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Alterations in human vitreous humour following cataract extraction

R.E. Neal*, F.A. Bettelheim, C. Lin, K.C. Winn, D.L. Garland, J.S. Zigler Jr.

National Eye Institute, National Institutes of Health, 7 Memorial Drive, MSC 0703, Bethesda, MD 20892-0703, USA

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This paper is dedicated to the memory of Dr Frederick A. Bettelheim, a distinguished researcher, teacher, and mentor.

Abstract

Cataract extraction is associated with the risk of posterior vitreous detachment, macular edema and retinal detachment possibly as a result of a disturbance to the vitreous body during surgery. While it is common for lens cortical fiber debris to leak into the vitreous humour during cataract extraction, the extent to which the vitreous humour is altered post-surgery is unknown. The current study examines the integrity of the vitreous humour of pseudophakic and phakic human donor eyes by comparing the proteome, the viscosity and the size distribution of macromolecules in different regions of the vitreous humour from human pseudophakic and phakic donor eyes. Major differences between the proteomes of anterior and posterior vitreous humour were observed in phakic and pseudophakic donor eyes. Seventeen spots identified as complete, modified or cleaved forms of αA-, αB-, βA_4-, βB_2, and γS-crystallins were present in the anterior vitreous humour of all pseudophakic eyes studied. Crystallins were not detected in the posterior vitreous humour of the pseudophakic eye or the vitreous humour of the phakic eye. Significant alterations in abundance and/or modification of transthyretin, alpha antitrypsin, and retinoic acid binding protein were observed in all locations of pseudophakic vitreous humour as compared to phakic samples. In addition, a significant decrease in the number and intensity of protein spots was observed for the posterior vitreous humour of pseudophakic eyes when compared to posterior vitreous humour of phakic eyes. Proteins which were affected include antioxidant proteins and enzymes such as carbonic anhydrase and trisephosphate isomerase. A reversal of the viscosity gradient, anterior to posterior, in the vitreous humour of pseudophakic eyes was observed concomitant with alterations in the distribution of 50 nm particles. These particles are likely primarily composed of hyaluronan. While varying degrees of vitreous degradation may have existed prior to surgery and may have contributed to the cataract formation, in no case did the phakic donor eyes exhibit the same alterations in the vitreous humour proteome, viscosity or particle sizes as did the pseudophakic donor eyes. The examination of phakic/pseudophakic donor eye pairs confirmed that the vitreous humour proteome and structural integrity were very similar in the matched phakic donor eye to eyes from donors with no history of cataract. Even though the number of samples for this study was limited, the observed changes support the hypothesis that alterations in the vitreous humour proteome occur in psuedophakic eyes with concurrent alterations in the structure of the vitreous humor. These modifications of the microenvironment of the retina may contribute to the development of retinal complications following cataract surgery.
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Keywords: phacoemulsification; proteome; vitreous; crystallin; dynamic light scattering; viscosity; cataract; surgery

1. Introduction

The vitreous body serves as a reservoir of nutrients for the lens and prevents the invasion of extra-ocular cells and proteins (Bishop, 2000). The mammalian vitreous body is composed of a network of collagen fibrils, interspersed with proteoglycans and glycosaminoglycans such as hyaluronan. These components collectively maintain the gel structure, viscosity, and transparency of the vitreous humour. In phakic donor eyes, the concentration of hyaluronan near the lens is lower than near the retina (Bettelheim and Zigler, 2004; Balazs and Denlinger, 1984; Sebag, 1998); however, the hyaluronan molecular size near the lens is larger. The net result is a higher total viscosity environment near the lens relative to the retina (Osterlin, 1977). The hyaluronan hydration layer is the main storage site for bound water in the vitreous humour (Bettelheim and Popdimitrova, 1992).

* Corresponding author. Dr R. E. Neal, Protein Biochemistry, National Eye Institute/National Institutes of Health, 7 Memorial Drive, MSC 0703, Bethesda, MD 20892-0703, USA.
E-mail address: nealr@nei.nih.gov (R.E. Neal).

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With aging, the bound water converts to free water due to syneretic processes leading to decreased viscosity of the hyaluronan, and thus decreased viscosity of the vitreous gel. Aging may also lead to alterations in the thickness of vitreous fibers, the vitreous volume, and collapse of the vitreous gel (Sebag, 1987). A recent study probing the association of vitreous liquefaction with cataract has documented an age dependent correlation of increased vitreous body liquefaction with nuclear cataracts, but not cortical or posterior subcapsular cataract, in human donor tissue (Harocopos et al., 2004).

The vitreous humour proteins are either serum derived, such as albumin; or synthesized within the eye by the ciliary body and retinal pigment epithelium, such as transthyretin (Bishop et al., 2002). The vitreous humour protein concentration is somewhat elevated in cataractous eyes (Watanabe et al., 1990). The abnormal presence of proteins in the vitreous humour has been implicated in inflammatory responses (Kim and Miller, 2002). In addition, pseudophakia has been linked to retinal detachment possibly through vitreous traction and alterations in the macromolecular composition of the vitreous humour (Osterlin, 1977). Thus, alterations in both the complement of vitreous humour proteins and biochemical properties of the vitreous humour have been linked to ocular disease.

Surgery is presently the only cure for vision loss due to cataract formation. With an increase in visual acuity for over 98% of patients and an immediate surgical complication rate below 5%, cataract surgery is a routine outpatient procedure (Lois and Wong, 2003). Standard surgical procedures include the removal of the lens material leaving the capsular bag intact and insertion of an artificial lens into the capsular bag creating a pseudophakic eye. Two of the most common surgical complications, posterior capsule rupture (approximately 4% of surgeries) and lens material dislocation (0·2% of surgeries) are risk factors for the development of uveitis and vitreous humour opacification, cystoid macular edema, glaucoma, and retinal detachment (Hansson and Larsson, 2002; Stefaniotou et al., 2003; Horiguchi et al., 2003). The most common delayed post-surgical complication (15–50% of surgeries) is posterior capsule opacification (Landers and Perraki, 2003). Nd:Yg laser treatment for this condition increases the risk of retinal breaks and detachment by 4-fold. Retinal detachment is also four to five times more likely in cases when vitreous humour is lost during phacoemulsification or when anterior vitrectomy is performed concurrent with cataract extraction (Landers and Perraki, 2003). Cataract surgery has been shown to decrease the concentrations of hyaluronan and alter the concentration gradient of hyaluronan (Sebag, 1987). It appears from these previous studies that disturbance of the vitreous humour integrity can have a serious impact on ocular health.

Recent studies have documented the presence of over 500 protein spots in the vitreous humour proteome which include multiple post-translationally modified forms of metabolic, antioxidant, angiogenic, and immune modulatory proteins (Bresgen et al., 1994; Koyama et al., 2003; Yamane et al., 2003; Nakanishi et al., 2002). These studies compared the vitreous humour proteome of patients with diabetic retinopathy and macular hole; unfortunately, a direct comparison to the normal vitreous humour proteome is unavailable. No study to date has examined alterations in the vitreous humour proteome following cataract surgery.

The current study aims to explore whether significant alterations in the vitreous humour proteome occur in human pseudophakic eyes as compared to human phakic donor eyes. This study also examines the spatial variation in the particle size distribution and the viscosity to examine whether the normal gel structure gradient is compromised in the pseudophakic donor eyes. Phakic/pseudophakic donor pairs aided in the determination of pre- and post-surgical alterations in the vitreous humour.

2. Materials and methods

Two pairs of phakic/pseudophakic donor globes (aged 77, 78), one pseudophakic globe (age 47) and five phakic donor eyes (aged 17 {left and right}, 66, 74, 77) were frozen on dry ice within 4–7 hr post-mortem. Donor history as to the type and degree of cataract as well as time since surgery was unavailable. The phakic eyes from the pseudophakic /phakic matched donor sets did not have visually evident cataracts however, an extensive examination of these lenses for optical clarity was not undertaken. The pseudophakic eyes contained IOL implants. Vitreous humour was dissected while still frozen and divided into six regions, three adjacent to retina and three in the vicinity of lens (Bettelheim and Zigler, 2004) without nasal to temporal location noted. Just prior to analysis, samples from each region were thawed and centrifuged at 14 000 rpm (Eppendorf, 5415) for 25 min at 4°C. We define the resulting supernatant samples as vitreous fluid.

Viscosity coefficients of whole vitreous from two sets of phakic/pseudophakic (77 and 78 years old) and one pseudophakic globe (47 years old) and several phakic donor eye pairs (74 and 77 years old) were obtained in a Cannon-Ubbelhode Calibrated Semi-Micro Viscometer measuring sample efflux times. The vitreous collected was diluted since the volume obtained was insufficient for direct measurement. Efflux times were obtained with three different dilutions (between 2·5- and 10-fold dilution) for each sample. The averaged values were plotted against dilution ratios and since the plots were linear, the original efflux time of the undiluted vitreous was determined by extrapolation to 1. In essence, we had a working definition for the viscosity of vitreous as the efflux time of the sample. From the ratio of the extrapolated efflux time of a sample to that of water, we obtained a relative viscosity coefficient in stokes. Viscosity coefficients are dependent upon the concentration of the macromolecules present.