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Antioxidant Effect of Polyoxidonium and Metaprot during Bronchopulmonary Inflammation in Rats

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The antioxidant effects of individual or combined application of polyoxidonium and metaprot were examined in rats with acute bronchopulmonary inflammation. By degree of antioxidant potency, polyoxidonium was inferior to metaprot, but their combined application produced more potent antioxidant effect. Polyoxidonium and metaprot in low concentrations increased and in high concentrations suppressed spontaneous biochemiluminescence in the model system of alveolar macrophages.

Key Words: *bronchopulmonary inflammation; polyoxidonium; metaprot; lipid peroxidation*

Disturbances accompanying bronchopulmonary diseases are closely related to mechanisms of unspecific resistance and immunological defense, oxygen supply system, and metabolism [3]. In light of this, complex therapy of bronchopulmonary inflammation should employ drugs with a broad profile of pharmacological activity that can correct disturbances in the energy metabolism, free radical processes, and immune system and stimulate the adaptive potencies in the organism. In most features, an antihypoxant metaprot and immunomodulator polyoxidonium (PO) meet these requirements.

Metaprote possesses the energy stabilizing and antioxidant properties, which are especially needed during hypoxia that develops in parallel with bronchopulmonary diseases [4,9]. PO activates the unspecific resistance of the organism and up-regulates the humoral and cellular immunity. An important feature of PO is its ability to stimulate the anti-infectious stability of the organism. PO affects all the elements of phagocytosis: it enhances the absorption capacity and bactericidal activity of the phagocytes, and augments clearance of alien particles from circulation. While participating in induction of synthesis of cytokines, PO plays the role of true immunomodulator by stimulating their spontaneous and inducible synthesis [6]. In complex therapy of various diseases, PO is a reliable partner to antiviral, antibacterial, antifungal, and other pharmacological agents [5]. The combined application of metaprot with PO should enhance effectiveness of bronchopulmonary therapy. Regretfully, there are few data on the antioxidant properties of PO in pulmonary tissues and immunocompetent cells during bronchopulmonary inflammation.

Our aim was to examine the antioxidant properties of PO, metaprot, and their combination during bronchopulmonary inflammation in rats.

MATERIALS AND METHODS

Experiments were carried out on 56 male Wistar rats (200-250 g) from Rappolovo Breeding Center. The animals were maintained on the standard ration with a 12-hour day-night cycle under artificial illumination. The experimental protocols were described elsewhere [8].

The acute bronchopulmonary inflammation was provoked under ether narcosis with 0.1 turpentine oil injected into the lumen of exposed trachea between two cartilage rings. The cervical wound had been sutured. Immediately after surgery and during following 5 postsurgery days, the physiological solution or tested agents were intraperitoneally injected to control and experimental rats, respectively. In 2 h after the last injection made on postsurgery day 5, the rats were

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decapitated to draw blood and isolate the lungs for biochemical assay.

The pulmonary tissue was homogenized in liquid nitrogen, thereupon the parameters describing LPO process (MDA) and activity of the antioxidant systems (SOD and reduced glutathione GSH) were essayed [7].

Spontaneous biochemiluminescence (BCL) of macrophages was used to examine the effects of examined agents on ROS production (including superoxide anion radical) by phagocytes. The alveolar macrophages were obtained from the bronchoalveolar lavage. Macrophage suspension contained 10^6 cell/ml. Macrophage adhesion was induced by 20-min incubation in a moist chamber at 37°C. According to staining with trypan blue (0.1%), the resulting suspension contained more than 98% living cells. The differential count in stained smears showed that macrophages amounted to about 90%.

The macrophagal effects of examined agents were assessed in a thermostabilized cuvette (37.0±0.5°C) incorporated into a Chemilum-1 chemiluminometer coupled with a computer. BCL was recorded according to the changes in lucigenin-dependent chemiluminescence augmented with lucigenin (bis-N-methylacridinium, Sigma). The cuvette contained 0.2 ml macrophage suspension with 10^7 cells, 0.1 ml lucigenin solution (100 μM), and 0.1 ml physiological solution with examined agents. The suspension was supplemented with 10 μl PO (1500 μg/ml) and/or 10 μl metaprot (30 mM). In control, the suspension was added with 0.1 ml physiological solution. The incubation time was 20 or 40 min. Spontaneous BCL was measured for 5 min under continuous stirring. An original software was employed to plot the data, calculate BCL intensity (an integral index), and to determine the luminescent maxima according to the peak magnitudes. The data were normalized to spontaneous BCL in the control group (macrophages in physiological solution).

The rats were randomized into 5 groups: group 1, intact; group 2, acute bronchopulmonary inflammation without therapy; group 3, acute bronchopulmonary inflammation treated with metaprot (Usolye-Siberian Chemical-Pharmaceutical Plant); group 4, treated with PO (Petrovax Pharm); and group 5, treated with metaprote+PO. All agents were injected intraperitoneally for 5 days in optimal effective dose (ED_{50}) obtained in previous studies [4-6]: PO, 0.75 mg/kg and metaprote, 25 mg/kg. In group 2, the rats received physiological solution in corresponding volume. In each group, the random sample was no less than 10 rats.

The data were analyzed statistically using 'Statistica' software and Student's t test at $p<0.05$. Normality of distribution was established with Kolmogorov-Smirnov fitting criterion.

RESULTS

Acute bronchopulmonary inflammation in rats was characterized with a 4-fold increase in MDA level, a decrease of SOD activity by 92%, and a drop in GSH level by 79%, all these parameters being assayed in the pulmonary tissue (Table 1). Injection of PO significantly decreased the level of MDA by 32%, increased SOD activity 3-fold, and increased GSH by 42%. Metaprot decreased the level of MDA by 59%, increased GSH by 51%, and enhanced SOD activity by 7 times ($p<0.05$).

A more pronounced antioxidant effect was observed during combined use of PO and metaprot, which decreased pulmonary MDA by 73%, increased GSH by 2.7 times, and restored SOD activity up to the normal level characteristic of intact animals.

Thus, during acute bronchopulmonary inflammation in rats, PO is less effective as metaprote in the degree of antioxidant action, while their combined application exerts a more pronounced effect reflecting potentiation of the individual therapeutic modalities.

In the development of acute and chronic inflammation, the phagocytes play the role of key effectors of homeostasis due to their potency to generate ROS underlying the microbicidal function of the granulocytes. During stimulation of phagocytes, ROS are produced with involvement of Ca^{2+} ions and NADPH

TABLE 1. Effect of Metaprote, PO, and Their Combination on LPO Parameters and Activity of Antioxidant Systems in Lungs of Rats during Acute Bronchopulmonary Inflammation ($M±m$, n=10)

Group	MDA, μmol/g tissue	SOD, act. U	GSH, μmol/g tissue
Intact	6.52±0.14	3.27±0.11	38.47±0.15
Bronchopulmonary inflammation	37.52±0.11*	0.25±0.08*	8.12±0.16*
+PO	25.33±0.15*+	1.10±0.13*+	11.56±0.14*+
+metaprot	15.23±0.12*+	2.12±0.11+	18.75±0.13*+
+meaprot+PO	10.11±0.14*+	2.99±0.14+	29.76±0.13*+

Note. *p<0.05 in comparison with *intact rats, +therapy-free inflamed rats.

TABLE 2. Effect of Metaprot, PO, and Their Combination on Spontaneous Lucigen-Dependent BCL (% Control) of Rat Alveolar Macrophages ($M\pm m$, n=10)

Agent	Incubation time, min	Luminescence maximum	Luminescence intensity
Control	–	100	100
	–	100	100
PO, 1500 μg/ml	20	84±4*	85±5*
	40	71±5*	69±4*
Metaprot, 30 mmol/liter	20	89±7*	77±6*
	40	72±8*	55±8*
PO, 1500 μg/ml+metaprot 20 mmol/liter	20	74±4*	66±4*
	40	97±3	85±5*
PO, 500 μg/ml	20	140±10*	180±16*
	40	128±9*	163±16*
Metaprot 0.1 mmol/liter	20	140±4*	169±5*
	40	129±3*	148±5*
PO 500 μg/ml+metaprot 0.1 mmol/liter	20	120±3*	196±7*
	40	127±5*	233±11*

Note. *p<0.05 in comparison with the control.

oxidase, which reduces the extracellular molecular oxygen to superoxide anion while oxidizing cytosolic NADPH до $NADP^+$ [1]. In contrast, ROS deficiency down-regulates the microbicide activity of the phagocytes. At the same time, an extra supply of superoxide anions triggers the downstream cascade of the free radical reactions and related phenomena resulting in destruction of the tissues in the inflammation focus.

It is an established fact that PO (500 μg/mL) increases the level of H_2O_2 in leukocytes, but does not change it in monocytes and lymphocytes [6]. However, PO can inhibit redundant production of extracellular ROS, which can be one of the mechanisms of its antioxidant action. It is a common knowledge that spontaneous lucigenin-enhanced BCL of the cells reflects the intensity of formation of extracellular ROS (mostly that of superoxide anion), which can be used to assess activity of the cells and their functional potential under the action of examined agent [2].

PO (1500 μg/ml) significantly diminished spontaneous lucigenin-enhanced BCL of macrophage cells. In 20 and 40 min incubation of PO in the model system, luminescence intensity of alveolar macrophages decreased by 15% and 31%, respectively (Table 2).

Metaprote (30 mM) decreased the intensity of luminescence by 23% and 45% after incubation for 20 and 40 min, respectively.

Combined incubation of PO (1500 μg/ml) and metaprot (30 mM) with alveolar macrophages for 20 and 40 min decreased intensity of luminescence by 34% and 15%, respectively.

The opposite effects were observed when PO and metaprot were used in concentrations of 500 μg/ml and 0.1 mM, respectively. When incubated with alveolar macrophages for 20 and 40 min, PO increased luminescence intensity by 80 and 63%, respectively. Similarly, metaprot increased the luminescence intensity by 69% (20 min incubation) and 48% (40 min incubation), respectively. The combined application of PO and metaprot for 20 and 40 min increased luminescence intensity by 96% and by 2.3 times, respectively.

Alveolar macrophages, aerobic cells depending on activity of oxidative phosphorylation processes are characterized with enhanced activity of SDH and cytochrome oxidase. Among all classes of macrophages, alveolar macrophages are most active in generating the superoxide anion, which maintains sterility of the alveoli and prevent infectious contamination of the lungs in normal antioxidant system. Evidently, the examined agents are dose-dependent in their potency to control the oxygen-determined metabolism of the phagocytic blood cells, which are important sources of the free radicals: when active, they produce 12-fold greater amounts of superoxide anion and H_2O_2 than at rest. The antioxidant (more precisely, anti-radical) action of PO and metaprote are manifested when these agents are used in rather high doses, while their low

doses exert a moderate pro-oxidant effects *in vitro*. The present *in vitro* data do not coincide with the *in vivo* results demonstrating antioxidant effects for both low and high doses of the examined agents [4].

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