CHANGES IN SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE ACTIVITY DURING INTENSIFICATION OF LIPID PEROXIDATION IN ISCHEMIA OF THE LIVER

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UDC 616.36-005.4-07 [616.36-008.939.15: 616.36-008.931]-074

Activation of free radical oxidation of lipids is known to play an important role in the onset of pathological changes associated with disturbances of the oxygen balance: hyperoxia and hypoxia. In hyperoxia, when an excess of oxygen enters the body, the fact that lipid peroxidation (LPO) is intensified can easily be explained. In hypoxia, however, and in particular in ischemia, against the background of a sharp decline in the partial pressure of oxygen, this phenomenon seems paradoxical.

However, the intensity of LPO processes is determined, not by the concentration of oxygen in the cell, but by other factors. Destruction of active forms of oxygen initiating LPO (superoxide anion-radicals) is brought about by the enzyme superoxide dismutase (SOD) [11]. Inactivation of free radicals and breakdown of lipoperoxides formed during LPO take place with the aid of biological antioxidants and glutathione peroxidase (GLP), respectively [3, 10].

Activation of LPO during ischemia of the liver, kidney, myocardium, and skeletal muscles, expressed as a rise in the level of lipid hydroperoxides and a fall in the antioxidant activity (AOA) of lipids, was demonstrated in [1, 2, 9]. In the present writers' view, under ischemic conditions the systems maintaining a constant low level of peroxidation reactions in the intact cell are distributed.

The object of the present investigation was accordingly to study changes in SOD and GLP activity and also in the AOA level of lipids and the content of lipid hydroperoxides during the development of ischemic liver damage.

EXPERIMENTAL METHOD

Experiments were carried out on 120 August rats. Ischemia was induced by application of a clamp to the vascular pedicle of a hepatic lobule for 15, 30, 60, 90, and 120 min, under intraperitoneal hexobarbital anesthesia. SOD activity in samples of the cytoplasm (supernatant after centrifugation at 6,000,000g for 1 min) of the ischemized liver was determined by inhibition of reduction of nitro-BT in a xanthine oxidase system on a "Leitz" spectrophotometer with automatic writer [8]. GLP activity in the same samples was determined from oxidation of NADPH in a conjugated glutathione reductase system, using tert-butyl hydroperoxide by the method in [12] in the writers' modification [4]. Lipids were extracted from liver homogenate by Folch's method. The AOA level of the lipids was judged from inhibition of oxidation of methyl oleate in a model system [3]. The quantity of lipid hydroperoxides was determined by iodometric titration [5]. The protein content in the samples was measured by Lowry's method.

EXPERIMENTAL RESULTS

As Fig. 1 shows, intensification of LPO took place in the earliest stages of ischemic liver damage. The content of lipid hydroperoxides in the homogenate increased 13-fold after 50 min of ischemia, after which it fell a little, and after 2 h of ischemia it was 6 times higher than initially. The level of AOA of the lipids in the same samples fell steadily throughout the period of ischemia studied (until 2 h), but it differed significantly from AOA of lipids of the intact liver only after 1 h of ischemia. The increase in the intracellular content of lipid peroxides in ischemia thus took place before the fall in the antioxidant level. Although

KEY WORDS: ischemia of the liver; peroxidation of lipids; superoxide dismutase; glutathione peroxidase.

Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 451-453, April, 1981. Original article submitted September 11, 1980.

 0007-4888/81/9104-0488$07.50

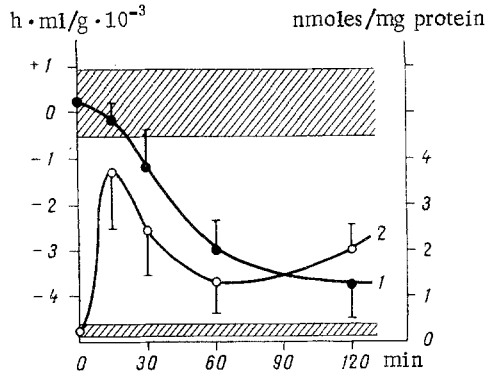

Fig. 1

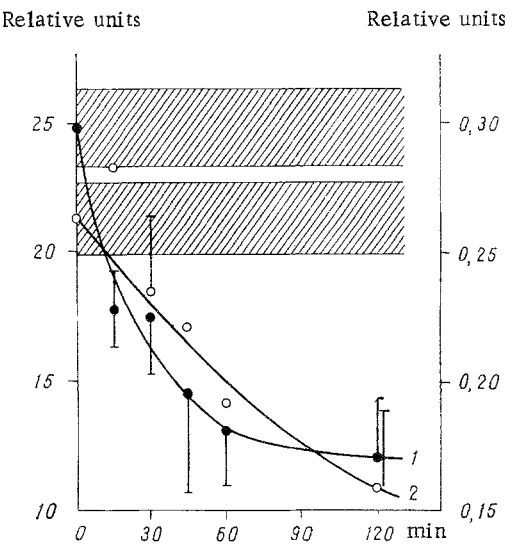

Fig. 2

Fig. 1. Changes in AOA of lipids (1) and content of lipid hydroperoxides (2) in ischemized rat liver. Abscissa, time (in min); ordinate: on left — AOA, on right — concentration of hydroperoxides.

Fig. 2. Change in SOD (1) and GLP (2) activity in ischemized rat liver. Abscissa, time (in min); ordinate: on left — activity of SOD, right — activity of GLP.

the lowered level of AOA of lipids undoubtedly facilitated the intensification of LPO, it is unlikely that under the conditions of this particular pathology it was the cause of this intensification.

Data in the literature show that the SOD level in the cell is closely linked with the partial pressure of oxygen. Under conditions of hyperoxia this enzyme is activated [6]. In hypoxia ("raising" animals in a pressure chamber) a decrease in SOD activity has been found [7].

In the present experiments SOD activity fell sharply during the development of ischemia, to reach values only half as high as in the control 2 h after the blood flow to the organ was stopped (Fig. 2). Maximal changes in enzyme activity were detected in the first 30 min of ischemia. Changes in GLP activity were similar, but its fall was greater — by 1.7 times compared with the control (Fig. 2). The temporal characteristics of the fall in activity of the "protective" enzymes and accumulation of lipid hydroperoxides thus coincide.

The results thus show that the sharp intensification of lipid peroxidation during the development of ischemic liver damage may be due to an upsetting of the balance or injury to the systems responsible for maintenance of LPO reactions in the cell at a low, stationary level.

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COMPUTER CLASSIFICATION OF MELTING PROFILES OF INTERPHASE CHROMATIN FROM HUMAN LYMPHOCYTES

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UDC 612.112.94.014.24.001.33

It was shown previously by the method of luminescence fluorometry with acridine orange labeling, in the writers' suggested [1] modification of the method of thermal denaturation of cell DNP [4], that the structure of the interphase chromatin in cells of patients with Down's syndrome has specific differences from that of identical healthy human cells [2, 3]. In melting profiles or temperature-dependent structural transitions of interphase chromatin of healthy human lymphocytes polymorphism was found.

The object of the present investigation was to study the structure of the chromatin complex of healthy human lymphocytes in interphase.

EXPERIMENTAL METHOD

The structure of the interphase chromatin of peripheral blood lymphocytes of 164 healthy persons (98 men and 66 women) aged from 20 to 45 years was studied by comparative analysis of the melting curves or temperature-dependent structural transitions in intracellular DNP. Readings were taken at intervals of 1-2°C. Changes in the structure of chromatin during heating were recorded as the quantity of luminescent label (acridine orange, AO) bound. The investigations were conducted on short-term cell cultures, i.e., on cells incubated for 1 h in Eagle's nutrient medium with the addition of 10% autologous serum.

The intensity of luminescence of AO bound with DNA of lymphocyte chromatin was measured on the MSP-0.5 microscope-photometer (from Opton). Luminescence was excited by light with λ = 365 nm and recorded at λ = 530 nm with the aid of an appropriate interference filter. To determine the degree of orderliness of DNA in the cell, or to determine the coefficient $\alpha = F_{640}/F_{530}$, parallel recordings were made of changes in the intensity of luminescence in the region λ = 640 nm. However, since the shape of the curve for α was practically independent of F_{640}, which was only 10-15% above the background level, data are given in this paper only for the change in F_{530}. The apparatus, details of the experiments, and method of isolation and culture of the lymphocytes were described previously [1].

The data on the intensity of fluorescence (F_{530}) of the intracellular DNP—AO complex within the temperature range 20-100°C were analyzed on a Sperry Univac 90-30-B computer, by means of a specially written program (by E. B. Voronov, All-Union Computer Center). The initial data for this program were numerical values of F_{530} of the DNP—AO complex at 24 points corresponding to temperatures of 20, 35, 40, 42, 45, 47, 50, 55, 60, 65, 76, 70, 75, 77, 78, 80, 82, 85, 87, 88, 89, 90, 92, and 95°C. Each curve was analyzed for the presence of a maximum in the vicinity of points corresponding to 45, 65, 78, 88, and 92°C. These neighborhoods were as follows: [42, 47], [60, 67], [77, 78], [85, 85], [90, 92]. Characteristics of the presence or otherwise of a maximum were established on the recorded curve for each of these neighborhoods: M) maximum present; N) no maximum present; V) indefinite (absence of data). For this purpose, the presence of a maximum was first analyzed at each point of the neighborhood separately. The analysis was carried out as follows: values of the curve at the point of analysis and two neighboring points were examined. If the value of the curve at the point

KEY WORDS: lymphocytes; chromatin; thermal denaturation; fluorescence microscopy.

Institute of Medical Genetics, Academy of Medical Sciences of the USSR. All-Union Computer Center, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 453-455, April, 1981. Original article submitted July 2, 1980.

 0007-4888/81/9104-0490$07.50