Effect of Polysaccharides on the Blood System in Rats

I. A. Sychev*, G. V. Poryadin, and V. M. Smirnov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 5, pp. 530-533, May, 2006
Original article submitted December 20, 2004

Natural plant polysaccharides stimulated hemopoiesis, increased the content of some macroglobulins in blood plasma, modulate the weight and cellular composition of lymphoid and hemopoietic organs, activated lymphopoiesis, and enhanced enzyme activity in healthy animals.

Key Words: *polysaccharides; hemopoiesis; bone marrow; globulins; phagocytosis*

Natural plant polysaccharides stimulate various systems in healthy animals. Previous studies showed that polysaccharides from yellow sweet clover increase exercise performance in healthy animals, stimulate hemopoiesis, and increase the number of antibody-producing cells in lymphoid organs of rats [3]. Similarly to other plant polysaccharides, these substances exhibit antiinflammatory activity, reduce edema of the inflamed limb, and normalize the blood system. They improve hemopoiesis and blood composition in anemic animals after treatment with benzene or lead acetate.

Activity of hemopoietic organs and composition and properties of the blood reflect the state of the organism and direction of vital processes. They are regulated by the nervous, endocrine, and immune systems. It is important to study the effect of polysaccharides from yellow sweet clover on the blood, hemopoiesis, and lymphoid organs.

MATERIALS AND METHODS

Polysaccharides isolated and purified by routine methods were dissolved in physiological saline to obtain 10% working solution. Experiments were performed on male Wistar rats weighing 150-250 g. The animals were kept in a vivarium under standard conditions. They received the test substances in a single dose of 0.1 g/kg (one time a day *per os*). Water-soluble polysaccharide complex (WSPC) and Pectin were given for 8 and 10 days, respectively. Control animals received an equivalent volume of physiological saline in the same periods.

The blood, lymphoid organs, and bone marrow were taken from animals receiving WSPC (days 2, 3, 5, and 8) and Pectin (days 1, 3, 5, 8, and 10).

The number of lymphocytes, monocytes, and leukocytes and T lymphocyte/B lymphocyte ratio in the blood were measured by the direct and indirect rosette-forming test. Activity of phagocytosis was determined by the method of Hamburger and Rite. The ratio between α_2-, β-, and γ-globulins in blood plasma was assayed chromatographically [1,4].

Structural changes in the spleen, thymus, and bone marrow were estimated by examination of samples stained with hematoxylin and eosin.

Blood smears and organ samples were stained with methyl green and pyronin to identify cellular RNA (purple to red-violet color). Blood smears and organ samples were stained with a mixture of α-naphthol and pyronin to reveal oxidases. Oxidase-containing structures appeared in various shades of dark blue [2,4].

RESULTS

The number of blood monocytes increased most significantly on days 2 and 1 of treatment with WSPC (by 2.2 times) and Pectin (by 2.6 times), respectively. Blood lymphocyte count was elevated in these periods. Blood lymphocyte count increa-

Russian State Medical University, Moscow; *Institute of Education Development, Ryazan'

0007-4888/06/1415-0592 © 2006 Springer Science + Business Media, Inc.

sed most significantly on days 5 and 3 of treatment with WSPC (by 21.5±0.1%) and Pectin (by 11.6± 0.3%), respectively. The number of monocytes and lymphocytes progressively decreased in the follow-up period and did not differ from the control on days 8-10 of the study ($p<0.01$ and $p\leq0.01$, respectively).

Administration of polysaccharides was accompanied by a decrease in the number of segmented leukocytes. Segmented leukocyte count was minimum on day 5 of WSPC treatment (38.6±0.2% below the control). On day 3 of Pectin treatment the decrease in the number of segmented leukocytes (by 41.7±0.2%) coincided with the maximum count of lymphocytes. The number of segmented leukocytes in the blood returned to normal on days 8 and 10 of treatment with WSPC ($p<0.01$) and Pectin ($p\leq0.01$), respectively.

Administration of the test substances was accompanied by the appearance of immature lymphocytes (day 3). The number of these cells reached the maximum on day 5 of treatment with WSPC and Pectin. Immature lymphocytes were revealed in the blood on day 8, but not on day 10 of the study (Fig. 1).

RNA concentration in rat monocytes reached the maximum on days 1-2 of polysaccharide treatment and returned to normal on days 8-10. RNA content in blood lymphocytes peaked on day 3 of polysaccharide treatment. WSPC and Pectin increased RNA content in these cells by 2.3 and 2.1 times, respectively, compared to the control. RNA content in lymphocytes decreased to normal on days 8-10.

Oxidase content in blood cells peaked on day 3 of treatment with the test substances. Oxidase content decreased in the following order: monocytes>segmented leukocytes>lymphocytes. Oxidase content slightly decreased on day 5 and returned to normal on days 8-10.

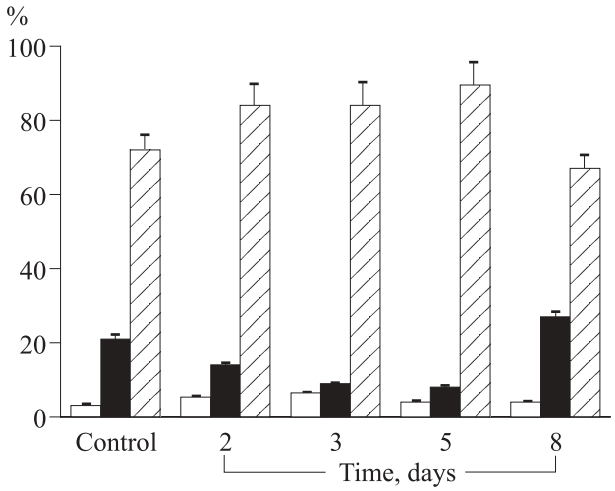

Fig. 1. Cellular composition of the blood (%) from rats receiving WSPC in a dose of 0.1 g/kg. Light bars, monocytes; dark bars, segmented leukocytes; shaded bars, lymphocytes.

Administration of polysaccharides was accompanied by an increase in the number of B lymphocytes in the blood. The number of these cells increased by 12.5±0.1 and 13.4±0.3% on day 3 of treatment with WSPC and Pectin, respectively. On day 10 of Pectin treatment the number of B lymphocytes surpassed the control by 10.5±0.2%.

The number of T cells in rat blood increased most significantly on day 3 of treatment with WSPC and Pectin (by 12.1±0.1 and 14.3±0.1%, respectively, compared to the control). The number of these cells progressively decreased in the follow-up period and only slightly surpassed the control on days 8-10 of the study (by several percents).

TABLE 1. Immunological Parameters of the Blood in Healthy Animals under the Influence of Polysaccharides from Yellow Sweet Clover (*M*±*m*)

Preparation, dosage	T rosette-forming cells, %***	T helper cells, %**	T suppressor cells, %*	B lymphocytes, %**	Phagocytosis, %***	Phagocytic number**
Control	55.10±0.11	44.12±0.31	11.2±0.3	26.2±0.2	60.66±0.31	4.1±0.3
Pectin						
1 dose	59.33±0.34	44.21±0.16	15.33±0.41	28.33±0.42	65.3±0.5	4.8±0.5
3 doses	66.38±0.42	55.23±0.41	11.1±0.3	35.95±0.36	71.3±0.3	6.23±0.13
5 doses	50.33±0.27	44.51±0.13	6.84±0.42	23.31±0.41	44.2±0.6	4.37±0.27
7 doses	55.33±0.15	43.67±0.51	11.67±0.38	33.01±0.21	60.33±0.48	5.31±0.61
10 doses	60.01±0.36	45.33±0.33	14.67±0.25	31.34±0.46	62.67±0.24	5.39±0.19
			WSPC			
2 doses	64.67±0.37	55.26±0.37	5.49±0.42	33.42±0.46	72.25±0.34	8.81±0.23
5 doses	62.51±0.54	52.49±0.24	7.64±0.24	25.14±0.27	56.14±0.41	8.35±0.12

Note. *$p<0.001$, **$p<0.01$, and ***$p\leq0.01$ compared to the control.

TABLE 2. Effect of Polysaccharides from Yellow Sweet Clover on Body Weight and Weights of Organs in Healthy Animals (*M*±*m*)

Preparation, dosage		Body weight, g*	Weight of the thymus, g**	Weight of the spleen, g***
Control		161.67±1.25	0.240±0.245	0.796±0.039
Pectin	1 doses	186.67±2.24	0.313±0.025	0.744±0.029
	3 doses	195.04±0.85	0.326±0.084	0.775±0.082
	5 doses	186.87±2.15	0.335±0.058	0.924±0.059
	7 doses	188.54±2.54	0.346±0.074	0.957±0.085
	10 doses	181.31±1.83	0.280±0.072	0.893±0.073

Note. *$p<0.01$, **$p\leq0.01$, and ***$p\leq0.05$ compared to the control.

The number of T helper cells increased most significantly on day 3 of treatment with WSPC and Pectin (by 14.02±0.2 and 11.6±0.3%, respectively, compared to the control). The number of T helper cells progressively decreased in the follow-up period and did not differ from the control on days 8-10 of the study. The number of T suppressor cells decreased most significantly on days 3 and 5 of treatment with WSPC (from 11.1±0.3 to 5.2±0.2%) and Pectin (7.3±0.4%), respectively. The number of T suppressor cells retuned to normal on days 8-10 of the study (Table 1).

Activity of phagocytosis in blood cells attained the maximum on day 3 of polysaccharide administration. WSPC and Pectin increased the number of phagocytizing cells by 12.22±0.15 ($p\leq0.01$) and 11.34±0.23% ($p<0.01$), respectively.

The number of blood microphages (segmented cells) decreased in treated rats. These changes contributed to the reduction of phagocytosis and decrease in the number of macrophages (by 14.30± 0.25%, day 5). This parameter returned to normal on days 8-10 of the study.

The phagocytic number increased most significantly on days 3-5 of polysaccharide administration (by 2 times compared to the control), which was associated with activation of the enzyme system in phagocytes. Oxidase activity increased on days 3 and 5 of treatment with WSPC (by 2.3 times) and Pectin (by 2 times), respectively.

The content of α_2-macroglobulins in blood plasma increased on days 2 and 3 of treatment with WSPC (by 55.6±0.3%) and Pectin (by 21.7±0.2%), respectively. The content of these regulatory proteins progressively decreased in the follow-up period and did not differ from the control.

The content of β-macroglobulins in blood plasma decreased on days 2 and 5 of treatment with WSPC (by 13.3±0.1%) and Pectin (by 22.8±0.2%), respectively. The content of β-macroglobulins returned to normal on days 8 and 10 of treatment with WSPC and Pectin, respectively.

The content of γ-globulins increased most significantly on days 5 and 3 of treatment with WSPC (by 52.8±0.2%) and Pectin (by 39.2±0.3%), respectively.

The content of γ-globulins decreased, but remained above the normal on day 8 of WSPC administration (by 29.7±0.5%). This parameter did not differ from the control on day 10 of pectin treatment.

Variations in the number of cells and content of regulatory proteins in the blood reflect changes in the weight, cellularity, and structure of the bone marrow, thymus, and spleen.

Body weight of animals increased most significantly on day 3 of Pectin administration (by 20.5± 0.3%). The weights of the thymus and spleen increased by 31.2±0.2 and 26.2±0.3%, respectively, on day 7 of treatment.

The number of bone marrow cells increased on day 1 of polysaccharide administration. This parameter increased most significantly on day 5 of treatment with WSPC and Pectin (by 1.6 and 1.4 times, respectively, compared to the control). We observed an increase in the number of erythrocytes, lymphocytes, and monocytes in the peripheral blood. The number of megakaryocytes increased in the spleen and bone marrow. Production and migration of cells into the blood underwent various changes. Cellularity of the bone marrow, thymus, and spleen reached the maximum on day 5, but slightly decreased on days 8-10 of study.

RNA content in macrophages reached the maximum on days 1-2 of polysaccharide administration. RNA content in macrophages and lymphocytes (lymphoblasts) reached the maximum on days 3-5. On days 8-10 of the study the intensity of staining decreased, but remained above the normal.

The number of secondary follicles increased in the spleen of treated rats. Administration of the test substances was accompanied by the appearance of primary follicles. On day 3, the mantle layer and germinal center of secondary follicles were densely

packed with cells. We observed a significant increase in the number of cells in the T-dependent zone of the white pulp (particularly at the boundary between the T and B zones). A considerable number of rosettes were found on day 3 and, particularly, on day 5 of the study. They consisted of a central macrophage and surrounding lymphocytes. The number of plasma cells increased. The number of spleen cells slightly decreased on days 8-10 of polysaccharide administration. These changes were accompanied by a decrease in the number of secondary follicles, rosettes, and plasma cells. However, these parameters remained above the control level.

The total cellularity of the bone marrow increased on day 1. The number of bone marrow cells increased most significantly on days 5-8. The number of erythroblastic islets in the bone marrow of treated rats exceeded the control by 1.5-2 times. The number of lymphoid cells increased most significantly on days 3-5 (by 1.5-2 times compared to the control). The number of mature leukocytes in the bone marrow of treated animals was minimum on days 3-5. This parameter approached the control level on days 8 and 10 of treatment with WSPC and Pectin, respectively.

Our results show that WSPC and Pectin increase blood lymphocyte count in healthy animals by 21.5 and 11.6%, respectively. These changes are accompanied by the appearance of immature lymphocytes. On days 1-2 of study WSPC and Pectin increase monocyte count by 2.2 and 2.6 times, respectively.

Polysaccharides activate phagocytosis, stimulate hemopoiesis, and increase cellularity of the bone marrow and lymphoid organs (WSPC, by 1.6 times; Pectin, by 1.4 times). WSPC and Pectin increase RNA concentration by 2.3 and 2.1 times, respectively. WSPC and Pectin increase the content of oxidases and number of blood cells and immune cells by 2.3 and 2 times, respectively. WSPC and Pectin increase the amount of γ-globulin, α_2-globulin, and β-globulin containing lymphokines and other factors of immune cell activation.

The test polysaccharides increase the number of secondary follicles, count of plasma cells, and size of the T zone in the spleen of animals.

REFERENCES

1. F. I. Komarov, B. F. Korovkin, and V. V. Men'shikov, *Biochemical Studies in Clinical Practice* [in Russian], Moscow (1984).
2. R. Lilly, *Histological Technique and Practical Histochemistry* [in Russian], Moscow (1969).
3. A. A. Podkolzin, V. I. Dontsov, I. A. Sychev, *et al*., *Byull. Eksp. Biol. Med*., **121**, No. 6, 661-663 (1996).
4. *Handbook on Clinical Laboratory Methods of Study* [in Russian], Ed. E. A. Kost, Moscow (1975).