INTRODUCTION

Understanding the pathogenesis of damage to the aqueous outflow pathway is important to prevent and treat glaucoma. Our previous studies in human eyes undergoing cataract and/or glaucoma surgery indicated increased intraocular molecular oxygen (\(pO_2\)) in vivo in eyes following vitrectomy and lens extraction. This increase of \(pO_2\) may be a source of reactive oxygen species leading to oxidative damage to the trabecular meshwork (TM). We hypothesized that increased \(pO_2\) in the region of the anterior chamber angle and TM would increase oxidative stress and lower antioxidant protection leading to damage or death of TM cells, thus initiating glaucoma development. In order to understand these pathological changes of the outflow pathway, we planned to establish an animal model of post-vitrectomy/lens extraction to replicate the altered oxygen environment documented in human patients. We successfully established this model in rabbits, but access to nonhuman primates is limited. In this study, we will assess whether rabbits may be a suitable animal model candidate for these intracocular surgeries. This information may lead to further studies of the effects of oxidative damage on TM cells and investigation of therapeutic interventions to prevent such damage.

DESIGN & METHODS

Experimental design: All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the approval of the Animal Studies Committee of Washington University School of Medicine. Eighteen adult Dutch belted rabbits underwent complete ocular examination prior to the experiment. The rabbits were divided into two groups and one eye will undergo surgery: 9 rabbits underwent partial vitrectomy (Lx-PPV). The fellow control eye underwent sham procedures.

Surgical procedures: Group 1: Partial vitrectomy (p-PPV). Modification of standard pars plana vitrectomy for Group 2 was not successful due to 1) large rabbit lens was difficult to remove without resulting in retinal detachment and/or excessive inflammatory reaction, 2) ProCare Plus Vitrectomy System used in this study did not effectively perform the lens extraction due to the density of the lens and poor function of the instrumentation. This surgical procedure was ceased following unsuccessful attempts with three rabbits. The observation periods were one month, 6 months and one year.

RESULTS

Surgery: Following the animal protocol, nine rabbits (Group 1) successfully underwent p-PPV on their right eyes. Post-surgery care including slit lamp, IOP and fundus measurements were performed and all rabbits recovered without any complications. However, lens extraction plus vitrectomy (Lx-PPV) for Group 2 was not successful due to large rabbit lens was difficult to remove without resulting in retinal detachment and/or excessive inflammatory reaction.

RNA extraction and qPCR analysis: Total RNA was extracted from each sample using a Qiagen RNeasy Microkit (Qiagen Technologies, Germantown, MD), reverse transcribed and amplified using the One Step Plus RT-PCR Kit (Qiagen Technologies, Neuss, Germany). qRT-PCR analysis was performed using a SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich, Saint Louis, MO) and an ECO Real-Time PCR System (Illumina, San Diego, CA) on 1.65 ng/μl of total RNA. qPCR primers are shown in Table 1.

CONCLUSIONS AND FUTURE STUDIES

1. The rabbit is not an ideal animal model for pars plicata vitrectomy/lens extraction procedures and induction of oxidative stress in the trabecular meshwork. Anatomical differences of the rabbit preclude replication of human procedures and physiological conditions of human patients.
2. Oxygen measurements in rabbit eyes also did not replicate the alterations noted in primates following vitrectomy surgery.
3. We were able to perform qPCR on trabecular meshwork tissue, identifying upregulation of ELAM1, a promising finding in this study and consistent with findings in the TM of patients with glaucoma.
4. Future studies will focus on identification of a more suitable large animal model to successfully perform this surgical intervention and determine if this can be a validated model of oxidative stress, an important factor in damage of the TM and glaucoma development.

REFERENCES


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