Investigating Subconjunctival Lymphatics for the Treatment of Glaucoma and Eye Disorders

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Purpose

The subconjunctival space of the eye is often used in the treatment of eye diseases. Drugs can be injected there. However, the drugs disappear or exit the subconjunctival space so that the drug-effects wear off. Glaucoma surgeries open the subconjunctival space to lower eye pressure by allowing fluid inside of the eye to exit. However, these surgeries can fail where the intraocular fluid becomes trapped and can no longer exit the subconjunctival space. Therefore, it is important to understand fluid outflow from the subconjunctival space. If this process can be slowed down, better drug delivery can be developed. If this process can be enhanced, glaucoma surgeries may work better. In this proposal, we proposed to understand the identity and characteristics of subconjunctival outflow. Specific Aim 1: To determine if subconjunctival lymphatics drain blebs. Specific Aim 2: To determine if subconjunctival lymphatics can be manipulated.

Methods

In this study, subconjunctival blebs were created in post-mortem pig and human eyes using various fluorescent tracers (fluorescein, ICG, and fixable/fluorescent dextrans). Live human subjects also received subconjunctival tracer during anesthesia (lidocaine + 0.005% ICG) prior to clinically-indicated intravitreal injections under UCLA IRB approval (20-001064). Outflow pathways were visualized using a combined angiographic camera with OCT (Heidelberg Spectralis HRA+OCT). To study lymphatic manipulation, mice with natively fluorescent lymphatics (Fig.1) (Prox1-EGFP or Prox1-TdTomato) received subconjunctival injections (non-injection control, balanced salt-solution [BSS], vascular endothelial cell growth factor C [VEGFC; 0.36mg/ml, a known lymphatic growth factor], 5-fluorouracil [5FU], and mitomycin C [MMC; 0.25% and 0.5%]). In each case, 3 superior subconjunctival injections were given (every other day) under anesthesia, and the eyes were harvested and fixed in 4% PFA on the 7th day. Anterior segment flat mounts were created and visualized using fluorescent microscopy. Sub-conjunctival lymphatic sprouts, length, or branch number were quantified in a blinded fashion.

Fig. 1 Fluorescent Lymphatic Reporter Mice (A) The nasal and (B) temporal sides of adult lymphatic reporter mouse eyes showing lymphatic sprouts (arrow) and nearby Schlemm's canal (S). C) More lymphatic sprouts were seen on the nasal (medial; me) compared to the temporal side of the eyes (n = 6).

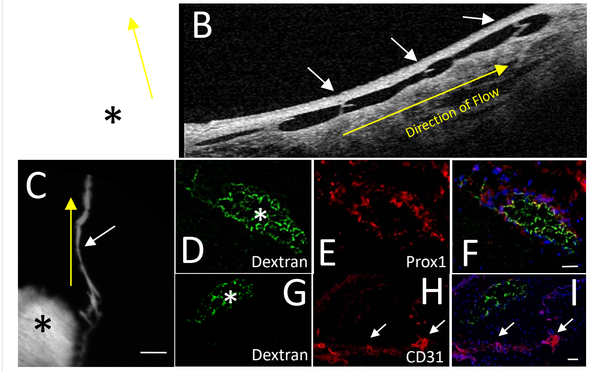

Fig. 2 Subconjunctival Lymphatics Drain the Subconjunctival Space. A/C) Fixable and fluorescent dextrans were injected under pig conjunctiva to make blebs (black asterisks) and outflow pathways (white arrows) having a direction of flow (yellow arrows). B) OCT on the outflow pathway showed bicuspid valves (white arrows) pointing in the direction of flow (yellow arrow). Lymphatics are known to have valves. D/G) The subconjunctival outflow pathways could also be identified on histological sections (white asterisks) owing to the trappable tracer and E-F) co-localized with PROX1 which labels lymphatics, G-I) but not CD31 which labels blood vessels (white arrows). Similar results were seen with podoplanin, another lymphatic marker (data not shown).

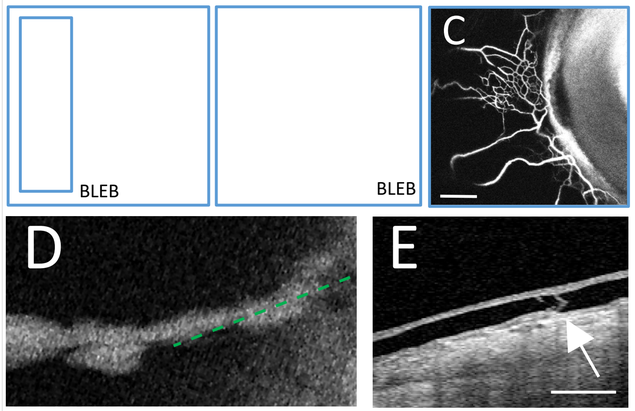

Fig. 3 Live Human Subjects Subconjunctival Outflow A/B/D) Subconjunctival lidocaine and ICG demonstrated a bleb and irregular outflow pathways with sausage-shaped patterns (arrows). C) This was in contrast to aqueous/episcleral vein outflow imaging. D) OCT on the bleb outflow pathway showed a lymphatic-like valve (E; white arrow).

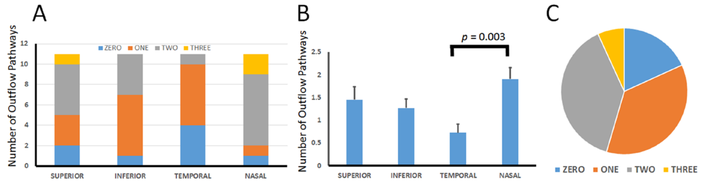

Fig. 4. Quantitation of Subconjunctival Outflow. A) Superior/inferior/temporal/nasal subconjunctival blebs were created in each of 11 porcine eyes (n = 44 blebs total) with the number of outflow pathways counted. B) A statistically significant difference was only seen comparing the temporal to the nasal quadrant, supporting mouse data (see Fig. 1). C) The distribution of the number of pathways across all blebs.

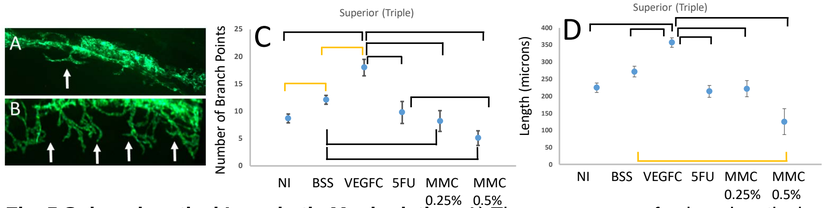

Fig. 5 Subconjunctival Lymphatic Manipulation. A) The appearance of subconjunctival lymphatics (arrows) after saline injection and B) after VEGFC injection. Arrows point out subconjunctival lymphatic branches. Superior subconjunctival lymphatic (C) branch number and (D) length were greater after VEGFC and lesser after anti-metabolite (5FU and MMC) treatment (n = 9-11 mice per condition).. Black comparisons $p<0.0033$ (After Bonferroni correction), orange comparisons $p=0.01–0.0033$).

Conclusions

- Subconjunctival lymphatics drain the subconjunctival space as evidenced by structural imaging and molecular study in post-mortem and live eyes of multiple species, including humans.
- Subconjunctival lymphatics can be positively (via VEGFC) and negatively (via 5FU and MMC) manipulated in a mouse model with natively fluorescent lymphatics.
- Future studies will be planned to evaluate subconjunctival lymphatic function after pharmacological manipulation.
- In the future, manipulation of subconjunctival lymphatics may be used to improve the treatment of eye diseases. Limiting subconjunctival lymphatics may improve drug delivery by allowing for maintained drug injection depots. Enhancing subconjunctival lymphatics may improve glaucoma surgery by promoting outflow from surgical blebs.

Publications: 1) Akiyama G., Saraswathy S., Bogarin T., Pan X., Barron E., Wong TT., Kaneko MK., Kato Y., Hong Y., and **Huang AS**. 2020. Functional, Structural, and Molecular Identification of Lymphatic Outflow from Subconjunctival Blebs. *Experimental Eye Research* Jul:196:108049. Doi: 10.1016/j.exer.2020.108049.
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Disclosures: Alex S. Huang (Aerie Pharmaceuticals: Consultant; Allergan/Abbvie: Consultant; Celanese: Consultant; Equinox: Consultant; Glaukos Corporation: Research Support and Consultant; Gore: Consultant; Heidelberg Engineering: Research Support; Qlaris: Consultant; Santen Pharmaceuticals: Consultant. Other authors: none relevant.

Grant Support: Funding for this work came from Glaucoma Research Foundation Shaffer Grant [AH]; National Institutes of Health, Bethesda, MD (Grant Numbers R01EY030501 [ASH] and R21EY026260 [YH]); unrestricted grants from Research to Prevent Blindness [UCLA and USC] (New York, NY).