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 (pO₂) in vivo in eyes following vitrectomy and lens extraction. This increase of pO₂ may be a source of reactive oxygen species leading to oxidative damage to the trabecular meshwork (TM). We hypothesized that increased pO₂ 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 mus musculus, but access to nonhuman primates is limited. In this study, we will assess whether rabbits may be a suitable animal model candidate for these intracranial 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.

RESULTS

Surgery: Following the animal protocol, nine rabbits (Group 1) successfully underwent p-PPV surgery on their right eyes. Post-surgery care included slit lamp, IOP and fundus measurements which 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, 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 instrument. This surgical procedure was ceased following unsuccessful attempts with three rabbits. The observation period was one month, 6 months and one year.

Oxygen measurements: At the end of the experiment, intraocular pO₂ was measured as shown in Figure 4, comparing human, monkey and rabbit measurements. During the surgical procedures, intraocular oxygen was maintained at room air oxygen levels (21%) with oxygen saturation (SaO₂) at ~97% simulating normal physiological conditions. There were no significant differences between control and p-PVV eyes at all locations. Comparison of pO₂ measurements at the anterior chamber angle on the vitreous port entrance vs. non-entrance side, revealed a slight but not significant increase in the region of the vitreous port.

TM laser dissection: Following euthanasia, fresh anterior segments were immediately frozen. Laser microdissection of the TM was performed (Figure 3). The specimens were prepared for RNA extraction.

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 Ovation Pico WTA System V2 (NuGEN Technologies, San Carlos, CA). qRT-PCR was performed using SYBR Green JumpStart TaqReadyMix (Sigma-Aldrich, Saint Louis, MO) and an Eco Real-Time PCR System (Ilumina, San Diego, CA) on 1.65 ng/µl of cDNA per sample. PCR primers are shown in Table 1.

REFERENCES

1. The rabbit is not an ideal animal model for pars plana vitrectomy/enucleation 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 identified in primates following vitrectomy surgery.
3. Novel techniques of laser microdissection of the trabecular meshwork were successful to excise this specific tissue for analysis.
4. We were able to perform qPCR on trabecular meshwork tissue, identifying upregulation of ELAM1, an interesting finding in this study and consistent with findings in the TM of patients with glaucoma.
5. 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.

CONCLUSIONS AND FUTURE STUDIES

ACKNOWLEDGMENTS

This project was supported by the Shaffer Grant of the Glaucoma Research Foundation, NEI R01 EY021515, Core grant NEI P30 EY02687 and an unrestricted grant from Research to Prevent Blindness, Inc.