NEW MEDICINAL PREPARATIONS

Pine Resin and Biopin Ointment: Effects on Nonspecific Resistance of Organisms

A. S. Simbirtsev, V. G. Konusova, G. Sh. Mchedlidze*, O. V. Markovskaya, E. Z. Fidarov, B. A. Paramonov*, and V. Yu. Chebotarev*

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We studied the effects of pine resin and Biopin ointment used for the therapy of burns, wounds, purulent and inflammatory diseases on blood leukocyte count, phagocytic activity of macrophages and neutrophils, and redox potential of peripheral blood neutrophils. The wound-healing effect of the test preparations is determined by their ability to activate phagocytosis.

Key Words: *pine resin; Biopin ointment; wound-healing effect; phagocytosis stimulation*

Galipot of various coniferous tress, including pine, fir, larch, and cedar, widely used in traditional medicine is now introduced into the composition of commercial medicinal preparations (ointments) [4]. For instance, Biopin ointment (BO) containing beeswax and pine resin (PR) holds much promise for the therapy of burns, wounds (phase I of wound process), purulent and inflammatory diseases of the skin and subcutaneous fat [3]. The mechanisms underlying the wound-healing effect of PR-containing preparations are poorly understood.

Inflammatory cells are mobilized over the first hours after burning or wound infliction. The counts of segmented and stab neutrophils and macrophages increase. During rejection of necrotic tissues and suppuration the intensity of phagocytosis increases, while its completeness decreases. The degree and duration of the decrease in neutrophil phagocytic activity determine the outcome of infections [1,2].

Here we studied the effects of PR and BO on phagocytic activity of macrophages and neutrophils (phagocytic index and phagocytic number) and redox potential of neutrophils characterizing their ability to ingest phagocytized cells (phagocytosis completeness).

Institute of Extrapure Biological Preparations; *St. Petersburg-Technology, St. Petersburg. ***Address for correspondence:*** elena@tech.spb.ru. Chebotarev V. Yu.

MATERIALS AND METHODS

In vivo effects of PR and BO on leukocyte count, phagocytic activity of peritoneal macrophages, and redox potential of peripheral blood neutrophils were studied on 230 male CBA mice weighing 16-18 g and obtained from the Rappolovo nursery (Russian Academy of Sciences). Thymogen and interleukin-1β (IL-1) were used as reference agents. Each group (n=46) included 5 animals. The test preparations were dissolved in physiological saline and injected intraperitoneally in doses of 0.5, 5, 50, 500, and 5000 μg/kg. Control mice received an equivalent volume of physiological saline. The animals were decapitated 4 and 24 h after treatment, and peritoneal cells were obtained. The count and ratio between leukocytes were estimated on peripheral blood smears stained by the method of Romanovsky.

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TABLE 1. *In Vivo* Effects of Test Preparations on Phagocytosis (Phagocytic Index, %) of Yeast Cells by Mouse Peritoneal Macrophages (*M*±*m*, *n*=5)

Time after treatment; dose, μg/kg		PR	BO	Thymogen	IL-1
4 h	0+	53.6±1.4	35.9±2.5	42.6±1.9	42.6±1.9
	0.5	46.8±2.7	41.7±2.6	49.0±5.3	43.2±1.7
	5	67.6±2.5	37.3±2.0	47.0±2.4	47.8±2.6
	50	50.6±7.3	39.8±2.5	44.0±4.1	49.8±1.0*
	500	48.8±1.5	38.1±4.3	43.4±4.7	56.1±2.5*
	5000	49.6±4.6	38.6±2.6	40.0±1.3	—
24 h	0+	53.6±1.4	59.2±2.6	39.6±6.5	39.6±6.5
	0.5	62.8±4.6	61.6±3.1	39.2±3.4	39.0±3.3
	5	70.9±3.8	64.8±3.2	39.1±7.7	47.6±2.3*
	50	67.9±2.8	67.8±3.9	44.9±4.2	55.2±2.4*
	500	70.4±4.1*	80.7±1.9*	42.9±8.0	58.5±4.0*
	5000	49.6±4.6	76.6±2.0*	47.7±2.8*	—

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

Phagocytic activity of macrophages was evaluated by the phagocytic index (ratio of phagocytizing cells). Bakers' yeast served as the object for phagocytosis. Peritoneal cells from each mouse were cultured in Petri dishes (40 mm) with medium 199 and 10% bovine serum, incubated with yeasts for 1 h, washed, fixed with methanol for 15 min, and stained by the method of Romanovsky. The reaction of 200 cells was estimated microscopically. Cells containing not less than 3 yeast cells were considered as phagocytizing cells.

The effects of preparations on the redox potential of neutrophils were studied by luminol-dependent chemiluminescence on a Lumina LKB-1252 system (LKB). Whole blood samples in heparin-containing buffer were placed in a cuvette, mixed with luminol, and incubated at 37°C for 40 min. The inductor (opsonized zymosan) was added.

We studied *in vitro* effects of BO on phagocytic activity and redox potential of peripheral blood neutrophils from 3 donors. Cells were isolated by centrifugation of heparinized blood in a Ficoll-Paque density gradient (Pharmacia). The water-soluble fraction of BO was added in doses of 0.001, 0.01, 0.1, 1, and 10 μg/ml. We performed 6 parallel measurements for 1 control and 5 experimental groups.

The effect of BO on phagocytic activity of neutrophils was estimated by the phagocytic index and phagocytic number (number of phagocytized particles). Bakers' yeast served as the object for phagocytosis. BO was placed in multiwell plates with yeast cells (2×10^7 cells/ml) and neutrophils washed with Eagle's medium (2×10^6 cells/ml), incubated at 37°C for 2 h, fixed, stained and the reaction was analyzed (similarly to macrophages). Two series of measurements with blood samples from various donors were carried out.

The effect of BO on the redox potential of neutrophils was evaluated by the reaction with nitroblue tetrazolium (NBT test). The ratio of NBT-positive leukocytes was estimated at various concentrations of BO.

The observed mean and dispersion were calculated for each group. The differences between the control and experimental groups were evaluated by Student's *t* test (probability 0.95).

RESULTS

Intraperitoneal administration of PR and BO had no effect on the total and differential count of peripheral blood leukocytes. Only PR in a dose of 5000 μg/kg caused a statistically significant increase in leukocyte count. However, IL-1 dose-dependently changed the test parameters.

Phagocytic activity of macrophages and leukocytes depended on the doses of PR and BO. These

TABLE 2. *In Vitro* Effects of BO on Phagocytosis of Yeast Cells by Human Peripheral Blood Neutrophils (*M*±*m*, *n*=6)

BO dose, μg/kg	Phagocytic index, %	Phagocytic number
0+	57.0±2.0	3.9±0.3
0.001	77.0±7.0*	4.1±0.1
0.01	71.0±5.0*	4.3±0.1*
0.1	57.0±7.0	4.0±0.1
1.0	66.0±2.0	4.1±0.1
10.0	67.5±6.5	3.9±1.0

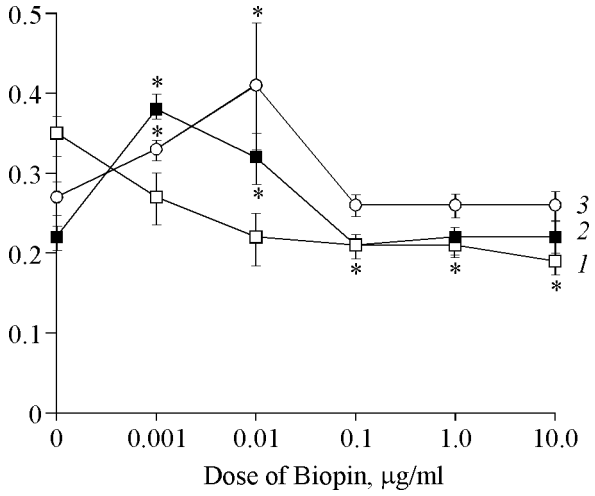

Fig. 1. Effect of Biopin *in vitro* ointment on redox potential of human peripheral blood neutrophils (ratio of NBT-positive cells): donors 1 (*1*), 2 (*2*), and 3 (*3*). **p*<0.05 compared to the control.

more rapidly, which can be explained by its biological functions in the organism.

NBT test showed that leukocytes from 3 donors had various baseline levels of oxidation-reduction metabolism (Fig. 1). BO decreased the redox potential of cells from donor 1 with intensive production of oxygen radicals. In donors 2 and 3 with normal baseline levels of oxygen radical generation BO dose-dependently increased, but then decreased the redox potential of neutrophils. It should be noted that BO in low doses activated these cells, which is typical of immunomodulators. BO in doses close to 0.01 μg/ml enhanced both phagocytic (Table 2) and degrading activities (Fig. 1) of neutrophils.

Our experiments show that preparations containing PR activate phagocytosis and hold much promise for the therapy of burns, wounds, purulent and inflammatory diseases.

TABLE 3. *In Vivo* Effects of Preparations on Oxygen Radical Generation (Intensity of Luminol-Dependent Chemiluminescence, mV/min) by Mouse Peripheral Blood Neutrophils (*M*±*m*, *n*=5)

Time after treatment; dose, μg/kg		PR	BO	Thymogen	IL-1
4 h	0⁺	25.0±0.6	25.0±0.6	29.2±1.3	29.2±1.3
	0.5	23.9±1.2	28.4±1.1	28.4±0.7	39.1±1.5*
	5	28.6±4.6	32.2±2.1	30.8±2.6	39.8±1.8*
	50	28.9±2.6	40.9±8.7	27.6±0.4	47.1±2.9*
	500	26.8±1.6	30.5±1.4	30.6±1.3	49.0±4.1*
	5000	27.3±2.1	33.3±2.6	29.1±1.4	—
24 h	0⁺	24.0±0.3	26.7±0.8	39.6±6.5	39.6±6.5
	0.5	23.9±0.3	28.6±2.1	30.9±2.3	39.0±3.3
	5	26.9±1.0*	33.5±5.7	28.5±7.7	47.6±2.3*
	50	27.3±1.3*	34.5±1.2*	46.9±4.2	55.2±2.4*
	500	26.1±1.1	34.5±0.3*	40.9±8.0	58.5±4.0*
	5000	26.6±1.8	26.6±0.2	47.7±2.8*	—

preparations in certain concentrations enhanced phagocytic activity of macrophages (Table 1) and neutrophils (Table 2). The phagocytic index and phagocytic number increased, particularly 24 h after intraperitoneal injection of preparations.

Activation of neutrophils (increase in their redox potential) was observed 24 h after intraperitoneal administration of PR and BO (Table 3). BO in various concentrations was more potent than PR in activating leukocytes. It should be emphasized that BO stimulate neutrophils as soon as 4 h after treatment. The stimulatory effect of IL-1 developed

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