The Effect of Glaucoma on Visual Search Tasks


Purpose: To investigate the effect of glaucoma on eye movements and performance of visual search tasks.

Methods: The sample comprised 13 glaucoma patients recruited from a hospital-based ophthalmology clinic, and 16 volunteer controls subjects with normal vision, who were enrolled in an ongoing study of risk factors for falls and motor vehicle collisions in glaucoma. Participants performed two visual search tasks. In the first task (serial search), a 6 x 5 array of optotypes was presented, where the target was a single O, and distractors were Landolt Cs. In the second task (parallel search), targets and distractors were reversed. Target presence, location within the array, and gap position of Landolt Cs were randomized over 60 trials. Eye movements were monitored with the EyeLink II (SR Research Ltd, Osgoode, ON). Main performance measures were mean number of fixations, errors and search time. Visual field impairment was measured with standard automated perimetry (SITA 24-2 MD [dB]; binocular Estarman [% detected]).

Results: Mean age was 68 years (SD, 7 years) and 67 years (SD, 8 years) for glaucoma patients and controls, respectively (p = 0.49). Visual field damage (MD) in the better eye of a glaucoma patients ranged from -1.1 dB to -6.9 dB (mean, -2.1 dB). In the serial search task with and without presence of a target, the search time of the glaucoma patients (median, 4.6 s and 7.6 s) was significantly greater than the controls (p<0.01, Mann-Whitney U [MWU]). In the parallel tasks, the findings were broadly similar; glaucoma patients tended to search longer and make more saccades, particularly during trials in which no target was present. In the glaucoma patients, visual field damage (MD) of the better eye was weakly but significantly related to an increase in search time (by 6% and 8% per 1 dB reduction of MD in target-present and -absent conditions, respectively, r-square 0.07, p<0.05).

Conclusions: Glaucoma patients made more fixations and took longer to perform a visual search task than persons with normal vision. Visual search performance decreased with increasing visual field damage. This challenges the assumption that early visual field losses in glaucoma do not impair real-world visual performance.


In vitro Detection of Neuronal Programmed Cell Death by Ultra High Resolution Optical Coherence Tomography

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Purpose: Major advances have been made in the understanding of the way in which cells undergo cell death. Neurons undergo prolonged periods of compromise and structural changes that precede the activation of the pathways that mediate cell death. If cell compromise could be identified, interventions could be timed to coincide with a therapeutic window that could lead to possible reversal in neuronal damage. If cell compromise could be identified, interventions can be timed to coincide with a therapeutic window that could lead to possible reversal in neuronal damage.

Methods: RGC-5 cells were cultured in growth medium of DMEM with 10% fetal calf serum (FCS), 4 mM Glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin in a humidified atmosphere with 5% CO2 at 37°C. Cells were seeded onto glass coverslips and cultured overnight. Cells were then washed with serum-free medium before incubation in DMEM growth medium containing 0% or 10% FCS for 24, 48 and 72 hours. Cells were imaged using UHR-OCT or incubated with 10 µg/ml JC-1 in incubation medium for 15 minutes at 37°C. Live cell images were obtained at a sampling rate of 20 Hz. Images were analyzed using OCT software or Image J.

Results: All cells were stained with JC-1, cells cultured with 10% serum and without serum for 24 hours showed both green and red fluorescence, however, cells serum deprived for 48 and 72 hours were devoid of red fluorescence indicating loss of mitochondrial membrane potential. Non serum deprived cells displayed a uniform intensity throughout the entire cell. Cells serum deprived for 24 hours showed both green and red fluorescence, however, cells serum deprived for 48 and 72 hours were devoid of red fluorescence indicating loss of mitochondrial membrane potential. Non serum deprived cells displayed a uniform intensity throughout the entire cell. Cells serum deprived for 24 hours showed both green and red fluorescence, however, cells serum deprived for 48 and 72 hours were devoid of red fluorescence indicating loss of mitochondrial membrane potential. Non serum deprived cells displayed a uniform intensity throughout the entire cell. Cells serum deprived for 24 hours showed both green and red fluorescence, however, cells serum deprived for 48 and 72 hours were devoid of red fluorescence indicating loss of mitochondrial membrane potential. Non serum deprived cells displayed a uniform intensity throughout the entire cell.

Conclusions: The optical properties of RGC5 serum deprived cells can be detected by UHR-OCT. Profiling the intensity distribution for cell populations may be a useful non-invasive technique for the quantification of neuronal health.

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Manganese-Enhanced MRI Studies of Intraretinal Ionic Activity in the DBA/2J Mouse Model of Glaucoma

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Purpose: Manganese-enhanced MRI (MEMRI) is a powerful non-invasive approach for studying intraretinal ionic regulation in vivo in models of ocular injury, diabetic retinopathy, retinopathy of prematurity or choroidal melanoma. In this study, we used MEMRI to evaluate changes in intraretinal ion activity, together with central retinal thickness and ocular circumference in DBA/2J mice with pigmentary glaucoma.

Methods: Four hours post i.p. injection of 66 mg/Kg MocCr2, high resolution MEMRI data were collected from light-adapted 3 mo C57BL/6 (n = 4) and DBA/2 (n = 9) mice, and 10 mo C57BL/6 (n = 7) and 11 month DBA/2 (n = 6) mice. Intraretinal ion activity, together with central retinal thickness and ocular circumference were measured from the MEMRI data. Eyes and optic nerves were harvested for histological comparisons.

Results: As C57BL/6 mice aged, ocular circumference increased 3% (p < 0.05), and total central retinal thickness decreased about 8% (p < 0.05). In addition, inner and outer retinal uptake of manganese increased (p < 0.05). In contrast, as DBA/2 mice aged, circumference increased 15% (p < 0.05), and both total and inner retinal thickness significantly decreased 7% and 8% (p < 0.05). However, the activity difference between inner and outer retina at 3 mo (14%) was significantly different from that at 11 mo (3%). This was largely due to a 6% decrease in inner retinal manganese uptake.

Conclusions: Measuring MEMRI, we readily detected differences in the age-dependent progression of changes in ocular circumference, retinal thickness and intraretinal ion activity between C57BL/6 and DBA/2 strains. Detection of changes in key ocular landmarks in the DBA/2 mouse highlights MEMRI as a powerful tool for measuring correlations in structural and functional ocular responses to glaucomatous stressors in vivo.

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343. New Ideas Organizing Section: GL

2879 - 12:15PM
Evidence of a New Uveolymphatic Outflow Pathway in Human and Sheep: Implications for Aqueous Humor Drainage and Glaucoma


Purpose: To determine whether a lymphatic circulation exists within the human and sheep ciliary body, and if present, its relationship to aqueous humor outflow.

Methods: Twelve normal human eyes with no history of ocular disease, attherosclerosis or stroke were obtained from the Eye Bank of Canada. Human eye sections were triple-immunostained with D2-40 (BD), CD31 (Abcam) and SMA (Abcam) antibodies for lymphatic vessels, blood vessels and smooth muscle cells respectively. Under general anesthesia, 50 μL fluorescent nanoparticles (Invitrogen) were injected intracameral in 3 sheep, with sacrifice 2 (n=2) and 4 (n=1) hours after the injection. Eyes were cryoprotected, serially sectioned and immunostained for a lymphatic endothelial marker (LYVE-1, Diagnostic Laboratories). Confocal laser microscopy was used to image ciliary body and capture images for 3-D construction (Microbrightfield Inc.).

Results: In normal human eyes, numerous fine D2-40 positive lymphatic channels were observed in circular, radial and longitudinal portions of the ciliary muscle, stroma, and ciliary processes. D2-40 positive lymphatic channels surrounded many blood vessels in the ciliary body. In the normal sheep, numerous channels containing fluorescent nanoparticles in the ciliary body were outlined by LYVE-1 immunoreactive lymphatic endothelial cells.

Conclusions: A uveolymphatic pathway is described for the first time in the human and sheep ciliary body. The flow of aqueous humor into these channels indicates a new mechanism by which aqueous humor flows out of the eye, and if altered in glaucoma, a possible novel therapeutic target.


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2880 - 12:30PM
CD44 Overexpression Causes Ocular Hypertension in the Mouse


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Purpose: CD44 plays major roles in multiple physiological processes including inflammation, phagocytosis, and cell survival pathways. Soluble CD44 (sCD44) is an ectodomain fragment of the cell-surface receptor CD44. sCD44 is cytotoxic to trabecular meshwork (TM) and retinal ganglion cells (RGC) cells in culture, and is elevated in primary open-angle glaucoma (POAG) vs. non-POAG patient aqueous humor. sCD44 concentration in aqueous humor correlates with the extent of visual field loss in POAG. This study examined the effects of adenoviral overexpression of either full-length or ectodomain fragment of CD44 on mouse IOP for establishment of a rodent model of POAG.

Methods: Human CD44 cDNA transcription variant 4 (CD44v4) cDNA (Origene #SC128160; RefSeq NM_001001391) was subcloned into the pacAd5.CMV.K-N.pA (U Iowa GTVC) shuttle vector for adenovirus production. A sCD44 version was also created by the introduction of a stop codon at the exon 5 (A>T, nt 1001) using the QuickChange XL mutagenesis kit (Stratagene). Expression of full-length and soluble CD44 was verified by ELISA. B6D2F1 (n=6) male BALB/c mice were injected intravitreally (one eye only per mouse) with 4.87×10^6 pfu adenovirus followed by IOP measurements (Tonolab). sCD44 levels in pooled mouse aqueous humor were assessed by ELISA.

Results: Elevated levels of CD44 were detected in cell lysate and medium from primary normal human TM cells transduced with either Ad5.CMV.CD44v4 or Ad5.CMV.sCD44 virus. In two separate studies, transduction of mouse eyes with either CD44v4 or sCD44 viral expression vectors caused ocular hypertension. The baseline IOP of the mouse was approximately 12 mmHg. At 8 days after vector injection, Ad5.CD44v4 significantly increased IOP to 28.3 ± 1.2 mmHg (mean ± SEM, n = 8; p < 0.001); Ad5.sCD44 increased IOP to 18.5 ± 2.6 mmHg (n = 8; p = 0.01), whereas the IOP of un.injected eyes at the same time point was 12.7 ± 0.2 mmHg (n = 16). The IOP elevation lasted more than 60 days. sCD44 levels were significantly elevated in the aqueous humor of Ad5.CMV.CD44v4 and Ad5.CMV.sCD44 eyes vs. paired un injected eyes (p<0.01). Elevated CD44 levels in CD44v4 eyes were more pronounced than in sCD44 eyes.

Conclusions: We have initiated the establishment of a rodent model of POAG by showing that viral overexpression of both full-length and truncated human CD44 variant 4 (sCD44) in mouse eyes is sufficient to cause ocular hypertension. Our data suggest that the elevated sCD44 levels seen in POAG vs. non-glaucoma aqueous humor are not just an epiphenomenon but may play an actual causative role in POAG pathogenesis.


Support: Alcon Research, Ltd.

2881 - 12:45PM
Multiphoton Imaging for Visualization and Ablation of the Trabecular Meshwork - A New Approach to Glaucoma Surgery


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Purpose: Currently, filtering surgery is limited by scarring and trabecular surgery which may allow for reduction of outflow resistance is lacking appropriate instrumentation. Multiphoton imaging combines diode imaging on a cellular level with the option of ablation in the micron range. We explored the potential application of multiphoton imaging for visualization and simultaneous ablation of trabecular tissue for non-invasive glaucoma surgery.

Methods: A compact solid-state mode-locked 90 MHz Ti:sapphire femtosecond laser with a wide wavelength range 715-930 nm and 140 fs pulse duration, connected to a modified multiphoton laser scanning microscope Zeiss Axiovert 510 Meta was used to visualize and ablate trabecular meshwork and anterior segment tissues in anesthetized mice and rabbits. Autofluorescent cellular structures were imaged at 750 nm incident wavelength, whereas extracellular collagen fibers were visualized at 850 nm. The images were collected in both tangential and sagittal levels using an 20 x 0.9 objective at 2 μm z-steps. For intrasutis ablation, the mean laser power was enhanced to about 210 mW. After imaging and ablation, animals were killed and the eyes were enucleated for histological analysis.

Results: Optical sections were obtained from corneal, scleral, and trabecular tissues. Multiphoton imaging provided high-resolution images of cornea, limbus and sclera at cellular level. The trabecular meshwork (TM) was identified through the sclera, avoiding opening of the eye. After femtosecond laser power enhancement, highly precise intrasutis ablation within the trabecular meshwork was performed. Histology confirmed not only the nature of the cellular images obtained by multiphoton imaging, but also the absence of detectable collateral damage adjacent to the ablation in the TM.

Conclusions: In vivo multiphoton-autofluorescence imaging and ablation by femtosecond laser pulses within TM is possible ab externo, and may prove to be a clinically applicable novel option for non-destructive trabecular surgery.

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