NEW MEDICAL PREPARATIONS

Pine Resin and Biopin Ointment: Effects on Free Radical Processes

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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 the content of lipid peroxidation products, including primary (conjugated dienes), secondary (malonic dialdehyde and ketodienes), and end products (Schiff bases), and content of isolated double bonds in the spleen and liver. Repeated application of Biopin did not modulate lipid peroxidation. The inhibitory effect of this preparation was observed only in the initial short period after application.

Key Words: *pine resin; Biopin ointment; lipid peroxidation*

Free radical reactions of lipid peroxidation (LPO) contribute to modification and chemical changes in membrane lipid layers and proceed under normal and pathological conditions. LPO determines normal functioning of cell membranes and modulates their transport, enzyme, receptor, and other properties [1]. The antioxidant system regulates the content of reactive oxygen species and LPO products and modulates compartmentalization of LPO substrates and catalysts in cels.

During wounds and burn diseases the skin and subcutaneous tissues serve as a potent source of free radicals triggering chain LPO reactions. Inflammatory cells are mobilized over the first hours after the incidence of burns and wounds and provide metabolic burst in neutrophils and macrophages, which is accompanied by generation of reactive oxygen species. Apart from antibacterial effects free radicals in high concentrations induce damage to cells and macromolecules (hyaluronic acid, collagen, glycoproteins, and immunoglobulins), which is paralleled by impairment by the antioxidant protective system.

The search for new preparations normalizing the intensity of LPO and stimulating antioxidant protective systems attracts much attention. Here we studied the effects of Biopin ointment (BO) produced from pine resin and used for the therapy of burns, wounds (phase I of wound process), purulent, and inflammatory diseases of the skin and subcutaneous fat [3] on LPO.

MATERIALS AND METHODS

Experiments were performed on 32 male outbred rats weighing 180-200 g and obtained from the Rappolovo nursery. The animals were divided into 4 groups (control and experiment, 2 series). Each group included 8 rats. Experimental rats were treated with 700 mg/kg BO (10 clinical doses). The preparation was applied to a sheared surface of the skin on the back (4 cm^2). Series I rats were decapitated 2 h after single treatment with BO. In series II, BO was applied 5 times at 12-h intervals, and the rats were decapitated 1 h after the last treatment. The contents of primary (conjugated

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dienes, CD), secondary (malonic dialdehyde, MDA; and ketodienes), and end LPO products (Schiff bases) and number of isolated double bonds (IDB) were estimated in spleen and liver tissues.

The concentration of CD, ketodienes, and Schiff bases and the number of IDB were determined in lipid tissue extracts [5]. The tissue was homogenized in a methanol-chloroform mixture (1:2 v/v) at 45°C for 15 min. The lipid fraction was washed with 0.75% KCl and evaporated in nitrogen flow at 38°C. Tissue samples were dissolved in a methanol-heptane mixture (4:1 v/v) to estimate the concentrations of CD and Schiff bases and number of IDB. Light absorption was measured on a Pue Unicam spectrophotometer at 232 (2.2×10^5 $M^{-1}cm^{-1}$), 278, and 220 nm, respectively [7]. To estimate the content of Schiff bases, tissue samples were dissolved in chloroform, and fluorescence was measured on a Hitachi-2M spectrofluorometer at 425 nm (excitation wavelength 365 nm) [4].

The concentration of MDA was determined by the reaction with 2-thiobarbituric acid (TBA) [1]. Tissues were homogenized in 100 mM Tris buffer (pH 7.4), proteins were precipitated with 10% trichloroacetic acid, and samples were centrifuged. HCl (0.6 N) and TBA (0.8% in 50% acetic acid) were added to the precipitate, samples were incubated in a boiling water bath for 10 min. Absorption was measured on a SF-16 spectrophotometer at 532 nm (1.56×10^5 $M^{-1}cm^{-1}$).

TABLE 1. LPO Content in Rat Tissues 2 h after Single Application of BO (*M*±*m*, *n*=8)

LPO products	Spleen		Liver	
	control	experiment	control	experiment
CD, nmol/mg protein	2.71±0.11	2.30±0.12* (-15)	3.92±0.19	3.33±0.18* (-15)
MDA, nmol/mg protein	0.106±0.022	0.073±0.003* (-31)	0.129±0.014	0.090±0.007* (-30)
Ketodienes, A_{278}/mg lipids	0.086±0.004	0.081±0.006 (-5)	0.236±0.008	0.202±0.009* (-14)
Schiff bases, arb. units/mg lipids	22.7±1.9	20.8±2.5 (-9)	26.7±1.8	21.6±1.2* (-19)
IDB, A_{220}/mg lipids	0.539±0.028	0.511±0.023 (-5)	0.837±0.029	0.684±0.028* (-18)

Note. Here and in Table 2: differences between control and experimental groups are shown in parentheses (%). A: light absorption*ml; 1 arb. unit: intensity of quinine sulfate fluorescence (1 mg/ml in 0.1 N H_2SO_4). *p<0.05 compared to the control.

TABLE 2. LPO Content in Rat Tissues 1 h after Repeated Application of BO (5 Times at 12-h Intervals, *M*±*m*, *n*=8)

LPO products	Spleen		Liver	
	control	experiment	control	experiment
CD, nmol/mg protein	2.32±0.10	2.42±0.18 (4)	3.57±0.09	3.78±0.18 (6)
MDA, nmol/mg protein	0.110±0.010	0.104±0.007 (-5)	0.111±0.008	0.122±0.008 (10)
Ketodienes, A_{278}/mg lipids	0.085±0.004	0.077±0.006 (-9)	0.217±0.015	0.200±0.019 (-4)
Schiff bases, arb. units/mg lipids	22.7±1.5	21.6±2.3 (-5)	23.7±1.0	19.0±1.3* (-18)
IDB, A_{220}/mg lipids	0.502±0.018	0.498±0.027 (-1)	0.793±0.030	0.761±0.029 (-4)

The concentrations of CD and MDA were standartized by total protein content (mg per mg tissue weight), which was estimated by the method of Lowry with modifications of Miller on a Pue Unicam spectrophotometer at 740 nm [6]. Calibration curves were constructed using crystalline albumin.

The concentrations of ketodienes and Schiff bases and number of IDB were standartized by total lipid content (mg per mg tissue weight), which was estimated by staining with phosphovanillin reagent on a SF-16 spectrophotometer at 540 nm [2]. Calibration curves were constructed using olive oil. This reagent was added to the lipid extract after its hydrolysis with strong H_2SO_4 in a boiling water bath for 15 min.

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).

RESULTS

The intensity of LPO markedly decreased 2 h after single application of BO (Table 1). The contents of primary (CD) and secondary LPO products (MDA) decreased in the spleen tissue. These changes were accompanied by a decrease in the concentration of various LPO products in the liver. This early effect of BO probably prevents destruction of membranes and contributes to the recovery of their permeability impaired after trauma or inflammation.

Repeated application of BO did not modulate LPO (49 h after treatment, Table 2). The concentration of LPO products in the spleen remained unchanged. In the liver we found a significant decrease in the content of Schiff bases.

Our results show that repeated application of Biopin does not modulate LPO. This preparation inhibited LPO only in the initial short period after treatment.

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