LIPID PEROXIDATION IN THE VITREOUS BODY AND RETINA FOLLOWING INTRAVITREAL HEMORRHAGE SECONDARY TO DITHISONE DIABETES

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An increase in the prevalence of diabetes in the last decades has led to a considerable increase in the number of patients with diabetic retinopathy [2]. The uncertainty of the pathogenesis of diabetic retinopathy still remains one of the main factors delaying the development of highly effective methods of its treatment.

One of the most frequent complications of diabetic retinopathy is hemorrhage into the vitreous body [5]. Studies of the pathogenesis of traumatic hemorrhage have shown that the escaping blood often leads to the formation of a permanent opacity of the vitreous body and adhesions, frequently ending with traction detachment of the retina, and ultimately, in atrophy of the eye [1, 3]. This indicates an important role for intravitreal hemorrhage in the aggravation of diabetic complications. According to the limited data available, in recent years free-radical lipid peroxidation has been included in the primary mechanism of the pathogenesis both of diabetic retinopathy and of intraocular hemorrhage, which is a frequent component of many forms of ocular pathology [7]. Intensification of LPO and a decrease in the concentration of endogenous antioxidants in the retina have been found in experimental diabetes [10]. In patients with intravitreal hemorrhages, just as in experimental animals, intensification of LPO has been found, on the basis of an increase in the malonic dialdehyde (MDA) concentration and intensification of chemiluminescence (ChL) [6]. However, the particular features of development and regulation of LPO in the vitreous body and retina during hemorrhages secondary to diabetes have not been studied. Accordingly, the aim of the investigation described below was to study changes in the intensity of LPO in the vitreous body and retina during intravitreal hemorrhage secondary to experimental diabetes produced in rabbits by dithisone.

EXPERIMENTAL METHOD

Experiments were carried out on 48 noninbred rabbits weighing 2.5-3.5 kg, which were kept on an ordinary diet. The rabbits were deprived of food but allowed water ad libitum for 24 h before the experiment. Experimental dithisone diabetes was induced with an alkaline solution of dithisone 40-50 mg/kg, which was injected slowly into the auricular vein. The development of diabetes was monitored by measuring sugar levels in the blood and urine, and also on the basis of the volume and specific gravity of the urine excreted and the results of qualitative tests for acetone. A full investigation of the eyes was carried out, including electroretinography and echography. In the course of development of diabetes for 4 weeks changes in the intensity of LPO in the vitreous body and retina secondary to intravitreal hemorrhage were studied. The intensity of LPO was judged by changes in the concentrations of hydroperoxides (HP) and MDA, determined by the method in [8]. The action of synthetic antioxidants — emoxipine (hydroxypyridine hydrochloride) and phenosan potassium — was investigated. Antioxidants were injected by the retrobulbar route on alternate days in a dose of 8 mg in 0.3 ml physiological saline. The results were subjected to statistical analysis by Student's test.

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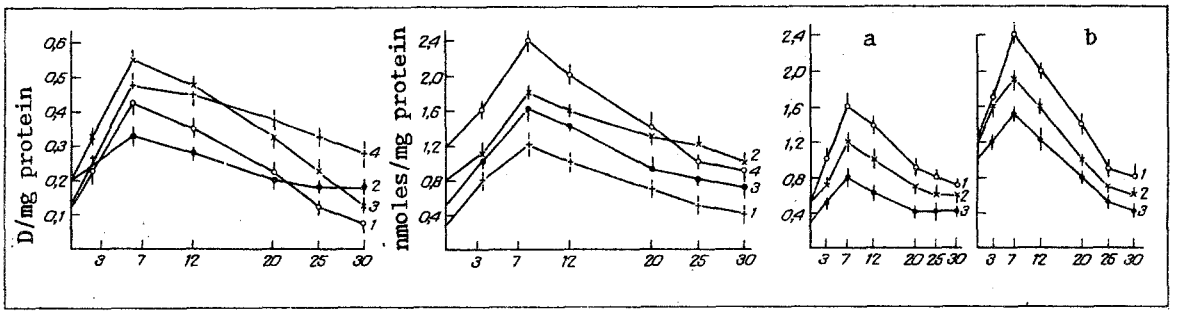

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Changes in HP content in vitreous body (1) and retina (2) in dithisone diabetes. 3 and 4) Vitreous body and retina respectively after intravitreal hemorrhage secondary to dithisone diabetes. In Figs. 2 and 3: abscissa, days of experiment.

Fig. 2. Changes in MDA content in vitreous body and retina in dithisone diabetes. Legend as to Fig. 1.

Fig. 3. Effect of synthetic antioxidants on changes in MDA content (in nmoles/ml protein) in vitreous body (a) and retina (b) after intravitreal hemorrhage secondary to dithisone diabetes. 1) Intravitreal hemorrhage secondary to dithisone diabetes, 2) the same, after preliminary injection of emoxypine, 3) the same, after preliminary injection of phenosan potassium.

EXPERIMENTAL RESULTS

Intravenous injection of dithisone increased the intensity of LPO. The HP content in the vitreous body 3 days after injection of dithisone showed a marked increase, to reach a maximum on the 7th day of the experiment, after which it fell to the initial level, which was reached on the 30th day. The action of dithisone in the retina also caused an increase in the HP content (Fig. 1). In the retina, however, the increase in the HP content after dithisone was smaller than in the vitreous body.

After injection of dithisone there was a significant increase in the MDA concentration also. The increase was significant on the 7th day after injection of dithisone, reached a maximum, and then decreased. The increase in the MDA concentration was greater in the retina than in the vitreous body (Fig. 2). Intensification of LPO after injection of dithisone was probably due to the action of toxic complexes formed between dithisone and metals, causing irreversible damage to the cell [4]. Like alloxan, the dithisone metabolite evidently is incorporated into a free-radical chain reaction, with the formation of a radical which causes intensification of LPO.

A no less important role in the intensification of LPO in dithisone diabetes is played by insulin deficiency, which develops as a result of damage to the pancreatic β-cells. Insulin is involved in the metabolism and utilization of lipid peroxides.

Reproduction of hemorrhage secondary to experimental dithisone diabetes increased even more the accumulation of HP in the vitreous body. The kinetics of changes in HP after hemorrhage obeyed the same rules as those observed previously after administration of dithisone (Fig. 1). Intravitreal hemorrhage was accompanied by elevation of the HP level in the retina also. Hemorrhage led to increased accumulation of MDA both in the vitreous body and in the retina compared with injection of dithisone. It is noteworthy that the MDA and HP concentrations in both these tissues remained high for a long period after development of intravitreal hemorrhage (3 weeks; Fig. 2). One possible mechanism of the significant increase in LPO in association with intravitreal hemorrhage is through the passage of heme products, metallic ions of variable valency, and the formation of radical oxygen intermediates, especially the superoxide anion-radical ($\cdot O_2^-$), singlet oxygen (IO_2), and the hydroxyl radical ($OH^{\cdot}$), into the vitreous body [9].

The experiments showed that intensification of LPO in the vitreous body and retina associated with intravitreal hemorrhage secondary to dithisone diabetes is controlled by synthetic antioxidants. Administration of emoxypine reduces the HP concentration in the vitreous body by a certain degree. For instance, on the 3rd day after injection of emoxypine the HP concentration in the vitreous body was 20% lower than in the presence of hemorrhage (Fig. 3a). A similar rule also was

observed in the retina. Admittedly, unlike in the vitreous body, accumulation of HP in the retina was not inhibited until the 7th day after injection of emoxypine. Emoxypine also caused a decrease in the MDA concentration in the vitreous body and retina during hemorrhage. Phenosan potassium, which inhibits LPO in the vitreous body and retina in the presence of hemorrhage secondary to dithisone diabetes, proved to be more effective than emoxypine. The results show that phenosan potassium inhibited HP and MDA accumulation equally effectively in the vitreous body and in the retina. After injection of phenosan potassium the MDA concentration in the vitreous body in the presence of hemorrhage was 2.0 times less on the 7th day, whereas in the retina it was 1.7 times less than the control level (Fig. 3b). Phenosan potassium also reduced HP accumulation in the vitreous body and retina. Their level on the 7th day of the experiment was 1.8 and 1.4 times lower respectively than in the control.

Dithisone diabetes thus increases accumulation of LPO products in the vitreous body and retina. Intravitreal hemorrhage secondary to dithisone diabetes intensifies LPO even more. The intensification of LPO in the vitreous body and retina associated with intravitreal hemorrhage can be corrected by administration of synthetic antioxidants. Of the synthetic antioxidants investigated, namely phenosan potassium and emoxypine, the former is more effective.

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