150 Pharmacology and Cellular Mechanisms
Sunday, May 07, 2017 3:15 PM–5:00 PM
Room 316 Paper Session
Program #/Board # Range: 848–853
Organizing Section: Glaucoma

Program Number: 848
Presentation Time: 3:15 PM–3:30 PM
Overexpression of ATF-4 in trabecular meshwork causes elevation of intra ocular pressure and reduction of outflow facility in a CHOP dependent manner
Prabhavathi Maddineni, Ramesh Kasetti, Gulab Zode. North Texas Eye Research Institute, University of North Texas Health Science Center, Fort worth, TX.
Purpose: Primary open-angle glaucoma (POAG) has been primarily associated with reduced aqueous humor outflow facility through trabecular meshwork (TM) and elevated intraocular pressure (IOP). Studies based on both human as well as mice models revealed that chronic endoplasmic reticulum (ER) stress in TM is one of the causative factors responsible for TM dysfunction and ocular hypertension. The purpose of this study is to examine whether forced expression of UPR downstream pro apoptotic molecules ATF4 and CHOP leads to reduced outflow facility and IOP elevation in normal C57 mice
Methods: Ad5.ATF-4/Ad5.CHOP/Ad5.empty virus (pfu=$2x10^7$) were injected intravitreally into C57BL/6J or CHOP KO ($CHOP^{-/-}$) mice. Conscious IOP of both the eyes was monitored once in a week until 7 weeks using rebound tonometer. Outflow facility was measured by constant-flow infusion technique. Also, we examined the expression levels of ATF-4 in the TM of age-matched normal and POAG donors by immunohistochemistry
Results: Forced expression of ATF-4 but not CHOP caused significant IOP elevation (23.97 mmHg in Ad5.ATF4 v/s 14.6 mmHg in Ad5.null mice) and reduced outflow facility (0.022µL/min/mmHg in Ad5.ATF4 v/s 0.04 in Ad5.null mice) in C57BL/6J mice. Elevation of IOP in C57BL/6J was prominent from 3 weeks post injection and sustained until 7 weeks. Interestingly Ad5.ATF-4 did not elevate IOP (17.7 mmHg) in $CHOP^{-/-}$ mice, indicating that ATF-4 interaction with CHOP is the prerequisite for ATF-4-induced IOP elevation. Also, ER stress-induced pro death marker, ATF-4 was significantly increased in human post-mortem glaucomatous TM tissues compared to normal TM tissues. Expression of ATF4 in primary TM cells induced oxidative and ER stress and also upregulated pro-apoptotic markers
Conclusions: This data indicates that chronic ER marker ATF4 is increased in the glaucomatous TM tissues, which may be associated with TM dysfunction, reduction of outflow facility and IOP elevation via induction of ER and oxidative stress. Furthermore, interaction of ATF-4 and CHOP is essential to carry out downstream signal transduction pathways
Commercial Relationships: Prabhavathi Maddineni, None; **Ramesh Kasetti**, None; **Gulab Zode**, None
Support: EY026177

Program Number: 849
Presentation Time: 3:30 PM–3:45 PM
Glucocorticoid receptor GRβ regulates glucocorticoid-induced ocular hypertension and glaucoma in mice
Gaurang C. Patel, Yang Liu, J Cameron Millar, Abbot F. Clark. North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX.
Purpose: Glucocorticoid (GC) induced ocular hypertension (OHT) is a serious side effect of prolonged GC therapy and if left untreated it can lead to iatrogenic glaucoma and permanent vision loss. The Alternatively spliced isoform of glucocorticoid receptor GRβ acts as a dominant negative regulator of GC activity. Our previous studies have shown that GRβ regulates GC responsiveness and that overexpressing GRβ in trabecular meshwork (TM) cells inhibits GC-induced and glaucomatous damage in TM cells. The purpose of this study was to determine whether increased expression of GRβ reversed GC- induced OHT in mice.
Methods: To generate GC-OHT, C57BL/6J mice received weekly bilateral periocular (administrated through conjunctival fornix) injections of dexamethasone acetate (DEX-Ac, 200ug/eye). Several weeks after DEX-Ac administration, mouse eyes were injected intravitreally with Ad5.null or Ad5.hGRβ expression vectors ($3x10^7$ pfu/eye) to transduce TM. Intraocular pressure (IOP) was measured using TonoLab rebound tonometer, and outflow facilities were measured in living mice using our constant flow infusion technique. Fibronectin and collagen I expression were evaluated using immunoblotting from mouse anterior segment tissues. Unpaired Student's t-test (2-tailed) and One-way ANOVA were used for statistical analysis.
Results: DEX-Ac significantly increased IOP from days 3-44 ($n=23$, $p<0.0001$), with a maximum IOP increase of 10 mmHg compared to vehicle control eyes. GRβ transduction of TM at day 19 after DEX-Ac induced OHT significantly lowered IOP ($n=14$, $p<0.001$) within 7 days to basline IOPs, thus reversing GC-OHT in mouse eyes. DEX-Ac significantly decreased the outflow facility ($n=10$, $p<0.01$) and GRβ transduction returned the outflow facility to normal levels ($n=9$, $p<0.05$). Increased expression of fibronectin and collagen I was observed in DEX-Ac treated mice compared to controls and GRβ transduced mouse eyes.
Conclusions: Overexpression of GRβ in the TM of mouse eyes reversed GC-OHT. GRβ gene therapy may be a useful therapeutic approach to treat glucocorticoid-induced ocular hypertension and glaucoma.
Commercial Relationships: Gaurang C. Patel, None; **Yang Liu**, None; **J Cameron Millar**, Shire Human Genetic Therapies, Inc. (F); **Abbot F. Clark**, NiCox Research Institute (F), Reata Pharmaceuticals (F), Western Commerce (C)
Support: NEI Grant R01EY016242

Program Number: 850
Presentation Time: 3:45 PM–4:00 PM
Does estrogen deficiency promote the development of glaucoma?
XIAOMIN CHEN, Yang Liu, Yi Zhang, Wendy R. Kam, Louis R. Pasquale, David A. Sullivan. Schepens/MEEI, Boston, MA.
Purpose: We hypothesize that an early estrogen deficiency accelerates the aging of the optic nerve and predisposes to glaucomatous damage. To begin to test our hypotheses, we sought to determine whether estrogen deprivation is associated with elevated intraocular pressure (IOP) and retinal ganglion cell (RGC) loss in male and female mice.
Methods: We obtained breeding pairs of C57BL/6J - aromatase knockout (ArKO) heterozygous mice (Dr. Nabil J. Alkayed; Oregon Health & Science University, Portland, OR) to generate homozygous ArKO mice and their wildtype (WT) controls. The homozygous ArKO mice harbored a targeted disruption of exon IX in the cyp19 gene and possess no aromatase activity. Aromatase catalyzes the conversion of androstenedione to estrone and the conversion of testosterone to estradiol. At 12 and 24 weeks of age, we measured in a masked fashion the IOP (n = 6 consecutive IOP measurements/value, 3 values/eye/day, 2 consecutive days) in the left and right eyes of conscious mice (n = 7-8/group/sex). Animals were then sacrificed and retinas were flat mounted and stained with Brn3a antibodies to

facilitate RGC counting using confocal microscopy. Unpaired t-tests were used for statistical analyses.
Results: The IOP levels in both 12- and 24-week old female ArKO mice were significantly ($p < 0.0001$) higher than those of age- and sex-matched WT controls. The mean increase in IOP ranged from 1.1 mmHg (7.9%) in the 12-week-, to 2.6 mmHg (19.7%) in the 24-week-old mice, respectively. These changes were accompanied by significant ($p < 0.05$, 12-week; $p < 0.05$, 24-week) decreases in RGC numbers in the ArKO female mice, relative to controls. In contrast, estrogen deficiency did not lead to an increased IOP in male mice. There was, however, a significant reduction in RGC counts in the 12- ($p < 0.05$), but not 24- ($p = 0.90$) week-old male ArKO mice, as compared to their age- and sex-matched WT controls.
Conclusions: Our results support our hypothesis that estrogen deprivation promotes the development of glaucoma.
Commercial Relationships: XIAOMIN CHEN, None; **Yang Liu**, None; **Yi Zhang**, None; **Wendy R. Kam**, None; **Louis R. Pasquale**, None; **David A. Sullivan**, None
Support: China Scholarship Council, the Harvard Glaucoma Center of Excellence, Glaucoma Research Foundation, Margaret S. Sinon Scholar in Ocular Surface Research fund, the Yong Zhang Research Fund, and the Guoxing Yao Research Fund

Program Number: 851
Presentation Time: 4:00 PM–4:15 PM
Increased IOP primes the NLRP3 inflammasome and increases IL-1β levels
Claire H. Mitchell, Farraj Albalawi, Wennan Lu. Anatomy and Cell Biology, University of Pennsylvania, Philadelphia, PA.
Purpose: The NLRP3 inflammasome is a multiprotein complex involved in the maturation of cytokine IL-1β. As IL-1β in turn drives the expression of many other cytokines, regulation of the inflammasome is a key upstream target. IL-1β has been implicated in the inflammatory response associated with glaucomatous neuropathy, but the links connecting elevated IOP and increased IL-1β levels are unclear. Here we examine the consequences of IOP elevation and mechanical strain on expression of inflammasome components.
Methods: Transient IOP elevation was induced in rats and mice by cannulating the anterior segment to a non-ischemic 50-60 mmHg for 4 hrs. Optic nerve head astrocytes were cultured from rats and mice. Levels of mRNA were compared using qPCR, levels of protein quantified from immunoblots, and location of protein changes identified with immunohistochemistry.
Results: Moderate elevation of IOP increased expression of message for inflammasome components IL-1β, NLRP3 and caspase 1 in rat retinas. Immunoblots showed transient IOP elevation increased IL-1β protein. The rise in IL-1β was inhibited by injection of P2X7 antagonist Brilliant Blue G, while intravitreal injection of P2X7 agonist BzATP induced a rise in IL-1β message. Expression of IL-1β, NLRP3 and caspase 1 were also increased in mouse retina after transient IOP elevations, while IL-1β was increased in 8 month old Tg-Myoc mice with chronic IOP elevation. Immunohistochemistry of rat eyes exposed to transient IOP elevation identified a rise in IL-1β protein in the inner retina, and in horizontal bands throughout the optic nerve head consistent with astrocytes. In isolated optic nerve head astrocytes, message for IL-1β increased with moderate stretch and swelling. The rise of IL-1β in astrocytes was prevented by P2X7 receptor antagonists BBG and A839977, but P2X7R agonist BzATP did not increase expression of IL-1β in astrocytes. Swelling, but not BzATP, activated transcription factor NFkB in isolated astrocytes, while the swelling activated rise of IL-1β in astrocytes was prevented by NFkB inhibitor Bay 11-7082.
Conclusions: Moderate elevation of IOP primes inflammasome components, particularly IL-1β. Stimulation of the P2X7R contributes to this increase, suggesting the IOP-induced release of agonist ATP may influence the inflammatory response.
Commercial Relationships: Claire H. Mitchell, None; **Farraj Albalawi**, None; **Wennan Lu**, None
Support: EY015537

Program Number: 852
Presentation Time: 4:15 PM–4:30 PM
P2RX4 facilitates Panx1-mediated neurotoxicity in ischemic and ocular hypertension injuries
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Purpose: A hemichannel-mediated ATP release is essential for glial and neuronal functionality and purinergic signaling in the retina. Molecular complex, comprised of P2X7 receptor (P2RX7) and pannexin-1 (Panx1) was postulated as the key component of purinergic signaling pathway in the retinal ganglion cells (RGCs). Compared to P2RX7, P2RX4 is much more sensitive to ATP and was shown to be co-activated with Panx1 in homeostatic brain, as well as in different injury conditions. In this work we explored whether a P2X4 receptor, is involved in this signaling and whether it is activated in ischemia and retinal injuries, induced by ocular hypertension.
Methods: Quantitative gene expression analysis, RNA scope in situ hybridization and immunofluorescence imaging were used to study gene expression and protein localization. Pharmacological inhibitors were applied to test the involvement of individual P2X receptors. Induced ocular hypertension injury was used in vivo and oxygen-glucose deprivation in vitro to model pathological conditions. Cell survival was assayed by measuring EGFP release in vitro and RGC density changes in vivo. Cell permeation tests were performed in stable Neuro2A cell lines expressing EGFP and different levels of the Panx1 protein.
Results: We showed that the P2X4 receptor is expressed in the retina at much higher levels, relative to P2RX7. In the inner retina, RNAscope in situ RNA hybridization and immunofluorescence imaging localized P2RX4 to neurons, particularly RGCs. P2RX4 blockade by 5-BDBD antagonist suppressed cell death from acute ischemic injury in vitro and from ocular hypertension injury in vivo. Significantly, the blockade of P2RX7 was only protective at high concentrations of extracellular ATP.
Conclusions: Our results show that P2RX4 is expressed in RGCs and is involved in extracellular ATP-induced and Panx1-mediated signaling in RGCs in physiological and mild pathological conditions.
Commercial Relationships: Valery I. Shestopalov; **Alexey N. Pronin**, None; **Vladlen Z. Slepak**, None; **Hailey D. Rooney**, None; **Anddre Valdivia**, None
Support: NIH Grants EY021517 and Core P30 EY014801, an unrestricted RPB and DOD #W81XWH-13-1-0048 grants to the Dept. Ophthalmology

Program Number: 853
Presentation Time: 4:30 PM–4:45 PM
Sustained dorzolamide release prevents axonal and retinal ganglion cell loss in a rat model of IOP-glaucoma
Ian Pitha[1, 2], Elizabeth Cone-Kimball[1], Ericka Oglesby[1], Mary Ellen Pease[1], Jie Fu[2], Yoo-Chun Kim[2], Julie Schaub[1], Qi Hu[2], Justin Hanes[2], Harry A. Quigley[1, 2]. [1]Ophthalmology, Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; [2]Ophthalmology, Center for Nanomedicine, Johns Hopkins University, Baltimore, MD.
Purpose: IOP-lowering eye drops are a mainstay of glaucoma treatment; however, they require patient adherence, can be difficult to administer, and can cause ocular surface toxicity. Previously, we developed a microparticle formulation of dorzolamide (DPP) that lowers IOP in normotensive rabbits for over 30 days. We hypothesize that IOP lowering provided by a single application of DPP would prevent retinal ganglion cell (RGC) loss in a rat model of glaucoma.
Methods: We injected either DPP or control microparticles intravitreally in Wistar rats. Two days later, unilateral ocular hypertension was induced by translimbal, diode laser treatment by a surgeon masked to treatment group. IOP and clinical exams were performed until sacrifice 6 weeks after laser treatment. RGC loss was measured by masked observers in both optic nerve cross-sections and RGC layer counts from retinal whole mounts.
Results: Cumulative IOP exposure was significantly reduced by DPP injection: 49 + 48 mm Hg days versus 227 ± 191 mm Hg days in control microparticle eyes (p=0.012, t test). While control-injected eyes increased in axial length by 2.4 ± 1.7%, DPP eyes did not significantly enlarge (0.3 ± 2.2%, difference from control, p = 0.03, t test). RGC loss was significantly less in DPP eyes compared to microparticle injection alone (axon count reduction: 21% versus 52%; RGC reduction: 25% versus 50% (beta tubulin labeling); p=0.02, t test).
Conclusions: A single injection of DPP microparticles, reduced IOP elevation and RGC loss in a rat model of glaucoma. The results demonstrate that a single injection of a controlled release IOP-lowering formulation can reduce glaucomatous RGC loss.
Commercial Relationships: Ian Pitha, None; **Elizabeth Cone-Kimball**, None; **Ericka Oglesby**, None; **Mary Ellen Pease**, None; **Jie Fu**, None; **Yoo-Chun Kim**, None; **Julie Schaub**, None; **Qi Hu**, None; **Justin Hanes**, Kala (P), GrayBug (S); **Harry A. Quigley**, None
Support: NIH/NEI K08EY024952, KKESH-Wilmer Collaboration