EXPERIMENTAL BIOLOGY

Free-Radical Status of Human Blood Leukocytes during Hyperbaric Exposure

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Submerging in a pressure chamber to a depth of up to 250 m was simulated in a 22-day experiment. The production of active oxygen forms in the blood leukocytes of divers was studied. The production of superoxide radical was 5-12 times decreased in the leukocytes stimulated with phorbol myristate acetate, which may be due to a 2- to 3-fold decrease in NADPH oxidase activity. A 5-fold increase in the ratio of luminol to lucigenin chemiluminescence indicates that production of active oxygen forms is shifted towards the forms other than superoxide. Increased incidence of infectious diseases in the divers after long deep submerging may result from disorders in primary nonspecific immunity in the presence of phagocytosing blood cells.

Key Words: *active oxygen forms; superoxide radical; leukocytes; hyperbaric exposure; oxygen-helium environment*

Study of the world ocean is impossible without deep diving. A diver is exposed to high environmental pressure. So far, the molecular and cellular mechanisms of hyperbaric effects on humans are unknown. The problems of the pathogenesis and therapy of aftereffects of short- and long-term deep diving are still to be solved. Toxic effect of oxygen is believed to be a pathogenetic factor of hyperbaric exposure. J. M. McCord and I. Fridovich in 1978 reported that the production of superoxide anion radical ($O_2^{\cdot-}$) — primary metabolite forming as a result of a single-electron reduction of oxygen by plasmalemma-bound NADPH oxidase — is increased in some forms of hyperbaric exposure [15]. High chemical aggression of active oxygen forms (AOF) and their contribution to the pathogenesis of various diseases are well known [4,5,13]. The role of AOF in clinical symptoms developing during diving and after it is still to be investigated.

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MATERIALS AND METHODS

Diving with the use of oxygen-helium medium to a depth of 250 m (2.5 MPa) in a pressure chamber was simulated for 22 days in accordance with the regime developed at the Institute of Biomedical Problems. Three healthy adult men aged 35-45 years acknowledged fit for diving after clinical examinations, participated in the study. Before the experiment they were subjected to pilot diving to a depth of 90-100 m. Blood was collected 1 day before the experiment, during it through a special lock from a depth of 10 m (0.1 MPa) on day 2, 100 m (1 MPa) on day 4, 200 m (2 MPa) on day 6, and 250 m (2.5 MPa) on day 10, and during decompression from the depth of 190 m (1.9 MPa) on day 13, 85 m (0.85 MPa) on day 17, and 12 m (0.12 MPa) on day 21, and immediately after lifting to the surface on day 22.

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Partial oxygen pressure in the pressure chamber varied from 0.021 to 0.05 MPa. Ten milliliters of venous blood with heparin (10 U/ml) was collected in each case. Blood decompression was carried out by the method of I. A. Voitsekhovich. The duration of blood decompression from the depth of 250 m was no more than 4 h. Under such conditions, neither hemolysis, nor decrease in blood cell viability occurred even at the maximum depths. Leukocyte mass was isolated by conventional method [11]. The cells were washed by double centrifugation in Hanks' solution (pH 7.4) without phenol red and stored on ice. Dead cells were counted after staining with 0.1% trypan blue. Cell composition was assessed by staining the smears by the method of Nocht. AOF production was evaluated by luminol- and lucigenin-dependent chemiluminescence (CL). Leukocyte CL was measured at 37°C in a PKhL-1 chemiluminometer (Russia) using glass cuvettes. Spontaneous CL of 10^6 cells with 10^{-4} M luminol or lucigenin in 1 ml of Hanks' solution was measured for 3-5 min. Then phorbol myristate acetate (PMA), an activator of CL (respiratory burst), in a concentration of 10^{-6} M was added, and the maximum intensity of CL flash was measured. The intensity of CL was estimated as the difference between the maximum intensity of PMA-activated and spontaneous CL. The activity of NADPH oxidase was assessed from the superoxide dismutase-sensitive reduction of cytochrome *c* by measuring light absorbance at 550 nm in an LKB Ultraspec-4050 spectrophotometer. A standard 1-ml specimen contained 50 μM cytochrome *c* and 10^6 cells in Hanks' solution without phenol red. The cells were activated with 10^{-6} M PMA. Then 2 mM sodium azide, 0.4% Triton X-100, and NADPH in concentrations 0.05-0.4 mM were added into the cuvette. The rate of cytochrome *c* reduction (activity of NADPH oxidase) was measured. The reference cuvette contained 45 μg superoxide dismutase [10].

RESULTS

We believed that some immunodepressive and allergic states developing in divers after prolonged deep diving and leading to reduction in the organism resistance and increase in the incidence of infectious diseases [3] are caused by disorders in primary nonspecific immunity at the level of blood phagocytes. The capacity of phagocytes to prime a normal immune response is related to their ability to produce AOF [5,6,13]. Inadequate production of AOF deteriorates the cytotoxic function of phagocytes. The production of prostaglandins, leukotrienes, and cytokines is decreased, there is no normal immune response, and immunodeficiency is forming [12-14]. Excessive production of AOF causes damage to proteins and nucleic acids, triggers the lipid peroxidation processes, which, in turn, can lead to cell damage, autoimmune states, allergic sensitization, alteration of the intercellular substance, and maintenance of chronic inflammation [4,13]. Cell composition of the leukocyte mass varied during the experiment: for lymphocytes it was 40±6%, polymorphonuclear leukocytes 54±7%, and monocytes 6±2%. There were 98±2% live cells in cell suspension. $O_2^{\cdot-}$ is formed in neutrophils and monocytes [5,9]. PMA-activated lymphocytes produce no AOF [16]. Lucigenin selectively reacts mainly with $O_2^{\cdot-}$ [8,14]. In a leukocyte suspension, the true oxidants of luminol are superoxide and hydroxyl radicals, hydrogen peroxide, singlet oxygen, and hypochlorions [7,9,13]. Phagocyte capacity to produce $O_2^{\cdot-}$ during diving decreases 7-fold *vs.* the norm as the depth is approximating 250 m (2.5 MPa). With the

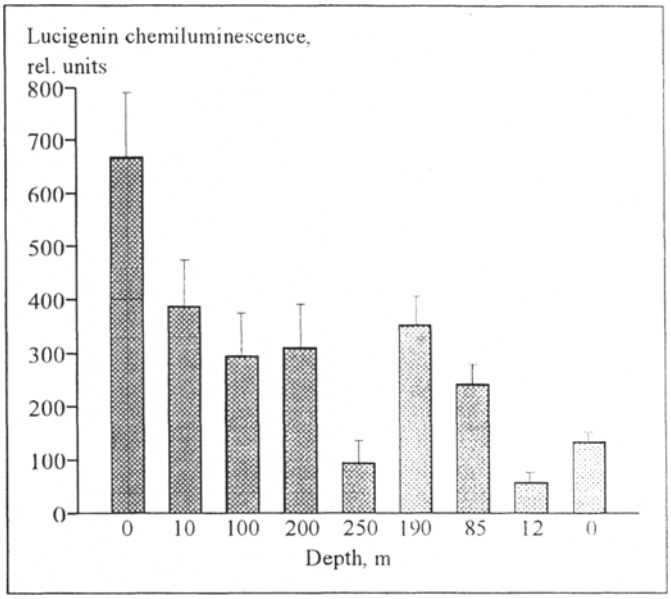

Fig. 1. Changed lucigenin chemiluminescence of blood leukocytes stimulated with phorbol myristate acetate during simulation of diving to a depth of 250 m (2.5 MPa) in oxygen-helium medium.

TABLE 1. Changes in the Activity of NADPH Oxidase in Blood Leukocytes during Simulated Diving to a Depth of 250 Meters in Oxygen-Helium Atmosphere (*M±m*)

Parameter	Norm	Sinking, m			
	0 m	100	250	12	0
Activity of NADPH oxidase, nmol $O_2^{\cdot-}$/min/10^6 cells	0.8±0.05	0.15±0.05	0.24±0.06	0.26±0.03	0.35±0.14

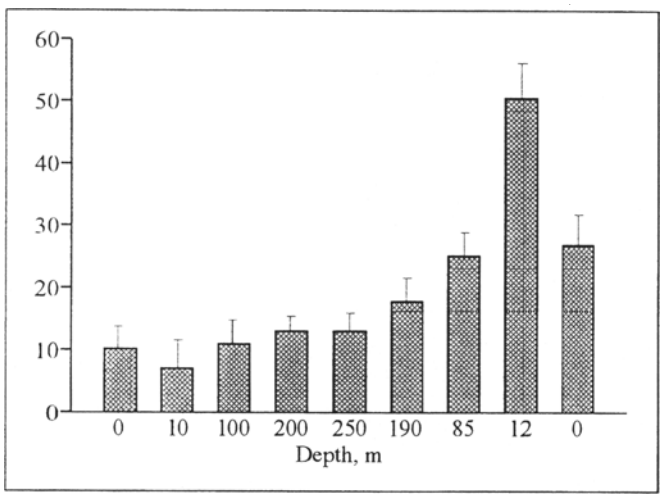

Fig. 2. Changed ratio of luminol to lucigenin chemiluminescence of blood leukocytes stimulated with phorbol myristate acetate during simulation of diving to a depth of 250 m (2.5 MPa) in oxygen-helium medium. Ordinate: ratio of luminol to lucigenin chemiluminescence.

beginning of decompression, the intensity of formation of radicals somewhat increases at depths of 190 m (1.9 MPa) and 85 m (0.85 MPa), but this process does not change general tendency toward a decrease in $O_2^{\cdot-}$ production in response to PMA over the entire period of hyperbaric exposure. On the depth of 12 m (0.12 MPa) this value is almost 12-fold lower and does not normalize even after the divers are lifted, remaining decreased 5-fold (Fig. 1). The low activity of NADPH oxidase can be one of the causes responsible for decreased production of $O_2^{\cdot-}$. Hyperbaric exposure in oxygen-helium medium changes physicochemical characteristics of biological membranes [2], which modifies the activities of all membrane-bound enzymes, including the plasma membrane NADPH oxidase. The activity of NADPH oxidase is decreased 3-fold on a depth of 250 m (2.5 MPa) and does not normalize even after decompression, remaining 50% decreased (Table 1). Changed AOF spectrum toward the forms other than superoxide at the expense of activation of enzymatic systems capable of producing other forms of active oxygen metabolites can be another cause. The ratio of luminol to lucigenin CL intensities shown in Fig. 2 demonstrates the ratio of total AOF production to production of $O_2^{\cdot-}$ alone. This value remains normal (10.1±3.5) over the entire period of diving (compression) and changes only during decompression, reaching the maximum by the 12th hour. This ratio did not normalize even after the volunteers quitted the pressure chamber. A fivefold increase of the ratio of luminol to lucigenin CL in the course of decompression means that the proportion of $O_2^{\cdot-}$ decreases and the proportion of other radicals increases. Leukocytes more actively produce AOF other than $O_2^{\cdot-}$ (hydroxyl radicals, haloid derivatives, or arachidonic acid derivatives). These AOF, particularly hydroxyl radicals, are more aggressive than $O_2^{\cdot-}$ and rapidly react with the adjacent molecules, modulating the normal immune response. The luminol to lucigenin CL ratio changes at the beginning of decompression and reaches the maximum at the end of it, at a time when the manifestations of decompression disease is the most probable [1]. Therefore, changed luminol to lucigenin CL ratio may be due to cell reaction to gas bubbles emerging during decompression. Fluctuations of oxygen concentration in pressure chamber can be another cause of changed AOF production. We have calculated empirical coefficients for correlations between changed partial oxygen pressure and changes in lucigenin CL (r=-0.1) and luminol CL (r=-0.3), the ratio of luminol to lucigenin CL being r=0.1. These data suggest that AOF production is not influenced by partial oxygen pressure in the chamber, but by other factors of hyperbaric exposure. Drugs correcting the free-radical status of the organism will probably improve the condition of individuals exposed to hyperbaric conditions for a long time. This methodological approach is prospective in choosing and assessing the most safe decompression regimens during test and experimental diving, for assessing the individual sensitivity of divers to decompression disease and their fitness for work.

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