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INHIBITION OF LIPID PEROXIDATION BY ANTIOXIDANTS IN THE VITREOUS BODY DURING HEMORRHAGE

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Hemorrhage into the vitreous body both in man and in experimental animals causes intensification of lipid peroxidation (LPO) [1, 2, 5]. It has been shown that an important role in the mechanism of LPO activation during hemorrhage is played by ions of metals with variable valency, hemoglobin, and lipids, introduced by autologous blood into the vitreous body [1]. As has been observed previously [1, 3, 5], intensification of LPO may be responsible for the severity of the course of traumatic hemophthalmia. Consequently, inhibition of LPO during hemorrhage may promote a favorable course of hemophthalmia and may prevent structural and functional disturbances arising both in the vitreous body and in the retina.

For this reason it was decided to study the possibility of preventing activation of LPO by antioxidants in rabbits with experimental hemorrhage into the vitreous body.

EXPERIMENTAL METHOD

Experiments were carried out on 148 male gray Chinchilla rabbits weighing 3 kg kept under ordinary animal house conditions. To reproduce intraocular hemorrhage, autologous blood was taken from the auricular vein and injected in a volume of 0.2 ml into the vitreous body of the rabbit's eye. The operation was performed on the animal's right eye and the left eye was left intact. The rabbits were killed 3, 8, 12, 20, and 30 days after hemorrhage into the vitreous body. The state of LPO was judged by the malonic dialdehyde (MDA) level, determined by the reaction with 2-thiobarbituric acid [6]. The test substances: phenosan potassium (phenosan K) and hydroxypyridine hydrochloride (HPHC), and also superoxide dismutase (SOD) and sodium diethyldithiocarbamate (DDTC), forming a complex with copper, were injected in two ways: by the intravitreous and retrobulbar routes. Phenosan K was injected by the intravitreous route in a dose of 2 mg and HPHC in a dose of 10 mg, both dissolved in 0.3 ml of physiological saline (PS); SOD was injected in a dose of 6.1 U and DDTC in a dose of 1.5 mg in 0.1 ml PS. The antioxidants, dissolved in PS, were injected in a volume of 0.1

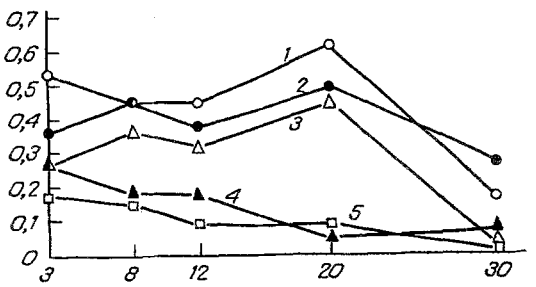

Fig. 1. Changes in MDA concentration in vitreous body after intravitreal injection of antioxidants and chelating agents. Abscissa, time of investigation (in days); ordinate, MDA level (in nmoles/mg protein). 1) 0.2 ml autologous blood; 2) HPHC; 3) phenosan K; 4) SOD by intravitreal and phenosan K by retrobulbar injection; 5) DDTC by intravitreal and phenosan K by retrobulbar injection.

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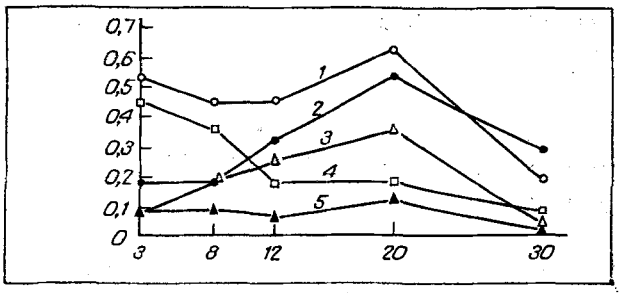

Fig. 2. Changes in MDA concentration in vitreous body after retrobulbar injection of antioxidants and chelating agents. 1) 0.2 ml autologous blood; 2) HPHC, 3) phenosan K; 4) SOD and phenosan K both by retrobulbar injection, 5) DDTC and phenosan K both by retrobulbar injection. **Remainder of** legend as to Fig. 1.

ml either together with autologous blood or separately, into the vitreous body. By the retrobulbar method of injection phenosan K and HPHC were used in a dose of 8 mg in 0.3 ml of PS, and DDTC in a dose of 1.5 mg in 0.2 ml of PS. The MDA level in the vitreous body with hemorrhage and without injection of antioxidants served as the control. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Injection of the antioxidants caused a marked fall in the level of LPO products in the vitreous body during hemorrhage (Figs. 1 and 2). After injection of phenosan K and HPHC, the MDA concentration was appreciably lower the whole time than in the control. These results are evidence that the effectiveness of antioxidants in inhibiting LPO depends on the mode of their administration. Phenosan K and HPHC were most effective by the retrobulbar route (Fig. 2). For instance, when phenosan K was injected by the retrobulbar route the MDA concentration on the 12th day of the experiment was less than half of that in the control, whereas after intravitreal injection it was 1.4 times less. A similar pattern was observed after injection of HPHC, although this was less effective than phenosan K. The difference in the kinetics of MDA accumulation after injection of antioxidants by the retrobulbar and intravitreal routes also is noteworthy. After intravitreal injection of phenosan K and HPHC the kinetics of MDA accumulation observed in experiments without antioxidants was repeated: the MDA level was high initially, then fell, and rose again to a maximum on the 20th day of the experiment. By contrast, when phenosan K and HPHC were injected by the retrobulbar route, the MDA concentration rose steadily but comparatively slowly until the 20th day of the experiment, and then fell. In the opinion of some workers, an important role in the intensification of LPO in experimental hemorrhage is played by the action of hemoglobin, of iron and copper ions, and lipids, introduced with the blood into the vitreous, and also by the action of radical oxygen intermediates, especially superoxide anion-radicals [6]. In view of these considerations, besides synthetic antioxidants other substances also were used in the present investigation: SOD, with a dismuting action on the superoxide anion-radical, and DDTC, which forms a complex with copper ions.

As was stated in the "Experimental Method" section SOD and DDTC were injected by the intravitreal and retrobulbar routes together with phenosan K. The results showed that a combination of phenosan K both with SOD and with DDTC caused a marked fall in the MDA level compared with that observed when phenosan K was injected without SOD or DDTC. The kinetics of the change in the MDA concentration after injection of DDTC and SOD differed from that both in the control and when HPHC and phenosan K were injected by the intravitreal and retrobulbar routes. After injection of SOD the MDA concentration fell steadily throughout the experiment. After intravitreal and retrobulbar injection of DDTC the MDA concentration fell until the 20th day of the experiment, then rose again, and fell sharply again until the 30th day. However, when DDTC was injected together with phenosan K the greatest fall in the rate of LPO was observed after their retrobulbar injection, but in the case of SOD, after intravitreal injection.

In experimental hemorrhage into the vitreous body, intensification of LPO may thus be weakened by administration of various antioxidants. Combined administration of antioxidants with chelating agents forming complexes with ions of metals of variable valency and SOD

increases their effectiveness appreciably in inhibiting free-radical oxidative processes in the vitreous body.

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ENDOGENOUS PROTEOLYSIS IN HUMAN ERYTHROCYTE MEMBRANE PREPARATIONS

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The problem of endogenous proteolytic activity in erythrocyte membranes has attracted the attention of many investigators engaged in the study of the structural and functional organization of cell membranes. This is because of the important role of various membrane-bound enzymes in the processes coordinating the formation of families of structurally interconnected membrane proteins and also in cell aging [8, 13]. Usually in the course of such investigations the need arises to determine the nature of activity discovered. This is a difficult task because of the high degree of contamination of erythrocyte membrane preparations by leukocytes, which may give rise to undesirable artefacts. The study of the protein components of human erythrocyte membranes also has a different aspect. In recent years extensive research has begun in a new field of biochemical genetics, known as "molecular anatomy," with the object of compiling a full catalog of erythrocyte membrane proteins on the basis of their mapping by two-dimensional electrophoresis [1, 9]. For and adequate and correct solution to many of the problems in this direction, the purity of the erythrocyte membrane preparations obtained and assessment of the degree of proteolytic degradation of the membrane proteins are of fundamental importance.

It was accordingly decided to study the effect of the method used to isolate human erythrocyte membranes on the endogenous proteolytic activity discovered in them and, at the same time, to study the degree of contamination of the preparations of erythrocyte "ghosts" by other forms of blood cells, primarily leukocytes. For this purpose, proteolytic degradation of erythrocyte membrane proteins under the influence of enzymes of both endogenous and leukocytic origin was investigated by electrophoretic methods, including the modified method of two-dimensional electrophoresis described in [11].

EXPERIMENTAL METHOD

Erythrocytes were obtained from 20 ml of heparinized blood by two methods. Method A: erythrocytes were separated from leukocytes and plasma by centrifugation 3 or 4 times at 1000g for 15 min. The white film of leukocytes was removed from the surface of the erythro-

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