Maitake D-Fraction: A Potent Mushroom Extract Product Against Human Malignancies

by Sensuke Konno, PhD
Department of Urology, New York Medical College, Valhalla

The medicinal aspects of mushrooms have been known for centuries in the Orient, and the Chinese and Japanese people have long consumed a wide variety of mushrooms. However, Western people have neither known nor gained from the potential benefits of these mushrooms until recently. One reason for this disparity arises from misunderstanding or misconception about the nature and properties of mushrooms. Since mushrooms belong to the family of “fungi,” many people have a general perception that mushrooms lack nutritional value while others have a less common but interesting thought that eating mushrooms may make one vulnerable to Candida or yeast infections. Neither view is now known to be valid, and to the contrary, mushrooms are rich in vitamins, minerals, amino acids, and fibers, but low in fat, cholesterol, and calorie content. In addition, scientific and clinical research on mushrooms over the past 20 years has revealed a number of their medicinal properties that might provide remarkable health benefits. A particular focus of such research has been on “maitake” (Grifola frondosa), an edible, tasty mushroom, which literally means “dancing mushroom.” It is a giant mushroom that often reaches 20 inches in diameter and may weigh up to 100 pounds. This mushroom has been available by cultivation since the mid-1980s, enabling scientists to study its medicinal properties and being widely available for public consumption. Many physiological benefits of maitake have been postulated, ranging from immunomodulatory and antitumor activities to treatment for hypertension, diabetes, hypercholesterolemia, obesity and hepatitis B infection. Its antiviral activity against human immunodeficiency virus (HIV)/AIDS was also confirmed by the US National Cancer Institute in 1992.

Maitake D-Fraction: Bioactive β-Glucan Extracted from Maitake

Most research on maitake mushroom has been performed using the bioactive extract product, namely “Maitake D-fraction,” to assess its potential efficacy on various human malignancies. The main active component of this unique D-fraction is the protein-bound polysaccharide, consisting of either β-1,6-linked glucan with β-1,3 branches or β-1,3 glucan branched with β-1,6 glucosides. It is a hot water-extractable fraction with a molecular weight of ~1 x 10^6 dalton and is prepared by a standardized procedure developed by Maitake Products, Inc. (Ridgefield Park, New Jersey). The D-fraction has demonstrated the most potent immune enhancement and antitumor activity regardless of the route of administration (oral or injection), resulting in the highest reduction rate in cancer proliferation. For instance, D-fraction has been shown to have an antitumor effect on tumor-bearing mice, with the enhanced cytotoxic activity of macrophages and the elevated interleukin-1 production leading to the activation of cytokine T-lymphocytes (CTL). These findings are highly suggestive that D-fraction acts not only through direct activation of various immune effectors (macrophages, CTL, natural killer cells, etc.) but also through potentiating the activity/production of various lymphokines.

### Table 1. Combined Effects of BCNU and D-fraction on Cell Viability and Gly-I Activity in PC-3 Cells

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Cell Viability at 24 h (%)</th>
<th>Gly-I Activity at 6 h (μmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>0.75 ± 0.02</td>
</tr>
<tr>
<td>+BCNU (50 μM)</td>
<td>48</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td>+D-fraction (60 μg/ml)</td>
<td>100</td>
<td>0.71 ± 0.03</td>
</tr>
<tr>
<td>+BCNU (50 / D-fraction (60)</td>
<td>11</td>
<td>0.14 ± 0.02</td>
</tr>
</tbody>
</table>

* Mean ± SD (standard deviation)

### Table 2. Effects of Various Natural Extracts on Cell Viability of PC-3 Cells

<table>
<thead>
<tr>
<th>Extracts / β-Glucan</th>
<th>Vitamin C (μM)</th>
<th>Cell Viability (% of Control) at 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-G** / ARBX (1 mg/ml)*</td>
<td>60</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>ARS (1 mg/ml)*</td>
<td>150</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>Y-G** / ABW (1 mg/ml)*</td>
<td>18.6</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>D-fractionb / PLMP (1 mg/ml)*</td>
<td>60</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>HC2 (1 mg/ml)*</td>
<td>200</td>
<td>100%</td>
</tr>
</tbody>
</table>

* Both “M-G” and “Y-G” are a mixture of β-glucan and Vitamin C at the specific ratio:
  Stock “M-G” containing “47.6 mg/ml β-glucan and 257 mM Vitamin C;
  Stock “Y-G” containing “370 μg/ml β-glucan and 48 mM Vitamin C.

b Thus, the concentrations of Vitamin C increase proportionally as those of β-glucan increase.

### Safety of D-Fraction

A critical and substantial question about Maitake D-fraction is its safety, which must be adequately addressed. D-fraction has been tested on mice, confirming its safety with no toxicity or adverse effects. A non-randomized clinical study of D-fraction on 165
patients with various types of advanced cancers showed the significant improvements in their clinical status without any side/adverse effects. In addition, side effects of patients receiving chemotherapy were ameliorated when D-fraction was given simultaneously. Adverse symptoms such as nausea, hair loss and leukopenia were alleviated in 90% of patients, while a reduction in pain was reported in 83% of patients. This finding suggests that D-fraction should be considered a valuable adjuvant in ongoing cancer chemotherapy.

Safety of D-fraction is further supported by the fact that the FDA has exempted D-fraction from a phase I study of toxicology. In 1998, the FDA granted Maitake Products, Inc. an Investigational New Drug (IND) Application to conduct a phase II pilot study using D-fraction on patients with advanced breast and prostate cancer. These studies are currently underway at several institutions/hospitals, and other independent institutions are also planning to conduct similar trials.

D-Fraction: Potential Apoptosis Inducer
As mentioned above, the antitumor effect of D-fraction appears to be primarily attributable to its potent immunostimulatory activity; however, the exact mechanism has not yet been completely elucidated. In fact, the recent study on prostate cancer (CaP) cells demonstrates that D-fraction is capable of inducing "apoptosis" (programmed cell death) in these CaP cells, which may also account for another antitumor mechanism of D-fraction.

To explore a more effective and alternative modality for CaP treatment due to the poor efficacy of conventional therapies, we have conducted a study of D-fraction on human prostatic cancer PC-3 cells (the most aggressive and metastatic cancer cells) in vitro. Such studies showed that D-fraction (480 pg/ml) was capable of inducing severe (>95%) cell death in PC-3 cells within 24 h. In situ hybridization then confirmed that D-fraction-induced cell death had resulted most likely from apoptosis (Fig. 1). Thus, this finding suggests that D-fraction could be considered a potential apoptosis inducer. Whether vitamin C might potentiate such an apoptotic ability of D-fraction was also examined, because Vitamin C had been proposed to modulate the D-fraction bioactivity. As little as 30 to 60 µg/ml of D-fraction combined with 200 µM of Vitamin C was found to be nearly as effective as 480 µg/ml of D-fraction alone, resulting in >90% cell death in 24 h. Because a given concentration (200 µM) of Vitamin C alone had no effect on cells, it might have primarily served to potentiate the bioactivity of D-fraction. These results show that even the relatively low concentrations of D-fraction can be synergistically potentiated with Vitamin C to become highly cytotoxic to PC-3 cells, ultimately inducing apoptosis. This is also consistent with several clinical reports (unpublished) describing that D-fraction appeared to work cooperatively with Vitamin C in certain cancer patients.

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Chemosensitizing Effect of D-Fraction

We also explored a possible chemosensitizing effect of D-fraction on anticancer drugs. Due to the total failure of chemotherapy in CaP patients, an improved efficacy of chemotherapy is urgently required. Possible cellular effects of several anticancer agents currently used in CaP treatment, including 5-fluorouracil (5-FU), methotrexate (MTX), etoposide (VP-16), cisplatin (CPL), mitomycin C (Mit.C) and carmustine (BCNU), were examined on PC-3 cells. Such studies showed that 5-FU, MTX and BCNU were found to induce an ~50% reduction in cell viability in 72 h, whereas VP-16, CPL, and Mit.C had no effect. Although the combinations of 5-FU, MTX and BCNU appeared to further (additively) enhance an individual cytotoxicity, no such potentiation was seen by any three combinations.

Yet to find an alternative way to improve the efficacy of three agents (BCNU, 5-FU and MTX), they were combined with D-fraction and their effects on PC-3 cells were assessed after 24 h. BCNU (50 μM) by itself caused an ~50% cell viability reduction while no cytotoxic effect was seen with D-fraction (60 μg/ml) alone. However, when BCNU was combined with D-fraction, cell viability then declined to ~10% (or ~90% cell death) (Table 1). In contrast, no cytotoxic sensitization was observed in 5-FU or MTX with D-fraction. Thus, only a cytotoxicity of BCNU was significantly potentiated with D-fraction, implying a selective chemosensitizing effect of D-fraction. In other words, D-fraction may help improve the efficacy of certain chemotherapeutic drugs/agents.

It was of interest to investigate the underlying mechanism of BCNU (with D-fraction)-induced cell death. BCNU is also known as a putative inhibitor of glyoxalase I (Gly-I), a vital detoxifying enzyme, and Gly-I has been postulated to play a regulatory role in the development/progression of prostate cancer. Accordingly, the possible effects of BCNU in combination with D-fraction on Gly-I activity were examined. After merely 6 h, Gly-I activity decreased by ~30% with BCNU (50 μM) alone and even further in combination with D-fraction (60 μg/ml), resulting in an ~80% loss in its activity (Table 1). These results show that BCNU by itself is capable of inactivating Gly-I to a certain extent but D-fraction can even further extend such an inactivation, leading to severe cell death in 24 h. Therefore, Gly-I appears to be critically involved in CaP growth/survival and its inactivation by BCNU/D-fraction may account primarily for cancer cell death.

Possible Effects of Other Natural Extracts on PC-3 Cell Growth

Currently there are various kinds of natural extracts besides D-fraction on the market, and it was tempting to examine how they might work on PC-3 cells. We obtained the following seven commercially available products for test:

- "M-G": another maitake β-glucan fraction available in US market
- "Y-G": β-glucan from yeast cell wall
- "ARBX": arabinoxylan from rice bran
- "ABSH": Agaricus blazei product offered by Company A in Japan
- "ABIW": Agaricus blazei product offered by Company B in Japan
- "PLMP": Meshima-koubo (Phellinus linteus) product from Japan
- "HC2": popular mycelial compounds from several mushrooms in Japan

PC-3 cells were cultured with varying concentrations of these extracts and their effects on cell growth were assessed in 72 h. D-fraction was also included serving as a positive control, capable of inducing >90% cell death. Five products, ARBX, ABSH, ABIW, PLMP, and HC2, were found to have no effects on cancer cell growth, whereas M-G and Y-G showed significant cytotoxic effects (Table 2). However, since both M-G and Y-G have been originally prepared with Vitamin C supplement (i.e. a mixture of β-glucan and Vitamin C), their data need to be interpreted with caution. For PC-3 cells, it is known that Vitamin C by itself could have an apparent cytotoxic effect when its concentrations go beyond 500 μM. With normalizing the conditions/concentrations of M-G, Y-G and D-fraction for comparison (Table 2), we estimate that M-G and Y-G required the concentrations of ~150 μM β-glucan with 720 μM Vitamin C and ~18.6 μM β-glucan with 2400 μM Vitamin C, respectively, in order to induce equivalent cell death (>90%) attained with D-fraction (60 μg/ml β-glucan with 200 μM Vitamin C). Separate study confirmed that such high Vitamin C concentrations (in M-G and Y-G) by

Figure Legend

Fig. 1. Induction of apoptosis by D-fraction in PC-3 cells. Control and D-fraction-treated PC-3 cells at 24 h were evaluated for apoptosis using in situ hybridization assay. Control cells show no brown stain (A), while >90% of D-fraction-treated cells have positive brown stains (B), indicating apoptotic cell death.
themselves indeed caused >90% cell death in PC-3 cells. Thus, it should be carefully assessed whether cytotoxic effects exerted by these two samples might have resulted primarily from their active ingredient (β-glucan) or from the exceeded amount of Vitamin C supplement. "Pure" β-glucans from M-G and Y-G are required and tested for their actual effects on PC-3 cells.

In contrast, as shown above, cell death (>90%) induced by "D-fraction (60 µg/ml β-glucan) with Vitamin C (200 µM)" is most likely due to a synergistic potentiation of β-glucan bioactivity by Vitamin C, because 200 µM Vitamin C alone is non-cytotoxic. However, it is peculiar that M-G with 60 µg/ml β-glucan and 280 µM Vitamin C had no effect (Table 2) when tested under similar condition to D-fraction. This discrepancy remains unknown but could be due to some difference in preparation of two products. Further studies are required for clarification. Nevertheless, under given conditions here, these results suggest that D-fraction thus far appears to have the most potent cytotoxic effect on PC-3 cells compared to other natural extracts tested. It should be noted that the effectiveness of various mushroom extracts/ polysaccharides is known to be cancerspecific despite the structural and functional similarities of these glucans. Similarly, some natural extracts besides mushrooms also demonstrate the cancer specificity. It cannot rule out the possibility that some of above extracts tested could be highly effective on certain human malignancies besides prostate cancer.

In the meantime, the findings of a potent cytotoxic activity of D-fraction, its potentiation with Vitamin C, and its chemosensitizing effect on certain anticancer drugs (e.g., BCNU) are significant and may have implications in clinical utility, particularly in a treatment of CaP patients.

Case Studies of D-Fraction on CaP Patients

Among ongoing clinical trials/studies of D-fraction on CaP patients, some of them deserve to be briefly mentioned here. These patients received 30 drops orally of D-fraction daily with 2000 mg Vitamin C and were evaluated for general condition, blood chemistry, and immune status (activities of natural killer cells and CTL). Additionally, the levels of serum prostate-specific antigen (PSA), a widely used biochemical marker for CaP, were measured for assessing the disease status of these patients.

The first case is a 68-year-old man diagnosed for CaP with the initial PSA of 20 ng/ml (a normal PSA range: <4.0 ng/ml) in 1998. His PSA kept steadily going up every year till 2000, indicating a possible progression of cancer. In 2001, after he started taking D-fraction (with Vitamin C), his PSA came down significantly (13 ng/ml) in 3 months. All parameters were also stable since then. This report suggests that cancer progression might have been slowed down or stopped by D-fraction.

In the second case, the initial PSA of 7 ng/ml (in 1995) in a 72-year-old patient went down to 1.7 ng/ml by 2000 following primary hormonal therapy. He appeared to respond well to a treatment and might be in "remission." However, 

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Recommended Dosage of D-Fraction

The following dosages of D-fraction for adults are recommended (but not established) at present:

- 5-6 drops of Maitake D-fraction (Grifron-Pro D-fraction®) 3 times daily for health maintenance, while 15-20 drops 3 times daily for therapeutic purpose. In addition, particularly recommended taking 1000-2000 mg of vitamin C daily with D-fraction for therapeutic purpose (e.g., cancer patients).

Conclusion

A number of basic science researches and limited clinical studies support a potent immunostimulatory, cytotoxic, apoptosis-inducing, and chemosensitizing activity of Maitake D-fraction, which appears to have a great potential in cancer treatment and prevention. Alleviation of various side effects with improved QOL is also reported in patients receiving chemotherapy in combination with D-fraction. However, since more comprehensive and controlled studies are required for the clinical assessment of D-fraction, the active participation of more health professionals and physicians managing a variety of human malignancies is advised to thoroughly evaluate this mushroom extract product in the near future.

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Correspondence:

Sensuke Konno, PhD
New York Medical College
Department of Urology
Munger Pavilion 4th Floor
Valhalla, New York 10595 USA
Fax 914-594-4428
Email: sensuke_konno@nymc.edu

References
