Corneal angiogenesis

Wednesday, May 06, 2015 11:00 AM–12:45 PM
Exhibit Hall Poster Session
Program #/Board #/Range: 4494–4519/A0194–A0219
Organizing Section: Cornea
Contributing Section(s): Multidisciplinary Ophthalmic Imaging

Program Number: 4494 Poster Board Number: A0194
Presentation Time: 11:00 AM–12:45 PM

Sphingolipid signaling in corneal neovascularization.
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**Purpose:** Sphingosine-1-phosphate (S1P) is a widely studied sphingolipid signaling mediator that acts as a ligand for five cell surface receptors, S1P1–5. Activation of these g-protein-coupled receptors is linked to cell proliferation, differentiation, inflammatory responses, angiogenesis, and neovascularization (NV). Sphingosine kinases (SphK) are responsible for the phosphorylation of sphingosine to S1P, yet their role in ocular neovascularization is currently unknown. The purpose of this study is to assess role of S1P in ocular neovascularization through the role of SphK. Docosahexaenoic acid (DHA), an ω-3 fatty acid is also known for its role in protecting from NV.

**Methods:** Mice that lack Sphk1 knockout mice have dramatically show protection from NV, therefore have elevated amounts of DHA when placed on a diet of 10% safflower oil containing only ω-6 PUFA. Litters of mice starting ω-6 PUFA; mice containing the transgene with the second generation of breeders on the diet were used for control of multiple pro-angiogenic factors and maintains angiostasis/avascularity. To further evaluate the contribution of miR-184 to corneal avascularity, we investigated the role of miR-184 as an angiostatic factor.

**Results:** miR-184 expression in corneal epithelium and HCEKs was 10- and 4-fold higher than in limbal epithelium and HLEKs, respectively. In situ hybridization confirmed that miR-184 was strongly expressed in the corneal epithelium compared to limbal epithelium. Scratch wounding of HDMECs demonstrated a 35% reduction in closure in media conditioned by HLEKs expressing miR-184 compared with control conditioned media. This indicates that miR-184-expressing cells secrete lower amounts of trophic factors including angiogenic mitogens than control cells. Phospho-Akt, which is involved in VEGF secretion and angiogenesis, was markedly decreased in HCEKs expressing miR-184 compared with controls. Soluble Flt-1 was reduced in HCEKs treated with anti-184 compared with an irrelevant control. Luciferase assay and protein data indicated that miR-184 targets multiple pro-angiogenic molecules, including splicing factor-1 (sf-1), platelet-derived growth factor beta (PDGF beta), friend of gata-2 (fog-2) and nuclear uncapreplin pyrophosphate synthase 1 (mus1).

**Conclusions:** miR-184 functions to inhibit angiogenesis via negative control of multiple pro-angiogenic factors and maintains angiostasis/vascularity. The use of miR-184 to modulate angiogenesis may be a novel therapeutic strategy to prevent neovascularization in the cornea.

Commercial Relationships: Jongkook Park, None; Olga V. Volpert, None; Robert M. Lavker, None.

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Presentation Time: 11:00 AM–12:45 PM

Tissue and cellular characterization of nucleolin in a murine model of corneal angiogenesis.
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**Purpose:** Nucleolin initially described as a nuclear protein, has been identified in the surface of endothelial cells of blood vessels where it participates in neo-vessels formation. To study a possible role of nucleolin in the development of blood vessels in the cornea, the aim of this work was to determine the nucleolin presence in corneal tissue with and without angiogenesis.

**Methods:** Following ARVO guidelines for animal research in visual science, suture induced corneal angiogenesis was performed in both BALB/c and C57BL/6 mouse strains. After clinical analysis, the corneal tissues were obtained at different time points and co-immunofluorescence assays were performed using different cellular

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compartments reference proteins. Control tissues were obtained from non-injured corneas.

**Results:** Nucleolin was observed in the nuclear compartment, principally inside the nucleoli of certain epithelial cells of the basal layer and lesser extent in few stromal cells in both mouse strains under healthy conditions. Interestingly, angiogenesis induced considerable changes in tissue as well as cellular localization of nucleolin, the nucleolin presence was detected in the cytoplasmic compartment and the cell membrane of epithelial cells, stromal cells, and corneal endothelial cells.

**Conclusions:** Nucleolin mobilization to the cell membrane during angiogenesis reveals a possible role as a receptor of proangiogenic molecules in the corneal tissue. These results suggest that nucleolin could be a target of study and treatment of corneal angiogenesis.

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**Commercial Relationships:** Fabio Bignami, None; Alessandro Rabiolo, None; Chiara Giacomini, None; Giulio Ferrari, None; Paolo Rama, None

**Program Number:** 4497 Poster Board Number: A0197

**Presentation Time:** 11:00 AM–12:45 PM

**Corneal neovascularization and its potential biomarkers in human tears.**

Fabio Bignami, Alessandro Rabiolo, Chiara Giacomini, Giulio Ferrari, Paolo Rama. Cornea Unit - Eye Repair Lab, San Raffaele Scientific Institute, Milan, Italy.

**Purpose:** To quantify expression of potential biomarkers of neoangiogenesis in the tears obtained from patients affected with corneal neovascularization (CNV) and to compare them with healthy, age-matched controls.

**Methods:** Tears were collected by minISP application from 19 eyes of 14 patients with CNV (average age: 52 years) and from 19 eyes of 16 healthy controls (average age: 48 years). Concentrations of Substance P (SP), Angiopoietin 2 (ANG2), VEGF-A and IL-1β in the tear samples were analyzed using a multiplex bead-based assays (Luminex Technology).

**Results:** No differences were observed between all CNV patients and all controls for the 4 analyzed biomarkers. When gender was taken into consideration, there was a significant difference (P<0.05) only for ANG2 in the CNV group and for VEGF-A in both groups (higher levels in males). Gender was not associated with any significant differences (patients vs. controls) for any of the biomarkers considered.

Although the two groups did not differ by age, SP was inversely related with age in controls (P<0.05), while IL1β, ANG2 and VEGF-A were positively correlated with age (P<0.05, P<0.001 and P<0.001, respectively). In the CNV group no correlations were observed between these biomarkers and age.

ANG2 was positively correlated with VEGF-A and IL1β (P<0.01) in both groups. VEGF-A and IL1β positively correlated with each other (P<0.05) in both groups. No correlation was found for SP vs. other biomarkers, except for VEGF-A in the control group (P<0.05).

When ocular cicatricial pemphigoid (OCP) patients were extrapolated from the CNV group (N=6), a significant increase (P<0.01) in SP concentration together with a reduction (P<0.05) in VEGF-A level was observed in comparison to CNV non-OCP patients.

CNV (clinically measured in quadrants) positively correlated with SP (P<0.01) and negatively with ANG2 (P<0.05). Inflamed eyes had higher values of these biomarkers (significance reached for IL1β).

**Conclusions:** ANG2, VEGF-A and IL-1β show a similar expression pattern in tears, which is different from SP. Tear levels of these biomarkers are related with age and some of them to gender or specific disease (OCP).

Finally, the expression levels of SP and ANG2 in the tear fluid are related to CNV severity, thus suggesting that these molecules may represent potential biomarkers to quantify CNV severity, and/or effective therapeutic targets.

**Commercial Relationships:** Fabio Bignami, None; Alessandro Rabiolo, None; Chiara Giacomini, None; Giulio Ferrari, None; Paolo Rama, None

**Program Number:** 4498 Poster Board Number: A0198

**Presentation Time:** 11:00 AM–12:45 PM

**Loss of tenascin X suppresses expression of VEGF in macrophages and of TGFβ1 in ocular fibroblasts in vitro; possible mechanism of inhibition of neovascularization in cornea**

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**Purpose:** To examine mRNA expression level of angiogenic growth factors, VEGF and TGFβ1, in macrophages and ocular fibroblasts derived from a tenasin X-null mouse. We previously reported that the loss of tenasin X suppress neoangiascularization with reduction of in vivo expression of angiogenic growth factors in a mouse cornea (ARVO 2014).

**Methods:** Peritoneal macrophages were obtained from tenasin X-null and wild types mice by using macrophage induction by oyster mycogen i.p. injection. Ocular fibroblasts were cultured from eye-shells of post-natal day 1 or 2 mice. The cultures were maintained for 24 hrs with or without exogenous TGFβ1 and processed for RNA extraction. Real-time RT-PCR was ran to examine the expression level of VEGF and TGFβ1.

**Results:** Loss of tenasin X suppresses mRNA expression of VEGF in macrophages and of TGFβ1 in fibroblasts in the absence of TGFβ1. Loss of tenasin X did not attenuate the TGFβ1 induction of VEGF and TGFβ1 in these cell types.

**Conclusions:** Tenasin X is involved in expression of angiogenic growth factors in macrophages and fibroblasts, partially explain the mechanism of inhibition of in vivo neovascularization in a tenacin X-null mouse cornea.

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**Presentation Time:** 11:00 AM–12:45 PM

**Effects of Sensory and Sympathetic Innervation on the Corneoinimal Neurovascular Complex**

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**Purpose:** The exact mechanisms of the interactions between corneal nerves and vasculature remain unknown. This study assessed the
corneolimb vascular complex and the specific effects of the sensory and sympathetic innervation on the corneolimb vascular structure.

**Methods:** Naive corneas, including the limbus, of BALB/c mice were excised, and whole-mounts were immunostained with anti-b-III tubulin, CD31, and Lyve-1 to evaluate the neurovascular anatomy of blood and lymphatic vessels, as well as nerves in the limbal area by confocal microscopy. Substance P (SP) and tyrosine hydroxylase (TH) antibodies were used to stain sensory and sympathetic nerve respectively. Imaris software (Bitplane) was used to reconstruct the 3D structure of corneolimb neurovascular complex. After unilateral trigeminal axotomy or superior cervical ganglionectomy (sympathectomy; SCGx), clinical changes in corneal transparency and neovascularization were evaluated by slit-lamp biomicroscopy. Axotomized, SCGx, contralateral, and sham-treated corneas were excised on postoperative days 1, 3, 7, and 14 and immunofluorescence was performed with anti-b-III tubulin, CD31, Lyve-1, SP, or TH. Analysis was performed with Neuron J to quantify nerve density.

**Results:** Sensory and sympathetic nerves were observed crossing, encircling, and running in parallel to blood and lymphatic vessels in the corneolimb area. Trigeminal axotomy resulted in near complete loss of corneal nerves in the limbal area compared to sham controls and contralateral corneas (p<0.0001). After unilateral SCGx, loss of sympathetic nerves were shown in limbal area (p<0.0001). Unilateral trigeminal axotomy resulted in bilateral neovascularization in 100% of axotomy eyes (p=0.001) and 17% of contralateral eyes (p=0.47), which corresponded with 1.7 fold enlarged vascular caliber in axotomized eyes (p=0.0001). Interestingly, unilateral SCGx resulted in neovascularization in 50% sympathectomized eyes (p=0.009) and in 40% of contralateral eyes (p=0.02).

**Conclusions:** The current study demonstrates the spatial features of murine corneolimb neurovascular complex. Unilateral trigeminal axotomy and sympathectomy led to loss of nerves in the limbal area and resulted in significant corneal neovascularization in both affected and unaffected eyes. Thus, sensory and sympathetic innervation may directly modulate blood flow and immune cells trafficking through regulation of the vasculature.

**Commercial Relationships:** Xiaodan Huang, None; Maria J. Lopez, None; Pedram Hamrah, None

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**Presentation Time:** 11:00 AM–12:45 PM

**Macrophages, Pericytes, and Smooth Muscle Cells in Corneal Angiogenesis and Regression**

**Jin Zhao, Takayuki Nagasaki.** Ophthalmology, Columbia University, New York, NY.

**Purpose:** Our previous studies showed that: 1) there are abundant macrophages in the vicinity of growing blood vessel fronts; 2) a periendothelial “pale” cell of unknown identity was found in an ML-9 induced angiogenesis model. The aim of this study is to examine the participation of macrophages, pericytes, and smooth muscle cells (SMCs) during pathological corneal vascular growth and subsequent regression.

**Methods:** A suture-induced angiogenesis model was used in the mouse eye. Vascular regression was studied after suture removal. Vasculature was monitored by angiography in vivo and analyzed by histology with transmission electron microscopy (TEM) and whole-mount immunostaining, using CD31 for endothelial cells, F4/80 for macrophages, NG2 for pericytes, and α-smooth muscle actin for SMCs.

**Results:** Following suture placement in the cornea, capillary vessels grew toward the suture at a rate of about 150 μm/day. TEM revealed the presence of “pale” cells with sparse cytoplasmic organelles near vessel tips and also at a branching point where they shared basement membranes with endothelial cells, suggesting a role in capillary branching or anastomosis. Varying lengths of growing capillary tips, ranging from 0 to 150 μm from the growth front, were free from pericytes. The largest distance is the length of two endothelial cells, or the distance that capillary grows in about 1 day, indicating that all growing capillaries older than one day were totally decorated with pericytes. SMCs were never found at the first 50 μm of capillary tips, and some vessels had no SMCs up to 300 μm from the growth front. These are the distances that extend beyond the first branching point of growing vessels. Some macrophages appeared to be in direct contact with endothelial cells in the pericyte-free zone. During vascular regression, remaining blood vessels were fully covered by pericytes and SMCs while abundant macrophages were present near them. Some macrophages maintained ghost shape of disintegrated blood vessels.

**Conclusions:** A growth front of capillary blood vessels is free from pericytes and SMCs for a maximum of one day for pericytes and two days for SMCs. Some macrophages in this area are in direct contact to endothelial cells. As such, this is a dynamic zone that endothelial tip cells incorporate macrophages, pericytes, and SMCs, which may be amenable to an intervention for controlling pathological vascular growth.

**Commercial Relationships:** Jin Zhao, None; Takayuki Nagasaki, None

**Support:** Research to Prevent Blindness, Eye Surgery Fund

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**Presentation Time:** 11:00 AM–12:45 PM

**Strain-dependent changes in the limbal lymphatic vasculature in BALB/c and C57BL/6 mice during aging**

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**Purpose:** In this study, our objective was to analyze age-related architectural changes in the endogenous limbal lymphatic vasculature of two frequently used mouse strains BALB/c and C57BL/6N.

**Methods:** Naive corneas from young BALB/c (n=19) or C57BL/6 (n=9) mice (8-10weeks) and old BALB/c (n=5) or C57BL/6 (n=5) mice (66-68weeks) were excised and whole mounts were stained with the lymphatic vessel marker LYVE-1. Mice were obtained from the animal facility at the Centre of Molecular Medicine, Cologne (CMMC). The vessel area was calculated semi-automatically by an algorithm using cellF software, the vessel length, number of branching points (BPs) and endpoints (EPs) was analyzed manually using cellF and related to the analyzed area. ANOVA was used for statistical analysis.

**Results:** a) In BALB/c mice the vessel area and vessel length did not change significantly between young and aged mice. In C57BL/6 the vessel area (1.77% versus 1.08%, p<0.001) and vessel length (0.85mm/mm2 versus 1.35mm/ mm2, p>0.001) significantly increased with age. The number of BPs and EPs did not change significantly in both strains during aging. b) Comparing the parameters between both strains showed that the vessel area in young C57BL/6 was increased compared to young BALB/c (1.77% versus 1.08%, p>0.001). This difference was also found when aged C57BL/6 were compared to aged BALB/c.

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This study has uncovered a unique lymphangiogenic podoplanin knockdown in LECs interfered with integrin activation. galectin-8- and VEGF-C-mediated LEC sprouting. Additionally, knockdown of VEGFR-3 did not affect galectin-8-mediated LEC proliferation. Sugaya
Noorjahan A. Panjwani1, Wei-Sheng Chen1, Zhiyi Cao1, Satoshi Sugaya, André Reis, None; Deniz Hos, None; Claus Cursiefen, None
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Program Number: 4502 Poster Board Number: A0202
Presentation Time: 11:00 AM–12:45 PM
Pathological lymphangiogenesis is regulated by galectin-8-dependent crosstalk among VEGF-C, podoplanin and integrin pathways. Noorjahan A. Panjwani, Wei-Sheng Chen, Zhiyi Cao, Satoshi Sugaya, Hakon Leffler, Ulf J. Nilsson, Lijun Xia. Ophthalmology, Tufts University Medical School, Boston, MA; Lund University, Lund, Sweden; Oklahoma Medical Research Foundation, Oklahoma City, OK.

Purpose: Lymphangiogenesis (LA) plays a vital role in diverse pathological conditions including corneal graft rejection, dry eye and glaucoma. The goal of the current study was to characterize the role galectin-mediated carbohydrate recognition system in the modulation pathological LA.

Methods: Lymphatic endothelial cell (LEC) sprouting assays, corneal micropocket assays, gene knockdown and antibody blocking assays, and galectin-8 and podoplanin knockout mice were used to assess the role and the mechanism of galectin-8-mediated LA.

Results: The study revealed that galectin-8 is a potent lymphangiogenic factor. Galectin-8 was markedly upregulated in inflamed human and mouse corneas, and inhibitors of galectin-8 reduced inflammatory LA. In corneal micropocket assays and 3D sprouting assays, galectin-8 promoted LA in a carbohydrate-dependent manner. Galectin-8 was identified as a key mediator of integrin-dependent crosstalk between VEGF-C and podoplanin lymphangiogenic pathways. Galectin-8 inhibitors reduced VEGF-C-induced LA. Conversely, exogenous galectin-8 markedly enhanced VEGF-C-induced lymphangiogenesis in a carbohydrate-dependent manner. Knockdown of podoplanin reduced not only galectin-8 but also VEGF-C-mediated LEC sprouting. Also, in corneal micropocket assays, VEGF-C-induced LA was significantly reduced in the galectin-8−/− and podoplanin−/− mice; likewise, galectin-8-induced lymphangiogenesis was reduced in podoplanin−/− mice. Interestingly, knockdown of VEGFR-3 did not affect galectin-8-mediated LEC sprouting. Instead, inhibiting integrins αβ1 and αβ1 curtailed both galectin-8- and VEGF-C-mediated LEC sprouting. Additionally, podoplanin knockdown in LECs interfered with integrin activation. Immunoprecipitation assays further confirmed galectin-8-dependent interactions between podoplanin and integrins α5 and β1.

Conclusions: This study has uncovered a unique lymphangiogenic pathway in which galectin-8-mediated interactions between PDPN and integrins α5β1 play a key role.

Commercial Relationships: Noorjahan A. Panjwani, None; Wei-Sheng Chen, None; Zhiyi Cao, None; Satoshi Sugaya, None; Hakon Leffler, None; Ulf J. Nilsson, None; Lijun Xia, None
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Program Number: 4503 Poster Board Number: A0203
Presentation Time: 11:00 AM–12:45 PM
Monocyte-derived Wnt Signal regulates Corneal Inflammatory Lymphangiogenesis. Roberto Sessa, Stephanie Wan, Tan N. Truong, Valerie Su, Preethi Padmanabhan, April Smith, Richard Lang, Lu Chen. Center for Eye Disease and Development, Program in Vision Science and School of Optometry, University of California, Berkeley, CA; Visual Science Group, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

Purpose: The Wnt system is known to be involved in multiple functions, such as cell fate determination and stem cell maintenance. However, to date, there is little information about its roles in lymphangiogenesis, a pathological process associated with many diseases. In this study, we investigated the effect of monocyte-derived Wnt signaling in inflammatory lymphangiogenesis in the cornea.

Methods: Wnt inhibitor (Wls) gene, which binds to Wnt proteins and facilitates Wnt sorting and secretion, was targeted for conditional deletion in murine monocytes. The transgenic mice were generated by cross breeding of Csf1R-cre and Wls-flox animals. Standard suture placement model was used to induce corneal inflammatory lymphangiogenesis in heterozygous mice or littermate controls. Whole-mount corneas were harvested for anti-LYVE-1 staining. Digital images from immunofluorescent microscopic assays were analyzed by NIH Image J software.

Results: Corneal lymphangiogenic response in heterozygous mice carrying monocytic specific Wls deletion was significantly suppressed compared to control littermates, as assessed by lymphatic invasion area.

Conclusions: This study shows that monocyte-derived Wnt signaling is critically involved in lymphatic vessel formation during inflammation. Further investigation on this observation may lead to the development of new cellular or molecular targeting strategies for lymphatic diseases occurring both inside and outside the eye.

Commercial Relationships: Roberto Sessa, None; Stephanie Wan, None; Tan N. Truong, None; Valerie Su, None; Preethi Padmanabhan, None; April Smith, None; Richard Lang, None; Lu Chen, None

Program Number: 4504 Poster Board Number: A0204
Presentation Time: 11:00 AM–12:45 PM
Nerve Growth Factor Mediates Corneal Lymphangiogenesis. Tatiana Ecoiffier, Pedram Hamrahi, Sammy Grimaldo, Gyeong Jin Kang, Roberto Sessa, Tan N. Truong, Deshea L. Harris, Ulrich von Andrian, Lu Chen, Lixin Zheng. Center for Eye Disease and Development, Program in Vision Science and School of Optometry, University of California, Berkeley, CA; Schepps Eye Research Institute, Massachusetts Eye & Ear, Department of Ophthalmology, Harvard Medical School, Boston, CA; Immune Disease Institute, Program in Cellular and Molecular Medicine at Children’s Hospital Boston, Harvard Medical School, Boston, CA.

Purpose: Lymphangiogenesis (LG), the growth of new lymphatic vessels, is a critical process involved in many pathological conditions, including cancer metastasis, tissue inflammation and graft rejection. This study was designed to investigate the critical role of nerve growth factor (NGF) in mediating corneal LG by using a repertoire of in vivo, in vitro and in real-time models and methods.

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Methods: A variety of in vivo corneal LG and nerve injury models (pellet implantation, cautery, trephination, axotomy and transplantation), ex vivo immunofluorescent microscopic assays, intravitral imaging technology, and in vitro lymphatic endothelial cell (LEC) culture systems were used to investigate NGF expressional changes during corneal LG, the role of NGF in inducing corneal LG in vivo and in real-time, and the role of NGF in mediating LEC functions in vitro.

Results: NGF was significantly increased during corneal LG and nerve damage and it mediated LG in both dose- and time-dependent manners. NGF also directly mediated LEC functions in vitro. Anti-NGF treatment suppressed NGF-induced LG in micropocket assay. However, it exacerbated LG after nerve injury. Accordingly, NGF administration inhibited LG during concurrent nerve injury.

Conclusions: Our study suggests that NGF has a dual function by maintaining nerve survival at a critical level, while inducing LG at higher concentrations. This study not only offers a direct link between NGF and LG, but also reveals a critical balance that is regulated by NGF between the lymphatic and nerve supplies within a tissue, which necessitates consideration in therapeutic approaches.

Commercial Relationships: Tatianna Ecoiffer, None; Pedram Hamrah, None; Sammy Grimaldo, None; Gyeong Jin Kang, None; Roberto Sessa, None; Tan N. Truong, None; Deshea L. Harris, None; Ulrich von Andrian, None; Lu Chen, None; Lixin Zheng, None

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Program Number: 4505 Poster Board Number: A0205
Presentation Time: 11:00 AM–12:45 PM
Interleukin-10 regulates corneal lymphangiogenesis and the resolution of inflammatory responses
Deniz Hos, Marie-Luise Dreisow, Franziska Bucher, Birgit Regenfuss, Felix Bock, Claus Cursiefen. Department of Ophthalmology, University of Cologne, Cologne, Germany.

Purpose: Aim of this study was to investigate whether Interleukin-10 (IL-10), an anti-inflammatory and immune-modulatory cytokine, is involved in the regulation of corneal hem- and lymphangiogenesis and inflammation.

Methods: IL-10 mRNA and protein expression was analyzed in healthy and inflamed murine corneas after suture placement. The in vitro effect of IL-10 on inflammatory and (lymph)angiogenic growth factor expression by macrophages (peritoneal exudate cells, PECs) was assessed by real-time PCR. Furthermore, suture placement was performed in wildtype (wt) and IL-10 deficient (IL-10 -/-) mice. Afterwards, corneal inflammatory and (lymph)angiogenic growth factor expression, corneal hem- and lymphangiogenesis and inflammatory cell numbers were determined.

Results: IL-10 mRNA was not measurable in healthy corneas. However, in inflamed corneas, IL-10 mRNA and protein were detectable. IL-10 was expressed by CD11b+ cells. IL-10 stimulation reduced IL-1B, TNF-α and VEGF-A expression in PECs, whereas VEGF-C expression was significantly upregulated. VEGF-D expression remained unaltered. After suture placement, IL-10 -/- mice showed similar hemvascularized areas, whereas lymphvascularized areas were reduced when compared to wt. Consistently, IL-10 -/- mice showed an increase in corneal VEGF-A levels after suture placement that was comparable to wt, whereas the increase in corneal VEGF-C and -D expression levels was significantly lower in IL-10 -/- mice compared to wt. IL-18 and TNF-α expression increased to significantly higher levels in IL-10 -/- mice compared to wt after suture placement, which was also accompanied by higher corneal CD11b+ cell numbers. Moreover, inflammatory cytokine expression and CD11b+ cell numbers in IL-10 -/- mice persisted on significantly higher levels even after the removal of corneal sutures.

Conclusions: IL-10 regulates corneal lymphangiogenesis, presumably via macrophage derived growth factor expression. Furthermore, reduced corneal lymphangiogenesis in IL-10 -/- mice is associated with more intense and prolonged inflammation, indicating a possible role of IL-10 and lymphatic vessels in the regulation and resolution of inflammatory corneal responses.

Commercial Relationships: Deniz Hos, None; Marie-Luise Dreisow, None; Franziska Bucher, None; Birgit Regenfuss, None; Felix Bock, None; Claus Cursiefen, None

Program Number: 4506 Poster Board Number: A0206
Presentation Time: 11:00 AM–12:45 PM
An in vitro model using corneal cells to test antiangiogenic activity
Javier A. Calles, Mario Crespo-Moral, Antonio Lopez-Garcia, Yolanda Diebold. IOBA (Institute of Applied Ophthalmobiology) - University of Valladolid, Valladolid, Spain.

Purpose: Vascular endothelial growth factor (VEGF) is widely viewed as the main proangiogenic stimulus in pathological neovascularization of eye tissues including the cornea. There is a growing interest in the discovery of new molecules that inhibit VEGF and only a few in vitro corneal models of neovascularization. This work aims at designing an in vitro model to study antiangiogenic activity of drugs based on VEGF production by corneal cells.

Methods: Human corneal epithelial (HCE) cells were grown in DMEM/F12 medium with or without serum as basal conditions. Then, cells were exposed to IL-6 (50 or 100 ng/ml) for 24 or 48 h. VEGF release was measured in cell culture supernatants by ELISA after 24, 48 and 72 h. In another set of experiments, cells were scratched with a pippette tip to simulate a wound and maintained in serum-free medium for 24 h. Immediately after the scratch, either same IL-6 concentrations or none were added, and release of VEGF by HCE cells during wound closure was measured. Conjunctival epithelium and retinal pigment epithelium cell lines were used as controls for unstimulated secretion of VEGF. Data are expressed as mean ± SEM, and were analyzed by the Student t test.

Results: HCE cells released detectable VEGF levels that were about 6 times lower than those produced by control cell lines. Basal release of VEGF by the three cell lines occurred in a time-dependent manner that was significantly affected by the presence of serum in culture medium. 50 ng/ml but not 100 ng/ml IL-6 significantly increased VEGF expression in HCE cells, being 85% and 66% higher for 24 and 48 h respectively (p < 0.05). A 20% reduction in VEGF release was observed in scratched cells (p < 0.05); however, the presence of IL-6 returned VEGF levels to those of the control uninjured cells.

Conclusions: Corneal cells responded to serum or IL-6 stimulation by increasing VEGF secretion. These cells responded with a reduction in VEGF secretion when they were wounded, and IL-6 partially compensated that reduction. These findings indicate that corneal cells are reactive in vitro to stimuli affecting VEGF production. This model could be useful to test antiangiogenic drugs for corneal neovascularization.

Commercial Relationships: Javier A. Calles, None; Mario Crespo-Moral, None; Antonio Lopez-Garcia, None; Yolanda Diebold, None
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Comparative Study of Tacrolimus and Bevacizumab on Corneal Neovascularization in Rabbits
Min ku Kang, Jin Heung Park, Sung Kun Chung. Ophthalmology, Catholic University of Korea College of Medicine, Seoul, Korea, Korea (the Republic of).

Purpose: To compare the antiangiogenic effects of tacrolimus and bevacizumab on corneal neovascularization in rabbits.

Methods: Neovascularization was induced in 32 eyes of 16 rabbits by placing suture placement in the corneal stroma. Seven days after suture placement, all rabbits were randomly divided into 4 groups and were treated four times daily with balanced salt solution (Group 1, 4 rabbits), topical 0.5% itraconazole (5 mg/mL, Group 2, 4 rabbits), topical 1% itraconazole (10 mg/mL, Group 3, 6 rabbits), and topical 2% itraconazole (20 mg/mL, Group 4, 4 rabbits). After one week, the area of surface area of corneal neovascularization was assessed on the digital photographs. In the corneal specimens, the concentration of VEGF A (vascular endothelial growth factor), VEGF R2, and PLGF (placental growth factor) mRNAs was measured by RT-PCR, and the concentration of ERK, p-ERK, Flk, and p-Flk was measured by Western Blotting.

Results: The area of induced corneal neovascularization was significantly smaller in Groups 2, 3, and 4 compared to the control group on day 14 (p<0.05). RT-PCR analysis showed that the mean concentration of VEGF and PLGF in Groups 2, 3, and 4 was significantly lower than that in the control group after 7 days of treatment. Western Blotting analysis showed that the mean concentration of p-ERK, Flk, and p-Flk in Group 3 was significantly lower than that in the control group after 7 days of treatment.

Conclusions: Topical itraconazole application was useful for effective inhibition of experimental corneal neovascularization.

Commercial Relationships: Kyung Euy Hong, None; Jae Yon Won, None; Sung Kun Chung, None

Localized AAV-PEDF gene transfer reduces corneal neovascularization significantly in rabbits in vivo via selective apoptosis
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Purpose: Corneal neovascularization (CNV) is a major cause of global blindness. We hypothesized that localized AAV-Figure Epithelium Derived Factor (PEDF) gene therapy would eliminate CNV in rabbits in vivo by restoring critical balance between pro-angiogenic and anti-angiogenic factors and causing apoptosis in neovessels via Fas-ligand signaling.

Methods: New Zealand White rabbits were used. Topical alkali (1N NaOH) application for 1min on the central cornea produced CNV. PEDF gene therapy was administered into rabbit stroma in vivo via topical AAV5 titer (100μl; 5x1012vg/ml) using defined technique: (a) 30min after alkali-burn (b) 3day after alkali-burn or (c) 1day prior to alkali-burn. Slitlamp biomicroscopy, H&E staining, stereomicroscope and immunofluorescence determined differential changes in CNV, vessels number, length and area, keratocyte density, apoptosis, and overall ocular health. Immunoblotting and qPCR quantified PEDF and Fas ligand expressions. Imaging data was analyzed with NIH ImageJ and Adobe Photoshop.

Results: Localized AAV-PEDF gene transfer into keratocytes significantly increased PEDF levels in rabbit cornea in vivo (>4 fold; p<0.01). AAV-PEDF therapy given eyes exhibited dramatically reduced vasculature, vessel density, vessel-size (length and thickness) in rabbit cornea. AAV-PEDF therapy given 30min after injury showed 86-89% (p<0.001), 3days after injury exhibited 63-69% (p<0.001), and 1day prior to injury 95% (p<0.001) in morphometric analysis. A remarkably less and small-diameter blood vessel in PEDF-delivered corneas than the no-PEDF controls were detected by H&E and immunofluorescence. Detection of 2.5 fold increased Fas-ligand (p<0.05) in PEDF-delivered rabbit corneas suggested that PEDF over-expression increases Fas-ligand significantly. Detection of double-labeled lectin+ and TUNEL+ cells in vessels suggested CNV resolution via apoptosis. Quantification of pro-and anti-angiogenic factors is underway.

Conclusions: Localized tissue-targeted AAV-PEDF gene therapy has translational potential to cure corneal neovascularization. Selective apoptosis in neovessels via Fas-Fas ligand pathway is the likely mechanism. More studies are warranted.

Commercial Relationships: Rajiv R. Mohan, None; Ajay Sharma, None; Elizabeth A. Giuliano, None; Prashant Sinha, None; Jason
Verteportin Photodynamic Therapy in Patients with Corneal Neovascularization

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Purpose:
To describe the effect of photodynamic therapy (PDT) using verteportin (Visudyne) on corneal neovascularization (CNV) in 20 patients.

Methods:
Twenty patients with corneal neovascularization were treated with a nonthermal laser light at 689 nm delivered 15 min after an intravenous infusion of verteportin. Postoperative outcome of neovascularization was followed clinically (inflammation, intraocular pressure, and visual acuity) and photographically [color photographs and corneal fluorescein and indocyanine green (ICG) angiography] for a minimum of 12 months.

Results:
Successful phototransformation of corneal neovascularization was obtained immediately after treatment in eighteen patients, and regression was verified by corneal fluorescein and ICG angiography. In eight cases, partial vessel recanalization was observed after 6 weeks, and treatment was repeated, with complete regression of new vessels. No relevant side effects were observed in our cases.

Conclusions:
PDT with verteportin is an effective and safe procedure indicated for patients with corneal neovascularization; however, multiple sessions may be required.

Commercial Relationships: Rodrigo Bolanos, None; ALEXANDRA PENA, None; Alejandro Navas, None; Enrique O. Graue, None; JUDITH SANDRA SARMINA, None; GUILLERMO DE WIT, None; Ericka P. Lopez, None

Program Number: 4510 Poster Board Number: A0210
Presentation Time: 11:00 AM–12:45 PM

Corneal graft rejection was prevented by conbercept eye drop in vascularized cornea

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Purpose: Corneal neovascularization is a distinctive sign of immune privilege crash of cornea, which is the main incentive to induce the graft rejection after corneal transplantation. To avoid the graft rejection after corneal transplantation of alkali burned cornea, conbercept eye drop was used to prevent the graft rejection following corneal transplantation.

Methods: The cornea of New Zealand rabbits was burned by placing a Whatman filter paper (8.00 mm in diameter) soaked in 1 N NaOH onto the cornea for 60 seconds followed by washing with PBS for 60 seconds and allowed the injured cornea to heal for 20 days prior to corneal transplantation using a donor cornea collected from Angola rabbit. After surgery, conbercept eye drop was applied to prevent the immune graft rejection and FK-506 eye drop was used as the control. Immunostaining was employed to label the neovascular vessels, inflammatory cells, and lymphatic vessels with the antibody of anti-CD31, vEGF, CD45, CD11b, lyve1, etc.

Results: After corneal alkali burn, central cornea changed to be cloudy and central corneal epithelium was broken; 2 weeks later, blood vessels proliferated to central cornea from limbus. Following corneal transplantation and application of eye drops, corneal blood vessels were significantly suppressed by treatment of conbercept eye drop and cornea was kept to be transparent; however, the corneas treated by FK-506 eye drop were vascularized and only 3 in 10 corneas were transparent 10 weeks after the treatment. Histology revealed that very few inflammatory cells were found in the corneas treated by conbercept eye drop in comparison with that of FK-506 eye drop at 10 week after corneal transplantation. Moreover, the expression of CD31 and vEGF was significantly reduced in the cornea treated with conbercept eye drop, which revealed by Western blot.

Conclusions: Application of conbercept eye drop may be a valuable treatment to prevent corneal graft rejection through the suppression of corneal neovascularization.

Commercial Relationships: Hongshan Liu, None; Xingwu Zhong, None

Program Number: 4512 Poster Board Number: A0212
Presentation Time: 11:00 AM–12:45 PM

Anterior Segment Angiography with 1050 nm Swept-Source Optical Coherence Tomography

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Purpose: To visualize anterior segment eye blood vessels with non-invasive, dyeless optical coherence tomography (OCT) angiography.

Methods: The ocular conjunctiva, sclera, and iris of human eyes were imaged in vivo using a swept-source anterior segment OCT system operating at 1050 nm wavelength and 100 kHz axial-scan repetition rate. Three-dimensional OCT angiography data was acquired over 6mm x 6mm and 9mm x 9mm regions with scan depth of 5 mm in tissue by using 3 repeated B-scans at 300 raster positions, each B-scan consisting of 300 axial-scans. Horizontal and vertical raster scan volumes were acquired and software motion correction was applied to reduce eye motion and combine the volumes. Split-spectrum amplitude-decorrelation angiography (SSADA) technique was used to detect flow and construct angiograms. En face angiograms were constructed by maximum flow projection.

Results: Anterior segment angiography was performed on 4 eyes (2 light-colored and 2 dark colored) of 4 normal subjects. Depth-resolved conjunctival, episcleral, and iris angiograms were generated for each eye. The OCT angiograms revealed a rich vascular system in conjunctiva and relatively sparse blood vessels in episclera (Figure 1). The iris angiogram exhibited radial iris vessel patterns in normal light-colored eyes (Figure 2). However, in dark iris the anterior pigment layer produced shadowing and flow artifacts that obscure deeper vasculature.

Conclusions: Depth-resolved anterior segment OCT angiography can visualize vascular patterns in conjunctiva, sclera and light-colored iris. It is potentially useful for the assessment of anterior eye vasculature. This is a first demonstration of OCT angiography in human iris and further studies are needed.

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The purpose of this study was to evaluate if Mab2F1 and its humanized antibody, H1L1, have inhibitory effects on corneal NV and investigate the underlying mechanism.

**Methods:** Corneal NV was induced by alkali burn in anesthetized rats which were then subconjunctivally injected with 50µg/50µl of Mab2F1 or H1L1, with nonspecific mouse IgG or human IgG as control, respectively (n=10 per group) on days 0, 2 and 4 after alkali burn. Corneal NV and inflammation were evaluated using quantification of area of corneal NV and inflammatory index by slit lamp on days 2, 4, 6 and 8 after alkali burn. The effect of Mab2F1 or H1L1 on expression of Wnt pathway components in corneal NV was determined by Western blot analysis and immunostaining. The anti-angiogenic effect of Mab2F1 in vitro was assessed using human umbilical vein endothelial cells (HUVECs) stimulated by Wnt3a conditional medium (WCM). The effect of Mab2F1 on Wnt signaling induced by WCM was examined in human corneal epithelial (HCE) cells by luciferase assay and Western blotting. Two-tailed Student’s t-test was used for statistical analysis.

**Results:** Areas of corneal NV and inflammatory index, levels of CD31, pro-angiogenic and pro-inflammatory factors were all significantly reduced in the Mab2F1 and H1L1-treated groups, compared with that in control IgG-treated groups on days 4, 6 and 8 after alkali burn (all p<0.01). Mab2F1 and H1L1 displayed similar effects on NV and inflammation (all p<0.05). Mab2F1 and H1L1 significantly suppressed Wnt signaling in the cornea with NV. Mab2F1 significantly inhibited proliferation, migration and tube formation of HUVECs in a dose-dependent manner. Mab2F1 displayed robust inhibition of Wnt signaling and downregulated the expression of pro-angiogenic and pro-inflammatory factors induced by WCM in HCE cells in a dose-dependent manner.

**Conclusions:** Mab2F1 and H1L1 are effective on inhibiting corneal NV and inflammation via blocking Wnt signaling, suggesting therapeutic potential for corneal NV.

**Commercial Relationships:** Fangfang Qiu, None; Younghwa Shin, None; Danyang Chen, None; Guotao Deng, None; Jian-Xing (Jay) Ma, None

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**Program Number:** 4514 **Poster Board Number:** A0214 **Presentation Time:** 11:00 AM–12:45 PM

**Corneal Angiogenesis, Sensitivity, and Visual Acuity: a Retrospective Study**

Giulio Ferrari, Giacinto Triolo, Fabio Bignami, giorgio paganoni, Paolo Rama. Ophthalmology -Cornea Unit-Eye Repair, San Raffaele Scientific Institute, Milan, Italy.

**Purpose:** To study a cohort of patients affected with different grades of corneal neovascularization (CNV) and define its etiology. To test whether CNV extent is correlated with (i) best spectacle corrected visual acuity and (ii) corneal sensitivity.

**Methods:** Medical records were searched for the presence of corneal neovascularization. Among these patients, best spectacle corrected visual acuity (BSCVA) and corneal sensitivity were also measured. Spearman correlation test was used to search for correlations between (i) BSCVA and CNV; (ii) BSCVA and sensitivity. Chi-square test for trend was used to quantify correlation between corneal sensitivity and CNV.
Results: Six hundred and fifty patients (803 eyes) who visited the San Raffaele Hospital Cornea Clinic between Jan 2004 and June 2012 were included in the study. Of these, 497 presented with monolateral corneal neovascularization and 153 with bilateral CNV. Mean patient age was 54±20 (M=53%; F=47%). CNV followed infectious keratitis in 31%, non-infectious keratitis in 57%; while the cause was unknown in 12% of cases. Among infectious keratitis cases, 74% were viral. Among non infectious keratitis cases, corneal dystrophies, including keratoconus (32%) were the most prevalent. CNV developed as a consequence of ocular surgery in 18% of cases. Patients affected with CNV involving 1 (P<0.01) and 4 (P<0.05) corneal quadrants were significantly more likely to have better and lower BSCVA respectively. BSCVA was significantly higher in patients with normal vs. absent corneal sensitivity (P=0.001). Finally, patients who maintained normal corneal sensitivity were significantly less vascularized (P=0.005).

Conclusions: Corneal neovascularization is common in patients presenting to a third-level cornea clinic. Its impact on visual acuity is, however, still under scrutiny. Our study shows that extensive corneal neovascularization is significantly associated with a reduction in BSCVA. We suggest that strategies aimed at controlling CNV should be improved. Additionally, the fact the CNV is associated with reduced corneal sensitivity, and, potentially, nerve dysfunction, confirms previous data obtained in animal models.

Commercial Relationships: Giulio Ferrari, None; Giacinto Triolo, None; Fabio Bignami, None; giorgio paganoni, None; Paolo Rama, None
Support: Italian Dept of Health Young Researcher grant

Commercial Relationships: Luis Fernando Nominato, None; Ana C. Dias, None; Lara Dias, None; Eduardo M. Rocha, None
Program Number: 4516 Poster Board Number: A0216
Presentation Time: 11:00 AM–12:45 PM
Quercetin suppress pathogenesis of inflammation on corneal alkaline burns in rabbits
Purpose: We investigated the effect of quercetin on inflammatory event in corneal alkaline burns rabbits.
Methods: Corneal neovascularization (NV) was induced by applying an 8-mm filter paper soaked in 1 N NaOH to the right central corneas of rabbits for one minute. Seven days later, the rabbits were randomly divided into three groups: the alkaline burn group (n=4, normal saline instilled four times per day), the 5 mg/mL Que group (n=5, 5 mg/mL quercetin instilled four times per day), and the 10 mg/mL Que group (n=5, 10 mg/mL instilled four times per day). The left eyes were used as controls. On the 10th day after therapy, we investigated the fibrosis, neovascularization (NV), inflammation and structural changes of the cornea using H&E staining, masson’s trichrome staining, immunohistochemistry and RT-PCR.
Results: The alkaline burn produced significant NV (2.9±0.5) and increased corneal thickness (931.06±39.6 μm). On day 10 after 10 mg/mL quercetin treatment, NV (1.8±0.5) and thickness (626.2±45.6 μm) of the cornea were markedly decreased in the quercetin group (p<0.05). In 5mg/mL Que group, NV was markedly decreased (2.3±0.3, p<0.05) but corneal thickness was not affected. In addition, the quercetin improved the healing of the cornea following alkaline burn, disrupting the corneal epithelial proliferation and reducing the fibrotic changes of the stroma. The hallmarks of angiogenesis and inflammation including VEGF, CD31, MMP9, macrophage, TNFα, ICAM-1, VCAM-1 and IL-1β were significantly induced in the cornea by the alkaline burn, and these expression were also suppressed by quercetin.
Conclusions: In this study, we demonstrated that quercetin was markedly effective in healing alkal-burned corneas by modulating the corneal opacity, NV, fibrosis and inflammation via blocking the NF-κB. Therefore, quercetin is possible promising material for treatment of ocular surface disease related inflammation.
Commercial Relationships: Hye Sook Lee, None; Yoon Jin Lee, None; Jihyun Lee, None; Min Hee Kim, 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 (grant #: HI12C0005)

Commercial Relationships: Luis Fernando Nominato, None; Ana C. Dias, None; Lara Dias, None; Eduardo M. Rocha, None
Program Number: 4516 Poster Board Number: A0215
Presentation Time: 11:00 AM–12:45 PM
Lacrimal gland gene transfer by recombinant adenovirus encoding human soluble VEGFR1 inhibits neovascularization in cornea alkaline burn model
Purpose: The aims of this study were (1) to determine the efficacy of adenovirus vector serotype 5 encoding human VEGF receptor 1 (Ad-VEGFR1) for delivering gene therapy to the lacrimal gland (LG), (2) to investigate whether expression of recombinant soluble VEGFR1 acts in corneal neovascularization induced by alkaline burn and (3) to evaluate the safety of those vectors to the target tissues and systemic spreading.
Methods: Ad-VEGFR1 viral vectors (25 μl, 1x10⁶ pfu/mL) of were injected in the right LG of Wistar male adult rats and evaluated in parallel with control animal that received Ad-Null vector (25 μl, 1x10⁶ pfu/mL) or saline (25μl), prior to ipsilateral cornea alkaline burn with NaOH 1M. After 7 days the animals were tested for changes in LG by histology and qRT-PCR, tear secretion were measured with phenol red thread, corneal neovascularization were observed in the slit lamp and human s-VEGFR1 was quantified in serum by Elisa.
Results: Ad-VEGFR1 successfully transduced the LG, as detected by qRT-PCR. The percentage of neovascularized area within the cornea was reduced in the group treated with Ad-VEGFR1 compared to the treated with Ad-Null and saline. It did not affect the LG histology nor tear secretion and s-VEGFR1 was not detected in serum.
Conclusions: These results not only support that adenoviral vectors was safe for LG structure and function but also demonstrate that using the LG as the target tissue, local expression of s-VEGFR1 might be a feasible approach for inhibiting the development of corneal neovascularization.
Methods: We used the established murine model of suture-induced inflammatory corneal neovascularisation assay. 14 days after suture placement we removed the sutures. The mice underwent corneal abrasion and received cross-linking treatment using 0.1% riboflavin-5-phosphat solution (Medicross H) (1x riboflavin-5-phosphat drop in 15 minutes) and subsequent UVA-light-illumination (370 nm; irradiance, 3 mW/cm2; dose, 5.4 J/cm2, 1x riboflavin-5-phosphat drop) for 15 minutes. After four days, corneal blood and lymphatic vessels were stained ex vivo using LYVE-1 and CD31 antibodies. Depth of crossing linking was assessed by measuring the nucleus free area between Bowman’s layer and corneal endothelium using Dapi staining and an ApoTome2 microscope.

Results: The cross linked corneas showed an enhanced rigidity as a sign of successful cross linking and were not perforated. The corneal stroma was cross linked to a mean depth of 23.96% (SD 18.5%) of total corneal stromal thickness. Furthermore we found a significant negative correlation between the depth of cross linking and the area covered by blood vessels (p=0.018; r²= 0.881). Epithelial healing was delayed in the crosslinked animals.

Conclusions: We demonstrate a novel murine model of corneal crosslinkink. Crosslinking in murine corneas is a new way to analyse the effect of this clinically often used method on corneal cells. This pilot study shows that crosslinking can regress pathological corneal blood vessel in a depth dependent manner. Therefore this is a promising model to develop a new strategy to regress preexisting corneal vessels prior to keratoplasty.

Commercial Relationships: Felix Bock, None; Gabór Tóth, None; Nora Szentmary, None; Franziska Bucher, None; Claus Cursiefen, None

Support: BMBS COST Action BM1302 “Joining Forces in Corneal Regeneration Research”

Program Number: 4518 Poster Board Number: A0218

Presentation Time: 11:00 AM–12:45 PM

Inhibitory Effect of Diospyros kaki on Alkaline Burn-Induced Corneal Neovascularization

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Purpose: The purpose of this study is to evaluate the effect of ethanol extract of Diospyros kaki leaves (EEDK) on corneal neovascularization (CoNV) in rats.

Methods: The dried leaves of D. kaki were extracted twice with ethanol at room temperature for 3 h and filtered. The combined filtrate was concentrated and dried by rotary evaporation. Twenty-four Sprague-Dawley rats were divided into 3 groups of 8 animals each. After producing alkali burns in the cornea by the application of a 3-mm diameter filter paper soaked with 1 mol/L NaOH, separate groups of rats received 100 mg/kg or 200 mg/kg EEDK daily by gavage. A third group was used as a positive control group. After 7 days, all subjects were examined using biomicroscopy and anterior segment photos were taken, with which the area of CoNV was compared between groups. The protein expression levels of vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), interleukin-6 (IL-6), and matrix metalloproteinase-2 (MMP-2) were determined by western blot analysis.

Results: One week after the chemical burn, the CoNV area coverage in the CoNV positive control group, 100 mg/kg EEDK group, and 200 mg/kg EEDK group was 43.3 ± 5.5%, 33.7 ± 2.5%, and 27.2 ± 4.3%, respectively. The areas of corneal neovascularization in the EEDK-treated groups were significantly different from that of the CoNV group. EEDK significantly attenuated the up-regulation of VEGF, FGF, IL-6, and MMP-2 protein levels.

Conclusions: Orally administrated D. kaki inhibited CoNV development in rats. Our result implies that the ethanol extract of Diospyros kaki does possess anti-angiogenic effect and anti-inflammatory effect which can be applied in various eye diseases.

Commercial Relationships: Sang Hoon Jung, None; Kui Dong Kang, None; Su Ah Kim, None; Hyoung Jo, None; Kyung-A Kim, None; Hong Ryul Ahn, None; Sung Jae Yang, None

Program Number: 4519 Poster Board Number: A0219

Presentation Time: 11:00 AM–12:45 PM

Combined Therapy with Verteporfin and Ranibizumab for Corneal Neovascularization

ALEXANDRA PENA1, 2, Rodrigo Bolanos1, 2, Alejandro Navas3, Enrique O. Graue2, JUDITH SANDRA SARMINA1, GUILLERMO DE WIT2, Erika P. Lopez2. 1OPHTHALMOLOGY SERVICE, REGIONAL HOSPITAL ADOLFO LOPEZ MATEOS ISSSTE, Mexico City, Mexico; 2CORNEA AND REFRACTIVE SURGERY, INSTITUTE OF OPHTHALMOLOGY CONDE DE VALENCIANA MEXICO, Mexico City, Mexico.

Purpose: To describe the effect of photodynamic therapy (PDT) using verteporfin (Visudyne) and subconjunctival Ranibizumab (Lucentis) on corneal neovascularization (CNV) in nine patients.

Methods: Nine patients with corneal neovascularization were treated with a nonthermal laser light at 689 nm delivered 15 min after an intravenous infusion of verteporfin and subconjunctival Ranibizumab 1.0mg (1.0 mg/0.10 mL) administration at the same time. Postoperative outcome of neovascularization was followed clinically (inflammation, intraocular pressure, and visual acuity) and photographically [color photographs and corneal fluorescein and indocyanine green (ICG) angiography] for a minimum of 6 months.

Results: Successful photothrombosis of corneal neovascularization was obtained immediately after treatment in all patients, and regression was verified by corneal fluorescein and ICG angiography. In all cases, complete regression of new vessels was observed after 6 weeks. No relevant side effects were observed in our cases.

Conclusions: Combined administration of PDT with Verteporfin and subconjunctival Ranibizumab is an effective and safe procedure for patients with corneal neovascularization.

Commercial Relationships: ALEXANDRA PENA, None; Rodrigo Bolanos, None; Alejandro Navas, None; Enrique O. Graue, None; JUDITH SANDRA SARMINA, None; GUILLERMO DE WIT, None; Erika P. Lopez, None

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