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ACTIVATION OF FREE-RADICAL REACTIONS AND CHANGES IN STATE OF THE ANTIOXIDATIVE PROTECTION SYSTEM IN THE BLOOD IN EXPERIMENTAL INFLUENZAL TOXICOINFECTION

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According to existing views acute virus infections are accompanied by profound changes of metabolism in the tissues [1, 2, 7], which can lead to activation of free-radical reactions [1], to accumulation of free-radical oxidation products ($O_2^{\cdot -}$, $HO^{\cdot}$, H_2O_2, nitric oxide, lipoperoxides, etc.) [1, 3], to a decrease in the content of natural antioxidants, to inhibition of enzymes of antioxidative protection, and to a change in the redox state and levels of hemoproteins and metallocomplexes (hemoglobin, cytochrome P-450, ceruloplasmin, transferrin, etc.), which may ultimately complicate the inflammatory processes and cause the development of hypoxia and toxicosis.

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The aim of this investigation was to estimate the intensity of free-radical reactions, concentrations of methemoglobin, transferrin, and $NO^{\cdot}$, and also the efficacy of the antioxidative protection system of the blood, which includes superoxide dismutase (SOD), catalase, ceruloplasmin (CP), and α-tocopherol, in the toxicosis which accompanied acute experimental influenzal infection.

EXPERIMENTAL METHOD

Altogether three series of experiments were conducted on male CBA mice. There were three groups of animals in each series (5-10 animals in each group): 1) control, 2) animals infected with an apathogenic virus, 3) animals infected with a pathogenic virus.

Influenza A/Victoria/35/72 (H3N2) virus, adapted (pathogenicity 4.2 log LD_{50}) and not adapted (apathogenic) to mice, were used and were applied in an equal dose. The peak of viral production in the lungs after infection with the pathogenic strain was observed after 48 h, and amounted to 8.3 log IED_{50}. Death of the animals was observed on the 6th day after infection.

Erythrocytes were isolated and blood samples prepared for EPR-spectroscopy after decapitation of the animals (on the 1st-6th day after infection). The erythrocytes were isolated in physiological saline in the presence of heparin. The blood samples were frozen in liquid solution.

The level of free-radical reactions and accumulation of $NO^{\cdot}$ in the blood were judged on the basis of the amplitude of the EPR signal in the region g = 2.00 and 2.041. The signal with a resolved triplet structure at g = 2.041 is characteristic of the mononitrosyl complex $NO^{\cdot}$ with endogenous Fe^{2+} in the presence of diethyl thiocarbamate (DETC) [2]. DETC was injected into the animals in the same way as in [2]. Spectra of the blood samples were recorded at 77°K and the power of the VHF radiation was 0.1 mW. The concentrations of CP, transferrin, and methemoglobin in the blood were estimated from the intensity of the EPR signals g = 2.05, 4.3, and 6.0 respectively. The spectra were recorded with VHF in a power of not more than 20 mW.

Lipid extracts were obtained, the concentration of lipid phosphorus determined, and concentrations of fluorescent lipid peroxidation (LPO) products in the erythrocytes were recorded as in [1]. The content of α-tocopherol in the erythrocytes was monitored by fluorescence analysis after preliminary isolation of the α-tocopherol from the lipid extract by thin-layer chromatography. Fluorescence spectra were recorded with excitation at 295 nm and emission at 320 nm (solvent – hexane), on a "Hitachi MPF-2A" spectrofluorometer (Japan).

Lysis of the erythrocytes was carried out in distilled water (1:1 by volume). The hemolysate was frozen and thawed three times. Activity of Cu,Zn-SOD was monitored polarographically with a mercury drop electrode [4].

The level of catalase activity was monitored polarographically, using a closed platinum electrode [6]. The volume of the electrolytic cell was 1.3 ml. A 50-mM phosphate buffer, pH 7.4, containing 1 mM EDTA and 0.9 mM H_2O_2, and hemolysate containing about $8 \cdot 10^{-5}$ g hemoglobin (Hb) were used in the experiments. The Hb concentration in the hemolysate was determined by the hemocyanide method.

The experimental data are presented with twice the standard error, and with a level of significance of $p < 0.05$.

EXPERIMENTAL RESULTS

The acute infectious process caused by the pathogenic strain of virus was accompanied by activation of free-radical reactions, expressed as almost doubling (on the 5th day after infection) of the intensity of the EPR signal g = 2.00 in the blood samples and accumulation of fluorescent LPO products in the erythrocytes (Fig. 1). Correspondingly, in cases of pathology, an increased concentration of CP (Fig. 2), possessing superoxide dismutase activity [8], was observed in the blood, in which it could be recorded until the 5th day after infection of the animals. On the 5th-6th days of development of the toxicoinfection a characteristic EPR spectrum due to superposition of signals of CP and nitrosyl complexes of heme iron (g = 2.03), was recorded in the region of the CP signal g = 2.05, and was evidently caused by $NO^{\cdot}$ generation by endothelial cells of the vessels in response to the development of hypoxia. Accumulation of $NO^{\cdot}$ in the blood of the animals with pathology was recorded by the use of an $NO^{\cdot}$ trap, namely DETC, starting from 4 days after infection (Fig. 1).

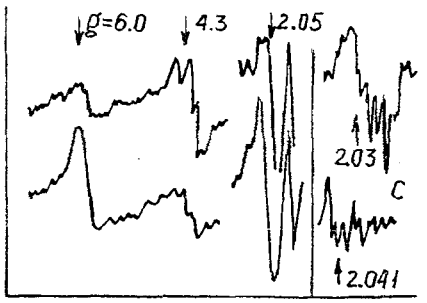

Fig. 1

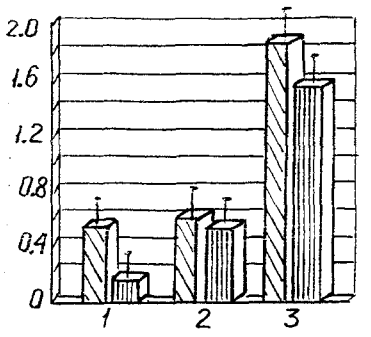

Fig. 2

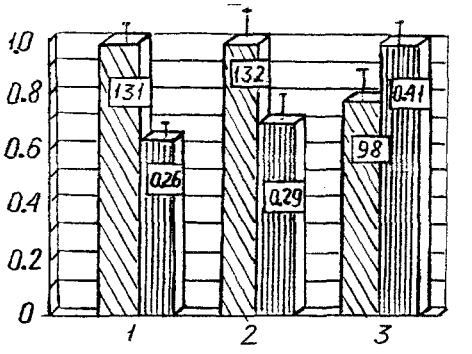

Fig. 3

Fig. 1. EPR spectra of blood samples from animals infected with influenza virus: a) apathogenic virus, b) pathogenic virus: g = 2.05 – signal of CP, 4.3 – signal of transferrin, 6.0 – signal of methemoglobin, C) EPR spectra of nitrosyl complexes of heme iron (g = 2.03) and mononitrosyl complexes of iron with DETC (g = 2.041) in blood of animals infected with pathogenic influenza virus.

Fig. 2. Concentrations of fluorescent LPO products (oblique shading) and ratio of EPR signals (vertical shading) of ceruloplasmin and transferrin in animals' blood. 1) Control, 2) infection by apathogenic virus, 3) infection by pathogenic virus.

Fig. 3. SOD (vertical) and catalase (oblique) activity in mouse eythrocytes. Legend as to Fig. 2. Numbers represent absolute values of activity (in sec^{-1}/g Hb).

Changes in metabolism in the tissues in the pathogenic form of influenzal infection were accompanied by intensification of iron metabolism, leading to a decrease in the blood transferrin concentration. The effect of the fall of transferrin during pathology was greatest on the 5th day (Fig. 1). Correspondingly, there was a marked increase in the ratio of the signals of CP and transferrin (Fig. 2).

Activation of LPO in their erythrocytes was accompanied by an increase in SOD activity from 0.27 ± 0.02 to 0.40 ± 0.06 mg/g Hb. In the case of infection with the apathogenic virus, SOD activity was 0.28 ± 0.05 mg/g Hb. Reduction of the superoxide radical ($O_2^{\cdot -}$) under the influence of SOD is known to be accompanied by hydrogen peroxide production. Inactivation of H_2O_2 in erythrocytes is performed by catalase and glutathione peroxidase, but if activity of these enzymes is insufficient, Hb, which also possesses catalase activity, is involved in the redox process [5, 9].

The results of these experiments showed (Fig. 3) that the toxic form of infection was accompanied by lowering of the catalase activity (98 ± 12 sec^{-1}/g Hb) in the erythrocytes compared with the corresponding activity of the enzyme in animals infected with the apathogenic virus (133 ± 14 sec^{-1}/g Hb). Catalase activity in the erythrocytes of animals of the control group was 131 ± 9 sec^{-1}/g Hb.

Against the background of activation of free-radical oxidation, increased SOD activity, and weakened catalase activity, an increase in the intensity of the EPR signal g = 6.0 (Fig. 1), characteristic of the Fe^{3+} of methemoglobin, was observed, this evidently accumulates during oxidation of Fe^{2+} of hemoglobin through the action of the H_2O_2 thus formed. The methemoglobin concentration in the blood of animals infected with the apathogenic virus remained at the control level. Similar changes in the blood methemoglobin level in influenza also were found in our clinical studies [3].

Activation of LPO in erythrocytes in the toxic form of influenza led to a fall in the α-tocopherol concentration in the membranes. This parameter amounted to 0.11 ± 0.05 $\mu g/\mu g$ lipid phosphorus in pathology, whereas under the influence of the apathogenic virus the α-tocopherol concentration was 0.26 ± 0.02 $\mu g/\mu g$, and in the control 0.31 ± 0.04 $\mu g/\mu g$ lipid phosphorus. The fall in the α-tocopherol content in the erythrocyte membranes and methemoglobin accumulation indicates a change in the efficiency of the corresponding NADPH-dependent reductase systems in experimental influenzal toxicosis.

The general conclusion can be drawn from these results that activation of free-radical reactions, an increase in the content of active forms of oxygen, of $NO^{\cdot}$, and of peroxidation products of lipids and proteins, may be among the causes of toxicosis and the development of hypoxia in the pathogenesis of acute influenzal infection.

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