quent restoration of the contractile function of the heart in acute, short-term hypoxia and subsequent reoxygenation [12, 13]. Preliminary administration of the antioxidant ionol guarantees more effective functioning of this system and, correspondingly, the more rapid recovery of the contractile function during reoxygenation.

LITERATURE CITED

1. L. M. Belkina, G. I. Markovskaya, and F. Z. Meerson, Kardiologiya, No. 8, 103 (1982).
2. F. Z. Meerson, A. A. Ugolev, and L. Yu. Golubeva, Kardiologiya, No. 11 (1980).
3. F. Z. Meerson, V. E. Kagan, Yu. P. Kozlov, et al., Kardiologiya, No. 2, 81 (1980).
4. F. Z. Meerson, L. M. Belkina, S. S. Dyusenov, et al., Kardiologiya, No. 10, 29 (1985).
5. F. Z. Meerson and L. M. Belkina, Patol. Fizol., No. 6, 1 (1986).
6. N. P. Meshkova and S. E. Severin (eds.), Textbook of Practical Biochemistry [in Russian], Moscow (1979), pp. 186-189.
7. O. L. Bricknell and L. H. Opie, Circulat. Res., 43, 102 (1978).
8. H. G. Hers, Adv. Metabol. Dis., 1, 44 (1964).
9. H. J. Heiner, E. Muller, and W. Bernauer, Basic Res. Cardiol., 83, 149 (1988).
10. D. Keppler and K. Decker, Methods of Enzymatic Analysis, H. U. Bergemeyer (ed.), Vol. 3, New York (1974), pp. 1123-1131.
11. V. V. Kupriyanov, V. L. Lakomkin, V. L. Kapelko, et al., Mol. Cell. Cardiol., 19, 729 (1987).

GENERATION OF ACTIVE BACTERICIDAL FORMS OF OXYGEN BY LEUKOCYTES CIRCULATING THROUGH THE LUNGS

A. Kh. Kogan, N. I. Losev, A. B. Tsypin, and B. M. Manuilov

UDC 612.262:[612.112.3:612.118.24

KEY WORDS: leukocytes; active forms of oxygen; free radicals

Phagocytic leukocytes (granulocytes and monocytes) generate active form of oxygen (AFO): the superoxide anion-radical ($O_2^{\cdot}$), singlet or electronically excited oxygen (I_{O_2}), the hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide, amounts of which increase sharply in response to stimulation of the leukocytes by biologically active substances [10, 13] and during phagocytosis [5, 6, 8, 10, 13]. In the absence of stimulation and phagocytosis AFO generation is miserly [9]. On the one hand, AFO are important bactericidal factors of phagocytosis [5, 8, 9, 11-13], whereas on the other hand they are powerful initiators of free radical lipid peroxidation [4, 5], which has an altering action [2, 4]. On account of these two effects, AFO generated by leukocytes perform a double function: they are involved in the formation of phagocytic antimicrobial defense and in the free radical status of the organism.

The aim of this investigation was to study the possible role of the lungs and other organs in the regulation of bactericidal AFO production by leukocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 18 intact dogs weighing 15-20 kg and two calves, anesthetized with thiopental, and receiving preliminary injections of droperidol, callipsol, trimeperidine, and atropine and maintained on artificial respiration. AFO generation by leuko-

I. M. Sechenov First Moscow Medical Institute. Moscow Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR, D. A. Kharkevich.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 6, pp. 688-690, June, 1989. Original article submitted December 23, 1988.

0007-4888/89/0006-0800$12.50

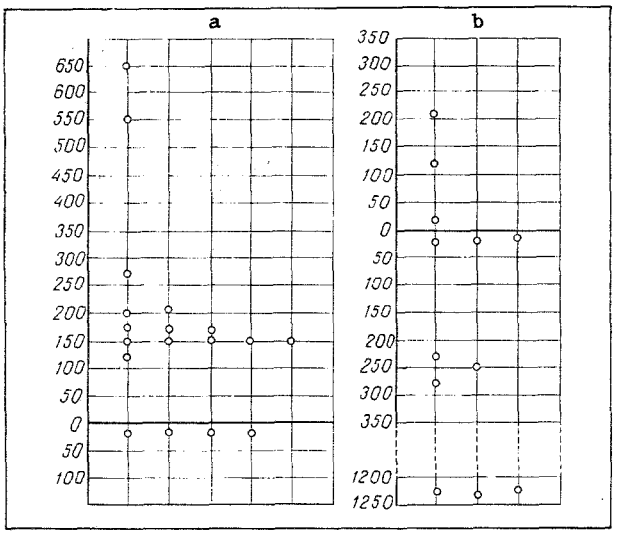

Fig. 1. Changes in AFO generation by leukocytes (neutrophils and macrophages) during passage through PVB (a) and SVB (b). Each circle represents one dog; p = 0.05 between number of dogs with increased and reduced AFO generation by leukocytes at exit from PVB or SVB. Ordinate, changes in AFO generation by leukocytes during passage through PVB or SVB (in %). Zero line – no change; above zero – increase, below zero – decrease.

cytes was investigated by a chemiluminescence (CHL) method [5-8, 11, 15] and by a chemical method – the nitro-BT test [3, 4]. The CHL method is based on a combination of AFO generation by leukocytes in very weak luminescence (CHL) [5-8, 11, 15]. CHL was potentiated by addition of a luminol (5-amino-2,3-dihydro-1,4-thalazine dione – 0.25 ml of a saturated iso-osmolar aqueous solution, pH 7.35), to 0.75 ml of packed leukocytes (PL); the luminol, during interaction with AFO, readily forms electronically-excited derivatives (aminophthalate ions and radicals) which are powerful luminescence emitters (the so-called luminol-dependent CHL, or LDCHL [6, 14]). Confirmation that LDCHL in fact reflects AFO generation was given by experiments which showed that superoxide dismutase (10 μg/ml) and catalase (100 μg/ml), which inactivate the superoxide anion-radical and hydrogen peroxide, inhibit LDCHL of leukocytes at rest by 90 and 80-100%, respectively [7] and, by a lesser degree, during phagocytosis [14]. To assess changes in AFO generation by leukocytes during their passage through the pulmonary vascular bed (PVB) AFO formation by leukocytes was compared at the entrance into the exit from PVB respectively, in the right ventricle (or posterior vena cava) and aorta; for comparison, a similar investigation was conducted at the entrance into and exit from the splenic vascular bed (SVB): in the splenic artery (or aorta) and splenic vein. The total PL fraction (granulocytes, monocytes, and lymphocytes) was isolated from heparinized blood (8-10 ml) by successive centrifugation at 500 and 1000 rpm and resuspended in isologous plasma (1.5 ml). Leukocytes in 1 mm^3 of PL were counted in a Goryaev's chamber, LDCHL of 0.75 ml of PL was measured, and the chemiluminescence criterion (CHLC) of AFO generation was calculated by the equation:

$$CHLC = \frac{\text{maximal CHL of PL} \cdot 10^6}{\text{Number of leukocytes in 1 mm}^3\ \text{PL} \cdot \text{total volume of PL in 1 mm}^3}$$

Determination of AFO by the nitro-BT test is based on the fact that nitro-BT in phagocytic leukocytes is reduced directly by the superoxide anion-radical [3, 4] into blue granules of diformazan, the concentration of which reflects AFO. The nitro-BT test is an "accepted indicator" of the "bactericidal function of phagocytes" [1]. The object for phagocytosis consisted of Staphylococcus aureus, strain Lepin (No. 201016). A mixture consisting of 0.1 ml of leukocyte suspension ($5 \cdot 10^6$ cells in 1 ml isologous plasma), 0.1 ml phosphate buffer (pH 7.4), and 0.1 ml of a suspension of heat-killed microbial cells ($2.5 \cdot 10^8$/ml), was incubated for 30 min at 37°C. The leukocytes were sedimented by centrifugation and

incubated with 1 ml of dimethylsulfoxide (DMSO). The DMSO containing extracted diformazan was separated by recentrifugation, and its diformazan concentration was measured spectrophotometrically in optical density units (λ = 492 nm). Phagocytosis was investigated by the traditional method. A mixture consisting of 0.15 ml of heparinized blood and 0.05 ml of a suspension of killed staphylococci ($1 \cdot 10^9$ cells/ml; ~50-60 microbial cells per leukocyte) was incubated for 30 min at 37°C, with periodic shaking. The average number of microbial cells ingested by one leukocyte was calculated from 100 leukocytes counted and the percentage of phagocytic leukocytes were determined in films stained by the Giemsa–Romanovsky method. The numerical results were subjected to statistical analysis (determination of p) the signs test.

EXPERIMENTAL RESULTS

During passage of the leukocytes through PVB (Fig. 1) CHLC of AFO generation in 14 of the 18 dogs (77.7%) was increased by 1.21-6.5 times, or on average by 2.4 times ($p = 0.05$). The mean absolute value of CHLC for AFO generation in all 18 dogs rose, irrespective of individual changes in particular animals, from 165.5 cps/leukocyte$\cdot 10^6$ in the right ventricle to 259.7 cps/leukocyte$\cdot 10^6$ in the aorta, i.e., by 1.6 times; CHLC for AFO generation by leukocytes in the calves also rose in the aorta to a higher level than in the right ventricle, from 80-308 to 364-853 cps/leukocytes$\cdot 10^6$, and on average, from 194 to 608.5 cps/leukocyte$\cdot 10^6$, i.e., by 3.66 times. A sample investigation showed that simultaneously with CHLC of AFO generation, the chemical criterion of AFO generation (intensity of the nitro-BT test) also was increased at the exit from PVB. In five of the six dogs in which CHLC of AFO was raised the intensity of the nitro-BT test (relative to the quantity of diformazan formed in the leukocytes) was increased in blood flowing from the lungs compared with blood flowing into the lungs from 0.023-0.065 to 0.046-0.112 optical density units, and on average from 0.0442 to 0.087, i.e., by 1.96 times. In four of the six dogs in which CHLC for AFO generation was increased at the exit from PVB, an increase in the intensity of the phagocytic reaction also was observed in blood flowing from the lungs, relative to the number of microbial cells ingested by leukocytes, but not relative to the percentage of phagocytic leukocytes; the number of microbial cells ingested by one leukocyte from 100 leukocytes counted was increased from 3-10.6 to 6.8-19.3, or on average from 6.25 to 10.15, i.e., by 1.6 times. Changes in CHLC for AFO generation by leukocytes during passage through SVB (Fig. 1) were unexpected. In eight of 11 dogs (72.8%) CHLC for AFO generation by leukocytes in blood flowing from the spleen was reduced compared with blood flowing into the spleen (or it was virtually unchanged) by 1.02-12.4 times ($p > 0.05$), or on average it was reduced by 4.1 times. The mean absolute value of CHLC for AFO generation by leukocytes at the exit from SVB was reduced in all 11 dogs, irrespective of changes in CHLC in individual animals, from 414.5 to 353.2 cps/leukocyte$\cdot 10^6$, i.e., by 1.17 times. The investigation showed that the percentage of neutrophils characterized by comparative high CHL activity in blood flowing into the lungs (in 11 of 15 dogs) (by 7.8-31%). Consequently, the rise of CHLC for AFO generation in blood flowing from the lungs was unconnected with any increase in the number of neutrophils in it. The results thus demonstrate on the whole that during passage of leukocytes through PVB, unlike through SVB, the intensity of their generation of active bactericidal forms of oxygen as a rule increases. Due to this phenomenon, it can be tentatively suggested, the lungs are able to take part in the formation of the free radical (oxygen and lipid) status and in phagocytic defense of the organism against infection.

LITERATURE CITED

1. World Health Organization, Clinical Immunology: Technical Publications Series No. 496 [in Russian], Moscow (1973), p. 28.
2. A. Kh. Kogan, N. I. Losev, A. N. Kudrin, and A. Miégombin, Byull. Éksp. Biol. Med., No. 5, 538 (1986).
3. A. N. Mayanskii and A. N. Galiulin, Reactivity of the Neutrophil [in Russian], Kazan' (1984), pp. 34-36.
4. M. N. Merzlyak and A. S. Sobolev, Biofizika, 5, 119 (1975).
5. R. C. Allen, R. L. Stjernholm, and R. H. Steel, Biochem. Biophys. Res. Commun., 47, 679 (1972).
6. R. C. Allen and L. D. Loose, Biochem. Biophys. Res. Commun., 69, 245 (1976).
7. C. D. Allred, J. Margetts, and H. R. Hill, Biochim. Biophys. Acta, 631, 380 (1980).
8. B. R. Andersen and A. M. Brendzel, J. Immunol. Meth., 19, 279 (1978).

9. H. Bielefeldt-Ohmann and L. A. Babiuk, Inflammation, 8, 251 (1984).
10. J. M. Goldstein, M. Cerqueira, S. Lind, and H. B. Kaplan, J. Clin. Invest., 59, 249 (1977).
11. T. D. Horan, D. English, and T. A. McPherson, Clin. Immunol. Immunopathol., 22, 259 (1982).
12. S. J. Klebanoff, Mononuclear Phagocytes: Functional Aspects, R. Van Furth (ed.), The Hague (1980), pp. 1105-1137.
13. F. Rossi, P. Bellavite, P. Dri, et al., Adv. Inflammat. Res., 1, 139 (1979).
14. P. Stevens, D. J. Winston, and K. Van Dyke, Infect. Immun., 22, 41 (1978).
15. L. S. Webb, B. B. Keele, and R. B. Johnston, Infect. Immun., 9, 1051 (1974).

INTERACTION BETWEEN SEROTONIN AND LIPID PEROXIDATION PRODUCTS DURING EXPERIMENTAL WOUND HEALING

V. V. Zakharov, L. A. Mamedov, S. A. Meshcheryakova, A. B. Shekhter, N. I. Kapitonov, E. N. Goncharenko, T. I. Gudz', Ch. R. Ragimov, and A. Yu. Agaev

UDC 616-001.4-003.9-092.9-07:[616.154:577.175.823]-074

KEY WORDS: serotonin; hydroperoxides; inflammatory process; wounds

Wound healing takes place in definite stages [6, 9]. The most complex changes (at tissue, cellular, and molecular levels) take place in stage I of wound healing, in the traumatic inflammation phase. The early manifestations of traumatic inflammation are due to the involvement of biogenic amines, monoamine oxidase (MAO), free oxygen radicals, and other biologically active substances [1, 5, 10, 11], which determine the abundance and intensity of this reaction and the possible development of a suppurative-inflammatory process [4].

The study of changes in biologically active substances and their inhibitors during wound healing may help with the discovery of ways of preventing wound infection and of stimulating repair processes. This investigation was devoted to the study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 220 male Wistar rats weighing 190-120 g. The experimental model consisted of full-thickness aseptic and infected skin wounds covering an area of 400 mm^2, created under sterile conditions by the method described previously [7]. Blood was taken from the femoral vessels of the rats under hexobarbital anesthesia.

Before the operation (initial data) and on the 1st-10th, 12th, and 15th days thereafter, the total serotonin concentration was determined in the blood by a fluorometric method [8]; concentrations of hydroperoxides were determined in the serum and tissues of the wound defect spectrophotometrically [12], and the total protein concentration was determined by the biuret method [16]. To determine the parameters 8-10 animals were used at each experimental point, and after sampling of the tissue, they were killed by decapitation. Samples of wound tissues at the same times were subjected to morphological examination (staining with hematoxylin and eosin, by Van Gieson's method, and with toluidine blue by Brachet's reaction, and various signs of inflammation and repair were evaluated, including the intensity of degranulation and lysis of mast cells (on a 5-point system).

Experiments to study the effect of an MAO inhibitor on wound healing were conducted in two series on 34 male Wistar rats with experimental linear wounds, produced by the method described previously [7], without observance of the rules of asepsis and antisepsis, and with

I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 6, pp. 690-693, June, 1989. Original article submitted June 23, 1988.