SUPRAMOLECULAR CHANGES IN PRESERVED CORNEAL TISSUE DURING TREATMENT WITH α-TOCOPHEROL

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UDC 612.841.1.014.46:615.357:577.161.3]-085.23

The kinetics of chemiluminescence of corneal tissue of isolated dogs' eyes induced with 1% hydrogen peroxide was compared with the result of determination of ultrastructural changes by the 30th day of preservation of the eye in a moist chamber at +4°C with or without filling of the anterior chamber of the eye with α-tocopherol (0.2 μg). The results indicate severe disturbances of free-radical processes in the structural components of the corneal cells with changes in the times and conditions of preservation. These disturbances can be regarded as basic for one of the trigger mechanisms of autolysis. Stabilization of the lipoprotein complexes of the cell during preservation is evidently determined by the ability of anti-oxidants, notably α-tocopherol, to support oxidative processes at a constant level.

KEY WORDS: α-tocopherol; cornea; preservation of the eye.

Great importance is attached in the literature to the state of the lysosomal enzyme systems in the study of postmortem changes in the membranous structures of the cell by physicochemical methods [5]. However, the initial disturbances of the cell membranes may be caused by lipid peroxides, the formation of which is probably the trigger mechanism of irreversible postmorten changes [1, 3, 9]. Processes of free-radical oxidation, inducing damage to thin cellular structures, are also observed in surviving and preserved tissue. Meanwhile the supramolecular processes taking place in preserved tissue during treatment with antioxidants have been inadequately studied.

The object of the present investigation was to study one mechanism of autolysis of corneal tissue and the result of treatment with antioxidants during preservation of the isolated dog eye.

EXPERIMENTAL METHOD

Experiments were carried out on 27 eyes enucleated from dogs immediately after death of the animals. In group 1 (9 dogs) the experiments were carried out 5-10 min after enucleation, in group 2 (9 dogs) after preservation of the eyes in the moist chamber at 4° C for 30 days, and in group 3 (9 dogs) after preservation of the eye in the moist chamber at 4° C, also for 30 days, but with the aqueous humor of the eye replaced by α-tocopherol.

The chemiluminescence of the corneal tissue induced by 1% hydrogen peroxide was determined on the FÉU-86 radiation detector. In a parallel series of experiments the cell membranes were studied in the IEM-100B electronmicroscope. For this purpose the cornea was fixed by Caulfield's method, dehydrated in alcohols, stained with uranyl acetate, and embedded in Durcupan. Sections 150-Å thick were cut on the LKB-8800 ultratome and stained with lead citrate by Reynolds' method.

EXPERIMENTAL RESULTS

Analysis of the kinetic curves of chemiluminescence of the cornea showed that after preservation of the eye in the moist chamber for 30 days the intensity of luminescence in the first 5 min reached 3601 counts,

Laboratory of Pathological Morphology and Laboratory of Pathological Physiology, Institute of Transplantation of Organs and Tissues, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 7, pp. 38-41, July, 1975. Original article submitted June 21, 1974.

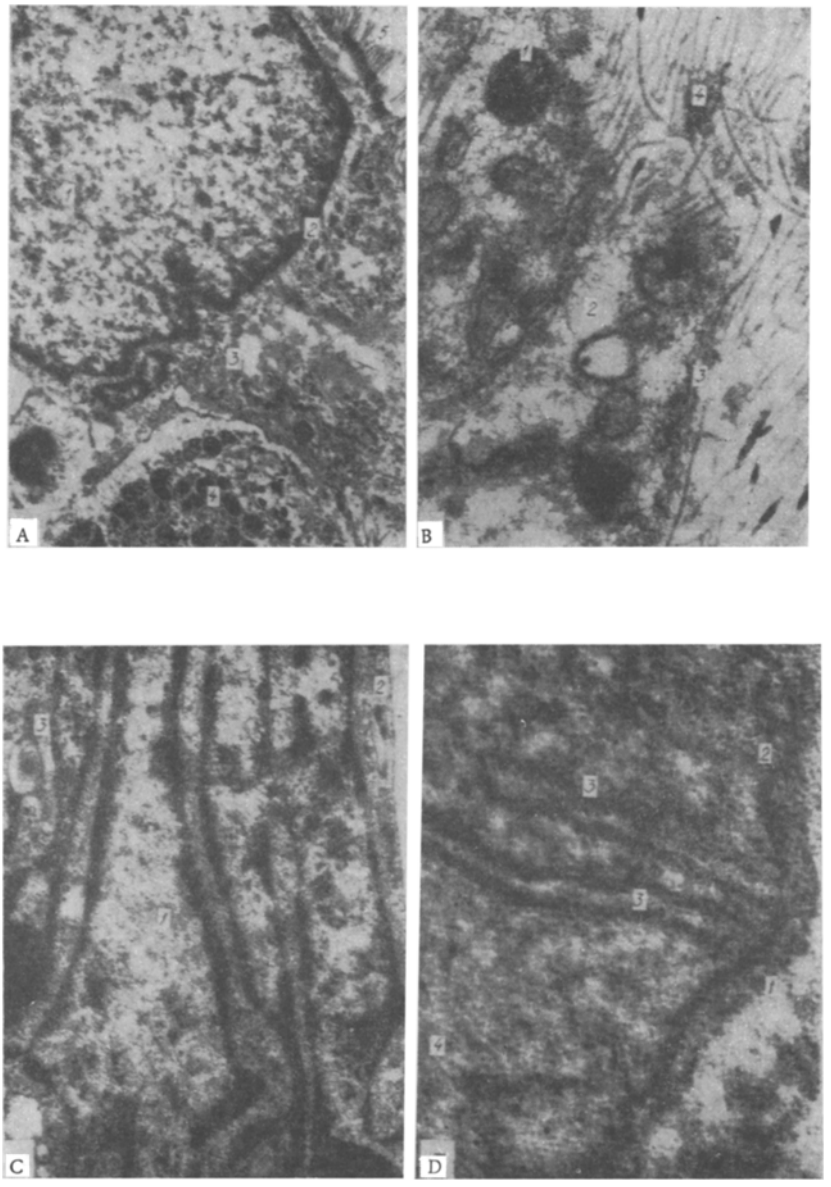

Fig. 1. Ultrastructure of fibroblasts in ground substance of dog cornea under normal conditions and after preservation. A) Control. Nucleus of fibroblasts (1), karyolemma (2), cytoplasm (3), lymphocyte (4), collagen (5); magnification 20,000 ×. B) Preservation in moist chamber at 4° C for 30 days. Mitochondrion (1), translucent cytoplasm with vaculated rough endoplasmic reticulum (2), destruction of membranes of cytolemma (3), decomposition of collagen (4); magnification 60,000 ×. C) Preservation in moist chamber at 4° C for 30 days with replacement of aqueous humor by α-tocopherol. Nucleus of fibroblasts (1), stabilized membranes of cytolemma (2) and mitochondria (3); magnification 25,000 ×. D) The same as C. Lipoprotein complexes of outer (1) and inner mitochondrial membrane (2), cristae (3), and translucent matrix (4). Magnification 500,000 ×. Fixation by Caulfield's and staining by Reynolds' methods.

falling during the next 25 min to 685 counts. Marked decomposition of the collagen fibers, with preservation of division into light and dark disks, were observed on the electron micrographs (Fig. 1B), accompanied by destruction of the cell membrane, the nuclear apparatus, and organelles of the fibroblasts and, in particular, of the lipoprotein complexes in the outer and inner mitochondrial membranes.

During preservation with the addition of α-tocopherol a much lower intensity of luminescence was observed at the beginning of the oxidative action of hydrogen peroxide on the corneal tissue, which was 2110 counts, falling after 25 min to 489 counts. Electron-microscopic investigation revealed signs of sterilization of the lipoprotein complexes of the fibroblast membranes (Fig. 1C) and their organelles. The mitochondria had an intact outer membrane, consisting of moderately swollen lipoprotein complexes (Fig. 1D), a slightly edematous and translucent matrix, and intact cristae. The collagen fibers of the ground substance of the cornea (by contrast with previous observations) had a regular arrangement of the fibrils and intact, slightly edematous, isotropic and anisotropic disks.

In the control group the intensity of luminescence of the cornea at the beginning of oxidative interaction between hydrogen peroxide and the corneal tissue was 703 counts, and 25 min later it was only slightly lower – 525 counts. Electron microscopic examination also revealed intact collagen fibers with the typical structure (Fig. 1A). The results thus demonstrate profound disturbance of free-radical oxidation and disorganization of the submicroscopic components of the fibrous and cellular components of the cornea of the isolated eye during preservation under hypothermic conditions.

The use of α-tocopherol as an antioxidant leads to preservation of the lipoprotein complexes of the cell membranes and, correspondingly, preservation of the product of free-radical oxidation, as reflected in a fall in the level of chemiluminescence.

The kinetic curves of this phenomenon reflect the state of the lipoprotein complexes in the cell membranes of the cornea. Injury to the membranes is accompanied by a shift of equilibrium between lipids and free fatty acids in favor of the latter [4], which leads to a sharp increase in chemiluminescence on oxidative interaction with hydrogen peroxide [6, 7]. α-Tocopherol inhibits accumulation of lipid peroxides [2] and protects the phospholipid base, which is the weakest link in the self-regulating system of the energy cycle of the cell [8]. Comparison of these results with data in the literature indicates that the integrity of the structural components of the cells during preservation is determined by the ability of antioxidants to support oxidative processes.

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