STATE OF LIPID PEROXIDATION AND ANTIOXIDANT SYSTEM IN HEPATIC AND PULMONARY MICROSOMES IN DIFFERENT PERIODS OF SENSITIZATION DEPENDING ON HISTAMINE CONTENT

Kh. Ya. Karimov, A. S. Komarin, T. E. Lysenko, L. N. Davletshina, and A. F. Alimova

UDC 616-056.3-092.9-07:[616.154:577-175.824+616.36-008.939.15-39

KEY WORDS: histamine; lipid peroxidation; sensitization; antioxidant system

Histamine occupies an important place in metabolic and structural disturbances in the liver and lungs in allergy [1, 4]. In many pathological states, a key role in the mechanism of tissue damage is played by intensification of lipid peroxidation (LPO) processes in the tissues [6, 7]. However, the role of histamine in changes in the intensity of LPO in the liver and lungs, organs actively participating in the pathophysiological manifestation of sensitization processes and the development of allergic reactions of immediate type, has not been adequately reflected in the literature [13, 14].

The aim of this investigation was to study the state of LPO and the antioxidant system (AOS) in hepatic and pulmonary microsomes at different times of formation of the sensitization process, and depending on the histamine content.

EXPERIMENTAL METHOD

Experiments were carried out on 210 male guinea pigs weighing 350-480 g. There were four series of experiments. In series I and II LPO activity in the hepatic and pulmonary microsomes was studied on the 1st and 21st days of the sensitization period (SP). The animals were sensensitized by subcutaneous injection of hen's egg albumin, diluted 1:5 with isotonic NaCl solution, three times on alternate days, and with an equal volume of mineral oil [1]. In series III, LPO activity was studied in experiments in vitro, in which histamine in doses of 10^{-7}, 10^{-5}, 10^{-3}, and 10^{-2} M was incubated with guinea pig hepatic and pulmonary microsomes on the 1st and 21st days of SP. Incubation of histamine with microsomes was carried out in a waterbath at 37°C for 10 min with continuous shaking in airtight tubes. In the experiments of series IV LPO activity in hepatic and pulmonary microsomes was studied after daily single injections of histamine intraperitoneally for 5 days in doses of 0.005, 0.05, 0.125, 0.25, and 0.5 mg/kg into five groups of intact animals. The mean number of animals in each group was 6-8.

Microsomes were isolated by differential centrifugation [8].

The intensity of LPO was determined: in microsomes – in spontaneous (SPO), NADPH-dependent (ND), and ascorbate-dependent (AD) systems by the thiobarbiturate reaction, based on the rate of accumulation of malonic dialdehyde (MDA) [5]; in the blood serum – based on the intensity of chemiluminescence (Chl), recorded on the KhLM Ts1-01 chemiluminometer [10]. The state of the AOS in the postmitochondrial supernatant of the liver and lungs was evaluated by measuring superoxide dismutase (SOD) [11] and catalase [9] activity. Protein in the samples was determined quantitatively by Lowry's method. The histamine concentration in the blood and hepatic and pulmonary homogenates was determined fluorometrically [12].

Central Research Laboratory, Tashkent Medical Institute. (Presented by Academician of the Russian Academy of Medical Sciences A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 145-148, August, 1992. Original article submitted September 20, 1991.

 0007-4888/92/0008-1106$12.50

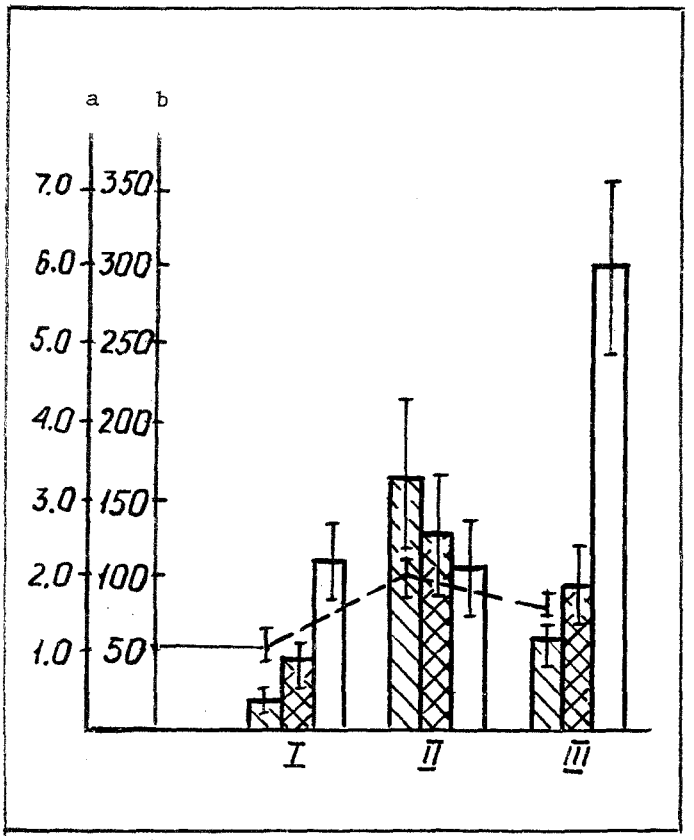

Fig. 1. Histamine concentration in blood, liver, and lungs (a) and chemiluminescence in blood (b) in different phases of sensitization: I) intact animals; II) 1st day of sensitization; III) 21st day of sensitization; oblique shading – blood (μg/ml), cross-hatching – liver (μg/g), unshaded – lungs (μg/ml); broken line denotes intensity of chemiluminescence (pulses/10 sec).

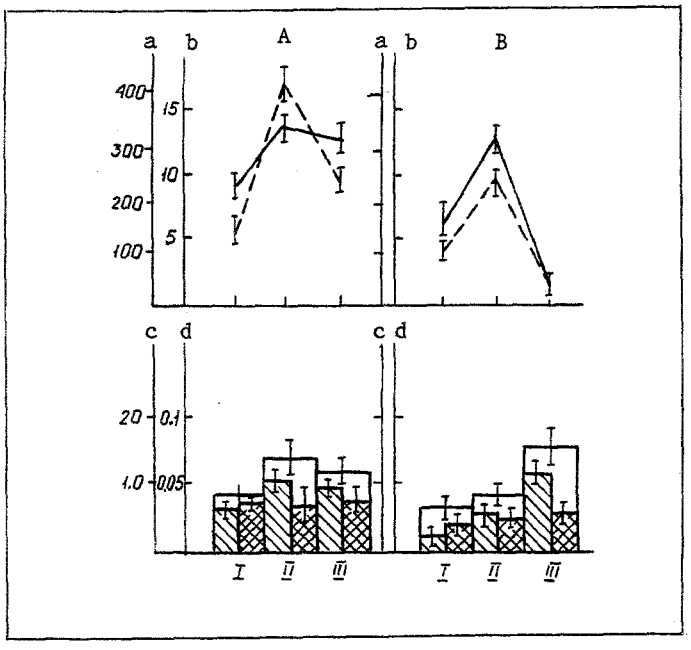

Fig. 2. State of antioxidant system (top) and LPO (bottom) in liver (A) and lungs (B) during sensitization; a) catalase activity, broken line (mmoles/min ·g protein); b) SOD activity (activity units/mg protein); c) NADPEI-dependent (oblique shading) and ascorbate-dependent LPO (crosshatching), nmoles MDA/mg protein ·min; d) spontaneous LPO (unshaded), nmoles MDA/ng protein ·min.

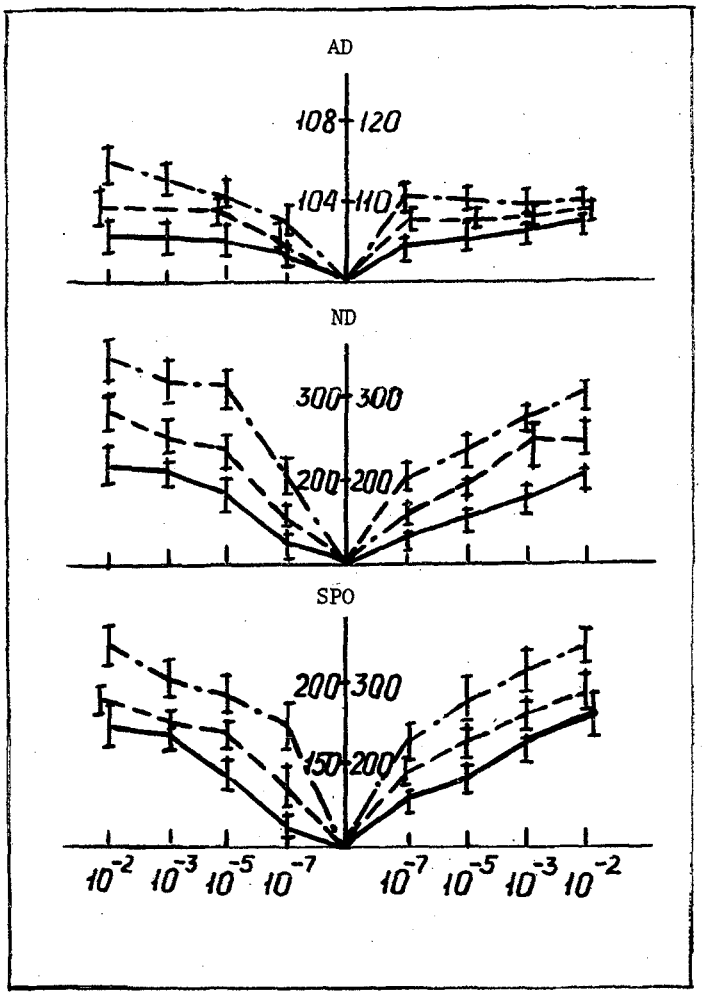

Fig. 3. Effect of histamine on state of LPO in hepatic and pulmonary microsomes in vitro. Abscissa, logarithm of histamine concentration; ordinate, MDA level, in percent; MDA content in hepatic (right) and pulmonary (left) microsomes, isolated from control and sensitized animals on 1st and 21st days of sensitization and incubated without histamine, taken as 100%; continuous line – control, broken line – 1st day of sensitization, line of dots and dashes – 21st day of sensitization.

Intact animals served as the control for all series of experiments. The numerical results were subjected to statistical analysis by Student's t test [2].

EXPERIMENTAL RESULTS

It will be clear from the data in Fig. 1 that on the first day of SP the histamine concentration in whole blood was 7.28 times higher than the control, 1.96 times in liver homogenates, but in the lungs it was unchanged. On the 21st day of the experiment the histamine concentration in the blood and liver homogenate was appreciably lower than in the previous period, but in the lungs it had risen to 277% of the control level. At the same time there was an increase in the intensity of very weak fluorescence of the blood serum on the first day of SP by 85%, and on the 21st day by 52%. The increase in the intensity of chemiluminescence of the blood was accompanied by intensification of free-radical lipid oxidation. On the first day of the investigation, for instance, the rate of MDA accumulation in the hepatic microsomes was 68 and 86% higher than the control, and 56 and 115% higher in the lungs in the SPO and ND systems of LPO respectively. On the 21st day of the investigation a tendency was noted for LPO activity to fall

in the SPO and ND systems of the liver microsomes, whereas in the lungs, on the contrary, it was increased by 183 and 497% respectively compared with the control. No significant changes were observed in the AD system of LPO in the hepatic and pulmonary microsomes on the 21st day of SPO.

Investigation of AOS showed that on the first day of SP there was a significant increase in SOD and catalase activity by 196 and 42% respectively in the liver and by 123 and 101% in the lungs compared with the control (Fig. 2). On the 21st day SOD and catalase activity in the liver cytosol remained high, but their activity in the lungs decreased.

The results in Fig. 3 show that the action of different doses of histamine in experiments in vitro was manifested as activation of LPO in the SPO and ND systems, the MDA level in the AD system remaining virtually unchanged. In response to an increase in the histamine concentration there was a greater increase in the rate of accumulation of LPO products in the hepatic and pulmonary microsomes on the 21st day of SP.

The results of experiments in vitro did not always correlate with the changes taking place in the animal. As the results of the experiments of series IV showed, with an increase in the dose of histamine injected there was an increase in the histamine concentration in whole blood. Meanwhile a rise of the histamine concentration from 0.005 to 0.125 mg/kg led to a decrease, whereas doses of 0.25 and 0.5 mg/kg led to an increase in the endogenous histamine content in the liver and blood homogenates.

Changes in LPO parameters in the hepatic and pulmonary microsomes also had particular features. With small doses of histamine injected, activation of LPO processes in the SPO and ND systems took place, but after injection of a dose of 0.5 mg/kg, it took place in the AD system also. Consequently, depending on the dose of histamine given, considerable changes take place in the histamine level in the test tissues and in the intensity of LPO in the hepatic and pulmonary microsomes of intact guinea pigs.

The main cause of the increase in LPO activity in the hepatic and pulmonary microsomes of sensitized guinea pigs, it must be assumed, was an increase in the histamine concentration in them, as was confirmed by experiments in vitro and in vivo. However, as will be clear from Fig. 2, besides an increase in the intensity of LPO in the microsomes, there was a sharp increase in activity of AOS, probably due to activation of protective and adaptive mechanisms.

Meanwhile, maintenance of a high histamine concentration in the lung tissues may lead to failure of adaptive processes, as shown by reduced activity of the antioxidant system and a regular increase in the intensity of LPO reactions in the pulmonary microsomes in all systems tested on the 21st day of SP (Fig. 2).

Weakening of activity of enzymes of antioxidant protection in sensitized animals toward the 21st day of the experiment may perhaps be one cause of the increase in sensitivity of isolated hepatic and pulmonary microsomes in the experiments in vitro to the pro-oxidant action of histamine.

Evidence of the involvement of histamine in LPO activation is given by the results of experiments in vivo, with a demonstration of different doses of histamine base to guinea pigs. After injection of small concentrations of histamine, activity of the nonenzymic pathway of LPO was inhibited in the hepatic and pulmonary microsomes, and this was accompanied by lowering of the concentration of endogenous histamine in homogenates of these organs. Later, with an increase in the dose of histamine, LPO processes were stimulated mainly through an enzymic mechanism – through the ND system of LPO. LPO activity in the SPO and ND systems during sensitization may perhaps be connected with potentiation of self-renewal or self-restructuring of the membrane structures, aimed at increasing activity of membrane-bound enzymes which participate in the biotransformation of endogenously formed toxic metabolic products [3, 6].

In the early period of sensitization (1st day of SP), for instance, elevation of the histamine level led to intensification of LPO and activation of antioxidant protection enzymes – SOD and catalase. In the later stage (21st day), in the lungs, besides a further increase in histamine concentration, the intensity of LPO also was increased due to marked inhibition of activity of the AOS. Under the conditions of formation of allergy in the lungs, by contrast with the liver, because of the insufficiently developed pathway of inactivation of histamine [13], it accumulated and, as a result, free-radical processes were activated.

Consequently, depending on the phase of allergization and the histamine concentration, changes take place in the intensity of LPO in microsomal membranes, leading to disturbance of the physicochemical properties of the phospholipid matrix of the membranes. This leads to increased permeability and accumulation of reactive biologically active compounds, which could affect activity of the detoxication system in the lungs and liver in the sensitization

period and could lead to reduction of the adaptive powers of these organs vis-a-vis the sensitizing action of the allergen.

REFERENCES

1. A. D. Ado, General Allergology [in Russian], Moscow (1978).
2. M. L. Belen'kii, Elements of Quantitative Evaluation of the Pharmacologic Effect [in Russian], Riga (1959).
3. E. B. Burlakova, Biochemistry of Lipids and Their Role in Metabolism [in Russian], Moscow (1981).
4. I. L. Vaisfel'd and G. N. Kassil', Histamine in Biochemistry and Physiology [in Russian], Moscow (1981).
5. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
6. Yu. A. Vladimirov, Patol. Fiziol., No. 4, 7 (1989).
7. P. V. Gulak, A. M. Dudchenko, V. V. Zaitsev, et al., The Hepatocyte: Functional and Metabolic Properties [in Russian], Moscow (1985).
8. J. de Pierre and G. Dallner, Biochemical Investigation of Membranes [Russian translation], Moscow (1979).
9. M. A. Korolyuk, L. I. Ivanova, I. G. Maiorova, et al., Lab. Delo, No. 1, 16 (1988).
10. Yu. M. Lopukhin, Yu. A. Vladimirov, M. N. Molodenkov, et al., Byull. Éksp. Biol. Med. No. 2, 61 (1983).
11. V. G. Mkhitaryan and G. E. Badalyan, Zh. Éksp. Klin. Med., No. 6, 7 (1978).
12. L. Ya. Proshina, Lab. Delo, No. 2, 90 (1981).
13. N. V. Syromyatnikov, V. A. Goncharova, and T. V. Kotenko, Metabolic Activity of the Lungs [in Russian], Leningrad (1987).
14. A. G. Khomenko, S. T. Müller, and W. Schilling, Exogenous Allergic Alveolitis [in Russian], Moscow (1987).

CHANGES IN SERUM LIPIDS AND LIPOPROTEINS OF RATS AFTER OVARIECTOMY AND ADMINISTRATION OF THE METHYL ESTER OF 6-OXY-D-HOMO-8-ISOESTRONE

I. Yu. Kameneva, A. G. Shavva, V. G. Lozovskii, and V. E. Ryzhenkov

UDC 618.11-089.87]-07:616.153.915-02:615.357:577.175.642

KEY WORDS: ovariectomy; lipoproteins; cholesterol; estrogen analogs

During estrogen replacement therapy an increase in the frequency of nonlethal venous thromboembolism and myocardial infarction is observed, and is associated with elevation of the blood triglyceride (TG) level [2]. It was accordingly decided to look for analogs of steroid hormones that possess sufficient estrogenic activity but, at the same time, have no adverse action on lipid metabolism [4]. Under these circumstances a model of hypercholesterolemia induced by ovariectomy is often used [13].

The aim of this investigation was to study lipid metabolism in ovariectomized rats and the possibility of correcting it by estradiol and an isoestrone analog.

St. Petersburg National University. Research Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg.

(Presented by Academician of the Russian Academy of Medical Sciences A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 148-150, August, 1992. Original article submitted January 9, 1992.

0007-4888/92/0008-1110$12.50