Efficiency of Trekrezan in Experimental Bronchopneumonia in Rats

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Trekrezan in a dose of 25 mg/kg normalized the lymphocyte immune status and produced an energy-stabilizing effect, which manifested in decreased levels of lactate, ADP, and AMP and increased content of pyruvate and ATP in blood lymphocytes and lung tissue of rats with experimental acute bronchopneumonia.

Key Words: *experimental bronchopneumonia; trekrezan; energy metabolism; immunity; rats*

The treatment of bronchopulmonary inflammations remains a pressing problem of medicine: the prevalence of bronchopneumonia (BP) is 2-15% and the BP-associated mortality reaches 30%, with a stable trend to increase [7]. Antibacterial therapy of BP is not always successful and sometimes leads to protracted course and chronic transformation of the disease [2]. Some drugs used for etiotropic therapy of pneumonias, *e.g.* penicillins and tetracyclines, are characterized by immunosuppressive effects. An important factor is suppression of proliferation of immunocompetent cells with antibiotics as a result of decreased content DNA and RNA in these cells. Among side effects of antibacterial therapy we have to pay special attention to bacteriolysis associated with endotoxin release, which suppresses the immune system. This necessitates the search for approaches to pathogenetic correction of the inflammatory process by means of immunomodulators [1]. A complex of disorders developing in BP, their relationship with nonspecific resistance mechanisms, energy metabolism and immune system status prompt the use of drugs with a wide spectrum of pharmacological activities modulating the basal cell processes and stimulating the adaptation potential of the body.

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Trekrezan (triethanolammonium salt of 2-methylphenoxyacetic acid) is a highly effective adaptogenic and immunostimulating drug exhibiting stress-protective effect on the models of immobilization and painful hypodynamic stress, stimulating reparation of damaged tissue (liver, myocardium, muscles), and protecting the viscera from the detrimental effects of toxins, microwave irradiation, and infections [5].

We studied the energy-stabilizing and immunotropic effects of trekrezan in experimental BP.

MATERIALS AND METHODS

The study was carried out on 106 albino rats (180-200 g). The animals were divided into 3 groups: 1) intact rats; 2) animals with experimental BP; and 3) rats with experimental BP treated with trekrezan. Bronchopneumonia was induced by injection of turpentine [5]. The trachea was surgically mobilized under ether narcosis and turpentine (0.1 ml) was injected into its lumen between the semirings. The incision was sutured and the animals were intraperitoneally injected with trekrezan (25 mg/kg) for 5 days. On day 6 the animals were decapitated. Morphological study showed macroscopically significant, more often lobar, inflammation of the lungs with typical infiltration and punctate hemorrhages in lung tissue in experimental animals injec-

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ted with turpentine. The pathological process was located in the diaphragmatic lobe of the right lung in the majority of cases. No appreciable differences in the extension and type of BP locus were detected.

Energy metabolism in lymphocytes and lung tissues frozen in liquid nitrogen, was evaluated by the content of pyruvate and lactate, ATP, ADP, and AMP, and by the adenyl system energy charge.

Lymphocytes for immunological studies were isolated from the whole blood in Ficoll-urotrast density gradient. Lymphocyte suspension containing 2×10^6 cell/ml was used in the study (cell viability was controlled). The study of cell immunity included lymphocyte migration inhibition test with concanavalin A and phytohemagglutinin characterizing the function of T cells. Nonspecific defense was evaluated by the level of neutrophilic phagocytosis in bacterial test culture after their co-incubation. The phagocytosis completeness index, phagocytic index (percent of phagocytes among counted neutrophils), and phagocytic number (mean number of bacteria phagocytosed by one active neutrophil) were evaluated. Activity of oxygen-independent bactericidal systems of the phagocyte was evaluated by the lysosomal cationic test. Oxygen-dependent antiinfectious systems of phagocytes were evaluated in NBT reduction test [9].

The sampling for each group was at least 12 rats. The results were processed using standard Statistica software and Student's *t* test.

RESULTS

The development of pulmonary inflammation is associated with significant disorders in the system of oxygen supply. Respiratory insufficiency in BP is endogenous and is paralleled by tissue hypoxia with shifts in characteristics of the mitochondrial enzymatic complex and disorders in the respiratory chain energy-producing function. Evaluation of the content of adenine nucleotides and the energy charge of the adenyl system is an informative method of investigation of bioenergetic hypoxia developing in BP [3].

Acute BP in rats was associated with increased levels of lactate, ADP, and AMP and decreased levels of pyruvate and ATP in lymphocytes and lung tissue. The metabolic changes were more pronounced in the lung tissue. Thus, in lymphocytes of rats with BP lactate content increased by 140%, ADP by 69%, and AMP by 86% (Table 1), while the content of pyruvate decreased by 81% and of ATP by 54% ($p<0.05$). In the lungs lactate level increased by 204%, ADP by 109%, and AMP by 143%; pyruvate level decreased by 92%, ATP by 69%. Changes in the adenine nucleotide pool lead to a reduction of the adenine nucleotide energy charge, this indicating the development of energy deficiency in lymphocytes and lung tissues of rats in BP (Table 2).

Trekrezan treatment reduced lactate content in rat lymphocytes by 40%, ADP content by 19%, and AMP by 16% ($p<0.05$). The level of pyruvate increased 3-fold, that of ATP by 62%. In the lung tissue the content of lactate, ADP, and AMP decreased by 49, 40, and 22%, while the level of pyruvate increased 5-fold and ATP by 98% ($p<0.05$) after trekrezan treatment. Changes in the content of adenine nucleotides were paralleled by an increase in their energy charge in blood lymphocytes and in lung tissues of rats.

The close relationship between energy metabolism disorders and LPO activation suggests that stabilization of energy metabolism after trekrezan treatment is due to its antioxidant effects. However, the mechanisms of energy-stabilizing effect of trekrezan in inflammatory bronchopulmonary diseases deserve further investigation.

The immune status and characteristics of lung inflammation processes are closely related, and therefore evaluation of the state of T and B cells and phagocytes in acute bronchopulmonary inflamma-

TABLE 1. Effect of Trekrezan on Parameters of Energy Metabolism in Blood Lymphocytes from Rats with Acute BP ($M\pm m$)

Parameter	Group		
	1	2	3
Lactate, μmol/g tissue	3.12±0.04	7.50±0.11*	4.51±0.14$^+$
Pyruvate, μmol/g tissue	0.26±0.01	0.05±0.01*	0.15±0.01$^+$
ATP, μmol/g tissue	2.84±0.04	1.30±0.06*	2.11±0.04$^+$
ADP, μmol/g tissue	0.71±0.02	1.20±0.04*	0.97±0.06$^+$
AMP, μmol/g tissue	0.44±0.01	0.82±0.03*	0.71±0.02$^+$
Energy charge	0.87±0.03	0.568±0.010*	0.808±0.020$^+$

Note. Here and in Tables 2, 3: $p<0.05$ compared to *group 1, $^+$group 2.

TABLE 3. Effect of Trekrezan on Immunological Parameters of Lymphocytes in Rats with Acute BP (*M*±*m*)

Parameter	Group		
	1	2	3
Lymphocyte migration inhibition test, %			
with concanavalin A	84±2	59.0±1.1*	68±2+
with phytohemagglutinin	54.6±3.4	41.4±2.4*	50.1±3.0+
Phagocytic index, %	94.5±0.1	80.3±1.3*	94.5±0.7+
Phagocytic number	13.4±0.5	18.9±0.5*	13.8±1.2+
Phagocytosis completeness index, %	22.5±1.4	28.3±0.9*	22.6±1.1+
Lysosomal cationic test, %	1.44±0.02	1.36±0.01*	1.44±0.03+
NBT test			
basal	0.31±0.02	0.43±0.02*	0.32±0.02+
stimulated	0.61±0.02	0.70±0.01*	0.61±0.02+

TABLE 2. Effect of Trekrezan on Parameters of Energy Metabolism in the Lungs of Rats with Acute BP (*M*±*m*)

Parameter	Group		
	1	2	3
Lactate, μmol/g tissue	2.81±0.60	8.55±0.11*	4.35±0.02+
Pyruvate, μmol/g tissue	0.37±0.01	0.03±0.01*	0.16±0.01
ATP, μmol/g tissue	3.51±0.09	1.08±0.03*	2.14±0.04+
ADP, μmol/g tissue	0.57±0.02	1.19±0.02*	0.71±0.04+
AMP, μmol/g tissue	0.28±0.02	0.68±0.05*	0.53±0.01+
Energy charge	0.87±0.03	0.568±0.010*	0.808±0.020+

tions includes immunological studies as accessory methods for the diagnosis and prediction of the course of these diseases, for evaluating activity and direction of inflammatory process and completeness of recovery.

Due to the presence of bronchus-associated lymphoid tissue, the lungs are an immunocompetent organ largely determining the type of immunological reactions in the respiratory system. Secondary immunodeficiency in BP can be of iatrogenic origin or caused by microorganism effects on the defense forces of the body. The immune status is closely related to the severity of BP, disease variant and outcome. As BP severity augments, the complex of detrimental factors of bacterial inflammation can have a more pronounced effect on the mechanisms of cellular and humoral defense. Different functional disorders are characteristic of each type of immunological response. An unfavorable factor is increased content of T-suppressers, associated with significant disorders in the ventilation-perfusion relationships and the hypokinetic type of central hemodynamics. The immune status, in turn, is essential for the pattern of BP. Severe and protracted BP develops against the background of suppressed complement and phagocytosis systems and functional insufficiency of cell immunity. The phagocytic cells play a special role in the development of lung inflammation [6]. The status of neutrophilic granulocytes and monocytes largely determine the emergence, course, and outcome of the pathological process in the bronchopulmonary system. Studies of phagocytosis revealed the dysfunction syndrome, manifesting by suppressed phagocytic and bactericidal activity of the blood neutrophils. The decrease in the complement and phagocytosis systems' values depends on the type of the process, its course and severity, the degree of reduction in phagocytosis values directly correlating with the severity of pneumonia.

Experimental acute BP in rats was associated with increased phagocytic activity of lymphocytes, suppression of T-lymphocyte function and activities of oxygen-independent bactericidal systems of phagocytes (Table 3).

Trekrezan treatment of rats with BP led to a significant increase in the lymphocyte lymphokine-producing function in the test with concanavalin A

(by 15%) and phytohemagglutinin (by 9%). The phagocytic activity of lymphocytes decreased by 21% during trekrezan treatment, while activities of oxygen-independent bactericidal systems increased by 9% ($p<0.05$). Trekrezan treatment led to normalization of the lymphocyte oxygen-dependent antiinfectious systems, characterizing the degree of the hexosomonophosphate shunt activation and free radical formation associated with it. Trekrezan treatment led to a reduction of the spontaneous NBT test by 27% in comparison with the values in BP, of induced NBT test by 11% ($p<0.05$). On the whole, trekrezan treatment led to normalization of the studied immunity values to levels characteristic of intact animals.

Hence, trekrezan used in experimental BP exhibited energy-stabilizing effect, manifesting in decreased levels of lactate, ADP, and AMP and increased levels of pyruvate and ATP in blood lymphocytes and lung tissue of rats. Treatment with this drug normalized the lymphocyte immune status in animals with acute BP.

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