NEW MEDICINAL PREPARATIONS

Pine Resin and Biopin Ointment: Effects of Water-Soluble Fractions on Functional Activity of Peripheral Blood Neutrophils

A. S. Simbirtsev, V. G. Konusova, G. Sh. Mchedlidze, B. A. Paramonov*, and V. Yu. Chebotarev*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 7, pp. 59-62, July, 2002
Original article submitted May 7, 2002

We studied the effects of water-soluble fractions of pine resin and Biopin-10 and Biopin-20 ointments used in the treatment of burns, wounds, and pyoinflammatory processes on the phagocytic activity and redox potential of human peripheral blood neutrophils *in vitro*. The test preparations contain bioactive substances producing opposite dose-dependent effects on phagocytosis, which suggests that the composition and dose of ointments should be selected individually in order to attain the optimal therapeutic effect.

Key Words: *pine resin; Biopin ointment; chromatographic fractionation; phagocytosis*

Pine resin (PR) is used in traditional medicine since ancient times. Now it is used as the basic component of commercial drugs (ointments). Biopin ointment containing 10 or 20% PR (BO-10 or BO-20, respectively) is highly effective in the treatment of burns, wounds, and pyoinflammatory processes of the skin and subcutaneous tissues [3].

It was previously shown that PR and BO-10 activate phagocytosis by stimulating functional activity of neutrophils and macrophages *in vivo* and *in vitro* [4]. The aim of the present study was to identify and analyse bioactive components of PR and BO. This is of particular importance for creating the optimal composition of the drugs and selecting effective therapeutic doses.

Neutrophilic leukocytes play an important role in the system of nonspecific immunity. Their mobilization during the very first hours after injury (burn or wound) determines the efficiency of the inflammatory reaction. Activity of phagocytosis sharply increases during rejection of necrotic mass and development of suppurative process, but its completeness decreases. At the same time the outcome of infectious process depends on the degree and duration of inhibition of neutrophilic functions [1,2].

We investigated the effects of chromatographic fractions of the initial water-soluble PR, BO-10, and BO-20 fractions on phagocytic activity (phagocytic index and number) and redox potential of neutrophilic leukocytes characterizing their capacity to digest phagocytosed cells (completeness of phagocytosis).

MATERIALS AND METHODS

Water-soluble fractions of PR and BO were obtained by the same protocol. In brief, 20 g PR, BO-10, or BO-20 was dissolved in 150 ml hexane, equal volume of acetonitrile:isopropanol (3:1) mixture was added, and the light (hexane) fraction was removed. The heavy

State Institute of Extra Pure Biological Preparations; *SPb-Tekhnologiya Firm, St. Petersburg. ***Address for correspondence:*** elena@tech.spb.ru. Chebotarev V. Yu.

0007-4888/02/1341-050$27.00

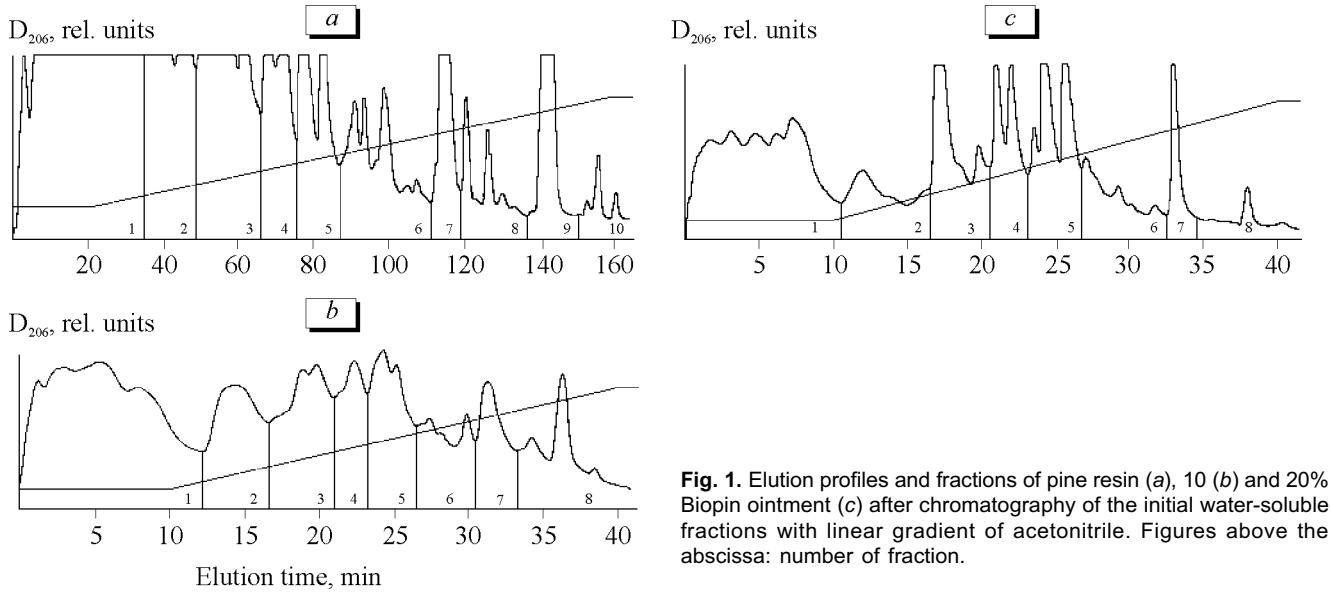

Fig. 1. Elution profiles and fractions of pine resin (*a*), 10 (*b*) and 20% Biopin ointment (*c*) after chromatography of the initial water-soluble fractions with linear gradient of acetonitrile. Figures above the abscissa: number of fraction.

fraction (150 ml) was mixed with 165 ml water, centrifuged at 3000*g* for 20 min, and the light (floating) fraction was removed. The heavy fraction was adjusted to 500 ml with 40% acetonitrile (initial preparation, fraction 0). Further fractionation was carried out on a Dynomax-60A C_{18} column (21.5×100 mm). Frac-

TABLE 1. Effects of Preparations on Phagocytosis of Yeast Cell by Human Peripheral Blood Neutrophils: Phagocytic Index (%; *M*±*m*, *n*=4)

Preparation; dose, μg/ml	Fraction No.								
PR	1	2	3	4	5	6	7	8	9
0					74±9				
0.001	67±7	77±5	76±8	70±4	79±7	79±10	81±5	71±3	78±8
0.01	68±4	70±2	78±6	63±5	66±2	75±3	73±6	83±3	86±6
0.1	71±3	72±4	66±8	69±9	75±5	64±4	80±4	84±6	75±5
1	71±5	82±4	77±5	64±3	75±10	76±9	78±9	81±4	82±6
10	82±9	65±7	75±5	67±5	73±4	77±9	74±4	82±10	80±3
100	76±5	81±3	83±4	64±3	64±4	75±5	76±4	76±9	73±13
BO-10	0+	1	2	3	4	5	6	7	8
0	57±2				60±3				
0.001	77±7*	74±8	74±1*	70±1*	62±2	59±3	63±3	67±4	65±7
0.01	71±5*	64±3	76±1*	74±1*	67±9	60±8	64±6	70±3	60±3
0.1	57±7	63±10	66±4	70±1*	62±2	68±4	68±5	70±6	69±4
1	66±2	58±3	65±3	65±3	55±4	76±6	50±5	63±3	78±1*
10	68±7	49±9	54±9	62±6	61±3	62±5	56±4	71±5	62±8
BO-20	0+	1	2	3	4	5	6	7	8
0	56±5				45±5				
0.001	75±9	49±3	58±4	48±3	41±9	58±8	45±15	44±6	50±9
0.01	74±7	41±3	55±5	53±4	35±5	40±10	48±4	33±9	60±9
0.1	66±5	25±1*	40±10	50±5	43±13	40±8	55±5	53±13	55±7
1	57±7	31±3	53±4	55±5	50±10	50±7	53±8	52±4	52±10
10	63±3	33±4	38±3	40±9	40±5	45±5	43±8	31±5	30±7

Note. Here and in Tables 2 and 3: *p<0.05 *vs.* control (dose 0). +n=6.

tion 0 (10 ml) was eluted with a linear gradient of acetonitrile in water (40-100%, 5 ml/min, 30°C). The elution profiles (UV detection at 206 nm) are shown on Fig. 1.

The effects of water-soluble fractions of PR and BO on phagocytic activity and redox potential of neutrophils were studied on blood samples from two donors. The cells were isolated from fresh heparinized blood by centrifugation in a Ficoll-Paque density gradient (Pharmacia). Water-soluble fractions of PR and BO were added in doses of 0.00001 (only BO-20), 0.0001 (only BO-20), 0.001, 0.01, 0.1, 1, 10, and (only PR) 100 μg/ml. Two series of experiments with blood from different donors were carried out in order to rule out accidental results.

Phagocytic activity of neutrophils was evaluated by the phagocytic index (percentage of phagocytosing cells) and phagocytic number (number of phagocytosed particles per leukocyte), using baker's yeast as the object of phagocytosis. The test fraction of the preparation was added to wells of microtitration plates containing yeast cells (2×10^7 cell/ml) and neutrophilic leukocytes washed with Eagle medium (2×10^6 cells/ml). The plates were incubated at 37°C for 2 h, fixed, stained, and the reaction was evaluated (microscopically). To this end, 200 cells were examined. Cells containing at least 3 yeast cells were considered phagocytizing.

Empirical means and dispersions were estimated for each group of data, after which the "control-experiment" pairs were tested for coincidence (with a probability of 0.95) of the true means with presumably equal true dispersions (Student's *t* test).

RESULTS

Experiments revealed a pronounced stimulatory effect of fractions 2-3, 5-6, and 8-9 on phagocytic index, phagocytic number, and redox potential of neutrophils in a wide dose range (Tables 1-3). Fraction 8 in doses of 0.1 and 1 μg/ml and fraction 9 in a dose of 0.001 μg/ml significantly increased the redox potential and phagocytic number and insignificantly increased the phagocytic index. It is noteworthy that stimulatory effect of low doses is characteristic of many immunomodulating agents. Fractions 1 and 4 slightly suppressed phagocytosis.

TABLE 2. Effects of Preparations on Phagocytosis of Yeast Cell by Human Peripheral Blood Neutrophils: Phagocytic Number (*M*±*m*, *n*=4)

Preparation; dose, μg/ml	Fraction No.								
PR	1	2	3	4	5	6	7	8	9
0				4.6±0.2					
0.001	5.0±0.3	4.9±0.1	4.9±0.3	4.6±0.5	4.8±0.3	4.7±0.3	4.6±0.2	4.5±0.1	5.4±0.1*
0.01	4.6±0.2	4.8±0.2	5.0±0.4	4.7±0.4	4.9±0.2	4.3±0.1	4.5±0.2	4.9±0.2	4.8±0.2
0.1	4.6±0.3	4.9±0.5	5.1±0.3	5.0±0.3	4.6±0.1	4.4±0.3	4.9±0.2	5.4±0.1*	4.8±0.2
1	4.7±0.4	4.6±0.3	4.6±0.1	4.2±0.2	4.8±0.5	4.3±0.3	4.7±0.2	5.6±0.1*	4.7±0.3
10	4.4±0.1	4.7±0.1	4.7±0.2	4.5±0.4	4.7±0.3	4.3±0.5	4.5±0.3	5.4±0.5	4.5±0.1
100	4.8±0.2	4.6±0.2	4.4±0.2	4.5±0.2	4.5±0.2	4.4±0.1	4.4±0.3	4.2±0.3	4.1±0.2
BO-10	0+	1	2	3	4	5	6	7	8
0+	3.9±0.3				3.9±0.3				
0.001	4.1±0.1	4.8±0.4	3.9±0.1	4.5±0.1*	4.2±0.2	4.0±0.2	3.6±0.3	3.5±0.3	4.0±0.2
0.01	4.3±0.1*	4.3±0.2	4.6±0.4	4.6±0.1*	4.1±0.2	4.0±0.3	3.4±0.5	3.8±0.7	3.9±0.5
0.1	4.0±0.1	4.0±0.3	4.5±0.3	4.8±0.1*	3.9±0.1	3.7±0.2	3.8±0.2	3.8±0.4	3.8±0.3
1	4.1±0.1	4.1±0.2	4.4±0.4	4.6±0.4	4.2±0.3	4.1±0.3	4.0±0.2	4.2±0.3	4.1±0.4
10	3.9±1.0	4.2±0.3	4.1±0.2	4.3±0.3	4.0±0.4	3.9±0.1	3.5±0.4	4.2±0.4	3.6±0.7
BO-20	0+	1	2	3	4	5	6	7	8
0	3.7±0.2				3.8±0.1				
0.001	3.8±0.1	3.5±0.2	3.6±0.3	3.5±0.3	3.9±0.1	3.6±0.2	3.3±0.3	3.8±0.2	4.2±0.7
0.01	4.2±0.3	3.7±0.1	3.9±0.2	4.0±0.2	3.5±0.3	3.5±0.3	3.5±0.2	3.3±0.3	4.0±0.1
0.1	3.5±0.2	3.4±0.1*	3.5±0.2	3.4±0.3	3.5±0.5	3.6±0.2	3.6±0.1	3.5±0.3	3.5±0.3
1	3.6±0.3	3.5±0.3	3.3±0.1*	3.7±0.1	3.6±0.2	3.6±0.4	3.7±0.1	3.5±0.2	3.5±0.2
10	3.7±0.1	3.8±0.2	3.4±0.3	3.4±0.4	3.4±0.4	3.7±0.1	3.6±0.5	3.4±0.3	3.4±0.4

TABLE 3. Effects of Preparations on Redox Potential of Human Peripheral Blood Neutrophils in NBT Test (%, *M*±*m*, *n*=4)

Preparation; dose, μg/ml	Fraction No.								
PR	1	2	3	4	5	6	7	8	9
0					41±1				
0.001	39±1	45±2	49±2*	42±2	48±1*	51±1*	45±2	54±2*	57±1*
0.01	42±1	44±1	48±3*	42±1	50±1*	51±1*	43±1	55±1*	56±2*
0.1	40±1	45±2	45±1*	43±1	45±1*	50±1*	44±1	59±1*	61±2*
1	40±3	47±1*	49±1*	44±1	46±1*	50±1*	44±1	61±1*	56±1*
10	39±1	47±1*	48±1*	45±2	44±1	51±1*	44±2	54±1*	56±1*
100	39±1	43±1	47±1*	39±1	44±1	45±1	42±1	44±2	55±1*
BO-10	0+	1	2	3	4	5	6	7	8
0	22±1				17±1				
0.001	38±2*	22±1*	24±1*	41±7*	19±1	18±1	20±1	15±1	15±1
0.01	32±3*	19±1	17±1	31±4*	15±1	17±1	20±1	15±1	16±1
0.1	21±1	16±1	25±2*	27±2*	13±1	16±1	19±1	17±1	16±1
1	22±1	18±1	17±1	30±2*	16±1	16±1	17±1	17±1	18±1
BO-20	0	1	2	3	4	5	6	7	8
0	—				24±1				
0.00001	—	21±1	21±1	24±1	26±1	26±1	26±1	24±1	25±4
0.0001	—	22±1	25±1	34±2*	33±1*	35±2*	31±1*	26±1	24±3
0.001	—	19±1*	31±1*	24±1	23±1	27±1	27±1	25±1	25±2
0.01	—	18±1*	22±1	21±1	23±1	24±1	28±1	25±1	24±1
0.1	—	22±1	24±1	26±1	25±1	25±1	34±2*	27±1	26±1
1	—	23±1	25±1	27±1	29±1	28±1	33±1*	31±1*	27±1

The initial fraction BO-10 (fraction 0) showed stimulatory activity in low doses (0.01 μg/ml): it increased all three parameters of phagocytosis. This activity was produced mainly by fraction 3 (in doses of 0.001-0.1 μg/ml it significantly increased all parameters of phagocytosis). Fractions 1-2 in low doses stimulated phagocytosis and in high doses slightly suppressed it, while fractions 7-8 showed a stimulatory effect in a wide range of doses (low-dose stimulation was characteristic of BO-10, similarly as with PR).

The effects of BO-20 fractions on parameters of phagocytosis were opposite. Fraction 1 in doses of 0.01-10 μg/ml and fractions 7 and 8 in high doses decreased the phagocytic index and number (fraction 1 in a dose of 0.1 μg/ml significantly decreased both parameters simultaneously). The redox potential significantly decreased after incubation with fraction 1 in doses of 0.001 and 0.01 μg/ml and increased after incubation with other fractions in different doses. The initial BO-20 fraction (fraction 0) in low doses slightly stimulated phagocytosis.

Hence, PR, BO-10, and BO-20 contain various bioactive substances producing opposite and dose-dependent effects on phagocytosis. This suggests that the composition and doses of the preparations should be carefully selected in order to attain the optimal therapeutic effect.

REFERENCES

1. B. V. Vtyurin and R. I. Kaem, *Vestn. Dermatol. Venerol.*, No. 8, 20-27 (1985).
2. N. I. Kochetygov, *Burn Disease* [in Russian], Leningrad (1973).
3. B. A. Paramonov and G. Sh. Mchedlidze, *Treatment of Burns, Wounds, and Trophic Ulcers by Biopin Ointment* [in Russian], St. Petersburg (2001).
4. A. S. Simbirtsev, V. G. Konusova, G. Sh. Mchedlidze, *et al.*, *Byull. Eksp. Biol. Med.*, **133**, No. 2, 168-171 (2002).