Chemiluminescence of Blood Plasma and Activity of Alveolar Macrophages in Experimental Pneumonia

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Acute experimental pneumonia in animals is accompanied by enhanced production of active oxygen forms by alveolar macrophages and increased plasma chemiluminescence. Low photosum of chemiluminescence and suppression of bactericidal activity of alveolar macrophages together with increased blood content of low-molecular-weight peptides are signs of lung destruction.

Key Words: *pneumonia; alveolar macrophages; chemiluminescence*

Disturbances in free radical oxidation (FRO), an obligatory component of metabolism, are early nonspecific signs of injury underlying various diseases, including diseases of the respiratory organs [5,7,13]. Most methods used for investigation of FRO in experiments cannot be used in clinical practice because of technological difficulties. A promising method providing objective information on free radical lipid peroxidation and production of free oxygen forms is registration of chemiluminescence (CL) of biological materials [9,10]. This method is used in practical pulmonology for elucidating the mechanisms of development of lung diseases [5,14], predicting their course and outcome [1, 15], studies of drug effects on FRO [12], and monitoring of the treatment efficiency [5,6].

We investigated FRO in experimental pneumonia in order to substantiate the use of plasma CL recording for evaluating the intensity of inflammatory process in lung tissue.

MATERIALS AND METHODS

Experiments were carried out on 100 random-bred albino rats weighing 180-200 g. A suspension of 24-h culture of group IIb pneumococcus in 0.5 ml normal saline (2 mln bacterial cells) was injected intrathoracically under ether narcosis through the chest wall between the second and third ribs into the right lung. Controls were injected with 0.5 ml sterile normal saline. Blood for CL was collected from the caudal vein, sodium citrate was used as the anticoagulant (5 mg/ml blood). Before measuring the fluorescence, 0.5 ml plasma was diluted in 18.5 ml phosphate buffer of the following composition (mM): 20 KH_2PO_4, 105 KCl, (pH was adjusted to 7.45 with KOH). Fluorescence was induced by adding 1 ml 50 mM $FeSO_4 \times 7H_2O$.

Chemiluminescence was studied on a CLM-003 device. The stability of its operation was verified by chemiluminescence of secondary reference sample with fluorescence intensity of 5.1×10^5 quanta/sec (1 arb. unit).

The generation of active oxygen forms by alveolar macrophages (AM) was evaluated in the test with nitroblue tetrazolium (NBT) test, spontaneous and induced with 18-h staphylococcal culture (sNBT and iNBT) [11]. For preparing bronchial lavage, the trachea was opened under narcosis, tracheobronchial tree was washed with medium 199 with heparin (10 U/ml) through a polyethylene catheter. The lavage fluid was centrifuged for 2-3 min at 1000 rpm. The precipitate containing AM was diluted in 0.05 ml medium. Phagocytic activity of AM was evaluated as described elsewhere [8]. Toxemia markers, medium-weight molecules (MWM) in the blood were measured spectrophotometrically [4]. The results were expressed in arbitrary optical density units. The animals were sacrificed

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under narcosis and cytoplasmatic fraction was isolated from cells of various organs by ultracentrifugation of the homogenates at 100,000*g*. Cytoplasmatic fraction and blood plasma were fractionated and MWM were isolated by gel filtration on Sephadexes G-200, 100, and 75 on a 100×2.5 cm column. In parallel, pathomorphological changes in the lungs were studied. All studies were carried out before the experiment and on days 1, 3, and 7 of inflammation. The data were processed by variation statistics using Statistica for Windows'95 software.

RESULTS

Before the experiment, parameters of plasma CL corresponded to the expected values, and the group was statistically homogenous (Table 1). The development of experimental acute pneumonia was associated with a 1.6-fold increase in spontaneous plasma chemiluminescence, which indicated intensification of lipid peroxidation (LPO) (Table 1). The values of AM sNBT test increased 10-fold and of iNBT test more than twofold. Coefficients of correlation between NBT test for AM and spontaneous plasma CL were high (r_1=0.71 and r_2=0.51 for sNBT and iNBT tests, respectively), which confirms the role of AM activation in the initiation of LPO in acute pneumonia. An objective parameter of inflammatory process in animals is photosum of blood CL. This parameter increased by 1.3 times during the first day of acute pneumonia, and then depended on the morphological changes in the lungs.

The animals were divided in 2 groups by CL photosum. In group 1, CL photosum remained high and sNBT and iNBT tests for AM were maximum on day 3 of acute pneumonia. Pathomorphological study showed predominance of infiltrative changes in lung tissue. In group 2, decreased CL photosum and lung destruction chemiluminescence were observed. On day

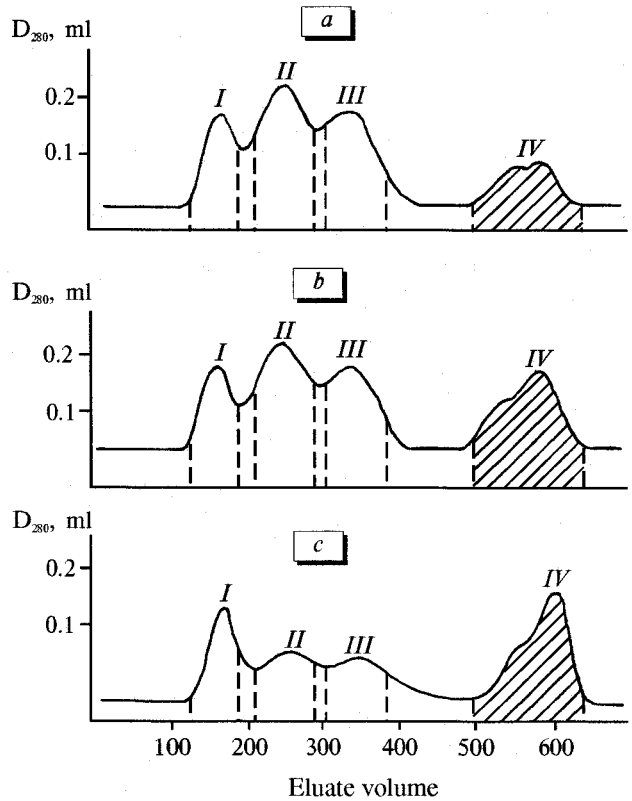

Fig. 1. Blood serum fractions (Sephadex G-200) in controls (*a*) and animals with lung tissue degeneration (*b*), and cytoplasmic fraction of lung homogenate (*c*). *I, II, III, IV*: fractions.

3 of experimental pneumonia their intensity decreased almost twofold, bactericidal and phagocytic activities of AM were appreciably suppressed. Later these animals developed generalized inflammatory process in the lungs.

It is known that suppressed plasma CL is characteristic of cell membrane degeneration and the release of intracellular contents into the blood flow; these components suppress the Fe^{2+}-induced chemiluminescence [2,3]. Plasma fractionation in these animals

TABLE 1. Plasma CL and Functional Activity of AM in Pneumonia Variants (*M*±*m*, *n*=10)

Parameter	Before experiment	Day of inflammation				
		1	3		7	
			PIC	PDC	PIC	PDC
SF	3.6±0.4	5.9±0.7	5.0±0.4	4.1±0.5	5.3±0.4	4.2±0.3
S	20.9±0.7	26.8±0.3	25.9±0.8	13.8±0.5*	16.9±0.8	10.2±0.4*
sNBT test	0.012±0.005	0.110±0.007	0.16±0.02	0.05±0.01*	0.06±0.01	0.030±0.002*
iNBT test	0.17±0.02	0.33±0.03	0.36±0.03	0.11±0.02*	0.10±0.01	0.07±0.01*
PN	58.1±5.5	58.0±8.5	75.2±2.4	34.8±8.1*	62.4±1.8	35.6±5.2*
PI	9.6±1.4	9.3±0.9	6.6±0.7	3.4±0.4*	5.4±0.3	3.8±0.5*

Note. **P* significant differences from initial values. SF: spontaneous chemiluminescence; S: photosum; PN: phagocytic number; PI: phagocytic index; PIC: predominance of infiltrative changes; PDC: predominance of degenerative changes.

showed increased content of fraction IV (1-10 kD polypeptides) (Fig. 1, cross-hatched area). Similar products were isolated from lung homogenate of intact animals, which confirmed the tissue origin of these polypeptides. One more proof is the increased plasma content of MWM (0.32±0.05 *vs.* 0.21±0.03 arb. opt. units or lower in the control), depending on the involved area of lung tissue. Increased blood content of polypeptides with molecular weight below 10 kD, LPO intensification, decreased CL photosum, and suppressed functional activity of AM were indicative of unfavorable course of pneumonia.

These findings demonstrated the pathogenetic role of FRO in acute pneumonia. Blood plasma CL objectively reflects the state of FRO, its intensity depends on peculiarities of inflammatory process in the lungs. Pneumonia is characterized by high oxygen-dependent metabolism in AM and enhanced plasma CL. Oxidative modification of biomembranes in lung degeneration leads to suppression of phagocyte activity, accumulation of MWM in the blood, which bind Fe^{2+} and reduce CL intensity.

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