Dietary ω-3 Polyunsaturated Fatty Acids Reverse Late Stage Pathologic Retinal Neovascularization

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Purpose: Retinal neovascularization, a prevalent cause of blindness and is characterized by two critical phases, vessel loss followed by hypoxia-driven destructive neovascularization. Our previous studies demonstrated that a lifelong ω-3 versus an ω-6 PUFA diet protected mice from oxygen-induced retinopathy by preventing neovascularization through revascularization of hypoxic retina. While long term treatment with dietary ω-3 PUFAs is clearly beneficial in preventing retinopathy it remains unclear what effect late stage treatment with dietary ω-3 PUFAs would have on reversing retinal neovascularization.

Our initial study showed that during the vessel loss phase of the disease (when mice are in high oxygen) dietary intake had no effect on vessel loss.

Methods: In the oxygen-induced retinopathy model, C57Bl/6 mothers were fed (postnatally) 0 (P0) a diet enriched with ω-3 PUFA or a diet elevated in ω-6 PUFA. ω-3 PUFA composition of the milk from the mothers reflected their diet. Alternatively, mice were treated with an ω-3 PUFA or an ω-6 PUFA diet at P12 or P15, after the initial vessel loss phase of oxygen-induced retinopathy. We then analyzed the role these diets played in reversing late stage vessel loss. Retinal RNA was isolated at the different time points. Mice isolated in normoxia only were controls.

Results: As seen previously, mice on a lifelong ω-3 (versus ω-6 enriched) PUFA diet had a significant increase in vessel re-growth as well as diminished neovascularization and a decrease in macrophage markers and associated inflammatory molecules. In mice given the diets after vessel loss, no change in vessel re-growth (vaso-obliteration) was observed at P17. However, mice on a ω-3 PUFA diet were significantly protected (~50%) from the pathological neovascular stage of the disease compared to their ω-6 PUFA fed counterparts. No change in macrophage markers was observed. However in the ω-3 PUFA fed mice there was a significant decrease (46%) in inflammatory molecules.

Conclusions: This data indicates that ω-3 PUFAs can reverse the pathological neovascularization phase of retinopathy after it has been initiated. This is associated with changes in the inflammatory response.

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Dietary ω-3 Polyunsaturated Fatty Acids Modulates Arachidonic Acid Cascade Enzymes and Protects Against Pathogenic Retinal Neovascularization

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Purpose: Lipid based molecules act as effectors of angiogenesis and inflammation; among the most potent are the eicosanoids, derived from the 20 carbon long chain polyunsaturated fatty acids (PUFAs); through the cyclooxygenase (COX) and lipoygenase (LOX) pathways. We have found that dietary intake of ω-3 PUFAs suppresses proliferative retinopathy in a mouse model of oxygen-induced retinopathy (OIR). Here we demonstrate that ω-3 PUFAs may inhibit pathologic neovascularization through various mediators in the COX and LOX pathways.

Methods: The diets of C57/Bl6 litters were enriched with ω-3 or ω-6 PUFA via maternal feed. Retinas of the pups reflected the change in ω-6/ω-3 ratio. To induce retinopathy the mothers and pups were placed in 75% oxygen for 5 days from P7 to P12 and then returned to room air. Isolated retinas were pooled across dietary groups, RNA was isolated, and converted to cDNA. Retinal mRNA expression was compared to cyclophilin and quantified using real time-PCR. Additionally, SC58236 (a specific COX-2 inhibitor) or NDGA (LOX inhibitor) was used to address the role of these enzymes in the pathogenesis of retinopathy through the modulation of downstream effectors.

Results: COX inhibition by NDGA resulted in a 7% reduction of retinal vaso-obliteration and a 22% reduction in neovascularization (NV). Similarly, low-dose COX-2 inhibition reduced NV by 50%. An ω-3 PUFA diet versus an ω-6 PUFA diet was associated with a 49% inhibition of retinal NV in the OIR mouse model at P17. During the hypoxia phase of the disease (P14), an ω-3 diet increased retinal mRNA levels of 12/LOX (~42%), 15-LOX (~66%), cytosolic phospholipase A2 (−38%) and cytosolic prostaglandin E2 synthase (~22%). Ceramide kinase and 5-LOX and did not show conclusive variation with diet at the level of mRNA.

Conclusions: These findings suggest that an ω-3 PUFA diet may inhibit retinopathy neovascularization in part through altering enzyme mRNA expression in eicosanoid lipid signaling pathways; thereby altering the levels of downstream lipid mediators.

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Differential Gene Expression in Ndpkα Knockout Mice in Retinal Development

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Purpose: Angiogenesis, the process of blood vessel development from an already existing vasculature, is essential for organismogenesis in order to provide oxygen and nutrition supply for still evolving structures. Mutations in the NDF gene seem to impair angiogenesis in the eyes of patients diagnosed with a type of blindness belonging to the group of exudative vitreoretinopathies. With this study, we wanted to investigate the primary defects caused by the absence of Ndpk (the NDF protein) in the developing mouse retina.

Methods: A comparative gene expression analysis was performed on p7 (postnatal day 7) retinas from a knockout mouse model for Nnr disease. Differential gene expression in wild type versus knockout mice was determined using Affymetrix microarrays (GeneChip® Mouse Genome 430 2.0 Array). Subsequently, results were confirmed by real time-PCR analysis. Hematoxylin and eosin staining was performed for the vascular permeability marker Dppa and collagen type IV.

Results: Our study identified transcriptional differences in Ndpkα vs. wild type mice retina at p7. Expression of the neutral amino acid transporter Slc38a5, apolipoprotein D (ApoD) and angiostatin receptor-like 1 (Agtr1l) were decreased in the knockout, whereas transcript levels for adrenomedullin (Adm) and the plasmalogenase vesiicle associated protein (Pip7) were increased in comparison to the wild type.

Conclusions: These data provide molecular evidence for a role of Nnr in the development of the retinal vasculature. Expression of genes Pip7 and Slc38a5 is considerably altered in retinal development of Ndpk knockout mice and reflect or may contribute to the pathogenesis of the disease.

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Antioxidants Reduce Retinal and Choroidal Neovascularization


Purpose: Oxidative damage has been implicated in the pathogenesis of age-related macular degeneration (AMD). Mice deficient in superoxide dismutase 1 (SOD1) have increased susceptibility to oxidative damage in the retina and develop a phenotype similar to that seen in patients with AMD including choroidal neovascularization (CNV). The purpose of this study was to investigate the role of SOD1 and SOD3 in the development of ocular neovascularization (NV).

Methods: Genetically engineered mice with altered expression of SOD1 or 3 (sod1-/-, sod3-/-, sod1 transgenic overexpressors, and sod3 transgenic overexpressors) were tested in models of ischemia-induced and VEGF-induced NV. Wild type mice were used in the same models to examine the effects of antioxidants.

Results: Compared to littermate control mice, mice deficient in SOD1, but not those deficient in SOD3 nor those that over-express SOD1 or 3, showed a statistically significant 3-fold increase in ischemia-induced retinal NV. Rho/VEGF transgenic mice that were deficient in SOD1 showed a 3-fold increase in subretinal NV compared to rho/VEGF mice with normal levels of SOD1. Since SOD1 and SOD3 mice have enhanced oxidative stress, we investigated the effects of antioxidants on NV. Compared to vehicle-treated controls, mice treated with a mixture of antioxidants (200mg/kg alpha-tocopherol, 250mg/kg ascorbic acid, and 100mg/kg alpha-lipoic acid) showed a significant reduction in ischemia-induced retinal NV, VEGF-induced subretinal NV in rho/VEGF transgenic, and laser-induced CNV.

Conclusions: These data suggest that oxidative stress creates a proangiogenic environment in the retina and choroid. These data may help to explain the reduced incidence of CNV in high-risk AMD patients taking antioxidants.

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Gene Profiles of Omega-3 and Omega-6 PUFA Fed Mice With Retinopathy

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Purpose: We have previously reported that mice with elevated levels of omega-3 polyunsaturated fatty acids, through either dietary or genetic means, are protected from retinopathy through enhanced vessel regression. Mice on a diet enriched in omega-3 fatty acids had decreased levels of microglial-derived TNP-alpha in a model of oxygen induced retinopathy. To further investigate the protective properties of omega-3 fatty acids in pathological neovascularization, we performed Illumina microarray analysis on retinas from mice on diets rich in either omega-3 or omega-6 polyunsaturated fatty acids (PUFAs).

Methods: Beginning at postnatal day 0 (P0), mothers were fed diets enriched with either omega-3 or omega-6 PUFAs. To induce vessel loss, and subsequent pathological neovascularization, nursing mothers and pups were exposed to 75% oxygen from P7 to P12 and returned to room air and sacrificed at P17. One eye from each mouse was isolated and lectin-stained for quantification of neovascularization and vaso-obliteration. Contralateral retinas from each group were isolated and flash frozen using RNase-free RNA later and used for Illumina microarray analysis, which showed a 2-8 fold upregulation of activated macrophage, inflammation, angiogenesis, and adhesion markers in omega-6 fed mice.

Conclusions: These findings suggest that upregulation of adhesion markers in omega-6 fed mice may be responsible for recruiting inflammatory cells and activated macrophages into the retina, resulting in an increased production of pro-angiogenic signals and more severe pathological neovascularization.

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3449 - A439
Cysteine-Rich 61, Cenil, as a Mediator of Ocular Angiogenesis

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**Purpose:** Cysteine-rich 61 (Cyr61) is a angiogenic factor. It is not clear that Cyr61 plays a role in ocular angiogenesis. We investigate Cyr61-mediated angiogenesis in vitro and in vivo to understand its role in ocular angiogenic disorders.

**Methods:** Cyr61 effects on monkey retinal endothelial cell (RF/6A) was evaluated with proliferative, chemotaxis assay and Matrigel capillary tube formation assay in vitro. Reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis were used to detect mRNA and protein expression of Cyr61 in RF/6A cells under hypoxic condition. Oxygen-induced retinopathy model of mice (OIR) was used to test the angiogenic property of Cyr61 in vivo. Cyr61 levels of vitreous samples from proliferative diabetic retinopathy (PDR) patients were measured by sandwich enzyme-linked immunosorbent assay (ELISA). Immunodepletion of Cyr61 in PDR vitreous samples by anti-Cyr61 monoclonal antibody was observed in endothelial cells chemotaxis assays.

**Results:** Cyr61 significantly induced proliferation and migration of RF/6A. Cyr61 induced formation of endothelial cell capillary tubes on Matrigel. Expression of Cyr61 was detected in RF/6A by RT-PCR and Western blotting under hypoxic condition. Cyr61 significantly expressed on new vessels in the mice of the OIR model. Intravitreal injection of anti-mouse Cyr61 antibody decreased retinal angiogenesis in the OIR model. Vitreous level of Cyr61 was elevated in PDR patients as compared to control. Immunodepletion of Cyr61 from PDR vitreous samples caused 50.5 ± 8.0% less migration of RF/6A.

**Conclusions:** Cyr61 is an angiogenic mediator in vitro and in vivo. Hypoxia could induce expression of Cyr61. Vitreous level of Cyr61 was elevated in PDR patients. Cyr61 may play an important role in ocular angiogenic disorders such as PDR.

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3450 - A440
Diabetes-Induced Changes of Morphology and Alpha Smooth Muscle Actin and Occludin Expression in the Rat Retinal Vasculature


**Purpose:** Diabetes causes functional changes in the retinal vasculature. These include alterations in the rate of blood flow and in the permeability. The key factors of these events are considered as contractile proteins and tight junction proteins. In this study, we investigated the morphological and immunohistochemical changes of the microvessels and their representative proteins in the rat retina following long-standing diabetes insulins, for better understanding on the pathophysiology of neovascularization.

**Methods:** Diabetic condition was induced by a single intravenous injection of streptozotocin in Sprague-Dawley rats aged of 8 weeks. The animals showing high blood glucose levels (above 300 mg/dl) were cared for 1, 4, and 12 weeks, respectively. The retinas were processed for alpha smooth muscle actin (α-SMA) and occludin immunohistochemistry and electron microscopy.

**Results:** In the whole-mounted retina of normal, arteries were differentiated with α-SMA immune-labeling and spirally wrapped smooth muscle cells, whereas venules with scanty of smooth muscle cells in their wall. The arterioles numbered 5 to 7 were extended radially from the optic disc to the periphery as branching, and gave rise to capillaries demarcated with α-SMA labeled pericytes penetrating into the inner retina throughout their courses. The venules were located in between the arteries. Occludin was labeled the boundary of the endothelial cells. During diabetes, the capillary networks immunolabeled by α-SMA in the inner retina were more remarkable due to reduce of thickness of the inner retina. The protein levels of α-SMA were gradually reduced, and those of occludin no large different. In the diabetic retina, the endothelial cells, pericytes and endothelial cells were showed degenerating morphology such as reduction of contractile activity in the smooth muscle cells and the pericytes, but also morphological changes including increase of adluminal endothetic vesiess in the endothelial cells of the retinal microvessels.

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3451 - A441
Plasminogen Activator Inhibitor-1 (PAI-1) Facilitates Angiogenesis in a Model of Oxygen Induced Retinopathy

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**Purpose:** The formation of new retinal blood vessels is a key feature of the pathology of diabetic retinopathy or retinopathy of prematurity. The aim of the present study was to investigate the role of the serine protease inhibitor PAI-1 in facilitating retinal neovessel formation.

**Methods:** The temporal expression of PAI-1 was examined by real time PCR, western blotting and immunohistochemistry in retinal tissues from mice with oxygen-induced retinopathy. The requirement for PAI-1 in facilitating the angiogenic response in this model was examined by quantitating the angiogenic response using both wildtype and PAI-1 null mice. The mechanism by which PAI-1 mediates angiogenesis was investigated using isolated human retinal vascular endothelial cells. The migratory activity of the cells was quantitated in the presence of a PAI-1 neutralizing antibody.

**Results:** PAI-1 expression is up regulated in the retina of mice with oxygen induced retinopathy. This coincides with a significant increase in the expression of vitronectin in the retina of the experimental mice. There was significant reduction in the angiogenic response of PAI-1/- mice as compared to wild type mice. PAI-1 promotes endothelial cell migration in vitro and facilitates migration of cells on a vitronectin substrate by regulating αv integrin cell surface expression.

**Conclusions:** These observations suggest a role for PAI-1 during retinal angiogenesis and point to a potential new therapeutic target in the prevention or treatment of retinal neovascularization.

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3452 - A442
Combined Intravitreal Ranibizumab and Verteporfin Photodynamic Therapy for Choroidal Neovascularization in Angioid Streaks: Preliminary Results


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**Purpose:** to assess the safety and efficacy of combined Verteporfin Photodynamic Therapy (PDT) and Intravitreal Ranibizumab (Lucentis) Injection in the treatment of extrafoveal choroidal neovascularization in angioid streaks over a short term.

**Methods:** Prospective interventional non-comparative pilot case study. Four eyes of 4 consecutive patients affected by extrafoveal choroidal neovascularization due to angioid streaks were involved. At baseline, each patient underwent Photodynamic Therapy with Verteporfin followed by the first intravitreal injection of 0,5 mg (0,05 ml) of Ranibizumab. The second injection was delivered 30 days later. Additional injections were carried out monthly, if needed. All patients underwent complete ophthalmological examination, fluorescein angiography (FA), indocyanine green angiography (ICGA), and optical coherence tomography (OCT3) at baseline and at each control visit. Follow-up visits were set at one week, four weeks and thereafter monthly.

**Results:** 3 patients were female, one male; mean age was 50 range 43-58. Mean follow-up was 8 months, range 6 - 10. Mean baseline visual acuity was 20/28, range 20/50 - 20/32. At month 6, mean visual acuity was 20/30, range 20/20 - 20/50. Visual acuity improved by one line in one patient, remained unchanged in two and decreased by two lines in one. Three patients received two injections and one patient received a third at month 5. All patients showed stabilization of the lesion during both angiographic and OCT examinations without foveal invasion.

**Conclusions:** In this pilot series of eyes with extrafoveal choroidal neovascularization in angioid streaks, combined PDT and intravitreal Ranibizumab therapy appears to be safe. Further follow-up is necessary to confirm long-term treatment benefits.

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3453 - A443
OCT Guided Re-Injection of 2.5mg Bevacizumab for Treatment of Macular Edema Due to Retinal Vein Occlusion

Purpose: To evaluate an OCT guided re-injection scheme of 2.5mg bevacizumab in patients with macular edema (ME) due to retinal vein occlusion.

Methods: Prospective study. After informed consent patients with persistent ME due to branch (BRVO) or central (CRVO) retinal vein occlusion received intravitreal bevacizumab (2.5mg/0.1ml) every 6 to 8 weeks until ME resolves. Ophthalmic examination, visual acuity (ETDRS) and Stratus OCT® are performed at baseline and every 6-8 weeks. Re-Injections are only performed if the OCT shows persistent or recuring ME.

Results: 78 patients (34 CRVO, 44 BRVO) have been included in the study so far with a mean follow-up of 31±22 weeks. In 43.6% (n=34) of patients ME had completely resolved 6 weeks after 1st injection (improvement in visual acuity 3.4±3.8 lines). 57.2% of these patients developed a relapse of ME within 13±3.4 weeks after first injection. In 42.3% of patients ME did not recur within 17±3.7 weeks. All patients with recurrence of ME received a 2nd injection that completely resolved ME within 6 weeks. Visual acuity gained the same level as after 1st injection (difference in visual acuity: 0.1±0.2 lines). Another relapse of ME in this group occurred in 62.9% of patients 13±5.7 weeks (6-25 weeks) after 2nd injection.

Patients with persistent ME after 1st injection (55.4%; n=44) received a 2nd injection which led to complete ME resolution in 62%. All of these patients had a recurrence of ME after 13±8.4 weeks. In 75% of patients ME persisted after the 2nd injection and were re-injected. Although no patient showed complete resolution of ME, there was a reduction of central retinal thickness during a follow up of 41.3±17.5 weeks and an average number of injections of 5.1±1.5 (3 to 8 injections). However there was no significant change in visual acuity in these patients.

Conclusions: OCT guided re-injections lead to anatomic and functional stabilisation or improvement even if transient recurrence of ME occurs and can minimize the number of injections needed in comparison to a re-injection scheme with fixed time intervals.

Persistent ME after 1st injection may indicate a poor response to treatment with bevacizumab. Patients with persisting ME after two injections never showed a complete resolution of ME.

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3454 - A444
Intravitreal Bevacizumab for the Treatment of CNV in Angioid Streaks - Long Term Results

Purpose: To evaluate visual acuity and anatomical outcomes after treatment with intravitreal Bevacizumab (Avastin) in patients with choroidal neovascularization (CNV) associated to angioid streaks.

Methods: Retrospective study on long term follow-up of 5 patients with a CNV associated to angioid streaks who were treated with one or more injections of Intravitreal Bevacizumab. Five patients were included in this study. Mean age was 57 years old with a range of 22-44 years. Best-corrected Snellen visual acuities, optical coherence tomography measurements, fluorescein angiography and ophthalmoscopic examination at baseline and at each follow-up visit were performed. Retreatment was performed if leakage and/or fluid were present associated or not with low visual acuity. Follow-up ranged from 15 to 28 months.

Results: All patients had a disciform scar in the other eye. Baseline visual acuities ranged in all patients was count finger from 1 to 3 m and final visual acuities ranged from 20/200 to 20/20. All of the eyes showed an improvement in visual acuity and one patient recovered to 20/20. Mean time to complete low visual acuity to get the injection was 46.4 days (7 - 60 days). All the eyes were treated at least 4 injections of Bevacizumab (4-13 injections) and all patients had to treated at least for 8 months. Mean baseline central foveal thickness by OCT was 336 microns, and mean final thickness was 267 microns. Reduction in membrane size by fluorescein angiography was noted in all patients. No ocular or systemic adverse events were found.

Conclusions: Reduction in CNV membrane size, leakage and foveal thickness by OCT were observed in all eyes. Intravitreal Bevacizumab appears to be effective in stabilizing and recover visual acuity in eyes with CNV associated to Angioid Streaks. Patients with early symptoms might get better benefits. Further studies must be done and should elucidate the potential benefit of this treatment.

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3455 - A445
Intravitreal Bevacizumab for Choroidal Neovascularisation in Pathological Myopia

Purpose: To evaluate the safety and efficacy of intravitreal bevacizumab as treatment for choroidal neovascularisation due to pathological myopia (mCNV).

Methods: Consecutive series of 23 patients with primary or recurrent mCNV treated with intravitreal bevacizumab 1.25mg after being thoroughly informed about the off-label character of the treatment. 17 patients received bevacizumab treatment only, 6 patients additional photodynamic therapy at the time of first injection. Best corrected visual acuity (EDTRS), ophthalmic examination and OCT were performed at baseline and at 6-week intervals. Fluorescein-angiography was performed depending on the clinical and OCT-findings. Re-Injections were given every 6 weeks if intra- or subretinal fluid persisted.

Results: Pre-injection mean visual acuity was 0.29 (0.52 logMAR±0.21). During a follow up of 17±14 weeks 1.39±0.8 injections were given. One eye received 2 injections, 2 eyes 3 injections, 2 eyes 2 injections and 18 eyes only one injection. Complete resolution of sub- or intraretinal fluid and inactivation of mCNV shown by angiography were achieved in all patients. Visual acuity (VA) improved by 2.8±5.3 lines on average. 9 eyes showed a significant improvement of at least 3 lines, 14 eyes remained stable and none lost more than one line. No intraocular or systemic side effects were observed.

Conclusions: In this so far largest series of patients with mCNV treated with intravitreal bevacizumab, the treatment seems to be effective and safe.

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3456 - A446
Intravitreal Injection of Bevacizumab in Subfoveal Choroidal Neovascularization Due to Pathological Myopia
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Purpose: To evaluate the efficacy of intravitreal bevacizumab in the treatment of choroidal neovascularization (CNV), in patients with pathological myopia, unresponsive to photodynamic therapy (PDT).

Methods: Retrospective chart review of 20 consecutive patients treated with intravitreal (IVT) bevacizumab (1.25 mg) for subfoveal choroidal neovascularization due to pathological myopia between February 2006 and October 2007. All patients were previously treated with PDT. Recurrence of CNV was confirmed by fluorescein and indocyanine green angiography. Outcome measures included visual acuity (VA), central retinal thickness with OCT, and degree of leakage on fluorescein and indocyanine green angiography.

Results: Twenty eyes of twenty patients were included. The mean age was 54.9 years (range 32-78). All patients were previously treated with PDT. The baseline mean VA was 2.6/10, the final VA was 3.9/10 with a mean follow up of 15.18 months (range 10-19). Overall, in 15 out of 20 (75%) eyes the CNV was successfully closed at last follow up visit. Seven of 20 eyes (35%) received 1 bevacizumab IVT, seven (35%) 2 injection, seven (35%) 4 injection and 1 eye (5%) received 5 IVT. All eyes (14), that received 1 or 2 treatments, had angiographic closure of lesion and 1 eye undergoing 4 bevacizumab injections had the same result.

There were no systemic or ocular complications.

Conclusions: In this small series of CNV due to pathological myopia intravitreal bevacizumab showed a good efficacy and safety. Further clinical trials are needed to evaluate a larger number of patient and a longer-term follow up.

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Support: None
Conclusions: To assess if the association of intravitreal rapamycin and bevacizumab have an additive effect in decreasing vitreous VEGF levels more than bevacizumab alone. To assess the safety of intravitreal rapamycin in a porcine model of choroidal neovascularization.

Methods: We included eight eyes in the study. A choroidal neovascularization was created using argon laser shots. We divided the eyes in four groups: Group A (N=2) was injected with 1 ml of intravitreal bevacizumab alone (2.25 mg/ml). Group B was injected with balanced saline solution, group C with 0.5 ml of rapamycin (1 mg/ml) and bevacizumab and group D just with rapamycin. The eyes were enucleated 21 days after the injections. We performed RT-PCR studies to all samples.

Results: A decrease in VEGF expression in group A, C and D was observed. The most important reduction was observed in group A. No structural damage was detected in eyes injected with intravitreal rapamycin or bevacizumab.

Conclusions: Intravitreal rapamycin is safe for intravitreal administration with no structural alterations. The combination of rapamycin and bevacizumab is not better than bevacizumab alone in decreasing VEGF vitreous levels.

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3459 - A449
Evaluation of Vascular Endothelial Growth Factor Levels After Intravitreal Bevacizumab in Retinal Disorders

Purpose: VEGF levels in both the aqueous and vitreous are elevated in patients with ocular ischemia secondary to exudative age-related macular degeneration, diabetes mellitus, and other retinal vascular diseases. We describe a unique assay for quantifying VEGF levels, that combines ELISA and flow cytometry with minimal quantity of aqueous aspirated at the time of intravitreal injection. Utilizing this sensitive technique, we investigated the effect of intravitreal injections of bevacizumab on VEGF levels in a variety of retinal disorders.

Methods: Aqueous samples were collected prior to and at monthly intervals at the time of intravitreal injection of bevacizumab (1.25 mcg in 0.05ml) for a variety of retinal disorders (N=24). Aqueous samples obtained from normal patients at the time of cataract surgery were used as controls (N=10). Detection of VEGF in the aqueous samples was performed at Rule Based Medicine Inc. using multiplex analysis. In brief, sample was incubated with capture microsphere multiplexes of the Human Antigen MAP for one hour at room temperature. Multiplexed cocktails of biotinylated reporter antibodies for each multiplex were then added, mixture incubated for an additional hour and multiplexed developed using streptavidin-phycocyrthrin solution. Analysis was performed in a Luminex 100 instrument and the data was interpreted using proprietary data analysis software (Quagen Instruments).

Results: Vascular endothelial growth factor concentration in the aqueous humor ranged from 326 to 12476 pg/ml (mean=SD, 1306±125 pg/ml) before intravitreal injection of bevacizumab and decreased to less than 180 pg/ml (P<0.00) in all eyes one month after injection. The concentrations in the control group ranged from 120 to 218 pg/ml (mean=SD, 162±42 pg/ml).

Conclusions: This study was also unique in that the assay employed to determine aqueous VEGF levels, utilized a combination of ELISA and flow cytometry, which afforded increased sensitivity in detecting VEGF, with the lowest detected value of 1.5 pg/ml. Estimation of VEGF in aqueous samples with ELISA is difficult as it requires large volume for reliable estimation. Our method utilizes only 100 mcL and overcomes this difficulty. Intracameral and intravitreal dose of bevacizumab is empirical at this time. Neutralization of all available VEGF is necessary for successful resolution of retinal pathology. Reliable estimation of VEGF and subsequent administration of appropriate dose of bevacizumab is ideal in treatment of proliferative retinovasculopathies.

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Microdose Intravenous Bevacizumab for the Treatment of Retinal Vascular Diseases Mediated by Vascular Endothelial Growth Factor (VEGF)
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Purpose: To demonstrate microdose (1.25-2.5mg) bevacizumab given intravenously has a bilateral salutary effect avoiding the need for intracocular injections or high dose systemic injections. Intravitreal bevacizumab in doses of 1.25 to 2.5 mg can produce a salutary effect on both eyes of patients with bilateral disease.

Methods: A retrospective chart review of thirty seven patients with either bilateral retinal vascular disease including macular degeneration, diabetic retinopathy and vein occlusions or who refused intracocular injection were treated microdose bevacizumab intravenously and followed by optical coherence tomography and fluorescein angiography to determine efficacy.

Results: Eleven patients had a complete response with resolution of cystic retinal changes in diabetic maculopathy and vein occlusions and neovascularization of the iris in patients with rubecosis. In age related macular degeneration, resolution of intraretinal fluid, subretinal fluid and sub-retinal pigment epithelial fluid was noted. Eleven eyes had a partial response with resolution but not resolution of these parameters and the remainder of eyes had no response at this low dose.

Conclusions: Selected patients with bilateral disease or who refused intracocular injection may benefit from microdose bevacizumab given intravenously. Larger doses, though much smaller than the systemic dose of 400 mg given for cancer treatment, may be necessary in recalcitrant cases.

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Role of MAP3K1 in Mouse Postnatal Eye Development

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**Purpose:** Mitogen-activated protein kinase kinase kinase 1 (MAP3K1) is involved in signal transduction in tissue morphogenesis during development. MAP3K1 ablation in mice results in defective eyelid development with characteristic eye open at birth (EOB) phenotype. Since the EOB inflicts secondary ocular tissue damages after birth, e.g., exposure keratitis, it remains unclear whether MAP3K1 ablation affects other ocular tissue development at postnatal stages.

**Methods:** To mimic the postnatal eyelid status of the wild type mice, we sutured the eyelid of 1-week-old mice immediately after birth and removed the suture at postnatal day 15. Conversely, we surgically opened the closed eyelids of wild type mice at birth to mimic the eyelid status of the Map3k1 ΔKD/ΔKD mice. At postnatal day 15, eyes were isolated and processed for paraffin embedding. H&E staining and immunohistochemistry were performed on 4 μm sections of the eyes to characterize the development of ocular tissues, including cornea, lens, meibomian glands and retina.

**Results:** In both wild type and Map3k1 ΔKD/ΔKD mice, an opened eye from P1-15 was associated with ocular surface pathologies, including thickening and vascularization of the corneal stroma with infiltration of inflammatory cells and disruption of stromal collagen fiber organization, lacking keratin 12 expression in corneal epithelial cells, and loss of the anterior chamber angle. There was also an adverse consequence of the opened eyelid on meibomian gland and lens development. The eyelid suture of the Map3k1 ΔKD/ΔKD mice had significantly improved clinical manifestations, e.g., keratitis, but did not completely restore normal ocular morphology. The Map3k1 ΔKD/ΔKD mice with and without eyelid suturing showed retinal abnormalities, including areas of folding between the rods and cones and the outer nuclear layers and increased cell numbers in the inner plexiform layer. Interestingly, the retina abnormalities were specific to the Map3k1 ΔKD/ΔKD, not seen in the wild type mice even if their eyelids were surgically opened at birth and developed ocular surface pathologies.

**Conclusions:** MAP3K1 plays a role in the postnatal retina development.

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Application of Laser-Targeted Drug Delivery Toward the Bench-Top to Bedside Transfer of Laser-Targeted Angiography for the Detection of Choroidal Neovascularization - A Phase I Dose Estimate

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**Purpose:** The detection and classification of choroidal neovascularization (CNV) is of paramount importance in assessing the efficacy of existing or new therapies for neovascular age-related macular degeneration (AMD). Presently, this is accomplished with fluorescein angiography. However, conventional angiography only provides information on leakage of dye from CNV. There is a need for a method capable of specifically visualizing the patent vascular lumens of CNV independent of whether or not there is active leakage. We have developed such a method of selective drug delivery that can be applied to perform targeted dynamic angiography. Following successful tests in animal models, a phase I study has been planned in patients with AMD to assess the safety of targeted angiography. This pilot study has been designed to estimate the lowest dose that will yield a visible angiogram.

**Methods:** Our method consists of encapsulating 6-carboxyfluorescein (CF) in thermo-sensitive nanoparticles which are injected intravenously. Due to fluorescence quenching, the nanoparticles are invisible during angiography. A safe, invisible laser beam is then directed to a retinal area suspected of harboring CNV. The laser beam causes the retinal tissues to warm-up, leading to the release of a bolus of dye from the nanoparticles. The progression of this bolus provides selective angiograms that highlight only the choroid, choriocapillaries, and CNV. Pigmented rabbits were injected with different fractions of the intended dose previously derived, and LTA was performed. The blue illumination used for visualization during angiography and the infrared beam used to activate release were set at 20% and 100%, respectively, of the maximal permissible exposure (MPE) set by standards (ANSI) for safe viewing. The digital camera was operated at high gain and a frame size reduced to 696 by 512 pixels.

**Results:** The dose of CF nanoparticles was reduced gradually from 100% to 13% of the intended dose. The dynamic angiograms acquired clearly showed a bolus progressing in the choroidal arteries, choriocapillaries, and veins. This sequence was visible down to the lowest dose.

**Conclusions:** This pilot study indicates that the choriocapillaries and small choroidal vessels may be visualized at all the escalation levels of the planned phase I study. Although encouraging, the study does not guarantee the same results in humans, as this pilot study was performed in animal eyes with heavy pigmentation, devoid of CNV or of any scar tissue.

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