505 Toxicology; hypoxia; ischemia; oxidative stress; corneal disease
Thursday, May 11, 2017 8:30 AM–10:15 AM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 5164–5188/B0051–B0075
Organizing Section: Physiology/Pharmacology

Program Number: 5164 **Poster Board Number:** B0051
Presentation Time: 8:30 AM–10:15 AM
Determination of a No Observable Effect Level (NOEL) for Endotoxin following a Single Intravitreal Administration to Cynomolgus Macaques
Brian J. Christian[2], Paul Miller[1], T Michael Nork[1], Carol A. Rasmussen[1], Thomas Larsen[4], Evan A. Thackaberry[3], Helen Booler[3], Vladimir Bantseev[3]. [1]OSOD (Ocular Services On Demand) LLC, Madison, WI; [2]Toxicology, Covance Laboratories Inc., Madison, WI; [3]Genentech Inc., South San Francisco, CA; [4]Pathology, Covance Laboratories Inc., Chantilly, VA.
Purpose: The presence of endotoxin in intravitreal ophthalmic formulations is a concern at all stages of therapeutic development. The objective of this study was to characterize the acute ocular inflammatory response and estimate the NOEL in the nonhuman primate to a range of endotoxin doses delivered by intravitreal injection.
Methods: Female cynomolgus macaques were treated with a single intravitreal dose at levels ranging from 0.01 to 0.5 endotoxin units/eye (EU/eye; USP standard), one animal/both eyes/dose level. Doses were administered in two 50 µL injections, given approximately 10 min apart for a 100 µL/eye total volume. Animals were monitored for 2 weeks post treatment by clinical ophthalmic exam using the modified standardization of uveitis nomenclature (SUN) Working Group Grading Scheme to score aqueous cell and flare. In addition, the posterior segment was evaluated at 1 and 2 weeks post treatment with color fundus photography and optical coherence tomography (OCT). Ocular histomorphology was evaluated at term.
Results: Intravitreal doses ≥ 0.05 EU/eye caused a generally dose-related acute inflammatory response characterized by an increase in aqueous flare and cell, and vitreal haze and cell. At 0.05 EU/eye, the anterior segment response was greatest by 2 days post dose and resolved by 1 week. Vitreal cell first observed after 4 days increased through 2 weeks and correlated with the presence of mononuclear cell infiltrates observed histologically at higher doses. Thickening of the retinal nerve fiber layer, wrinkling of the inner limiting membrane and mildly swollen optic nerve were noted at ≥ 0.1 EU/eye at 1 week post dose, findings which improved at 2 weeks. With the exception of mild vitreal haze noted by OCT there was no evidence of ocular inflammation at a dose level of 0.01 EU/eye.
Conclusions: Intravitreal administration of a formulation containing endotoxin corresponding to 0.01 EU/eye to the nonhuman primate, was shown to elicit a subclinical inflammatory response identified with OCT. Results from this study compare well with those determined in a similarly designed study conducted in rabbits where 0.01 EU/eye was the NOEL (Streit T, et al, IOVS 2015;56:(7):3368).
Commercial Relationships: Brian J. Christian, None; **Paul Miller**, None; **T Michael Nork**, None; **Carol A. Rasmussen**, None; **Thomas Larsen**, None; **Evan A. Thackaberry**, None; **Helen Booler**, None; **Vladimir Bantseev**, None

Program Number: 5165 **Poster Board Number:** B0052
Presentation Time: 8:30 AM–10:15 AM
Toxic keratopathy due to zoanthid coral palytoxin
Asim V. Farooq[1], Allister Gibbons[2], Matthew Council[3], George J. Harocopos[4], Simon Holland[5], Jeffrey Judelson[6], Bradley Shoss[4], Eric Schmidt[3], Umi Md Noh[5, 7], Alexander D'Angelo[3], Rao Chundury[3], Richard Judelson[6], Victor L. Perez[2], Andrew J. Huang[4]. [1]Ophthalmology and Visual Science, University of Chicago, Chicago, IL; [2]University of Miami, Miami, FL; [3]St. Louis University, St. Louis, MO; [4]Washington University in St. Louis, St. Louis, MO; [5]University of British Columbia, Vancouver, BC, Canada; [6]Pasqua Hospital, Regina, SK, Canada; [7]National University of Malaysia, Bangi, Malaysia.
Purpose: To describe a series of patients that developed toxic keratopathy shortly after exposure to zoanthid coral.
Methods: Retrospective, multi-center case review.
Results: The corneal findings in seven patients exposed to aquarium coral palytoxin ranged from a mild reversible epitheliopathy to severe keratolysis and perforation. Treatment most commonly included topical steroids and topical antibiotics; in some cases, oral doxycycline, oral prednisone, amniotic membrane transplantation, corneal gluing, and/or corneal transplantation were employed. All patients provided a history of exposure to zoanthid corals, although in some cases this history was not elicited at the time of initial presentation. A review of the literature revealed that palytoxin is one of the most lethal substances in the world, and exposure can lead to severe systemic effects including respiratory failure and death.
Conclusions: Zoanthid coral palytoxin is a potential cause of severe toxic keratopathy. In patients presenting with signs suggesting a toxic exposure but without a clear etiology, a thorough history should include whether there has been recent contact with zoanthid coral species. Treatment should include aggressive anti-inflammatory medications, with a high index of suspicion for secondary infection. We recommend the use of personal protective equipment, including goggles and gloves, while handling zoanthid coral species.
Commercial Relationships: Asim V. Farooq, None; **Allister Gibbons**, None; **Matthew Council**, None; **George J. Harocopos**, None; **Simon Holland**, None; **Jeffrey Judelson**, None; **Bradley Shoss**, None; **Eric Schmidt**, None; **Umi Md Noh**, None; **Alexander D'Angelo**, None; **Rao Chundury**, None; **Richard Judelson**, None; **Victor L. Perez**, None; **Andrew J. Huang**, None

Program Number: 5166 **Poster Board Number:** B0053
Presentation Time: 8:30 AM–10:15 AM
Ocular side effects of novel and traditional chemotherapeutic agents
Anne Kunkler, Kari Kendra, Colleen M. Cebulla. The Ohio State University, Columbus, OH.
Purpose: Traditional and novel chemotherapeutics improve the prognosis and quality of life for a variety of malignancies. However, limited data are available regarding the ocular side effects of many of these novel agents. This retrospective, observational study aims to examine the ocular side effects of chemotherapeutic agents in patients treated for their malignancy at The Ohio State University.
Methods: Approval was obtained from the institutional review board at the Ohio State University. An ICD-9 search of the electronic medical record was performed for patients treated with high risk medications who were seen in the department of ophthalmology between 1/1/2010 and 2/2/2015. This search yielded 3,531 cases. The first 2,000 charts were reported previously (ARVO 2016). The last 1,531 charts were analyzed herein. A temporal association between

chemotherapeutic use and development of the side effect was deemed necessary for inclusion.
Results: Eight of the 1500 patients analyzed were oncologic patients who developed ocular side effects. The patients were being treated with Veliparib, ARRY-438162, Pimasertib/SAR245409, Ibrutinib, Ipilimumab, Paclitaxel, and Pemetrexed. The most common presenting symptoms were anterior segment side effects (red eye, dry eye, epiphora, and/or eyelid edema (4/8 or 50%)). The most common medication with anterior side effects was Ibrutinib (2/8 patients), which also induced photophobia and headache. Posterior ocular side effects were noted in a patient treated with a MEK inhibitor (ARRY-438162) who developed subretinal fluid and a patient treated with Ipilimumab developed bilateral macular edema. Pemetrexed, an antimetabolite, was associated with development of a visual field defect and suspected ischemic optic neuropathy. Every patient remained on treatment; 4 patients had resolution without changing the drug, 1 experienced complete resolution after decreasing the dose, and 3 continued to have ocular effects while on drug.
Conclusions: The ocular side effects found in this review were generally mild to moderate, with most either resolving while remaining on treatment or at a reduced dose.
Commercial Relationships: Anne Kunkler, None; **Kari Kendra**, None; **Colleen M. Cebulla**, None
Support: Ohio Lions Eye Research Foundation; K08EY022672 from the National Eye Institute of the National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding institutions. Clinical trial support from Bristol-Myers Squibb.

Program Number: 5167 **Poster Board Number:** B0054
Presentation Time: 8:30 AM–10:15 AM
Reduction of macular pigment as early feature of Tamoxifen retinopathy: a pilot study
Chiara Preziosa, Isabella D'Agostino, Ugo Nava, Mariano Cozzi, Mario V. Cigada, Marco Pellegrini, Giovanni Staurenghi. Sacco Hospital, Eye Clinic, University of Milan, Milan, Italy.
Purpose: Tamoxifen retinopathy is a condition characterized by cavitations occurring in the central macula at Spectral Domain - Optical Coherence Tomography (SD-OCT) imaging; these alterations appear similar to changes occurring in macular telangiectasia type 2 where a reduction of macular pigment has been already established with toxicity primarily affecting retinal Muller cells. In this study we investigated macular pigment density in patients treated with Tamoxifen for breast cancer.
Methods: A transversal observational study to compare the macular pigment optical density (MPOD) in 30 patients treated with Tamoxifen (20 mg daily) and 16 healthy subjects matched by age. The MPOD was measured by means of the two-wavelengths autofluorescence (AF) technique (488 and 514 nm) using the Heidelberg HRA + OCT Spectralis (Heidelberg Engineering, Heidelberg, Germany). MPOD maps were generated by automatic subtraction of the log AF images using the inbuilt Eye Explorer software (HEYEX).
Results: Mean MPOD at peak was 0,69 ± 0,18 density units (DU) and 0,78 ± 0,14 respectively in Tamoxifen and control group (P = 0,07). In Tamoxifen group the mean MPOD at 0,5° eccentricity was 0,58 ± 0,14, at 1° was 0,54 ± 0,13 and at 2° was 0,34 ± 0,1 while in the control group it was 0,62 ± 0,16 at 0,5° eccentricity, 0,55 ± 0,13 at 1° and 0,32 ± 0,06 at 2° (P > 0,05). The radius at half peak of MPOD was located at 1,4 and 1,1 degrees from fovea respectively in Tamoxifen group and in healthy subjects (P = 0,0076). A linear regression investigated the relation between the duration of treatment (mean 32 months, min 10 max 60) and the MPOD at peak, showing a weak inverse correlation (r = -0,35, P = 0,051).
Conclusions: In our series patients treated with Tamoxifen likely showed a reduction of MPOD levels at peak, 0,5° and 1° eccentricity compared to control group; nevertheless these differences were not statistically significant. The radius at half peak of MPOD in Tamoxifen group was statistically significant farther from the fovea compared to control group, suggesting an alteration in macular pigment distribution in patients treated with Tamoxifen probably due to a reduction of macular pigment nearby the fovea.
Commercial Relationships: Chiara Preziosa, None; **Isabella D'Agostino**, None; **Ugo Nava**, None; **Mariano Cozzi**, Alcon (F), Heidelberg Engeneering (F), Bayer (F); **Mario V. Cigada**, None; **Marco Pellegrini**, Optovue (R); **Giovanni Staurenghi**, Alcon (R), Alcon (C), Optovue (F), Zeiss (F), Boehringer Ingelheim (C), Genentec (C), Novartis (C), Optos (C), Roche (C), Novartis (R), Bayer (C), Heidelberg Engineering (C), Bayer (R), Novartis (F), Heidelberg Engineering (R), Ocular Instrument (P), Zeiss (C), Allergan (C)

Program Number: 5168 **Poster Board Number:** B0055
Presentation Time: 8:30 AM–10:15 AM
Safety and tolerability of trabodenoson and latanoprost in dogs following a 39 week ocular (topical) instillation
Adam Brockman, David Albers, William K. McVicar. NonClinical Development, Inotek Pharmaceuticals Inc, Lexington, MA.
Purpose: To determine ocular toxicity of 3% and 6% trabodenoson alone or in the presence of latanoprost when administered twice daily in one eye for 39 weeks in beagle dogs.
Methods: Topical ocular formulations of trabodenoson (TRABO - 3%: 1500 µg/dose; 6%: 3000 µg /dose) were administered twice daily alone or in combination with once daily latanoprost (LAT) (0.005%: 1.5 µg/dose) for 39 weeks in the right eye of beagle dogs (8-9 month old: 3 males and 3 females per treatment group. Animals were monitored daily, with several in-life procedures conducted, including ophthalmoscopic examinations, slit-lamp biomicroscopy and Hackett-McDonald scoring on all animals pretest, monthly, and at the end of study. Electrocardiography (ECG) examinations were performed twice monthly two hours after the first daily dose. Blood was collected on days 1, 28, and 273, and plasma was analyzed for TRABO and its primary metabolite using validated bioanalytical methods. Toxicokinetic (TK) parameters were estimated using a non-compartmental approach consistent with an ocular route of administration. A complete necropsy was performed and representative tissues from all organ systems were preserved for histopathological evaluation.
Results: No dose-related clinical signs or changes in body weight, ECG, calculated heart rate were noted. No TRABO-related findings were noted from external or internal ocular examinations. Pupillary constriction and hyperemia were noted in LAT-treated animals only. TK evaluation estimated maximum plasma concentration of TRABO was reached within the first 15-60 min post dose when dosed alone or in combination with LAT; $AUC_{0\text{-}8hr}$ and C_{max} increased with increasing dose in a less than dose proportional manner. No organ weight or gross pathological findings were noted in any of the treatment groups. No histopathological findings were noted in any ocular tissue with TRABO alone or in combination w LAT, and any microscopic findings in other tissues were considered incidental and unrelated.
Conclusions: TRABO given by twice daily ocular instillation for 39 weeks at 1500 or 3000 µg/dose with or without once daily 1.5 µg/dose LAT to beagle dogs was well-tolerated and produced no evidence of ocular or systemic toxicity. Based on these results, the no-observed-adverse-effect level for TRABO was considered to be

603-655 µg/kg/day alone, or in combination with 0.15-0.163 µg/kg/day LAT.
Commercial Relationships: Adam Brockman, Inotek Pharmaceuticals Inc (E); **David Albers**, Inotek Pharmaceuticals Inc (E); **William K. McVicar**, Inotek Pharmaceuticals Inc (E)

Program Number: 5169 **Poster Board Number:** B0056
Presentation Time: 8:30 AM–10:15 AM
Preclinical safety assessment of novel pharmaceuticals following intravitreal biologics in rabbits
Yong Li[1, 2], Joanna Marie Busoy[1], Ben Alfyan Achirn Zaman[2], Jay Ji-Ye Wei[1, 2], Wanjin Hong[2], Tien Yin Wong[1, 3]. [1]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore, Singapore; [2]Institute of Molecular and Cell Biology, A*STAR, Singapore, Singapore, Singapore; [3]Duke NUS Medical School, Singapore, Singapore, Singapore.
Purpose: Intravitreal injections of anti-angiogenic drugs have become a dominant approach to restore sight in the blindness of the angiogenic and neovascular diseases. However, the intravitreal biologics is often complicated by immunogenicity and inflammation. The purpose of this study was to evaluate safety and tolerability of a novel anti-angiogenic small peptide (SIPRAD 0276) after a single intravitreal (IVT) injection in naïve Dutch-Belted (DB) rabbits.
Methods: 4 DB rabbits were randomly divided into 2 groups (each group, n=2). The small peptide of SIPRAD 0276 was formulated in a fixed volume (50 µl), and concentrations ranged from 0.2 to 5mg per eye in single-dose. Ocular inflammation was evaluated in preclinical observation by one ophthalmologist using slit lamp for anterior segment screen. Standard grading from 0-4 was applied according to the Standardization of Uveitis Nomenclature (SUN) grading scheme. Retinal toxicity was determined by the profile of fundus photographic images, fluorescein angiography (FA) and optical coherence tomography (OCT). All imaging and intraocular pressure (IOP) measurements were performed at baseline and scheduled time points over 4 weeks in conscious animals that were manually restrained.
Results: No anterior segment inflammation was observed at the low dose group (n=2) of 0.2mg/eye. The eyes were received with high dose of 5mg/eye (n=2) which showed an slight uveitic reaction (aqueous flare of 1+ to 2+ and 0.5+ for 1–5 cells per field) peaked at 3 days after the injection, and slowly resolved by 2 to 3 weeks subsequently. There was no significant change in IOP or retinal toxic effects were identified such as vitreous clouding, retinal cloudy or edema by fundus photography, OCT as well as FA during the study period in any of the eyes.
Conclusions: A single intravitreal administration of anti-angiogenic small peptide included an early onset uveitic reaction in a dose-dependent fashion by slit-lamp examinations in DB rabbits.
Commercial Relationships: Yong Li; **Joanna Marie Busoy**, Singapore Eye Research Institute, Singapore National Eye Centre, Singapore (E); **Ben Alfyan Achirn Zaman**, Institute of Molecular and Cell Biology, A*STAR, Singapore (E); **Jay Ji-Ye Wei**, Institute of Molecular and Cell Biology, A*STAR, Singapore (E), Singapore Eye Research Institute, Singapore National Eye Centre, Singapore (S); **Wanjin Hong**, Institute of Molecular and Cell Biology, A*STAR, Singapore (E); **Tien Yin Wong**, Duke NUS Medical School, Singapore (E), Singapore Eye Research Institute, Singapore National Eye Centre, Singapore (E)

Program Number: 5170 **Poster Board Number:** B0057
Presentation Time: 8:30 AM–10:15 AM
Ocular safety of high doses Polyhexanide (PHMB) in healthy volunteers
Vincenzo Papa[1], Ivanka J. van Der Meulen[2], Sylvie Rottey[3], Guy Sallet[4], Iolanda Overweel[5], Margreet op 't Hof[5], Antonino Asero[1], John K. Dart[6]. [1]SIFI SPA, Lavinaio, Italy; [2]Academic Medical Center (AMC), Amsterdam, Netherlands; [3]DRUG Research Unit, Ghent, Belgium; [4]O.L.V. Research Centre, Aalst, Belgium; [5]PSR Group, Amsterdam, Netherlands; [6]Moorfields Eye Hospital and the UCL Institute of Ophthalmology, London, United Kingdom.
Purpose: PHMB 0.02% eye drops are widely used in the treatment of *Acanthamoeba* keratitis. However, higher concentrations of PHMB may be required for patients with *Acanthamoeba* invading the deep stroma. To evaluate the safety and tolerability of three dose levels (0.04%, 0.06% and 0.08%) of preservative-free PHMB ophthalmic solution, a double-masked, placebo-controlled, parallel-group multicenter Phase I study was performed in healthy volunteers
Methods: Ninety volunteers (44 M/46 F, age 18-55 years) were randomised to 1 of the 4 study arms: 0.04%, 0.06%, 0.08% PHMB and placebo, in a 3:3:3:1 ratio respectively. Subjects were dosed 12 times daily (1 drop every hour, daytime only) for 7 consecutive days followed by 6 times daily (1 drop every 2 hours) for 7 days.
The primary outcome measure was the rate of dose limiting events (DLEs) leading to interruption of dosing. The frequency of treatment emergent adverse events (TEAEs) as well as serious AEs (SAEs) starting after the first administration of the study drug were also computed. The rate of occurrence of SAEs, DLEs and TEAEs were compared between groups by Fisher's exact test. Statistical tests were performed 2-sided and with a 5% significance level. A 95% confidence interval for the difference between each respective dose and placebo and p-value was calculated.
Results: During the study no SAEs occurred. The global DLE rate was 5.6%. DLEs occurred in five subjects (2 dosed with 0.06% PHMB and 3 with 0.08% PHMB). No statistically significant differences between treatment groups were observed. Events causing DLEs (burning, itching, conjunctival hyperaemia, corneal staining) ranged from mild to moderate intensity. The global TEAE rate was 58.9%. TEAEs occurred in 53 subjects with a frequency of 55.6%, 38.5%, 78.6% and 59.3% in the placebo, 0.04%, 0.06% and 0.08% PHMB dose groups respectively. Only the difference between the 0.04% and 0.06% PHMB groups was statistically significant (p=0.0051). Most frequently reported events were conjunctival staining, corneal staining, pain after instillation and conjunctival hyperemia. Most TEAEs were of mild intensity.
Conclusions: Only mild to moderate ocular AEs occurred after intensive dosing with high concentrations of PHMB. Therefore, it is considered safe to investigate 0.08% PHMB eye drops in a Phase III study in patients with *Acanthamoeba* keratitis.
Commercial Relationships: Vincenzo Papa, SIFI SPA (E); **Ivanka J. van Der Meulen**, None; **Sylvie Rottey**, None; **Guy Sallet**, None; **Iolanda Overweel**, None; **Margreet op 't Hof**, None; **Antonino Asero**, SIFI SpA (E); **John K. Dart**, None
Support: European Union under the Seventh Framework Programme (GA N 305661)
Clinical Trial: NCT02506257

Program Number: 5171 **Poster Board Number:** B0058
Presentation Time: 8:30 AM–10:15 AM
Safety of Polyhexanide (PHMB) 0.08% ophthalmic solution after 26-week repeated-dose ocular administration in rabbits
Antonino Asero[1], Michela Salvador[2], Silvana Venturella[2], Germano Oberto[2], Anna Rita Blanco[1]. [1]SIFI SPA, Lavinaio - Aci S. Antonio, Italy; [2]RTC SPA, Pomezia - ROMA, Italy.
Purpose: The objective of the study is to evaluate the safety of PHMB 0.08% following repeated ocular instillation for 26 weeks in rabbits.
Methods: Eight male and eight female New Zealand White rabbits were treated into the right eye with 1 drop of PHMB 0.08%: 16 times/day at approximately 1 hour-intervals from day 1 to day 5; 8 times/day at approximately 2-hour intervals from day 6 to week 3 (day 21) and 4 times/day at approximately 4-hour intervals from week 4 to week 26. Another identical group of animals, acting as a control, was similarly treated with placebo. The left eye in both the two groups remained untreated.
Ocular irritation assessment was performed daily, before first dosing, in all animals for 28 days then weekly up to the end of the study. Slit-lamp examination and corneal epithelium fluorescein staining of both eyes of all animals were performed prior to allocation and on day 1 before the first treatment. Subsequently, all animals were examined weekly prior to the first dosing. In addition, main target tissues of all animals sacrificed at the end of the 26 weeks were histologically examined.
Results: No irritation of the conjunctivae, iris or cornea was observed on any day of treatment in all animals treated with placebo. In the animals treated with PHMB 0.08%, slight conjunctival redness was generally noted in the majority of animals starting from day 2 up to day 4. Slight conjunctival chemosis was noted on day 2 and 3 in a few numbers of treated animals as well as slight discharge in few animals on the first days of the treatment period. Afterwards, in the eye treated with PHMB 0.08% slight conjunctival redness and discharge were observed only in a sporadic number of animals during the study. No lesions were observed with fluorescein staining examination in all animals treated with PHMB 0.08% or with placebo. No ocular abnormalities were detected throughout the study with slit-lamp examination. No treatment-related changes were reported after histopathological examination of the eyes and their annexa as well as in the remaining organs/tissues.
Conclusions: A 26-week repeated instillation of PHMB 0.08% in rabbits did not show any relevant treatment-related effect. These favourable results strengthen our plan to develop PHMB 0.08% ophthalmic solution as a novel orphan drug for the treatment of *Acanthamoeba* keratitis.
Commercial Relationships: Antonino Asero, SIFI SPA (E); **Michela Salvador**, RTC SPA (E); **Silvana Venturella**, RTC SPA (E); **Germano Oberto**, RTC SPA (E); **Anna Rita Blanco**, SIFI SPA (E)
Support: European Union under the Seventh Framework Programme (Grant Agreement number 305661)

Program Number: 5172 **Poster Board Number:** B0059
Presentation Time: 8:30 AM–10:15 AM
Dual enkephalinase inhibitor (DENKI) PL265: a novel topical treatment for ocular pain?
Annabelle Reaux-le Goazigo[1], Hervé PORAS[2], Tanja OUIMET[2], Christophe BAUDOUIN[1, 3], Stéphane MELIK PARSADANIANTZ[1], Michel WURM[2]. [1]Therapeutic Department, INSERM, UMR_S968, Vision Institute, Paris, France; [2]PHARMALEADS, PARIS, France; [3]INSERM-DHOS CIC 1423, Quinze-Vingts National Ophthalmology Hospital, PARIS, France.
Purpose: The peripheral endogenous opioid system is critically involved in neuropathic and inflammatory pain. Here, we tested the antinociceptive effect of a highly specific DENKI prodrug, PL265, using experimental models of corneal pain.
Methods: Adult male C57BL/6 mice (8 weeks old) were used. Under anesthesia, a corneal scraping was performed on one eye with a trephine (1.5 mm) at day 0 (D0). Some operated animals received a drop of LPS (50 μg/2 μl) after the corneal injury and again on D3. Non-operated and operated mice were treated twice a day either with a drop of PL265 (10 mM) or with PBS (control animals) in right eye for 5 days. The ocular surface was evaluated by *in vivo* confocal microscopy at D5. Mechanical and chemical sensitivity were evaluated with von Frey filaments at D3 and D5 and capsaicin treatment at D5, respectively. Data were analyzed using Prism® software.
Results: In non-operated mice (without corneal injury), topical instillation with PL265 for 5 days did not change ocular surface and mechanical sensitivity compared to PBS-treated mice. In mice with corneal injury, we observed that topical daily instillation of PL265 significantly increased mechanical threshold at D5 compared to PBS-treated mice (49.00 ± 4.58 mg versus 29.60 ± 4.43 mg, p= 0.0186; n=10 per group). In addition, the palpebral closure time induced by topical capsaicin was significantly decreased (97 versus 210 seconds, p= 0.0013; n= 10 per group) after PL265 treatment. In experiments using corneal injury plus LPS instillation, we found that topical treatment with PL265 has potent antinociceptive effects. Mice treated with PL265 exhibited an increased mechanical threshold (61.00 ± 14.70 mg versus 24.00 ± 4.00 mg, p = 0.0413; n=5 per group) at D5 and a significantly shorter palpebral closure time (70 seconds compared to 170 seconds in PBS-treated mice, p= 0.0079; n = 5 per group).
Conclusions:
This study provides the first evidence that PL265, a highly specific DENKI prodrug, is highly effective to decrease corneal sensitivity. This prodrug appears as a promising treatment for ocular pain alleviation.
Commercial Relationships: Annabelle Reaux-le Goazigo, None; **Hervé PORAS**, PHARMALEADS (E), PHARMALEADS (P); **Tanja OUIMET**, PHARMALEADS (E); **Christophe BAUDOUIN**, None; **Stéphane MELIK PARSADANIANTZ**, None; **Michel WURM**, PHARMALEADS (E), PHARMALEADS (P)
Support: Grant Pharmaleads - UPMC C16/1069

Program Number: 5173 **Poster Board Number:** B0060
Presentation Time: 8:30 AM–10:15 AM
Effects of botulinum toxin type A for the treatment of dry eye syndrome and tear biomarkers
Joonhyung Yeo, Jae Chan Kim. Ophthalmology, Chung-Ang university Hospital, Seoul, Korea (the Republic of).
Purpose: Injection of botulinum toxin type A (BTX-A) in the medial part of the eyelid was previously reported to decrease the amount of tear volume ejected at each blink and the ability to drain tears, favoring the maintenance of the teardrop which may benefit patients with dry eye. This study tested the hypothesis that BTX-A injection can not only improve dry eye signs and symptoms, but also reduce a level of tear matrix metalloproteinase (MMP)-9 and serotonin.
Methods: In this retrospective study, 13 patients who treated with BTX-A for intractable severe dry eye (level 3 or 4) and 13 age-matched controls were included. In BTX-A group, patients were injected with BTX-A (2.5units/0.1mL per site) in each medial pretarsal orbicularis muscle of the upper and lower eyelid. In a control, only eye drops were administered. Before and at two weeks, one, two, four months after injection, dry eye symptoms (Ocular

Surface Disease Index, OSDI), signs (tear film break-up time, TBUT; Schirmer I test; corneal fluorescein staining score, CFS) were assessed. A level of tear MMP-9 and serotonin was measured before and at one month after injection.
Results: Twenty-six patients were included with a mean age of 57.8 years, and 80.8% (n=21) of patients were female. At baseline, no statistically significant differences were detected between BTX-A and control groups. Up to two months after injection, the eyes in BTX-A group showed lower OSDI scores compared with control group(25.5±14.9, 48.2±13.3, respectively; p=0.019). TBUT was found to be increased up to two months after injections in BTX-A group (p=0.006). The CFS were significantly lower in BTX-A group at one and two months (1.1±0.6; p=0.005, 1.0±0.6; p=0.001), and the Schirmer I test showed better measurement in the same group at two weeks and one month (6.9±1.0; p=0.03, 7.5±1.5; p=0.004). In addition, BTX-A reduced a level of tear MMP-9 and serotonin significantly one month after injection (p<0.001). There were no complications during the observations.
Conclusions: Our results are consistent with our hypothesis that BTX-A injection in the medial part of the lower and upper eyelid is an effective procedure that improves signs and symptoms of patients with dry eye and reduces a level of tear cytokines. However, the effect of BTX-A injection lasted only about two months. Further studies with a larger population will be needed to clarify the effect of BTX-A on dry eye syndrome.
Commercial Relationships: Joonhyung Yeo, None; **Jae Chan Kim**, None

Program Number: 5174 **Poster Board Number:** B0061
Presentation Time: 8:30 AM–10:15 AM
Exploring the effects of an ophthalmic solution containing high concentration hyaluronic acid (0.4%) and taurine 0.5% on the ocular surface of glaucoma patients under topical hypotensive therapy
Francesca Berardo[1], Manuela Ferrazza[1], Gloria Roberti[1], Luca Agnifili[2], Lucia Tanga[1], Gianluca Manni[4], Michele Figus[3], Manuele Michelessi[1], Francesco Oddone[1]. [1]IRCCS FONDAZIONE G.B.BIETTI, Rome, Italy; [2]University of Chieti-Pescara, Chieti, Italy; [3]University of Pisa, Pisa, Italy; [4]University of Rome Tor Vergata, Rome, Italy.
Purpose: Glaucoma patients (GLP) under preserved topical hypotensive therapy are at increased risk of reduced number of goblet cells (GC) and dry eye. Hyaluronic acid (HA) eye drops have been found to be effective in the treatment of dry eye. Taurine (TAU) is an aminonacid with osmoprotectant and antioxidant properties. The aim of this pilot, randomized, prospective, parallel study was to evaluate the effect of an ophthalmic solution containing HA 0.4% and TAU 0.5% (Oftaial Plus, Alfaintes srl) on ocular surface signs, symptoms and morphological parameters in GLP under chronic preserved hypotensive eye drops.
Methods: Twenty-eight GLP were included in this study. A complete ophthalmological examination was performed at baseline including Shirmer I and tear break-up time (BUT) tests and conjunctival confocal laser microscopy with the Rostock Cornea Module of the HRT3 (Heidelbergh Engineering, Germany). GLP were asked to fill the Ocular Surface Disease Index (OSDI) and Glaucoma Symptoms Score (GSS) questionnaires. GLP were then randomized to use either Oftaial Plus QID (G1) or an ophthalmic solution of hyaluronic acid 0.2% QID (G2) in both eyes and were followed-up at 30 and 90 days. Multivariate analysis of variance for repeated measures and Dunnet post-hoc test were used to analyze between and within groups changes throughout the follow-up.
Results: At baseline all the parameters were similar between G1 and G2: Shirmer test, (p=0.87), BUT (p=0.13), OSDI (p=0.31), GSS (p=0.28), GC (p=0.52).
Changes over time of GC, BUT, but not of Shirmer test, GSS and OSDI, were statistically different between groups (p=0.01 and p<0.001, p=0.19, p=0.28 and p=0.89).
GC and BUT significantly improved at 90 days from baseline in G1 (+20.4±32.6 cells, p=0.035 and +5.6±2.6 sec, p<0.0001) but not in G2 (+0.8±2.9 cells, p=0.033 and +0.9±2.0 sec, p=0.10). A trend of improved GSS score was observed at 90 days in G1 (+8.5±15.4, p=0.06) but not in G2 (+2.1±6.1, p=0.2). No significant changes of OSDI score were observed at 90 days in either groups.
Conclusions: The use of an ophthalmic solution containing HA at high concentration (0.4%) and TAU 0.5% in GLP under chronic preserved hypotensive topical therapy seems to be associated with an increase of conjunctival goblet cells number and with an improvement of BUT and dry eye symptoms.
Commercial Relationships: Francesca Berardo, None; **Manuela Ferrazza**, None; **Gloria Roberti**, None; **Luca Agnifili**, None; **Lucia Tanga**, None; **Gianluca Manni**, Omikron (R), Santen (S), Allergan (S), Alcon (S); **Michele Figus**, None; **Manuele Michelessi**, None; **Francesco Oddone**, Alcon (R), Omikron (R), Allergan (R), Santen (R)

Program Number: 5175 **Poster Board Number:** B0062
Presentation Time: 8:30 AM–10:15 AM
Moxifloxacin, voriconazole and chlorhexidine eye-drops: a new combined treatment for *Acanthamoeba* keratitis
Á Ortillés[1, 2], Ekaterina Gámez[1], Marta Sierra[2], Encarna Rubio[3], María Benito[3], Maria T. Fernández[4], Jose Á. Cristóbal[5], Begoña Calvo[2], Pilar Goñi[3]. [1]Animal Pathology, University of Zaragoza, Zaragoza, Spain; [2]Aragón Institute of Engineering Research (i3A), University of Zaragoza, Zaragoza, Spain; [3]Microbiology, Preventive Medicine and Public Health, University of Zaragoza, Zaragoza, Spain; [4]Physiatry and Nursery, University of Zaragoza, Zaragoza, Spain; [5]Ophthalmology, "Lozano Blesa" University Clinic Hospital, Zaragoza, Spain.
Purpose: Treating *Acanthamoeba* keratitis (AK) is often complicated and remains a major challenge because this disease cause prolonged or resistant infection to the conventional therapies. In the present study, the *in vivo* response to 0.5% moxifloxacin, 1% voriconazole and 0.02% chlorhexidine was assessed in a rabbit model.
Methods: Fourteen male New Zealand white rabbits (1.8-2.2 kg) were used. *Acanthamoeba* keratitis was induced through the corneal epithelial debridement with a diamond burr unit and infected contact lenses ($3.8x10^5$ cells/ml, 90% trophozoites) with an environmental strain (genotype T4; GenBank no. KY038362). Control eyes remained intact. The following drugs were applied three times a day, waiting 5 minutes between each, always in the same order: 0.5% moxifloxacin, 1% voriconazole, 0.02% chlorhexidine. All the agents were maintained for 42 days and 0.3% nepafenac was included on the 15^{th} day. For monitoring the treatment effectiveness, a clinical evaluation was performed every 7 days. Corneal cytology and culture, polymerase chain reaction and histopathological analysis were used to confirm the infection.
Results: The keratitis severity was classified as severe in a 50% (n=7) of animals, as moderate in a 42.9% (n=6) and as mild in a 7.1% (n=1). A significant improvement due to the topical treatment was observed in all animals. On the 42^{th} day, five animals were positive for PCR; however, only one corneal culture was positive after 30 days. Therefore, the treatment was considered amoebicidal in 13 of 14 rabbits. No toxicity signs were observed as a consequence of the therapy applied.

Conclusions: The medical treatment protocol based on moxifloxacin, voriconazole and chlorhexidine eye-drops has showed interesting effectiveness in this rabbit model, even in severe cases of AK. The dosing schedule established, three times a day, reduces significantly the standard protocol used up to date (hourly at the beginning), increasing the patient's comfort and reducing the possibilities of the drug-induced toxicity. Further *in vivo* investigations through clinical trials would help to establish its potential utility in humans.
Commercial Relationships: Ángel Ortillés, None; **Ekaterina Gámez**, None; **Marta Sierra**, None; **Encarna Rubio**, None; **María Benito**, None; **Maria T. Fernández**, None; **Jose Á. Cristóbal**, None; **Begoña Calvo**, None; **Pilar Goñi**, None
Support: Spanish Ministry of Economy and Competitiveness (DPI2014-54981R), Government of Aragón (FSE-DGA T88 and B124)

Program Number: 5176 **Poster Board Number:** B0063
Presentation Time: 8:30 AM–10:15 AM
Minimum inhibitory concentration of corneal storage media
Talita Mizushima. ISCMSP, Sao Paulo, Brazil.
Purpose: The purpose was to evaluate the minimum inhibitory concentration and the minimum bactericidal concentration of corneal storage media.
Methods: Minimum inhibitory concentration (MIC) was analyzed using macrodilution method and minimum bactericidal concentration (MBC) was analyzed by microbial culture of different standard strains of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus aureus* and *Streptococcus pneumoniae*. Samples of corneal storage media (Life4$0^{®}$, Optisol-GS®, Eusol- C®) were diluted for 100%, 50.00%, 25.00%, 12.50%, 6.25%, 3.12%, 1.60%, 0.80%, 0.40%, 0.20% and 0.10 %. Half milliliter of each diluted solution was placed into sterile micro tube and mixed with 0,5 mL of strain studied. After incubation, microbial growth was evaluated based on its turbidity of the corneal media. The main value considered in our study was based on the minimum concentration value of the sample without turbidity for each microorganism. And the MBC considered was the lowest concentration that demonstrates less than 99,9% of bacterial growth.
Results: The Life4$0^{®}$ presented the smaller MIC to *Staphylococcus epidermidis* 12, 5% and the smaller MBC to *Pseudomonas aeruginosa* 3,12%, *Staphylococcus epidermidis* 25% and *Staphylococcus aureus* 6,25%. The Optisol-GS® presented the smaller MIC to *Staphylococcus epidermidis* 12,5% and the smaller MBC to *Staphylococcus epidermidis* 25%. The Eusol- C® presented the smaller MIC to *Escherichia coli* 1,6%, *Pseudomonas aeruginosa* 0,8%, *Staphylococcus aureus* 1,6% and *Streptococcus pneumoniae* 0,40% and the smaller MBC to *Escherichia coli* 1,6%, *Staphylococcus aureus* 6,25% and *Streptococcus pneumoniae* 3,12%.
Conclusions: *In vitro*, the Life4C® and the Optisol-GS® presented an effective antimicrobial activity with MIC 12,5% and MBC 25%. The Eusol-C® presented an effective antimicrobial activity with MIC 25% and MBC >50%.
Commercial Relationships: Talita Mizushima

Program Number: 5177 **Poster Board Number:** B0064
Presentation Time: 8:30 AM–10:15 AM
Corneal neovascularization and inflammation in pterygium mouse model induced by subconjunctival injection of human pterygium epithelial cells
Minsup Lee[1], Seohyeon Yun[1], So Yeon Choi[1], JaeWook Yang[1, 2]. [1]T2B Infrastructure Center for Ocular Disease, Inje University Busan Paik Hospital, Busan, Korea (the Republic of); [2]Department of Ophthalmology, Inje University College of Medicine, Busan, Korea (the Republic of).
Purpose: The pathogenesis of pterygium is well-studied using human pterygium epithelial cells (hPEC), however, it is still unclear in mouse experimental model. As part of our ongoing effort to develop mouse experimental model, we induced pterygium-like lesion in athymic nude mouse by hPEC subconjunctival injection. And we further identified pathological protein expression in the pterygium-like lesion of cornea.
Methods: We induced pterygium in male athymic nude mouse (8 weeks, n=8) by nasal subconjunctival injection of hPEC (1×10^5 cells in 10 μL of DPBS). Cornea was observed by retinal microscopy with OCT and histochemistry. Cornea lesion was photographed with macroscopy for 10 days. Corneal thickness and lesion size were measured. Corneal neovascularization (NV) was scored from 0 through 3 where 0=no NV, 1=NV confined to the corneal periphery, 2=NV extending up to the pupil margin, and 3=NV extending beyond the pupil margin into the central cornea. Protein expression in cornea was analyzed by immunostaining and immunoblotting. Student's *t*-test was used for statistical analysis.
Results: For 10 days, pterygium-like lesion was increased in the hPEC-injected mouse. Pterygium-like lesion grew over the cornea in the hPEC-injected mouse. OCT photograph showed that corneal thickness was significantly increased in the lesion (235.3±22.1 μm), compared with normal cornea (163.1±14.8 μm). Corneal NV was extended into the central cornea and scored 2.1±0.6. Histochemistry figure showed neovascularization and macrophage infiltration in epithelial layer of cornea. Protein expressions of VEGF, ICAM-1, VCAM-1, fibronectin, MMP-2, and MMP-9 were increased in the lesion area. Pro-inflammatory cytokines including TNFα, IL-1β, and IL-6 were produced and co-localized with macrophage in the lesion area.
Conclusions: Pterygium-like lesion in mouse cornea after hPEC injection showed similar clinical features of human pterygium. Furthermore, the lesion showed to increase protein expressions related with neovascularization and inflammation in cornea. The hPEC-injected athymic nude mouse may be a useful *in vivo* model for studying human pterygium.
Commercial Relationships: Minsup Lee; **Seohyeon Yun**, None; **So Yeon Choi**, None; **JaeWook Yang**, None
Support: This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare Affairs, Republic of Korea.

Program Number: 5178 **Poster Board Number:** B0065
Presentation Time: 8:30 AM–10:15 AM
Granular corneal opacities in workers of BCMP manufacturing factory: a suspicious occupational eye injury
Xiao Lin[1], Yen-Chiao Wang[2], Hao Zhou[1]. [1]Ophthalmology and visual science, Fudan University, Shanghai, China; [2]School of Optometry, Indiana University, Bloomington, IN.
Purpose: Corneal opacities are a common sign of corneal dystrophies. We report two cases of granular opacities in the corneas of two patients, after one year exposure to 4,4'-Bis(chloromethyl) -1,1'-biphenyl(BCMP), which was produced in the factory where the

patients worked. To the best of our knowledge, this is the first report of granular corneal opacities that may be due to long term exposure in an occupational setting.

Methods: At presentation, they were examined with a slitlamp examination, confocal microscopy, anterior segment OCT and Pentacam. Refractive surgery was not acceptable for one patient, while another one had LASEK and PTK on her right eye in our hospital. Follow-up examinations were scheduled for 1 week, 2 week and 3 months after surgery, including UCVA, BCVA and slit-lamp biomicroscopy.

Results: Both patients denied any family history of corneal opacities. They had symptoms of gradual visual reduction, mild conjunctival congestion and corneal opacities. The upper eyelids were everted to exclude the presence of a foreign body. The opacities were in the subepithelium, Both confocal microscopy (Fig. 2A, B) and anterior segment OCT (Fig. 2C, D) demonstrated large amounts of round, hyper-reflective spots deposited in the subepithelium, Bowman layer and antierior stroma in both eyes of the cases. The patient who was without surgery had UCVA of 0.05 in both eyes. The one who had refractive surgery on her right eye had BCVA of 0.6 in the right eye and 0.5 in the left eye before surgery. Post-operatively, BCVA of the right eye was to 0.3, 0.3 and 0.5 at two weeks, six weeks and three months respectively. The BCVA of left eye was 0.25 at three months. Corneal topography was performed at 1 week. Slit-lamp biomicroscopy showed a significant decrease in total amount of the granular opacities, especially around the optic zone(Fig. 1C). The vision recovery process was slow but stable.

Conclusions: Corneal granular opacities may appear not only in corneal dystrophies, but the opacities may be associated with exposure to BCMP overtime, and therefore, may have prognostic significance and contribute to the study of the mechanism of corneal dystrophy. Workers in BCMP production factories should be aware of such potential occupational disease.

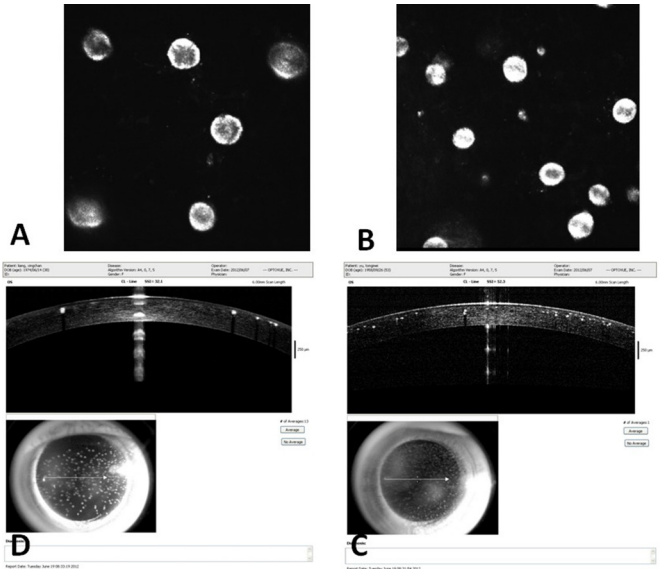


Fig.2

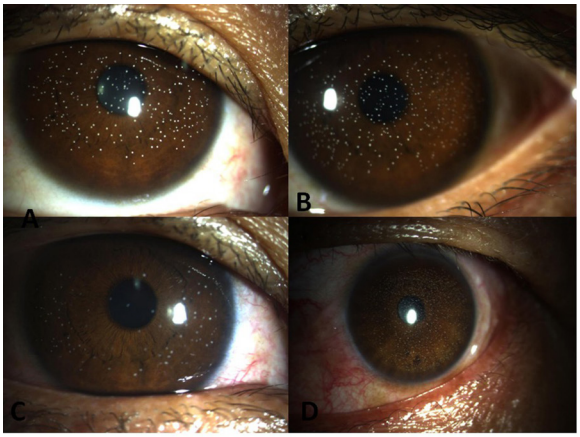


Fig1.

Commercial Relationships: Xiao Lin, None; **Yen-Chiao Wang**, None; **Hao Zhou**, None

Program Number: 5179 **Poster Board Number:** B0066
Presentation Time: 8:30 AM–10:15 AM

Distinct effects of trehalose and chloroquine on acute inflammatory response in human ocular surface and immune cells

Trailokyanath Panigrahi[1], Rohit Shetty[2], Shivapriya Shivakumar[1], Nallathambi Jeyabalan[1], Arkasubhra Ghosh[1]. [1]GROW Research Laboratory, Narayana Nethralaya Foundation, Bangalore, India; [2]Cornea Department, Narayana Nethralaya, Bangalore, India.

Purpose: Ocular surface inflammation is a vital immune mechanism, activated in response to a variety of stress and a key driver of disease pathology. Inflammation and autophagy are often implicated in disease conditions but the molecular link between them is poorly studied. The aim of this study is to understand the regulation of inflammatory signaling pathway by using autophagy regulators trehalose (TRE) and chloroquine (CQ) on human corneal cells and blood derived immune cells.

Methods: Acute inflammatory stress was simulated by treating human corneal epithelial cells (HCE) and peripheral blood mononuclear cells (PBMC) with recombinant TNFα for 2 hours. The regulation of TNFα induced inflammatory and autophagy gene expression and protein activation by TRE(80mM) and CQ(60nM) was studied and compared with Cyclosporine A (CsA). Optimal drug treatment concentrations were determined by dose escalation cytotoxicity studies. Gene expression was evaluated by quantitative PCR, while protein expression and functions were tested by immunoblotting and fluorescence imaging (Cyto-ID, Lysotracker Red).

Results: TRE treatment reduced inflammation induced gene expression of IL-6(19.7%), MCP-1(24.8%), IL-8(58.3%), MMP-9 (2.02%), LC3(44.37%) and LAMP-1(56.8%) in HCE. In PBMCs, we did not observe significant changes in gene expression levels of inflammatory or autophagy markers by TRE. However, PBMC cells treated with CQ showed a decreasing trend in the gene expression levels of TNFα (37.5%), IL-6(37.9%), MCP-1(14.13%), MMP-9(7.14%), when compared to controls and CsA treatment. But, the CQ treatment did not alter autophagy genes significantly. Similar results were observed in HCE cells treated with CQ. LAMP1 and LC3 protein levels were altered significantly by TRE and to a lesser extent by CQ in both control and TNFα stimulated cells.

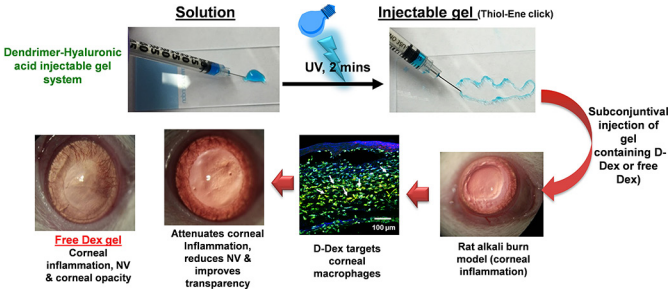
Conclusions: The data demonstrate that TRE and CQ have distinct anti-inflammatory mechanisms in corneal cells and primary immune cells. This regulatory effect of TRE was more pronounced in HCE rather than PBMCs. Interestingly, CQ, an autophagy blocker, had a similar inflammation dampening effect in both HCE and PBMCs without significant changes in autophagy markers. Thus CQ might regulate inflammation in an autophagy independent manner. The data suggest new modalities for treating ocular surface inflammation.
Commercial Relationships: Trailokyanath Panigrahi, None; **Rohit Shetty**, None; **Shivapriya Shivakumar**, None; **Nallathambi Jeyabalan**, None; **Arkasubhra Ghosh**, None
Support: Narayana nethralaya Foundation, Bangalore, India; Micro Labs Limited, Bangalore, India; FDC Limited, Mumbai, India

Program Number: 5180 **Poster Board Number:** B0067
Presentation Time: 8:30 AM–10:15 AM
Effect of a low concentration of desonide disodium phosphate on inflammatory parameters in a model of endotoxin-induced uveitis
Francesco Giuliano[1], Valeria Vitale[1], Giuseppe De Pasquale[1], Maria Grazia Mazzone[2]. [1]Research and Preclinical Development, S.I.F.I. S.p.A., Lavinaio-Aci S. Antonio, Italy; [2]Business and Portfolio Development, S.I.F.I. S.p.A., Lavinaio-Aci S. Antonio, Italy.
Purpose: A viable approach to an effective, yet safer, use of glucocorticoids in mild/moderate ailments of the ocular surface may be represented by eyedrops containing low concentrations of this class of molecules. Thus, we set out to characterize the effects of three different concentrations of desonide disodium phosphate (DES) in a model of endotoxin (LPS)-induced uveitis seeking to identify the one that could spare intraocular structures while exerting antiinflammatory activity on the ocular surface.
Methods: NZW male rabbits (7-11 w) were anesthetized, injected intravitreally with 100 ng of LPS (055:B5) and treated topically with 55 µl of DES 0.25%, 0.025%, 0.0025% or vehicle (VHC) immediately after, and then at 3, 6, and 9 h following LPS injection. The corneal thickness was measured by pachimetry prior to injection and then 9 and 24 h later. Then, animals were suppressed and the aqueous humor (AH) withdrawn for total leukocytes and prostaglandin E_2 (PGE_2) determination. Data were gathered from at least 10 animals per group. Significant differences between groups were sought by one-way ANOVA.
Results: Nine and 24h following LPS injection, VHC animals showed an increase of corneal thickness by 9% and 11% over baseline values (359±16 µm), respectively. The corneal edema induced by LPS was inhibited by DES 0.25% and 0.025% on average by more than 70% ($p<0.001$) both at 9 and 24 h, while DES 0.0025% was found to lack any significant effect ($p>0.05$). Similarly, when compared to VHC animals, DES 0.0025% did not inhibit accumulation of leukocytes or PGE_2 in AH, whereas DES 0.25% and 0.025% on average produced an inhibition of both parameters by more than 20% and 40%, respectively. However, only DES 0.25% did produce effects on accumulation of leukocytes and PGE_2 attaining statistical significance ($p<0.05$).
Conclusions: Here we have shown that DES, a medium-potency glucocorticoid, administered at concentrations matching those used in clinical practice is effective in inhibiting inflammation in a rabbit model of LPS-induced uveitis. Most importantly, we have shown that DES 0.025%, a concentration 10-times lower than that used in clinical practice, retained a notable degree of activity that may well prove effective in treating or preventing inflammatory conditions of the ocular surface while sparing intraocular tissues from adverse effects.
Commercial Relationships: Francesco Giuliano, S.I.F.I. S.p.A. (E); **Valeria Vitale**, S.I.F.I. S.p.A. (E); **Giuseppe De Pasquale**, S.I.F.I. S.p.A. (E); **Maria Grazia Mazzone**, S.I.F.I. S.p.A. (E)

Program Number: 5181 **Poster Board Number:** B0068
Presentation Time: 8:30 AM–10:15 AM
Effect of Recombinant Human Heat Shock Protein 27 on the Ultraviolet B-Irradiated Pterygial-derived Fibroblast
Yoo Jeong Park, Chan Hee Moon, Jin A Shin, Soon Suk Kang, Hungwon Tchah, Jae Yong Kim. Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea (the Republic of).
Purpose: To investigate the effect of recombinant human heat shock protein 27 (rhHSP27) on the differentiation of human pterygial fibroblast
Methods: Human conjunctival and pterygial fibroblasts were isolated and cultured from specimens of normal conjunctiva and pterygium head, which were collected during pterygium excision. Cultured conjunctival and pterygial fibroblasts were exposed to 20 mJ/cm^2 of UVB irradiation with and without 4 ug/mL rhHSP27 pre-treatment. Western blot analyses of α-smooth muscle actin (α-SMA), a marker for myofibroblast, were performed in each group and quantified using Image Gauge 4.0 software. The ratio of α-SMA / GAPDH was compared
Results: Pterygial fibroblasts showed 2.85 ± 0.11 fold greater α-SMA level than normal conjunctival fibroblasts ($P<0.001$). When normal conjunctival fibroblasts were exposed to UVB irradiation without rhHSP27 pre-treatment, the level of α-SMA was increased by 1.92 ± 0.02 times ($P<0.001$). However, when normal conjunctival fibroblasts were exposed to UVB irradiation with rhHSP27 treatment, the fibroblasts demonstrated 0.82 ± 0.04 fold smaller α-SMA level than control ($P=0.04$)
Conclusions: The rhHSP27 had inhibitory effects on UVB irradiation-induced differentiation from normal conjunctival fibroblasts to pterygial myofibroblast. The rhHSP27 might be used as a preventive treatment for pterygium
Commercial Relationships: Yoo Jeong Park, None; **Chan Hee Moon**, None; **Jin A Shin**, None; **Soon Suk Kang**, None; **Hungwon Tchah**, None; **Jae Yong Kim**, None
Support: the Basic Science Research Program through the National Research Foundation of Korea (NRF) and funded by the Ministry of Education, Science, and Technology (MEST) (NRF-2010-0025662) and a grant (2016-464) from the Asan Institute for Life Sciences, Seoul, Korea.

Program Number: 5182 **Poster Board Number:** B0069
Presentation Time: 8:30 AM–10:15 AM
PAMAM dendrimer based injectable gels for the treatment of corneal inflammation
Siva Pramodh Kambhampati[2, 3], Uri Soiberman[2, 3], Tony Wu[1, 3], Samuel C. Yiu[2, 3], Abdul Towerki[4], Walter Stark[2], Kannan Rangaramanujam[2, 3]. [1]Biomedical engineering, Johns Hopkins University, Baltimore, MD; [2]Opthalmology, Johns Hopkins School of Medicine, Baltimore, MD; [3]Center for Nanomedicine, Johns Hopkins School of Medicine, Baltimore, MD; [4]King Khaled Eye Specialist Hospital, Riyadh City, United Arab Emirates.
Purpose: Corneal inflammation is often encountered as a post-surgical complication or as a key pathological event in many corneal diseases. Current treatments involve frequent topical corticosteroids which often causes corneal toxicity, elevated IOP and corneal discoloration. Hence, new interventions that can provide sustained efficacy and reduce side effects will be highly beneficial. In this study, we evaluate our novel injectable dendrimer-dexamethasone

(D-Dex) hydrogels for targeted, sustained attenuation of corneal inflammation in rat mild alkali burn model.
Methods: D-Dex incorporated injectable gel was prepared using PAMAM dendrimer (G4-OH) and hyaluronic acid (HA) using click chemistry. Dendrimer-Cy5 (D-Cy5) was used to evaluate the biodistribution in alkali burn rat model. The efficacy of D-Dex or free Dex in the gel was evaluated for both clinical (POD 0, 3, 7 and 14) and biochemical parameters (POD 7 and 14). The rats were assessed for corneal thickness (CCT), corneal edema, corneal opacity and neovascularization using clinical observation, and IOP was measured using tonometry. Attenuation of corneal inflammation was evaluated using IHC, and PCR for cytokine expression.
Results: Alkali burn results in macrophage accumulation, increased corneal thickness, opacity, and edema in central cornea. Subconjuntivally-administered dendrimers showed pathology dependent biodistribution, targeting macrophages in the central cornea, with minimal dendrimer uptake in healthy corneas. Subconjunctival D-Dex gel treatment resulted in favorable clinical outcomes with reduced CCT ($p< 0.05$) and improved corneal clarity ($p< 0.01$) compared to free Dex and untreated positive controls. The extent of corneal NV was significantly reduced in D-Dex groups with no signs of elevated IOP. Compared to free Dex, D-Dex gel treatment attenuated corneal inflammation more effectively by suppressing the expression of inflammatory cytokines in a sustained manner.
Conclusions: Subconjunctival Dendrimer-dexamethasone gel treatment was associated with lower CCT, inflammatory cytokine expression, and macrophage recruitment to the central cornea. These findings suggest that the novel injectable D-Dex gel may be a potential drug delivery platform for the treatment of many inflammatory ocular surface disorders such as dry eye, microbial keratitis and post-surgical complications where frequent dosage are required.



Commercial Relationships: Siva Pramodh Kambhampati, Johns Hopkins University (P); **Uri Soiberman**, Johns Hopkins University (P); **Tony Wu**, None; **Samuel C. Yiu**, None; **Abdul Towerki**, None; **Walter Stark**, Johns Hopkins University (P); **Kannan Rangaramanujam**, Johns Hopkins University (P)
Support: Kwok cornea research funds, and NEI RO1 EY025304(RMK)

Program Number: 5183 **Poster Board Number:** B0070
Presentation Time: 8:30 AM–10:15 AM
D-Arginine as a Novel Excipient Resulted in Retinal Degeneration Following Intravitreal Administration to Male New Zealand White Rabbits
Roxanne Andaya, Helen Booler, Charly Sioson, Vladimir Bantseev. Genentech, Inc., South San Francisco, CA.
Purpose: High osmolality and high viscosity solutions are common problems in ophthalmology intravitreal (ITV) drug development. With a majority of drug excipients excluded from ITV ophthalmic drugs because of safety concerns unique to the eye, investigation into novel excipients for ITV drug administration is warranted. This study aimed to characterize the tolerability and toxicity of D-arginine as a novel excipient following ITV administration to rabbits.
Methods: Rabbits received a single ITV dose of buffered D-arginine-HCl solution ranging from 3.5 to 8.7 mg/eye (375-816 mOsm/kg). Another set of rabbits were given a single ITV dose of vehicle formulations with matched levels of tonicity. The duration of the in-life phase was 8 days, with gross clinical observations and body weights recorded daily. Intraocular pressure (IOP) measurements, indirect ophthalmoscopic examination and slit lamp biomicroscopic examination were performed. Clinical pathology and macroscopic and microscopic anatomic pathology were performed at interim and terminal necropsies, Day 2 and Day 8, respectively.
Results: ITV administration of D-arginine resulted in retinal toxicity at doses of 3.5 mg/eye and higher. The observed changes noted on Day 2, were irreversible and still progressing at Day 8, indicating that the lesions identified would eventually lead to loss of sight. High tonicity vehicles induced retinal changes at 534 mOsm/kg and above. The majority of retinal changes, including vacuolation of the photoreceptor layer were reversible; however, irreversible changes were identified at tonicities of 534 mOsm and above. Although no toxicity was observed during the in-life ophthalmic examinations, the histopathology revealed that intravitreally administered D-arginine is not tolerated in rabbits.
Conclusions: These data show that D-arginine is not a suitable excipient for ITV drug administration due to the severe retinal toxicity observed following ITV administration in rabbits. Further work is required to determine the mechanism of toxicity.
Commercial Relationships: Roxanne Andaya, None; **Helen Booler**, None; **Charly Sioson**, None; **Vladimir Bantseev**, None

Program Number: 5184 **Poster Board Number:** B0071
Presentation Time: 8:30 AM–10:15 AM
The Influences of Smartphone Use on the Tear film and Oxidative Stress
Yung Hui Kim[1], Won Choi[1], Ying Li[1], Lian Cui[1, 2], Ji Suk Choi[1], In Cheon You[3], Kyung Chul Yoon[1, 2]. [1]Department of Ophthalmology, Chonnam National University Medical School and Hospital, Gwangju, Korea (the Republic of); [2]Department of Biomedical Sciences and Center for Creative Biomedical Scientists, Chonnam National University, Gwangju, Korea (the Republic of); [3]Department of Ophthalmology, Chonbuk National University Medical School and Hospital, Jeonju, Korea (the Republic of).
Purpose: To investigate the influences of smartphone use on ocular symptoms, quantity and quality of the tear film, and oxidative stress.
Methods: In 50 eyes of 25 healthy volunteers, tear film break-up time (TBUT), Schirmer score, keratoepitheliopathy (KEP), and non-invasive TBUT (NIBUT) and tear meniscus height (TMH) measured by Keratograph 5M were evaluated. Subjective ocular fatigue evaluated by visual analogue scale (VAS), computer vision score (CVS), ocular surface disease index (OSDI), and accommodative power were evaluated before and after the use of the smartphone. Oxidative stress markers such as 8-oxo-2'-deoxyguanosine (8-OHdG), hexanoyl lysine (HEL), 4-hydroxy-2-nonenal (HNE), and malondialdehyde (MDA) in the tear film were measured by enzyme-linked immunosorbent assay (ELISA), and reactive oxygen species (ROS) in the conjunctival epithelium were measured by 2',7'-dichlorodihydrofluorescein diacetate (DCFDA). All measurements were evaluated at baseline, 1 hour, and 4 hours after the use of smartphone.
Results: TBUT and NIBUT significantly decreased 4 hours after the use of smartphone (p=0.019, p=0.023). Schirmer score, KEP, TMH, and accommodative power showed no significant changes.

Scores of OSDI, VAS, and fatigue, burning sense, and dryness of the CVS showed a significant increase at 1 and 4 hours after the use of smartphone compared with baseline (all $p<0.01$). In the analysis of oxidative stress markers, a concentration of HEL significantly increased 4 hours after the use of smartphone ($p=0.026$). The concentrations of the 8-OHdG, 4-HNE, and MDA showed no significant change. After 4 hours of smartphone use, the level of ROS significantly increased ($p<0.001$).
Conclusions: The use of smartphone could not only aggravate subjective symptom indices such as OSDI, VAS, and CVS but also deteriorate the tear film quality and induce the oxidative stress in the tear film.
Commercial Relationships: Yung Hui Kim; **Won Choi**, None; **Ying Li**, None; **Lian Cui**, None; **Ji Suk Choi**, None; **In Cheon You**, None; **Kyung Chul Yoon**, None

Program Number: 5185 **Poster Board Number:** B0072
Presentation Time: 8:30 AM–10:15 AM
Paradoxical neuroprotective effect induced by a single intravitreal injection of TNF-α following optic nerve crush
Moran Friedman Gohas[1, 2], Shirel Weiss[1, 2], Nitza Goldenberg-Cohen[1, 3]. [1]The Krieger Eye Research Laboratory, Felsenstein Medical Research Center, Petach Tikva, Israel; [2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [3]Ophthalmology Department, Bnai Zion Medical Center, Haifa, Israel.
Purpose: Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine produced by macrophages and T-cells. It plays an important role both in inflammation and apoptosis. In the eye, TNF-α appears to enhance apoptosis following trauma or inflammation. As predicted, TNFR1/2 knockout mice showed resistance to optic nerve crush (ONC) damage. Here we report a paradoxical neuroprotection effect of a single intravitreal injection (IVT) of TNF-α following ONC induction.
Methods: Histological analysis of wild type (WT) mice, with/without IVT TNF-α injection following ONC (n=5 each). Retinal thickness and the number of RGCs were measured. ONC was performed to the right eye only and the left eye served as control. Immunofluorescent staining with CD45, Vimentin and GFAP was used to demonstrate inflammatory reaction.
Results: Histologically, RGCs count revealed preserved RGCs in the retinae of the TNF-α injected group as compared to the control ONC induced mice. WT group dropped by 6.6% in RGC's count in comparison to the healthy eye, whereas the TNF-α injected group showed similar count and lost only 1.6%. Furthermore, retinal thickness of the control group lost 18.6% as compared to the treated group which lost 6.4%.
Conclusions: Previously, we showed that TNFR1/2 KO mice were resistant to ONC damage. Molecularly we showed increased TNF-α expression levels We assumed the lack of receptors blocks its proapoptotic effect. In this study, against expectations, IVT injection of TNF-α induced a neuroprotective effect. A possible mode of action is associated with decreased inflammation response. We assume that high dosage of TNF-α might suppress TNF-α receptors function and can cause a paradoxical nueroprotective effect. The immunomodulatory role in the inflammatory response following ONC is based on the balance between too low to over expression of TNF-α. As TNF-α inhibitors are used as neuroprotective drugs, the benefits and risks of the use of TNF-α or TNF-α blockers is not completely understood and yet to be explored.
Commercial Relationships: Moran Friedman Gohas, None; **Shirel Weiss**, None; **Nitza Goldenberg-Cohen**, None

Program Number: 5186 **Poster Board Number:** B0073
Presentation Time: 8:30 AM–10:15 AM
Ophthalmic artery occlusion causes endothelin-1 mediated vasoconstriction 48 hours post ischemia
Frank W. Blixt[1], Karin Warfvinge[2], Lars Edvinsson[1, 2]. [1]Department of Clinical Sciences, Lund University, Lund, Sweden; [2]Department of Clinical Experimental Research, Glostrup Research Institute, Glostrup, Denmark.
Purpose: Retinal ischemia is a major cause of blindness in the world. Researchers in the last couple of decades have focused on protecting the retina after ischemia by focusing on the retina itself. However, even today, few therapies are available. Within cerebral ischemia, vasculature has become a new focus for treatment approaches. After ischemia cerebral vasculature exhibits an increase in vasoconstrictive receptor expression which exacerbates cerebral damage after reperfusion. In the retina, few studies have evaluated the vascular effects following ischemia (Blixt et al 2016). This project evaluates the ophthalmic artery after the commonly known filamentous middle cerebral artery occlusion model which due to the immediate proximity of the ophthalmic artery to the MCA, also occludes the ophthalmic artery.
Methods: The vascular properties of the ophthalmic artery were evaluated with wire myography while immunohistochemistry coupled with fluorescence microscopy was used to visualize endothelin-1 receptor expression in the artery in both ischemic and control arteries.
Results: Results show an ET_B mediated vasoconstriction in MCAO operated animals which does not occur on controls. Furthermore, ET_B receptors are shown to be expressed in the smooth muscle cell layer after ischemia while controls express ET_B receptors only in endothelial cell.
Conclusions: This study shows an increase in ET_B mediated vasoconstriction in the ophthalmic artery after 48 hours induced by 2 hours of ophthalmic artery occlusion. This increase can lead to further decreased blood flow to the retina after occlusion/reperfusion and might thus become a valid target for future therapies.
Commercial Relationships: Frank W. Blixt, None; **Karin Warfvinge**, None; **Lars Edvinsson**, None
Support: Swedish Heart-Lung Foundation and Royal Physiographic Society of Lund

Program Number: 5187 **Poster Board Number:** B0074
Presentation Time: 8:30 AM–10:15 AM
Hypoxia protects horizontal cells from low glucose-induced Ca^{2+} dysregulation in the anoxia-tolerant goldfish
Michael W. Country, Benjamin F. N. Campbell, Michael G. Jonz. Department of Biology, University of Ottawa, Ottawa, ON, Canada.
Purpose: Intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ rises in neurons during ischemia or hypoxia, and induces cell death. The retina of anoxia-tolerant fish from the genus *Carassius* is resistant to hypoxia, but the underlying mechanisms at the cell level have not been investigated. Horizontal cells (HCs) of goldfish (*C. auratus*) exhibit spontaneous Ca^{2+} action potentials (APs), which may provide insights into HC Ca^{2+} dynamics during ischemia in the anoxia-tolerant retina. We tested the hypotheses that during hypoxia or ischemia Ca^{2+} APs in goldfish are reduced, and baseline $[Ca^{2+}]_i$ is maintained, in a novel *in vitro* model.
Methods: Dissociated HCs were loaded with the Ca^{2+} indicator, Fura-2, and superfused for 40 min with HEPES-buffered extracellular solution (ECS). HCs were treated for 20 min with hypoxic ECS (N=6), glucose-free ECS (N=6), oxygen-glucose-deprivation (OGD) ECS (N=10), or no treatment (N=10). Baseline $[Ca^{2+}]_i$ was monitored and spontaneous Ca^{2+} transients were analyzed for amplitude,

time-to-peak, area-under-the-curve (AUC), duration, and frequency. The fold-change for each parameter was obtained by normalizing data to pre-application values. The Kruskal-Wallis test and Dunn's post test were used for statistical analysis. Means ± S.E.M. are presented.

Results: Baseline $[Ca^{2+}]_i$ was significantly higher following administration of 0 glucose ECS, and was 3.2±0.6-fold higher than controls ($p<0.01$). Interestingly, when 0 glucose was combined with hypoxia during OGD application, baseline was unaffected. Hypoxia alone had no effect upon $[Ca^{2+}]_i$ baseline. Moreover, AP frequency was significantly affected ($p<0.05$). 0 glucose ECS abolished APs in nearly all cells, but frequency was preserved in hypoxic ($p<0.05$) and OGD conditions ($p<0.05$) over 0 glucose ECS. No significant changes in other parameters were observed.

Conclusions: Our results are consistent with the hypothesis that $[Ca^{2+}]_i$ baseline is maintained in OGD and hypoxic conditions, but do not support the hypothesis that Ca^{2+} APs are down-regulated in hypoxic or OGD conditions. The data suggest that glucose deprivation (which occurs during ischemia) causes Ca^{2+} dysregulation in goldfish HCs, but hypoxia preserves low $[Ca^{2+}]_i$ in spite of the concurrent low-glucose challenge. Further research will be needed to clarify the mechanisms by which hypoxia maintains Ca^{2+} homeostasis in the anoxia-tolerant goldfish.

Commercial Relationships: Michael W. Country, None; **Benjamin F. N. Campbell**; **Michael G. Jonz**, None
Support: NSERC Grant 342303

Program Number: 5188 **Poster Board Number:** B0075
Presentation Time: 8:30 AM–10:15 AM

Effects of hypobaric hypoxia on rat retina and protective response of resveratrol to the stress

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Purpose: The aim of current investigation was to explore the influence of hypobaric hypoxia on the rat retina and determine whether resveratrol has a protective efficacy on hypoxic damage to retina.

Methods: 54 Sprague-Dawley(SD) rats were randomly divided into the control, hypoxia group and resveratrol +hypoxia group. The hypoxia group and the resveratrol +hypoxia group were maintained at low pressure oxygen cabin (simulation for 5000m above sea level), and the resveratrol +hypoxia group was given daily intraperitoneal injection(30mg/kg,per day). Retina was removed after rats were treated for 7 days. Immunohistochemisty was processed for detecting the expression of thioredoxin 1 (TRX1), thioredoxin 2 (TRX2) and thioredoxin-interacting protein (TXNIP). The levels of superoxide dismutase(SOD)and glutathione peroxidase(GSH-Px)in retina were measured by ELISA. RT-PCR was performed to evaluate the mRNA expression of hypoxia inducible factor-1 (HIF-1),caspase 3, caspase 9, heat shock protein 70 (HSP70), and heat shock protein 90 (HSP90).

Results: Increased TXNIP, TRX1 and TRX2 expression presented in hypoxia group compared with controls, resveratrol treatment reversed these changes and especially significantly suppressed TRX1 and TRX2 expression in retinas, compared with those of the hypoxia group($P = 0.023$, $P = 0.034$ respectively). Hypobaric hypoxia reduced protein level of GSH-Px(41.55±2.04pg/ml) and SOD(137.21±16.10ng/ml) as compared to unexposed group(50.22±2.30pg/ml, 164.47±25.31ng/ml respectively), the administration of resveratrol attenuated hypoxia-induced alterations by enhancing the activity of two proteins(P=0.01, P>0.05 respectively). In comparison with controls, hypoxia up-regulated the gene expression levels of caspase3($P < 0.001$), caspase9 (P =0.007), HSP70(P=0.025), HSP90 ($P < 0.001$) and HIF-1($P = 0.034$). Resveratrol administration caused a significant decreases in the gene expression of caspase3($P < 0.001$), HSP90($P = 0.024$) and HIF-1 mRNA (P =0.001) as well as an increase in HSP70 mRNA when compared with the hypoxia group.

Conclusions: Hypobaric hypoxia poses a pathological impact on rat retina. The intervention of resveratrol elicits a protective role on hypobaric hypoxia-induced retina impairment by increasing antioxidant production and regulating the expression of hypoxia-related genes.

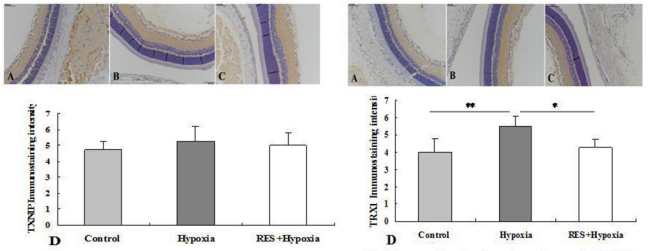

Fig1. Immunostaining of TXNIP expression in rat retina. (A) Control group; (B) Hypoxia group; (C) Resveratrol (RES) + hypoxia group; (D) Positive immunostaining intensity of TXNIP expression. Scale bar = 100 μ m.

Fig 2. Immunohistochemistry photomicrograph of TRX1 expression in rat retina. (A) Control group; (B) Hypoxia group; (C) Resveratrol (RES) + hypoxia group; (D) Positive immunostaining intensity of TRX1 expression (*P < 0.05;**P < 0.01). Scale bar = 100 μ m.

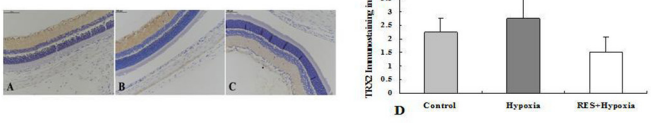

Fig 3. Immunohistochemical images of TRX2 expression in rat retina. (A) Control group; (B) Hypoxia group; (C) Resveratrol (RES) + hypoxia group; (D) Positive immunostaining intensity of TRX2 expression (*P < 0.05); Scale bar = 100 μ m.

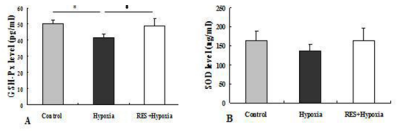

Fig 4. Effect of resveratrol (RES) on antioxidant levels. (A) GSH-Px; (B) SOD; Results are presented as mean ± standard deviation (SD) (n =6). (*P < 0.05)

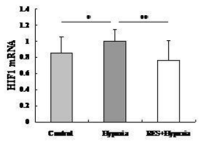

Fig 5. Effect of resveratrol (RES) on HIF-1 mRNA expression in retina. Results are presented as mean ± standard deviation (SD) (n =6). (*P < 0.05, **P < 0.01)

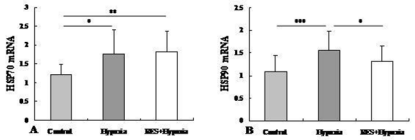

Fig 6. Effect of resveratrol (RES) on HSP70 mRNA (A) and HSP90 mRNA (B) expression in retina. Results are presented as mean ± standard deviation (SD) (n =6). (*P < 0.05, **P < 0.01 and ***P < 0.001)

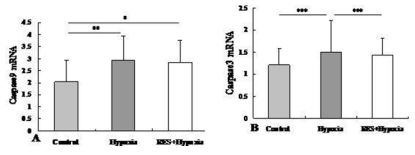

Fig7. Effect of resveratrol (RES) on caspase9 mRNA (A) and caspase3 mRNA (B) expression in retina. Results are presented as mean ± standard deviation (SD) (n =6). (*P < 0.05, **P < 0.01 and ***P < 0.001)

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