Comparison of Giant Vacuoles in the Inner Wall Endothelium of Schlemm's Canal between Normal and Glaucomatous Human Donor Eye

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Abstract

Primary open-angle (POAG) glaucoma is an eye disease that is often linked to an increased fluid pressure inside the eye (Intraocular pressure – IOP) that can damage the optic nerve, leading to blindness. Previous studies reported decreased pores in the endothelium of Schlemm's canal (SC) in POAG eyes, which may contribute to the increased outflow resistance. However, these studies used scanning electron microscopy (SEM), which is limited to surface view of the cells. This study investigated giant vacuoles (GV) in SC using serial block-face scanning electron microscopy (SBF-SEM) and 3D reconstruction. SBF-SEM images were obtained and visualized in the computer program Reconstruct. GVs in high- and non-flow regions of a glaucomatous eye perfusion-fixed at 15mmHg were traced and its volume, type, and wall thickness were analyzed to determine factors that may contribute to the decrease pore formation in POAG eyes. The results were compared with published data of normal eyes. The data showed GVs with I-pores decreased in high-flow regions of a POAG eye compared to normal eyes. More than half of the GVs were type 2 and the GVs with pores had larger volumes which is consistent with analysis done on normal eyes (4). In addition, the span of GVs in POAG eyes were greater than those in normal eyes which suggests GVs in POAG eyes are flatter. However, due to the small sample size (one eye), statistical significance could not be performed. In the future, more eyes should be analyzed to increase sample size and perform significance tests.

Introduction

Glaucoma affects over 3 million people in the United States and 76 million people globally (5). This number is projected to increase to 111 million by 2040. Current treatment for glaucoma is to lower IOP for slowing the progression of the disease, but there is no cure. POAG is usually associated with elevated pressure, resulting from increased resistance to the outflow of the fluid in your eyes (aqueous humor). Previous studies reported there were decreased pores in POAG eyes, which may contribute to the increased outflow resistance. However, these studies used scanning electron microscopy (SEM), which had certain limitations, such as; it only imaged the surface of the cells and could not distinguish the endothelial cell nuclei from GVs. However, this study utilized serial block-face scanning electron microscopy (SBF-SEM) and 3D Reconstruction. By examining thousands of serial images of cross-sections of the inner wall endothelial cells of SC, the percentage of GVs with pores and basal openings, GV shapes, and volume were analyzed. In addition, the inner wall endothelial cells of SC can be reconstructed in 3D to investigate the role of their cellular connectivity with neighboring inner wall endothelial cells and with underlying matrix and cells on pore formation. Although the factors that may contribute to pore formation have been studied in normal eyes, it has not been studied in POAG eyes. In this study, we investigated GV volume, type, thickness of cellular lining of GVs, and pore count in glaucomatous eye and compared the results to the published data in normal eyes (4). The results of this research will help to understand the reasons why glaucomatous eyes decrease pore formation. This allows to find a potential new target for decreasing outflow resistance and lower IOP in glaucoma by increasing pore formation.

Methods

Serial Block-Face Scanning Electron Microscopy Imaging (SBF-SEM): One human glaucomatous donor eye was perfusion-fixed at 15 mmHg and the high and non-flow regions of the eye were dissected, embedded in plastic resin, and sent to Cleveland Clinic to be serially sectioned and imaged using SBF-SEM. Each tissue sample (block) yields about 1500-2000 serial SEM images.

3D Reconstruction: Using the software Reconstruct, two blocks of images on which the GVs were previously identified were analyzed(3). GVs (range: 10 to 300 images) from high- and low-flow regions of the eye are manually traced using Reconstruct software. Their volumes and wall thickness were calculated using Excel.

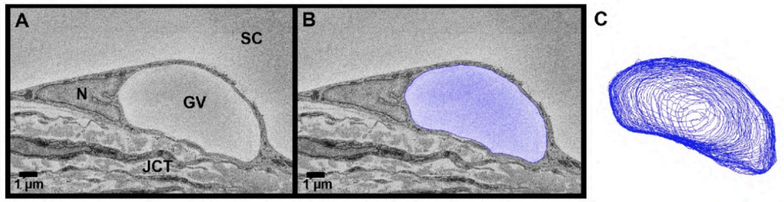

Figure 1: Method of giant vacuole 3D-reconstruction from serial block-face scanning electron micrographs
Giant vacuoles (GVs) are identified throughout each block (**A**) of serial electron micrographs and traced (*purple*) (**B**) to reconstruct their geometries in 3-dimensions (**C**) and to calculate their volumes and surface areas.

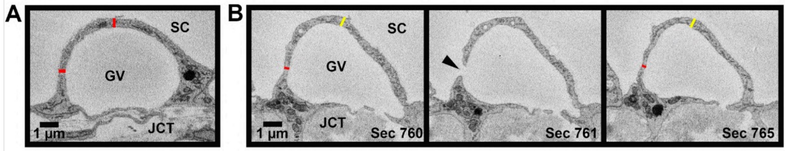

Figure 2: Method of measurement of thickness of the cellular lining of the 3D-reconstructed GVs
Thickness of the cellular lining of the giant vacuoles (GV) was measured two ways. **A:** Thickness of the cellular lining of a GV (*red*) measured on top of the GV and ≥ 90° to the side on the largest cross-sectional area section and sections away from the largest area. **B:** Peri-pore measurement (*red*) of thickness of cellular lining of a GV measured on the section before and after I-pore (*arrowhead*). Away measurement (*yellow*) taken on the same sections ≥90° away from peri-pore measurement.

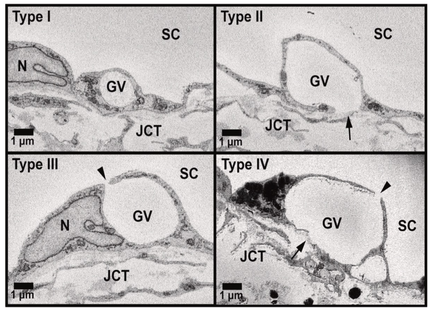

Figure 3: Types of GVs
GVs were categorized into four types based on their attributes. **Type I:** no basal opening or luminal I-Pore. **Type II:** has only basal openings (*arrow*). **Type III:** has only luminal I-Pore (*arrowhead*). **Type IV:** has basal openings and luminal I-Pore.
GV: giant vacuole
JCT: juxtacanalicular connective tissue
SC: Schlemm's canal
N: inner wall endothelial cell nucleus

Results

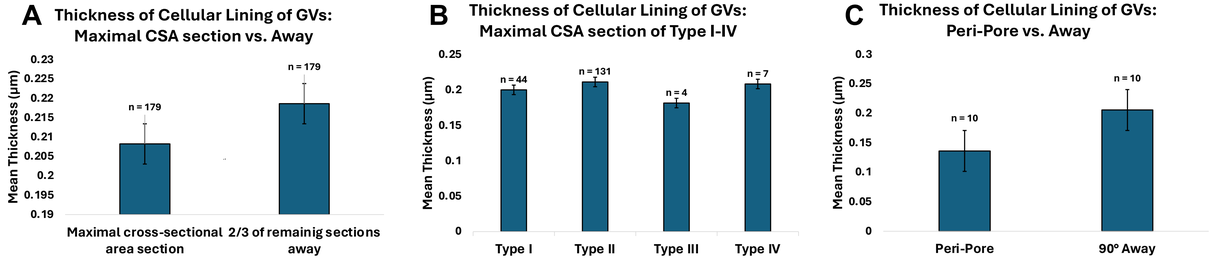

Figure 4: Various Comparisons of Thickness of Cellular Lining of Giant Vacuoles
Thickness of the cellular lining of 179 3D-reconstructed giant vacuoles. **A:** Mean thickness of the cellular lining of the GVs was significantly thinner on sections with maximal cross-sectional area (CSA) of GVs than away (away section = [(last section) – (max CSA section)]*(0.67)). **B:** Mean thickness of the cellular lining of GV types measures on the section with maximal CSA. Mean thickness was thinnest in Type I and III. **C:** Mean thickness of the cellular lining of GVs in the peri-pore region on the section before and after an I-pore was noticeably thinner than ≥90° away from the I-pore area on the same sections. *Error bars*: standard error based on mean.

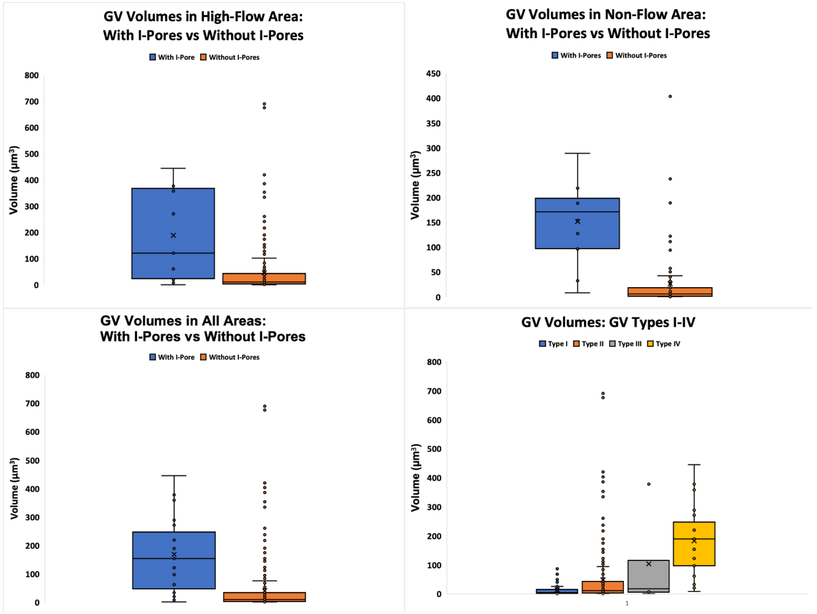

Figure 5: Various comparisons of GV volumes
Quadrant I: In the non-flow area, median volume of GVs with pores is significantly higher than GVs without pores. **Quadrant II:** In high-flow area, median volume of GVs with pores is significantly higher than GVs without pores. **Quadrant III:** Overall median volumes of GVs with pores is significantly larger than GVs without pores. **Quadrant IV:** When compared four types of GVs, median volume of Type IV GVs is significantly larger than the rest. Type 2 has a higher median volume than type 1. All results are in an agreement with normal eyes data(4).

Conclusion

In general, giant vacuoles (GV) with I-pores decreased in high-flow regions of glaucomatous eyes compared to normal. More than half of the GVs were type II and those with I-pores has larger volumes, consistent with data based on normal eyes (1). Span of GVs in glaucomatous eyes are greater than normal suggesting GVs in POAG eyes were flatter. The thickness of cellular lining of GVs at the max cross-sectional area is noticeably thinner than that of away sections and Type III has the thinnest cellular lining compared to the other three types.

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Acknowledgments

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