Effect of Na-,Fe-,Co-,Cu-Polygalacturonate on Hemopoietic Function in Laboratory Animals

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The effects of water-soluble Na-,Fe-,Co-,Cu-polygalacturonate on hemopoiesis were studied in laboratory animals of various species, age, and functional groups. The compound had a strong positive effect on hemopoiesis, which manifested in an increase in hemoglobin concentration and erythrocyte count and rapid recovery of blood parameters after acute blood loss.

Key Words: *pectin polysaccharides; metal complexes; hemoglobin; erythrocytes; microelements*

Water-soluble polymetal complex (PC) obtained on the basis of pectin biopolymers (Na-,Fe-,Co-,Cu-polygalacturonate [1]) of high biological value in a bioavailable form contains microelements essential for hemopoiesis [1]. The effects of these compounds on hemopoietic function in animals of various species and functional groups is important for the creation for new potent antianemic preparations, which is an urgent problem due to high incidence of anemias associated with deficiency of biologically available iron [5] and other microelements [6,7].

MATERIALS AND METHODS

PC was synthesized as described elsewhere [1]. This complex contains 6.05% Na, 0.68-1.10% Fe, 0.76-0.89% Co, and 0.77-0.85% Cu.

Experiments were performed on animals of the same sex and age groups. Experiments on rats were performed on adult males and females aging 3-4 months and weighing 150-200 g, intact and after single blood loss (1.5-2.0 ml blood from the tail end) and on young growing animals. Young rats initially received PC with breast milk, and then with water (from the 1st month of life). Experiments on mice involved adult males weighing 25-30 g. The animals received 1% solution of PC diluted with distilled water by 2 or 4 times (instead of drinking water). Controls received pure water.

We measured hemoglobin concentration (Hb, hemoglobin cyanide method), hematocrit, erythrocyte count (Goryaev chamber, light microscopy), Hb content per erythrocyte (computational method), and osmotic resistance of erythrocytes in NaCl solution of various concentrations. Blood parameters in rats were estimated weekly and in mice not more than 1 time in 2 weeks.

The results were analyzed by Student's *t* test (Origin 6.1 software).

RESULTS

In adult male rats receiving PC in a dose of 120 mg/kg Hb concentration and erythrocyte count increased by 13-17 and 21-33%, respectively. Osmotic resistance of erythrocytes also increased under these conditions (Table 1). Hb content per erythrocyte and hematocrit did not differ from the control.

In female rats receiving PC in a dose of 300 mg/kg, hemoglobin concentration increased by 20.3% ($p<0.001$, to 187.9±3.0 g/liter *vs.* 156.2±2.7 in the

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TABLE 1. Blood Parameters in Male Rats (*M*±*m*, *n*=5)

Parameter		Control	PC, 120 mg/kg
Hb concentration, g/liter		159.7±3.9	187.5±4.4**
Erythrocyte count, mln/μl		8.53±0.42	11.36±0.29**
Hb content per erythrocyte, pg		17.63±1.00	16.51±0.90*
Hematocrit, %		47.21±3.01	48.12±0.90
Erythrocyte resistance, % NaCl	maximal	0.31±0.01	0.24±0.03*
	minimal	0.45±0.01	0.48±0.01

Note. Here and in Tables 2 and 3: *p<0.05 and **p<0.001 compared to the control.

TABLE 2. Average Daily Increase in Blood Parameters in PC-Receiving Male Rats after Blood Loss (*M*±*m*, *n*=3)

Parameter	Control	PC, 250 mg/kg
Hb concentration, g/liter/day	1.11±0.24	1.90±0.14*
Erythrocyte count, mln/μl/day	0.077±0.009	0.14±0.01*

TABLE 3. Effect of PC on Blood Parameters in Young Growing Rats (*M*±*m*)

Parameter	Control (*n*=24)	PC, 250 mg/kg (*n*=10)
Hb concentration, g/liter		
5-6 weeks	121.0±4.8	153.7±8.0**
9-10 weeks	145.7±9.4	175.1±8.4*
Erythrocyte count, mln/μl		
5-6 weeks	4.53±0.60	6.84±0.99*
9-10 weeks	6.63±0.31	7.14±0.33

control) and erythrocyte count increased by 4.7% (9.62±0.28 *vs.* 9.19±0.23 mln/μl in the control). Hb content per erythrocyte increased by 16.4% (19.70± 0.80 *vs.* 16.92±0.35 pg in the control, p<0.01).

The effect of PC in males and females was most pronounced on days 20 and 35-42, respectively, and persisted over 2 weeks after withdrawal.

Blood loss in male rats was followed by a decrease in Hb concentration and erythrocyte count by 10-18 and 10-15%, respectively, compared to the basal levels. Hb content per erythrocyte remained unchanged under these conditions. One week after the start of PC administration, Hb concentration increased by 15% (139.1±5.0 *vs.* 121.0± 1.0 g/liter in the control, p<0.05) and erythrocyte count by 30% (9.05±1.36 *vs.* 6.94±0.76 mln/μl in the control, p<0.05). These parameters progressively increased over the following 5-6 weeks. In this period Hb concentration and erythrocyte count exceeded the basal level practically by 55% (189.6±5.1 g/liter and 11.51±0.49 mln/μl, respectively, p<0.001). Evaluation of the average daily increase in blood parameters showed that treated animals recovered more rapidly than controls (Table 2).

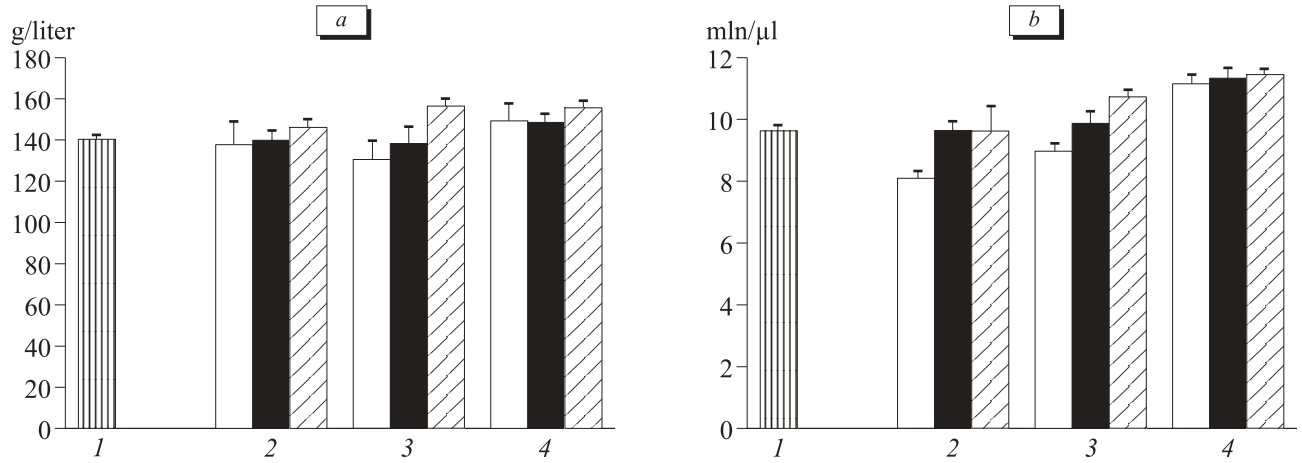

Fig. 1. Effect of PC on blood parameters in laboratory mice: Hb concentration (*a*) and erythrocyte count (*b*). Basal level (*1*); days 10-14 (*2*), 30-35 (*3*), and 70 (*4*). Light bars, control group; dark bars, mice receiving 360 mg/kg PC; slant shading, mice receiving 480 mg/kg PC. *p<0.05, **p<0.01, and ***p<0.001 compared to the basal level.

The test parameters in young animals aging 5-6-weeks were much lower than in adult specimens (Table 3). These differences can be explained by increased functional load due to growth and, therefore, high demands for nutrient substances and trace elements. Blood parameters in treated animals were higher than in control specimens. Starting from the age of 5-6 weeks, blood parameters in young animals of the treatment group practically did not differ from those in adult animals.

PC also had a positive effect on hemopoietic function in mice. Probably, blood sampling (even several tens of microliters) in control animals performed at fixed time intervals can be considered as a blood loss. This procedure was sufficient for the decrease in blood parameters (Fig. 1). The animals were not traumatized over 35 days before the study. On day 70 of the study, blood parameters in mice retuned to normal and even exceeded the basal level. These changes were mediated by compensatory mechanisms for the regulation of hemopoiesis. The test parameters did not decrease in animals of the PC group. Hb concentration in these mice increased on day 30-35. PC produced a dose-dependent effect (Fig. 1). PC had little effect on erythrocyte count.

Our study showed that PC has a positive effect on blood parameters in laboratory animals of various groups. PC produced no negative effect on animals. Hence, PC holds much promise for the development of new antianemic preparations.

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