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Antitumor and Immunostimulating Activities of *Acanthopanax sessiliflorus* Fruits

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Abstract – The antitumor and immunostimulating activities of *Acanthopanax sessiliflorus* fruits were investigated. Polysaccharide isolated from this plant, when administered consecutively for 9 days at 50 and 100 mg/kg i.p. in mice, caused a significant increase in the life span and a significant decrease in the tumor weight and volume in mice inoculated with Sarcoma-180 tumor cells. Polysaccharide was also demonstrated to exhibit phagocytosis-enhancing activity as measured by the carbon clearance in mice. Polysaccharide, when administered i.p. at 50 and 100 mg/kg/day for 3 consecutive days, exhibited a significant RCtr/RCc [the rate of regression coefficient of the animals treated (RCtr) to that of the control (RCc)], being 1.44 (PI = 1), 1.52 (PI = 2) which was approximately the same with that of enhancement of phagocytosis, its potency as expressed by the regression coefficient ratio of zymosan (RCtr/RCc = 1.55, PI = 2), a typical phagocytosis enhancer. Polysaccharide also caused a significant increase in the acid phosphatase activity representing lysosomal enzymes in macrophages at 1-100 ìg/ml *in vitro* in compliance with *in vivo* results. These results suggest that the antitumor activity of polysaccharide might be related to the immunostimulating function.

Keywords – *Acanthopanax sessiliflorus*, Araliaceae, polysaccharide, antitumor activity, immunostimulating activity

Introduction

Several polysaccharides from *Angelica gigas*, *Lentinus edodes*, *Phellinus linteus*, *Platycodon grandiflorum* and *Schizophyllium commune* have been previously shown to have immunostimulating activities (Borchers *et al.*, 1999; Han *et al.*, 1998; Han *et al.*, 2001; Kim *et al.*, 1996). Several of the immunomodulatory activities of polysaccharides from *Acanthopanax* species have also been reported. A polysaccharide from *A. senticosus* increased the *in vitro* proliferation of total spleen cells and *in vivo* T-dependent antibody response (Shen *et al.*, 1991). A polysaccharide from *A. obovatus* also increased *in vivo* T-dependent antibody response (Wang *et al.*, 1991; Wang *et al.*, 1993).

Acanthopanax species are herbaceous genus of the family Araliaceae. They are distributed in Korea, Japan and China. They have traditionally been used as a tonic and a sedative, as well as in the treatment of rheumatism and diabetes (Perry, 1980). Among fifteen species of *Acanthopanax* growing in the Korean peninsula, *A. sessiliflorus* is one of the most abundant species. To date, investigations on the compounds have revealed the presence of iriodendrin from the cortex of *A. sessiliflorus* forma *chungbunensis* (Ro *et al.*, 1977), stigmasterol, β-sitosterol, campesterol and (+)-sesamin from the root bark of *A. sessiliflorus* (Yook *et al.*, 1977) and sessiline from the fruits of *A. sessiliflorus* (Lee *et al.*, 2002). 6-Hydroxyhexadeca-12Z-enoic, 6-hydroxydeca-9Z,12Z-dienoic, and the isomeric 6- and 7-hydroxyoctadeca-12Z-enoic, and 6- and 7-hydroxyoctadeca-9Z,12Z-dienoic acids were isolated from the fruits of *A. sessiliflorus* (Asilbekova *et al.*, 1991). Only a few compounds including saturated and unsaturated fatty acids have been isolated from the seeds of *A. sessiliflorus* (Kim and Kim, 1987).

This study aims at the evaluation and comparison of antitumor and immunostimulating activities of polysaccharide from this plant fruits.

Experimental

Materials – The fruits of *Acanthopanax sessiliflorus* Nakai were collected at Kong Ju, Korea in October 1999 and authenticated by Prof. Seon Haeng Cho, Kong Ju University of Education, Korea. A voucher specimen of this plant was deposited at the Herbarium of Natural Products Research Institute (NPRI), Seoul National University, Korea. The air-dried powdered fruits (100 g) of *A. sessiliflorus*

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were defatted with ether at room temperature and extracted 1 hr with 100°C water. The combined supernatant was filtered to remove insoluble materials and the supernatant was concentrated to a small volume and precipitated with ethanol at 4°C overnight. The precipitate was obtained by centrifugation at 15,000 rpm at 4 for 30 min and dialyzed using molecularporous membrane (Spectrum, MWCO 6-8000) for 2 days against water. The nondialyed portion was lyophilized to give a brownish residue (2.5 g).

Cells and animals – Sarcoma-180 tumor cell line was supplied from the Korea Cell Line Bank, Seoul National University and maintained by inoculating 1×10^6 cells intraperitoneally in ICR mice. The viable cells were counted by haemocytometer using trypan blue exclusion method. Four-week-old and specific pathogen-free male ICR mice weighing 23±2 g were supplied from the Daehan Animal Center and the maintenance and animal experiments were carried out in the SPF barrier zone of Seoul National University Hospital. They were fed on lab. chows. and water *ad lib.* and were housed at 23±0.5°C and 10% humidity in a 12 hr light-dark cycle.

Measurement of carbon clearance *in vivo* – Phagocytosis activity was estimated by the carbon clearance test in mice established by Wagner *et al.* (1985). Mice were injected intraperitoneally with polysaccharide once a day for 3 consecutive days. 24 hr after the last treatment, diluted carbon suspension was injected at 0.1 ml/10 g body weight into the tail vein of mice. Carbon suspension was centrifuged at 5,000 rpm for 15 min, and the supernatant was diluted three-fold with sterile 1.5% gelatin in saline to bring the carbon concentration to about 30 mg/ml. Blood was drawn from the orbital vein at 3 min intervals up to 15 min. The blood was hemolyzed with the addition of 1 ml 0.1% Na_2CO_3 solution and measured its absorbance at 600 nm. PI (Phagocytosis Index) was expressed as the rate of regression coefficient of the animals treated (RCtr) to that of the control (RCc).

Measurement of acid phosphatase in murine macrophages *in vitro* – Murine peritoneal macrophages were elicited by i.p. injection of 2 ml sterile 2.98% Brewers thioglycollate medium into the cavity of ICR mice, peritoneal exudates cells were obtained 3 days after injection by peritoneal lavage with ice-cold RPMI 1640 medium. Cells were washed twice, and resuspended in RPMI 1640 medium supplemented with 5 mM Hepes, 10% FBS, penicillin (100 unit/ml) and streptomycin (100 μg/ml). On average, $15\text{-}30\times10^6$ macrophages can be obtained from each mouse. The total cell number was counted with hematocytometer and cell viability was examined by trypan blue exclusion. The activity of acid phosphatase in macrophages was assayed by the procedure of Suzuki *et al.* (1990). A 200 μl of the cell suspension containing 2×10^5 cell/well was placed in 96 well culture plate. The cells were allowed to adhere for 2 hr at 37°C in humidified 5% CO_2 incubator. Non-adherent cells were removed by washing twice with RPMI-1640 medium. Fresh media, LPS and polysaccharide were added to each well and the plates were incubated with macrophages at 37°C for 24 hr. The medium was discarded by aspiration and the macrophage monolayer in each well was solubilized by addition of 25 μl of 0.1% triton X-100. After addition of 200 μl of *p*-nitrophenyl phosphate/0.1 M citrate buffer (pH 5.0) to each well, the plate was incubated at 37°C for 60 min. The reaction was stopped by addition of 50 μl of 0.2 M borate buffer (pH 9.8) and then the optical density at 405 nm was measured.

Measurement of antitumor activity – The effects of polysaccharide on tumor growth and host survival in Sarcoma-180 tumor in mice were estimated by evaluating the tumor volume, tumor weight, and percentage increase in the life span of tumor (ILS) hosts, respectively. On day 0, groups of seven mice were inoculated i.p. with 1×10^6 Sarcoma 180 cells/mouse and treatment of polysaccharide (50 and 100 mg/kg, i.p.) was started 24 hr after tumor cell inoculation and continued for nine consecutive days. The control group was treated with saline only. The anti-tumor activities of polysaccharide was estimated by measuring ILS and expressed as median survival time (MST). For solid tumor development, mice were injected with 0.1 ml of Sarcoma-180 suspension into the right hind limbs. Six days after tumor transplantation, mice randomized into six groups were injected i.p. with polysaccharide (50 and 100 mg/kg, i.p.) and krestin (30 mg/kg) once a day for 9 days. Ten days later, animals were killed by cervical dislocation, solid tumor was removed and the wet weight was weighed. The tumor volume was measured with digital caliper.

Statistical analysis - Data were expressed as the mean ± S.E.M. Statistical analysis was performed using an unpaired student's *t*-test; Value at $p<0.05$ was considered to be statistically significant.

Results and Discussion

The phagocytosis-enhancing activity of polysaccharide isolated from *A. sessiliflorus* fruits was estimated by measuring the carbon clearance in mice and the result was shown in Table 1. Polysaccharide with 3 daily consecutive i.p. administrations at a dose of 50 and 100 mg/kg was shown to exhibit a very strong enhancement of phagocytosis. The potency of phagocytosis of polysaccharide at 100 mg/kg as indicated by the regression coefficient ratio

Table 1. Effects of polysaccharide from *A. sessiliflorus* on the carbon clearance in mice

Treatments	Dose (mg/kg, i.p.)	Regression coefficient (RCtr/RCc)	PI
Zymosan	50	1.55	2
Polysaccharid	50	1.44	1
e	100	1.52	2

PI: (RCtr/RCc); >1.5 = 2 (very active); <1.5 = 1 (active); ≤ 1.0 = 0 (not active).

Table 2. Effects of polysaccharide from *A. sessiliflorus* on the acid phosphatase activity in peritoneal macrophages

Treatments	Concentration (μg/ml)	Acid phosphatase activity[a)]	Increasing ratio (%)
Control	–	0.50±0.01	–
LPS	1	1.42±0.06**	284
Polysaccharide	10	1.25±0.05*	250
	20	1.24±0.03*	248
	50	1.15±0.03*	230
	100	1.03±0.05*	206

[a)]Data were expressed as mean ± S. E. M.
Significantly different from the control; $^{*}p < 0.05$, $^{**}p < 0.01$.

(RCtr/RCc = 1.52, PI = 2) revealed to be similar with that of zymosan (RCtr/RCc = 1.55, PI = 2), a typical phagocytosis enhancer, whereas, it at 50 mg/kg exhibited moderate enhancing activity (RCtr/RCc = 1.44, PI = 1). Polysaccharide also caused a significant increase in the acid phosphatase activity in macrophages *in vitro* in concentration dependent manner in compliance with *in vivo* results as shown in Table 2. Similar enhancements of the acid phosphatase activity representing lysosomal enzymes in macrophages were shown at 10-100 μg/ml of polysaccharide. Polysaccharide showed twice more strong activity than control in the acid phosphatase activity.

The effects of polysaccharide on the survival time in Sarcoma-180 ascitic tumors in mice were tested and the result was summarized in Table 3. The median survival time (MST) for the Sarcoma-180 control group was 23.0 days, while the MST were dose dependently increased with the treatments of polysaccharide at doses of 50 and 100 mg/kg for nine consecutive days, their MST, being

Table 3. Effects of polysaccharide from *A. sessiliflorus* on the survival time in Sarcoma-180 ascitic tumor

Treatments	Dose (mg/kg)	Median survival time (Days)	Increase in life span (%)
Control	–	23	–
Krestin	30	45	195.7
Polysaccharide	50	31	134.8
	100	39	169.6

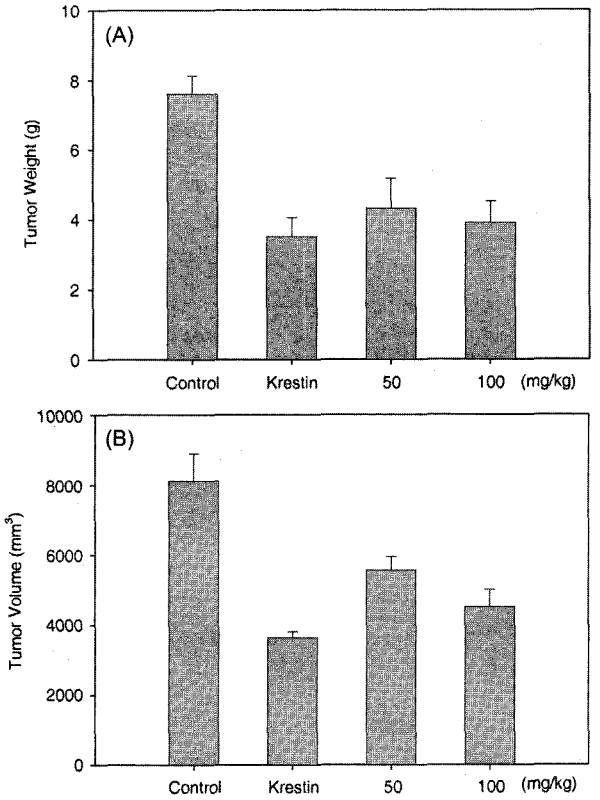

Fig. 1. Effects of polysaccharide from *A. sessiliflorus* on the tumor weight **(A)** and volume **(B)** in Sarcoma-180 tumor in mice. Solid-type Sarcoma-180 was prepared by subcutaneous transplantation of 1×10^6 cells into the right groins of mice on day 0. Six days after tumor cell inoculation, treatment with saline, polysaccharide (50 and 100 mg/kg) and krestin (30 mg/kg) were commenced for 9 days. Ten days later after treatment, solid tumor was removed and its wet weight was measured. Tumor volume was measured by digital-caliper. Data were expressed as the mean±S. E. M. of nine mice. Significantly different from the control; $^{*}p<0.01$.

31.0 and 39.0 days, respectively. The animal groups treated with 30 mg/kg dose of krestin, as a positive drug showed a much stronger enhancement of MST by 45 days.

The results of the effects of polysaccharide on solid tumors induced by Sarcoma-180 tumor cells in mice as measured by the tumor weight and tumor volume were shown in Fig. 1. As shown in Fig. 1, polysaccharide when treated i.p. with 50 and 100 mg/kg caused decrease in the tumor weight by 39.3% (4.6±0.86 g) and 48.7 (3.9±0.61) respectively, while krestin at 30 mg/kg inhibited the tumor weight by 53.8% (3.51±0.54 g) compared with the control (7.6±0.53 g). And the average tumor volume in the control group was 8112±775 mm^3. The level of the tumor volume in animals treated with krestin injection was decreased by 55.2% (3632±170 mm^3) compared with the control level. Polysaccharide at the dose of 50 and 100 mg/kg caused inhibition of the tumor volume by 31.6% (5552±386 mm^3)

and 44.5 (4505±490).

Several polysaccharides have previously been shown to possess reticuloendothelial system potentiating activity and these polysaccharides exhibited phagocytosis enhancement activity (Da Silva *et al.*, 2000). From the present experiments, it has also been demonstrated that polysaccharide exhibit a significant immunostimulating activities *in vivo* as measured by the carbon clearance and the acid phosphatase activity, a lysosomal enzyme produced by activated macrophages *in vitro*.

Recently, immuno-modulatory polysaccharides are rapidly emerging as promising immunotherapeutic agents in the treatment of cancer. Preclinical studies of several polysaccharides isolated from higher plants, mushrooms and seaweeds have demonstrated anti-tumor activity against transplantable tumors in mice (Wong *et al.*, 1994). Most of the clinical evidence comes from the commercial polysaccharides lentinan, krestin, and schizophyllan from mushroom. Krestin has remarkable immune-enhancing activity and a broad anti-neoplastic scope. It has been shown to prolong the survival time of radiated mice, stimulate phagocytic activity of macrophages, and improve the functions of the reticuloendothelial system (Zhu, 1987). With regard to its anti-tumor properties, it acts directly on tumor cells, as well as indirectly in the host to boost cellular immunity (Wasser, 2002). It has shown anti-tumor activity in animals with sarcoma, carcinoma, colon, lung cancer, *etc.* (Sugimachi *et al.*, 1997). Unlike conventional chemotherapeutics, many are relatively nontoxic and stimulate the immune system.

It has been reported that activated macrophages, NK cells, and cytotoxic T lymphocytes were generally involved with anti-tumor activity (Wang *et al.*, 1993). Macrophages may play a role in anti-tumor activity in part due to the production of effecter molecules such as NO, TNF-α and IL-1. A large body of experimental and clinical evidence demonstrates the beneficial results of known polysaccharides for the following purpose; 1) immuno-potentiating activity against tumors in conjunction with chemotherapy, 2) preventive effects on tumor metastasis, and 3) direct anti-tumor activity against various allogeneic and syngeneic tumors.

Although the exact mechanism of anti-tumor actions has not been clearly elucidated, polysaccharide might contribute to the anti-tumor activity of *A. sessiliflorus* and the antitumor effects may be related to the immunostimulating activity of polysaccharide from *A. sessiliflorus*.

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References

Asilbekova, D. T., Gusakova, S. D. and Glushenkova, A. I., New hydroxy fatty acids of *Acanthopanax sessiliflorus*. *Khim. Prir. Soed.* **5**, 616-619 (1991).

Borchers, A. T., Stern, J. S., Hackman, R. M., Keen, C. L. and Gershwin, M. E., Mushrooms, tumors, and immunity. *Proc. Soc. Exp. Biol. Med.* **221**, 281-293 (1999).

Da Silva, B. P., Tostes, J. B. and Parente, J. P., Immunologically active polysaccharides from *Centrosema pubescens*. *Fitoterapia* **71**, 516-521 (2000).

Han, S. B., Kim, Y. H., Lee, C. W., Park, S. M., Lee, H. Y., Ahn, K. S., Kim, I. H. and Kim, H. M., Characteristic immunostimulation by angelan isolated from *Angelica gigas* Nakai. *Immunopharmacol.* **40**, 39-48 (1998).

Han, S. B., Park, S. H., Lee, K. H., Lee, C. W., Lee, S. H., Kim, H. C., Kim, Y. S., Lee, H. S. and Kim, H. M., Polysaccharide isolated from the radix of *Platycodon grandiflorum* selectively activates B cells and macrophages but not T cells. *Int. Immunopharmacol.* **1**, 1969-1978 (2001).

Kim, C. W. and Kim S. K., Studies on the constituents of seeds of *Acanthopanax sessiliflorus* Seemann. *Kor. J. Pharmacogn.* **18**, 184-187 (1987).

Kim, H. M., Han, S. B., Oh, G. T., Kim, Y. H., Hong, D. H., Hong, N. D. and Yoo, I. D., Stimulation of humoral and cell mediated immunity by polysaccharide from mushroom *Phellinus linteus*. *Int. J. Immunopharmacol.* **18**, 295-303 (1996).

Lee, S., Ji, J., Shin, K. H. and Kim, B.-K., Sessiline, a new nitrogenous compound from the fruits of *Acanthopanax sessiliflorus*. *Planta Med.* **68**, 939-941 (2002).

Perry, L. M., "Medicinal Plants of East and Southeast Asia", MIT press, Cambridge, Massachusetts and London, 1980, p. 41.

Ro, H. S., Lee, S. Y. and Han, B. H., Studies on the lignan glycoside of *Acanthopanax* cortex. *J. Pharm. Soc. Kor.* **21**, 81-86 (1977).

Shen, M. L., Zhai, S. K., Chen, H. L., Luo, Y. D., Tu, G. R. and Ou, D. W., Immunopharmacological effects of polysaccharides from *Acanthopanax senticosus* on experimental animals. *Int. J. Immunopharmacol.* **13**, 549-554 (1991).

Sugimachi, K., Maechara, Y., Ogawa, M., Kakegawa, T. and Tomira, M., Dose intensity of uracil and tegafur in postoperative chemotherapy for patients with poorly differentiated gastric cancer. *Cancer Chemother. Pharmacol.* **40**, 233-238 (1997).

Suzuki, I., Tanaka, H., Kinoshita, A., Oikawa, S. and Yadomae, T., Effect of orally administered β-glucan on macrophage function in mice. *Int. J. Immunopharmacol.* **12**, 675-684 (1990).

Wagner, H., Proksch, A., Riess-Maurer, I., Vollmar, A., Odenthal,

S., Stuppner, H., Jurcic, K., Le, T. M. and Fang, J. N., Immunostimulating polysaccharides of higher plants. *Arzneim-Forsch* **35**, 1069-1075 (1985).

Wang, J. Z., Mao, X. J., Ito, H. and Shimura, K., Immunomodulatory activity of polysaccharide from *Acanthopanax obovatus* roots. *Planta Med.* **57**, 335-336 (1991).

Wang, J. Z., Tsumura, H., Ma, N., Shimura, K. and Ito, H., Biochemical and morphological alterations of macrophages and spleen cells produced by antitumor polysaccharide from *Acanthopanax obovatus* roots. *Planta Med.* **59**, 54-58 (1993).

Wasser, S. P., Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl. Microbiol. Biotechnol.* **60**, 258-274 (2002).

Wong, C. K., Leung, K. N., Fung, K. P. and Choy, Y. M., Immuno-modulatory and anti-tumor polysaccharides form medicinal plants. *J. Int. Med. Res.* **22**, 299-312 (1994).

Yook, C. S., Lee, D. H., Seo, Y. K. and Ryu, K. S., Studies on the constituents in the root bark of *Acanthopanax sessiliflorus* Seemann. *Kor. J. Pharmacogn.* **8**, 31-34 (1977).

Zhu, D., Recent advances on the active components in Chinese medicines. *Abstr. Chin. Med.* **1**, 251-286 (1987).

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