POSSIBLE MECHANISMS OF ACTIVATION OF THE LIPID PEROXIDATION REACTION IN THE VITREOUS BODY AFTER HEMORRHAGE

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UDC 617.747-003.215-07:617.
747-008.939.15-39

KEY WORDS: hemorrhage; vitreous body; malonic dialdehyde; chemiluminescence; metals of variable valency; hemoglobin

It has been shown [3, 4] that when autologous blood escapes into the vitreous body of the eye, lipid peroxidation (LPO) is intensified. Analysis of metabolic reactions and physicochemical processes taking place in the vitreous body after hemorrhage show that the intensification of LPO can be attributed mainly to the action of hemoglobin. Meanwhile, during hemorrhage ions of metals of variable valency (iron and copper) which, according to some workers [1, 5], catalyze LPO to an appreciable degree, enter the vitreous body. However, in the few available publications describing the investigation of changes in the intensity of LPO in the vitreous body during hemorrhage, no attempt was made to examine the action of hemoglobin, of ions of metals of variable valency (iron and copper), or of an emulsion of phospholipids on the development of the LPO reaction. Yet the study of the character of LPO in the vitreous body under the influence of ions of metals of variable valency, hemoglobin, and lipids, or of autologous blood introduced into it, would explain some mechanisms of the intensification of LPO observed during hemorrhages of varied nature.

With these considerations in mind, it was decided to study the character of the LPO reactions under the influence of ions of metals of variable valency (Fe^{++} and Cu^{++}), of hemoglobin, and of phospholipid emulsion on the vitreous body.

EXPERIMENTAL METHOD

Experiments were carried out on gray Chinchilla rabbits and on the isolated vitreous body removed from the rabbit's eyes. Solutions of $FeSO_4$ (10^{-4}M), $CuSO_4$ (10^{-4}M), hemoglobin (24 mg/ml), emulsion of phospholipids (1.2 mg/ml), and autologous blood (0.5 ml) either were injected into the vitreous body of the animal or were added directly to the removed vitreous body in the model. After parenteral administration of the LPO catalyst, namely Fe^{++}, Cu^{++}, and hemoglobin, changes in the intensity of LPO were monitored for 50 days. The animals were killed at definite time intervals and the concentration of LPO products was determined in the isolated vitreous body. Changes in the intensity of LPO were judged from the intensity of chemiluminescence (CHL) and the malonic dialdehyde (MDA) level [5, 6].

EXPERIMENTAL RESULTS

The experiments showed that addition of $FeSO_4$ caused intensification of LPO in the vitreous body. The MDA concentration reached a maximum 7 days after addition of the iron.

These changes correlated with changes in the intensity of CHL (Fig. 1b).

The experiments showed that when copper sulfate was injected into the vitreous body the intensity of LPO also was increased: CHL was intensified and the MDA level raised. The kinetics of the change in the parameters studied in this case differed from that following injection of $FeSO_4$. In the course of 14 days the intensity of CHL and the MDA level fell throughout the rest of the experiment, and fell below the initial level after 50 days. Incidentally, the maximal MDA concentration and CHL intensity in the vitreous body were 1.5 times less than under the influence of Fe^{++}.

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 0007-4888/87/0005- 0646$12.50

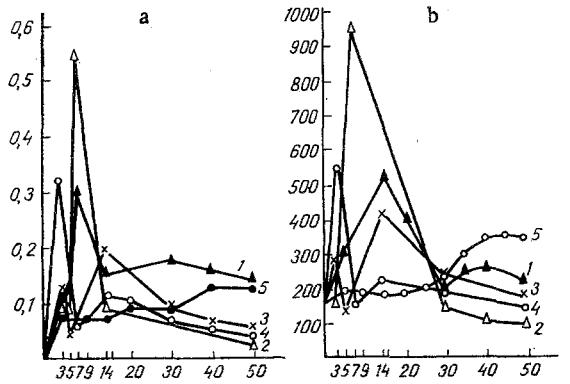

Fig. 1

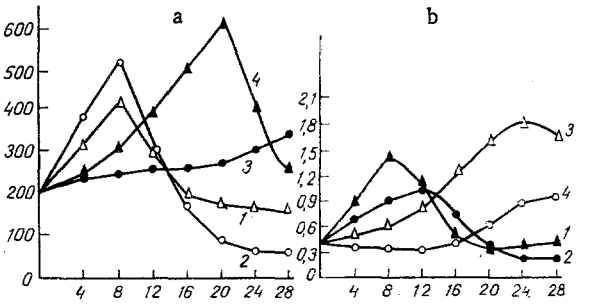

Fig. 2

Fig. 1. Changes in intensity of CHL (a) and DMA level (b) in vitreous body after injection of various LPO catalysts. 1) 0.5 ml of autologous blood; 2) 10^{-4}M $FeSO_4$; 3) 10^{-4}M $CuSO_4$; 4) 25 mg/ml hemoglobin; 5) 1.2 mg/ml of phospholipid emulsion. Here and in Fig. 2: abscissa, time after injection of substances (in days); ordinate: a) intensity of CHL (in counts per 10 sec); b) MDA level (in nmoles/mg protein).

Fig. 2. Changes in intensity of CHL (a) and MDA level (b) of vitreous body after addition of various catalysts of LPO to model system. 1) 24 mg/ml hemoglobin; 2) 10^{-4}M $FeSO_4$; 3) 10^{-} M $CuSO_4$; 1.2 mg/ml phospholipid emulsion.

The effect of hemoglobin on the LPO reaction of the vitreous body differed somewhat from the action of metals with variable valency. After addition of hemoglobin the MDA content and CHL intensity reached a maximum on the 3rd day of the experiment, whereas after addition of the metals of variable valency, the maximum was reached rather later (on the 7th-14th day). However, there was also a similarity in the action of hemoglobin, Fe^{++}, and Cu^{++}, namely that the effect of all these factors were accompanied by the appearance of one maximum, occurring on the 3rd, 7th, and 14th days respectively of the experiment.

After injection of phospholipid emulsion into the vitreous body the kinetics of the changes in the intensity of LPO differed significantly from the kinetics of LPO induced by Fe^{++}, Cu^{++}, and hemoglobin. In this case, during the 25 days of the experiment the intensity of LPO rose but not significantly. By the 35th day of the experiment the DMA concentration and CHL intensity had risen appreciably to a maximum, but its level was lower than after addition of autologous blood. The intensity of LPO thereafter decreased, and by the end of the experiment it has fallen to the control level.

These experiments showed that an almost identical kinetics with injection of autologous blood can be produced by the addition of a combination of Fe^{++} or Cu^{++} with hemoglobin and phospholipid emulsion. In this case, the kinetic curve has two maxima: the first and highest is observed on the 6th day, the second on the 30th day. However, it must be pointed out that after combined injection of the three LPO catalysts, the first maximum of accumulation of LPO products appeared much faster than after injection of autologous blood.

In the later experiments the action of Fe^{++}, Cu^{++}, hemoglobin, and phospholipid emulsion on LPO was intensified, as is shown by an increase in the MDA concentration and the intensity of CHL.

The velocity of LPO in the model system was shown to be largely determined by the state of the vitreous body. For instance, after addition of the LPO catalysts (hemoglobin, Fe^{++}, and Cu^{++}) to the unhomogenized vitreous body the MDA concentration reached a maximum after 8, 12, and 24 h, respectively (Fig. 2), whereas after homogenization of the vitreous body, it occurred after 2 h. A second addition of the metals of variable valency, such as Fe^{++}, caused another small increase in the intensity of LPO, confirming the view that the decrease in the intensity of LPO after reaching a maximum was due to the absence of reducing agents, converting Fe^{+++} into Fe^{++}.

These experiments showed that addition of phospholipid emulsion to the vitreous body *in vitro* also causes accumulation of LPO products, but by a much lesser degree, and after a longer time interval that after injection of phospholipid emulsion into the vitreous body.

Analysis of the results thus shows that of all the components of blood, Fe^{++}, Cu^{++}, hemoglobin, and phospholipids lead to intensification of LPO of the vitreous body, both when injected into the eye and when added directly to the isolated vitreous body. Hemoglobin and Fe^{++} act most rapidly on LPO reactions. The results are evidence that the first maximum of intensity of LPO which was observed is connected with the action of hemoglobin and Fe^{++}. Comparison of the kinetics of LPO after injection of autologous blood with its kinetics after injection of an emulsion of blood phospholipids, both *in vivo* and in the model, leads to the conclusion that the appearance of the second maximum is probably associated with oxidation of the lipids of autologous blood.

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USE OF PARAMETERS OF LIPID PEROXIDATION ACTIVITY TO STUDY HUMAN ADAPTATION TO NEW CLIMATIC AND GEOGRAPHIC CONDITIONS

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UDC 613.11-07:[612.014.49-08:612.397.2

KEY WORDS: lipids; peroxidation; adaptation

During adaptation to stress situations, including to new climatic and geographic conditions, various changes of a systemic character take place in the body, and are manifested as changes in various physiological and biochemical parameters. At the whole-body and cellular levels, this phenomenon is considered to be based on changes in activity of lipid peroxidation (LPO) of biological membranes. A promising way of studying the process of adaptation is accordingly to study parameters characterizing LPO activity [2, 3, 5, 10].

This paper gives the results of a study of the concentrations of LPO products in lipids of blood erythrocytes obtained from workers in the southern districts of the USSR in the course of their adaptation to conditions of life in Western Siberia.

EXPERIMENTAL METHOD

Altogether 250 clinically healthy individuals aged from 20 to 50 years were investigated during the period of adaptation from the 1st day until 40-50 weeks. The control group consisted of 50 local inhabitants of the corresponding age groups.

The optical density of solutions of lipid extracts, with absorption at 232 nm, is known to characterize the concentration of conjugated dienes, including diene perixides, whereas absorption at 268 nm is considered to be due to absorption of conjugated trienes [9]. Venous blood was taken by the standard method [1]. The analysis was carried out by the writers' modification of Placer's method [7]. Central blood, in a volume of 0.9 ml, was stabilized with 0.01 ml of a 3.8% solution of sodium citrate and centrifuged for 10 min at 3000 rpm. The plasma was removed and the pellet was washed twice in a 1 mm tube with 0.9% solution of NaCl and was centrifuged twice for at 3000 rpm for 5 min. The supernatant was discarded and 0.3 ml of erythrocyte suspension (ES) was diluted with phosphate buffer (pH 7.4) in the ratio of 1 : 33, and 1 ml of its was transferred into a tube with ground glass stopper, mixed with 0.9 ml of a mixture of heptane and isopropyl alcohol

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(Presented by Academician of the Academy of Medical Sciences of the USSR, S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 5, pp. 571-573, May, 1987. Original article submitted June 16, 1986.

0007-4888/87/0005- 0648 $12.50