAM usage was approximately 26%.6

Shiitake mushrooms are among the most consumed mushrooms in the world and have been used in traditional medicine in Asia for more than 2000 years.7 Active polysaccharide compounds...
have been isolated from the fruiting body, mycelia, and culture medium of various preparations of *Lentinus edodes* (shiitake mushroom). Shiitake mushrooms and cultured *Lentinus edodes* mycelium contain lentinan and krestin (PSK), beta-1,3-glucans. Their antitumor mechanisms are proposed to be mediated primarily by T cells and macrophages.\(^8\)

We selected a shiitake mushroom extract (SME) to test objectively as a CaP treatment. This selection was based on our review of the existing research data,\(^8\) in light of the mushroom’s popularity as a CAM treatment for cancer, and because SME was available through the Internet for the treatment of CaP at approximately $300 per month.

### MATERIAL AND METHODS

#### NUDE MICE STUDIES

Sixteen 5-week-old PC-3-bearing nude mice were divided into two groups and used in this study. After housing for 1 week, all 16 mice were inoculated subcutaneously with PC-3 cells (3 million per mouse in 0.2 mL phosphate-buffered saline mixed with 0.1 mL matrigel). From day 1, 8 mice in the SME group were treated daily with water containing 10% SME at a volume of 0.1 mL/10 g body weight. The company had showed that this dose induced activation of natural killer cells (macrophages, lymphocytes and lymphokine-activated killer cells) to produce tumor necrosis factor-alpha, interferon-gamma, interleukin-1, interleukin-2, lymphokine-activated killer cells). SME was given in hard-shell capsule form on a weight basis. The dose chosen was 8 g/day (16 capsules) for a 70-kg man according to the results of Japanese clinical studies. The dose was increased or decreased at the rate of 1 g/10 kg body weight. The company had showed that this dose induced activation of natural killer cells (macrophages, lymphocytes and lymphokine-activated killer cells) to produce tumor necrosis factor-alpha, interferon-gamma, interleukin-1, interleukin-2, and interleukin-12. Patients returned to the clinic at 1, 2, 4, and 6 months to be monitored.

### HUMAN STUDIES

This study was a nonrandomized, open-label trial in patients with a diagnosis of CaP. Patients were eligible for study if they had histologic confirmation of CaP and an elevated prostate-specific antigen (PSA) level on two consecutive readings. Sixty-two men with the diagnosis of CaP and elevated PSA levels were entered in the study. The demographic data, any previous treatment, Gleason score (an index of CaP), and SME dosage are shown in Table I. The localized disease category included men in whom radical prostatectomy or radiotherapy had failed on the basis of elevating PSA levels. It also included 8 patients who were on active surveillance, 19 patients receiving some form of hormonal therapy, and 10 who had received more than one treatment modality. In all cases, the PSA level was elevated. The aim of the study was to lower, not stabilize, the PSA readings, and the endpoint criteria for PSA response was similar to that used in routine clinical trials. The criteria used were a complete response, represented by an undetectable PSA level; partial response, represented by a decrease of greater than 50%; stable response, represented by a 50% change or less in PSA level; and progression, represented by an increase of 50% or more. PSA levels were obtained at 0, 1, 2, 4, and 6 months.

### PRODUCTION STANDARDIZATION

The preparation of SME from shiitake mycelium was produced by culture in a liquid medium that included rice bran for 3 weeks followed by the addition of enzymes that break down the walls of the basidiomycetes and result in the release of the active ingredients from the mycelia of shiitake. The SME includes oligosaccharide (alpha-1,4-glucan), polysaccharide (beta-1,3-glucan), and proteins. The SME is manufactured under the strict control of HACCP and ISO9000. SME standardization is monitored by keeping gene-stable seeds of shiitake mushrooms. All the final products are measured for active components (8% to 10% oligosaccharide, 4% to 5% polysaccharide, and 11% to 14% proteins).

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### STATISTICAL ANALYSIS

The observed proportion of men with a clinical response was calculated and an exact 95% two-sided confidence interval obtained. In the secondary analyses, exact confidence intervals were also obtained for observed proportions for other characterizations of outcome. Finally, the longitudinal patterns of change in PSA for the duration of the study were characterized using random-effects regression models for repeated measures. This approach allowed use of all available data for each patient, including the pretreatment PSA measurements. The models allowed for both differing pretreatment levels and different overall rates of change in PSA, by including person-specific random effects that were assumed to follow a bivariate normal distribution. Because the PSA measurements had a skewed distribution, they were log-transformed before analysis, and the results were interpreted as the percentage of change. Two possible models for SME extract

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**TABLE I. Patient demographics and dosage**

<table>
<thead>
<tr>
<th>Status</th>
<th>Patients (n)</th>
<th>Mean Age (yr)</th>
<th>Gleason Score</th>
<th>Mean PSA</th>
<th>Mean Capsules/Day*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localized disease</td>
<td></td>
<td></td>
<td>2–4</td>
<td>5–7</td>
<td>8–10</td>
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<tr>
<td>After RRP</td>
<td>15</td>
<td>70.1</td>
<td>0</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>After RT</td>
<td>19</td>
<td>70.0</td>
<td>1</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Surveillance</td>
<td>7</td>
<td>77.6</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off-cycle (hormonal)</td>
<td>5</td>
<td>70.8</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Hormonal refractory</td>
<td>15</td>
<td>71.2</td>
<td>0</td>
<td>7</td>
<td>4</td>
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<tr>
<td>Surveillance</td>
<td>1</td>
<td>83.7</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Key: PSA = prostate-specific antigen; RRP = radical retropubic prostatectomy; RT = radiotherapy.

*One capsule = 500 mg.
were examined. In the first, the effect was assumed to take effect in the first month and provide a fixed change for the entire duration of the follow-up (constant effect while receiving treatment). In the second model, the effect was assumed to increase steadily during follow-up, affecting the person's rate of change in PSA level. Finally, the random effects models were repeated, adjusting for disease status, age, and Gleason score.

RESULTS

PC-3–BEARING NUDE MICE

All 16 mice were alive at day 50. The results are shown in Figure 1. From day 19, when SME treatment was stopped, to day 33, when treatment was started again, tumors grew at approximately the same rate for the control and treated animals. From day 33 to day 50, after therapy reintroduction, the growth of the tumors in the treated animals was considerably slower.

In follow-up experiments, we examined the results of PC-3 cell lines to treatment with SME and a genistein-containing compound alone or in combination with regard to their ability to alter production of vascular endothelial growth factor or p21. Although neither agent alone had a substantial effect on vascular endothelial growth factor or p21 production, the results of the combination were dramatic. A major reduction in vascular endothelial growth factor production and a marked increase in the production of p21 (unpublished data) were noted.

The production of apoptosis was marked for the genistein-containing compound but not for SME (data not shown). These same data were obtained after immunoblot analysis of the treated PC-3 tumor tissues. The results with regard to p21 and vascular endothelial growth factor production mimicked those found in the PC-3 cell lines treated in vitro. The apoptosis results were also similar.

HUMAN STUDIES

Primary Outcome. Of 62 men enrolled in the study, 61 were assessable at 6 months. One patient withdrew from the study after an unrelated operation. At 4 months, of 61 patients, 7 had progression and discontinued the study; none had an objective response. Of the 54 remaining patients at 6 months, 16 had progression and none had responded. Thus, of the 61 patients, none had responded (95% confidence interval 0% to 5.9%) and 23 had progression (38%, 95% confidence interval 26% to 51%).

Secondary Analyses. In the secondary analyses, the patients were analyzed with regard to their extent of disease at presentation and considering other outcome measures. Of the 62 patients, 41 had localized CaP and 21 had metastatic disease (Table II). Seven of the patients with localized CaP were on active surveillance; the remaining 34 were roughly equally divided between radical prostatectomy only and radiotherapy only. Only 1 of the 21 patients with metastatic disease was on surveillance; most of the others had hormone-refractory disease. No patient, regardless of disease status at presentation, had a reduction in PSA level greater than 50% at either 4 or 6 months.

Random effects regression models using all 61 analyzable patients showed an overall increase in PSA of about 6.4%/mo, on average (P <0.001), during the time of observation for this study, including before treatment. No appreciable differ-
ence was found in level of PSA after treatment initiation or in the rate of change in the PSA level after treatment, with the pretreatment rate of increase about 0.3 percentage points lower (6.4% versus 6.7%, \( P = 0.78 \), not statistically significant). The patients' baseline levels of PSA and their estimated overall rates of change during the study varied significantly, with higher levels at baseline generally associated with more rapid increases. Additional models looked at the extent to which treatment might have slowed a pre-existing rate of change after accounting for other patient characteristics. Some of the variation in the rate of increase in PSA could be accounted for by the extent of disease or age of the patient. The rate of increase in PSA dropped as the patient age went up and was substantially greater for patients with metastatic disease or higher Gleason scores. However, the estimated impact of treatment on the rate of change was not materially affected by including these terms in the model and remained small and not statistically significant (results not shown).

**COMMENT**

The need for an effective nontoxic therapy for patients in whom local therapy fails or who are pursuing active surveillance is highly desirable. The prevalence of CAM use for cancer treatment and the testimonial but nonobjective reports from Japan are what stimulated this study. Regarding CAM prevalence, the total 1997 out-of-pocket expenditures for CAM were estimated at $27 billion, a level comparable to the total projected out-of-pocket expenses for all physician services in the United States.

Widespread use of nutritional and/or botanical supplements for CaP treatment and prevention is not surprising. Two well-controlled and highly publicized clinical studies have reported benefits from supplementation of vitamin E\(^\text{10,11}\) and selenium.\(^\text{12}\) Suggestive evidence has also been found for botanical supplements of garlic,\(^\text{13}\) soy,\(^\text{14}\) lycopene,\(^\text{15}\) and saw palmetto.\(^\text{16}\)

Although not spectacular, the nude mice results with SME and data from other studies both supported the efficacy and safety sufficiently that an open-label clinical trial appeared warranted. The test material was indeed safe, and we had no adverse reactions. The endpoint response criteria used to test this SME were as strict as those used to test any other new chemotherapeutic agent or regimen. It was our belief that if activity were evidenced in this pilot study, a dose escalation and randomized clinical trial would be warranted. However, without such evidence of effectiveness, the expense of such a study did not appear justified.

Using the criterion of a decrease in PSA level by greater than 50% (partial or complete response), no patients met the criterion. Although this might have been anticipated for our patients with relatively advanced disease, it was especially disappointing that none of the patients with presumed low-volume disease responded.

Having rejected the initial hypothesis, a secondary analysis was conducted to determine whether patients who had a rising PSA at the time of entry had stabilized during their 6 months of treatment. Four patients (7%) did so.

A quality-of-life (QOL) questionnaire based on that used in the Southwest Oncology Group studies was given at initiation and 4 and 6 months later. All the initial QOL questionnaires were completed. However, the 4 and 6-month QOL questionnaires had sporadic compliance. This sporadic compliance corresponded with the patients' reluctance to perform the follow-up questionnaires after they became aware that their PSA levels had not altered during the course of the study. We believed that these QOL data were not valid and therefore did not report them.

### TABLE II. Baseline vs. 6-month PSA level

<table>
<thead>
<tr>
<th>Status</th>
<th>Patients (n = 41)</th>
<th>Range (ng/mL)</th>
<th>Mean (ng/mL)</th>
<th>Patients (n = 38)</th>
<th>Range (ng/mL)</th>
<th>Mean (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline PSA</strong></td>
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<tr>
<td>Localized disease</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>After RRP</td>
<td>15</td>
<td>0.3–72.9</td>
<td>10.5</td>
<td>14</td>
<td>0.3–69.7</td>
<td>12.8</td>
</tr>
<tr>
<td>After RT</td>
<td>19</td>
<td>0.7–55</td>
<td>12.3</td>
<td>17</td>
<td>0.52–61</td>
<td>14.7</td>
</tr>
<tr>
<td>Surveillance</td>
<td>7</td>
<td>3.8–35.8</td>
<td>13.9</td>
<td>7</td>
<td>3.9–30.5</td>
<td>14.1</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off-cycle (hormonal)</td>
<td>5</td>
<td>0.8–26.8</td>
<td>10.7</td>
<td>5</td>
<td>1.02–22.4</td>
<td>15.2</td>
</tr>
<tr>
<td>Hormonal refractory</td>
<td>15</td>
<td>1.1–293</td>
<td>41.5</td>
<td>9</td>
<td>2.3–91.1</td>
<td>29.3</td>
</tr>
<tr>
<td>Surveillance</td>
<td>1</td>
<td>23.3</td>
<td>—</td>
<td>1</td>
<td>30.6</td>
<td>—</td>
</tr>
</tbody>
</table>

**Abbreviations as in Table I.**
CONCLUSIONS

The results of this study show that SME did not reduce PSA levels by 50% in any patient and thus is not an effective treatment for CaP when given alone. In addition, the results demonstrate that claims for CAM, particularly for herbal and food supplement remedies can be easily and quickly tested. To do so should be mandatory before patients spend large sums of money on unproven treatments.

REFERENCES