Free-Radical Oxidation during Experimental Pneumonia

U. R. Farkhutdinov and R. R. Farkhutdinov

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Lipid peroxidation in the lungs and plasma of albino rats was studied under normal conditions and during experimental pneumonia. In intact rats the content of lipid peroxidation products and the rate of their accumulation in lung homogenate is lower than in homogenates of other organs. Bivalent iron ions added to blood plasma did not induce chemiluminescence characteristic of lipid peroxidation. Experimental pneumonia intensifies production of active oxygen forms by alveolar macrophages and blood neutrophils and increases the content of lipid peroxidation products in lung homogenate. Combined application of antibiotics and antioxidant (vitamin E) during experimental pneumonia promotes normalization of free-radical oxidation and diminishes morphological alterations in the lungs.

Key Words: *pneumonia; lipid peroxidation; chemiluminescence*

Recently considerable attention was focused on the key role of free radical oxidation in the pathogenesis of nonspecific pulmonary diseases [2,6,11,14]. All conditions necessary for oxidative stress are available in the lungs: direct contact with atmospheric oxygen, high concentrations of oxidation substrates (unsaturated fatty acids [1,8,12]), availability of oxidation catalyst Fe^{2+} (in hemoglobin), and alveolar macrophages (AM) producing the active oxygen forms (AOF): siglet oxygen $O_2^{\bullet}$, superoxide anion-radical $O_2^{\bullet-}$, hydrogen peroxide H_2O_2, hydroxyl radical $OH^{\bullet}$, hypochlorite $ClO^{\bullet}$ [5,13], and other active metabolites during phagocytosis. The active oxygen forms determine the microbicidal potential of the phagocytes, but under pathological conditions accompanied by incompetence of the antioxidant defense system they can induce lipid peroxidation and damage cell membranes. Both excessive and deficient AOF production by phagocytes cells may affect the course of inflammation.

Recording of chemiluminescence (CL) appearing during free radical interaction [3,4,9] is a simple and reliable method evaluating the state of free radical oxidation, which helps to elaborate pathogenetic therapy and control effectiveness of the treatment in nonspecific pulmonary diseases.

Our aim was to study free radical oxidation in experimental pneumonia, to reveal the dependence of this process on therapy, and to evaluate diagnostic value of CL recording for monitoring of the course of the disease.

MATERIALS AND METHODS

Experiments were carried out on 340 outbred Wistar rats weighing 180-200 g. Suspension of 1-day-old culture of group IIB pneumococcus (2 million microbial cells in 0.5 ml physiological saline) was intrathoracically injected into the right lung through the wall in 2-3 intercostal space under light narcosis. Control rats (n=20) were treated with sterile physiological saline. The animals with experimental pneumonia were subdivided into 4 groups of 80 animals per each: group 1 rats were not treated; group 2 rats were injected intramuscularly with penicillin (10,000 U/day); group 3 rats were injected with vitamin E (daily dose 100 mg/kg); group 4 rats were injected with vitamin E and penicillin in the above doses. Sodium citrate (5 mg/kg) was added to plasma samples as the anticoagulant.

To study lipid peroxidation in organs, they were extirpated in cold, homogenized for 5 min in cold sa-

Bashkir State Medical University, Aviation Technical University; Interinstitute Laboratory of Technical Systems for Medico-Biological Investigations, Ufa. ***Address for correspondence:*** FarhutdinovRR@soros.bashedu.ru. Farkhutdinov R. R.

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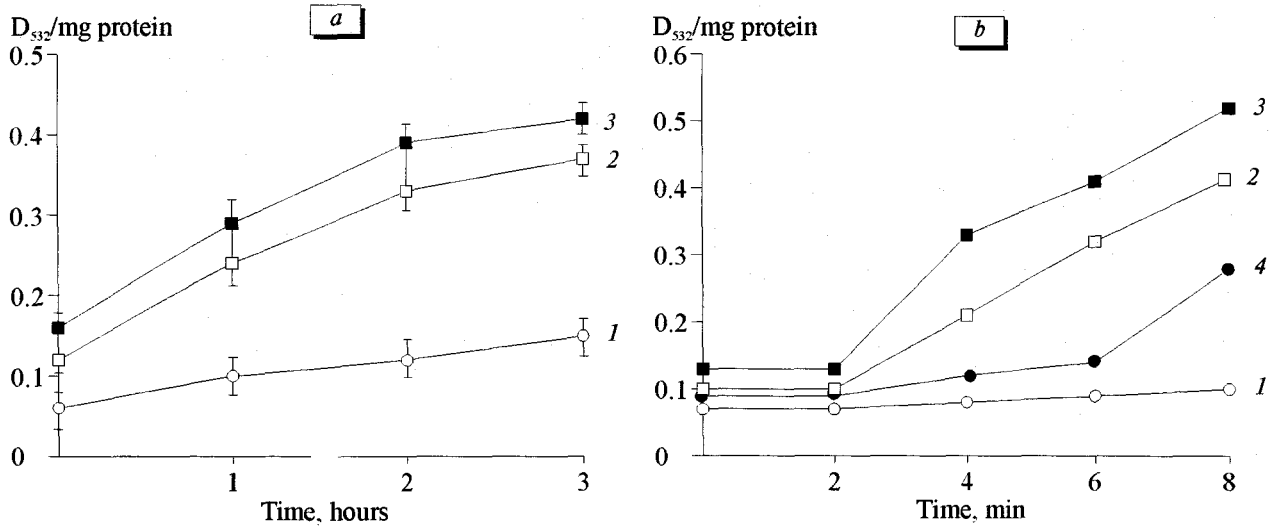

Fig. 1. Accumulation of MDA in lung, kidney, and liver homogenates during aerobic incubation (*a*) and in the presence of Fe^{2+} (*b*). Here and in Fig. 2: homogenate of the lungs (*1*), kidneys (*2*), liver (*3*), and lungs with liver (*4*).

line containing 20 mM KH_2PO_4 and 105 mM KCl; pH was adjusted to 7.45 with KOH (phosphate buffer). The homogenate was used to determine the content of protein, malonic dialdehyde (MDA, by color reaction with 2-thiobarbituric acid TBA [1]), and its accumulation after a 3-h aerobic incubation at 37°C.

Chemiluminescence was recorded on an KhLM-003 spectrofluorimeter. Stability of experimental setup was tested by CL of a secondary calibration standard emitting 5.1×10^5 quantum per sec (1 mV) considered as 1 rel. units. Before recording, plasma and homogenate (0.5 ml) were dissolved in phosphate buffer (18.5 ml). CL was induced by adding 1 ml 50 mM $FeSO_4\times7H_2O$.

Generation of AOF in AM and blood neutrophils (BN) was assessed by spontaneous nitroblue tetrazolium (sNBT) and induced (iNBT) by 18-h pneumococcus culture tests [10]. To obtain bronchial lavage, the trachea was opened in narcotized rats, and the tracheobronchial tree was rinsed via a polyethylene catheter with medium 199 containing heparin (10 U/ml). The lavage fluid was centrifuged at 1000 rpm for 2-3 min, the precipitate with AM was resuspended in 0.05 ml medium. The phagocytic activity of AM and BN was measured as described previously [7]. Comparative analysis of pathomorphologic alterations in the lungs during pneumonia was performed.

RESULTS

The content of MDA in lung homogenate was 2-3 times lower than in liver and kidney homogenates (Fig. 1), while accumulation of this product during aerobic incubation was 3-4-fold slower. In the presence of lipid peroxidation inductor Fe^{2+}, the content of MDA in lung homogenate increased insignificantly, and in contrast to homogenates of other organs, no CL typical for lipid peroxidation was observed (Fig. 2). Addition of lung homogenate to liver homogenate suppressed CL and MDA accumulation. These data confirmed high antioxidant activity in the pulmonary tissue.

TABLE 1. Accumulation of MDA in Lung Homogenate and Plasma CL in Rats during Acute Experimental Pneumonia (*M±m*)

Group	MDA, D_{532}/mg protein		Plasma CL, rel. units	
	initial	final	spontaneous	induced
Control	0.089±0.012	0.154±0.013	3.6±0.4	20.9±0.7
Acute pneumonia				
day 1	0.20±0.02*	0.32±0.02*	5.9±0.7*	21.4±0.5
day 3	0.20±0.02*	0.35±0.03*	5.0±0.4*	25.9±0.8*
day 7	0.14±0.02*	0.21±0.01*	5.3±0.4*	16.9±0.8*
day 14	0.14±0.02*	0.32±0.01*	3.6±0.4*	13.5±0.8*

Note. Here and in Tables 2 and 3: $p<0.05$ *compared to the control.

TABLE 2. Functional Activity of AM and BN in Rats with Experimental Pneumonia (*M±m*)

Group	sNBT		iNBT		Phagocytic number		Phagocytic index	
	AM	BN	AM	BN	AM	BN	AM	BN
Control	0.012±0.005	0.007±0.005	0.17±0.02	0.13±0.02	58.1±5.1	58.3±6.5	9.6±1.4	8.7±1.4
Acute pneumonia								
Day 3: 1st	0.16±0.02	0.02±0.01	0.37±0.02	0.18±0.02	75.2±2.4	71.4±8.0	6.6±0.67	7.4±0.9
2nd	0.050±0.007*	0.006±0.008	0.18±0.03*	0.08±0.02*	59.3±3.9*	41.8±7.6*	8.9±1.2	9.7±2.7
3rd	0.04±0.02*	0.02±0.01	0.18±0.02*	0.04±0.02*	66.0±3.7*	63.7±7.2	7.9±1.9	6.2±0.6
4th	0.020±0.007*	0.004±0.003*	0.09±0.01*	0.03±0.02*	54.7±4.7*	40.2±11.4*	9.4±2.8	10.20±1.72
Day 7: 1st	0.06±0.01	0.06±0.005	0.10±0.01	0.19±0.04	51.2±4.7	79.3±6.5	8.5±1.1	7.0±1.7
2nd	0.03±0.01*	0.004±0.005*	0.050±0.009*	0.12±0.05	44.3±3.5*	36.0±5.9*	6.3±0.9*	9.7±1.5
3rd	0.050±0.004*	0.200±0.006*	0.08±0.01	0.040±0.006*	56.0±5.6	68.0±9.5	6.8±0.7*	5.9±0.9
4th	0.010±0.005*	0.002±0.004*	0.020±0.005*	0.08±0.03*	30.5±7.4*	34.0±7.5*	7.0±1.6	8.70±0.97
Day 14: 1st	0.040±0.009	0.04±0.01	0.13±0.01	0.09±0.03	50.0±5.5	49.7±6.4	6.9±0.9	6.1±0.7
2nd	0.020±0.007*	0.006±0.008*	0.12±0.02	0.07±0.05	64.7±2.6*	47.2±4.4	10.9±2.7*	9.9±2.3
3rd	0.030±0.007	0.002*	0.070±0.007	0.03±0.01*	46.4±4.2	37.3±5.6*	5.7±0.6	5.50±0.96
4th	0.005±0.004*	0.002±0.004*	0.11±0.01	0.02±0.01*	69.0±4.1*	32.8±3.4*	10.6±1.2*	9.6±0.5

The development of experimental pneumonia was accompanied by an increase in MDA content in lung homogenate (Table 1). Simultaneously, CL in the blood plasma increased: on day 1, spontaneous luminescence and the photosum of Fe^{2+}-induced CL increased 1.6- and 1.3-fold, respectively. These indices remained at a high level during 3-7 days and correlated with MDA content in lung homogenate (r_1=0.63 and r_2=0.59 for spontaneous CL and photosum, respectively), which attests to a dependence of the changes in plasma CL on lipid peroxidation intensity in the lungs.

Activation of phagocytes and generation of AOF also contribute to activation of lipid peroxidation during inflammation. On pneumonia day 1, sNBT test with AM increased 10-fold and iNBT test more than 2-fold and achieved the maximum on pneumonia day 3 (Table 2). Similar changes were observed in BN, but the maximum values of NBT test were observed only on pneumonia day 7. The correlation coefficient for NBT test with AM and MDA content in the lung homogenate was high (r=0.99), which supports the role of AM activation in induction of lipid peroxidation during acute pneumonia.

Preinjection with vitamin E decreased MDA content in lung homogenate, intensity of plasma CL, sNBT and iNBT tests for AM and BN, and phagocytic activity of these cells (the phagocytic number and phagocytic index) in rats with experimental pneumonia in comparison with untreated rats. Therefore, vitamin E inhibited generation of AOF by phagocytes and decreased their microbicidal potential, which modulated the development of inflammation: starting from

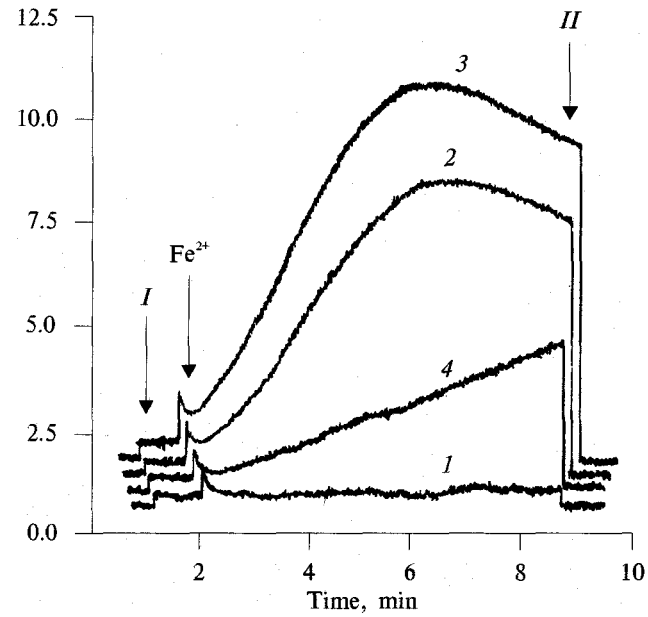

Fig. 2. Fe^{2+}-induced chemiluminescence in homogenates of visceral organs. Ordinate: chemiluminescence intensity, mV. The arrows mark beginning (*I*) and end (*II*) of chemiluminescence recording.

TABLE 3. Plasma CL in Various Periods of Experimental Pneumonia in Rats during Therapy (*M±m*)

Group	Days			
	1	3	7	14
1st				
spontaneous luminescence	5.9±0.7	5.0±0.4	5.3±0.4	3.6±0.4
photosum	21.4±0.5	25.9±0.8	16.9±0.8	13.5±0.8
2nd				
spontaneous luminescence	4.6±0.3*	4.0±0.4*	4.9±0.4*	3.1±0.5
photosum	19.6±0.7*	12.3±0.9*	12.4±0.9*	12.2±0.6*
3rd				
spontaneous luminescence	5.9±0.9	3.6±0.3*	4.2±0.8*	4.2±0.7
photosum	20.3±0.9	14.6±0.6*	17.3±0.8	11.2±0.7*
4th				
spontaneous luminescence	4.7±0.5*	2.6±0.6*	4.3±0.6*	3.4±0.3
photosum	19.2±0.8*	10.7±1.4	13.9±1.4*	11.4±0.6*

pneumonia day 7, generalization of inflammation and destructive alterations in the lungs were observed. In rats treated with penicillin, MDA content in lung homogenate, intensity of plasma CL, and NBT test were lower than in untreated rats, but higher than in rats treated with vitamin E. Intensive fibrosis was observed in the lung tissue of penicillin-treated rats. Antibacterial therapy, which suppresses the phagocytic subdivision of the immune system, can affect the outcome of acute pneumonia and promote its chronization. Combined therapy with penicillin and vitamin E was characterized by minimal content of MDA in the lung tissue and decreased plasma CL (Table 3).

In this group the values of NBT tests for AM and BN were low, although phagocytic index remained at a high level attesting to the maintenance of phagocyte function. Pathomorphologic alterations in the lungs of the rats receiving combined therapy were limited, and no fibrosis was observed. In this case, α-tocopherol inhibited activation of free radical oxidation (protective effect), while a decrease in phagocyte microbicidal activity was compensated by the effect of the antibiotic.

Thus, normal lung tissue is characterized by high antioxidant activity, which is extremely important under conditions of persistent contact with inductors of free radical oxidation. The development of pneumonia is accompanied by accumulation of lipid peroxidation products in the lungs.

The inductors of oxidative stress during experimental pneumonia are AM and BN, whose functional state correlates with lipid peroxidation intensity in the lung and plasma CL. Vitamin E inhibits free radical oxidation and microbicidal activity of phagocytes. Combined administration of vitamin E and antibiotics normalizes free radical oxidation, alleviates pathomorphologic alterations in the lungs, and prevents fibrosis.

REFERENCES

1. Yu. A. Vladimirov and A. N. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972).
2. E. V. Gembitskii and V. G. Novozhenov, *Klin. Med.*, No. 5, 7-12 (1994).
3. N. A. Didkovskii, Yu. A. Vladimirov, N. V. Reshetova, *et al.*, *Pul'monologiya*, No. 1, 24-34 (1995).
4. N. K. Zenkov, E. B. Men'shikova, and S. M. Shergin, *Oxidative Stress: Diagnostics, Therapy, and Prevention* [in Russian], Novosibirsk (1993).
5. M. B. Keniya, A. I. Lukash, and E. P. Gus'kov, *Usp. Sovr. Biol.*, **113**, No. 4, 456-470 (1993).
6. A. N. Kokosov, Yu. M. Goldenberg, and V. P. Mishchenko, *Pul'monologiya*, No. 1, 38-43 (1995).
7. E. A. Kost and M. I. Smenko, in: *Proceedings of S. P. Botkin Hospital* [in Russian], Moscow (1947), pp. 253-256.
8. N. V. Syromyatnikova, V. A. Goncharova, and T. V. Kotenko, *Metabolic Activity of the Lungs* [in Russian], Leningrad (1987).
9. R. R. Farkhutdinov and V. A. Likhovskikh, *Chemoluminescent Methods to Study Free Radical Oxidation in Biology and Medicine* [in Russian], Ufa (1995).
10. M. G. Shubich and V. G. Mednikova, *Lab. Delo*, No. 9, 515-518 (1978).
11. J. Braun, M. Pein, H. Djonlagic, and K. Dalhoff, *Intensive Care Med.*, **23**, No. 2, 170-176 (1997).
12. Ch. I. Bryan and S. I. Jenkinson, *Clin. Chest. Med.*, **9**, No. 1, 141-152 (1988).
13. S. J. Klebanoff, in: *Inflammation. Basic Principles and Clinical Correlates*, New York (1992), pp. 541-588.
14. K. Moussa, H. J. Michie, I. A. Cree, *et al.*, *Thorax*, **49**, No. 2, 107-111 (1994).