

208 Retina/RPE 1

Monday, May 08, 2017 8:30 AM–10:15 AM

Room 301 Paper Session

Program #/Board # Range: 1202–1208

Organizing Section: Physiology/Pharmacology

Program Number: 1202

Presentation Time: 8:30 AM–8:45 AM

The role of Fc portion in the intravitreal half-life of anti-VEGF drugs

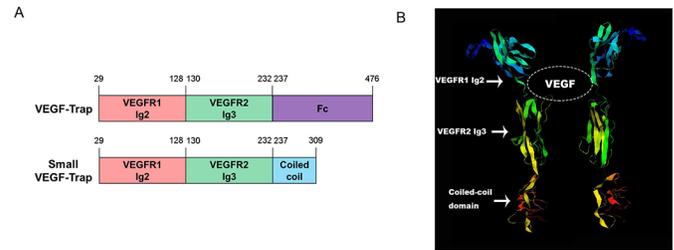
Kwangsic Joo¹, Sang Jun Park¹, Young Mi Na¹, Hye kyung Hong¹, Yewon Choi², Jung Eun Lee¹, Hyuncheol Kim³, Kyu Hyung Park¹, Ho Min Kim⁴, Jae Yong Chung², Se Joon Woo¹. ¹Department of Ophthalmology, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam-si, Korea (the Republic of); ²Department of Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine and Bundang Hospital, Seongnam-si, Korea (the Republic of); ³Department of Chemical and Biomolecular Engineering, Sogang University, Seoul, Korea (the Republic of); ⁴Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea (the Republic of).

Purpose: To identify the role of Fc portion in intraocular pharmacokinetics (PK) of protein drugs using the two identical protein drugs except the Fc portion

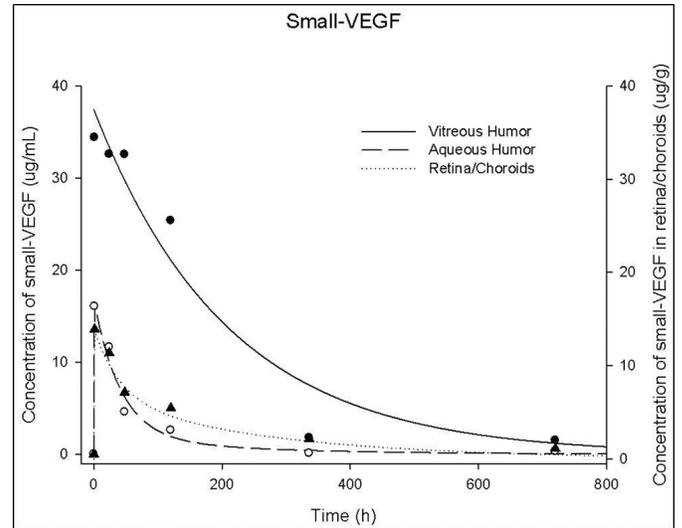
Methods: We generated a new VEGF-Trap without Fc portion (small VEGF-Trap, MW=100kDa) by replacing the Fc portion of conventional VEGF-Trap (MW=145kDa) with a dimerized coiled-coil domain. A total of 42 eyes of 42 rabbits were injected intravitreally with VEGF-Trap (n=21) and small VEGF-Trap (n=21). Eyes were harvested at 6 time points (1h and 1, 2, 4, 14 and 30 days after injections). The concentrations of VEGF-Trap and small VEGF-Trap in the vitreous, aqueous humor, and retina/choroid were measured by an indirect enzyme-linked immunosorbent assay, and pharmacokinetic properties of the drugs were analyzed under hypothetical compartment model system. One-compartment model was applied as the final model for the vitreous whereas two-compartment model was applied for aqueous humor and retina/choroid tissue.

Results: In all three tissues, the maximum concentrations of both small VEGF-Trap and VEGF-Trap were observed 1 hour after intravitreal injection. The estimated half-lives of small VEGF-Trap in the vitreous and retina/choroid were 1.4 and 2.3 times longer (145.02 and 102.12 hours, respectively) than those of VEGF-Trap (103.99 and 44.42 hours, respectively). The total exposure of small VEGF-Trap in the aqueous humor and retina/choroid were approximately 13.2% and 39%, respectively, of the vitreous exposure whereas VEGF-Trap concentrations were approximately 25.2% and 26.2%, indicating that small VEGF-Trap show a preference for the posterior excretion.

Conclusions: The vitreous and retina/choroid half-lives of small VEGF-Trap are significantly longer than that of VEGF-Trap in rabbit eyes despite the lower molecular weight of small VEGF-Trap. This suggests that Fc receptors in ocular tissues contribute to the elimination of anti-VEGF drugs. Truncation or mutation of Fc portion can partially prolong the residence time of VEGF-Trap and have the possibility of reducing the number of VEGF-Trap injections.



Protein structures of VEGF-Trap and truncated VEGF proteins. (A) Small VEGF-Trap contains a dimerized coiled coil domain instead of Fc domain of VEGF-Trap. (B) A protein 3D structure of small VEGF-Trap was predicted by I-TASSER.



Small VEGF-Trap concentration in the eye of rabbit. Points represent observed concentrations and lines represent estimated concentrations by models

Commercial Relationships: Kwangsic Joo, None; Sang Jun Park, None; Young Mi Na, None; Hye kyung Hong, None; Yewon Choi, None; Jung Eun Lee, None; Hyuncheol Kim, None; Kyu Hyung Park, None; Ho Min Kim, None; Jae Yong Chung, None; Se Joon Woo, None

Program Number: 1203

Presentation Time: 8:45 AM–9:00 AM

QRX-411, an Antisense Oligonucleotide (AON) Directing Splice Correction in *USH2A* mRNA Caused by the Frequent Deep-Intronic c.7595-2144A>G Mutation Associated with Retinitis Pigmentosa in Usher Syndrome Type 2

Hester van Diepen¹, Heelam Chan¹, Janne Turunen¹, Ma'ayan Semo², Anthony Vugler², Ralph Slijkerman³, Erwin van Wijk³, Peter Adamson^{1,2}. ¹ProQR Therapeutics, Leiden, Netherlands; ²Institute of Ophthalmology, UCL, London, United Kingdom; ³Department of Otorhinolaryngology and Donders Institute for Brain, Cognition and Behaviour, Radboudumc, Radboud University, Nijmegen, Netherlands.

Purpose: The recently discovered intronic c.7595-2144A>G mutation in the *USH2A* gene is associated with Usher syndrome. Usher syndrome is a genetic orphan disease presenting as a combination of deafness and blindness. Mutations in *USH2A* gene are the most frequent cause of Usher syndrome accounting for up to 50% of patients worldwide. There is no therapy available to address the blinding aspect of this disease. The c.7595-2144A>G mutation creates a splice donor site that leads to inclusion of a

pseudoexon into the mature mRNA. QRX-411 is a single stranded fully phosphorothioated and 2'-O-methyl modified antisense oligonucleotide targeting the frequently found intronic *USH2A* c.7595-2144A>G mutation. QRX-411 is designed to correct the splicing pattern and restore wild-type mRNA and *USH2A* function.

Methods: Fibroblasts of an *USH2A* patient, with compound heterozygous *USH2A* mutations (c.7595-2144A>G and c.10636G>A), were transfected with QRX-411 and mRNA levels were assessed. Labeled QRX-411 was injected by intravitreal injection into mouse eyes to measure localization in the retina.

Results: QRX-411 demonstrated the ability to restore the wild-type mRNA transcript profile via repair of mutant *USH2A* mutant mRNA. Localization of labelled QRX-411 in the outer nuclear layer of the retina was shown after intravitreal injection into mouse eyes.

Conclusions: QRX-411 was shown to be effective in restoration of wild-type mRNA in patient-derived fibroblasts carrying the intronic c.7595-2144A>G mutation in the *USH2A* gene, and could be successfully delivered to the outer nuclear layer of the mouse retina.

Commercial Relationships: Hester van Diepen, Heelam Chan, ProQR Therapeutics (E); Janne Turunen, ProQR Therapeutics (E); Ma'ayan Semo, ProQR Therapeutics (F); Anthony Vugler, ProQR Therapeutics (F); Ralph Slijkerman, ProQR Therapeutics (F); Erwin van Wijk, ProQR Therapeutics (P); Peter Adamson, ProQR Therapeutics (E)

Program Number: 1204

Presentation Time: 9:00 AM–9:15 AM

OCX063, A Novel Small Molecule Drug, Exhibits Anti-Inflammatory and Anti-Fibrotic Properties in Retinal Cultures

Roy Chze Khai C. Kong¹, Alison J. Cox¹, Sylwia Glowacka¹, Darren J. Kelly^{1,2}, Fay Khong^{1,2}. ¹Medicine, The University of Melbourne, Melbourne, VIC, Australia; ²OccuRx Pty Ltd, Melbourne, VIC, Australia.

Purpose: Retinal microglia are the resident immune cells in the central nervous system, which undergo activation and release inflammatory cytokines in injury. During injury, Muller cells can also become activated and exhibit a fibroblast-like phenotype. Since it has been shown that microglia and Muller cells interact functionally, we hypothesize that inhibiting inflammatory cytokine release from activated microglia can have anti-inflammatory/anti-fibrotic effects on Muller cells. We tested this using OCX063 which we have shown to be anti-inflammatory in mesenchymal and immune cells.

Methods: To assess OCX063's effect on activated microglia, primary rat retinal microglia were induced using low pH6.8 medium, in the presence of 10, 30 and 100µM OCX063. Low pH induction was chosen to mimic an acidified extracellular environment, representative of metabolic abnormality as seen in inflammatory/proliferative retinal diseases. We then co-cultured microglia under these conditions with non-activated Muller cells in a Transwell system. Cells were analyzed for inflammatory and fibrotic markers via RT-qPCR/ELISA.

Results: pH6.8 caused an inflammatory phenotype in microglia as shown by the significantly elevated expression of interleukin (IL) 6 and IL1β gene and protein, compared to physiological pH7.6. Excitingly, the gene expression of IL6 and IL1β was decreased by 100µM OCX063 (both $p<0.05$) while their protein expression was decreased by OCX63 at concentrations as low as 10µM (IL6, $p<0.01$; IL1β, $p<0.05$). Subsequent co-culture of Muller cells with activated microglia also led to significant up-regulation of inflammatory and fibrotic gene markers in Muller cells including IL6, IL1β and inducible nitric oxide synthase (iNOS) as well as collagen type IV (ColIV) and matrix metalloproteinase-9 (MMP9), respectively.

As hypothesized, these pathological effects were reduced when the Muller cells were co-cultured with OCX063-treated (30µM) microglia (IL6, $p<0.01$; IL1β, $p<0.05$; iNOS, $p<0.05$; ColIV, $p<0.05$; MMP9, $p<0.001$).

Conclusions: Our findings show that OCX063 not only exerts anti-inflammatory effects on retinal microglia, but also has protective anti-inflammatory/anti-fibrotic effects on other cells, like Muller cells, which exhibit pathological phenotypes due to microglial activation. Thus, OCX063 may be a strategy for treating proliferative retinal diseases.

Commercial Relationships: Roy Chze Khai C. Kong, OccuRx Pty Ltd (F); Alison J. Cox, None; Sylwia Glowacka, None; Darren J. Kelly, OccuRx Pty Ltd (E); Fay Khong, OccuRx Pty Ltd (E)

Program Number: 1205

Presentation Time: 9:15 AM–9:30 AM

Distinct Cellular Uptake and Clearance Patterns of Nanoceria in the Retina

Lily L. Wong¹, Swetha Barkam², Sudipta Seal^{2,3}. ¹Department of Ophthalmology, College of Medicine, University of Oklahoma Health Sciences Center & Dean McGee Eye Institute, Oklahoma City, OK; ²Department of Materials Science and Advanced Materials Processing and Analysis Center, University of Central Florida, Orlando, FL; ³NanoScience Technology Center and College of Medicine, University of Central Florida, Orlando, FL.

Purpose: Our stable water-dispersed, catalytic nanoceria have anti-oxidative effects in rodent models of retinal degeneration. A single intravitreal injection of nanoceria delays disease progression from weeks to months. Even though we have shown that our synthesized nanoceria are rapidly taken up by retinal cells, retained in the retina for over a year, and are non-toxic, we still do not know which cell types in the retina preferentially take up these nanoparticles. In this study, we examined the temporal and spatial distribution of fluorescently-labeled nanoceria in retinal sections to better understand the cellular action of nanoceria.

Methods: We compared the *in situ* distribution of the fluorescent dye, Alexa647 NHS ester (Al647), and Alexa647-conjugated nanoceria (Al647-CeNPs) in retinal sections at various time points post intravitreal injection. We delivered 1 µl of either Al647 or Al647-CeNPs to the vitreous of Balb C mice. We detected the dim fluorescent signals using the ultra-high sensitivity detector equipped in our Olympus FV1200 confocal system. Quantitative intensity analyses were performed using the Fluoview software.

Results: We detected minute autofluorescence in the neurofiber layer (NFL), inner plexiform layer (IPL), outer plexiform layer (OPL), and rod inner and outer segments (IS/OS) of uninjected Balb C mice. In Al647 injected mice, we detected robust signals in vascular cells in the intra-retinal vasculature and detectable signals in the NFL and IPL from 1 day to 2 months post injection (pi) with decreasing intensity over time. In Al647-CeNPs injected ones, we failed to detect signals in 7 dpi but detected the highest signal level at 14 dpi with decreasing signal intensity from 1 to 3 months pi. We observed signals in the NFL, IPL, OPL, IS/OS, apical region of retinal pigment epithelium (RPE), with the highest signals found in photoreceptor (Pr) OS. Unlike Al647 injected animals, we did not observe enhanced signals in retinal vascular cells.

Conclusions: We conclude that nanoceria follow distinct uptake and clearance pathways from other small molecules after delivery to the vitreous. They are taken up by the six major neuronal cell types and Muller Glia, and accumulate in the OS of Pr cells. They are likely to be eliminated by RPE through the shedding of Pr OS. Consistent with

prior retention studies, we observe fluorescent signals in retinal cells months after a single intravitreal injection.

Commercial Relationships: Lily L. Wong, US 7347987 (P), US 8703200 (P), US 7727559 (P); Swetha Barkam, None; Sudipta Seal, US 7347987 (P), US 8703200 (P), US 7727559 (P)
Support: NIH Grants: R01 EY02211, P30EY021725; Presbyterian Health Foundation (<http://www.phfoc.com/>); Research to Prevent Blindness (<http://www.rpbusa.org/rpb/>.)

Program Number: 1206

Presentation Time: 9:30 AM–9:45 AM

Adverse ocular effect of sertraline use: case series of sertraline-associated maculopathy

Hedayat Javidi, Konstantinos Balaskas, Tariq Aslam, Sajjad Mahmood. Manchester Royal Eye Hospital, Manchester, United Kingdom.

Purpose: To report on two cases of possible sertraline related maculopathy, one shortly after treatment commenced and one after long term usage. Sertraline, a selective serotonin re-uptake inhibitor (SSRI), is one of the most prescribed antidepressants in the United States. Its use for the management of depression, anxiety, eating disorders and premenstrual dysphonic disorder has become a mainstay of treatment. Only a small number of cases of maculopathy associated with sertraline use have previously been reported.

Methods: Report ocular examination findings, features on spectral domain OCT scanning and review of available literature.

Results: The first case describes that of a 27 year old female presenting with a 2 week history of paracentral scotoma just above the fixation point. This patient had commenced sertraline 4 weeks prior to her symptoms for treatment of panic attacks but otherwise had no significant past medical history. Her visual acuity was reduced to 6/9.5 bilaterally and Optical Coherence Tomography (OCT) showed disruption of the photoreceptor layer just below the centre of the fovea. Symptoms resolved on discontinuation of sertraline.

The second case describes a 36 year old female presenting with a generalised deterioration in vision and who had become acutely symptomatic approximately 3 months before presentation. Past medical history included anxiety, depression, mood swings and chronic back pain. She had been taking sertraline for 5 years prior to the onset of symptoms. Visual acuity was 6/36 bilaterally and this did not improve 1 year after discontinuation of sertraline. OCT scan demonstrated similar architectural disturbance in the outer retina to the first case.

Conclusions: Given the prevalence of sertraline use, it is important for physicians to be aware of the possibility of effects on vision and macular adverse effects. With only a small number of reported cases, we advocate a wider awareness of this condition.

Commercial Relationships: Hedayat Javidi, None; Konstantinos Balaskas, None; Tariq Aslam, None; Sajjad Mahmood, None

Program Number: 1207

Presentation Time: 9:45 AM–10:00 AM

Effect of Bevacizumab, Ranibizumab and Aflibercept on retinal pigment epithelium phagocytosis

Patricia Fernandez^{1,2}, Maria Hernandez^{1,2}, Sergio Recalde^{1,2}, Manuel Saenz de Viteri¹, Idoia Belza¹, Maite Moreno¹, Jaione Bezunartea¹, Alfredo Garcia-Layana^{3,2}. ¹Experimental Ophthalmology Laboratory, Clinica Universidad de Navarra, Pamplona, Spain; ²IdISNA, Navarra Institute for Health Research, Pamplona, Spain; ³Ophthalmology, Clinica Universidad de Navarra, Pamplona, Spain.

Purpose: Controversy exists regarding the safety of vascular endothelial growth factor (VEGF) inhibitors in the retinal pigment epithelium (RPE) and it is currently unknown whether the development of geographic atrophy in patients with age-related macular degeneration (AMD) treated with these drugs is a toxic effect, or otherwise responds to the natural course of the disease. The aim of this work was to evaluate if phagocytosis, one of the most important functions of the RPE, is affected by Bevacizumab, Ranibizumab and Aflibercept and their effect on the main molecular regulators of that function.

Methods: Assays were performed *in vitro* using the human ARPE-19 cell line. Polarized cell monolayers were treated with clinical concentrations of Bevacizumab, Ranibizumab and Aflibercept during 24 hours and phagocytosis of fluorescent microspheres was assessed at 48 hours by confocal microscopy. We also evaluated the effect of anti-VEGFs in MerTK, phospho-MerTK and $\alpha 5\beta 1$ integrin by western blot, as mediators of the phagocytosis process. Moreover, the structure of the cytoskeleton labelled with fluorescent phalloidin (F-actin) was also analyzed by confocal microscopy.

Results: Compared to untreated control cells, exposure to VEGF produced a statistically significant decrease in ARPE-19 cells phagocytosis of fluorescent microspheres ($p=0.036$). On the contrary, exposure to Bevacizumab and Aflibercept ($p=0.016$) but not for Ranibizumab ($p=0.286$) increased the phagocytosis. The expression of MerTK, phospho-MerTK or $\alpha 5\beta 1$ integrin was not modified by VEGF treatment and also by any of the anti-VEGFs drugs compared to untreated cells. However, exposure to VEGF produced a disruption of the normal distribution of actin F-actin cytoskeleton fibers that was not observed in cells treated with anti-VEGFs or untreated controls.

Conclusions: Our results suggest that inhibition of VEGF with antiangiogenics treatments increases phagocytosis in RPE cells, while exposure to VEGF has the opposite effect. These results were not explained by differences in the expression of the main molecular regulators of phagocytosis; however, our experiments suggest that they could be related to changes in the normal architecture of the cytoskeleton that was found to be disrupted by exposure to VEGF.

Commercial Relationships: Patricia Fernandez, None; Maria Hernandez, None; Sergio Recalde, None; Manuel Saenz de Viteri, None; Idoia Belza, None; Maite Moreno, None; Jaione Bezunartea, None; Alfredo Garcia-Layana, Thea (C), Novartis (C), Bayer (C), Alcon (C), Allergan (C)

Program Number: 1208

Presentation Time: 10:00 AM–10:15 AM

Expression and Inhibition of Peptidyl arginine Deiminase (PAD) Enzymes in Eye Injury

Royce Mohan, John Wizeman. Neuroscience, University of Connecticut Health Center, Farmington, CT.

Purpose: We investigated the expression of peptidyl arginine deiminase (PAD) enzymes in the injured retina after alkali injury to the cornea.

Methods: The corneal alkali injury model was employed using equal numbers of 129SvEv male and female mice as described (Mol Vis. 2016 22:1137). Injured mice recovered for different periods of time and RNA and protein extracted from retina of noninjured and injured cohorts. Quantitative PCR was performed for GAPDH, PAD2, PAD3 and PAD4 transcripts. Tissue sections were subjected to immunohistochemistry (IHC) and protein analyzed by western blot (WB) using antibodies against GFAP, PAD2, PAD4, β -III Tubulin, GAPDH and F95 antibody for citrullinated proteins. In other experiments, mice were injured *in vivo* and the enucleated eye was subjected to intravitreal injection with PAD4 inhibitor streptonigrin. Mouse eyes were maintained in buffered saline for 5 h at 37 °C in 5%

ARVO 2017 Annual Meeting Abstracts

CO₂ and retinal extracts subjected to WB. Statistical differences were identified by two-tailed t-test.

Results: PAD2 transcripts detected in the uninjured retina decreased by 7 days post-injury. PAD3 transcript was absent in the retina at all stages. PAD4 transcript was detected in both the injured and uninjured retina, showing a 4-fold increase 1 h post-injury and greater than 24-fold by 7 days. By WBs, PAD4 protein showed increased expression (Unj = 1.91 ± 0.38 ; 7d injury = 3.59 ± 0.42 ; $P < 0.05$). PAD4 immunoreactivity by IHC was not detected in the uninjured retina, but at 7 days post-injury, PAD4 expression was observed throughout the layers of the inner retina. In injured retina, PAD4 expression aligned with GFAP expression in Müller glial cells and exhibited a filament like expression pattern, with PAD4-positive processes beginning at the vitreous. GFAP expression was restricted to the

ganglion cell layer astrocytes in the uninjured eye, but extended to the outer nuclear layer in the injured retina. In the injured enucleated eye model, 25 nM streptonigrin inhibited citrullination as detected by the F95 antibody, as well as, reduced GFAP expression by WB (Veh = 2.78 ± 0.57 ; streptonigrin = 1.56 ± 0.12 ; $P < 0.05$).

Conclusions: PAD4 expression increases in a timely manner after retinal injury and its expression localizes to Müller glial cells where GFAP is a major target of citrullination. PAD4 may be a likely target for attenuation of glial reactivity, especially where chronic gliosis can lead to scar formation.

Commercial Relationships: Royce Mohan; John Wizeman, None
Support: R01EY016782; John A. and Florence Mattern Solomon Endowed Chair