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Enzyme-induced posterior vitreous detachment in the rat produces increased lens nuclear pO_2 levels☆

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ABSTRACT

It has been proposed that disruption of normal vitreous humor may permit O_2 to travel more easily from the retina to the center of the lens where it may cause nuclear cataract (Barbazetto, I.A., Liang, J., Chang, S., Zheng, L., Spector, A., Dillon, J.P., 2004. Oxygen tension in the rabbit lens and vitreous before and after vitrectomy. Exp. Eye Res. 78, 917–924; Harocopos, G.J., Shui, Y.B., McKinnon, M., Holekamp, N.M., Gordon, M.O., Beebe, D.C., 2004. Importance of vitreous liquefaction in age-related cataract. Invest. Ophthalmol. Vis. Sci. 45, 77–85). In the present study, we injected enzymes intravitreally into guinea pigs (which possess an avascular retina) and rats (which possess a vascular retina) to produce either vitreous humor liquefaction plus a posterior vitreous detachment (PVD) (with use of microplasmin) or vitreous humor liquefaction only (with use of hyaluronidase), and 1–2 weeks later measured lens nuclear pO_2 levels *in vivo* using a platinum-based fluorophore O_2 sensor (Oxford-Optronix, Ltd.). Experiments were also conducted in which the animals were allowed to breathe 100% O_2 following intravitreal injection with either microplasmin or hyaluronidase in order to investigate possible effects on O_2 exchange within the eye. Injection of guinea pigs with either of the two enzymes produced no significant differences in lens pO_2 levels 1–2 weeks later, compared to controls. However, for the rat, injection of microplasmin produced a 68% increase in O_2 level in the center of the lens, compared to the controls (5.6 mm Hg increasing to 9.4 mm Hg, $p < 0.05$), with no corresponding effect observed following similar use of hyaluronidase. Treatment of guinea pigs with microplasmin dramatically accelerated movement of O_2 across the vitreal space when the animals were later allowed to breathe 100% O_2 (for example, O_2 traveled to a location directly behind the lens 5× faster than control; $p < 0.01$); however, the effect following treatment with hyaluronidase was significantly less. When microplasmin-injected rats breathed 100% O_2, the time required for O_2 to reach the center of the lens was 3× faster than control (0.4 min compared to 1.4 min, $p < 0.01$). The results have implication with regard to the occurrence of age-related PVD in the human, and a possible acceleration of maturity-onset nuclear cataract. In addition, enzymatic creation of a PVD to increase the rate of O_2 exchange within the vitreal space may have potential application for treatment of retinal ischemic disease.

1. Introduction

The past few years have been marked by heightened interest in the measurement of O_2 levels in vitreous humor and lens, and the linking of increased levels of O_2 in the lens nucleus with formation of cataract. Two groups have recently reported partial pressures of O_2 existing in the nucleus of the normal bovine lens *in vitro* (1.5 mm Hg) (McNulty et al., 2004) and the rabbit lens *in vivo* (10.4 mm Hg) (Barbazetto et al., 2004). O_2 levels have been found to climb in the vitreal space following vitrectomy surgery in both the rabbit (Barbazetto et al., 2004) and the human (Holekamp et al., 2005), and vitrectomy has long been known to induce nuclear cataract (Cherfan et al., 1991; Margherio et al., 1985; Michels, 1984). A hypothesis was presented nearly 20 years ago that vitrectomy-induced nuclear opacification is caused by an infusion of O_2 into the vitreal space, leading to oxidation of lens proteins (Ogura et al., 1991). It is also known that exposure of experimental animals or human patients to elevated pressures of O_2 can induce loss of transparency in the lens nucleus (Gesell and Trott, 2007; Giblin et al., 1995; Palmquist et al., 1984; Schocket et al., 1972), and oxidation of lens proteins is recognized as being strongly associated with nuclear cataract (Simpanya et al., 2005; Truscott, 2005). Recently, an association between age-related vitreous humor

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liquefaction and human nuclear cataract was demonstrated, and the proposal made that degeneration of the vitreous gel may allow more O_2 from the retinal circulation to reach the lens, causing nuclear cataract (Beebe, 2008; Harocopos et al., 2004). Our laboratory has shown that enzymatic liquefaction of vitreous humor, combined with a posterior vitreal detachment (PVD), causes an increase in pO_2 levels in the mid-vitreous humor of rats and cats (Quiram et al., 2007). However, to our knowledge, experiments have not been conducted previously to determine whether enzymatic liquefaction of vitreous plus a PVD will lead to increased lenticular O_2 levels.

There is also considerable interest in comparing rates of O_2 exchange and O_2 diffusion in normal and liquefied vitreous humor. Vitrectomy surgery is known to improve oxygenation of the inner retina, and thus this surgery can relieve retinal hypoxia in cases of diabetic retinopathy and other ischemic retinopathies (Stefansson et al., 1990; Stefansson and Loftsson, 2006). Experiments conducted by Maurice (1959) and Barton et al. (2007) have indicated that the rate of diffusion of molecules in intact vitreous gel is about the same as that in water. Any observed increase in the rate of redistribution of molecules in liquefied vitreous has been attributed by these investigators to an increase in fluid circulation. However, Stefansson and Loftsson (2006) have suggested that, based on the Stokes–Einstein equation and previously reported measurements of the viscosity of vitreous humor (Lee et al., 1992), rates of diffusion in vitreous should increase many-fold upon liquefaction.

In this study, we have liquefied vitreous humor of rats and guinea pigs by intravitreal injection of either of two enzymes, microplasmin or hyaluronidase. Microplasmin is a recombinant protein, possessing a molecular mass of 28 kDa and containing the catalytic domain of human plasmin (Nagai et al., 2003). Intravitreal injection of microplasmin is known to produce vitreous humor liquefaction, as well as a PVD (Gandorfer et al., 2004), whereas injection of hyaluronidase causes only vitreous liquefaction (Hikichi et al., 2000). Plasmin is a serine protease which hydrolyzes various glycoproteins including laminin and fibronectin which are important for vitreoretinal attachment (Gandorfer et al., 2004). Both microplasmin and hyaluronidase have been shown to be non-toxic to the retina (Gandorfer et al., 2004; Gottlieb et al., 1990; Sakuma et al., 2005). In the current study, we have made *in vivo* measurements of lens nuclear O_2 levels in guinea pigs and rats having either normal or enzymatically liquefied vitreous humor. Guinea pigs, in contrast to rats, possess a naturally avascular retina (Cringle et al., 1996), such that the partial pressure of O_2 in the innermost regions of the retina (at the interface of the retina and vitreous humor) is nearly zero, whereas at the same location in the retina of the rat, the pO_2 level is about 22 mm Hg (Yu and Cringle, 2001). In this study, we have allowed guinea pigs and rats to breathe 100% O_2 and determined whether liquefaction of the vitreous humor, or liquefaction combined with a PVD, can act to accelerate the time required for O_2 molecules to reach the center of the lens.

2. Materials and methods

The following species of animals were used in this study: cats (female, 4–5 months old, Liberty Laboratories, Waverly, NY, USA), Brown Norway rats (male, 9–10 months old, 400 g, Charles River Laboratories, Wilmington, MA, USA), New Zealand white rabbits (2 kg, Kuiper Rabbit Ranch (Indianapolis, IN, USA)) and Hartley guinea pigs (male, 16–17 months old, Kuiper Rabbit Ranch). All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the Oakland University animal care committee.

2.1. pO_2 measurement

Measurement of pO_2 in vitreous humor and lens *in vivo* was accomplished in anesthetized animals (employing ketamine and xylazine as anesthetics) using a fiber optic detection system (Oxylab, Oxford-Optronix, UK), as described previously by us (Quiram et al., 2007) and other vision researchers (Holekamp et al., 2005; McNulty et al., 2004; Shui et al., 2006). The O_2-sensing probe consisted of a 250-μm-wide optic fiber with a platinum-based fluorophore at the tip. Since O_2 is not consumed by the probe during analysis, it is possible to make accurate measurements of pO_2 from 0 to 100 mm Hg. The pO_2 resolution of the probe is 0.1 mm Hg, and the measurements are insensitive to sample viscosity (McNulty et al., 2004). Probes arrived pre-calibrated by the company; however, calibrations were routinely checked before and after use using a 3% solution of sodium sulfite ($pO_2 = 0$ mm Hg), as well as water bubbled with 1, 3 and 5% O_2 (pO_2's = 7.6, 22.8 and 38.0 mm Hg, respectively).

For pO_2 measurements made with cats and rabbits, anesthetized animals were intubated and maintained by a ventilator on room air. Measurements with anesthetized guinea pigs and rats were made while the animals breathed room air without use of a ventilator. In certain experiments, measurements were made with guinea pigs and rats while the animals breathed 100% O_2 delivered with the use of a face mask. For delivery of the probe into the vitreous space or into the center of the lens, a 25-gauge needle was used to first create an insertion track behind the limbus and through the conjunctiva and sclera. However, for the lens, in order to avoid introducing oxygen into the tissue artifactually, a needle was not used to create an insertion track in the lens tissue itself. Instead, the probe was inserted directly into the lens at the equator, and then advanced to the lens center. No difficulty was experienced with insertion of the probe into the lens centers of any of the species studied. For rabbits, cats, and guinea pigs, pO_2 measurements were made in the center of the lens and in two locations in the vitreous humor, including close to the retina over a retinal vessel, and directly behind the lens. For rats, pO_2 measurements in the current study were made only in the center of the lens.

2.2. Intravitreal injection of enzymes

The enzymes hyaluronidase (bovine, Sigma–Aldrich, St. Louis, MO, H3631) and microplasmin (human recombinant, obtained as a gift from ThromboGenics, Ltd., Dublin, Ireland) were injected intravitreally into guinea pigs [25 IU hyaluronidase in 20 μl; 0.6 units microplasmin (0.05 mg) in 20 μl] and rats [12.5 IU hyaluronidase in 10 μl; 0.3 units microplasmin (0.025 mg) in 10 μl]. The injections were carried out under an operating microscope into the mid-vitreous cavity of anesthetized animals with use of a 30-gauge needle. The injections were well-tolerated and gave no evidence of anterior segment inflammation, vitritis or increase in intraocular pressure. Experiments conducted with sham intravitreal injections of 20 μl saline into guinea pigs showed no increase in vitreal pO_2 levels, measured 1–2 weeks later, as compared with vitreous humor of non-injected control animals.

2.3. SEM analysis

Scanning electron microscopy (SEM) analysis was conducted to evaluate the vitreoretinal interface for the presence of a PVD in eyes of intravitreally-injected guinea pigs and rats. Eyes were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, overnight. Corneas and lenses of the fixed eyes were removed one day later and the whole eye cup with the retina and vitreous humor intact was fixed for one additional day, washed with 7% sucrose, dehydrated in ethanol, dried to the critical point,