NEW MEDICINAL PREPARATIONS

Pine Resin and Biopin Ointment: Effects on Cell Composition and Histochemical Changes in Wounds

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

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We studied the effects of Biopin ointment containing pine resin and used for the therapy of burns, wounds, and purulent and inflammatory diseases on the cell composition of wounds and histochemical changes in the granulation tissue during the therapy of extensive third-degree burns. The preparation stimulated the nonspecific immune response, normalized hemodynamics in damaged regions, and stimulated cell proliferation in the squamous epithelium.

Key Words: *pine resin; Biopin ointment; composition of wound smears; histochemical assay; granulation tissue*

Galipot from various coniferous tress, including pine, fir, larch, and cedar, is widely used in traditional medicine and is now included into the composition of industrial medicinal preparations (ointments) [3]. Biopin ointment (BO) contains pine resin and beeswax and holds much promise for the therapy of burns, wounds (phase I of wound process), and purulent-and-inflammatory diseases of the skin and subcutaneous fat [2]. Here we studied the effects of BO on the cellular composition of smears from the wound area and histochemical changes in the granulation tissue during the therapy of severe burns.

MATERIALS AND METHODS

BO was used for the therapy of a patient with extensive thermal damages (flambeau, degree IIIa). For the comparative analysis we used Levosin (LV) and Shostakovsky balsam (SB). Bioptates from adjacent injured skin were used for comparative analysis.

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

Cytological assay of smears was performed using a 5-point semiquantitative scale [1]. The cells were counted, and cytograms were constructed. The observed mean and dispersion were calculated for each group. The differences between control and experimental groups were evaluated by Student's *t* test (probability 0.95).

Histochemical examination of slices from the granulation tissue (depth 2 mm) was performed using immunoreactive staining. T-cell serum reacts with CD4 and CD8 subpopulations of T lymphocytes and myelocytic granulocytes and monocytes (B cells and natural killer cells are unstained). B-cell serum (CD45RA) reacts with most B cells (rarely with monocytes). LCA serum (CD45RB) reacts with most normal lymphoid cells, histiocytes and macrophages (rarely), plasma cells (weakly), and tumor cells of non-Hodgkin's lymphomas, T- and B-cell leukemias, and malignant lymphomas of the thyroid gland. A-1-A serum (alpha-1-antichymotrypsin) reacts with histiocytes, monocytes, and macrophages of the intestine, skin, and tonsils. Immunoglobulins (IgG) are found in tonsils, lymph nodes, Peyer's patches, and spleen under normal conditions.

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TABLE 1. Cell Composition of Wound Surface (%, $M \pm m$, n=4; Brackets: 5-Point Semiquantitative Scale) Analyzed after Staining with Hematoxylin and Eosin (Numerator) and Azure and Eosin (Denominator)

Parameter	Before therapy (control)	Day 3			Day 5		
		BO	LV	SB	BO	LV	SB
Neutrophils	78±12 (+++) / 33±8 (++)	57±10 (++) / 73±12* (+++)	— / 74±12* (+++)	(++) / 4±2*	65±13 (+++) / (±)	65±10 (+++) / (—)	(±) / 47±7 (++)
Macrophages	11±3 (+) / 12±3 (+)	(++) 47±9* / 30±5* (++)	— / 24±7* (++)	0* (—) / 22±7 (++)	31±9* (++) / (±)	34±8* (++) / (±)	0* (—) / 6±3 (±)
Lymphocytes	2±2 (±) / 29±7 (++)	(—) / 0* (—)	— / 0* (—)	(—) / 5±3* (±)	(—) / (±)	(—) / (±)	(—) / 43±11 (++)
Fibroblasts	2±2 (±) / 4±3 (±)	(—) / (—)	— / (—)	(—) / (—)	4±2 (±) / (±)	(—) / (±)	(—) / (—)
Epithelium	5±2 (±) / 5±2 (±)	(++) / (+)	— / (—)	(—) / (±)	(++) / (++)	(+) / (+)	(—) / (+)
Erythrocytes	Hemolysis (+++) / Hemolysis (+++)	(—) / (—)	— / (++)	(+++) / (+++)	(+++) / (++)	(+++) / (+++)	(+++) / (++)
Eosinophils	(—)/1±1 (±)	(—)/(—)	—/(—)	(—)/(—)	(—)/(—)	(—)/(—)	(—)/(—)
Mast cells	(—)/1±1 (±)	(—)/(—)	—/(—)	(—)/(—)	(—)/(—)	(—)/(—)	(—)/(—)
Bacterial flora	c, r (++) / c, r (++)	(—) / r (±)	— / r, a (±)	(—) / c (±), r (+), a (+)	(—) / r, a (±)	r, a (+) / r, a (±)	(—) / a (+)
Phagocytosis	Neutrophils (±) / Macrophages (±)	(—) / (—)	— / (—)	(—) / (—)	(—) / (—)	(—) / (—)	(—) / (—)
Toxic signs	Karyorrhexis (++) / (±)	(—) / (—)	— / Karyorrhexis (++)	(—) / (—)	(—) / (—)	(++) / (++)	(—) / (±)
State of bioptates	Degenerative changes (*e.g.*, nucleolysis and vacuolated cytoplasm) in the epithelium	Pronounced proliferation of the squamous epithelium	(—)	Regions with epithelium neutrophilic exudates (+++)	Pronounced proliferation of the squamous epithelium	(—)	Pronounced proliferation of lymphocytes

Note. For cells and signs: none (—), individual and weak (±), small and moderate (+), moderate and pronounced (++), many and severe (+++). c: cocci; r: rod-shaped bacteria; a: anaerobic bacteria. *p<0.05 compared to the control.

TABLE 2. Histochemical Examination of Granulation Tissue (cells/mm^2)

Staining	Before therapy (control)	Day 3			Day 5		
		BO	LV	SB	BO	LV	SB
T-cell	0	326	191	276	0	0	0
B-cell	0	7	0	6	0	0	0
LCA	1265	228	720	—*	411	1996	671
A-1-A	—*	165	410	283	664	796	484
IgM	(2)***	365 (2)	397 (3)	83 (2)	109 (3)	256 (3)	135 (3)
IgG	51 (3)	398 (3)	487 (4)	432 (3)	509 (4)	(4)***	269 (3)
IgA	(3)***	—*	549 (3)	371 (3)	(2)***	783 (3)	409 (2)
Hematoxylin and eosin	1739	1203	1845	3793**	3960**	2574	1882

Note. Intensity of staining is shown in brackets. *Insignificant results due to the small size of slices; **slice contains only inflammatory infiltrate (may not correspond to morphological signs of the bioptate); ***exact calculations are impossible due to background staining.

RESULTS

Cytological assay of the wound area showed that BO stimulated the specific immune response, promoted migration of neutrophilic granulocytes and macrophages into the inflammatory focus (day 3 of application), and enhanced proliferation of epithelial cells (Table 1). Antibacterial activity of BO was similar to that of LV. However, BO was less toxic to burned tissues than LV. BO was more potent than other preparations in blocking migration of erythrocytes from the vascular bed. These data indicate that BO normalized hemodynamic parameters in inflammatory focus.

Histochemical examination of wounds and quantitative assay (Table 2) showed that alterative changes were least pronounced after therapy with BO (these changes were degenerative, but not necrotic), which attested to less pronounced intoxication. After treatment with BO signs of serous and fibrinous exudative inflammation were less pronounced than in other bioptates. Cell infiltration was less deep, and hemorrhages were absent. BO markedly increased the count of macrophages (by 4 times) and lymphocytes in bioptates. The cell infiltrate contained mainly macrophages, their derivatives (histiocytes and epithelioid cells), and lymphocytes. The number of neutrophils was low. Microabscesses were not found.

Our results show that BO used in the therapy of burns stimulates the nonspecific immune response, normalizes hemodynamics in damaged regions, and stimulates proliferation of epithelial cells.

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