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Antitumor Activity of Cultured Mycelia of *Ganoderma lucidum*

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Abstract – The cultured mycelia of fungus *Ganoderma lucidum* were investigated for the inhibitory effect on the growth of s.c. transplanted Lewis lung carcinoma (LLC) in BDF-1 mice by intraperitoneal (i.p.) administration. The cultured mycelia showed antitumor activity with T/C values of 89.6 and 50.3 % at doses of 100 and 500 mg/kg, respectively, compared to adriamycin, which was used a positive control, with T/C value of 54.6 % at 2 mg/kg.
Key words – *Ganoderma lucidum*, antitumor activity, cultured mycelia, Lewis lung carcinoma

Introduction

Ganoderma lucidum KARST (Polyporaceae) is a well-known Chinese crude drug, which has been used clinically in Korea, Japan, and China. It was considered as a natural medicine for promoting longevity and maintaining the vitality of human being. Nowadays, this mushroom is used for leukopenia and has been paid much attention as a home remedy (Jiangsu New Medical College, 1978). The cultured mycelia of *G. lucidum* contained highly oxygenated lanostane-type triterpenes and sterols without polysaccharides and glycoprotiens.

Over 130 highly oxygenated and pharmacologically active lanostane-type bitter triterpenes, and related compounds have been isolated from the cultured mycelia, fruiting bodies, and spores of *G. lucidum*. Some of them have been reported to have cytotoxic activity against hepatoma HTC (ganoderic acids U, V, W, X and Y) (Toth *et al.*, 1983), hepatoma PLC/PRF/5 and KB (ganoderic aldehyde A) (Lin *et al.*, 1991), Meth-A (ganodermanondiol) and Lewis lung carcinoma (LLC) (lucidumol A) cells *in vitro* (Min *et al.*, 2000), and anti-histamine releasing activity in rat mast cells (ganoderic acids C and D) (Kohda *et al.*, 1985), inhibitory activity against angiotensin converting enzyme (ganoderic acid F) (Morigiwa *et al.*, 1986), hepatoprotective activity (ganoderic acid A) (Kim *et al.*, 1999), inhibitory effect on farnesyl protein transferase (ganoderic acid A and methyl ganoderate A) (Lee *et al.*, 1998), anticomplement activity (ganoderiol F, ganodermanondiol, and ganodermanontriol) (Min *et al.*, 2001), anti-HIV-1 activity (ganoderiol F and ganodermanontriol) (El-Mekkawy *et al.*, 1998), and inhibitory effect on protease of HIV-1 (ganoderic acid β) (Min *et al.*, 1998).

The polysaccharides and glycoproteins isolated from the fruiting bodies of *G. lucidum* showed the antitumor and immunomodulatory activities (Miyazaki and Nishijima, 1981; Maruyama *et al.*, 1989; Lieu *et al.*, 1992; Kino *et al.*, 1989). Many polysaccharides including branched (1→3)-β-D-glucans were highly effective against solid tumor of sarcoma 180 by intraperitoneal administration (Mizuno *et al.*, 1988; Sone *et al.*, 1985). Herein we investigated the antitumor effect of cultured mycelia of fungus *G. lucidum* on LLC cells *in vivo*.

Materials and Methods

Plant materials – The cultured mycelia of fungus *G. lucidum* were obtained from Prof. Masao Hattori, Toyama Medical and Pharmaceutical University, Japan.

Chemicals and media – Roswell Park Memorial Institute (RPMI) 1640 was purchased from ICN Biomedicals Inc. (Ohio, USA) and fetal bovine serum from JRH Biosciences Co. (Lenexa, USA). Streptomycin and penicillin G potassium obtained from Wako Pure Chemical Co. (Osaka, Japan), and adriamycin from Sigma Chemical Co. (St Louis, USA).

Tumor cells – Lewis lung carcinoma (LLC, mouse) cells were purchased from RIKEN Cell Line Bank (Tsukuba, Japan). The cells were maintained as monolayer cultures in RPMI 1640 medium supplemented with 7% fetal bovine serum, sodium bicarbonate (2 g), penicillin G (100,000 units), and streptomycin (100 mg).

Animal – Specific pathogen-free female BDF-1 mice,

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purchased from Japan SLC, Inc. (Shizuoka, Japan), used at 4 weeks of age, weighing 14 to 16 g. The animals were fed a commercial pellet chow (Clea Japan Inc., Tokyo, Japan) in a temperature-controlled room at 25±2°C and water *ad libitum*.

Tumor transplantation – LLC cells were maintained in cell culture. A suspension of 5×10^5 cells in 0.9% NaCl solution (0.2 ml) was inoculated subcutaneously into the left flank of mice for the subcutaneous tumor assay (Kato *et al.*, 1994).

Treatment of sample – The cultured mycelia of *G. lucidum* were suspended in 0.9% NaCl solution (0.1 ml), and administered intraperitoneally (*i.p.*) once daily for consecutive 2 weeks to mice. Control animals were given 0.1 ml of 0.9% NaCl solution by i.p. injection.

Estimation of tumor volume – Tumors were measured each alternate day using a vernier caliper from the initiation of treatment to the time when grosses ulceration of the tumor was developed in control mice. The tumor size was calculated as:

$$\text{tumor vol. } (mm^3) = 0.5\times a\times b^2$$

where a is the longest diameter and b is the shortest diameter (Kato *et al.*, 1994). The effects by treatments were represented as follows:

$$T/C\ (\%) = (\text{mean value of a treated group/mean value of a control group}) \times 100$$

Statistical analysis – The significance of differences between the experimental group was calculated by Dunnett's t test. $P<0.05$ was considered significant.

Results and Discussion

The cultured mycelia of *G. lucidum* were evaluated for antitumor activity against subcutaneous transplanted LLC cells, *in vivo*. The fungus, when given intraperitoneally once daily for the two weeks at dosages of 100 and 500 mg/kg, inhibited tumor growth with T/C values of 89.6 and 50.3%, respectively, compared with that of the control (Fig. 1). When the sample was intraperitoneally administrated once daily for the two weeks, the body weights continued to until 5th day after injection, following gradual increase and weighing almost same as body weights in control group (Table 1).

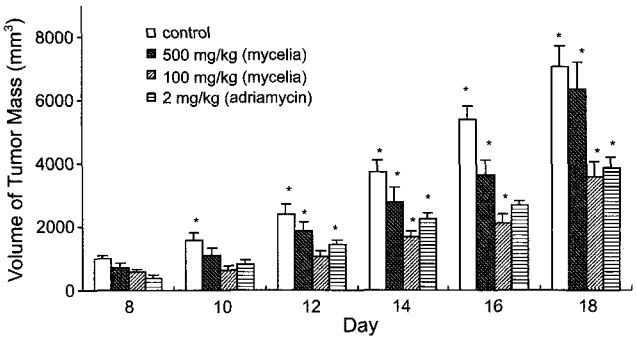

Fig. 1. *In vivo* antitumor activity of cultured mycelia of G. lucidum against LLC tumor cells. Statistical significance: *P<0.05, vs normal group. Points, mean±SEM.

The mycelia, which were almost existed triterpenes, showed antitumor activity, although we did not know the precise content of the total lanostane-type triterpenes contents. On the other hand, polysaccharide fraction extracted with hot water from fruiting bodies of *G. lucidum* contained α- and β-D-glucans, and heterogalactans. Examination by the solid form of Sarcoma 180 in ICR mice, either *i.p.* or *p.o.* method has revealed that all polysaccharides having remarkable antitumor activities contained (1→3)-β-D-glucose residues as backbone chains, some of which were attached mostly to single D-glucose and a very few two or three (1→6)-linked D-glucose residues (Sone *et al.*, 1985). Antitumor activities of these glucans, which appeared to be minor constituents of the fruiting bodies, were significantly dependent on the molecular weight, degree of branching, and conformation.

When compared with the chemical constituents included

Table 1. Inhibitory effects of mycelia of *G. lucidum* on *s.c.* transplanted LLC Cells

Day	Control			Mycelia, 100 mg/kg			Mycelia, 500 mg/kg			Adriamycin, 2 mg/kg		
	Tumor size[a]	W.[b]	S.M.[c]	Tumor size	W.	S.M.	Tumor size	W.	S.M.	Tumor size	W.	S. M.
8	999 ± 120	16.8	6/6	718 ± 156	16.6	6/6	559 ± 111	15.7	6/6	382 ± 88	15.8	6/6
10	1593 ± 238*	17.5	6/6	1072 ± 238	17.4	5/6	639 ± 100	15.8	6/6	815 ± 128	16.1	6/6
12	2358 ± 343*	18.6	6/6	1868 ± 280*	18.4	5/6	1058 ± 188	17.1	6/6	1447 ± 143*	16.6	6/6
14	3693 ± 412*	19.0	5/6	2761 ± 496*	18.9	5/6	1655 ± 224*	17.1	6/6	2233 ± 213*	16.5	6/6
16	5378 ± 435*	20.0	4/6	3614 ± 479*	20.0	5/6	2103 ± 282*	18.5	6/6	2648 ± 161	17.1	6/6
18	7053 ± 661*	20.5	3/6	6320 ± 880*	21.3	5/6	3551 ± 504*	19.3	6/6	3849 ± 339*	18.1	5/6
				T/C: 89.6%			T/C: 50.3%			T/C: 54.6%		

[a]Standard ± SEM. [b]Weight of mouse. [c]Survival mice.
*P < 0.05 (compared to normal group).

the fruiting bodies and cultured mycelia of *G. lucidum*, the antitumor activity of this mushroom might be depended on not only the content of carbohydrate such as α-D-glucan, β-D-glucans, and heterogalactans, but also the highly oxygenated lanostane-type triterpenes.

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