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An Antitumor Component of *Laetiporus sulphureus* and its Immunostimulating Activity*

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Abstract□ A protein-polysaccharide fraction was prepared from the carpophores of *Laetiporus sulphureus*. This fraction suppressed growth of sarcoma 180 in A-strain mice when administered *i.p.* To investigate the mechanism of antitumor action of this fraction, plaque assay was conducted by administrating *i.p.* to the mice at a dose level of 50mg/kg for five days. Ten days later, the mice were immunized with 1×10^7 sheep red blood cells. The number of hemolytic plaque forming cells was significantly greater than that of the control mice. Three monosaccharides and fifteen amino acids were identified in the protein-polysaccharide fraction.

Keywords□ A protein-polyaccharide fraction, *Laetiporus sulphureus*, Basidiomycetes, Antitumor action, Plaque assay, Immunostimulation.

Although many anticancer drugs have been developed and are clinically used, they have serious disadvantages because of their direct cytotoxicities even to the normal cells. It is desirable, therefore, to develop new nontoxic anticancer agents from natural resources such as fungi[1], actinomycetes[2], and higher plants[3].

In our laboratory, the efforts to develop the safe and nontoxic anticancer agents from fungi are approached in two avenues. Firstly, antitumor components of the carpophores of basidiomycetes in Korea are screened and secondly, the selected basidiomycetes are shake-cultured and the antitumor components of the cultured mycelia are studied.

As a result, antitumor components were found in the carpophores of *Coriolus versicolor*[4], *Pleurotus ostreatus*[4], *Lentinus edodes*[4], *Russula pseudodelica*[5], *Microporus affinis*[5] and *Ganoderma lucidum*[6]. Among these fungi *Coriolus versicolor*[7], *Lentinus edodes*[8] *and Ganoderma lucidum*[9] were found to produce antitumor components when shake-cultured.

In this experiment the carpophores of *Laetiporus sulphureus* were extracted with hot water and from the extract a protein-polysaccharide fraction (=PPF) was prepared. The antitumor activity of PPF was tested against sarcoma 180 implanted in A-strain mice. To investigate the mechanism of antitumor action of PPF, hemolytic plaque assay was conducted.

EXPERIMENTAL METHODS

Materials

The carpophores of *Laetiporus sulphureus*(Fr.) Bond. et Sing. which were collected in the area of Gwangneung, Gyunggi Province during the Summer in 1980 were used in the experiments.

Animal

The animals used were male A-strain mice,

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weighing 25g, purchased from Seoul National University Experimental Animal Farm.

Extraction and Isolation of Antitumor Component

The dried carpophores (73g) of *L. sulphureus* were homogenized in a blender and extracted with 4 l of 0.1 N NaOH by refluxing for seven hours.

After filtration, the residue was re-extracted. The extract was dialyzed with Visking tube (Visking Co.) at 5°C for 3 days and concentrated. The resulting concentrated extract was mixed with 4-5 volumes of ethanol and allowed to stand at 4°C overnight.

The precipitates were collected by centrifugation and dried by lyophilization. Approximately 8.1g of tasteless and odorless protein-polysaccharide fraction(PPF) was obtained.

Characterization of PPF

1) Polysaccharide Analysis: The polysaccharide content of PPF was determined by anthrone test as described previously[6]. To determine the identity and content of each monosaccharide, 10mg of PPF was dissolved in 2ml of 0.1N HCl and hydrolyzed for 5 hrs in an ampoule filled with nitrogen gas. After filtration, the filtrate was dried by lyophilization.

The dried sample was analyzed by H.P.L.C. using a Bondapak carbohydrate analysis column. The eluent used was AcCN/H_2O/BuOH (80/20/15). The identification and content of monosaccharides of the sample were determined by comparing retention times and peak areas with those of standard monosaccharides.

2) Protein Analysis: The protein content of PPF was determined by Lowry-Folin method[11] using bovine serum albumin as a standard. To determine the identity and content of each amino acid which constitutes PPF, the hydrolysate of PPF was analysed as previously described[6].

Assay of Antitumor Activity of PPF

1) Inhibition Ratio against Sarcoma 180: On day 0, 1×10^6 cells of sarcoma 180 were subcutaneously implanted into the mice at the right axilla. Intraperitoneal administration of PPF was started on day 3 at a dose of 20mg/kg daily for ten days. On day 30 the mice were sacrificed and solid tumors were excised and weighed. Inhibition ratio (=I.R.) was calculated as an index of antitumor activity according to the following formula:

$$I.R.=\frac{C_w-T_w}{C_w}\times100(\%)$$

where C_w is average tumer weight of the control group and T_w is that of the treated group.

2) Life Span Test: Another evaluation of antitumor activity of PPF was conducted by observing the life span prolongation effect. Five million cells of sarcoma 180 were intraperitonealy implanted into into A-strain mice on day 0. Starting on day 1, PPF was injected *i.p.* at a dose of 20mg/kg, and 100mg/kg daily for 10 days. The number of survivors of each group was observed for 30 days.

Effects of PPF on Immune Response[10]

PPF was injected *i.p.* at a dose of 50mg/kg daily for 5 consecutive days.Ten days after the last administration of PPF, the mice were immunized by *i.p.* administration of 1×10^7 of sheep red blood cells(=SRBC). Five days later, the mice were sacrificed and hemolytic plaque forming cells(=PFC) in their spleens were assayed by Cunningham's method.[10]

To observe PFC, 30 μl of BSS, 20 μl of complement (M.A. Bioproducts), 10μl of 20% SRBC and 100μl of spleen cell suspension (10^5 cell) were mixed well, transferred into a slide chamber, sealed and incubated for 2 hrs at 37°C. Plaque forming cells were counted under a dissecting microscope with substage illumination.

RESULTS AND DISCUSSION

Chemical Properties of PPF

Since PPF gave positive reactions to Molish, anthrone, ninhydrin, biuret and Lowry-Folin tests, analysis of polysaccharide and protein was performed.

As shown in Table I, PPF was found to consist mainly of a polysaccharide and a small amount of protein. The result of amino acid analysis is shown in Table II. Because ammonia was detected, it might be deduced that asparagine and glutamine were contained in the portion of aspartic acid and glutamic acid. The results of the monosaccharide analysis are shown in Table III. It is uncommon that the content of fucose was 94.5% among the monosaccharides. This fact indicates that the polysaccharide is unique one.

Although PPF and the antitumor components from other basidiomycetes[4~9] have similarity in that they consist of a polysaccharide and a protein, they differ in amino acid and monosaccharide compositions.

Table I: Polysaccharide and protein contents of PPF.

	Polysaccharide	Protein	Water-insoluble fraction
Content/PPF(%)	84	5	6

Table II: Content of amino acids in the protein moiety of the antitumor fraction.

Amino acid	Content(%)	Amino acid	Content(%)
Asp(+Asn)	10.9	Val	6.4
Thr	8.5	Met	0.9
Ser	10.0	Ile	4.9
Glu(+Gln)	10.3	Leu	6.8
Pro	3.9	Tyr	0.9
Gly	13.7	Phe	3.3
Ala	10.1	Lys	4.9
Arg	4.4	(NH_3	+)

Table III: Contents of monosaccharides in the polysaccharide moiety of the antitumor fraction.

Monosaccharide	Content(%)
Glucose	3.2
Galactose	2.3
Fucose	94.5

Antitumor Activity of PPF

The antitumor activity of PPF was recognized by the suppression of the tumor growth and the prolongation of the life span of the sarcoma 180-bearing mice. As shown in Table IV, the results represented as inhibition ratio indicated that PPF exerted a high antitumor effect. The prolongation of life span of the PPF-treated group as shown in Figure 1 also indicated that PPF exerted a high antitumor effect and a low toxicity. It was considered that antitumor effect of PPF was similar to those of the previously reported antitumor components of basidiomycetes[4~9].

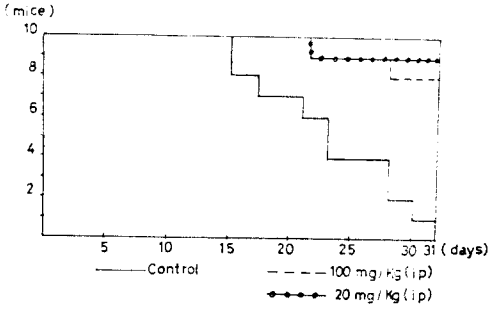

Fig. 1: Effects of PPF on the life span of mice inoculated with sarcoma 180.

Effects of PPF on the Immune Response

In order to elucidate the mechanism of the antitumor action of PPF, it was examined whether or not PPF potentiates immunity. In this experiment, a dose of 1×10^7 SRBC which evokes no immune response in normal mice was used as antigen. The PPF-treated group produced a significantly higher number of PPF than the non-treated group did. The result indicates that PPF stimulates humoral immunity. However

Table IV: Effects of PPF on the sarcoma 180-bearing mice.

	No. of mice	Average tumor weigh(g) (Mean±SE)	Inhibition ratio(%)	Complete regression
Control	10	9.04±0.40	—	—
20mg/kg(*i.p.*)	10	2.61±0.24[a]	71.1	4/10
100mg/kg(*i.p.*)	10	2.43±0.24[a]	73.2	3/10

a : $p<0.001$ (highly significant)

Table V: Hemolytic plaque-forming cells(PFC) in the spleen of mice immunized with sheep red blood cell(SRBC).

	SRBC	No. of mice	Spleen cell count ($\times 10^7$) (mean±SE)	PFC/10^6 spleen cells (mean±SE)	PFC/ spleen ($\times 10^3$) (mean±SE)
PPF-treated	1×10^7	6	34.9±4.0	397±30[a]	134±12[a]
Control	1×10^7	6	27.0±3.3	16±3.6	6.6±1.2
PPF-treated	—	6	29.0±1.9	9.6±3.1	4.8±0.8
Control	—	6	24.4±1.9	8.0±3.1	2.1±1.9

a : $p<0.01$ (highly significant)

it is not clear from this experiment which type of the immune cells was potentiated, because the production of PFC in the spleen of mice involves both T-cells and B-cells.

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CONCLUSION

The protein-polysaccharide fraction (PPF) of the carpophores of *Laetiporus sulphureus* exerted antitumor activity against sarcoma 180 in mice. PPF was found to consist of a polysaccharide (84%) and a protein (5%). PPF increased the number of plaque forming cells in the spleen of mice immunized with SRBC. This result indicates that PPF is an immunopotentiator.

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