вання хронічного абактеріпльного простатиту з супутнім осеохондрозом попереково-крижового відділу хребта з використанням сучасних методів фізіотерапії: магнітолазерної, хромомагнітної, МХТ – рефлексотерапії, пневмовібромасажу передміхурової золози, фармакоакупунктури Sol.Traumel – S. Наведено результати клінічного дослідження та порівняльно характеристику застосування цих методів в порівнянні з традиційною фізіотерапією: діадинамічні токи, сінусоідально – модульовані токи, ультразвукова терапія; доведена більш висока клінічна ефективність сумісного використання методів сучасної фізіотерапії.

УДК 612.014.464:611-018.7:678.048

HIGH OXYGEN LOAD CAUSES DAMAGE TO LENS EPITHELIUM WHICH IS REDUCED BY ANTIOXIDANTS

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This study was supported in part by the Guzik Ophthalmology Research Fund

Introduction

An adult lens contains two morphologically distinct compartments, the epithelium and the fiber-cell mass. The fiber-cell mass provides the lens with its functional phenotype and transparency. Metabolically, the epithelium is the more active compartment of the ocular lens. This single layer of cells, in addition to acting as a metabolic engine that sustains the physiological health of this tissue, also works as a source of stem cells, providing precursor cells, which through molecular and morphological differentiation give rise to fiber cells. Morphological simplicity, defined developmental history and easy access to the researcher make this epithelium a material for investigation of universal questions of cell growth, development, epithelial function, cancer and aging. There are two important aspects of the lens epithelium that make it highly relevant to the modern biologist. Firstly, there are no known clinically recognizable cancers of the ocular lens. The lack of vascular system may explain the absence of tumors in this tissue, but this provides only a teleological basis to a very important question for which the answers must reside in the molecular and physiology of the lens epithelial cells. Secondly, lens epithelium as a morphological entity in the human lens is first recognizable in the 5th-6th week of gestation. It stays in this morphological state as the anterior epithelium of the lens for the rest of life, making it an attractive tissue for the study of the effects of aging on epithelial function (1).

Studies on human patients and experimental animals indicate that hyperbaric O_2 can damage the lens nucleus and the lens epithelium in vivo. When the cells were exposed to 50 atm O_2 (99% O_2 + 1% CO_2) for 3 hr, there were no immediate effects on lens morphology, viability and transport processes (uptake of ^{86}Rb and ^{14}C-alpha AIB). In addition, the O_2 treatment did not lower the high level of reduced glutathione or increase the low level of oxidized glutathione. However, 50 atm O_2 did produce a near doubling in the glycolytic rate which maintained ATP at levels only slightly lower than normal (2). In previous studies we found that high oxygen load has a toxic effect on bovine lenses in organ culture. Changes marking toxicity follow the route of oxygen diffusion into the lens, from the periphery to the center (3). The current study investigated the mechanisms of hyperbaric oxygen on lens epithelial enzymes and the effects of the antioxidant Zinc-desferrioxamine (Zn-DFO) using a lens organ culture system.

Methods

Experimental treatments

Intact bovine lenses (one year old) in organ culture conditions were included in the present study. We divide the lenses into3 groups:

(1) **Hyperbaric oxygen (HBO) exposure group**: 25 lenses exposed daily to HBO for 4 days. Each exposure session consisted of 120 minutes 100% oxygen in a pressure chamber at 2.5 ATA. During the exposure lenses were kept in PBS.

(2) **HBO exposure group with Zn-DFO:** 25 lenses exposed daily to HBO for 4 days. Each exposure included 120 minutes of 100% oxygen in a pressure chamber at 2.5 ATA. During HBO exposure lenses were kept in a Zn-DFO 2.5 mg/liter in PBS.

(3). **Control group.** 50 lenses incubated daily for 4 days in PBS for 120 min.

Organ Culture System

Each lens was placed in a glass and silicon rubber chamber containing 24ml of culture medium (M 199) with Earl's balanced salt solution, supplemented with 5.96g/L HEPES, 3% dialyzed fetal calf serum and antibiotics (penicillin 100 U/ml and streptomycin 0.1 mg/ml). Lenses were completely immersed in culture medium both

below and above. The medium was changed daily. The lenses were incubated at 35°C. Experimental treatments started after pre-incubation of 24 hours.

Pressure Chamber

Bovine lenses in the specially designed culture chambers were exposed to hyperbaric oxygen in a sealed hyperbaric oxygen pressure chamber. Pressure was raised to 2.5 ATA over 20 minutes. The duration of each exposure was 120 minutes. During the exposures, the temperature inside the chamber remained constant. Oxygen saturation inside the pressure chamber was monitored and kept constant throughout the exposure session.

During the exposure to hyperbaric oxygen the culture medium was changed to PBS for 120 minutes in all study and control lenses. Lenses treated with Zn-DFO were put in PBS containing 2.5mg/liter Zn-DFO. Study lenses were exposed to oxygen at the pressure chamber and control lenses kept at room air during the exposure. Medium was changed back to original medium immediately after exposure.

Lens optical quality monitoring

Lens optical quality was monitored daily throughout the 7 days of the culture period. Lens optical measurements were determined by an automated scanning laser system that recorded both relative transmission and focal length across the lens (The Scan-ToxTM In Vitro Assay System, Harvard Apparatus, Holliston, MA).

Lens epithelium morphology and enzyme analysis

On day 7 of the culture period, lenses were taken for morphological and enzyme analysis. Total flat preparations of the front capsule epithelium monolayer of lenses from the different treatments were evaluated. When evaluating the different grade of its differentiation was taken into account, i.e. topographical features of central intermediate and equatorial zones (fig. 1).

We followed the histochemical localizations of the enzymes: succinic dehydrogenase (SDH), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PD), hexokinase (HK) and adenosine triphosphatase (ATPase). For analysis we used the classical methods of Pearse (4). Ouabain (10^{-4} M), the ATPase inhibitor was used as control samples for ATPase activity.

Quantitative analysis of the intensity of the reactions at epithelial central and equatorial zones was done by Image-Pro Plus program, Version 4.0 for Windows, by measuring optical density in each cell, following by mathematical processing in Microsoft Excel. A change was defined as significant if the difference between control and treated groups reached value of $P < 0.05$.

Results

Figures 2a and 2b demonstrate the activities of G6PD at the center and equators of the lens epithelium of control lenses, HBO treated lenses and lenses treated with HBO and Zn-DFO.

There is not much difference of G6PD activities at the center of the lens epithelium for the different treatments however at the equators after exposure to HBO, G6PD activity increased by almost 10% and in the presence of Zn-DFO the activity increased by 25%. (Fig. 2b).

Cells were well stained and diformazan grains were situated on the whole area of the cytoplasm (Fig. 2a). The area of the cells exposed to HBO did not change much but was increased in the presence of the antioxidant Zn-DFO. The changes in cells area were higher at the equators of the lens epithelium (Fig. 2c).

The diformazan grains in the cells after HBO treatment did not show much contrast compared to controls and lenses treated with antioxidant.

SDH activity is demonstrated in Fig 3a. Diformazan location in the cells demonstrated by blue product formed after recovering of the ditetrazolium salts (fig. 3a).

Treatment by HBO decreased SDH activities in central zone of lens epithelium by 17.9% and in equatorial zone by 21.5%. In lenses treated with HBO in the presence of the antioxidant, SDH activities were similar to the activities of the control group (fig. 3b).

The activity of LDH is demonstrated in Figure 4a.

Activity of LDH (enzyme with high substrate

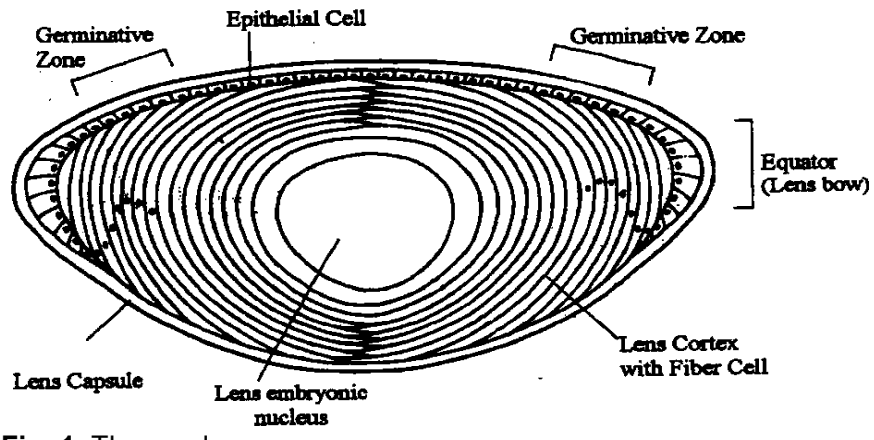

Fig. 1. The eye lens.

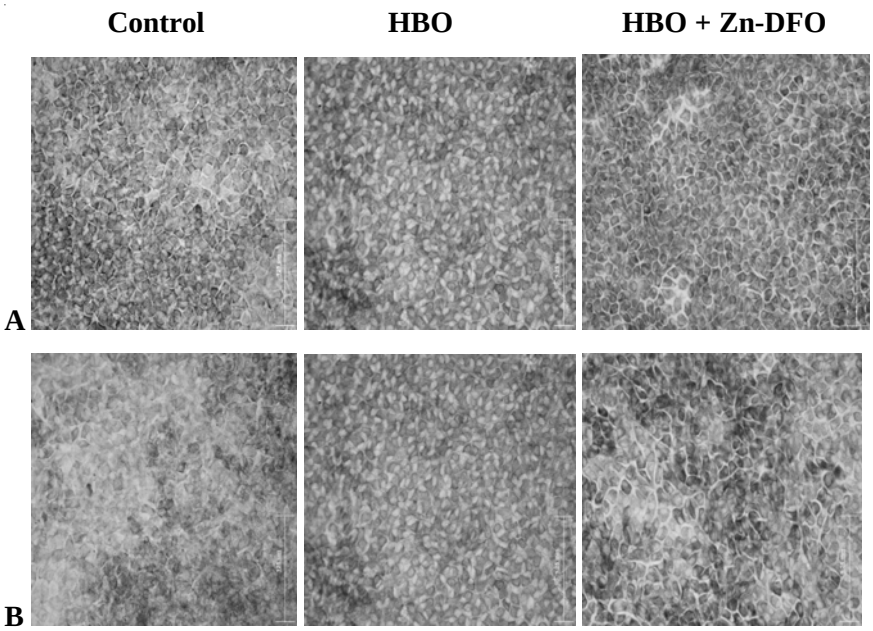

Fig. 2a. G6PD activities of control lenses, HBO treated lenses and HBO+Zn DFO treated lenses: (A) Center of lens epithelium (B) Equators of lens epithelium.

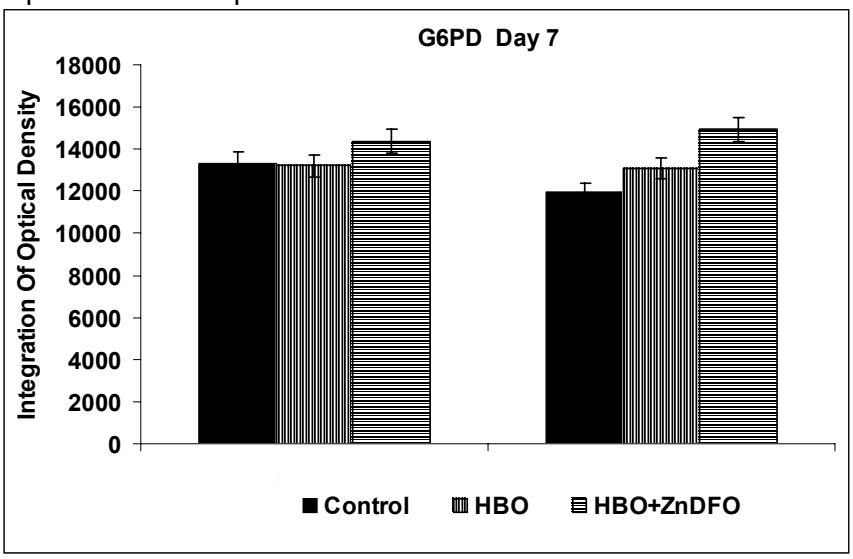

Fig. 2b. G6PD activities of lens epithelial cells in control lenses and lenses incubated with HBO and HBO with Zn-DFO.

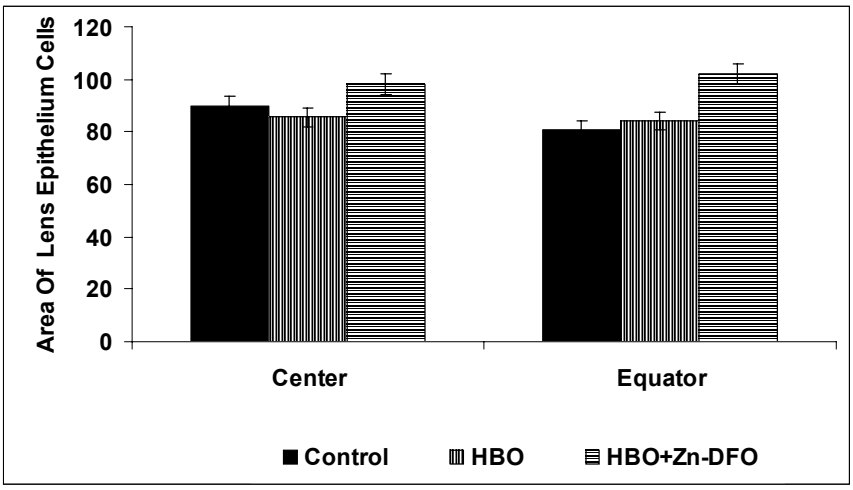

Fig. 2c.-Changes in epithelial cells area, after HBO treatment and after treatment with HBO in the presence of the antioxidant (Zn-DFO) – G6PD activity.

specificity), is reduced by HBO treatment and also by HBO treatment in the presence of the antioxidant. Activity of LDH was decreased markedly (32.7%) in equatorial zone after treating with HBO and antioxidant (Fig. 4b).

Distinctly expressed changes of hexokinase activities were found in the epithelial monolayer after HBO treatment (Fig.5b).

Hexokinase activities increased almost twice in both center and equators. The localization of hexokinase in the cells is demonstrated in Fig.5a. Hexokinase is displaced to the peripheries of the cells. The size of the cells (Fig 5c), show increased area after HBO treatment.

The observable reinforcement of the ATPase activities after HBO and HBO+ Zn-DFO treatments was expressed by increased quantity of the black deposits in the cells (Fig.6), in comparison to control group.

Discussion

In our studies lenses in organ culture conditions were subjected to high oxygen load and in some experiments the antioxidant Zn-DFO was added to the oxygen treated lenses for checking the possible ability to protect the lenses from oxygen damage. We demonstrated that the lens epithelium reacted differently with different enzymesal: also Zn-DFO presence during oxygen exposure did not always prevent the oxygen changes.

All enzyme activities were analyzed in lens epithelium after 7 days in culture. Hexokinase activities increased as a result of HBO treatment. Increased hexokinase activity means increased glucose phosphor-

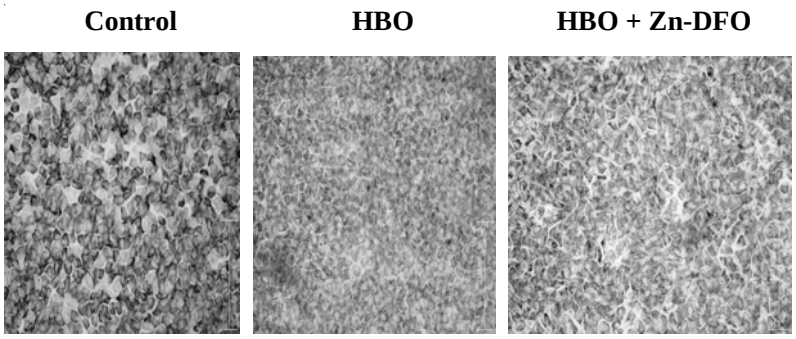

Fig. 3a. SDH activities in cells of lens epithelium equator zone at three different treatments: Control, HBO exposed lenses and HBO treated lenses in the presence of Zn-DFO.

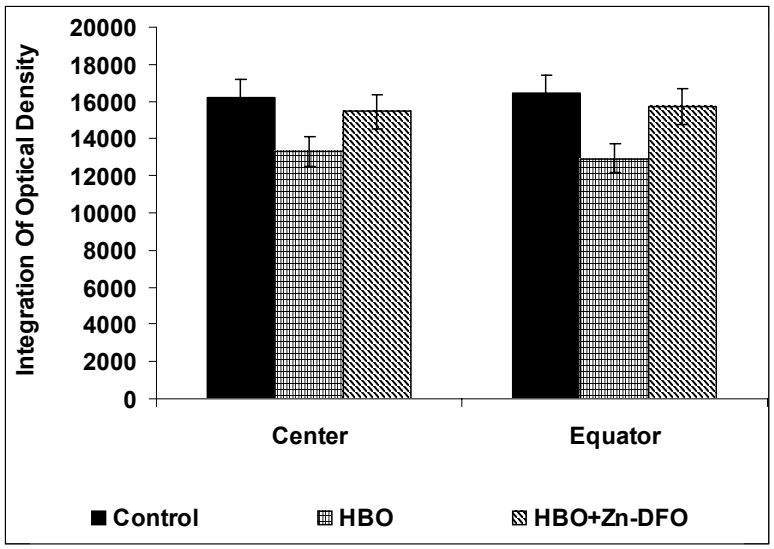

Fig. 3b. Integration of optical density of SDH activities in lens epithelial cells under influence of the HBO and the antioxidant (Zn-DFO)..

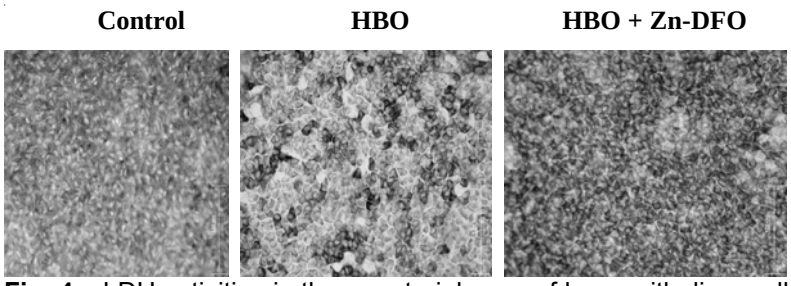

Fig. 4a. LDH activities in the equatorial zone of lens epithelium cells

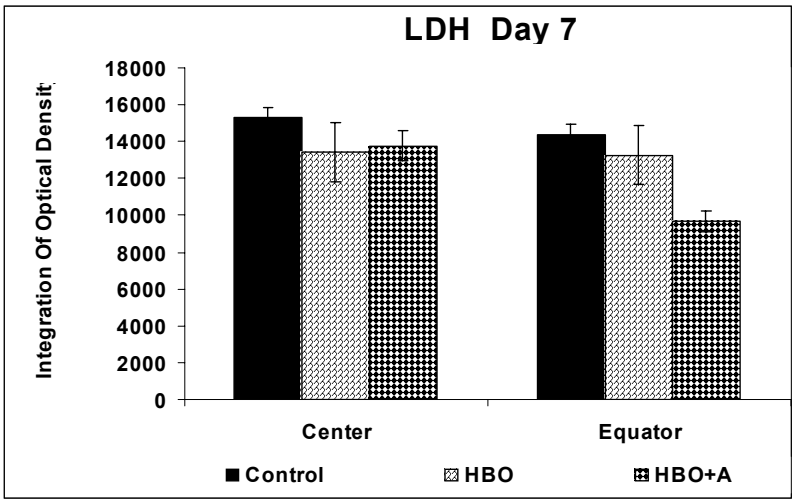

Fig. 4b. Integration of optical density of LDH activities in the cells of lens epithelium under influence of the HBO and HBO+Zn-DFO.

ylation. Also G6PD showed increase in activity which demonstrate activation of hexose monophosphate shunt.

In the study of Marsili et al (5) it was found that the oxidative damage may be the part of the response to elevated levels of the glucose. Giblin et al.(6) exposed lenses to 50 atm of O_2 and produced a three-fold stimulation of hexose mono-phosphate shunt activity, equal to that which has been reported for treatment of lenses with 0.06 mM H_2O_2.

The studies of Cappiello et al.fe: (7) by chromatography method on Matrex Orange resin allowed the separation of glutathione modified and native aldose reductase in crude extracts of bovine lens. The analysis of hyperbaric oxygen treated lenses revealed the formation in the intact cultured lens of an enzyme form displaying affinity column binding properties, specific activity, sensitivity to inhibition and susceptibility to activation by thiol reducing agents, all comparable to glutathione modified aldose-reductase. The extent of the enzyme modification increased with the time of the oxidative treatment and was maximal in the lens nucleus.

Recent studies of Giblin (8) have indicated an important hydroxyl radical-scavenging function for GSH in lens epithelial cells, independent of the cells' ability to detoxify H_2O_2. Depletion of GSH or inhibition of the redox cycle allows low levels of oxidant to damage lens epithelial targets such as Na/K-ATPase, certain cytoskeletal proteins and proteins associated with normal membrane permeability. The level of GSH in the nucleus of the lens is relatively low, particularly in the aging lens. Combined with low activity of the glutathione redox cycle in this region, makes the nucleus especially vulnerable to oxidative stress, as has been demonstrated with use of in vivo experimental animal models such as hyperbaric oxygen, UVA light

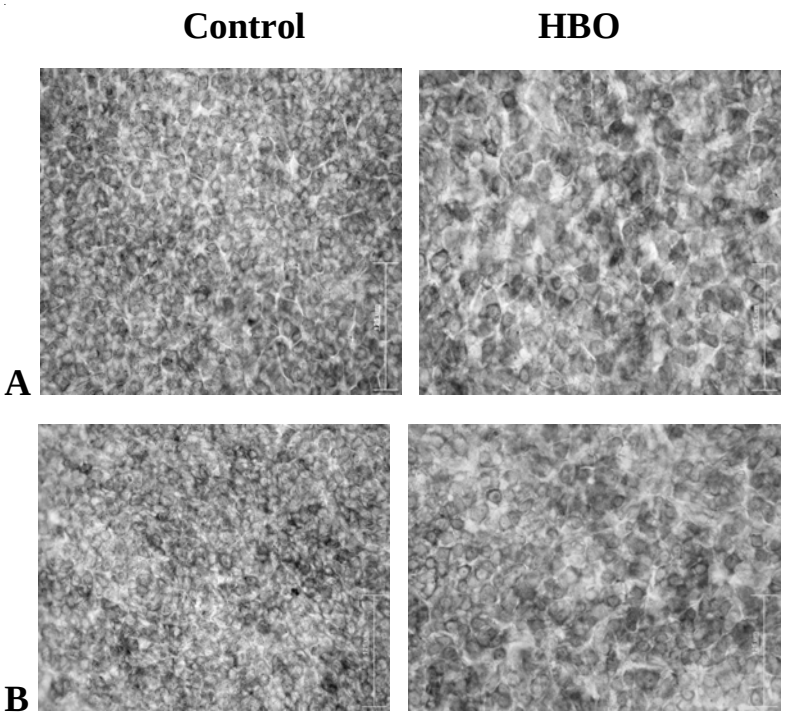

Fig. 5a. Activities of hexokinase at the center (A) and equators (B) of lens epithelial cells of control and HBO treated lenses.

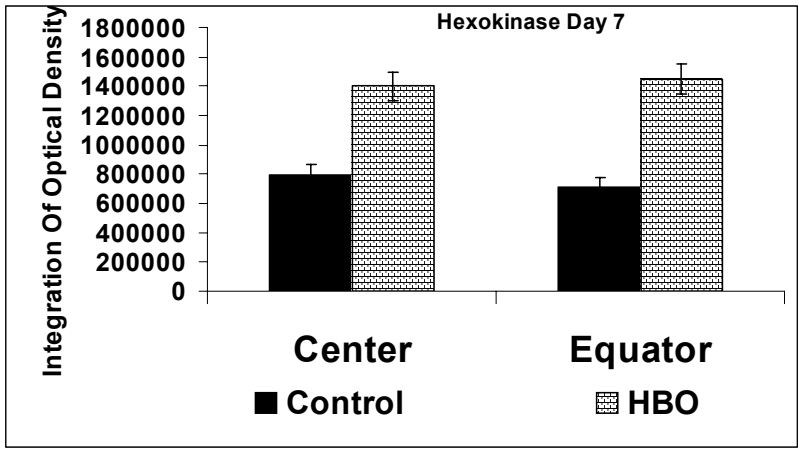

Fig. 5b. Integration of optical density of hexokinase activity in lens epithelium of control and HBO treated lenses.

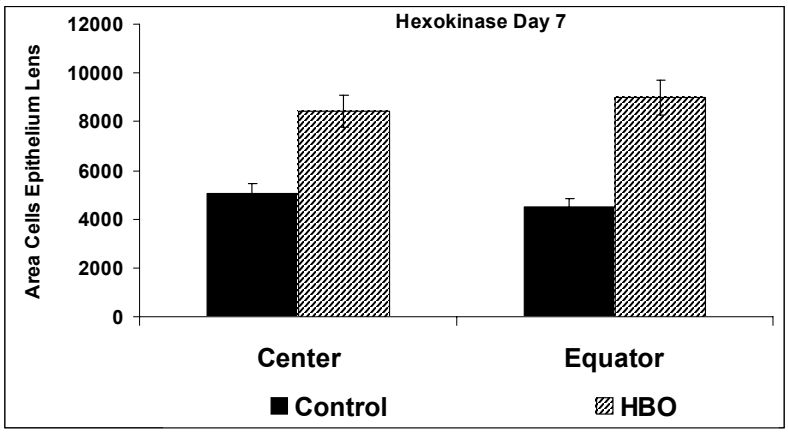

Fig. 5c. Cells area analysis in lens epithelium of control and HBO treated lenses (Hexokinase activity)

and the glutathione peroxidase knockout mouse. Effects observed in these models, which are currently being utilized to investigate the mechanism of formation of human senile nuclear cataract, include an increase in lens nuclear disulfide, damage to nuclear membranes and an increase in nuclear light scattering. A need exists for development of therapeutic agents to slow age-related loss of antioxidant activity in the nucleus of the human lens to delay the onset of cataract.

Azzam et al. (9) investigated morphological changes in the eye lens caused by UV-A. Analysis by scanning electron microscopy found irregularity of fiber morphology in lenses exposed to UV-A irradiation (but not in control lenses). They concluded that UV-A caused damage to cell membranes of the lens and alterations in lens optics, which may subsequently lead to senile cataract formation. Lens NaK-ATPase activity can recover from damage caused by UV-A at 365 nm. When the lenses received irradiation of 33 J/cm^2, NaK-ATPase activity recovered from the damage during the culture period only at the center and not at the equators of the epithelium (10).

HBO enhanced metabolic activity of lens epithelial cells that accompanied with changes of the cells size. The same fact is known from the work of Slaaf et al. (11). They detected the relative number of capillaries. Occlusion is unity at low local oxygen, and diminishes with increasing local oxygen to become 0 at an oxygen tension of about 70 mm Hg.

Three doses of daily x-ray irradiation caused 1 week later, mild changes in the lens such as uneven height of epithelial cells, irregular bow structures, and swelling of cortical fibers were observed. Eight weeks later, irregular bow configuration, posterior dislocation of nuclei, severe epithelial loss and marked swelling of cortical fibers

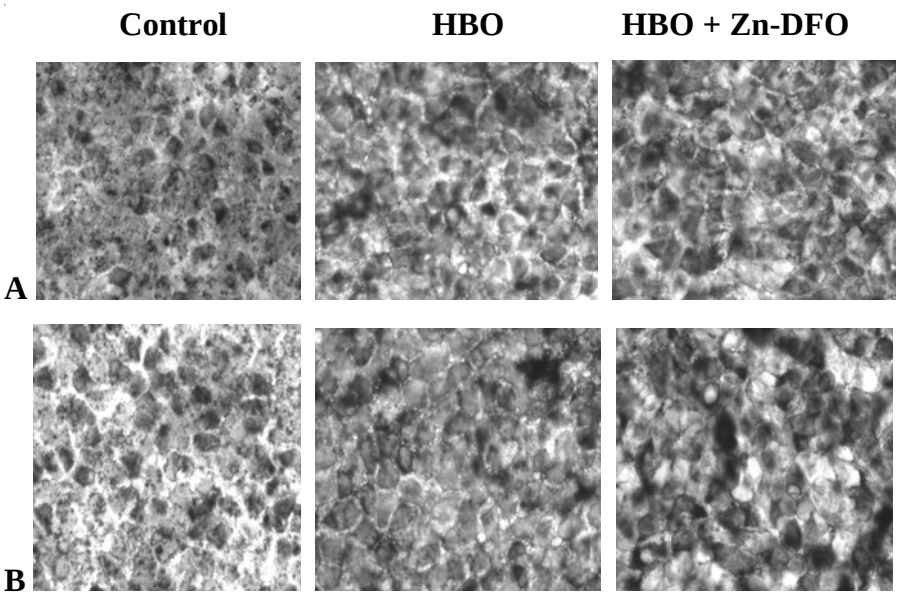

Fig. 6. Na-K-ATPase activities at the center (A) and equators (B) of lens epithelial cells exposed to three treatments: control, HBO exposure and exposure to HBO in the presence of the antioxidant ZN-DFO (15x40). ATPase (Day 7)

were observed at the equatorial area. Epithelial loss and deformed nuclei of the epithelium were observed in the central area (Morita et al.12). Struck et al (13) studied hematoxylin-eosin stained anterior central lens capsules with attached lens epithelial cells by light microscopy for cell parameters such as cell density (morphometry), nucleus area (A0), nucleus volume (V), cell area (A) and nucleus-plasma-ratio. The mean cell density in type-II diabetics (group I) is 3691 +/- 346 cells/mm^2 and in non-diabetics (group II) 4162 +/- 504 cells/mm^2, respectively ($p = 0.001$). The total female mean cell density (4036 +/- 525 cells/mm^2) was not significantly higher than the male (3788 +/- 412 cells/mm^2). A decrease of the mean cell density could be attributed to age only in the non-diabetic group. With regard to the type of cataract the posterior sub-capsular cataract shows the lowest mean cell density (3620 +/- 333 cells/mm^2) and the nuclear cataract (4250 +/- 513 cells/mm^2) the highest, respectively. The medium nucleus area and -volume and cell area are in the type-II diabetic group significantly larger than in non-diabetics.

The results of our observations, demonstrated positive effects of Zn-DFO during HBO treatment. This factor under the right conditions can serve as an effective protector for reactivation and normalization of the different functions of the visual organ. It is knowledge that the change of glycolysis pathway directed to reduction of the “effect of Paster” that is caused of oxygen insufficiency of cells. May be possible that in smaller dose and intensities HBO treatment, can be used in combination with Zn-DFO as conductor of the drug to the eye with different pathology , which are accompanied the breaches oxidation-reconditioning process.

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Summary

HIGH OXYGEN LOAD CAUSES DAMAGE TO LENS EPITHELIUM WHICH IS REDUCED BY ANTIOXIDANTS

Elvira Bormusov, Shlomit Schaal and Ahuva Dovrat

Purpose: To investigate the mechanisms involved in the effects of oxygen on the eye lens and the possible protective effects of Zinc-desferrioxamine (Zn-DFO) using lens organ culture system.

Methods: Bovine lenses, kept in an organ culture system, were exposed to high oxygen load in the presence or absence of Zn-DFO complex (20 μM). Lens optical quality was assessed throughout the 7 days of the culture period. At the end of the culture, lenses were taken for morphological and enzyme analysis.

Results: Decreased lenticular optical quality and changes in lens epithelium enzymatic activities were observed in lenses exposed to high oxygen concentration. The enzymes analyzed were from Krebs cycle, glycolysis pathway and membrane bound ATPase. Addition of Zn-DFO to the culture before the exposure to oxygen eliminated most of the oxygen-induced damage.

Conclusions: The present results may indicate a possible role of Zn-DFO as a protective agent against oxygen-induced cataract formation.

Реферат

ЗНАЧИТЕЛЬНЫЕ КИСЛОРОДНЫЕ НАГРУЗКИ ВЫЗЫВАЮТ ПОВРЕЖДЕНИЯ ЭПИТЕЛИЯ ХРУСТАЛИКА, КОТОРЫЕ УСТРАНЯЮТСЯ АНТИОКСИДАНТАМИ

Эльвира Бормусова, Шломит Шааль, Ахува Доврат

Цель: Изучить механизмы воздействия кислорода на хрусталик и возможный защитный эффект цинк-дезферриоксамина (Zn-DFO).

Методы: Хрусталики, полученные из глаза быка, хранили на культуре, полученной из тканей данного органа. Хрусталик подвергали воздействию значительной кислородной нагрузки либо в присутствии, либо без комплекса Zn-DFO (20 μM). Оптические свойства хрусталика оценивали через семь дней от начала выращивания культуры. По завершении периода, хрусталик извлекали и проводили его морфологический и ферментный анализ.

Результаты: В хрусталиках, подвергнутых воздействию больших концентраций кислорода, наблюдали уменьшение оптических свойств и изменение активности энзимов эпителия изучаемого органа. При анализе активности энзимов изучали цикл Кребса, течение гликолиза и состояние АТФ пограничных мембран. Добавление Zn-DFO к культуре клеток до начала воздействия кислородом, элиминировало большую часть кислород-зависимых повреждений.

Выводы: Полученные результаты могут указывать на возможную роль Zn-DFO как защитного агента при возникновении кислород-индуцированных катаракт.

УДК: 616.711-007.234-07

ОСТЕОПОРОЗ В ПРАКТИКЕ НЕВРОПАТОЛОГА

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Остеопороз метаболическое заболевание скелета, сопровождающееся снижением минеральной плотности костной ткани (МПКТ), превосходящее возрастную и половую нормы, приводящее к возникновению переломов различных отделов скелета, в том числе позвонков, ухудшению качества жизни людей, значительным материальным затратам на лечение и реабилитацию [1,2,5,6,9]. В США ежегодно увеличиваются затраты на лечение и реабилитацию больных, связанные с переломами вследствие остеопороза. Так, в течение 5 лет эта сумму возросла с 10 до 13,8 млр долларов в год [4,10]. По предварительным данным остеопороз встречается у 11-12% населения Европейских стран. Существует тенденция к распространению заболевания в связи с увеличением численности на-