Maitake D-Fraction: a promising natural agent for alternative cancer treatment

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Introduction

Despite the medicinal aspects of several mushrooms having been passed down through folklore in the Orient, the scientific study of these mushrooms to explore their biological properties has been initiated only within the past 20 years. A number of researchers have particularly focused their attention on one of these mushrooms called maitake (Grifola frondosa) mushroom. "Maitake" (pronounced "my-tah-keh") is an edible, tasty mushroom and literally means "dancing mushroom." Characteristically, it is a giant mushroom that often reaches 20 inches in diameter and may weigh up to 100 pounds. This mushroom has been available through cultivation since the mid-1980s, enabling scientists/researchers to study its medicinal properties as claimed in anecdotes and folklore. Numerous physiological benefits of maitake have been postulated, ranging from antitumor effects to treatment for hypertension, diabetes, hypercholesterolemia, obesity, and hepatitis B infection. (1-9) Maitake's antiviral activity against HIV (AIDS) was also confirmed by the US National Cancer Institute in 1992. (10)

Maitake D-Fraction

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 component of this unique D-fraction is the protein-bound polysaccharide, consisting of either [beta]-1,6-linked glucan with [beta]-1,3 branches or [beta] 1,3 glucan branched with [beta]-1,6 glucosides. It is a hot water-extractable fraction with a molecular weight of ~1 x [10.sup.6] dalton (1,11) and is prepared by a standardized procedure. 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. (12,13) For instance, D-fraction has been shown to have an antitumor effect on tumor-bearing mice, (12) with the enhanced cytotoxic activity of macrophages and the elevated interleukin-1 production leading to the activation of cytotoxic T-lymphocytes (CTL). (14) These fi ndings are highly suggestive that D-fraction acts not only through direct activation of various immune effectors (macrophages, CTL, natural killer cells, etc.) targeting tumor cells, but also through potentiating the activity/production of various lymphokines.

D-Fraction-Induced Apoptosis in Cancer Cells

While the antitumor effect of D-fraction may result primarily from its potent immunostimulatory activity, the recent study on prostate cancer (CaP) cells demonstrates that D-fraction is capable of inducing apoptosis (programmed cell death) in these CaP cells, (15) which may also account for another antitumor mechanism of D-fraction.

CaP is the most common malignancy with high mortality in elderly men in the United States--approximately 140,000 new cases and more than 39,000 deaths are expected annually. (16) Unfortunately, conventional therapies for CaP, such as androgen ablation, brachy-therapy (radioactive seed implants), external radiotherapy, chemotherapy, etc., are currently available but have not been able to achieve the expected level of efficacy. (17) To explore a more effective and alternative modality for CaP treatment, we have recently conducted a study of D-fraction on human prostatic cancer PC-3 cells (the most aggressive and metastatic cancer cells) in vitro. (15)

Such studies showed that D-fraction (480 mg/ml) caused severe (>95%) cell death in PC-3 cells, via elevated oxidative stress leading subsequently to DNA fragmentation. In situ hybridization (Fig. 1) then confirmed that D-fraction-induced cell death had resulted most likely from apoptosis. This finding suggests that D-fraction could be considered a possible apoptosis inducer. In addition, since vitamin C has been proposed to modulate the bioactivity of D-fraction, (18) whether vitamin C might potentiate the apoptotic ability of D-fraction was examined. As little as 30-60 mg/ml of D-fraction combined with 200 mM of vitamin C was found to be nearly as effective as 480 mg/ml of D-fraction alone, resulting in >90% cell death. Because vitamin C (200 mM) alone had no effect on cells, it might have primarily served to potentiate the bioactivity of D-fraction. Therefore, these results show that D-fraction can be synergistically potentiated with vitamin C to become highly cytotoxic to PC-3 cells, inducing apoptosis. Th is is consistent with several clinical reports (unpublished) describing that D-fraction appeared to work cooperatively with vitamin C in certain cancer patients.

Chemosensitizing Effect of D-Fraction

We were also interested in exploring a possible chemosensitizing effect of D-fraction on anticancer drugs. Due to the total failure of chemotherapy in the CaP treatment, (17) an improved efficacy of chemotherapy is urgently required. Possible cellular effects of several anticancer agents currently used in CaP treatment, (19) such as 5-fluorouracil (5-EU, 5 mg/ml), methotrexate (MTX, 100 mM), etoposide (VP-16, 100 nM), cisplatin (CPL, 100 mM), mitomycin C (Mit.C, 300 nM) and carmustine (BCNU, 50 mM), were examined on PC-3 cells. After cells were treated with these agents for 72 hours, 5-EU, MTX and BCNU were found to induce an ~50% reduction in cell viability, whereas VP-16, CPL, and Mit.C bad no such effect. Since these results suggested that the combinations of 5-FU, MTX and BCNU might further potentiate an individual cytotoxicity, such possibility was tested next. However, any combinations of 5-EU, MTX and BCNU failed to enhance their cytotoxicity, and cell viability yet remained at ~50%.

To find an alternative way to improve the efficacy of three agents (BCNU, 5-FU and MTX), we examined the combined effects of D-fraction and these agents. PC-3 cells were cultured with three agents in combination with 30 or 60 mg/ml of D-fraction for 24 hours. No cytotoxic effect was seen with D-fraction (30 or 60 mg/ml) alone; however, when BCNU (50 mM) was combined with them, cell viability declined from ~50% (BCNU alone) to -10% (with 60 mg/ml D-fraction) (Table 1). In contrast, no such sensitization of cytotoxicity was observed in 5-FU or MTX with D-fraction. Thus, only a cytotoxicity of BCNU was significantly potentiated or

enhanced 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.

We further explored the underlying mechanism of BCNU (with D-fraction)-induced cell death. BCNU is also known as a putative inhibitor of glyoxalase I (Gly-I), (20) a detoxifying enzyme, and Gly-I has been postulated to play a key role in the development/progression of prostate cancer. Accordingly, the possible effects of BCNU and its combination with D-fraction on Gly-I activity were examined. After merely 6 h, Gly-I activity decreased by ~30% with BCNU (50 mM) alone and even further in combination with D-fraction (60 mg/ ml), resulting in an ~80% loss in its activity (Table 1). Thus, BCNU by itself is capable of inactivating Gly-I to certain extent but D-fraction can even further extend such Gly-I inactivation, leading to severe cell death in 24 hours. These studies demonstrate that Gly-I is critically involved in CaP growth/survival, and its inactivation by BCNU/D-fraction may primarily account for cancer cell death. Moreover, as BCNU is also used clinically in patients with brain tumor, (21) the BCNU/D-fra ction combination could even be applicable to treatment of patients with prostate cancer, brain tumor, or other human malignancies. Such a clinical feasibility deserves further investigations.

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 P0-3 cells. We obtained the following seven commercially available products for test:

* "M-G": another maitake [beta]-glucan fraction available in US market

* "Y-G": [beta]-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-kobu (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 hours. 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 [beta]-glucan and Vitamin C), their data need to be interpreted with caution. For PC-3 cells, it is found that vitamin C by itself could have an

apparent cytotoxic effect when its concentrations go beyond 500 mM. With normalizing the conditions/concentrations of M-G, Y-G and D-fraction for comparison (Table 2), we estimated that M-G and Y-G had required the concentrations of 150 mg/ml [beta]-glucan with 720 mM vitamin C and 18.6 mg/ml [beta]-glucan with 2400 mM vitamin C, respectively, in order to induce equivalen t cell death (>90%) attained by D-fraction (60 mg/ml [beta]-glucan) with 200 mM vitamin C. Our separate study also confirmed that such high vitamin C concentrations (720 mM in M-G and 2400 mM in Y-G) by themselves indeed caused nearly 100% cell death in PC-3 cells. Therefore, it should be carefully assessed whether cytotoxic effects exerted by these two samples might have resulted exclusively from their active ingredient ([beta]-glucan) or from the excess amount of vitamin C supplement. "Pure" [beta]-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 mg/ml [beta]-glucan) with vitamin C (200 mM) is most likely due to a synergistic potentiation of [beta]-glucan bioactivity by vitamin C, because 200 mM vitamin C alone is indeed non-cytotoxic. It is yet peculiar that M-G with 60 mg/ml [beta]-glucan and 280 mM vitamin C had no effect (Table 2) when tested under similar condition to D-fraction. This discrepancy remains unexplained but could be due to some difference in preparation of two products. Further investigations are required for clarification. Taken altogether, under given conditions here, these results indicate 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 yet noted that the effectiveness of various mushroom extracts or polysaccharides is known to be cancer-specific despite the structural and functional similarities of these glucans. (22) Similarly, some natural extracts besides mushr ooms also demonstrate cancer specificity. The possibility cannot be ruled out that some of above extracts tested could be highly effective on certain human malignancies besides prostate cancer.

In the mean time, 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 clinical implications, particularly in a treatment of prostate cancer patients.

Safety of D-Fraction

Maitake D-fraction has been tested on mice to assess its potential toxicity, confirming its safety with no toxicity or adverse effects. (23) Moreover, a non-randomized clinical study of D-fraction was conducted on 165 patients with various types of advanced cancers. Some patients also received it with chemotherapeutic drugs. Overall, the significant improvements in the clinical status of these patients were seen without any side/adverse effects of D-fraction. (23) Interestingly, many side effects of chemotherapy on patients were found to be 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. (23) 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 phase I study of toxicology. In 1998, the FDA granted Maitake Products, Inc. an Investigational New Drug (ND) Application to conduct a phase II pilot study using D-fraction on patients with advanced breast and prostate cancer. (24) These studies are currently underway at several institutions/hospitals and other independent institutions are also planning to conduct similar trials.

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[R]) 3 times daily for health maintenance

* 15-20 drops 3 times daily for therapeutic purpose

In addition, it is particularly recommended to take 1,000-2,000 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, cyto toxic, apoptosis-inducing, and chemosensitizing activity of maitake D-fraction, which appears to have a great potential in cancer treatment and prevention. Amelioration of various side effects with improved quality of life is also reported in patients receiving chemotherapy in combination with D-fraction. However, since more comprehensive and controlled trials are required for its clinical demonstration, the active participation of more health professionals and physicians managing a variety of human malignancies might be advised to thoroughly evaluate this promising natural agent in the near future.

Table 1

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

Conditions	Cell Viability at 24 h
Control	100%
+BCNU (50 [micro]M)	48%
+D-fraction (60 [micro]g/ml)	100%
+BCNU (50) / D-fraction (60)	11%

Gly-I Activity at 6 h

Conditions	([micro]mol/mg protein. (a)
Control	0.75 [+ or -] 0.02
+BCNU (50 [micro]M)	0.54 [+ or -] 0.04
+D-fraction (60 [micro]g/ml)	0.71 [+ or -] 0.03
+BCNU (50) / D-fraction (60)	0.14 [+ or -] 0.02

(a) Mean [+ or -] SD (standard deviation)

Table 2

Effects of Various Natural Extracts on Cell Viability of PC-3 Cells

Extracts	[beta]-Glucan: (mg/ml)	Vitamin C (mM)	Cell Viability (% of Control) at 72 h
M-G (a)	60	280	100%
M-G (a)	150	720	<10
Y-G (a)	18.6	2400	<10
D-fraction (b)	60	200	<10
ARBX (1 mg/ml) (c)			100
ARSH (1 mg/ml) (c)			100
ABIW (1 mg/ml) (c)			100
PLMP (1 MG/Ml) (c)			100
HC2 (1 mg/ml) (c)			100

(a)Both "M-G" and "Y-G" are a mixture of [beta]-glucon and Vit.C at the specific ratio: Stock "M-G" conaining "47.6 mg/ml [beta]-glucan and 227 mM Vit.C" Stock "Y-G" containing "370 [micro]g/ml [beta]-glucan and 46 mM Vit.C" Thus, the concentrations of Vit.C increase proportionally as those of [beta]-glucan increase.

(b)"D-fraction" is run as a positive control (reference).

(c)Due to a significant insolubility of these extracts, the concentrations used here should be considered the "estimated" values.

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REFERENCES

(1.) Mizuno T, et al. "Maitake, Grifola frondosa: Pharmacological effects." Food Rev Int, 1995; 11:135-149.

(2.) Preuss H, et al. "Syndrome X, hypertension, and maitake mushroom." Int J Integrative Med. Nov/Dec 1999; 1:42.

(3.) Kubo K, et al. "Anti-diabetic activity present in the fruit body of Grifola frondosa (maitake.)." Biol Pharm Bull, 1994; 17:1106-1110.

(4.) Kubo K, et al. "The effect of maitake mushrooms on liver and serum lipids." Altern Ther Health Med, 1996; 2:62-66.

(5.) Jones K. "Maitake, A potent medicinal food." Altern Complemnt Ther, 1998; 4:420429.

(6.) Kabir Y, et al. "Effect of shiitake and maitake mushrooms on blood pressure and plasma lipids of spontaneously hypertensive rats." J Nutr Sci, 1987; 33:341-346.

(7.) Adachi K, et al. "Blood pressure-lowering activity present in the fruit body of Grifola frondosa." Chem Pharm Bull, 1988; 36:1000-1006.

(8.) Nakai R, et al. "Effect of maitake (Grifola frondosa.) water extract on inhibition of adipocyte conversion of C3HI0T1/2B2C1 cells." J Nutr Sci Vitaminol (Tokyo.), 1999; 45:385-389.

(9.) Wu S, et al. "Therapeutic effect of Grifola polysaccharides in chronic hepatitis B." International Programme and Abstracts, International Symposium on Production and Products of Lentinus Mushroom, Quingyan, China, Nov 1994; P-18 (Abstract.),

(10.) Developmental Therapeutics Program, National Cancer Institute. "In-vitro anti-HIV drug screening results." NSC: F195001. Jan 1992.

(11.) Nanba H, et al. "The chemical structure of an antitumor polysaccharide in fruit bodies of Grifola frondosa (maitake.)." Chem Pharm Bull, 1987; 35:1162-1168.

(12.) Hishida I, et al. "Antitumor activity exhibited by orally administered extract from fruit body of Grifola frondosa (maitake.)." Chem Pharm Bull, 1988; 36:1819-1827.

(13.) Nanba H. "Antitumor activity of orally administered D-fraction from maitake mushroom." J Naturopathic Med. 1993; 1:10-15.

(14.) Adachi K, et al. "Potentiation of host-mediated antitumor activity in mice by [beta]-glucan obtained from Grifola frondosa (maitake.)." Chem Pharm Bull, 1987; 35:262-270.

(15.) Fullerton SA, et al. "Induction of apoptosis in human prostatic cancer cells with [beta]-glucan (maitake mushroom polysaccharide.)." Mol Urol, 2000; 4:7-11.

(16.) Landis SH, et al. "Cancer statistics, 1999." CA Cancer J Clin, 1999; 49:8-31.

(17.) Kreis W. "Current chemotherapy and future directions in research for the treatment of advanced hormone-refractory prostate cancer." Cancer Invest, 1995; 13:296-312.

(18.) Morishige F. "The role of vitamin C in tumor therapy (human.)." In: Vitamins and Cancer: Human Cancer Prevention by Vitamins and Micronutrients. (eds.) Meyskens FI Jr and Parasad KN. Humana Press, Clifton, NJ, 1986; pp 399-427.

(19.) Yagoda A and Petrylak D. "Cytotoxic chemotherapy for advanced hormone-resistant prostate cancer." Cancer, 1993; 71:1098-1109.

(20.) Vanhoefer U, et al. "Carbamoylation of glutathione reductase by N,N-bis(2-chloroethyl.)-N-nitrosourea associated with inhibition of multidrug resistance protein (MRP.) function." Biochem Pharmacol, 1997; 53:801-809.

(21.) Shapiro JR, et at. "Chromosome number and carmustine sensitivity in human gliomas." Cancer, 1993; 71:4007-4021.

(22.) Borchers AT, et at. "Mushrooms, tumors, and immunity." Proc Soc Exp Blot Med. 1999; 221:281-293.

(23.) Nanba H. "Maitake D-fraction: Healing and preventive potential for cancer." J Orthomol Med, 1997; 12:43-49.

(24.) Maitake Products, Inc. "Maitake D-fraction obtained IND for clinical study" (corporate publication.), Feb 1998.

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Dr. Sensuke Konno is an Assistant Professor and Director of Molecular Urology Research Laboratory at the Department of Urology, New York Medical College. His research focuses on various urological malignancies, including prostate cancer, renal cell carcinoma, and bladder cancer. Also, his laboratory is conducting biochemical studies on renal disorders! diseases, such as acute renal failure due to nephrotoxicity and ischemia, diabetic nephropathy, and nephrolithiasis (kidney stone). Specific aims of such studies are set to elucidate the underlying mechanisms of these malignancies/disorders and to establish more effective prophylactic/therapeutic modalities. Particularly his work on prostate cancer has been well recognized and published in the major urology journals. Recently, he has become interested in alternative and complementary medicine offering natural remedies using medicinal mushrooms and natural antioxidants. Clinical and basic science studies of these natural agents on urological cancers and renal di sorders are currently in progress