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Desferrioxamine and zinc–desferrioxamine reduce lens oxidative damage

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Abstract

Our purpose was to investigate the quality and morphology of cultured bovine lenses after exposure to hyperbaric oxygen (HBO) in the presence or absence of desferrioxamine (DFO) or zinc–desferrioxamine (Zn–DFO). Intact bovine lenses were cultured and exposed to HBO of 100% oxygen at 2.5 ATA for 120 min. One hundred and fifty lenses were included in the present study. Lenses were divided into study groups of 25 lenses each: (1a) HBO-exposed lenses; (1b) control lenses extracted from the contralateral eyes of group 1a and exposed to normal room air. (2a) HBO-exposed lenses treated with DFO; (2b) control lenses extracted from the contralateral eyes of group 2a exposed to normal room air in the presence of DFO (3a) HBO-exposed lenses treated with Zn–DFO; (3b) control lenses extracted from the contralateral eyes of group 3a, exposed to normal room air in the presence of Zn–DFO. Lens optical quality and structural changes were assessed. Oxygen toxicity to lenses was demonstrated by decreased light transmission, increase in focal length variability and a decrease in morphological integrity. Light intensity measurements showed a distinct pattern in control lenses. A different pattern was noticed for hyperbaric oxygen-exposed lenses. Focal length variability values were stable in control lenses and increased significantly in oxygen-exposed lenses. Structural damage to lenses was demonstrated by the appearance of bubbles between lens' fibers possibly demonstrating failure of lens tissue to cope with oxygen load. All measured parameters showed that both Zn–DFO and DFO attenuated the oxidative damage. The effect of DFO was small whereas Zn–DFO demonstrated a significantly stronger effect. Treatment of hyperbaric oxygen-exposed lenses with DFO only marginally reduced the oxidative damage. Treatment with Zn–DFO was superior in reducing the oxidative damage to lenses. These results indicate a possible role for Zn–DFO in the prevention of cataracts.

Keywords: oxygen toxicity; eye lens; lens organ culture; hyperbaric oxygen; desferrioxamine; zinc–desferrioxamine; reactive oxygen species; free radicals

1. Introduction

The "*Free Radical Theory of Aging*", revised by Denham Harman (2003), suggests that aging results from accumulation of changes caused by reactions in the body initiated by highly reactive molecules known as "free radicals." Free radicals result in changes, which are believed to be major causes of cell damage, pathology, aging and finally cell death (Rosini et al., 2005).

There are an increasing number of studies that stress the role of free radicals in the development of many chronic, age-related diseases including cataract. Continuous oxidative stress is a known causative factor for reduced lens clarity and cataract formation (Marsili et al., 2004). Oxygen damage

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to lenses is thought to be mediated through reactive oxygen species (ROS). These are highly reactive compounds that alter lens configuration possibly by lipid peroxidation (Babizhayev et al., 2004). A major route for ROS formation is the Fenton reaction, which converts oxygen into toxic free radicals, the most important of which are the powerful hydroxyl radicals (OH•). Metal ions participate in this reaction by undergoing univalent reduction (Korbashi et al., 1986). Iron is a key catalyst in this reaction and as such plays a major role in oxidative tissue damage. This fact has led researchers to undertake efforts to diminish the catalyzing ability of iron in order to decrease toxic ROS formation. Desferrioxamine (DFO) is a compound capable of chelating the iron (Zigman et al., 1995; Avunduk et al., 1999). DFO has been used in several lens studies as a means of reducing tissue oxidative stress (Bhuyan et al., 1991; Reddan et al., 1992, 1999; Wang et al., 1992; McGahan et al., 1994).

Zinc–desferrioxamine (Zn–DFO) is a novel metal complex that inhibits the catalysis of iron (and copper) in the formation of free radicals. The protective effects of Zn–DFO can be interpreted as the removal of redox active iron that is responsible for the production of the hydroxyl radicals through chelation by the DFO component and replacing the iron in the catalytic binding sites with the relatively inert zinc ion that is liberated during the exchange of iron within the complex (Chevion, 1991; Karck et al., 2001). The spatial structure of this complex is markedly different from that of DFO alone, enhancing its ability to penetrate cells and tissues (Banin et al., 2003) and allowing it to reach higher effective concentrations within tissues and cells. To the best of our knowledge, the ability of Zn–DFO to protect lenses from oxidative stress has not been previously examined.

Young lenses allow transmission of light rays with almost no decrease in their intensity, and are able to focus these rays on a single point. Older lenses lose their ability to focus the light rays on a single point (scattering) and become increasingly opaque. This aging process of the lens ultimately leads to cataract formation.

The intensity of light can be measured before light rays enter the lens and just after leaving it. The range of intensity loss across the lens is a good estimation of lens clarity (Wahlman et al., 2003).

The variation in focal point for light rays can be measured and represents the focusing ability of the lens. Young lenses typically focus all rays at one point and hence show no variation in focal distance, while older lenses scatter the rays producing variation in focal distance. In fact, this method has proved to indicate the occurrence of lenticular damage even before any opaqueness could be observed (Sivak et al., 1990; Kuszak and Al-Ghoul, 2002; Schaal et al., 2003). In a previous study we have used focal lens variability to demonstrate oxidative damage to cultured lenses exposed to HBO (Schaal et al., 2003). The aim of the present study was to demonstrate the oxygen-induced structural and functional damage and to investigate the ability of DFO and Zn–DFO to protect lenses from the adverse effects of oxygen.

2. Materials and methods

2.1. Experimental groups

One hundred and fifty bovine lenses were included in the present study. Lenses were divided into 3 study groups and 3 control groups of 25 lenses each. Lenses' culture medium was changed into phosphate buffered saline (PBS) during exposure to HBO (experimental groups 1a, 2a, and 3a) or to room air (control groups 1b, 2b, and 3b).

(1a) *Hyperbaric oxygen (HBO) exposure group:* 25 lenses exposed daily to HBO for 4 days. Each exposure session consisted of 120 min 100% oxygen in a pressure chamber at 2.5 ATA. This group will be referred to as the "HBO" group.

(1b) *Control group 1:* 25 lenses extracted from the contralateral eyes of study group 1 and exposed to normal room air (1 ATA, 160 mm Hg oxygen partial pressure) for 120 min, in PBS.

(2a) *HBO exposure group with DFO:* 25 lenses exposed daily to HBO for 4 days. Each exposure included 120 min of 100% oxygen in a pressure chamber at 2.5 ATA. During HBO exposure lenses were cultured in the presence of DFO 2.5 mg/l. This group will be referred to as the "HBO/DFO" group.

(2b) *Control group 2:* 25 lenses extracted from the contralateral eyes of study group 2 exposed to normal room air (1 ATA, 160 mm Hg oxygen partial pressure) in the presence of DFO 2.5 mg/l for 120 min.

(3a) *HBO exposure group with Zn–DFO:* 25 lenses exposed daily to HBO for 4 days. Each exposure included 120 min of 100% oxygen in a pressure chamber at 2.5 ATA. During HBO exposure lenses were cultured in the presence of Zn–DFO 2.5 mg/l. This group will be referred to as the "HBO/Zn–DFO" group.

(3b) *Control group 3:* 25 lenses extracted from the contralateral eyes of study group 3 exposed to normal room air (1 ATA, 160 mm Hg oxygen partial pressure) in the presence of Zn–DFO 2.5 mg/l for 120 min.

The 4 sessions of exposure to HBO or room air took place at the beginning of the culture period starting at the second day of the culture. Each lens was exposed once each day on days 2–5 of the culture. Lens optical quality was assessed daily throughout the 14 days of the culture period. Lenses were examined by inverted microscopy on days 4, 8 and 14 of the culture period and the results were documented by digital photography.

2.2. Lens organ culture system

Lenses were excised from 1-year-old male calves' eyes obtained from an abattoir under sterile conditions, 2–4 h after enucleation. Each lens was placed in a glass and silicon rubber chamber containing 24 ml of culture medium (M 199) with Earl's balanced salt solution, supplemented with 5.96 g/L