EFFECT OF CHLORGYLINE ON INTENSITY OF LIPID PEROXIDATION AND STABILITY OF ERYTHROCYTE MEMBRANES IN HYPEROXIA

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UDC 612.273.1

KEY WORDS: hyperoxia; chlorgyline; lipid peroxidation

Activation of lipid peroxidation (LPO), which leads to changes in the state of membrane structures and to modification of the properties of membrane enzymes, is a very important triggering mechanism in the development of oxygen poisoning during exposure to hyperbaric oxygen. The writers showed previously that hyperoxia is characterized by changes in activity and substrate specificity of type A mitochondria monoamine oxidase (MAO) [3]. Chlorgyline [N-(2,4-dichlorophenoxy)propyl-N-methyl-2-propinylamine·HCl], a specific inhibitor of type A MAO, has a protective action in hyperoxia, for it delays the onset of oxygen convulsions in animals and increases their survival rate [12].

In the investigation described below the antioxidant properties of chlorgyline and its effect of membrane stability in hyperoxia were investigated. The effect of chlorgyline on the intensity of LPO was studied by determining concentrations of primary and end products of LPO (diene conjugates and Schiff bases, respectively) in the brain and blood plasma and stability of erythrocyte membranes. The results of determination of serum levels of extraerythrocytic hemoglobin (EEHb), total peroxidase activity (TPA), and glucose-6-phosphate dehydrogenase (G6PDH) activity were used as indicators of stability of the erythrocyte membranes.

EXPERIMENTAL METHOD

Adult noninbred male rats weighing 150-180 g were used. Chlorgyline was injected intraperitoneally in a dose of 5 mg/kg, and type A MAO activity was inhibited under these circumstances by 87%. The effect of oxygen under a pressure of 0.7 MPa on the animals were investigated. An intact animal and one receiving chlorgyline 30 min before the session of hyperoxia were placed simultaneously in the pressure chamber. The experiment ended when the intact animals began to have convulsions, on average 30 min after the beginning of exposure. Rats protected with chlorgyline did not develop convulsions. In a separate series of experiments the animals were kept in the pressure chamber until rats protected with chlorgyline developed convulsions, on average after 63 min. Intact animals and animals 60 min after injection of chlorgyline served as the controls. Lipids were isolated from brain and blood plasma by the method in [10]. Concentrations of primary LPO products (diene conjugates) were determined by the method in [9]. Concentrations of Schiff bases were estimated from the intensity of fluorescence of the lipid extract at 440 nm, with an excitation wavelength of 360 nm [11]. A solution of quinine sulfate in 0.1 N H_2SO_4 (1 mg/ml) was used as the standard. Fluorescence spectra were recorded on a Hitachi 650-60 spectrofluorometer (Japan). Total lipids were determined by the phosphovanillin method [7], EEHb by the method in [6], PTA as in [8], G6PDH activity as in [4], and protein by a modified Lowry's method [14]. The chlorgyline (May and Baker, England) was generously provided by Dr. D. Sket (Ljubljana University, Yugoslavia).

EXPERIMENTAL RESULTS

In the convulsive phase of oxygen poisoning the brain level of diene conjugates was raised by 200% and of Schiff bases by 210% (Fig. 1). Elevation of the plasma levels of diene conjugates and Schiff bases amounted to 270 and 230%, respectively. Injection of chlorgyline into the animals sharply reduced the intensity of LPO in the brain and blood

Biological Research Institute, M. A. Suslov Rostov University. Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 1, pp. 54-56, January, 1987. Original article submitted April 14, 1986.

 0007-4888/87/0103-0062$12.50

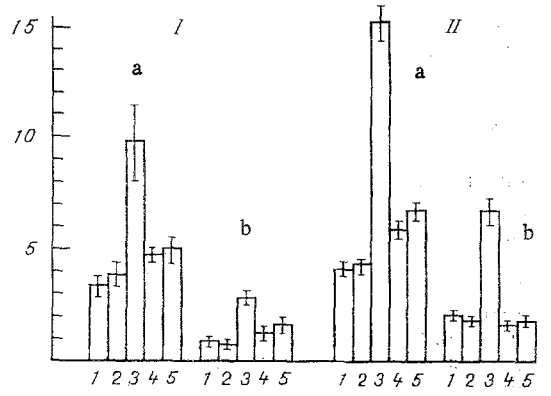

Fig. 1

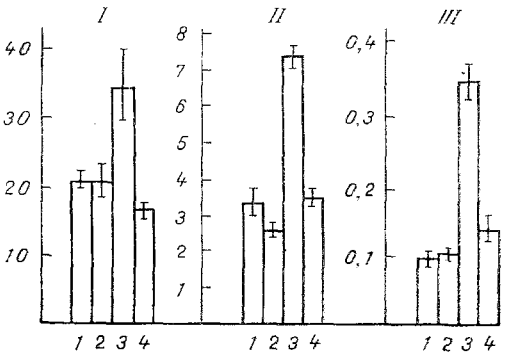

Fig. 2

Fig. 1. Effect of chlorgyline on brain (I) and plasma (II) levels of diene conjugates (a) and Schiff bases (b) under normal and hyperoxic conditions. Ordinate, Level of LPO products, of diene conjugates (in μmoles/mg lipid), and of Schiff bases (in relative units/mg lipid). 1) Control; 2) control + chlorgyline; 3) hyperoxia (0.7 MPa, convulsions); 4) chlorgyline + hyperoxia (0.7 MPa, 20-40 min; 5) chlorgyline + hyperoxia (0.7 MPa, 60-90 min).

Fig. 2. Effect of chlorgyline on EEHb (I) and TPA (II) concentrations and G6PDH activity (III) in blood serum under normal and hyperoxic conditions. Ordinate: I) EEHb concentration (in mg%); II) TPA (in relative units/ml); III) G6PDH activity (in nmoles $NADPH_2$/mg protein/min). 1) Control; 2) control + chlorgyline; 3) hyperoxia (0.7 MPa, convulsions); 4) chlorgyline + hyperoxia (0.7 MPa, 20-40 min).

compared with that in unprotected animals. The brain level of diene conjugates in animals protected with chlorgyline was only 45% higher, and the plasma level only 42% higher than in the controls. Brain and plasma levels of Schiff bases were indistinguishable from those in intact animals Brain and plasma levels of primary and end products of LPO in animals protected with chlorgyline, for whom the session of hyperoxia continued until they developed convulsions or until an agonal type of breathing appeared, were the same as in protected animals tested at the time of appearance of convulsions in the control rats. This indicates that the antioxidant effect of chlorgyline is connected with its direct influence on LPO, and not with any improvement in the animals' general condition.

Evidence of a direct effect of chlorgyline on the LPO system was given by data obtained by the writers previously [2]. Addition of chlorgyline (15-75 μg/ml) to brain homogenate depressed the formation of malonic dialdehyde, an end product of LPO, by 25-34% in the course of incubation for 30 min.

Activation of LPO increases membrane permeability. The convulsive phase of oxygen poisioning was characterized by labilization of the erythrocyte membranes, as reflected in elevation of serum levels of EEHb by 60%, of PTA by 120%, and of G6PDH activity by 250% (Fig. 2). This agrees with results obtained in the writers' laboratory previously [1]. The EEHb level is entirely determined by erythrocyte membrane permeability. An increase in the EEHb concentration is the principal, but not the only, cause of the increase of TPA. A definite contribution to elevation of the serum TPA level and G6PDH activity may perhaps be made by the outflow of myeloperoxidase and G6PDH, respectively, from leukocytes, whose permeability must evidently also be increased during hyperoxia.

Injection of chlorgyline into intact animals did not affect EEHb and TPA levels or G6PDH activity. If chlorgyline was injected into the animals immediately before they were placed in the pressure chamber, it prevented destabilization of the blood cell membrane structures. In animals protected by chloryline, none of the parameters studied (EEHb, TPA, G6PDH activity) differed from those in intact animals.

The results are evidence that chlorgyline has a marked antioxidant effect in hyperoxia, one result of which is to stabilize membrane structures. The ability of chlorgyline to inhibit LPO may perhaps be connected with the fact that, by blocking the catalytic centers of

type A MAO, it prevents qualitative changes (transformation) of the enzyme in hyperoxia. According to data in the literature [5], if catalase efficiency is present, marked stimulation of LPO takes place in the source of reactions catalyzed by transformed MAO. Chlorgyline, by preventing transformation of type A MAO, thereby exerts an antioxidant effect. Inhibition of MAO also reduces the formation of hydrogen peroxide, which intensifies peroxidation [13-15]. In view of the leading role of activation of LPO in the mechanism of oxygen poisoning, it can be tentatively suggested that the protective action of chlorgyline in hyperoxia is based on its antioxidant effect.

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HIGH INTERINDIVIDUAL RESTRICTION FRAGMENT LENGTH AND COPY NUMBER OF POLYMORPHISM OF A TVRI FAMILY IN MODERATE HUMAN DNA REPEATS

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UDC 612.6.052.014.24:577.212.3

KEY WORDS: interindividual polymorphism; structure of genome; molecular marker

Studies of genome structure have revealed the existence of genetic elements with an increased frequency of aberrations in somatic or sex cells in some living organisms [1]. In man, these genetic elements may be families of multi-copy, dispersed DNA repeats [5-7]. However, it is impossible to establish the presence of essential interindividual polymorphism for distribution in the genome for the members of these DNA families, due to some extent to technical difficulties, caused by the high repetitiveness, but, at the same time, the dispersed distribution of these DNA families in the human genome.

An attempt was accordingly made to isolate other classes of sequences — with low or moderate copy numbers in the genome. In this paper we describe the selection of cloned human DNA sequences, with a copy number not exceeding 1000 copies per diploid genome, and their

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 1, pp. 57-58, January, 1987. Original article submitted May 19, 1986.

0007-4888/87/0103-0064$12.50