863 - D681
Neuroprotection of Laser-Injured Retina by Intravitreal Saline Injection
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Purpose: To investigate the neuroprotective effect of intravitreal saline injection on reducing the spread of laser-induced retinal damage in animals that served as control groups for other studies.

Methods: Standard argon laser lesions (514 & 544 nm, 200 μm, 0.1 W, 0.05 second) were created in 36 DA pigmented rats that received saline either by intravitreal (5μl, n=18) or intravenous (0.5 ml, n=18) injection seven days before the laser session. The intravitreal injection was performed with a 30-gauge needle of a Hamilton syringe through the temporal posterior scera and retina. The laser-induced lesions were evaluated histologically and morphometrically 3, 20 and 60 days after exposure to laser.

Results: After intravitreal injection of saline the laser induced cell loss was reduced (P < 0.05) and the laser lesion diameter decreased (P < 0.05), as compared to intravenous treatment at all the time-points examined.

Conclusions: The results show that pre-treatment by intravitreal saline injection has a neuroprotective effect in the rat retina. The mechanism of action should be evaluated and the clinical applicability of this effect should be tested both morphologically and functionally.

Support: None

863 - D683
Neuroprotective Roles of Lutein in Endotoxin-Induced Uveitis

Purpose: To investigate the neuroprotective effect of lutein on retinal neural damage due to inflammation using a murine model of endotoxin-induced uveitis (EIU).

Methods: EIU was induced by an intraperitoneal injection of lipopolysaccharide (LPS). Animals were subcutaneously injected with lutein or vehicle 3 times, at the same time with and 3 hours before and after LPS injection. Twenty-four hours after EIU induction, the retinal protein levels of rhodopsin were analyzed by immunoblotting.

Results: Compared to non-treated mice, the amplitude of a-wave was reduced together with a significant decline of rhodopsin in EIU animals. Lutein treatment led to significant suppression of these functional and molecular changes in EIU.

Conclusions: The present data revealed the neuroprotective role of lutein in ocular inflammation, suggesting a potential validity of lutein supplementation as anti-inflammatory treatment to suppress neural damage in the retina.

Support: None

864 - D682
High Intracocular Pressure (IOP)-Induced Ischemia Elevates Extracellular Glutamate in the Retina of Rat: Pharmacological Evidence to Implicate Derangement of Glutamate Transporters

Purpose: To study by ocular microdialysis the extracellular changes of glutamate in high IOP-induced ischemia in vivo, in the presence of DL-threo-beta-benzoxypaspartate (DL-TBOA), a non-selective, non-transportable blocker of excitatory amino acid transporters (EAATs) or a solution (coenzyme Q10 (0.05%) and Vitamin E (0.05%) (CoQun, Visufarma, Italy)) with documented free-radical scavenger and anti-apoptotic properties (Nucci et al., 2007 Int. Rev. Neurobiol.).

Methods: retinal ischemia was induced in the right eye of anaesthetised, male, Wistar rat (250 g) by acutely increasing the IOP (see Osborne et al., 2004, Prog Ret Eye Res) and extracellular glutamate monitored during and after ischemia using microdialysis (see Nucci et al., 2005, Neurotoxicology). DL-TBOA or Coenzyme Q10 + Vitamin E solution were administered intravitaly via the microdialysis probe.

Results: extracellular glutamate increases during the first 10 min of ischemia though this reach statistical significance 10 min and 150 min after the reperfusion had started.

Conclusions: Administration of DL-TBOA (500 μM) during ischemia showed a trend towards glutamate increase at 10 min after reperfusion and a more sustained glutamate increase after 150 min of reperfusion without reaching, however, statistical significance whereas Coenzyme Q10 + Vitamin E solution ablated the peak increases typically induced by ischemia/reperfusion sequence.

Support: None

864 - D684
Comparison of the Ocular and Systemic Pharmacokinetics of Besifloxacin, a Novel Fluoroquinolone Antibiotic, With Moxifloxacin and Gatifloxacin in Pigmented Rabbits

Purpose: Besifloxacin is a novel fluoroquinolone antibiotic in clinical development for the treatment of bacterial conjunctivitis. Information for the direct evaluation of the in vivo efficacy of besifloxacin with other commonly marketed fluoroquinolones is currently unavailable. The present study was designed to compare the ocular and systemic pharmacokinetics of besifloxacin with moxifloxacin (Vigamox®) and gatifloxacin (Zymar®) following topical ocular administration to pigmented rabbits.

Methods: Dutch Belted rabbits (32/group) were arbitrarily assigned to one of the three dose groups: Group 1: Vigamox® (moxifloxacin HCl, 0.5%), Group 2: Zymar® (gatifloxacin, 0.3%), Group 3: Besifloxacin, (0.6%). Animals received a single instillation of 50 μL/eye of the appropriate formulation. Ocular tissue and plasma samples were collected at various intervals up to 24 hr after instillation. Drug concentrations were determined by LC/MS/MS.

Results: All fluoroquinolones exhibited rapid absorption following topical ocular administration (Tmax < 1 hr). Maximal concentrations of besifloxacin in tears and conjunctiva were generally higher when compared with those obtained with moxifloxacin or gatifloxacin; while concentrations in cornea were comparable for all three compounds. Systemic exposure to besifloxacin was found to be considerably lower when compared with the other fluoroquinolones.

Support: None
Phytoestrogens as Neuroprotectants in Optic Neuropathies

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Purpose: Phytoestrogens are a class of plant flavonoids that act as free radical scavengers utilizing hydroxyl groups on a conjugated ring structure. In this study we tested whether estrogen, apigenin and genistein might be neuroprotective.

Methods: Transforming rat retinal ganglion cells (RGC-5) were treated with varying concentrations of IAA from 2-10 μM. Antioxidants were administered at concentrations of 5-50 μM along with IAA. Cell viability was assessed by Calcein/AM assay. Involvement of reactive oxygen species (ROS) was determined by H2DCF (dichlorofluorescein)-EDA assay, caspase-3 activity by fluor assay, and changes in mitochondrial membrane potential (MMP) were determined by JC-1 fluorescent dye.

Results: IAA is cytotoxic to RGC-5 cells and induces the generation of ROS in vitro. 17-estradiol rescues RGC-5 cells from death in the presence of IAA, as well as generation of ROS. Apigenin and genistein do not rescue RGC-5 cells from IAA induced cytotoxicity. Genistein rescues RGC-5 cells in the presence of IAA, however, it also increases caspase activation and does not inhibit the generation of ROS. Kaempferol and genistein rescues RGC-5 cells from IAA induced cell death as well as reduce caspase activity and ROS generation.

Conclusions: IAA is cytotoxic to retinal ganglion cells. IAA cytotoxicity was reversed in the presence of exogenous antioxidants, kaempferol, genistein and genistin. IAA treatment results in a loss of MMP of RGC-5 cells. DCF assay suggested generation of ROS by IAA treatment, which was reversed by the phytoestrogens kaempferol and genistin. Caspases were activated in the presence of IAA, which was rescued by kaempferol and genistin. Taken together these results suggest that through these compounds may be neuroprotectively similar but functionally differ due to their chemical structure.

Support: None

867 - D668
Upregulation of Retinal NTDPase I and Vitrilux gene expression in an Experimental Rat Glaucoma Model

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Purpose: ATP is released in numerous tissues to signal a change in pressure or other mechanical perturbation. The increased intraocular pressure that can accompany mechanical perturbation. The increased intraocular pressure that can accompany mechanical perturbation. The increased intraocular pressure can lead to production of ATP as a result of mechanical perturbation. The increased intraocular pressure that can accompany mechanical perturbation. The increased intraocular pressure can lead to production of ATP as a result of mechanical perturbation. The increased intraocular pressure can lead to production of ATP as a result of mechanical perturbation. The increased intraocular pressure can lead to production of ATP as a result of mechanical perturbation. The increased intraocular pressure can lead to production of ATP as a result of mechanical perturbation. The increased intraocular pressure can lead to production of ATP as a result of mechanical perturbation. The increased intraocular pressure can lead to production of ATP as a result of mechanical perturbation. The increased intraocular pressu

Methods: Elevated IOP was induced in one eye of adult Brown Norway rats by injection of hypertonic saline into an episcleral vein. The IOP was monitored with TonoLab tonometer 4-6 x weekly. After 14 days, animals were sacrificed and eyes were fast frozen. The vitreous was dissected on dry ice and samples assayed for ATP content using the luciferase assay. Retinas were dissected, with proteins purified using standard techniques and run on a SDS-PAGE. Gels were blotted with a polyclonal antibody to rat NTDPase 5. Staining was quantified and the mean ratio from experimental to control retinas determined from 3 independent immunoblots for each retina.

Results: The injection of hypertonic saline, while challenging to learn, did lead to elevation of IOP in the treated eye of 6 rats. During the 14 days after injection, treated eyes had a mean IOP of 30.1 ± 1.4 mm Hg, while pressure in the controlateral control was 18.8 ±0.3 mm Hg. Pressure generally was higher in the first 5 days after injection, while that on the final day 26.2 ± 2 mm Hg. Levels of NTDPase1 protein were higher in eyes had a mean IOP of 30.1 ± 1.4 mm Hg, while pressure in the contralateral control was 18.8 ±0.3 mm Hg. Pressure generally was higher in the first 5 days after injection, while that on the final day 26.2 ± 2 mm Hg. Levels of NTDPase1 protein were higher in eyes had a mean IOP of 30.1 ± 1.4 mm Hg, while pressure in the contralateral control was 18.8 ±0.3 mm Hg. Pressure generally was higher in the first 5 days after injection, while that on the final day 26.2 ± 2 mm Hg. Levels of NTDPase1 protein were higher in

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868 - D666
Age-Dependent Neuroprotection of Retinal Ganglion Cells by Tempol C8-acyl Ester in a Rat NMDA Toxicity Model

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Purpose: Tempol (4-hydroxy-2,6,6-tetramethylpiperidin-1-oxyl), a superoxide dismutase mimetic, and Tempol acetyl esters were shown previously to be neuroprotective on retinal ganglion cells (RGC) in rat partial optic nerve crush (PONC) model. Here, we compare the efficacy of Tempol and its most effective acyl derivative (Tempol-C8) as retinoprotective agents in a rat model of NMDA (N-methyl-D-aspartate) toxicity, using sexually immature and mature animals.

Methods: Tempol at doses of 116, 290 and 580 μmol/kg (equivalent to 20, 50 and 100 mg/kg) or Tempol-C8 at doses 2.9, 5.8 and 29 μmol/kg were administered intraperitoneally to male Brown-Norway rats (6 weeks of age, 100-120 g BW) 9-10 weeks of age, 190-210 g BW) 24 hours and 30 minutes before the intravitreal NMDA injection then once daily for 6 consecutive days. Control rats were treated with vehicle (5% dextran in PBS, pH 7.2). RGC were retrogradely labeled with the fluorescent tracer Fluorogold 5 days after NMDA injection. Eyes were enucleated 2 days later, RGC counting was performed on retinal wholemounts.

Results: In vehicle-treated animals NMDA injection reduced RGC counts by about 90% independent of age. Tempol administration did not affect significantly RGC counts in any group of animals in any dose. In young animals Tempol-C8 showed a neuroprotective effect in a dose-dependent manner with peak activity at a molar dose equivalent to 1 mg/kg Tempol (increasing RGC counts by approx. 75%). No significant neuroprotection was observed in older animals in doses up to molar doses equivalent to 5 mg/kg of Tempol.

Conclusions: Tempol did not show significant neuroprotective effect on RGC in NMDA toxicity model contrary to the strong protective effect in PONC. Interestingly, Tempol-C8 acyl ester is neuroprotective in both PONC and NMDA toxicity models. In NMDA model, however its neuroprotective effect was only evident in immature rats.

Support: None

870 - D688
N-Methyl-D-Aspartic Acid Causes Relaxation of Preconstricted Porcine Retinal Arterioles Through an Adenosine Receptor Dependent Mechanism

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Purpose: Disturbances in retinal perfusion due to impaired regulation of vascular tone are believed to be involved in the pathogenesis of a number of vision threatening retinal diseases. In two recent studies, we have shown that the glutamate receptor agonist, N-methyl-D-aspartic acid (NMDA), and adenosine induce relaxation of porcine retinal arterioles. However, it remains to be elucidated whether the action of the two substances are separate or coupled.

Methods: Porcine retinal arterioles with preserved perivascular retinal tissue were mounted in a myograph for isometric tone measurements. Changes in tone were induced by increasing concentrations of NMDA in the presence of blockers of adenosine receptors and of ATP hydrolysis. Additionally, changes in tone were also induced with increasing concentrations of adenosine in the presence of the NMDA receptor blocker, DL-APV. All experiments were repeated after the perivascular tissue was removed.

Results: NMDA produced a concentration-dependent relaxing effect on retinal vessels with preserved perivascular retinal tissue (p<0.001) which disappeared after removal of the perivascular tissue. Blocking of the NMDA receptor, adenosine receptors and hydrolysis of ATP significantly reduced the vasorelaxing effect of NMDA in the presence of perivascular retinal tissue (p<0.05 for all three comparisons). However, adenosine-induced vasorelaxation was not significantly affected after blocking the NMDA receptor with DL-APV, neither on isolated arterioles (p=0.08) nor on arterioles with preserved perivascular tissue (p=0.22). Ibuprofen blocked the vasorelaxing action of NMDA but did not affect the vasorelaxing effect of the other substances tested.

Conclusions: The findings suggest that the vasorelaxing effect of NMDA is mediated by hydrolysis of ATP to adenosine in the perivascular retinal tissue.

Support: None

867-870
Role of Nitric Oxide in Regulation of Retinal Blood Flow in Response to Hyperoxia in Cats


**Purpose:** To investigate whether nitric oxide (NO) contributes to regulation of retinal circulation during and after induction of hyperoxia in cats.

**Methods:** NC-nitro-L-arginine-methylester (L-NAME; n=6), a NO synthase (NOS) inhibitor; NG-nitro-D-arginine-methylester (D-NAME; n=7), an inactive isomer; BQ-123 (n=7), an endothelin (ET) A receptor antagonist; BQ-788 (n=6), an ETB receptor antagonist; or phosphate-buffered saline (PBS; n=7) was injected intravitreously into feline eyes. A selective neuronal NOS inhibitor, 7-nitroindazole (7-NI; n=7), was injected intraperitoneally. Hyperoxia was induced for 10 minutes by inhalation of 100% oxygen. We measured the vessel diameter and blood velocity simultaneously in large retinal arterioles in cats by laser Doppler velocimetry and the retinal blood flow (RBF) was calculated during and after hyperoxia.

**Results:** In the PBS group, the vessel diameter (-18.3%±2.1%, P<0.05), and RBF (-48.4%±2.6%, P<0.05) decreased during hyperoxia. These parameters increased and recovered to baseline within 10 minutes after cessation of hyperoxia. In the L-NAME and BQ-788 groups, no changes occurred in RBF during hyperoxia compared with the PBS group. However, RBF recovery after cessation of hyperoxia was attenuated significantly until 20 minutes after cessation of hyperoxia in both groups compared with the PBS group (P<0.05). In the BQ-123 group, the decreased RBF during hyperoxia was significant (P<0.05) compared with the PBS group, whereas the RBF returned to baseline over the same time course as the PBS group. 7-NI did not affect changes in RBF in response to hyperoxia.

**Conclusions:** NO contributes to RBF recovery after hyperoxia. Further, the NO synthesized by the action of endothelial NOS via the ETB receptor in the retinal endothelium may regulate RBF after hyperoxia. These findings suggest that the RBF response to hyperoxia may be evaluated by the endothelial function of the retinal arterioles.

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## The Role of Calcium Activated Potassium Channels With Small (SKCa) and Intermediate (IKCa) Conductance in NO-Release in Porcine Retinal Arterioles

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**Purpose:** Endothelial dysfunction leads to changed release of vasodilating agents from the endothelium, which may be involved in the pathogenesis of retinal vascular diseases. In this study, we investigated the role of nitric oxide (NO) synthase, cyclooxygenase, and a NO scavenger for vasodilation induced by bradykinin and NS309, an opener of calcium activated potassium channels with small (SKCa) and intermediate (IKCa) conductance in porcine retinal arterioles. In addition, we investigated the role of SKCa and IKCa channels in the bradykinin- and NS309-evoked dilation by NO-release.

**Methods:** Retinal arterioles (diameter ~112 μm, N = 122) were mounted in wire myographs for isometric tension recordings. The arterioles were pre-contracted with the thromboxane analogue, U46619, and dose-response curves for bradykinin and NS309 were performed in the presence of inhibitors of the above mentioned enzymes and channels.

**Results:** Bradykinin and NS309 induced concentration-dependent vasodilation in the pre-contracted arterioles. Bradykinin-induced vasodilation was significantly higher in intact vessels than in vessels without endothelium (P < 0.001, n = 10). Inhibition of NO synthase and/or cyclooxygenase reduced bradykinin- and NS309-induced vasodilation (P < 0.0001, n = 10 and P < 0.05, n = 12), where NO synthase inhibition had the most pronounced effect (P < 0.0001, n = 9). NO synthase and cyclooxygenase inhibition together with a NO scavenger abolished the bradykinin-evoked vasodilation (P < 0.0001, n = 7). This treatment only attenuated the NS309-evoked vasodilation (P < 0.01, n = 9), indicating no involvement of an EDHF type relaxation. Inhibition of SKCa and IKCa channels in the presence of a cyclooxygenase inhibitor, markedly reduced NO-mediated bradykinin- and NS309-induced vasodilation (P < 0.0001, n = 8 and P < 0.0001, n = 11), where inhibition of SKCa channels alone (P < 0.01, n = 7) blunted the response most.

**Conclusions:** Mainly NO, but also prostacyclin, were found to be involved in bradykinin and NS309-evoked vasodilation in porcine retinal arterioles. SKCa and IKCa channels were involved in the bradykinin- and NS309-induced release of NO, whereas EDHF was not involved in endothelium-dependent vasodilation. These data suggest that modulation of SKCa and IKCa channels can lead to an increased release of NO from the endothelium and increase retinal blood flow. This may be involved in the development of retinal diseases, where hyperperfusion is part of the pathogenesis.

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