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Peripapillary Blood Flow After Use of Anti-glaucoma Medications: An OCT Angiography Study

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OPTOVUE

EXHIBIT A
PROTOCOL

Title of Study: Changes in Peripapillary Blood Flow After Use of Antiglaucoma Medications: A Prospective, Quantitative OCT Angiography Study
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1) **Protocol Title**

Changes in Peripapillary Blood Flow After Use of Antiglaucoma Medications: A Prospective, Quantitative OCT Angiography Study

2) **Objectives**

The primary objective of this study is to prospectively and quantitatively measure the acute changes in peripapillary blood flow with instillation of antiglaucoma medications in patients with primary open angle glaucoma (POAG), normal tension glaucoma (NTG), or ocular hypertension (OHTN) using Optical Cohere Tomography (OCT) angiography.

Hypothesis

Use of timolol will result in a decrease in peripapillary blood flow compared to brimonidine that is detectable on quantitative split spectrum amplitude decorrelation angiography (SSADA) using commercially available spectral domain OCT angiography imaging.

Secondary Hypothesis

Patients with normal tension glaucoma will have vascular dysregulation leading to greater decreases in peripapillary blood flow with the use of timolol compared to patients with POAG or OHTN.

3) **Background**

Reduction of intraocular pressure (IOP) with topical antihypertensive medications is the mainstay of initial treatment in patients with OHTN, POAG, and NTG. Many patients, however, continue to experience disease progression despite IOP reduction. Alternative mechanisms of neurodegeneration, including vascular dysregulation and structural susceptibility of the lamina

cribrosa, have been proposed as important mechanisms in progression, particularly in cases of NTG.

The Low-pressure Glaucoma Treatment Study found a significant correlation between the use of systemic antihypertensive medications and lower mean ocular perfusion pressure with visual field progression in NTG patients. This further implicated the possible role of ocular perfusion in the progression of NTG. Patients with NTG undergoing treatment with brimonidine, an alpha-2 agonist, was shown to have lower rates of visual field prognosis compared to treatment with timolol, a non-selective beta blocker. Mechanisms, including differences in medication mediated vascular regulation or neuroprotection have been proposed, though no definitive evidence currently exists to support either mechanism.

Prior studies have also found decreased calculated mean ocular perfusion with the use of timolol compared to other antiglaucoma medications in patients with normal tension glaucoma. Visual field deterioration has also been shown to be associated with systemic nocturnal arterial hypotension in patients with NTG, POAG, and after anterior ischemic optic neuropathy. The use of ophthalmic topical beta-blockers has been shown to lower nocturnal diastolic blood pressure and heart rate. Thus, topical beta blockers are often avoided in the treatment of NTG due to the potential risk of reduced optic nerve head perfusion.

Studies evaluating optic nerve head (ONH) perfusion are limited. Earlier studies evaluated indirect measurements, such as calculated mean ocular perfusion pressure or systemic hypotension, as indications of optic nerve hypoperfusion. Direct measurements of ocular perfusion have been attempted using retrobulbar color Doppler imaging, which demonstrated decreased short posterior ciliary artery flow velocity in patients with glaucomatous visual field progression. This technique, however, has yielded inconsistent results in other studies, and is only capable of detecting gross changes to ocular blood flow.

Direct observation of peripapillary retinal blood flow has been demonstrated with fluorescein angiography and Heidelberg retina flowmetry, though these techniques are only capable of detecting gross blood flow of large surface retinal vessels. Laser Doppler flowmetry and laser speckle flowgraphy are capable of evaluating fine capillary blood flow. These techniques, however, evaluate blood flow at small locations of the optic nerve head, yielding variability depending on the location of the sampled area.

Optical Coherence Tomography Angiography (OCTA) is a novel technique first introduced in 2014 using a custom swept-source OCT system. Since then, a commercially available spectral OCT system (RTVue-XR; Optovue, Inc) has been developed capable of obtaining two repeated B-scans at 400 locations, each comprised of 400 A-scans. Each of the two B-scans obtained at each location are compared to each other using a split-spectrum amplitude decorrelation angiography (SSADA) algorithm to detect flow using motion contrast. Measurements of capillary perfusion is quantified as the vascular density, defined as the proportion of area covered by perfused vessels within the area of interest, and flow index, defined as the mean decorrelation value, on an en face retinal angiogram.

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Most studies with OCTA have focused on fo,veal vascular perfusion. Recent studies on peripapillary vascular perfusion with OCTA have demonstrated sectoral qualitative and quantitative decreases in patients with NTG, POAG, and multiple sclerosis. No studies currently exist to evaluate the effects of antigluaocma medications on peripapillary blood flow using OCTA.

4) **Risks/Discomforts**

Timolol Eye Drops

Possible side effects or discomfort to the eyes associated with these drops are skin allergies around eyes, eyelid drooping, eye redness, eye irritation or itchiness, eyes feeling dry and blurred or double vision. Other possible side effects are asthma, worsening of asthma or other lung disease, difficulties breathing, slow or irregular heart beat, low or high blood pressure, worsening of heart failure, worsening of angina, sudden stop of heart beat, dizziness, fainting, psychic disturbances, fatigue, dry mouth, masking of symptoms from low blood sugar or from thyroid gland overactivity, decreased libido or impotence.

Brimonidine Eye Drops

Possible side effects or discomfort to the eyes associated with these drops are itching/redness/burning/stinging, feeling like something is in your eye, blurred vision, redness of the eye or eyelid, swollen or puffy eyes, sensitivity to light, nausea, upset stomach, headache, dizziness, muscle pain, dry nose or mouth, drowsiness, tiredness, sleep problems (insomnia), or unusual or unpleasant taste in your mouth. Unlikely, but serious side effects include fast or pounding heartbeats, persistent headache, eye pain or swelling, extreme sensitivity to light, or vision changes.

Eye Pressure Test: Having your eye pressure taken with a tonometer can cause minor discomfort. In rare cases, the eye could experience an abrasion (or scratch) if you were to rub it while the eye was still numb or if the tonometer was applied with too much pressure by the physician.

OCTA: There are no risks associated with this testing. It is painless and non-invasive.

5) **Inclusion and Exclusion Criteria**

Inclusion criteria:

- Diagnosis of ocular hypertension, primary open angle glaucoma, or normal tension glaucoma in the study eye(s)
- Age 18-90
- Best corrected visual acuity of 20/60 or better

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Exclusion Criteria:

- Current use of either brimonidine or timolol
- Other disease, ophthalmic or systemic, that is likely to significantly affect the OCT test in the study eye(s) including:
    - More than moderate grade cataract that significantly reducing OCTA scan signal level
    - Macular degeneration other than mild drusen or pigmentary changes
    - Diabetic retinopathy other than mild background non proliferative retinopathy
    - Prior or current macular edema
    - Prior laser treatment to the retina
    - Prior retinal detachment
    - Prior central serous retinopathy
    - Prior retinal vein or artery occlusion
    - Prior inflammatory retinopathy or choroidopathy
    - Keratoconus or other corneal ectasia
    - Corneal scarring in central 4 mm
    - Prior penetrating keratoplasty
    - Ischemic optic neuropathy
    - Dementia beyond early/mild memory loss
    - History of cerebrovascular accident
    - History of severe carotid stenosis
    - History of previous ocular surgery other than non-complicated cataract extraction

6) **Number of Patients to Enroll**
    - Brimonidine Group
        - 5 ocular hypertension patients
        - 5 POAG patients
        - 5 NTG patients
    - Timolol Group
        - 5 ocular hypertension patients
        - 5 POAG patients
        - 5 NTG patients

7) **Study Timeline**

Patient enrollment with begin after IRB approval is received. We plan to complete enrollment and data acquisition within 1 year of IRB approval.

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8) **Study Endpoints**

Acute quantitative change in peripapillary vessel density and/or blood flow index detected by OCT Angiography using SSADA algorithm with the use of topical brimonidine or timolol.

9) **Procedures Involved**

Patient records will be reviewed from the private practice of Drs. Lee, Fudemberg, Katz, Mantravadi, Myers, Ophthalmic Partners of Pennsylvania, and the Wills Eye Hospital Glaucoma and Cataract and Primary Eye Care service. Patients with a diagnosis of OHTN, or mild-to-moderate POAG or NTG will be included in the study based on Visual Fields, both Octopus and Humphrey Visual Fields will be used. Patients may be undergoing current treatment with any topical medications other than brimonidine or timolol.

Patients meeting study criteria will be selected and contacted prior to their scheduled office appointments by members of the research study team. Patients agreeing to participate in the study will be instructed to arrive 3 hours prior to their scheduled appointment on the morning of their visit. Informed consent discussion will take place and consent to participate in the study will be obtained. Patients will undergo two consecutive 4.5 x4.5 mm HD Disc scans with the commercially available spectral domain OCT angiography device for repeatability and reliability. Poor quality scans with a signal strength index less than 55 or registered image sets with residual motion artifacts (discontinuous vessel pattern or optic disc boundaries) will be excluded and a third additional scan will be performed. All eyes will undergo imaging using the standard 4.5 mm HD Disc scan protocol. In addition, a regular ONH structural scan (along with a 3D-Disc baseline scan) and a regular GCC structural scan will be acquired which provide comparison to normative database of the device to characterize the RNFL and GCC thickness status of the study eyes.

Patients will then be instructed to instill 1 gtt of an artificial tear OU, patients will wait 2 hours and be scanned again in both eyes. Patients will proceed with their scheduled office visit during this 2 hour interval prior to this third scan. Upon completion of the third scan, patients will be instructed to use a drop of either brimonidine or timolol. A final set of two (up to three) scans will be obtained with OCT angiography 2 hours after instillation of the topical antiglaucoma medications. Peak action was previously reported to be within 1-2 hours of administration of timolol, and 0.5 to 4 hours of administration of brimonidine. All patients will undergo IOP measurements with Goldmann Applanation Tonometry after each scan. The time of application of medication will be recorded.

10) **Data analysis**

<u>OCT Analysis</u>

After confirming the images were of sufficient quality based on signal strength rating as well as clinical evaluation, the peripapillary region will be defined as a 1000 um wide elliptical annulus , centered on the optic disc identified on the en face angiogram.

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Within-visit repeatability of peripapillary flow index will be calculated with 2 sets of sufficient quality images obtained sequentially before and after administration of antiglaucoma medications. All scans with a signal strength index of less than 55 or with residual motion artifacts will be excluded from the study.

Overall Analysis

Changes in the peripapillary vessel density and/or flow index 2 hours after administration will be defined as an acute change related to administration of antiglaucoma medications. As a feasibility pilot study, the goal of statistical analysis will be the estimation of the effect size. 95% confidence interval for the difference in means will have a width of approximately 1.5 standard deviations. Subgroup analysis will be performed to determine differences in the change in peripapillary blood flow related to the class of medication used, and the underlying diagnosis. Segmentation analysis will be performed using the research software provided by Optivue, Inc. to determine sectoral changes in peripapillary vasculature. Patients with high myopia and anomalous optic nerves will be included to evaluate baseline microvascular perfusion features.

11) **Setting**

All patient records will be reviewed daily from the practices of Drs. Katz, Myers, Fudemberg, Mantravadi, Lee, Ophthalmic Partners of Pennsylvania, and the Wills Eye Hospital glaucoma and CPEC service. All patients meeting inclusion criteria be contacted and evaluated for inclusion into study. Patient will undergo imaging with OCT angiography in the Wills Eye Diagnostic Imaging Center.

12) **Confidentiality**

Every effort will be made to protect the confidentiality of the data for participants of the study. **The site assigned ID# will be used to track individual participants. The information will be collected into a study database. All identifying information will be removed (name, etc.) beforehand. The database is password protected and is kept in a locked research room.**

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