COMPARATIVE STUDY OF THE BIOLOGICAL ACTION OF THE POLYSACCHARIDE GLUCAN AND LAMINARIN

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The intensification of nonspecific resistivity of macroorganisms caused by polysaccharides is a well established fact [3, 9, 15]. This protective action, due mainly to stimulation of the reticulo-endothelial system, occurs in different pathological conditions, especially in experimental infections and transplanted tumors in animals. There is however a lack of conclusive data on the relationship between the chemical structure and the biological action of the polysaccharides.

A marked macromolecular heterogeneity of the microbial heteropolysaccharides has been found by a number of investigators [11, 14]. It has been shown that molecules of relatively large dimensions are necessary for the appearance of biological activity of polysaccharides. Dissociation of polysaccharide macromolecules with the formation of smaller subunits is accompanied by considerable loss of biological activity. With reaggregation of the macromolecular complex the biological activity is restored [12].

A study of the fine chemical structure of the heteropolysaccharides presents great difficulties, and therefore we have restricted ourselves to an investigation of the biological action of 2 homopolysaccharides, glucan and laminarin, the structure of which can be considered to have been interpreted. Both the polysaccharides are polyglucosides in which glucose residues are associated mainly with β-1,3- and to a smaller extent β-1,6-bonds.

Glucan, a polysaccharide from the cellular membranes of the yeast fungi Saccharomyces cerevisiae, [5], is insoluble in cold and boiling water, diluted acids and alkalis, and organic solvents. Laminarin,* a polysaccharide from the alga Laminaria cloustoni, is readily soluble in boiling water [6]. It is assumed that the glucan molecule is an unbranched chain in which groups of glucose residues joined by β-1,3-bonds interchange with glucose residues joined by β-1,6-bonds. The latter comprise 10-20% of the total number of bonds [13]. The laminarin also is an unbranched chain consisting of glucose residues joined by β-1,3-bonds. On the ends of the chains are mannitol residues. The individual short chains are joined by β-1,6-bonds. The polymerization coefficient of laminarin is 20 and that of glucan 40 [10]. Apart from this, it is known [8] that the glucan molecules in the membrane of the yeast cells are connected by acetylglucosamine residues. In our preparation there is about 3% hexosamine; it can therefore be assumed that the polysaccharide in question is a complex aggregate of molecules.

EXPERIMENTAL METHODS

The study was on crossbred white mice weighing 16-18 g. The polysaccharides (glucan and laminarin) were injected into the animals once at different periods before inoculation intravenously or intraperitoneally at a rate of 5 to 30 mg/kg. The mice of the control group received intravenously or intraperitoneally 0.5 ml physiological solution. The ability of polysaccharides to increase the resistivity of the organism was tested on mice in conditions of experimental sepsis caused by intraperitoneal injection of 1 DLM of the following microorganisms: S. typhi

* The laminarin preparation was supplied by Dr. M. A. Bacon, The Macaulay Institute for Soil Research, Aberdeen, Great Britain.

TABLE 1. Relation of the Antibacterial Effect to Polysaccharide Dose

Preparation	Dose (in mg/kg)	Percentage survival (M + m)
Glucan	5	23,3±7,7
	10	36,6±8,8
	20	83,3±6,8
	25	80,0±7,3
	30	86,3±8,1
Laminarin	5	31,4±8,4
	10	30,0±8,1
	20	76,3±7,8
	30	70,6±8,5
Control	Physiological solution	10,0±5,5

TABLE 2. Effect of Time of Injection with Glucan (20 mg/kg) on Its Protective Effect

Time of injection	Percentage survival (M ± m)
Before infection:	
2 h	63.3 ± 8.8
4 h	70.7 ± 8.4
24 h	76.6 ± 7.8
48 h	80.0 ± 7.3
72 h	61.7 ± 8.9
1-3 h after infection	22.4 ± 7.6
Control	11.0 ± 5.6

abdominalis (strain No. 4446) B. dysenteriae Sonne (strain No. 4604,- Escherichia coli (strain No. 94), Proteus mirabilis (strain No. 2), Pseudomonas aeruginosa (strain No. 273), Staph. aureus (strain Gure) sensitive to penicillin, Staph. albus (strain No. 186) resistant to penicillin, and D. pneumoniae type 1. The results were evaluated by the difference in survival rate of the animals of the trial and control groups (from means obtained in 4 trials). The observation period was 10 days.

The antitumorigenic action of glucan and laminarin was studied with patterns of transplanted Ehrlich tumors and Croker sarcoma (C-180) in mice. The polysaccharides were injected intravenously and intraperitoneally at a rate of 20 mg/kg once on the 6th day after tumor implantation or repeatedly 24 h after infection with intervals of 4 days. The antitumorigenic activity was evaluated by the difference in weight of the tumors in the animals of the control and trial groups.

To elucidate the relation of protective effect to the doses of polysaccharides a pattern of experimental staphylococcal sepsis caused by intraperitoneal injection of 1 DLM Staph. albus (strain No. 186) was used. For this the preparations were injected intraperitoneally in doses of 5, 10, 20, and 30 mg/kg once 24 h before infection. It was found that the optimum dose is 20 mg/kg which secures a survival rate of 76-83% of the mice with 90% deaths in the control group of infected animals (Table 1).

On further increasing the polysaccharide dose there was no marked intensification of the antibacterial effect. In connection with this, a dose of 20 mg/kg was used in subsequent studies of the antibacterial action of the preparations. The data in Table 1 also indicate that different doses of glucan and laminarin have different activities in staphylococcal sepsis. Intraperitoneal injection of polysaccharides at a rate of 20 mg/kg 24 h before infection prevented the development of infection and ensured 76-83% survival whereas 90% of the mice in the control group died in the first 2 days.

On the same pattern of staphylococcal sepsis a relation was established between glucan activity and the time of injection. The protective effect of glucan (20 mg/kg intraperitoneally) was most marked when given 24-48 hr before infection. The antibacterial action of the preparation was also considerable when given 2-4 hr before infection. Later use of glucan was ineffective; injection 1-3 h after infection did not prevent losses of animals (Table 2).

In pneumococcal sepsis both preparations were ineffective: the animals given the polysaccharides died earlier than the controls.

Significant differences in the action of glucan and laminarin were apparent in infections caused by Gram-negative microbes. Thus laminarin at a rate of 20 mg/kg given intraperitoneally 24 h before infection had no protective effect in typhoid, Proteus and coli sepsis. In contrast to laminarin,glucan, 20 mg/kg, increased the resistance of mice to those infections and protected 65-83% of the animals from death. Marked antibacterial activity of glucan was also seen in sepsis caused by intraperitoneal administration of Bacillus pyocyaneus and dysentery bacilli. The preparation ensured 66-86% survival of mice whereas 90% of the control mice died (Table 3).

TABLE 3. Antibacterial Action of Glucan and Laminarin in Experimental Sepsis (Single Intraperitoneal Injection with 20 mg/kg)

Microorganism	Preparation	Percentage survival (M + m)
Staph. aureus	Glucan	83,3±6,8
	Laminarin	86,6±8,1
	Control	6,6±4,5
Staph. albus	Glucan	86,5±8,1
	Laminarin	76,6±7,8
	Control	10,0±5,5
B. typhi abdominalis	Glucan	76,6±7,8
	Laminarin	24,4±7,7
	Control	16,6±6,8
Escherichia coli	Glucan	83,3±6,8
	Laminarin	34,0±8,6
	Control	16,6±6,8
Proteus mirabilis	Glucan	65,0±8,9
	Laminarin	24,0±7,1
	Control	10,0±5,5
D. pneumoniae	Glucan	10,0±5,5
	Laminarin	3,0±3,05
	Control	10,0±5,5
Pseudomonas aeruginosa	Glucan	66,6±8,7
	Control	6,6±4,5
B. disenteriae sonne	Glucan	86,6±8,1
	Control	10,0±5,5

TABLE 4. Antibacterial Action of Glucan and Laminarin in Experimental Sepsis (Single Intravenous Injection with 20 mg/kg)

Microorganism	Preparation	Percentage survival (M + m)
Staph. albus	Glucan	16,6±6,8
	Laminarin	10,0±5,5
	Control	13,3±6,2
B. typhi abdominalis	Glucan	23,3±7,6
	Laminarin	16,6±6,8
	Control	20,0±9,2
Escherichia coli	Glucan	13,3±6,2
	Laminarin	6,6±4,5
	Control	10,0±5,5

The protective action of glucan and laminarin in experimental infections occurred only with intraperitoneal injection. With intravenous injection of the same dose (20 mg/kg) the preparations were ineffective and, in a number of cases, even aggravated the course of the infection (Table 4). Preliminary trials showed that intravenous administration of the doses of glucan used had no toxic effect.

The most significant differences between the preparations were observed in a study of their action on growth of tumors. Glucan at a rate of 20 mg/kg injected once on the 6th day after implantation of an Ehrlich tumor or of sarcoma 180 inhibited development of those tumors by 58.6-60%. Under the influence of glucan, large areas of necrosis appeared in the tumors accompanied by a fibroblastic reaction in the surrounding tissues, and hyperplasia of the liver and spleen. When glucan injection was repeated at intervals of 4 days at the same dose rate, there was no further intensification of the antitumor effect. The percentage inhibition of tumor growth, both with single injection or repeated 4 times, was in the range 54-60.

Diller et al. [7] obtained data on the high antitumor activity of glucan with regard to sarcoma 37 in mice. Glucan, injected intravenously in nontoxic doses caused regression of the tumor in 92-98% of the animals. In our trials the antitumor action of glucan was demonstrated with different experimental neoplasma (S-180, Ehrlich tumor, Walker carcinosarcoma [2]). However, the degree of growth inhibition of these tumors using considerably larger doses of the preparation was less.

In similar experimental conditions laminarin possessed practically no antitumor activity. The average inhibition of growth of Ehrlich tumor and S-180 after intravenous or intraperitoneal injection with the preparation at a rate of 20 mg/kg did not exceed 25.3% (Table 5).

The somewhat greater antitumor activity of glucan when injected intravenously compared with its effect when injected intraperitoneally (54-60.1 and 40.7-47.3%, respectively) and the absence of any protective effect of the intravenously administered preparation with regard to bacterial infections deserve attention, especially in studying the mechanisms of the antitumor and antibacterial effect of polysaccharides.

The data show that laminarin and glucan differ in their action on nonspecific resistivity and on the growth of malignant neoplasms. The 2 preparations have a similar composition and types of bonds in the molecule (β-1,3 and β-1,6). Consequently, these 2 factors do not determine the biological activity of the polysaccharides. In all

TABLE 5. Action of Glucan and Laminarin on Different Transplanted Tumors in Animals

Preparation	Tumor	Total dose (mg/kg)	Meth. of injec.	No. of injec.	Inhibition of tumor growth (in %)	P
Glucan	Sarcoma 180	80	i.p.	4	42,0	0,87
		20	„	1	47,3	0,95
		80	i.v.	4	57,9	0,99
		20	„	1	60,1	0,999
	Ehrlich tumor	80	i.p.	4	41,7	0,70
		20	„	1	43,2	0,75
		80	i.v.	4	54,0	0,96
		20	„	1	59,6	0,98
Laminarin	Sarcoma 180	80	i.v.	4	22,0	0,60
		20	„	1	25,3	0,50
		20	i.p.	1	19,7	0,40
	Ehrlich tumor	80	i.v.	4	18,4	0,40
		20	„	1	21,9	0,55
		20	i.p.	1	16,8	0,40

Notes: i.v. = intravenous; i.p. = intraperitoneal.

probability, the size and configuration of the macromolecule of a polysaccharide plays the main role in the development of biological action. The reduction in the splitting of part of the glucose residues under the influence of an acid or enzyme confirms this hypothesis [1, 4].

SUMMARY

Polysaccharides glucan and laminarin produce a practically identical preventive effect in experimental staphylococcic sepsis and secure the survival of 76-83% of mice, whereas in control experiments the death rate is 90%.

Substantial differences have been revealed in the action of these preparations in sepsis caused by gram-negative microorganisms (E. coli., B. typhi abdominalis, Proteus mirabilis). Glucan in a dose of 20 mg/kg infected intraperitoneally averted the death of animals, whereas laminarin in the same dose proved ineffective.

Glucan injected intraperitoneally or intravenously in a dose of 20 mg/kg produced a pronounced antitumor effect against Ehrlich's tumor and sarcoma 180, inhibiting the tumor growth by 53-60%. No inhibitory activity of laminarin with regard to S-180 and Ehrlich's tumor was found under analogous conditions.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
