Studies on an Enzymatically Synthesized Antitumor Polysaccharide SPR-901

Hisao KADO,* Yasuo YONETA, Suguru TAKEO, Masaru MITSUI, and Nobuhiro WATANABE

Pharmaceutical Research Laboratories, Sapporo Breweries Ltd., 10, Okatohme, Yaizu, Shizuoka 425, Japan. Received January 14, 1991

An antitumor polysaccharide SPR-901 was found in a fermented broth of a kind of lactic acid bacteria isolated from rice bran. SPR-901 is a high molecular α -glucan and its linkages are almost linear α -1,6 glucosidic ones with a small amount (ca. 5%) of branches at C-3 positions. It is a highly purified α -glucan and it contains no protein and no inorganic salts. SPR-901 showed significant antitumor activities against murine allogeneic and syngeneic tumors by both intraperitoneal and oral administration, and enhanced carbon clearance ability in mice, while it showed no direct cytotoxicities *in vitro*. The mechanism of antitumor activities of SPR-901 is supposed to be a host-mediated one, and this substance is classified as one of the biological response modifiers. These properties of SPR-901 were identical to those of RON, which was obtained from rice bran, therefore we concluded that these two polysaccharides were the same substance.

Keywords RBS; RON; dextran; α -glucan; polysaccharide; SPR-901; *Leuconostoc mesenteroides* subsp. *dextranicum*; antitumor; Meth-A; BRM

In previous papers we reported on the antitumor polysaccharides RBS¹⁾ (rice bran saccharide) and RON.²⁾ RBS is obtained from the hot water extract of rice bran and RON is a purified main fraction of RBS. Their antitumor and immunomodulating activities were studied well.^{3,4)} Recently, we found that a certain polysaccharide produced by a kind of lactic acid bacteria, isolated from rice bran, possessed antitumor activities.⁵⁾ Therefore we studied the physico-chemical properties and biological activities of this polysaccharide, comparing it with those of RON.

Materials and Methods

Preparation of SPR-901 SPR-901 is enzymatically synthesized by the extracellular enzyme of such bacteria as *Leuconostoc mesenteroides* subsp. *dextranicum* using sucrose as a substrate, purified by the alcohol precipitation method several times, and then dried and recovered as a white powder (Fig. 1).

Measurement of Antitumor Activities Ten mice (6 weeks, female) were used in each group. Tumor cells were inoculated subcutaneously on day 0. SPR-901 was administered on days 1—10 (30 mg/kg/d) and only saline for the control group. Tumor weight or area was measured on day 33 or day 21, respectively, and the inhibition ratio was calculated from the

average tumor growth of the test group vs. the control group. Statistical significance was evaluated by applying Student's *t*-test.

Carbon Clearance Test Ten mice (BALB/c, 7 weeks female) were used in each group. SPR-901 was administered orally on days 1—10 (30 mg/kg/d). Lipopolysaccharide (LPS) (Difco, *E. coli* 055:B5) was administered intraperitoneally on day 8 (1 mg/kg) as a positive control. Mice of the control group were administered with only saline orally for 10 d. Phagocytic ability against carbon particles was measured on day 11, and phagocytic index was calculated with the method of Biozzi *et al.*⁶⁾

Results

The physico-chemical properties of SPR-901 are as follows: it is a pure α -glucan composed solely of glucose and contains no protein and no inorganic salts; its linkages are almost linear α -1,6 glucosidic ones and a small amount of branches (ca. 5%) at C-3 positions; infrared (IR) spectrum and ¹³C nuclear magnetic resonance (¹³C-NMR) spectrum

Fig. 1. Process of Synthesis and Purification of SPR-901

Fig. 2. IR Spectrum of SPR-901

Fig. 3. ¹³C-NMR Spectrum of SPR-901

TABLE I. Antitumor Effect of SPR-901 on Murine Allogeneic and Syngeneic Tumors

Tumor	Inoculum size	Mice	Route ^{a)}	Tumor growth (mean $\pm$ S.D.)		Inhibition ratio (%)
				Control group	Test group	
S-180	1×10^7	ICR (CRJ)	i.p.	6.7 ± 3.3 (g)	2.5 ± 2.1 (g)	63 ^{c)}
Ehrlich	1×10^6	ddY (SLC)	i.p.	5.7 ± 1.6	3.1 ± 2.0	46 ^{c)}
Meth-A	6×10^4	BALB/c (CRJ)	i.p.	3.8 ± 0.9	1.9 ± 0.9	50 ^{d)}
Meth-A	6×10^4	BALB/c (CRJ)	p.o.	277 ± 46 (mm ²)	101 ± 58 (mm ²)	64 ^{c)}
3LL	1×10^5	BDF1 (CRJ)	p.o.	414 ± 73	267 ± 93	36 ^{c)}
Colon 26	1×10^4	BALB/c (CRJ)	p.o.	397 ± 55	297 ± 45	25 ^{c)}
B-16	25 mg ^{b)}	BDF1 (CRJ)	p.o.	549 ± 87	498 ± 31	9

a) Administration route of SPR-901. b) B-16 melanoma cells (25 mg/kg) were inoculated subcutaneously. Significant difference from the control. c) $p < 0.01$, d) $p < 0.001$.

TABLE II. Effect of SPR-901 on Carbon Clearance Ability in Normal and Tumor Bearing Mice

Sample	Normal mice		Tumor bearing mice ^{a)}	
	Phagocytic index mean $\pm$ S.D. ($\times 10^2$)	Activation rate ^{b)}	Phagocytic index mean $\pm$ S.D. ($\times 10^2$)	Activation rate ^{b)}
Control	3.48 ± 0.65 ^{c)}		3.50 ± 0.86	
SPR-901	4.53 ± 0.78	130 ^{d)}	4.21 ± 0.83	120 ^{c)}
LPS	5.57 ± 1.75	160 ^{d)}	5.88 ± 0.69	168 ^{d)}

a) Meth-A cells (6×10^4) were inoculated subcutaneously into mice on day 0. b) Versus control (%). Significant difference from the control; c) $p < 0.05$, d) $p < 0.01$.

spectrum are shown in Figs. 2 and 3, respectively; $[\alpha]_D^{20}$ is ca. $+185^\circ$ (H₂O), ca. $+210^\circ$ (formamide); intrinsic viscosity is 1.6–2.4 (25°C, H₂O); molecular weight is approximately 1×10^7 daltons (lightscattering method). Although its molecular weight is high, it is soluble in water giving a viscous solution. The contamination of LPS is less than 1 ng/mg.

SPR-901 showed antitumor activities against murine allogeneic tumors such as sarcoma 180 (S-180) and Ehrlich carcinoma, moreover, it showed significant antitumor activities against murine syngeneic tumors such as Meth-A fibrosarcoma (Meth-A) and Lewis lung carcinoma (3LL) not only by intraperitoneal administration (i.p.) but also by oral administration (p.o.) (Table I). Its effective doses were 10–300 mg/kg/d, and an optimum dose was supposed to be around 30 mg/kg/d. Moreover, orally administered SPR-901 enhanced the reticuloendothelial system in both normal and tumor bearing mice (Table II). But SPR-901 showed no direct cytotoxicities against several tumor cell lines including Meth-A tumor cells *in vitro* (data not shown). These results suggest that the mechanism of antitumor activity of SPR-901 is to be a host mediated one, and the substance is classified as one of the biological response modifiers (BRMs).

Discussion

From these results, since both the physico-chemical and the biological properties of SPR-901 are identical to those of RON reported previously,²⁾ we concluded that these two polysaccharides were the same substance. Also, it is presumed that RON had been produced in the intact rice

bran microbiologically by existing lactic acid bacteria and then extracted with hot water.

Recently, some polysaccharides are being used for clinical purposes as anticancer drugs based on their immunomodulating activities, for example lentinan,⁷⁾ schizophyllan,⁸⁾ and so on. These polysaccharides are all β -glucan and the relationship between their structures and biological activities has been studied.⁹⁾ But in the case of SPR-901, its structure is not a β -glucan but an α -glucan, and its linkages are almost linear α -1,6 glucosidic ones with a small amount of branches at C-3 positions. There are few reports on the antitumor and immunomodulating activities of dextrans.¹⁰⁾ Such dextrans are, however, all highly branched ones and no report could be found on antitumor or immunomodulating activities of almost linear dextran like SPR-901. The structure of SPR-901 is very similar to that of NRRL B-512 dextran whose structure and general properties have been well studied,¹¹⁾ and its down-sized substances are now being used clinically as a plasma expander. But NRRL B-512 dextran shows neither antitumor activities nor immunomodulating activities in our assay systems. The difference between these two dextrans, though still not clear, is very interesting and worthy of investigation. The active site of the antitumor action of SPR-901 and the mode of action are currently being studied.

References and Notes

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