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Effect of *Agaricus blazei* Murrill Extract on HT-29 Human Colon Cancer Cells in SCID Mice *In Vivo*

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Abstract. *Agaricus blazei Murrill (ABM) popularly known as 'Cogumelo do Sol' in Brazil, or 'Himematsutake' in Japan, is a mushroom native to Brazil and widely cultivated in Japan for its medicinal uses and is now considered one of the most important edible and culinary-medicinal biotechnological species. This study is the first tumor growth model to evaluate the amelioratory effect of ABM extract using HT-29 human colon cancer cells in severe combined immunodeficiency (SCID) mice. Forty SCID mice were inoculated with HT-29 cells to induce tumor formation and were then divided into four groups. All the four groups (control, low, medium and high concentration treatment) of mice were separately orally administered 0 mg, 1.125 mg, 4.5 mg or 45 mg ABM extract daily. After six weeks of treatment, 8 out of the 40 mice had not survived including one mouse which scored +++ (tumor up to 15 mm diameter) and four mice which scored ++++ (tumor over 15 mm diameter) in the control group and three mice which scored ++++ on the low-dose ABM treatment. After high- or medium-dose treatment, all ten mice in each group survived. The oral administration of ABM does not prevent tumor growth, as shown by increased tumor mass, but compared with the control group, the tumor mass seems to grow more slowly depending on the ABM dose.*

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For males and females in Taiwan, the colon/rectum is the third leading primary cancer site because of the poor prognostic outcome of colorectal cancer due to its resistance to current therapies, it is the leading cause of cancer-related death.

A number of phytochemicals present in medicinal plants are known to possess substantial anticarcinogenic and anti-mutagenic activities (1-8). Successful treatment with chemotherapeutic agents is largely dependent on their ability to trigger cell death in tumor cells; therefore, novel inducers of apoptosis provide a new anticancer therapeutic approach, and certain phytochemicals present in medicinal herbs have demonstrated apoptosis induction in cancer cells (9-10).

Mushrooms and primarily basidiomycetous fungi are a popular and valuable food, low in calories and high in minerals, essential amino acids, vitamins and fiber (11); some 'medicinal' mushrooms produce substances having potential medical effects. Among the mushroom species of *Basidiomicetes*, *Agaricus blazei* Murrill (ABM), a species native to Brazil, where it is generally known as 'sun

mushroom', is often consumed as food and tea in different parts of the world recently (12-13). It has traditionally been used for the prevention of a range of diseases, including cancer, hepatitis, atherosclerosis, hypercholesterolemia, diabetes and dermatitis (14-19).

ABM antitumor activity has been shown in some reports (20-22), but there are few reports regarding HT-29 human colon cancer in severe combined immunodeficiency (SCID) mice. The purpose of this research was to examine whether ABM extract was effective against HT-29 tumor-bearing SCID mice and to determine whether the treatment effect was dependent on the concentration of ABM extract.

Materials and Methods

Experimental animals and housing conditions. SCID male mice, specific pathogen-free and 5 weeks old, were obtained from the Animal Medicine Center, College of Medicine, National Taiwan University (our own breeding colony). The animals were kept in polypropylene cages (five animals/cage) covered with metallic grids in a room maintained under constant environmental conditions, with air filter tops in a filtered laminar air flow-controlled room, with an ambient temperature of 20±2˚C, relative humidity 75±15%, and with a 12-h light-dark cycle. The mice were raised and cared for on laboratory pellet chow, given autoclaved water and fed *ad libitum* following the animal procedures approved up by the National Science Council of the Republic of China. The experiments were performed according to the law, regulations and guidelines for animal experiments in Taiwan, which are in agreement with the Helsinki declaration.

ABM extract and administration. Powdered ABM was obtained from S. Canaan Biotechnology Development Co. (Taipei, Taiwan, ROC) and 22.5 and 90 or 900 mg were separately suspended in 6 mL distilled water at 60˚C for 10 min, then cooled to room temperature and left for 5 h with stirring at 200 rpm to form the low, medium and high concentration aqueous extracts.

Colon cancer formation and experimental treatment. Forty mice in this study were inoculated with HT-29 human colon cancer cells in the dorsal area (2×10^7 cells/mouse). Around 2-5 weeks after inoculation, the mice with carcinomas of 3 mm in diameter as measured by using caliper were divided into 4 groups of 10 mice each. The mice were fed with regular diet and double-distilled water. All the four groups (control, low, medium and high concentration treatments) of mice were separately orally administered 0 mg, 1.125 mg, 4.5 mg or 45 mg ABM extract daily. After 6 weeks of treatment, all the survivors were sacrificed under anesthesia by CO_2. The colon tumor size was scored under gross examination. The tumor status was categorized into: +, tumor diameter up to 5 mm; ++ and +++, tumor diameter up to 10 mm and 15 mm respectively, and over 15 mm diameter scored ++++.

Results

Effect of ABM extract on colon cancer formation. HT-29 cells inoculation induced 3 mm diameter tumors after around 2-5 weeks in all forty mice. After 6 weeks' treatment, 8 out of the 40 mice had not survived, including one mouse scored +++ and four mice scored ++++ in the control group and three mice scored ++++ in the low-dose ABM treatment. After the high- or medium-dose treatments, all ten mice in each group survived.

By naked eye, the 40 mice had obvious tumor masses in the colon without full dissection (Figure 1). All ten mice of the control group were scored +++/++++, showing that the size of the tumor mass increased sharply to around 10 mm to over 15 mm during the 6 weeks of incubation. After low-dose ABM treatment, five mice still scored ++++, showing that low-dose treatment was not very effective. After the high- or medium-dose treatments, no mouse was scored ++++ (Table I). The oral administration of ABM did not prevent tumor growth since the tumor masses increased, but compared with the control group, the tumor mass seemed to grow more slowly with ABM treatment. Tumor growth inhibition was dependent on the ABM dose.

Discussion

Whole-mushroom extracts contain compounds including an α-1,6- and α-1,4-glucan complex and α glucomannan with a main chain of β-1,2-linked D-mannopyranosyl residues that may modulate tumorigenesis and carcinogenesis at different stages and/or may act at the same stage through different mechanisms (23, 24). A combination of distinct downstream responses involving different cell subsets could conceivably provide greater tumor inhibition than could be induced by a single polysaccharide. However, the wide number of different and only partially homogeneous ABM extracts used represents a difficult challenge for establishing the best extract and active substances. Moreover additive, or even synergistic, effects may occur while increasing fractionations of one ABM extract enhanced some biological activities, but abolished others (25).

Whilst to have anticlastogenic properties with a 100% reduction of chromatid and 144.4% reduction of isochromatid breaks, is apparently really important for cancer prevention in humans since it is usually consumed in its natural form as tea or as food (26). A aqueous extracts of ABM have variously demonstrated no clastogenic activity. While an *n*-butanolic extract was both anticlastogenic and clastogenic (26) and at different concentrations, hexane extracts were genotoxic, cytotoxic and anticlastogenic, suggesting further studies are needed (27). A strong protective effect (28-30), activation, of apoptosis (31) and no protective effect have been shown in some cell types (32-34). Additionally, differences in the cultivation, storage and extract preparation might influence the effectiveness of aqueous extracts of ABM (34).

Thus lineages and pre-treatment influence the pharmacological anticancer activity of ABM extracts and as confirmed by Manzi and Pizzoferrato (35), β-glucans,

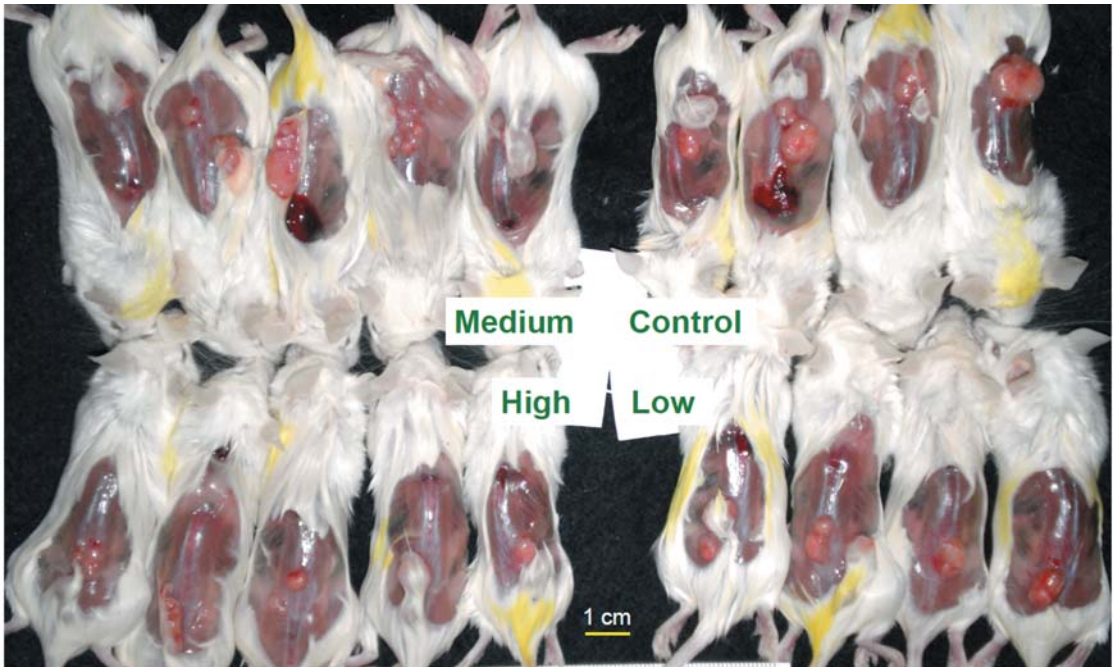

Figure 1. *The size of tumor mass varied widely after 6-week treatment with or without ABM. Representative mice of the four groups are shown.*

Table I. *Tumor size score at death or after 6-week treatment of SCID mice with or without ABM. Tumor status categorized as: +: up to 5 mm; ++: up to 10 mm, +++: up to 15 mm; ++++: over 15 mm. Low: 1.125 mg; medium: 4.5 mg; high: 45 mg ABM orally daily.*

Treatment	+		++		+++		++++	
	Survived	Died	Survived	Died	Survived	Died	Survived	Died
Control					5	1		4
Low			1		4		2	3
Medium	1		4		5			
High	2		5		3			

apparently the most important constituent, are distributed variably in mushrooms, both in the soluble and in the insoluble fraction. Luiz *et al*. (33) also suggested that fatty acids (especially linoleic and eicosapentanoic acid) in ethanol and chloroform/ methanol extracts could have a role in the antimutagenic activity.

In the present study, while ABM treatment did not stop the growth of HT-29 tumor masses, it did inhibit rapid growth. Aqueous ABM extracts given in the drinking water have demonstrated protection at the initiation step of liver carcinogenesis in rats and mice, but were ineffective when administered in the post-induction period (32, 36-37). Our next study may investigate ABM treatment prior to cancer induction. ABM fed in dry powdered form to Wistar rats exhibited significant chemopreventive influence on the promoting phase of chemical hepatocarcinogenesis (38). Among nearly 40 articles directly associated with ABM in animal studies, this study was the first to evaluate the effect of ABM extract on HT-29 cell colon cancer growth in SCID mice, and this tumor growth model performed well because colon cancer was successfully induced in all forty mice.

Although the results point to effective treatment by ABM, one should be very cautious about applying these data to human clinical studies, because this study was performed on a specific strain of mice using a HT-29 colon cancer cell line. Which substance in ABM inhibits the cells needs to be established. Moreover, a more detailed study of its specificity to organs or any other cell inoculation may be valuable in terms of clarifying its mode of action, and its antitumor effectiveness should be studied in different tumor inoculation

models. Careful clinical studies comparing the activity of isolated compounds, whole-mushroom extracts and epidemiological data are still necessary to determine whether ABM can provide real clinical benefits. Dose–response studies and isolation, as well as chemical identification and quantification of specific compounds responsible for the potential benefit from ABM should be fully developed.

In conclusion, ABM extracted by hot water led to tumor growth rate decline in the tumor growth model, *in vivo*. The treatment effect and survivor rate are dependent on the ABM dose used.

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