Pathological alterations in the trabecular meshwork following vitrectomy and lens extraction: A model of oxidative stress

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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 hypothesize 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 rhesus macaques, but access to nonhuman primates is limited. In this study, we will assess whether rabbits may be a suitable animal model candidate for these intraocular 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 23-gauge pars plana vitrectomy (p-PPV) in one eye; 9 were planned to undergo concurrent lensectomy and vitrectomy (Lx-PPV). The fellow control eye underwent sham procedures.

Surgical procedures: Group 1: Partial vitrectomy (p-PPV). Modification of standard vitrectomy was required for the unique anatomy of rabbit eyes with its large lens. A 23-gauge p-PPV was performed through a ProCare Plus Vitrectomy System (VisionCare Ophthalmic Technologies, CA, USA). In order to avoid damage to the lens, the vitrector was directed posteriorly leaving the anterior vitreous intact. Approximately 0.5 ml of vitreous was extracted during the procedure. (Figure 1A). Group 2: Lens extraction plus vitrectomy (Lx-PPV). We planned to remove the vitreous and lens concurrently via 23-gauge pars plana vitrector to create a unichamber eye, as in patients following vitrectomy and cataract surgeries (Figure 1B).

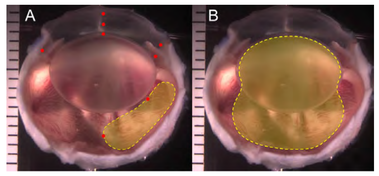

Figure 1. Photos represent actual rabbit eye size. The lens is in the center of the eye and fills approximately 50% of the posterior segment. Yellow regions designate locations of (A) p-PPV and (B) Lx-PPV. The red dots indicate the locations for intraocular oxygen measurements.

Oxygen measurements: Intraocular oxygen levels in the rabbit anterior chamber and vitreous cavity were performed with a fiberoptic probe (Oxylab™, United Kingdom) as in our previous studies with rabbits, monkeys and humans. Oxygen measurement procedures required approximately two minutes.

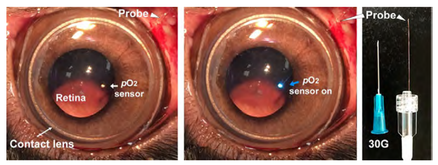

Figure 2. *In vivo* oxygen measurement inside vitreous cavity as visualized through the contact lens (left, center). Fiberoptic probe size compared to 30 G needle (right).

DESIGN & METHODS

Trabecular meshwork laser microdissection: Fresh anterior segment ocular tissue was embedded in optimal cutting temperature media (O.C.T.) and stored at -80^{0}C. Frozen sections of 10 µm thickness were transferred to glass polyethylene naphthalate (PEN) foil slides and stained with Eosin Y. Careful identification of anterior chamber angle structures was performed, outlined and excised by laser microdissection (Figure 3; Leica Microsystems LMD 6000 and CTR 6500, Wetzlar, Germany).

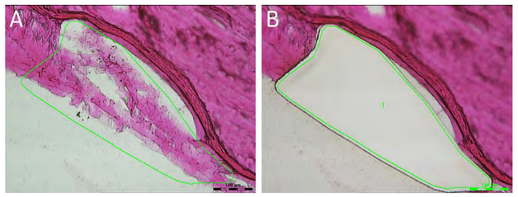

Figure 3. Rabbit TM precisely dissected by laser microdissection technique from anterior chamber angle. A: Eosin-stained anterior chamber angle section and TM region precisely delineated (green). B. Excision of TM tissue with laser microdissection. Bar: 100 µm.

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 amplification was performed using a SYBR Green JumpStart TaqReadyMix (Sigma-Aldrich, Saint Louis, MO) and an Eco Real-Time PCR System (Illumina, San Diego, CA) on 1.65 ng/µl of cDNA per sample. PCR primers are shown in Table 1.

RESULTS

Surgery: Following the animal protocol, nine rabbits (Group 1) successfully underwent p-PPV surgery on their right eyes. Post-surgery care including slit lamp, IOP and fundus measurements was performed and all rabbits recovered without any complications. However, lens extraction plus vitrectomy (Lx-PPV) 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 lensectomy 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.

Oxygen measurements: At the end of the experiment, intraocular pO_2 was measured as shown in Figure 4, comparing human, monkey and rabbit measurements. During the surgical procedures, inhaled oxygen was maintained at room air oxygen levels (21%) with oxygen saturation (SaO_2) at ~97% simulating normal physiological conditions. There were no significant differences between control and p-PPV eyes at all locations. Comparison of pO_2 measurements at the anterior chamber angle on the vitrectomy port entrance vs. non-entrance side, revealed a slight but not statistically significant increase in the region of the vitrectomy 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.

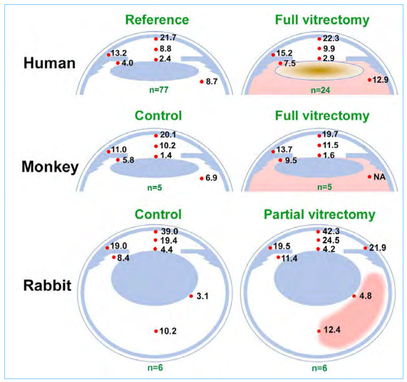

Figure 4. Comparison of pO_2 (mmHg) distribution in human, monkey (unpublished data), and rabbit eyes at noted locations before and after vitrectomy. Human reference group underwent glaucoma/cataract surgery (anterior segment)[1] and patients undergoing pars plana vitrectomy (posterior segment).[2] Red area in the vitreous cavity indicates vitrectomy area.

RESULTS (Cont.)

Gene name	Primer sequence	Product size	Fold change (by ACTB)	Fold change (by GAPDH)
ELAM1 (SELE)	F: 5' GTGTGCAATTTTGCATGTCC 3'	113 bp	282.37	300.78
	R: 5' GCAATGGGTTCTTCACAGGT 3'			
fibronectin	F: 5' TGATTTGGTTTGGGGTGAAT 3'	128 bp	0.31	0.32
	R: 5' GGCCAGAGTTCTGTGAAAGC 3'			
COL1A1	F: 5'CTCCTGGCAAAGATGGACTC 3'	102 bp	0.46	0.11
	R: 5'GAAGGGGGCTACGTACAACA 3'			
COL4A6	F: 5' TTCCCTTTGGGACACTTCCT 3'	150 bp	0.04	0.03
	R: 5' ACTGCTAAACGGCTCTGTGG 3'			
LAMA5	F: 5' CATTCGCTTCGGCTTCAACC 3'	144 bp	0.25	0.22
	R: 5' TTCACGTAGCGGAAGACGAG 3'			
LAMB1	F: 5' CTGATCTGGACAGCATCGAA 3'	116 bp	0.23	0.21
	R: 5' GAGGCTCTCGACTCGTTCAC 3'			
LAMC1	F: 5' CAACCAGGAGCTTCGAGAAC 3'	88 bp	0.22	0.21
	R: 5' GGCCACCATCTCAATGCTAT 3'			
NF-kB	F: 5'TGCGTTCCCACTGTCATTTA 3'	133 bp	0.58	0.59
	R: 5'TGCACACCAAGTGACTGTCC 3'			

qPCR analysis: PCR primers of 8 genes designed for qPCR analysis. Housekeeping genes ACTB and GAPDH were also analyzed. qPCR indicated > 2 fold change only in ELAM1 (SELE).

CONCLUSIONS AND FUTURE STUDIES

1. The rabbit is not an ideal animal model for pars plana vitrectomy/lensectomy 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. Our 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.

References

1. Siegfried CJ, Shui YB, Holekamp NM. et al. Oxygen distribution in the human eye: relevance to the etiology of open angle glaucoma after vitrectomy. Invest Ophthalmol Vis Sci. 2010;51:5731–5738.
2. Holekamp NM, Shui YB, Beebe DC. Vitrectomy surgery increases oxygen exposure to the lens: a possible mechanism for nuclear cataract formation. Am J Ophthalmol. 2005;139:302–310.

ACKNOWLEDGMENTS

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