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Hyperbaric Induction of Ocular Hyperuricosis

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Ehler WJ, Bonney CH, Lam K-W, Cissik JH. Hyperbaric induction of ocular hyperuricosis. J Hyperbaric Med 1987; 2(2):69–73.—Hyperbaric oxygen (HBO) treatment in humans has been associated with visually impairing changes in the lens of the eye. In this study the source of nutrition for the lens, the aqueous humor, was analyzed from 27 New Zealand white rabbits exposed to 2.4 ATA of 95 to 96% oxygen for 90 min for a total of 60 exposures. Ascorbate levels did not change significantly, whereas urate levels were significantly increased ($P < 0.001$). An alteration of aqueous humor biochemistry related to HBO is a new finding and may be related to the lens changes reported in HBO patients.

hyperuricosis, aqueous humor, ascorbate, uric acid

Introduction

Multiple exposures to hyperbaric oxygen (HBO) have been reported to cause the development of myopia with or without the formation of nuclear cataracts (1, 2). Myopia is an immediate response and is considered to be due to reversible changes occurring in the lens of the eye while the nuclear cataract development is a delayed (6 mo. to 1 yr) and nonreversible phenomenon (1). Other ocular structures are known to be affected by HBO. Experimental exposures to HBO will produce changes in the corneal endothelium in guinea pigs (3) and in the lens epithelial in both guinea pigs and mice (4).

The maintenance of intraocular pressure and the nutrition of the cornea and the lens depend on the formation and composition of the fluid filling the anterior segment, the aqueous humor. In this study, aqueous humor was taken from rabbits after exposure to HBO and analyzed for ascorbate and uric acid.

Methods and Materials

Adult, mixed-sex, New Zealand white rabbits in a weight range of 2 to 3 kg were used. Twelve rabbits were in the control group and were exposed to ambient air only; 15 rabbits were in the treatment group. The treatment group was exposed to 2.4 ATA of 95 to 96% oxygen for 90 min for a total of 60 dives.

The rabbits, in individual wire cages, were placed into a Rheem diving chamber. No medications were administered. The chamber was flushed with 100% oxygen until an oxygen concentration of 95 to 96% was reached. This took approximately 25 to 30 min to attain. When the final oxygen concentration was reached, the chamber pressure was increased to 45 ft of sea water (13.72 m). At this point the timing of the dive began. Oxygen concentration within the chamber was monitored with a Beckman oxygen analyzer. The relative humidity within the chamber was kept in the range of 35 to 60%. After a 90-min exposure, the chamber was depressurized in approximately 10 min. This procedure was repeated daily, 5 d a wk, until a total of 60 dives had been conducted. No animals received more than 1 dive/d.

Collection of aqueous humor samples was conducted in the following manner: anesthesia was induced with an i.m. injection of ketamine (200 mg/kg) and topical anesthetic was applied to the corneas (1% proparacaine). Samples were collected by paracentesis, under a Zeiss surgical microscope at $\times 25$, using a 30-gauge needle attached to a 1-ml syringe. Aqueous humor was taken from each eye, and the syringes immediately placed on ice and maintained for analysis. Thus the statistical analysis of the control group was based on 24 samples and the treatment group on 30 samples.

The aqueous humor was analyzed for uric acid and ascorbate levels using high performance liquid chromatography (HPLC). The column was a microBondapak-NH_2 column for the separation of biochemical constituents in aqueous humor. The column was equilibrated in 10 m*M* NH_4–H_2–Po_4 and eluted with the same buffer. One microliter of standard ascorbate solution (1 to 2.0 mmol/liter) was injected into the column to identify the retention time of ascorbate and the relationship of UV absorption peak height to ascorbate concentration. Based on experience, the UV absorbance is directly proportional to ascorbate concentration between 0.05 and 10.0 m*M*. An aliquot of 1 μl of aqueous humor was injected into the column and eluted in the same way as the standard. The eluate was analyzed at 254 and 280 nm by a UV monitor (Waters Associates, model 441). The UV absorption peak height in the location of ascorbate was compared to the peak height of the standard ascorbate solution to calculate the ascorbate concentration of the sample.

Results

None of the animals was found to have lens opacities as viewed at the time of aqueous humor sample collection.

The control animals showed a mean ascorbate level of 22.83 mg/dl ($\pm$ 1.12) as compared to the treatment group value of 19.76 mg/dl ($\pm$ 1.14). The mean uric acid level for the control group was 0.12 mg/dl ($\pm$ 0.02) and 0.32 mg/dl ($\pm$ 0.06) for the treatment group (Table 1). The control and treatment group means were analyzed using the two-tailed *t* test. No significant difference was found between the ascorbate levels for the control and treatment groups. The mean uric acid levels were found to be significantly different ($P < 0.001$).

Table 1: Mean Ascorbate and Uric Acid Levels in Rabbit Aqueous Humor

	n	$\bar{X}$, mg/dl	SEM
Control group			
Ascorbate	24	22.83	1.12
Uric acid	24	0.12	0.02
Treated group			
Ascorbate	30	19.76	1.14
Uric acid	30	0.32	0.06

Discussion

The work was not designed to study nuclear cataracts. HBO exposures, as used in this study, might show such changes if the animals were held for a number of months.

Ascorbate

It is thought that the eye maintains its concentration of ascorbic acid through blood flow to the ciliary processes (5). In this study, no significant change occurred in the ascorbate level, indicating that the blood flow to the ciliary processes was not reduced appreciably.

Uric Acid

In man, uric acid is the final metabolic product of the metabolism of the purines, adenine and guanine. These compounds may be found within cells linked to desoxyribose-5-phosphate, forming two of the four basic units of chromosomal DNA, and they combine with ribose-5-phosphate to form two of the structural units of RNA. Hormonal effects are mediated by cyclic AMP which contains these purines. Purines may be liberated by enzymatic degradation of tissue (6).

Normally, aqueous humor contains only trace amounts of uric acid (7). The initial report of significant levels of uric acid in aqueous humor was from eyes with glaucoma that had been treated medically for years before surgery (8). The question raised by the initial discovery of uric acid in aqueous humor addressed the source of uric acid and its role in either the physiology of the eye or the pathology of glaucoma. In an experimental model of hyperuricemia, Yonetani et al. (9) found evidence that production of uric acid was associated with stimulation of alpha adrenergic receptors by the systemic administration of epinephrine. Similar data for alpha receptors in the eye do not exist. The source of intraocular uric acid has been addressed in two previous experimental studies. In the first, hyperuricemia was produced in the rat, and aqueous humor was sampled; no significant elevation of uric acid was found (10). A second study evaluated epinephrine topically applied to the cornea of

rabbits (11). The rabbit aqueous humor samples were found to have a significant elevation of uric acid. These two studies indicate a blood-aqueous barrier to uric acid, and that the uric acid arises from reactions within the eye.

Uric acid within the eyes of rabbits treated with HBO is a new finding. Aqueous samples were taken several hours after the last dive. The mean values reported in this study may represent lower figures than actually produced. A diminished value could be the result of either enzymatic activity or uricase, which would degrade uric acid, or an intraocular turnover. Washout of the uric acid by the constant formation and outflow of aqueous humor would produce a decay of the uric acid unless it was being added to the aqueous humor on a continual basis. In the case of HBO, it is not known whether the appearance of uric acid is related to the increase in tissue oxygenation or to a regional ischemia secondary to the oxygen vasoconstrictive effects on blood vessels and resulting decreased perfusion (12). Nor is it yet known if the production of uric acid is from altered biochemistry or from cell death. If the former is the cause, it is suspected that it would be a transient event, perhaps with permanent ocular tissue changes.

In neither the pharmacologically produced nor the HBO-produced ocular hyperuricosis is the presence of uric acid understood in terms of ocular physiology. The known changes, myopia and nuclear cataracts, may be related to changes in the composition of the aqueous humor.

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