1308 - B77
Transient Ischemia of the Retina Results Three Months Later in Altered Erg, Degeneration of Inner Retinal Layers, and Deafferentation of the Contralateral Superior Colliculus. Neuroprotection With Brimonidine
Eye Research Institute, Oakland University, Rochester, MI.
Abstract:
Purpose:
Commercial Relationships are noted at the end of each abstract by “None” or with codes.

1309 - B78
Transplantation Thrombolytic Therapy (TTT) Induces Heat Shock Protein (HSP) 70 in the Rat Optic Nerve Head
J.H. Kim, Y.J. Kim, D.M. Kim
Ophthalmology, Seoul National University College of Medicine, Seoul, Republic of Korea.

Abstract:

Purpose:

Cytotoxicity was induced in primary culture of adult rat RGC by glutamate treatment

Methods:

The primary culture of retinal cells used in these studies contained mainly retinal

Results:

The primary culture of retina was enriched with RGC. By immunocytochemistry, 30-50% of all cells expressed

Conclusions:

In the confocal scanning laser ophthalmoscope and SEM findings, morphologic change of optic nerve head was observed. Photocauterization of the optic nerve head and peripapillary tissue was also found at 2 minutes and above with 100 mW. 

Conclusions:

Transplantation thrombolytic irradiation of 100 mW power with 60 seconds induces Hsp 70 expression in the retina. The induction of Hsp 70 by TTT may provide a preliminary data for future neuroprotective experiments in glaucoma by enhancement of natural cytoprotective stress response.

Support:

CR: None.

CR: None; J.M. Kim; H.J. Park; Y.J. Kim; D.M. Kim; None.

Support: BK2-2004

1311 - B81
Protective Effects of Pigment Epithelium-Derived Factor on Cultured Adult Rat Retinal Ganglion Cells

Abstract:

Results:

Light (NF-L) and calretinin and retrograde labeling studies. Apoptotic cell death in both
tPA knockout mice and their normal littermates was determined by TUNEL staining

Conclusions: Retroinal ischemia results 3 months later in alterations of the ERGs and degeneration of inner retinal layers as well as degeneration of retinofugal afferents. BMD has a strong neuroprotective effect in preserving retinal function, as observed with the ERG, cytoarchitecture of inner retinal layers, as well as the retino-tectal projection.

Support:

CR: None.

Support: NIH Grant 1R15EY13643

1311 - B80
Neuroprotective Effect of a-2 Adrenergic Receptor Agonist Against ER-stress Induced Apoptosis in Rat Retinal Ganglion Cells

Abstract:

Results:

ERG b-wave amplitudes in vehicle- or BMD-treated animals at 1, 8 and 12 weeks represented

Conclusion: Excitotoxicity was induced by injection of kainic acid (KA), a non-NMDA type

Support:

CR: None.

Support: None

1310 - B79
Inhibition of Tissue Plasminogen Activator Attenuates Non-NDMA Retinal Degeneration
S.K. Chinata, R.S. Malik
Eye Research Institute, Oakland University, Rochester, MI.

Abstract:

Results:

Localization of tPA in the retina was determined by immunohistochemistry. Activation of

Localization of tPA in the retina was determined by immunohistochemistry. Activation of

Conclusion: tPA knockout mice showed attenuated retinal degeneration after intravitreal injection of KA.

Conclusions: Results support that inhibition of IPA activity might protect retina in blinding retinal diseases in which over-stimulation of glutamate receptors is believed to be responsible for retinal degeneration.

Support:

CR: None.

Support: B2H102-03742, IROC-CO/C13; Allergan inc/unv

1308 - B77
Transplantation Thrombolytic Therapy (TTT) Induces Heat Shock Protein (HSP) 70 in the Rat Optic Nerve Head
J.Kim, J.H. Park, Y.J. Kim, D.M. Kim
Ophthalmology, Seoul National University College of Medicine, Seoul, Republic of Korea.

Abstract:

Purpose:

Protective Effects of Pigment Epithelium-Derived Factor on Cultured Adult Rat Retinal Ganglion Cells

Abstract:

Results:

Light (NF-L) and calretinin and retrograde labeling studies. Apoptotic cell death in both
tPA knockout mice and their normal littermates was determined by TUNEL staining

Conclusion: tPA knockout mice showed attenuated retinal degeneration after intravitreal injection of KA.

Conclusions: Results support that inhibition of IPA activity might protect retina in blinding retinal diseases in which over-stimulation of glutamate receptors is believed to be responsible for retinal degeneration.

Support:

CR: None.

Support: None

1310 - B79
Inhibition of Tissue Plasminogen Activator Attenuates Non-NDMA Retinal Degeneration
S.K. Chinata, R.S. Malik
Eye Research Institute, Oakland University, Rochester, MI.

Abstract:

Results:

Localization of tPA in the retina was determined by immunohistochemistry. Activation of

Conclusion: tPA knockout mice showed attenuated retinal degeneration after intravitreal injection of KA.

Conclusions: Results support that inhibition of IPA activity might protect retina in blinding retinal diseases in which over-stimulation of glutamate receptors is believed to be responsible for retinal degeneration.

Support:

CR: None.

Support: B2H102-03742, IROC-CO/C13; Allergan inc/unv

1312 - B81
Protective Effects of Pigment Epithelium-Derived Factor on Cultured Adult Rat Retinal Ganglion Cells
Glaucoma Research, Alcon Research Ltd.

Abstract:

Results:

Localization of tPA in the retina was determined by immunohistochemistry. Activation of

Conclusion: tPA knockout mice showed attenuated retinal degeneration after intravitreal injection of KA.

Conclusions: Results support that inhibition of IPA activity might protect retina in blinding retinal diseases in which over-stimulation of glutamate receptors is believed to be responsible for retinal degeneration.

Support:

CR: None.

Support: None

1312 - B81
Protective Effects of Pigment Epithelium-Derived Factor on Cultured Adult Rat Retinal Ganglion Cells
Glaucoma Research, Alcon Research Ltd.

Abstract:

Results:

Localization of tPA in the retina was determined by immunohistochemistry. Activation of

Conclusion: tPA knockout mice showed attenuated retinal degeneration after intravitreal injection of KA.

Conclusions: Results support that inhibition of IPA activity might protect retina in blinding retinal diseases in which over-stimulation of glutamate receptors is believed to be responsible for retinal degeneration.

Support:

CR: None.

Support: None

1313 - B82
Neuroglial Expression is Increased in the Retina of Human Eyes With Chronic Glaucoma
R.Rajendram*, E. Gonzalez*, N.A. Rao*.
"Ophthalmology, "Pathology, "Doheny Eye Institute, Los Angeles, CA.

Abstract:

Results:

Expression of Ngb was not seen in the ganglion cell layers of the glaucoma or normal retina. Ngb was present in the astrocyte and microglial cell layers in both groups. 

Conclusions: In chronic glaucoma, the presence of increased neuroglial protein in the plexiform layers which are rich in astrocytes, as well as the ganglion cell layer, is consistent with its putative function as a neuroprotectant under conditions of increased oxidative stress. The absence of neuroglial in the ganglion cell layer may contribute to the relative inability of these cells to survive the hypoxia and increased oxidative stress, which are thought to be major factors in the pathogenesis of chronic glaucoma.

Support: None.

CR: None; N.A. Rao, None.

Support: EY 05174; EY131253; EY 03040.
1314 - B83 Neurorheoprophic Effects of Bax-inhibiting Peptides on Hypoxic Damages in Purified Cultured Retinal Ganglion Cells Y. Chen,a M. Alhara,a M. S. Araie,a,b M. Saita,c M. Salinas,a,b,c,c S. Bolz,a,b,c,c, S. Mayor-Torroglosa,c,c A. Triviño,a,b,c,c 1Department of Ophthalmology, Faculty of Medicine, University of Tokyo, Tokyo, Japan; 2Department of Radiation Oncology and Pharmacy, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

**Purpose:** Hypoxia induces deleterious effects on neural cells. Bax-inhibiting peptide ((BIP)) is a membrane protein, susceptible to transactivation by dysfunction of mitochondria (mitochondria) designed from the Bax-binding domain of K17. It inhibits Bax-mediated translocation of cytochrome c and suppresses Bax-dependent apoptosis. We investigated the effect of BIP on hypoxic damage of cell death in cultured retinal ganglion cells (RGCs).

**Methods:** Purified RGCs were obtained from retina of 6 to 7-day-old rats utilizing the new technique of microdissection. Cells were cultured in serum-free medium. After being preincubated with VPMK (10, 50 and 200 μM) for 2 hrs, the RGCs were then incubated under hypoxic condition (5% O2, 2% CO2, 37°C) for 12 hours. Using the calcein-AM assay, the number of the viable cells was counted as a percentage of the total cells. All experiments were repeated 6-8 times.

**Results:** The viability increased in a dose dependent manner with exposure to VPMK (10 μM: 55.1%, NS; 50 μM: 57.1%; p<0.01, 200 μM: 61.3%; p<0.01, n=8). There was no significant increase of viability in the KLPVM-addings groups.

**Conclusions:** BIP has the protective effect against hypoxia-induced cell death of the purified RGCs. This finding suggests that Bax-mediated apoptosis plays a role in hypoxia-induced damage.

**CR:** Y. Chen, None; M. Alhara, None; M. Araie, None; S. Matsuayama, None. Support: None.

1316 - B85 Immunoreactivity of Erythropoietin and Its Receptor in Retina and Optic Nerve in an Experimental Rat Model of Glaucoma J. Li,a W. Liu,a,a A. I. Ramirez,a,c J. Cao,a,b,c,c J. Cao,a,b,c,c F. S. Schuettauf,a,b,c,c A. Triviño,a,b,c,c 1Department of Ophthalmology, Columbia University, New York, NY; 2Eye Research, Regeneron Pharmaceuticals, Inc., Tarrytown, NY.

**Purpose:** To assess the ocular histological changes by episceral venous caviar (EVC) in a rat model of glaucoma and to describe the expression pattern of erythropoietin (EPO) and erythropoietin receptor (EPO-R) in retina and optic nerve following intraocular administration of exogenous EPO.

**Methods:** The EVC method was performed to create an ocular hypertensive model of glaucoma. The intraocular injection of EPO or normal saline (NS) was administered at the end of the EVC procedure. The eyes were collected for histological and immunohistochemistry (IHC) evaluation after 21 days of elevated intraocular pressure. Immunohistochemistry was assessed in retinal and optic nerve specimens, focusing on EPO and EPO-R reactivity, as well as staining for Thy-1, a specific marker for retinal ganglion cell (RGC).

**Results:** Compared to normal eyes, morphological changes were observed in the EVC eyes, including dilation of the aqueous humor drainage pathways and iridial and choroideal vascular beds. Histological changes encompassed vaculization in the retina (especially in the photoreceptor outer segment, RGC layer, and inner limiting membrane), as well as reduced RGC density. In the EVC-treated eyes, the extent of vaculization as well as the reduction of RGC density was attenuated. No significant changes were observed in EPO immunohistochemistry between EVC-EPO and the normal contralateral eyes. In terms of EPO-R reactivity, stronger positive signals were observed in the RGC layer, inner nuclear layer, and photoreceptor inner segment in the EVC-EPO eyes when compared to normal eyes. For Thy-1, positive reactions were localized to the photoreceptor outer segment, RGC layer, and retinal nerve fiber layer in control eyes, and were increased in the EVC-EPO eyes. In the optic nerve specimens, intense immunoreactivity of EPO-R and Thy-1 were observed in the EVC-EPO eyes.

**Conclusions:** In the EVC caviar model of glaucoma, widespread histological changes were observed throughout the aqueous drainage and surrounding vascular systems. Increased immunoreactivity of EPO-R and Thy-1 were observed in EVC-EPO eyes. These immunohistochemical changes might contribute to the increased RGC survival observed with intraocular administration of EPO.

**CR:** L. Wu, None; J. Cao, None; M. Forbes, None; J. C. Tsai, None. Support: Eye Surgery Fund; G. and Richard Siegal, Research to Prevent Blindness, Inc.

1318 - B87 Metabolic Analysis of the Retinal Astrogliosis in an Ocular Hypertension Model J.-M. Bázquez,a,c,c J. A. Alvarez,a,c,c J. S. Sánchez,a,c,c J. L. Salazar,a,c,c J. G. Rojas,a,c,c J. J. De Hoy,a,c,c M. Villegas-Pérez,a,c,c S. Mayor-Torroglosa,a,c,c S. Salinas,a,c,c M. Vidal-Sanz,a,c,c A. Triviño,a,c,c 1Institut Invest Oftalmol Ramon Carbonell. Hospital Clinic, University of Barcelona, Universitat Pompeu Fabra, Barcelona, Spain; 2Department of Ophthalmology, Laboratory of Ophthalmology, University of Murcia, Murcia, Spain.

**Purpose:** To study the effects of chronic ocular hypertension on astrocytes in adult rat retinas.

**Methods:** The intracocular pressure of the left eye in adult Sprague-Dawley rats was chronically elevated by laser photocoagulation (532 nm, 500 mW, 200 μs, 250 pulses, degree 0 mmHg) in the nerve fiber layer. The number of astrocytes in the glaucomatous eyes decreased overall. These cells decreased towards the retinal periphery and the decrease was statistically significant. Also, the astrocytes associated with the blood vessels showed higher immunoreactivity than the control. Additionally, the Müller cells in these retinas exhibited increased GFAP immunoreactivity.

**Conclusions:** Chronic elevation of the intracocular pressure resulted in reduced retina ischemia 3 weeks after photocoagulation and reduced immunoreactivity of the retina in control and experimental astroglioses. These alterations of the glial cells could have induced axonal degeneration.

**CR:** J. Ramírez, None; J. J. Salazar, None; A. J. Ramírez, None; B. Rojas, None; R. De Hoy, None; M. Villegas-Pérez, None; S. Mayor-Torroglosa, None; S. Salinas, None; M. Vidal-Sanz, None; A. Triviño, None. Support: INCIB-IEC-SIN-13-136481-09003.
1320 - B90
Retinal Volume and Pericyte Number Decrease Over Time in the DBA/2 Mouse Model of Glaucoma

D.K. Cathcart, S.A. Ferguson, R.K. Clark, 1, M.B. Amsden, 2, B.M. 2, 1, D.J. Calkins, 2, N.Agarwal
1,2, None; 1,2, None.

Purpose: There is a tight relationship between vascular volume/flow and the metabolic and inflammatory state of the CNS. Capillary size is tightly associated with metabolic demand, inflammation, and the presence of MMPs in the extracellular matrix (ECM). MMPs are involved in the degradation of NMDA excitatory synapses, suggesting a role for MMPs in a neurodegenerative conditions. Astrocytes and pericytes are the vascular flow and the tightness of the blood-retina barrier. Using the DBA/2 mouse model of glaucoma, in which the model of the glaucoma is retinopathy, we predict that often with increased intracranial pressure and eventual retinal ganglion cell loss, we sought to determine if there is an angsogenic response to MMP. In DBA/2 mice, we indicated increased inflammatory response. We also sought to determine if there was evidence of vascular flow dysfunction in response to the glaucoma.

Methods: FITC-dextran perfused eyes obtained from DBA/2 and C57Bl/6 mice were used to evaluate MMPs in the retinal vasculature. Vascular and pericyte were counted in 2-11 month-old DBA/2 and C57Bl/6 retinal whole mounts using an unbiased stereological approach. Results: DBA/2 retinal vasculature significantly decreased its carrying capacity with age while that of the C57Bl/6 did not change. The DBA/2 mouse had significantly fewer pericytes than age-matched C57Bl/6, and DBA/2 mice lost pericytes with age. The ratio of pericytes to vascular elements was significantly reduced compared to that of C57Bl/6 mice. A loss in the total vascular network of the DBA/2 retinas–which reaches significance at 11 months–indicates that a large peripheral immune response is absent that but metabolic activity is decreased, likely due to the documented loss of RGCs. Vasculature in the DBA/2 remained patent, with few lobulated instances of FHF-dextran tests being the vascular elements. Conclusions: Given that pericytes are probably responsible for local control of blood flow and may participate in maintaining the blood-brain barrier, the loss of fewer pericytes and decreased vessel volume suggest that the DBA/2 retina is likely less able to react to changes in pressure or metabolic demand and this is exacerbated as the mice age.

1321 - B90
Modulation of Glutamate Toxicity in Retinal Ganglion Cells by Extracellular Matrix K. Chatzipanteli, M.E. Fini, F. Orthopthalmology, University of Miami School of Medicine, Miami, FL;

Purpose: Previous studies have reported that kainic acid-induced apoptosis of retinal ganglion cells (RGCs) occurs with specific death receptors of high mobility group box 1 (HMGB1) and loss of the limiting membrane by an increased gelsolin-like metalloproteinase activity. This study evaluated the effects of matrix and matrix components on retinal ganglion cell line (RG5-S) growth and viability. 5 µM ionomycin (a Ca2+ ionophore) and 500 µM glutamate (Glu) in glaucoma we studied the effects of different inner limiting membrane components on RG5-S viability in the presence of glutamate (Glu). RG5-S cells were cultured in media containing 10% fetal calf serum. Attached viable cells were quantified using a neutral red assay. Proliferation was quantified by 5-bromo-2’-deoxyuridine (BrdU) incorporation into RG5-S cells. Following exposure to glutamate, BrdU and neutral red assays were performed. Components evaluated included type IV collagen, fibronectin, laminin, and matrigel. Infiltrated with growth factors. We tested the effect of glutamate on RG5-S cells grown in a 10% serum media and on the above media in the presence of 5 µM glutamine, incubated for 24 hours.

Results: Analysis of the BrdU incorporation into RG5-S cells suggested that RGCs increased growth by accelerated cell proliferation apoptosis viability in comparison to inclusion. The efficacy of neuroprotection by the different matrix components and matrix itself in the presence of glutamate were as follows: fibronectin 95.87±7.2; collagen 90.42±3.5; anecot 89.5±5; laminin 77.96±5; matrigel 72.34.

Conclusions: These results suggest that retinol is superior in laminin in promoting RG5-5 survival and growth. However, it also potentially lacks glutamate toxicity.

1322 - B91
Effects of Novel Free Radical Scavengers on Intraperitoneal Pressure and ERGs in Rat Glaucoma Model

Center for Ocular Pharmacology, Eastern Virginia Med School, Norfolk, VA.

Introduction: The retinal ganglion cell (RGC) is the final common pathway for the visual information from the retina. Free radicals are implicated in the pathogenesis of glaucoma. The retinal and optic nerve involve extensive amounts of free radical damage to retinal ganglion cells (RGC). Novel free radical scavengers with a methoxyphenylethyl glycol backbone and varying free radical scavenging groups were evaluated as agents in terms of IOP reduction and RGC neuroprotection. Methods: Rats were treated topically with various free radical scavengers and the compound that showed the greatest excitotoxicity was evaluated. Results: MP compounds significantly reduced MP compounds to control animals (p<0.05). MP protected against glutamate induced MP compounds to control animals (p<0.05). MP protected against glutamate induced MP compounds to control animals (p<0.05). MP protected against glutamate induced MP compounds to control animals (p<0.05).

Conclusions: Given that pericytes are probably responsible for local control of blood flow and may participate in maintaining the blood-brain barrier, the loss of fewer pericytes and decreased vessel volume suggest that the DBA/2 retina is likely less able to react to changes in pressure or metabolic demand and this is exacerbated as the mice age.

Support: None; 1,2, None; 1,2, None; 1,2, None. Supported: Glaucoma Research Foundation and the Kerns Foundation.

1323 - B92
Mechanism of Apoptosis in Retinal Ganglion Cell Line RG5-S Cells Involving Calpain-Mediated Proteolysis: Calpain Inhibition Provides Functional Neuroprotection


1Department of Neurology, 2Psychology, "Medical University of Charleston, Charleston, SC; 3Center for Ocular Pharmacology, Eastern Virginia Med School, Norfolk, VA.

Purpose: An understanding of the role of calpains in retinal ganglion cell (RGC) apoptosis under pathological conditions may provide novel approaches to the treatment of glaucoma. Calpain-mediated proteolysis is involved in apoptosis of many cell types. Calpain is a Ca2+-dependent neutral protease that can cleave a wide variety of substrates. The aim of this study was to determine the role of calpains in retinal ganglion cell apoptosis induced by excitotoxic stimuli.

Methods: Calpain-mediated proteolysis was studied using a rat retinal ganglion cell line RGC-5 following exposure to ionomycin (a Ca2+ ionophore) and interferon-gamma (IFN-g) and also tested the functional neuroprotection with calpeptin, a cell-permeable inhibitor of calpain. We used Wright staining and ApopTag assay to detect apoptotic death, fura-2 assay to measure intracellular free Ca2+-levels, Western blotting for detection of specific protein bands, and electrophysiological recordings for determining whole-cell membrane potential.

Results: Wright staining and ApopTag assay detected apoptosis morphologically and biochemically, respectively, following exposure of RG5-S cells to 250 µM tunicamycin or 500 µM CuCl2 for 24 h. Apoptosis in RG5-S cells was associated with a significant increase in intracellular free Ca2+-levels, as determined by fura-2 assay. A pretreatment of RG5-S cells with 2 µM calpeptin for 1 h prevented Ca2+- influx. Western blot analyses showed an increase in Bcl-2 ratio, release of cytochrome c from mitochondria, and an increase in the activities of calpain, caspase-2, caspase-9, and caspase-3, during apoptosis. Pretreatment with calpeptin attenuated all these apoptosis related molecular events and prevented apoptosis of RG5-S cells. Most importantly, pretreatment with calpeptin maintained whole-cell membrane potential and thus provided functional neuroprotection.

Conclusions: Intracellular free Ca2+ result in activation of calpain, triggering apoptotic death in RGCs. Calpeptin, a cell-permeable inhibitor of calpain, provides functional neuroprotection. Our results imply that calpeptin may serve as a potential therapeutic agent for functional neuroprotection in glaucoma and other calpain-dependent diseases.

Support: None; 1,2, None; 1,2, None; 1,2, None; 1,2, None; 1,2, None. Supported: Grants from the NIH and the State of SC.

1324 - B93
Neuroprotective Effect of Tropisetron in Pig RGCs: Role of the AKT Signaling Pathway

V. Srinivasan, A. Depina, R. Kinsel, N. Agarwal, P. Williams, T.R. Lee
Support: In part by American Health Assistance Foundation and the Commonwealth Health Research Board

1325 - B94
Vitreal Concentration of Topically Applied Brimonidine-Purite 0.15%: In Vitro Study

L.B. Bartholomew, A.R. Kent, J.King

Ophthalmology, Storm Eye Institute/MUSC, Charleston, SC; 2Eyeare Physicians and Surgeons, LLC, Charleston, SC; 3Retina Center of Charleston, Charleston, SC.

Purpose: To determine the vitreous brimonidine concentration of topically applied brimonidine-Purite (Alphagan-P®) Methods: Patients scheduled for pars plana vitrectomy were invited to participate in this study after IRB approval was obtained. Patients were asked to place brimonidine 0.15%-Purite (Alphagan-P®) in the operated eye either bid or tid for at least one week prior to surgery, 2 or 8 hours respectively prior to the vitrectomy. Four patients served as controls and did not receive any drops. Vitreous (~ 0.5 - 1.0 cc) was aspirated prior to opening the infusion line. Three blank vitreous samples from cadaver eyes were included in the samples as a control for micro contamination lab.

Results: Nine and ten patients received brimonidine 0.15%-Purite in a bid and tid dosing regimen. The mean concentration of brimonidine in the bid patients was 18.5 µg/mL (range 2.0-120 µg/mL); the mean concentration of brimonidine in the tid patients was 48.5 µg/mL (range 0.5-370 µg/mL).

Conclusions: Brimonidine 0.15%-Purite topically applied both bid and tid obtain vitreous concentrations of brimonidine that are consistent with the ocular beta receptor.

Support: Unrestricted research grant from Allergan, Inc.

CR: L.B. Bartholomew, None; A. Astrakos, None; D.P. Garner, None; D.M. Kent, None; J.J. Woodward, None; D.M. Kumar, None; N. Agarwal, None; S.K. Ray, None.

Support: Grants from the NIH and the State of SC.
1326 - B95
ACR Receptor Activation Links Two Separate Signaling Proteins to Neurontoprecipitation Against Glutamine-Induced Excitotoxicity in Isolated Pig Retinal Ganglion Cells
C. L. Grant, A. S. M. Asomugha. Biological Sciences, Western Michigan University, Kalamazoo, MI.

Purpose: Acute retinal ischemia is the process by which excess excitatory neurotransmitter is released in the CNS, ultimately destroying neurons through an apoptotic mechanism. In mammalian retina, excess glutamate release has been shown to be involved in retinal ganglion cell (RGC) death. ACR deficiency, with a mouse of retinal disease states including retinal ischemia, diabetic retinopathy and glaucoma. Previous studies from this lab have found that acetycholine (ACh) increases retinal blood flow and protects against glutamine-induced excitotoxicity in isolated cultured pig RGCs. In this study, ELISA text was used to determine if activation of nicotinic ACh receptors (nAChRs) effect phosphorylation of specific signaling proteins that could be linked to neuroprotection against glutamine-induced excitotoxicity.

Methods: Two separate signaling proteins were targeted for ELISA studies; including ACR and p38 MAP kinase. ACR has been associated with cell survival pathways in a number of different preparations, whereas p38 MAP kinase has been implicated in apoptotic pathways. In these experiments, total and phosphorylated protein content was measured under control untreated conditions, after treating isolated RGCs with 500 µM glutamate to induce excitotoxicity, after treating cells with 5 µM ACh, and after treating cells with 5 µM ACh before 500 µM glutamate to induce neuroprotection. Results: MAP kinase ELISA results demonstrated that there was a significant increase of phosphorylated p38 MAP kinase when cells were treated with glutamate (37% ± 4%) compared to control conditions. This increase was eliminated when cells were treated with 5 µM ACh before glutamate. In addition, percent phosphorylation of p38 MAP kinase significantly decreased (25% ± 2%) when cells were treated with only ACh. ACR ELISA results demonstrated that ACh alone induced a significant increase in phosphorylated ACR compared to control conditions (33% ± 3%). Glutamate had no effect on the ACh-induced increase in ACR protein content. Conclusions: These results suggest that only ACR kinase is involved in glutamate-induced excitotoxicity, and that both ACR and p38 MAP kinase may be likely to be involved in ACh-induced neuroprotection. Identification of the enzymes involved in ACh neuroprotection can ultimately lead to therapeutic treatment for a variety of diseases associated with excitotoxicity throughout the CNS.

CR: C.L. Linn, None; C.D. Asomugha, None.
Support: NIH Grant EOY04866

1328 - B97 Reductins in Retinal Ganglion Cell Loss by Repetitive Hypoxic Preconditioning in a Mouse Model of Glaucoma
J.F. Schmidt1A, Y.Zhu1B, Y.Zhang1B, K.Hashimoto 1A, K.M. Leahy

Methods:

Results:

Conclusions:

RACE:

CR:

Support:

1330 - B90 Racial Differences in Ocular Hemodynamics Between African-American and Caucasian Patients With Glaucoma
B.A. Siesky1A, A.Harris1B, R.Abbasi1A, L.Cantor1A, Y.Catoira1, H.J. Garzozi1, L.Kageman1, R.L. Cantor1A.

Supported by:

Purpose:

Methods:

Results:

Conclusions:

CR: B.A. Siesky, None; A. Harris, Merck; R. Abbasi, None; C. Yung, None; Y. Catoira, H.J. Garzozi, L. Kageman, R. L. Cantor, None.
Support: None; B.A. Siesky, None; A. Harris, None; R. Abbasi, None; C. Yung, None; Y. Catoira, None; H. J. Garzozi, None; L. Kageman, None; R. L. Cantor, None.

1331 - B100 Ocular Blood Flow in the Rat During Acute Elevation of Intracranial Pressure

Methods:

Results:

Conclusions:

CR: L. Wang, None; B. Fortune, None; G. Cull, None; B. Bu, None; G. A. Coull, None.
Support: Legacy RAC, NIH R38

Purpose:

RACE:

Support:

CR:

Support:

Copyright 2005 by the Association for Research in Vision and Ophthalmology, Inc. All rights reserved. Permission to reproduce any abstract, contact the ARVO Office at pubsub@arvo.org.

Commercial Relationships are noted at the end of each abstract by “None” or with codes.
1332 - B101 Effects of Dorzolamide and Timolol on Optic Nerve Head Blood Flow Assessed With the HRF - Comparison of Data Evaluation G. Fuchsjaeger-Mayr1, B. Wally2, G. Rainer3, O. Findl4, C. Fass5, L. Schmetterer1,6,7
1Clinical Pharmacology, 2Ophthalmology, 3Biomedical Technical and Physics, "Medical University of Innsbruck, Innsbruck, Austria.

Purpose: Several techniques to evaluate data as obtained with the Heidelberg Retina Flow Meter (HRF) were proposed. The aim of the present study was to compare the standard procedures with the recently described AFFPIA algorithm for automatic evaluation of data on a set of HRF images taken in a clinical trial comparing the effects of dorzolamide and timolol on ocular blood flow.

Methods: In 140 patients with either normal or glaucomatous optic nerves, the effect of a 6 months treatment period of dorzolamide versus timolol on ocular hemodynamic parameters was studied. The design of the trial was randomized (1:1), double-masked in two parallel groups. Blood flow in the rim of the optic nerve head was measured with scanning laser Doppler flowmetry using the HRF. Data were evaluated by either using a 20x20 pixel area at the neuroretinal rim and applying standard software or by using the AFFPIA algorithm and automatic data evaluation.

Results: For both methods, comparison data on the neuroretinal rim were similar, with no significant difference between the effects of dorzolamide and timolol on ocular blood flow. Using the AFFPIA algorithm, the effect of timolol was +2.1 ± 1.5% and using the standard procedures, the effect of dorzolamide was +8.5 ± 1.6% and 10.9 ± 1.7% with the conventional and the AFFPIA algorithm, respectively.

Conclusions: The results indicated that the AFFPIA algorithm can be used to evaluate HRF data on the neuroretinal rim.

CR: G. Fuchsjaeger-Mayr1, M.F. Bally2, M. F. Rainer3, M. F. Findl4, M. F. S. Schmetterer5, M. F. F.
Support: M. F. medical school grant

1334 - B103 Vasodilatory Mechanism of Levobunolol on Isolated Rabbit Ciliary Artery T. Ishikawa1, N. Chiba2, K. Kashiwagi2, A. Morimoto2, A. Ishida1
1Department of Ophthalmology, Akita University, Akita, Japan; 2Laboratory for Neuroinformatics, RIKEN Brain Science Institute, Saitama JAPAN, Japan; 3Department of Ophthalmology, Kitasato University, Tokyo, Japan.

Purpose: Topical application of levobunolol, a beta antagonist used for treatment of glaucoma, reportedly increases ocular blood flow. To investigate the vasodilatory mechanism of this drug, we have investigated the effect of this drug on isolated rabbit ciliary artery in vitro.

Methods: Under the dissecting microscope, ciliary arteries were prepared from albino rabbit eyes and mounted in a myograph. The effects of levobunolol and other agents were investigated using isometric tension recording methods.

Results: Levobunolol induced a dose-dependent relaxation in ciliary arteries that were pre-contracted with high-K solution, 10μM histamine, 10μM phenylephrine or 100μM endothelin-1. Levobunolol was more effective in relaxing histamine-induced contraction (EC50: 17.0±1.4 μM) compared to high-K solution induced contractions (EC50: 80.3±12.6 μM). Application of N-nitro-L-arginine methyl ester (30μM), a nitric oxide (NO) synthase inhibitor, or denudations of endothelium by rubbing the inner surface with a scarp hair did not affect this relaxation. Pretreatment with levobunolol (100 μM) (NO) synthase inhibitor, or denudations of endothelium by rubbing the inner surface with a scarp hair did not affect this relaxation. Pretreatment with levobunolol (100 μM) inhibited histamine-induced transient contractions in Ca2+-free solution by 48.2±14.6 %. Calcium-induced pre-contracted vascular smooth muscle relaxation by levobunolol does not depend on endotelium or NO production. Relaxation may be mediated by inhibition of Ca2+ release from intracellular stores.

CR: T. Yoshihito, N. Takaseki, N. Hayami, N. Kajami, N. Ishikawa, N.
Support: Grant-In-Aid for Scientific Research, Japan #126719

1336 - B105 Systemic Arterial Stiffness in Glaucoma Patients T. Kawai1, H. Ishikawa1, N. Chiba1, T. Sakahara1,2, O. Findl3, M. R. Lesk4, L. Authier1, J. Couture1
1Ophthalmology, Saint Elisabeth Hospital Rodalben (Palatinate), Pirmasens, Germany; 2Ophthalmology, University of Yamashina, Yamato, Japan.

Percutaneous coronary intervention is one of possible risk factors in glaucoma. Infractal ankle pulse wave velocity (baPWV) is a marker of systemic arterial stiffness that deeply effects on the systemic or local circulation. In this study, we determined baPWV for investigating the relationship between systemic arterial stiffness and glaucoma.

Methods: Forty six patients with glaucoma (31 normal tension glaucoma: NTG and 15 primary open angle glaucoma: POAG) and 47 normal subjects were enrolled in the study. There was no significant difference in age distribution among groups and all subjects had no known factors influencing arterial sclerosis such as hypertension, diabetes mellitus and hyperlipidemia. The baPWV was determined using a volume-plethysmographic apparatus (Colin Co., Komaki, Japan). Parameters investigated for correlation with baPWV were mean deviation (MD), nerve fiber layer thickness of optic disc program 30-2, age, and other systemic or local factors. Results: The baPWV is positively correlated with age in all groups (NTG: r=0.51, POAG: r=0.28, Normal: r=0.24). Whereas, the baPWV was significantly correlated with MD only in the NTG group (NTG: r=0.27, POAG: r=0.02). Other parameters did not show any significant correlation. Conclusion: These results indicated that the systemic arterial stiffness may be associated with patients with NTG.

CR: T. Chiba; N. Kashiwagi; N. Chiba; N. Tsukahara; M. F. O. R. Conclusion: We evaluated the effect of treating systemic arterial hypertension on the flow of the optic nerve head neuroretinal rim and afferent blood flow in patients with normal tension glaucoma.

Methods: 40 patients with systemic high blood pressure underwent Scanning Laser Dopper Flowmetry (SLDF) examination of disc rim before and at least 3 weeks after the normalization of blood pressure levels with different medications, as prescribed by a family medicine clinic. Each patient was classified into one of four groups: 1) normotensive (n=2), 2) mild, 3) moderate, and 4) severe, based on risk factors as follows: duration of hypertension, diabetes, dyslipidemia and smoking.

Results: The cohort of 40 patients had a mean arterial blood pressure decrease after treatment of 15.7±9.8 to 9.4±5.7 mmHg. The mean pressure significantly (217.9±50a to 237.4±66a, p<0.009). Mean temporal and nasal retinal blood flow did not change significantly (p=0.735 and p=0.513). For group 1 (lowest risk for atherosclerosis, n=17) the neuroretinal blood flow significantly increased from (241.5±33.2a to 243.5±59.7a; p=0.001) while the retinal blood flow was unchanged (p=0.572). There was no significant difference between the groups with higher risk factors. Moreover, we did not show a statistically significant blood flow change. In the group of smokers (n=8) the mean retinal nerve retinal blood flow decreased from (221.2±51.2a to 212.3±68.8 a; p=0.066) and it increased in the non-smokers group (n=32) from (217.1±50.4a to 237.4±64a; p<0.000). This difference between the two groups was statistically significant (repeated ANOVA p=0.044).

Conclusion: Following normalization of systemic blood pressure, the neuroretinal blood flow increases in patients at low risk for atherosclerosis. The peripapillary retinal blood flow did not change significantly. In patients at low risk for atherosclerosis, particularly smokers, no significant change in neuroretinal rim blood flow was observed.

CR: P. Jeansson, M. F. R. Descovich, N. A. Hafez, N. St. Olivier; M. R. Lesk; N. Gervais, N. A. Author; N. Coulure; N. Fournier, N.
Support: None.
1338 - B107

Asymmetric Visual Field Loss and Retrolubar Blood Flow Velocities in Primary Open-Angle Glaucoma
M. Kaup, N. Plange, O. Aренд, A. Remky. Dept of Ophthalmology, Aachen University, Aachen, Germany.

**Purpose:** To investigate retrolubar blood flow velocities in patients with asymmetric glaucomatous visual field loss. **Methods:** Forty patients with primary open-angle glaucoma and asymmetric visual field loss (difference of mean deviation between the eyes >6dB) were included in this prospective study. Blood flow velocities (peak systolic velocity PSV and end-diastolic velocity EDV) and resistive indices of the ophthalmic artery (OA), central retinal artery (CRA) and nasal and temporal posterior ciliary arteries were measured by means of color Doppler imaging. **Results:** Mean deviation of eyes with more severe glaucomatous visual field loss was -1.18 ± 0.21 versus -0.18 ± 0.34 dB (p<0.001). Intraocular pressure and perfusion pressure were not significant different between eyes. The PSV of the ophthalmic artery (30.5 ± 8.6cm/s) versus 32.9 ± 9.6cm/s, p=0.014) and the EDV of the central retinal artery (2.2 ± 0.4cm/s versus 2.5 ± 0.5cm/s, p=0.005) were significant decreased in eyes with more severe glaucomatous field loss. The other blood flow data of the retrolubar vessels were not significantly different.

**Conclusions:** Patients with asymmetric glaucomatous visual field loss exhibit asymmetric blood flow velocities of the OA and CRA in primary open-angle glaucoma. The nasal and temporal posterior ciliary arteries did not show any significant differences between the more and less affected eye.

**CR:** M. Kaup, None; N. Plange, None; O. Aренд, None; A. Remky, None. **Support:** None.

1339 - B108

The Pulsatile Ocular Flow in Primary Open Angle Glaucoma Patients Undergoing Trabeculectomy

**Purpose:** To compare the influence of pulsatile ocular flow (POBF) and intraocular pressure (IOP) on mean defect (MD) of visual field (VF) in patients with primary open angle glaucoma (POAG) before and after operation. **Methods:** Fifty-nine POAG patients who planned to receive trabeculectomy at Xiamen Eye Center were assessed by POBF tonometry, Goldmann tonometer and Humphrey perimeter before and after operation, serially for at least 6 months. Linear regression analysis was applied to determine the correlation among the change of POBF, IOP and MD of VF prior to and after therapy. **Results:** In all 59 cases, the mean follow up was 13.0 ± 4.2 months (range: 7-21 months). There was a statistically significant relation between MD of VF and POBF (r= -0.23, p=0.031), while an insignificant relation was found between IOP and MD of VF (r=-0.068, p=0.210). Visual field and optic disc were stable in 34 cases and a highest statistically significant relation between MD of VF and POBF was found in these cases (r=0.34, p=0.017). POBF decrease in all those eyes (25 eyes) with VF loss and increase in cup-disc ratios during the follow-up period with an average value of 3.34±1.96 u/100 (Mean±SD). **Conclusions:** POBF might be a better determinant of VF defect in POAG cases.

**CR:** D.P. Fan, None; Z. Fu, None; M. Zhang, None; X. Liu, None; X. Wu, None. **Support:** None.

1340 - B109

Simulation of Dynamic Contour Tonometry on a Non-linear, Non-spherical Eye Model Using Finite Element Methods

**Purpose:** To evaluate the influence of different corneal properties on the results obtained with Dynamic Contour Tonometry (DCT) we generated numerical models of the eyehatchetsoner matches living human eyes. **Methods:** We enhanced the spherical eye model of Gullstrand using an ellipsoid approach. Both corneal surfaces were fitted using ellipse equations. To describe the mechanical behavior of the cornea we used the non-linear material model presented by Kanngiesser et al., ARVO 2004. We simulated Goldmann Applanation Tonometer (GAT) measurements to verify our eye model. We used a modified Newton iteration method, to determine the initial eye geometry needed to give the desired ellipsoid shape to the bulbus under IOP. Using a force driven tip with a planar surface we calculated the fully inflated cornea with increasing apopositional forces. We applied the precise capillary force, as calculated by the formulas presented by Kanngiesser et al., ARVO 2003. We generated the model for the DCT tip using an incompressible pressure sensor element molded into a rubber-like material having the desired contoured shape. Using the eye model we simulated DCT measurements using different appositional forces. We changed the central corneal thickness (CCT), corneal radius (CR) and the stiffness of the eye. We simulated measurements with DCT using a fixed force of 10mN and measurements with GAT using a constant applanation diameter of 3.06mm to determine the maximum possible error in a close to clinical use setup.

**Results:** We used an ellipsoid model with an anterior surface asperity of Q=1.75. The ratio between anterior and posterior radius of 0.81 and an asperity ratio of 1.1 as proposed by Dubbelman et al., Acta Ophthalmologica Scandinavica 2002. Simulations of GAT showed similar correlation with CCT and CR as reported in the literature on a cornea with mechanical properties described by Young’s modulus E=42.66*10^10 [N/m²] and Poisson ratio ν=0.46. Simulations of measurement errors obtained with DCT agree well with measurements obtained by the in vitro study presented by Kanngiesser et al., ARVO 2003. Changes in corneal properties influence DCT to a lesser degree than GAT.

**Conclusions:** The new non-spherical finite element model confirms the results obtained by Kanngiesser et al., ARVO 2004. In contrast the results are stable over a larger range of corneal properties. The model is ready to predict the behaviour of DCT on abnormal corneas like on keratocous, scars, extreme astigmatisms and other irregularities.

**CR:** H.E. Kanngiesser, Swiss Micro Technology E; C. Inversini, SIS Surgical Instrument Systems Ltd E; V.L. Ducry, Swiss Micro Technology E. **Support:** None.