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Anticarcinogenicity of Ganoderma Lucidum

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INTRODUCTION

Cancer still remains a disease which can not be cured completely even though much research has been done.[1,2] Recently some efforts have grown to develop chemopreventive agents from natural food.[3~6]

Ten thousands species of mushrooms are reported including 1,500 species in Japan, 2,500 species in US and 800 species in Korea.[7] *Ganoderma lucidum* (GL) is a popular edible mushroom and is known as miraculous or auspicious herb for its medicinal value.

We established a new medium-term *in vivo* model (Yun's model) using benzo(a)pyrene for evaluating the anticarcinogenicity of various natural products.[8~10] The fruiting body of GL showed significant inhibition of lung tumors in Yun's model when it was ground and mixed in the diet,[11] and its water extract was given as drinking water.[12]

In this study, we examined whether mycelia of GL which makes a shortened period of cultivation and is suitable for mass production, has a similar anticarcinogenic effect in Yun's model. For the purification of active compounds, we partially fractionated, and also compared their efficacy along with several other natural products.

MATERIALS AND METHOD

1) Mice

Non-inbred N : GP(S) mice were from National Cancer Institute (MCI, USA). Newborn mice less than 24 hours old were used. Diet pellet was made by the prescription of NIH 7-open formula diet.

2) Experimental design

Newborn mice less than 24 hours old were injected subcutaneously in the scapular region with 0.02 ml of a suspension, 0.5 mg or 1 mg of benzo (a) pyrene (BP, Sigma Chemical Co., USA) in 1% aqueous gelatin, once. The carcinogen was used within 1 hour of emulsification. The following materials were administered for 6 weeks after weaning: Total fraction of GL mycelia, high molecular fraction and low molecular fraction of GL mycelia were provided from Il-Yang Pharm. Co., LTD. and dissolved in tap water at 2 mg or 10 mg. Authentic Korean honey was purchased from Kang-Won Do, Korea and given 80 mg/ml in drinking water. Sea cu-cumber extract from *Holothuria forskalii* containing cholo-turinosides A (non-sulphated triterpenoid glycosides), cholo -thurinosides B (nonsulphated pnetosacchride saponin), holo-thrinosides C and D (di or tetrasacchride), and desholothuric A (tetras -acchride) and was given 10 mg/ml in drinking water. Red ginseng extract (Office of Monopoly, Korea) was given in the drinking water at a concentration of 1 mg/ml and beta- carotene (Sigma Chemical Co., USA) was mixed in diet to be 0.5 mg/g diet. Drinking water was changed every other day and diet was prepared every other week.

3) Scoring of lung tumor

All mice were sacrificed at the 9th week after birth. Lungs were excised, fixed in Tellyesniczky's solution, and the adenoma was counted with the naked eye. To obtain an index of tumor incidence, the percentage of tumor bearing mice per total number of mice in each group was calculated. Tumor multiplicity was defined as the average number of tumors per mice obtained by dividing the total number of tumors by the total number of mice per group including non tumor-bearing animals. Statistical comparisons were made using the Chisquare test for tumor incidence and Student's t test of multiplicity. A null hypothesis was rejected whenever a P value of 0.05 or less was found.

RESULTS

There was no significant difference in body weight

and lung weight at the end of experiment. Histopathological analysis revealed that all lung tumors were pulmonary adenoma. Table 1. showed the incidence and multiplicity of lung adenoma induced by BP. Lung adenoma incidence was 59.2% at a concentration of 0.5 mg. The incidence and multiplicity of lung tumors in total extract of *Ganoderma lucidum* mycelia were significantly inhibited by 32.4% and 29.6% at a concentration of 10 mg/ml and 2 mg/ml, respectively. High molecular fraction also inhibited lung adenoma incidence significantly by 21.9% and 28.4% at a dose of 10 mg/ml and 2 mg/ml, respectively. However, low molecular fraction showed significant inhibition only at higher dose concentration (10 mg/ml) (Table 1.). In the case of red ginseng extract, it also showed significant inhibition of lung tumor (Table 2.). In contrast to these results, sea cucumber extract, beta-carotene and authentic Korean honey did not show any significant inhibition of lung tumors.

DISCUSSION

In this paper, mycelia of *Ganoderma lucidum* showed anticarcinogenicity in Yun's model, as well as its fruiting body and this activity was concentrated into its high molecular weight fraction.

Ganoderma lucidum is a species which belongs to Poly-poraceae of Aphyllophorales. In Shen Nong Ben Cao Jing, *Ganoderma lucidum* has effects on liver, circulatory system, respiratory system and has been used for hypertention, athritis, nerasthenia, leukopenia, dermatomycositis, various cancers, alopecia, and scleroderma.[16] There are many scientific reports about *Ganoderma lucidum*. Polysacchride has more than 40,000 molecular weight which had anti tumor effects on the sarcoma 180 transplanted mice[17,18] and this anti tumor mechanism is due to immunostimulation.[19] The fruiting body of *Ganoderma lucidum* showed significant inhibition of lung tumors in Yun's model when it was ground and mixed in the diet,[11] and its water extract was given as drinking water.[12] Kim et al., also reported that the water extract of *Ganoderma lucidum* inhibited GST-P Positive foci in diethylnitorsamine treated rats.[17]

Since, the goal of 5-year cancer survival rate was to achieve one out of two patients, by 1970, but it was not successful,[20] established a 9 week medium term in vivo bioassay system (Yun's model) to develop the anticarcinogenic agents[8~10] and to evaluate this model, water extract of red ginseng, ascorbic acid, carrot, caffeine, soybean lechitin, caffeine, *Ganoderma lucidum* powder, S*esamum indicum,* spinach,[9] water extract of *Ganoderma lucidum* fruiting body, 13-cis retinoic acid,[10] biochanin A,[21] and capsaicin[22] were tested. The results indicated that, water extract of red ginseng, caffeine, soybean lechitin, caffeine, *Ganoderma lucidum* Powder, water extract of *Ganoderma ludicum,* 13-cis retinoic acid, biochanin A, and capsaicin had anti-carcinogenic effects, but, carrot, spinach, *Sesamum indicum,* beta-carotene and 13-cis retinoic acid did not.

In these results, mucelia of *Ganoderma lucidum* also had anti-carcinogenic effects in Yun's model and active compounds might be concentrated into high molecular fraction. Sea cucumber extract which has reported anti-tumor effects on L1210 leukemia and KB cells[13] and anti-viral effects,[14] and authentic Korean honey did not show any anti carcinogenic effect. Therefore, *Ganoderma lucidum* mycelia might be a useful candidate for a chemopreventive agent and further study is necessary for the detection of anti carcinogenic active components.

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Table 1. The incidence and multiplicity of lung tumors in mice

Groups and Treatment	Dose	Number of mice		Incidence (%)	Multiplicity (mean±S.D)
		Sex	No.		
Normal control		M	25	1(4.0)	0.04±0.20
		F	25	1(4.0)	0.04±0.20
		M+F	50	2(4.0)	0.04±0.20
Benzo(a)pyrene	0.5 mg/head	M	30	16(53.3)	0.90±1.12
		F	30	19(63.3)	1.10±1.16
		M+F	60	29(59.2)	1.00±1.13
BP+IY009 Total Fraction	0.5 mg/head +10 mg/ml	M	30	11(36.7)	0.60±0.91
		F	30	13(43.3)	0.84±1.14
		M+F	60	24(40.0)*	0.72±1.03
BP+IY009 Total Fraction	0.5 mg/head +2 mg/ml	M	30	13(43.3)	0.52±0.65
		F	30	12(40.0)	0.64±1.11
		M+F	60	25(40.0)*	0.58±0.91
BP+IY009 High Mol. Fraction	0.5 mg/head +10 mg/ml	M	30	12(40.0)	0.60±0.96
		F	30	13(43.3)	0.60±0.82
		M+F	60	25(41.7)*	0.60±0.88
BP+IY009 High Mol. Fraction	0.5 mg/head +2 mg/ml	M	30	10(33.3)	0.40±0.58
		F	29	15(51.7)	1.20±1.66
		M+F	59	25(41.7)*	0.80±1.29
BP+IY009 Low Mol. Fraction	0.5 mg/head +10 mg/ml	M	30	12(40.0)	0.60±0.87
		F	30	13(43.3)	0.52±0.71
		M+F	60	25(41.7)*	0.56±0.79
BP+IY009 Low Mol. Fraction	0.5 mg/ml +2 mg/ml	M	30	14(46.7)	0.64±0.76
		F	30	14(46.7)	0.88±1.27
		M+F	60	28(46.7)	0.76±1.04
IY009 Total Fraction	0.5 mg/head +10 mg/ml	M	25	0(0.0)	0
		F	25	1(4.0)	0.04±0.20
		M+F	50	1(2.0)	0.02±0.14
IY009 Total Fraction	2 mg/ml	M	25	0(0.0)	0
		F	25	0(0.0)	0
		M+F	50	0(0.0)	0
IY009High.mol Fraction	10 mg/ml	M	25	0(0.0)	0
		F	25	0(0.0)	0
		M+F	50	0(0.0)	0
IY009High.mol Fraction	2 mg/ml	M	25	0(0.0)	0
		F	25	0(0.0)	0
		M+F	50	0(0.0)	0
IY009Low.mol Fraction	10 mg/ml	M	24	0(0.0)	0
		F	25	2(8.0)	0.08±0.28
		M+F	49	2(4.0)	0.04±0.20
IY009Low.mol Fraction	2 mg/ml	M	25	1(4.0)	0
		F	25	0(0.0)	0
		M+F	50	1(2.0)	0

*P < 0.05

Table 2. The incidence and multiplicity of lung tumors in mice

Groups and Treatment	Dose	Number of mice		Incidence (%)	Multiplicity (mean±S.D)
		Sex	No.		
Normal control		M	25	1(4.0)	0.04±0.20
		F	25	1(4.0)	0.04±0.20
		M+F	50	2(4.0)	0.04±0.20
Benzo(a)pyrene	0.5 mg/head	M	24	13(54.2)	0.93±1.55
		F	25	16(64.0)	1.72±2.55
		M+F	49	29(59.2)	1.32±2.12
Red ginseng ext.	2 mg/ml	M	25	0(0.0)	0
		F	25	0(0.0)	0
		M+F	50	0(0.0)	0
BP+Red ginseng ext.	0.5 mg/head +2 mg/ml	M	25	11(44.0)	0.43±0.73
		F	23	10(43.5)	0.65±0.75
		M+F	48	21(43.8)*	0.54±0.74
Sea cucumber	10 mg/ml	M	25	1(4.0)	0.04±0.20
		F	25	2(8.0)	0.08±0.28
		M+F	50	3(6.0)	0.06±0.24
BP+Sea cucumber	0.5 mg/head + 10 mg/ml	M	30	17(56.7)	0.87±0.98
		F	30	21(70.0)	0.94±1.14
		M+F	60	38(63.3)	0.91±1.05
Authentic honey	80 mg/ml	M	25	1(4.0)	0.04±0.20
		F	25	1(4.0)	0.04±0.20
		M+F	50	2(4.0)	0.04±0.20
BP+Authentic honey	0.5 mg/head + 80 mg/ml	M	30	15(50.0)	0.77±0.90
		F	29	15(51.7)	0.83±1.20
		M+F	59	30(50.8)	0.80±1.05
β-Carotene	0.5 mg/gdiet	M	25	0(0.0)	0
		F	25	0(0.0)	0
		M+F	50	0(0.0)	0
BP+β-Carotene	0.5 mg/head + 0.5 mg/gdiet	M	30	14(46.7)	0.85±1.42
		F	29	14(48.3)	0.83±1.81
		M+F	59	28(47.5)	0.84±1.62

*P < 0.05