

Antitumor Activity of Polysaccharide Fractions from Pine Cone Extract of *Pinus Parviflora Sieb. et Zucc.*

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Abstract. Hot water extract of pine cone (PCE) of *Pinus parviflora Sieb. et Zucc.* dose-dependently suppressed both solid and ascites tumor cells transplanted into various mice. Acidic polysaccharides of PCE significantly increased the survival time of mice bearing ascites tumor cells, and activity increased with acidity. One of the four polysaccharide fractions obtained by NaOH extraction showed the most potent antitumor activity. This fraction significantly suppressed the growth of solid tumor cells, with occasional tumor regression and necrosis, and with little or no cytotoxic effect on cultured tumor cells. All acidic polysaccharides were able to activate mouse macrophage-like cell line J774.1. There did not appear to be any correlation between the antitumor activity of these polysaccharides and their content of arabinose (or fucose), mannose, galactose, glucose, or uronic acid.

Folk wisdom maintained that oral administration of hot water extract of pine cones (PCE) of *Pinus parviflora Sieb. et Zucc.* significantly improved the condition of patients who had stomach cancer, and that many recovered from the disease. However, no investigator has yet reported a biochemical analysis of the substance(s) that might be responsible for these effects. We therefore initiated a study to identify various components of PCE that might be helpful in cancer treatment. We have reported partial purification of a novel substance that induced differentiation of a human myeloblastic leukemic cell line, ML-1, into macrophage-like cells (1). Recently, this substance was purified to homogeneity and found to contain PAS-stainable substance(s) in the molecule (Sakagami *et al*, unpublished data). Based on these findings,

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we investigated the possible antitumor activity of 10 different polysaccharide fractions of pine cone extract.

Materials and Methods

Mice. Male ddY mice (5 weeks old, 24-26g), female BALB/c (6 weeks old, 17-18 g) and ICR mice (5 weeks old, 23-25 g) were obtained from Sankyo Labo. Service Co. The mice were used for experiments at 6-7 weeks of age.

Cell culture. ML-1 and the mouse macrophage-like cell line, J774.1, were cultured in RPMI1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco), as described previously (1).

Tumors. Sarcoma-180 and Meth A fibrosarcoma were maintained serially in ascites form by weekly *in vivo* transfer in ICR mice.

Fractionation of polysaccharides. Polysaccharides were fractionated as shown in Figure 1. A 250 g sample of pine cone of *Pinus parviflora Sieb. et Zucc.*, collected at Nagasaki in October 1986, was washed successively, twice with methanol and twice with 85% ethanol, and then extracted for 4 hours, three times with boiling hot water. After removal of insoluble materials by filtration, the extract (PCE) was concentrated under reduced pressure, and precipitated with 6 volumes of ethanol. The precipitate was dissolved in distilled water, dialyzed against distilled water and applied to a DEAE-cellulose column (2.5×40 cm, Cl⁻ form) (Brown Co) equilibrated with distilled water (Figure 2). Stepwise elution was performed with water (Fr. I, fraction no. 15-95), 0.5 M NaCl (Fr. II, fraction no. 144-200), 2 M NaCl (Fr. III, fraction no. 220-260) and 0.15 M NaOH (Fr. IV (fraction no. 297-303) + Fr. V (fraction no. 304-325)). Fractions (10 ml/tube) were collected and 50 µl portions were used to determine carbohydrates. Since 0.15 M NaOH eluate contained two different peaks, these peaks were collected separately as Fr. IV and V. The latter predominant peak was further fractionated into Fr. V-1 and V-2 on a Sepharose CL-4B column (Figure 3).

Residue that was not extracted with hot water was further extracted twice with 1% NaOH for 4 hours at room temperature (Figure 1). After centrifugation for 20 minutes at 10000 ×g, the pH of the clarified supernatant was adjusted to 5.0 with acetic acid. The precipitate was collected by centrifugation (Fr. VI), and the supernatant was successively precipitated with 1, 2 and 5 volumes of ethanol (Fr. VII, VIII, and IX). All of these fractions were extensively dialyzed against distilled water and lyophilized.

Antitumor activity of polysaccharides. (a) Effect on ascitic tumor growth: Female ICR mice were inoculated intraperitoneally with 0.2 ml of a saline