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Studies on Antitumor and Immunopotentiating Activities of Polysaccharides from Trichosanthes Rhizome

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Abstract□The polysaccharide fraction from the rhizome of *Trichosanthes kirilowii* (Cucurbitaceae) showed marked antitumor and cytotoxic activity with immunopotentiating activity. It was evidenced by the increase in the number of circulating leucocytes and peritoneal exudate cells and the recovery of lowered antibody forming activity in mice. The polysaccharide was mainly composed of glucose, galactose, fructose, mannose and xylose and a samll amount of protein.

Keywords□Polysaccharide, antitumor activity, immunopotentiation, *Trichosanthes kirilowii*, Cucurbitaceae.

Several polysaccharides from natural sources were reported to show potent biological activities, such as zymosan from yeast for antitumor activity by Bradner *et al.*,[1] lentinan from *Lentinus edodes* for antitumor and immune activation aganist sarcoma 180 cells by Chihara *et al.*[2,3] and Harumo *et al.*,[4] several phytopolysaccharides for antitumor activity by Kang *et al.*[5] and Moon *et al.*[6,7] The antitumor and immune activity of polysaccharide from the rhizome of *Trichosanthes kirilowii,* which has long been used for tumor therapy in oriental countries, were studied.

METHODS

The chemicals used were all G.R. grade. UV and IR spectra were taken with Shimadzu UV-240 and Shimadzu IR-400, respectiveity. Gas chromatogram was determined on Shimadzu GC-R1A. Animals were healthy male ICR mice weighing 18-22g which were cultivated in our laboratory.

Extraction of polysaccharides

The rhizome was purchased in a local market. The rhizome defatted by refluxing with n-hexane for 24 hours (300g) was extracted with 0.5 M-NaOH solution (2.0l) in a refrigerator overnight. The filtered extract was added with three volumes of 95%-ethanol and then placed in refrigerator for 24 hours. The precipitation obtained by recentrifugation (5,000 rpm, 10 min) of the mixture was dissolved in small amount of water. Then protein was precipitated by the same volume of cold 15%-trichloroacetic acid solution and by centrifugation (12,000×g, 1 hr). The precipitate was dissolved in 2%-sodium acetate soln and the soluble part was added again with two volume of 95%-ethanol to stand in a refrigerator overnight. The final mixture was centrifuged (12,000×g) for 15 min to separate the precipitate and the crude polysaccharide fraction (Tri-K) was purified by consequent dialysis and lyophilization[7].

Chemical analyses of components

Polysaccharide content of the fraction was determined by anthrone test of Herbert[8], and monosaccharide constituents was analyzed by gas chromatographic method of Mitruka[9] using methylsilanization. The protein content was determined by Lowry-Folin method[10] with bovine serum albumin (Sigma Fr.V) as a standard.

Antitumor activity test

Mice were injected with 0.1 m*l* suspension of sarcoma-180 tumor cells (1×10^6 cells) intraperitoneally to examine the effects on life span and subcutanously into left groin for the effects on solid tumor[11]. In 24 hrs after the inoculation of tumor cells, sample solution was administered intraperitoneally for 10 days. Then observation was continued for 35 days to obtain life span prolongation ratio and for 26 days to calculate inhibition ratio of solid tumor growth, respectively. To examine direct cytotoxic effects *in vitro* by dye exclusion method[12-14], 1×10^5 cells (0.2 m*l* suspension) were incubated with sample in Eagle's medium and Hank's

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solution to make 5 m*l* as total volume for 24 hrs and finaly added with trypan blue solution to stain the non-viable tumor cells.

Immune function tests

The blood of the Tri-K treated mice were collected from eye venous plexus to test effects on number of circulating leucocytes[15]. The peritoneal exudate were also collected to examine effects on number of total peritoneal cells[16]. Tumor cell suspension (10^6 cells in 0.1 m*l*) were inoculated subcutanously into the left groin of mice sensitized with Freund's complete adjuvand followed by sample injection 24 hrs later to test effects of Tri-K treatment on Arthus reaction and delayed-type hypersensitivity[17-20]. In the above tests, change in thickness of footpad of mice challenged with heat aggregated bovine serum albumin was examined three hrs (Arthus reaction) and 24 hrs (delayed-type hypersensitivity) later, respectivity. And also Tri-K treated mice were injected with colloidal carbon suspension to study effects on phagocytosis of macrophage[21-23]. In the test, blood was collected from eye venous plexus to obtain the phagocytic index and weights of body, spleen and liver were measured to calculate the corrected phagocytic index.

RESULTS AND DISCUSSION

Chemical composition of the polysaccharide (Tri-K)

The total yield of the polysaccharide from 300g of the rhizome of *Trichosanthes kirilowii* was 2.87g with 89.35% purity. The main impurity was found as protein (1.46%) due to the incomplete removal by trichloroacetic acid. The presence of protein should be further studies as compared with the earlier report showing an increase of antitumor activity[24]. The gas chromatogram showed three main monosaccharides (fructose; 4.99 min retention time-15.08% constituent ratio, galactose; 15.75 and 16.22 min-15.04% and glucose; 16.89 min-68.23%) and two trace monosaccharides (xylose; 13.15 min and mannose; 14.12 min). The IR spectra showed O-H (3300-400 cm^{-1}) and C-H (2900 cm^{-1}) streching frequencies which are usual in polysaccharides and also C-H and O-H bending frequencies near 1000-1100 cm^{-1}. A weak β-linkage also appeared at 890 cm^{-1}.

Antitumor activity

As shown in Table I, the dose of 10 mg Tri-K/kg showed significant inhibition on solid type tumor but not on survival days. The dose of less than 10 mg/kg showed non-significant weak activity and the dose of more than 50 mg/kg showed severe toxicity in the mice. The effects on life span prolongation were in agreement with the results of the earlier reports with ethyl acetate extract of the rhyzome[25,26].

Table I. Antitumor activities of Tri-K against sarcoma-180 solid tumor type and on survival of mice with sarcoma-180 ascites tumor

Treatments*	Dose (mg/kg)	Solid tumor type inhibition ratio (%)	Survival days prolongation ratio (%)
Tri-K	5	33.46 (n.s.) †	
Tri-K	10	54.80 ‡	
Tri-K	30	82.31#	22.22‡

*Each group consists of eight animals.
Significance: † non-significant, ‡ $p<0.05$ and # $p<0.01$.

Viabilities of sarcoma-180 cells by Tri-K showed decreasing tendency (73.1, 64.3 and 60.8%, control group; 76.9%) as the dose of the sample (1,2,6 mg) was increased. Such results suggest direct cytocidal or cytotoxic effects of this polysaccharide on tumor cells but further studies should be carried out.

Effects on immune function

Among several methods to study immunopotentiating activities, effects of the polysaccharide on circulating leucocytes, total peritoneal exudate cells, Arthus reaction and delayed-type hypersensitivity were carried out. As shown in Table II, the Tri-K treated group manifested an increase in the number of leucocytes which can protect mice from foreign invaders. But the total number of peritoneal exudate cells were not significantly increased after fourth day of Tri-K treatment.

Tri-K treatment normalized the decreased formation due to inoculation of sarcoma-180 tumor cells shown by antibody-mediated hypersensitivity (Arthus reaction) test. Such phenomenon implies the activation of complements in antigen-antibody reactoin by the polysaccharide. As shown in Table III, the treatment also restored the T-lymphocyte reactivity in cell-mediated hypersensitivity test[27,28], which implies the presence of some mechanism between macrophage and anitigen conjugation.

But the results of the study to elucidate the mode of immune activation with macrophage did not show any significant difference in phagocytic and corrected phagocytic indices in carbon clearance activity test with Tri-K treatment *in vivo* (Table IV).

These results suggest that the antitumor effects of

Table II. Effects of Tri-K on the number of circulating leucocytes and peritoneal exudate cells in mice

Treatments*	Leucocytes** in blood				Exudate cells**		
	Day 1†	2	4	7	Day 1†	2	4
Control	6.88±1.10	6.98±1.19	7.12±0.97	7.20±1.07	8.9±2.8	10.2±3.2	11.2±2.1
Tri-K	10.23±0.92	10.16±0.75	10.20±0.45	9.79±1.11	30.6±9.2	22.6±4.1	12.1±3.8

*30 mg/kg of sample was administered intraperitoneally to 12 animals for 10 days (in leucocytes test) or to nine animals for three days (in exudate cells test). Control group was administered vehicle only.
**Units: 10^3 cells/mm^3 of blood or 10^5 cells/ml exudate.
†Days after the final treatment of sample.

Table III. Effect of Tri-K on the Arthus reaction (antibodymediated hypersensitivity, AMH) and delayed-type hypersensitivity (DTH) in tumor bearing mice

Treatment	Increase in footpad thickness (10^{-1} mm)	
	AMH	DTH
Negative control	8.82	5.95
S-180 control	5.45	3.26
S-180 Tri-K	7.45	4.75

*30 mg/kg of sample was treated to six animals for 10 days. Control group was treated with vehicle only.

Table IV. Effects of Tri-K on th carbon clearance activity (Phagocytic activity) in mice

Treatments*	Phagocytic index	Corrected phagocytic index
Control	0.011±0.004	3.096±0.308
Tri-K	0.012±0.002	3.059±0.173

*30 mg/kg of sample was treated to six animals for 10 days. Control group was treated with vehicle only.

polysaccharide of the rhizome of *Trichosanthes kirilowii* may be due to restoration of immune function to suppress tumor cell rather than direct cytotoxicity.

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