Blindness; The Abraham and Phyllis Katz Foundation; W81XWH1210255; NIH P30EY006360; Research to Prevent Blindness: Jeffrey H. Boatright, None; Eldon E. Geisert, None;rip

Conclusions: The effect of HIOC is mediated by activation of BDNF/TrkB receptors. HIOC for 1 week preserves visual function for at least 4 months. The protective effect of HIOC against blast-induced vision loss was completely blocked by ANA-12, a selective TrkB antagonist (Cazorla et al., J Clin Invest 2011;121:1846-1857), 2.5 hr before each HIOC or vehicle injection. The protective effect of HIOC was delayed by 24 hr after blast, the protective effect on visual function was not observed. Four months after exposure to blast, axon numbers in the optic nerve were significantly reduced in vehicle-treated mice (p<0.001), but not in HIOC treated mice. Pretreatment with ANA-12 completely blocked the protective effect of HIOC against blast-induced vision loss.

Conclusions: The effect of HIOC is mediated by activation of BDNF/TrkB receptors.

Commercial Relationships: P. Michael Iuvone, None; Polina Lyuboslavsky, None; Curran Siddhu, None; Li He, None; Jeffrey H. Boatright, None; Eldon E. Geisert, None

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Program Number: 738 Poster Board Number: B0371
Presentation Time: 1:30 PM–3:15 PM

Effects of blast overpressure on the retina in a rat model of primary blast injury

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Purpose: Primary blast injury (PBI) following blast overpressure exposure can affect the eyes and other organs (polytrauma). Yet, relatively little is known about the primary effects to the retina per se. We evaluated the consequences of blast overpressure exposure to the retina using a novel rat model of PBI, under conditions simulating mild traumatic brain injury (mTBI).

Methods: Group 1: Adult male rats exposed to a blast pressure of ~63 kPa (~190 dB pSPL), using a custom-built shock tube, with blast wave applied symmetrically (face-on); unexposed age/gender-matched rats were controls (N=3/group). One wk later, one eye per rat was processed for H&E histology and immunohistochemistry, while the contralateral retina was subjected to Western blot (WB) analysis. Tissue sections were probed with primary and fluor-conjugated secondary antibodies, counterstained with DAPI, and imaged by confocal fluorescence microscopy. Group 2: An asymmetrical blast was applied (left side of head); 2 wk post-blast, visual function was assessed by electroretinography (ERG) and optokinetic tracking (OKT: visual acuity (VA) and contrast sensitivity (CS)), and histological analysis of eyes was performed.

Results: Group 1 (1 wk post-blast): GFAP+ staining was observed in the inner limiting membrane (ILM) and radially throughout the neural retina; only ILM GFAP+ staining was observed in controls. WB analysis of retinas showed ~2-fold increase (p<0.03, t-test) in GFAP in blast-exposed vs. control retinas. Heme oxygenase-1 (HO-1)+ staining was elevated in the RPE, as well as in patches of neural retina, in blast-exposed vs. control rats; however, overt retinal damage was not evident. Group 2 (2 wk post-blast): VA and CS reductions (rel. to controls) were comparable in both eyes, despite blast asymmetry. VA thresholds trended toward reduction (~8-10%) in blast-exposed eyes, reaching statistical significance (p<0.05; 1-way ANOVA) with combined data. ERGs showed no apparent blast-induced rod or cone dysfunction; retinal histology was normal.

Conclusions: Exposure of rats to mTBI-like PBI conditions results in production of molecular signatures of oxidative stress (HO-1) and reactive gliosis (GFAP) with visual function deficits prior to obvious retinal damage. Involvement of oxidative stress suggests that antioxidants may provide a therapeutic intervention for blast overpressure-induced retinal injury if applied within an appropriate time frame.

Commercial Relationships: Steven J. Fliesler, None; Bruce A. Pfeffer, None; Megan Prunty, None; Michalle T. Pardue, None

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Program Number: 739 Poster Board Number: B0372
Presentation Time: 1:30 PM–3:15 PM

Activation of the immune system following blast injury to the eye

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Purpose: Exposure to blast radically increases circulating levels of pro-inflammatory cytokines, along with the release of tryptamines, which can activate BDNF/TrkB receptors.
Purpose: Our group at Emory is characterizing the effects of a 48psi blast injury to the eye. The present study examines the activation of the innate immune system and the chronic infiltration of T-cells into the compromised retina.

Methods: Blast injury was produced using a modified paintball gun (Hines-Beard et al. Exp. Eye Res 2012, 99:36-41) in the BXD recombinant inbred (RI) strain set. Expression datasets were generated 5 days after blast and compared with normal retinal microarray datasets respectively constructed from 30 mouse strains. The dataset is presented on GeneNetwork (genenetwork.org). To further examine the activation of the innate immune system and the infiltration of T-cells, retinal whole mounts and sections of retina from control animals and retinas that had received a 48psi blast were examined using immunohistochemical methods.

Results: The innate immune network and microglia are activated following blast with significant upregulation of C4b (p=0.01), Cx3cr1 (p=0.067), and Il-10 (p=0.001). In addition there is a clear indication that T-cells are invading the retina by the upregulation of CD4, FoxP3 and CD8 (known markers of lymphocyte subtypes). To investigate the possibility that lymphocytes were invading the retina, we examined a blast eye 10, 21 and 30 days following the initial injury. Surprisingly, we found a significant number of invading CD4, FoxP3 and CD8 positive T-cells. In addition, treating the animals for 1 week with Meloxicam (modulating the inflammatory response) or with Fingolimod (decreasing the invasions of lymphocytes) caused a significant increase in contrast sensitivity as measured by an optokinetic tracking system.

Conclusions: Following a 48psi blast injury to the mouse eye, the innate immune system is activated and subsequently T-cells invade the retina. These potentially deleterious events may be countered by treatments with FDA approved drugs. Currently we are examining the timing and effectiveness of potential therapeutics.

Commercial Relationships: Ying Li, None; Rebecca King, None; Felix L. Struebing, None; P. Michael Iuvone, None; Eldon E. Geisert, None
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Program Number: 740 Poster Board Number: B0373
Presentation Time: 1:30 PM–3:15 PM

Acute aqueous edema in survivable primary blast induced ocular injury in rabbits

J. David Rios1, Jae-Hyek Choi2, Jennifer McDaniel1, Brian Lund1

1:30 PM–3:15 PM
Poster Board Number: B0374

Optic nerve changes after repeated closed-head traumatic brain injury in wild type and htau mice

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Purpose: We have reported previously effects on the visual system from a novel, closed-head impact injury mouse model at sub-chronic (3 weeks) and chronic (3 months) time points post mild TBI (mTBI) injury. The purpose of the current work is to extend these findings into acute time points (less than 3 weeks) post injury.

Methods: Adult C57BL/6 mice (n = 5-7/group, 10 weeks of age) and htau mice (15 weeks of age, or 65 weeks of age, n=5-7/group) were assigned to either repeated mTBI (r-mTBI) or repetitive sham treatment (r-sham; anesthesia only) groups and five consecutive hits were applied according to an established protocol. Mice were euthanized at 24 hours after the last injury and optic nerves were examined histologically. Specifically, hematoxylin and eosin (H&E) staining was applied and the distribution of the nuclei within the nerve was assessed in three regions of 1,000 microns span along the length of the nerve. Myelin content was estimated with Luxol Fast Blue (LFB) staining and microglial activation with staining for Iba-1.

Results: In all three groups, H&E staining demonstrated increased cellularity in the optic nerve at 24 hours post injury. However, the distribution of the increased cellularity along the length of the nerve was not uniform, but demonstrated a trend for a peak in the region closest to the chiasm in wild type and older htau mice (55.1 and 125.2% increase vs. r-sham, respectively), while it diminished in the second and third region further away from the chiasm (28.5 and 48.5% in second region; -8.6 and 24.1% in third region). In contrast, younger htau mice showed much less of a change in cellularity across the three regions (37.3%, 18.2%, 29.6%). In both young and old htau mice the difference in cellularity for the region closest to the chiasm was significant compared to the sham treated group. This study suggests that repeated mild TBI leads to chronic changes in the optic nerve.
Biology Center, Georgia Regents University, Augusta, GA; VA Medical Center, Augusta, GA; Department of Pharmacology and Toxicology, Georgia Regents University, Augusta, GA; Culver Vision Discovery Institute, Georgia Regents University, Augusta, GA; College of Allied Health Sciences, Georgia Regents University, Augusta, GA.

Purpose: Traumatic ocular injury is frequently associated with degeneration of retinal ganglion cells (RGCs) due to primary trauma to their axons in the optic nerve and may also involve degeneration of other retinal neurons secondary to oxidative stress, vascular dysfunction, ischemia and edema. Our previous studies have shown that deletion of the arginase 2 (A2) gene significantly reduces neuronal injury in models of retinopathy of prematurity and ischemia/reperfusion injury. To evaluate whether A2 could be a therapeutic target for limiting traumatic retinal injury, we determined the effect of A2 deletion on neuronal cell loss and gliosis in a mouse model of optic nerve crush (ONC).

Methods: ONC surgery was performed on the left eye of wild type (WT, C57Bl6J) and A2-/ mice. After incision of the limbal conjunctiva and deflection of the intraocular muscles, self-closing N7 forceps were used to clamp the optic nerve at 2 mm from the eyeball for 3 seconds. Sham control surgery was performed on the right eye. At 7 days after the surgeries retinas and optic nerves were harvested for analysis. Confocal immunofluorescence imaging of NeuN-positive cells in the ganglion cell layer (GCL) was used to determine the effects of ONC and A2 deletion on neuronal survival. Immunofluorescence imaging of retinal or optic nerve sections was used to examine expression of glial fibrillary acidic protein (GFAP) and microglia/macrophage markers Iba1 and CD68.

Results: ONC injury caused a 60% reduction in numbers of NeuN-positive GCL neurons in the WT retinas as compared to the controls (p<0.01). This neuronal cell loss was blunted to 40% in the A2-/ mice (p<0.01). GFAP expression was increased in WT ONC retinas and this was attenuated in the A2-/ animals. Following ONC the microglial/macrophage population (Iba1 and CD68 positive) was increased in the optic nerve of WT but not in the A2-/ mice.

Conclusions: A2 deficiency reduces the loss of GCL neurons and limits the activation of retinal glial cells following ONC injury. Deletion of A2 also blocks the increases in numbers of microglia/macrophage cells in the injured optic nerves. These data demonstrate that A2 plays an important role in neuronal degeneration and gliosis after ONC. Inactivation of A2 may offer a therapeutic strategy for preventing neuronal cell death in traumatic retinal injury.

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Caspase-2 mediates retinal ganglion cell apoptosis in traumatic optic neuropathy caused by blunt ocular trauma

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Purpose: Traumatic optic neuropathy (TON) is a major cause of visual loss after brain and eye injury. TON can be direct – where the optic nerve is crushed or cut – or more commonly indirect – where brain or ocular injury is associated with secondary retinal ganglion cell (RGC) degeneration. After blunt ocular trauma, RGC degeneration occurs at and around the injury site, contributing to visual loss.

The cell death protease caspase-2 has features of both initiator and executioner caspases and its inhibition is RGC-neuroprotective in a number of animal models of RGC death. We hypothesised that caspase-2 mediates RGC apoptosis after blunt ocular trauma and its inhibition would reduce RGC death.

Methods: Anaesthetised adult rats were subjected to blunt ocular trauma and caspase-2 activity assessed by immunohistochemistry and western blotting. After blunt ocular trauma, caspase-2 expression was inhibited by unilateral intravitreal injection of chemically modified small interfering RNA molecule (siCASP2) with contralateral injection of siGFP as control. RGC survival was assessed by Brn3a positive cell counts on sagittal retinal sections. n=4-8 rats /analysis

Results: Cleaved caspase-2 immunolocalised to RGC at 5 and 48 hours in injured but not intact retina (Fig 1). Retinal levels of full length caspase-2 (30kDa) increased up to 24 hours after injury (p<0.002) and a cleaved fragment (12kDa) was elevated at 48 hours (Fig 2; p=0.035). Caspase-2 inhibition by intravitreal siCASP2 injection increased RGC survival peripheral but not central to the injury site (p<0.001; Generalised Estimating Equations).

Conclusions: Caspase-2 is active in RGCs after blunt ocular trauma and may contribute to RGC degeneration by apoptosis. Caspase-2 inhibition protects apoptotic RGC peripheral but not central to the injury site, as central cells are more likely to be necrotic. siCASP2 has the potential to be used therapeutically as a neuroprotective treatment in TON caused by blunt ocular trauma.

Figure 1. Cleaved Caspase-2 co-localised with Brn3a, confirming RGC localisation. Cleaved Caspase-2 is present 5 and 48 hours after injury, but not in intact retinae.

Figure 2. On western blots, full length retinal Caspase-2 (30kDa) increased up to 24 hours after injury (*p<0.005, ANOVA). Cleaved Caspase-2 fragment (12kDa) was elevated at 48 hours (***p=0.035, ANOVA).

Commercial Relationships: Chloe N. Thomas, None; Ann Logan, None; Zubair Ahmed, None; Richard J. Blanch, None

Support: Fight for Sight PhD Studentship (Grant code: 1560/1561)
Neurovascular Injury after Retinal Ischemia Reperfusion Insult: Contrastive Roles of Arginase Enzyme Isoforms

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Program Number: 746 Poster Board Number: B0379
Presentation Time: 1:30 PM–3:15 PM

Purpose: Our previous studies have demonstrated involvement of the ureohydrolase enzyme arginase in potentially blinding disease conditions characterized by retinal inflammation and neurovascular injury. Whereas the cytosolic isoform, arginase 1 (A1) is implicated in inflammation and vascular dysfunction in models of uveitis and diabetic retinopathy, the mitochondrial isoform arginase 2 (A2) is involved in neurovascular injury in oxygen-induced retinopathy model. The present study was undertaken to determine and compare the distinct roles of A1 and A2 in neurovascular damage following ischemia/reperfusion (I/R) injury.

Methods: Studies were performed with mice lacking one copy of arginase 2 and wild type (WT) controls (C57BL/6J). I/R insult was conducted on the right eye and the left eye underwent sham surgery. Retinas were collected for analysis at different times (3h-4wk post injury). Neuronal and microvascular degeneration were evaluated using NeuN staining and vascular digests, respectively. Glial activation was evaluated by GFAP expression. Microglial activation was measured by Iba-1 staining and confocal microscopy. Necrotic cell death was studied by propidium iodide (PI) labelling and western blot for RIP-3. Arginase expression was determined by western blot and quantitative RT-PCR. Retinal function was determined by electroretinography (ERG).

Results: I/R injury caused significant increases in A2 expression along with thinning of the neural retina, decreases in NeuN+ GCL neurons and formation of acellular capillaries. Increases in PI labeling and RIP-3 expression showed that cell death occurred by necroptosis. Neurovascular injury was accompanied by microglial activation along with increased expression of GFAP and impairment of the ERG. Neuronal cell loss, capillary degeneration, necroptosis, gliosis and ERG impairment were all significantly reduced by deletion of A2, whereas A1 expression was significantly increased. On the other hand, A1 deletion exacerbated I/R-induced neuronal and vascular injury and further increased necroptosis and gliosis as compared with WT retinas.

Conclusions: This study shows for the first time that arginase isoforms play different roles in retinal neurovascular injury and repair after I/R insult. I/R-induced necrotic cell death and gliosis are mediated by A2, whereas upregulation of A1 may play a role in limiting the pathology.

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Program Number: 747 Poster Board Number: B0380
Presentation Time: 1:30 PM–3:15 PM

Establishment of retinal ganglion cell-specific gene recombination murine models using tet system

Yasaku Katada1,2, Maki Miyasuchi1,2, Yukihiro Miwa1,2, Xiaoyan Jiang1,2, Kiwako Mori2,2, Hidefumi Torii2,2, Kenji F. Tanaka1, Kazuo Tsubota2, Toshihide Kurihara1,2, 1Laboratory of Photobiology, Keio University School of Medicine, Tokyo, Japan; 2Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan; 3Department of Neuropsychiatry, Keio University School of Medicine, Tokyo, Japan.

Purpose: It is generally difficult to obtain an efficient gene recombination in a specifically targeted cell population in order to generate conditional transgenic mice. To establish both highly specific and sufficient gene recombination simultaneously, we utilize a tetracycline-controllable gene expression system (tet system, Fig. 1) in which the amount of gene expression have been much improved and previously reported (KENGE-tet system). In the present study we establish retinal ganglion cell (RGC)-specific gene recombination murine models with high efficiency using this system.

Methods: We employed two different mouse lines which express the gene encoding tetracycline transactivation (tTA) protein under the control of a cell-type-specific promoter, muscarinic acetylcholine receptor M4 or serotonin receptor 5B control region. Those mice were further crossed with another transgenic mouse line which contains the yellow cameleon (YC) fluorescent gene connected into the downstream of the tet operator (tetO) promoter. The YC gene expression was induced only by the presence of tTA protein in the double transgenic mice (M4-tTA::tetO-YC or 5B-tTA::tetO-YC). The expression of YC was observed in the double transgenic mouse retina with a fluorescence microscope.

Results: In the M4-tTA::tetO-YC mouse retina we identified the expression of YC mainly in RGC and in some population of amacrine cells, whereas RGC specific expression of YC was observed in the 5B-tTA::tetO-YC mouse retina.

Conclusions: Using the tet system, RGC was visualized and the cell-type-specific gene recombination was confirmed. These results suggest that the current tetO-based system is useful to modify gene expression specifically in RGC.
Purpose: Selective inhibition of Rho-associated kinase (ROCK) is suggested as an efficient strategy to achieve neuroprotection and axonal regeneration in CNS injury models. However, it still remains elusive how ROCK signaling exactly precludes both processes. To elucidate how ROCK affects neuronal cell death, retinal cell cultures and retinal explant models from post-mortem pig eyes are optimized and validated, while mouse retinal explants are used to further explore ROCK as a negative modulator for axonal regeneration. Understanding the pathological mechanisms underlying neurodegeneration is essential to support novel therapeutic strategies for CNS injuries.

Methods: Adolescent pig Müller glia, microglia and neuronal cultures are optimized, validated via immunocytochemistry (ICC) and subjected to various stress conditions. ICC and Western blotting (WB) are applied to unravel the cellular source and expression level of ROCK in healthy and stressed retinal cell cultures. Pig retinal explant cultures and subsequent sectioning are optimized to histologically study neuronal survival. Furthermore, an operational postnatal mouse retinal explant model was used to study the effects of ROCK inhibition on axonal outgrowth.

Results: Glia cultures have been established and purity was evaluated via ICC for glutamine synthetase and Iba-1. ICC and WB revealed increased ROCK expression after stress in Müller glia, but not in microglia. Sectioning and immunohistological stainings of pig explants showed survival of inner retinal neurons up to 1 week in culture with no increase in glial reactivity. These retinal explants are being further exploited to assess neuronal survival after ROCK inhibition. Application of ROCK inhibitors on mouse explants showed a clear outgrowth-promoting effect - rather than an effect on elongation - which is even more pronounced when combined with growth factor supplementation.

Conclusions: Mouse ex vivo approaches already confirmed that ROCK inhibition has a strong potential to support initial axonal outgrowth. To evaluate the neuroprotective effect of ROCK inhibitors, a set of various in vitro and ex vivo models has been established. This set of complementary models will allow a thorough understanding of the pathological mechanisms underlying neurodegeneration and restricted regeneration.
Support: FWO - L’oreal/UNESCO for women in science

Program Number: 750 Poster Board Number: B0383
Presentation Time: 1:30 PM–3:15 PM

IGFBP1 regulates axon growth through IGF-binding domain
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Purpose: Activation of axonal growth program is a critical step in successful nerve regeneration following injury. As a standard model of CNS neurons, retinal ganglion cells (RGCs) shut down the intrinsic axon growth program during the perinatal period in mice. We reported that IGFBP1 functions as a secretory factor that regulates the intrinsic program for RGC axonal outgrowth. However, the biological activities of the two functional domains of IGFBP1, immunoglobulin (IG) and IGF-binding (IB) domain remain to be elucidated.

Methods: We generated mice deficient in IG domain (KO). RGC and axon counts were carried out in retinal flat-mounts and optic nerve sections, respectively, and compared with that of B6 wild type mice. Axon growth capacities of their RGCs were also assessed in culture. In addition, the functions of IG and IB domains were investigated by overexpressing whole IGFBP1 protein, IG domain, or IB domain in an RGC cell line.

Results: Absence of IG domain led to increased levels of expression of IB and IGF-1 in IG KO mice. Moreover, we noted increased number of RGC axons in optic nerves of KO mice, when examined at both P0 and adult mice. Correspondingly, the expression of Tuj1 was increased for ~14-fold, while the expression of GFAP decreased by 2 folds in KO mice as compared to B6 WT mice. Consistently, fluorescence intensity of GFAP in optic nerve sections was largely reduced in KO mice. RGCs isolated from KO mice exhibited increased axon length and improved survival in culture. Overexpression of IG domain resulted in dramatically decreased neurite length while expression of IB domain increased neurite outgrowth.

Conclusions: Our results suggest that the axon growth-promoting effect of IGFBP1 is mediated primarily by the IB domain, while the IG domain plays an opposing role in RGC axon growth.

Commercial Relationships: Yingqian Li, None; Kin-Sang Cho, None; Chenying Guo, None; Tor P. Utheim, None; Dong F. Chen, None

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Program Number: 751 Poster Board Number: B0384
Presentation Time: 1:30 PM–3:15 PM

Retinal Iron Overload during Diabetic Retinopathy Accelerates Ganglion Cell Death
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Purpose: Iron is required for normal function of many proteins in retina but excess iron can be toxic. So stringent mechanisms maintain iron levels by regulating proteins involved in iron homeostasis. Surprisingly, the potential role of impaired iron metabolism in diabetic retinopathy (DR) has not been elucidated. During DR, retina may accumulate iron due to inflammation and hemorrhage. Although there are reports on positive association between iron levels and proliferative retinopathies, the mechanism by which iron exacerbates this process is unknown. HFE, an important iron regulatory protein, senses cellular iron status and regulates iron uptake by competitively inhibiting transferrin receptor. We have previously reported that HFE is expressed in basolateral membrane of retinal pigment epithelium and HFE KO mice accumulate iron in retina with significant retinal degeneration by 18 months of age. In the present study, we induced diabetes in HFE knockout mice to understand the role of iron overload in the pathogenesis of DR.

Methods: Type I diabetes was induced in C57BL/6 mice by intraperitoneal injection of streptozotocin. C57BLKS-Leprdb mice were used for type II diabetes. Iron levels in retina were measured by quantifying tissue levels of ferritin using ELISA, immunostaining and western blot. HFE WT and KO mice were made diabetic using streptozotocin. A group of WT and KO diabetic mice were treated orally with iron chelator deferiprone. Retinal phenotype was characterized using morphometric analysis, electroretinogram and immunostaining.

Results: We found a marked increase in iron storage protein, ferritin, by ELISA, western blot and immunofluorescence in diabetic retina compared to control retina. Consistently, we found that HFE is upregulated in diabetic mice as well as in diabetic human retina. Also, diabetic HFE knockout mice had increased ganglion cell loss compared to diabetic wildtype mice by morphometric analysis, electroretinogram and immunostaining. Treatment with iron chelator, deferiprone, prevented neuronal cell death significantly.

Conclusions: Increase in retinal iron accumulation was evident in diabetic retina. Increased expression of HFE may indicate a regulatory mechanism in response to increase in retinal iron levels. Increased neuronal cell death in diabetic HFE knockout mice model of iron overload is an extremely novel finding indicating that iron overload can exacerbate the progression of DR.

Commercial Relationships: Jaya P. Gnana-Prakasam, None; Kapil Chaudhary, None; Wanwisa Promsote, None; Sylvia B. Smith, None; Vadivel Ganapathy, None; Pamela M. Martin, None; Alan Saul, None

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Program Number: 752 Poster Board Number: B0385
Presentation Time: 1:30 PM–3:15 PM

The role of telomere-associated proteins in retinal ganglion cell apoptosis after axotomy
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Purpose: Telomeres are DNA-protein complexes that cap the ends of eukaryotic chromosomes. They have a dynamic secondary structure that binds and interacts with a large network of telomere-associated proteins that protect the genomic integrity and maintain the proliferative success of cells. When telomeres are destabilized, chromosome ends are misrecognized as DNA double stranded breaks, eliciting DNA damage responses that lead to cellular apoptosis. There is sparse evidence that telomere destabilization plays a role in the pathology of CNS diseases. In the present study we examined whether telomere dynamics play a role in retinal ganglion cell (RGC) degeneration after axotomy.

Methods: In order to evaluate the role of the Telomerase enzyme in RGC apoptosis after axotomy, rats received intraocular (IO) (4µL) or optic nerve (ON) (10µL) injections of either Trichostatin A (TSA: TERT up-regulator and Telomerase activator, 3 & 8 days post-axotomy), TAG-6, PIPER, or MST-312 (Telomerase Inhibitors III, IV, or IX, respectively), or a short hairpin RNA (shRNA) plasmid directed against TERT (catalytic subunit of Telomerase). In order
to further examine the role of telomere-associated proteins in RGC apoptosis after axotomy; rats received ON injections (10µL) of Dicer short interfering RNAs (DsiRNAs) directed against TRF1, TRF2, TCAB1, DNA PKcs, Ku70, and EST1A. RGC survival was quantified in fixed flat-mounted retinas at 7 or 14 days post-axotomy.

**Results:** IO delivery of TSA increased RGC survival by fourfold (p<0.001) at 14 days, while ON delivery of a TERT shRNA plasmid decreased RGC survival (p<0.001) at 7 days. However, ON delivery of Telomerase Inhibitors III, IV, and IX had no effect on RGC survival at 7 days. Furthermore, in vivo transfection of axotomized RGCs with TRF1 (p<0.01), TCAB1 (p<0.001), Ku70 (p<0.001), and EST1A (p<0.001) DsiRNAs decreased RGC survival at 7 days, while TRF2 or DNA PKcs DsiRNAs had no effect on RGC survival at 7 days.

**Conclusions:** Our results show that TERT gene expression promotes RGC survival while Telomerase enzymatic activity has no role in RGC survival after axotomy. Additionally, the TRF1, TCAB1, Ku70, and EST1A telomere-associated proteins play an important role in RGC survival after axotomy, while the TRF2 and DNA PKcs proteins do not.

**Commercial Relationships:** Meghan D. Lysko; Philippe M. D’Onofrio, None; Paulo D. Koeberle, None

Support: CIHR MOP 119309

**Program Number:** 753 **Poster Board Number:** B0386

**Presentation Time:** 1:30 PM–3:15 PM

**Relative susceptibility of individual neuron classes to different insults in rat retinal cultures**

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**Purpose:** To assess the relative susceptibility of different classes of cultured retinal neuron to distinct insults: metabolic compromise, excitotoxicity and oxidative stress.

**Methods:** Retinal cultures were prepared from neonatal rat pups via a mechanical and enzymatic digest; cultures were allowed to grow for 7 days before treatment and each treatment lasted 16 hours. Neurons were classified according to immunolabelling for combinations of discrete markers (GABA, tau, βIII-tubulin, MAP2, calretinin, syntaxin, PGP9.5, synaptophysin, NeuN). Cells were treated with sodium azide (NaN3 ; 500µM) to simulate metabolic compromise, kainic acid (KA; 50µM) to induce excitotoxicity or t-butyl hydroperoxide (t-bH; 1µM) to bring about oxidative stress. After treatments, surviving cells were quantified by immunocytochemistry, Western blot or the absence of apoptosis (TUNEL); cell loss for analysed markers was quantified in each case either by cell/protein quantification or by using ImageJ, where appropriate.

**Results:** All neurons in the cultures (except for photoreceptors) labelled positively for tau; 35±5% of tau-positive neurons remained after treatment with KA, 53±7% after treatment with t-bH and 19±4% after treatment with NaN3. Different subsets of the tau-positive neurons labelled with combinations of GABA, βIII-tubulin, MAP2, syntaxin, PGP9.5, synaptophysin or NeuN; interestingly, tau/calretinin neurons did not label with any other marker. GABA/tau- and calretinin/tau-labelled neurons were the most susceptible to KA, being reduced to 5±3% and 13±5% of control values respectively; syntaxin/tau neurons were the least susceptible, with 43±11% remaining after insult. PGP9.5/tau neurons were the most affected by NaN3, with just 8±5% remaining after treatment; syntaxin/tau neurons were the least affected (72±7% remaining). Calretinin/tau neurons were the most susceptible to t-bH (14±6% remaining after treatment); βIII-tubulin/tau neurons were the least susceptible (68±8% remaining).

**Conclusions:** Cultured retinal neurons display differential susceptibilities to both specific (excitotoxic, KA) and to more general (oxidative stress, t-bH; metabolic compromise, NaN3) toxic agents. Translation of the present data to the in vivo situation may allow predictions of specific retinal neuron class susceptibility to specific injurious situations and diseases.

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