

Issue 327

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This free weekly bulletin lists the latest published research articles on macular degeneration (MD) and some other macular diseases as indexed in the NCBI, PubMed (Medline) and Entrez (GenBank) databases.

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Drug treatment

Antiinflamm Antiallergy Agents Med Chem. 2017 May 1. [Epub ahead of print]

Designed Ankyrin Repeat Proteins: A look at their evolving use in medicine with a focus on the treatment of chorioretinal vascular disorders.

Smithwick E, Stewart MW.

BACKGROUND: Antibodies constitute an important drug development platform for drugs to treat several ophthalmic, oncologic, and immunologic conditions, but due to limitations inherent in antibody production and structure, a wide range of other protein binding scaffolds are being investigated. Designed ankyrin repeat proteins (DARPs) are simple to produce and offer a range of advantages over antibodies because of their stability, high binding affinity, and rigid structure.

OBJECTIVE: DARPs are being developed for a wide variety of medical applications, and the most studied molecule, abicipar pegol, is used to treat chorioretinal vascular diseases. This mini-review will discuss the current state of DARPin technology and will summarize drug development with a focus on abicipar.

METHODS: PubMed searches with keywords "DARPin" and "designed ankyrin repeat proteins" were performed and the reference lists of identified articles were examined for additional research material. Studies using DARPin molecules were identified at Clinicaltrials.gov. Non-peer reviewed data were found through Google searches of pertinent websites.

RESULTS: Abicipar prevents angiogenesis by binding all isoforms of vascular endothelial growth factor (VEGF)-A with single-digit picomolar affinity. Abicipar has a long intraocular half-life in rabbits and has produced promising results in pre-clinical studies. Pivotal phase III registration trials for the treatment of neovascular age-related macular degeneration are ongoing and a phase II/III trial for the treatment of diabetic macular edema has been announced.

CONCLUSION: Abicipar pegol has the potential to effectively treat chorioretinal vascular conditions with an extended duration of action beyond those of currently used anti-VEGF drugs.

PMID: 28464780

Ocul Immunol Inflamm. 2017 May 4:1-7. [Epub ahead of print]

Pharmacogenetic Aspect of Intravitreal Ranibizumab Treatment in Neovascular Age-Related Macular Degeneration: A Five-Year Follow-Up.

Sengul EA, Artunay O, Rasier R, Kockar A, Afacan C, Hancer VS, Yuzbasioglu E.

PURPOSE: This study aims to evaluate the role of complement factor H (CFH) in response to intravitreal ranibizumab (IVR) treatment, which is administered to patients with neovascular age-related macular

degeneration (nAMD).

METHODS: In this retrospective study, 90 nAMD patients' 90 eyes were evaluated. IVR was injected once a month for three consecutive months, and then, patients were followed up for five years by using pro re nata method.

RESULTS: Average visual acuity (BCVA) values in TT group for the third, fourth and fifth years were found to be significantly higher than those in TC and CC groups, while average BCVA values in TC group were significantly higher than those in CC group (all $p = .000 < .0167$).

CONCLUSION: Patients with CFH TT genotype responded significantly better to treatment after third year, while patients with CC genotype had a poorer response to IVR.

PMID: 28471284

Korean J Ophthalmol. 2017 Apr 24. [Epub ahead of print]

Comparison of Ranibizumab and Bevacizumab for Macular Edema Associated with Branch Retinal Vein Occlusion.

Son BK, Kwak HW, Kim ES, Yu SY.

PURPOSE: To assess the effectiveness and safety of intravitreal ranibizumab compared with bevacizumab for the treatment of macular edema associated with branch retinal vein occlusion (BRVO).

METHODS: This was a retrospective study of 80 eyes with macular edema associated with BRVO. Patients received either 0.5 mg of ranibizumab ($n = 24$) or 1.25 mg of bevacizumab ($n = 56$) intravitreally. Both groups received three initial monthly injections followed by as-needed injections. The best-corrected visual acuity, central subfield thickness, mean number of injections, and retreatment rate were evaluated monthly for 6 months after the initial injection.

RESULTS: The best-corrected visual acuity significantly improved from logarithm of the minimal angle of resolution (logMAR) 0.55 ± 0.26 at baseline to 0.24 ± 0.26 at 6 months in the ranibizumab group ($p < 0.001$) and from logMAR 0.58 ± 0.21 at baseline to 0.29 ± 0.25 at 6 months in the bevacizumab group ($p < 0.001$), which is not a statistically significant difference ($p = 0.770$). The mean reduction in central subfield thickness at 6 months was $236 \pm 164 \mu\text{m}$ in the ranibizumab group ($p < 0.001$) and $219 \pm 161 \mu\text{m}$ in the bevacizumab group ($p < 0.001$), which is not also a statistically significant difference ($p = 0.698$). The mean numbers of ranibizumab and bevacizumab injections were 3.25 ± 0.53 and 3.30 ± 0.53 , respectively ($p = 0.602$). In addition, after the three initial monthly injections, the retreatment rates for ranibizumab and bevacizumab injections were 20.8% and 26.7%, respectively ($p = 0.573$).

CONCLUSIONS: Both ranibizumab and bevacizumab were effective for the treatment of BRVO and produced similar visual and anatomic outcomes. In addition, the mean number of injections and the retreatment rates were not significantly different between the groups.

PMID: 28471106

Korean J Ophthalmol. 2017 Apr 24. [Epub ahead of print]

Treatment of Bilateral Retinal Angiomatous Proliferation with Anti-vascular Endothelial Growth Factor: 12-Month Outcome.

Kim JM, Kim JH, Chang YS, Kim JW, Kim CG, Lee DW.

PURPOSE: To evaluate the 12-month outcome of intravitreal anti-vascular endothelial growth factor therapy in eyes with bilateral retinal angiomatous proliferation (RAP).

METHODS: This retrospective observational study included 38 eyes of 19 patients with stage 1 or 2 bilateral RAP at diagnosis. The eyes of patients who exhibited different baseline best-corrected visual acuity (BCVA) values in both eyes were assigned to one of two groups-the better (n=13) and worse (n=13) visual acuity groups. The BCVA values in both groups were compared to those at baseline and at 12 months. In addition, the 12-month changes in BCVA were compared between the two groups. The association between the optical coherence tomography findings at diagnosis and the 12-month BCVA was also analyzed.

RESULTS: The values of mean baseline and 12-month BCVA in the better visual acuity group (13 eyes) were 0.48 ± 0.19 and 0.58 ± 0.29 , respectively, and those in the worse visual acuity group (13 eyes) were 0.83 ± 0.20 and 0.90 ± 0.31 . The 12-month changes in BCVA were not significantly different between the two groups ($p=0.786$). Among the six patients with equivalent baseline BCVA in both eyes, four patients (66.7%) exhibited 1 to 2 lines or ≥ 3 lines of difference in BCVA between eyes at 12 months. Eyes without pigment epithelial detachment (PED) at diagnosis exhibited significantly better BCVA at 12 months than eyes with PED ($p=0.021$).

CONCLUSIONS: Better baseline visual acuity was associated with better BCVA at 12 months posttreatment in patients with bilateral RAP. However, equivalent baseline visual acuity in both eyes might not guarantee similar treatment outcomes. In addition, the absence of PED is predictive of better visual outcome.

PMID: 28471100

Int J Retina Vitreous. 2017 May 1;3:6. eCollection 2017.

Aqueous vascular endothelial growth factor and clinical outcomes correlation after single intravitreal injection of bevacizumab in patients with neovascular age-related macular degeneration.

Cabral T, Lima LH, Polido J, Duong J, Okuda É, Oshima A, Serracarbassa P, Regatieri CV, Belfort R Jr.

PURPOSE: To evaluate the concentration of vascular endothelial growth factor (VEGF) in aqueous humor after a single intravitreal injection of bevacizumab (IVB) in eyes with neovascular age-related macular degeneration (AMD).

METHODS: In this prospective interventional case series study, 24 eyes of 24 patients with types 1 and 2 choroidal neovascularization secondary to neovascular AMD were treated with a single intravitreal injection of bevacizumab. Aqueous humor samples were obtained before the intravitreal injection and at one week, one month, and three months follow-up periods. Best-corrected visual acuity (BCVA) and three spectral-domain optical coherence tomography parameters (central retinal thickness, macular volume and macular area) were also analyzed and correlated with VEGF expression at the baseline and each follow-up period.

RESULTS: All of the ninety-six aqueous humor study taps were well tolerated by the study patients without adverse events. Increased VEGF levels (mean $\pm$ SD = 179.7 ± 88.3 pg/mL) were observed in the aqueous humor of all study patients before the intravitreal injection of bevacizumab. At all follow-up periods, compared to baseline, levels of VEGF significantly reduced ($P < 0.0001$), and BCVA significantly improved ($P < 0.005$). The lowest VEGF expression was observed at 1 week, and the greatest BCVA improvement occurred 1 month after treatment. At 1 month, central retinal thickness (CRT), macular volume (MV), and macular area (MA) significantly reduced compared to baseline ($P < 0.0001$, $P = 0.0005$, $P = 0.007$, $P = 0.009$, respectively). At 1 week and 3 months, although without statistical significance ($P > 0.005$), CRT, MV and MA also reduced in comparison to baseline.

CONCLUSIONS: Single intravitreal bevacizumab injection in eyes with neovascular AMD resulted in a substantial decrease of aqueous VEGF levels 1 week after treatment with the greatest improvement of clinical outcomes occurring at 1 month follow-up.

PMID: 28469938 PMCID: PMC5410688

Int Med Case Rep J. 2017 Apr 18;10:139-141.

Taguchi M, Sakurai Y, Kanda T, Takeuchi M.

BACKGROUND: Tuberculosis (TB)-associated uveitis presents periphlebitis, occasionally causing central retinal vascular occlusion (CRVO). Intravitreal injection of ranibizumab (IVR) is an effective treatment for CRVO, which improves macular edema (ME) by reducing vascular permeability and prevents progression of retinal nonperfusion in CRVO. We report a case of CRVO due to TB-associated uveitis, which initially remitted by repeated IVR as an adjunct to anti-TB therapy and systemic corticosteroids, but subsequently led to severe vitreous hemorrhage (VH).

CASE PRESENTATION: A 28-year-old man was referred to our hospital with a 2-week history of uveitis in his right eye. Ophthalmoscopic examination of the right eye revealed fine keratoprecipitates and moderate cell infiltration into the anterior chamber and vitreous. No obvious retinal lesion was observed. Despite initiation of topical corticosteroids, CRVO developed a few weeks later in the right eye. TB-associated uveitis was diagnosed based on a positive tuberculin skin test and interferon- γ release assay in addition to the ocular findings. Anti-TB therapy together with IVR and systemic corticosteroids was initiated. Although fundus findings associated with CRVO gradually improved, CRVO with VH recurred before the fourth IVR. Although IVR was continued, VH progressed to obscure fundus observation. Therefore, vitrectomy and panretinal photocoagulation were performed. After surgery, ocular inflammation was controlled, and anti-TB therapy was continued for 6 months and was suspended.

CONCLUSION: In addition to anti-TB therapy with or without corticosteroids, panretinal photocoagulation for retinal nonperfusion area in TB-associated uveitis should be performed for preventing neovascularization that may cause VH, and this role of panretinal photocoagulation cannot be replaced by anti-VEGF therapy.

PMID: 28458584 PMCID: PMC5403006

Clin Ophthalmol. 2017 Apr 18;11:715-721. eCollection 2017.

Evaluation of contrast sensitivity and other visual function outcomes in neovascular age-related macular degeneration patients after treatment switch to aflibercept from ranibizumab.

Nixon DR, Flinn NA.

PURPOSE: This study evaluated visual function and anatomic and vision-related quality-of-life outcomes in recalcitrant neovascular age-related macular degeneration (AMD) subjects switched to aflibercept (Eylea®) from ranibizumab (Lucentis®).

METHODS: In a single-center study conducted in Barrie, ON, 40 patients with persistent fluid despite previous ranibizumab treatment were switched to aflibercept with 3 consecutive monthly doses. Main outcome measure was mean change from baseline to week 12 in Pelli-Robson contrast sensitivity (CS). Secondary outcomes were mean change in best corrected visual acuity (BCVA), central retinal thickness (CRT), and National Eye Institute 25-Item Visual Function Questionnaire (NEI VFQ-25) score. A two-sided paired t-test was used in the statistical data analysis to compare the means of continuous variables.

RESULTS: Forty-nine eyes (baseline visual acuity [VA] $>6/120$) were evaluated. Ranibizumab injections (mean $\pm$ standard deviation [SD] 28.2 ± 22.1 [range 3-86]) were administered prior to treatment switch. Mean CS improved from 1.32 at baseline to 1.40 log units at week 12. VA was stable throughout. Mean CRT decreased from 354 μ m at baseline to 332 μ m at week 12 (-22 μ m, $P=0.004$). Twenty-six (65%) patients experienced an overall improvement in NEI VFQ-25 score. Interestingly, a correlation was observed between improvement in log CS and CRT change ($P=0.00046$).

CONCLUSION: Contrast sensitivity improved statistically and significantly, and CRT decreased from baseline to week 12 after a switch to aflibercept from ranibizumab. Analysis of CS as an independent outcome end point in neovascular AMD treatment switch studies may provide a more complete

understanding of visual response.

Graefes Arch Clin Exp Ophthalmol. 2017 May 3. [Epub ahead of print]

Ranibizumab versus aflibercept for macular edema due to central retinal vein occlusion: 18-month results in real-life data.

Călugăru D, Călugăru M.

PMID: 28470435

Other treatment & diagnosis

Clin Ophthalmol. 2017 Apr 20;11:745-752. eCollection 2017.

Intersession test-retest variability of 10-2 MAIA microperimetry in fixation-threatening glaucoma.

Wong EN, Morgan WH, Chen FK.

PURPOSE: To determine the intersession test-retest variability (TRV) of CenterVue Macular Integrity Assessment (MAIA) microperimeter in glaucoma patients with fixation-threatening field defects.

METHODS: This is a prospective case-control study of 27 participants consisting of 13 patients with stable primary open-angle glaucoma and 14 control subjects including 5 healthy individuals and 9 retinal patients (5 with non-neovascular age-related macular degeneration and 4 with inherited retinal disease). Each participant underwent three microperimetry tests in one eye at 1-month intervals. Each test used an identical test strategy of 10-2 Cartesian grid and 4-2 staircase algorithm. We investigated TRV by calculating the coefficient of repeatability (CR) for mean sensitivity (MS) and point-wise sensitivity (PWS) for glaucomatous subjects and retinal and normal subjects. 95% confidence intervals (CIs) for CRs were calculated.

RESULTS: There was no significant change in MS, and the median durations of microperimetry sessions were 9'26", 8'52", and 8'46" across the three study visits. The intersession CRs for MS were 1.1, 2.5, and 1.8 dB, and the average CRs for PWS were 3.5, 7.4, and 8.6 dB for healthy controls and retinal and glaucoma patients, respectively. For test loci with 25-34 dB at baseline, CRs for PWS were 8.2 (95% CI: 7.5-8.9) and 4.3 (95% CI: 4.0-4.6) dB for glaucoma and control subjects, respectively.

CONCLUSION: We found differences in TRV of test loci depending on the baseline sensitivity value. Glaucoma patients had significantly worse TRV for loci that had sensitivity values within the normal range at baseline. The estimated CR has implications for sample size calculation in future glaucoma treatment trials using microperimetry as a clinical endpoint.

PMID: 28461736 PMCID: PMC5404802

Ophthalmology. 2017 Apr 26. [Epub ahead of print]

The Evolution of Outer Retinal Tubulation, a Neurodegeneration and Gliosis Prominent in Macular Diseases.

Dolz-Marco R, Litts KM, Tan ACS, Freund KB, Curcio CA.

PURPOSE: To document outer retinal tubulation (ORT) formation in advanced retinal disorders.

DESIGN: Retrospective, observational study.

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PARTICIPANTS: Consecutive cases with retinal diseases showing outer retinal disruption and atrophy of the retinal pigment epithelium (RPE) associated with ORT on spectral-domain (SD) optical coherence tomography (OCT) at the final available visit.

METHODS: Cross-sectional SD OCT scans showing ORT at the last available visit were compared with eye-tracked baseline scans. Only patients showing the formation of ORT over time with absence of ORT at baseline were analyzed.

MAIN OUTCOME MEASURES: Steps in ORT formation based on shapes of the external limiting membrane (ELM) descent (flat, curved, reflected, and scrolled) at the border of outer retinal and RPE atrophy, ORT characteristics (open, closed), and time between steps through a long-term follow-up.

RESULTS: From 170 eyes of 86 patients with ORT, 38 eyes of 30 patients (11 men, 19 women) with a mean age of 78.87 years (range, 56-96 years) met inclusion criteria. Of these 38 eyes, 23 (60%) had geographic atrophy secondary to age-related macular degeneration (AMD) and 2 eyes (5%) had geographic atrophy secondary to pattern dystrophy. Twelve eyes (32%) had neovascular AMD and 1 eye (3%) had neovascularization secondary to pseudoxanthoma elasticum, all showing similar ORT formative steps. Seventy-three different retinal areas (1434 cross-sectional images) were analyzed over a mean follow-up of 69.5 months (range, 21-93 months). At 73 borders, grading of eye-tracked follow-up SD OCT line scans showed a flat ELM descent at least once at 34 borders (47%), a curved ELM at 47 borders (64%), a reflected ELM at 37 borders (51%), and a scrolled ELM at 24 borders (33%). Of 81 ORTs, 73 (90%) were closed and 8 (10%) were open. The mean time for ORT formation was 14.9 months (range, 1.4-71.3 months).

CONCLUSIONS: We propose progressive steps in the development of ORT and analyze the time of progression between these steps. Analyzing the borders of atrophy to determine the origin of ORT provides new insights into the pathophysiology of advanced retinal disease highlighting a role for Müller cells and may inform future therapeutic strategies.

PMID: 28456420

Retina. 2017 Apr 28. [Epub ahead of print]

Imaging of Exudative Age-Related Macular Degeneration: Toward a Shift in the Diagnostic Paradigm?

Cohen SY, Mrejen S.

PMID: 28463905

Pathogenesis

Nat Protoc. 2017 Jun;12(6):1136-1150.

Comprehensive analysis of mouse retinal mononuclear phagocytes.

Lückoff A, Scholz R, Sennlaub F, Xu H, Langmann T.

Abstract: The innate immune system is activated in a number of degenerative and inflammatory retinal disorders such as age-related macular degeneration (AMD). Retinal microglia, choroidal macrophages, and recruited monocytes, collectively termed 'retinal mononuclear phagocytes', are critical determinants of ocular disease outcome. Many publications have described the presence of these cells in mouse models for retinal disease; however, only limited aspects of their behavior have been uncovered, and these have only been uncovered using a single detection method. The workflow presented here describes a comprehensive analysis strategy that allows characterization of retinal mononuclear phagocytes in vivo and in situ. We present standardized working steps for scanning laser ophthalmoscopy of microglia from

MacGreen reporter mice (mice expressing the macrophage colony-stimulating factor receptor GFP transgene throughout the mononuclear phagocyte system), quantitative analysis of Iba1-stained retinal sections and flat mounts, CD11b-based retinal flow cytometry, and qRT-PCR analysis of key microglia markers. The protocol can be completed within 3 d, and we present data from retinas treated with laser-induced choroidal neovascularization (CNV), bright white-light exposure, and Fam161a-associated inherited retinal degeneration. The assays can be applied to any of the existing mouse models for retinal disorders and may be valuable for documenting immune responses in studies for immunomodulatory therapies.

PMID: 28471458

Oncotarget. 2017 Apr 13. [Epub ahead of print]

Dry age-related macular degeneration like pathology in aged 5XFAD mice: Ultrastructure and microarray analysis.

Park SW, Im S, Jun HO, Lee K, Park YJ, Kim JH, Park WJ, Lee YH, Kim JH.

Abstract: Age-related macular degeneration (AMD) is a leading cause of blindness in the elderly. The two types of AMD are: dry and wet AMD. While laser-induced choroidal neovascularization has been used extensively in the studies of wet AMD, there is no established mouse model that fully recapitulates the cardinal features of dry AMD. A lack of appropriate mouse model for dry AMD has hampered the translational research on the pathogenesis of the disease and the development of therapeutic agents. We hypothesized that 5XFAD mice, an animal model for the study of Alzheimer's disease, can be used as a mouse model for dry AMD with regard to the amyloid beta (A β) related pathology. In this study, the ultrastructure of the retinal pigment epithelium (RPE) of 5XFAD mice was analyzed using transmission electron microscopy. Of importance, the aged 5XFAD mice show ultrastructural changes in the RPE and Bruch's membrane (BM) that are compatible with the cardinal features of human dry AMD, including a loss of apical microvilli and basal infolding of the RPE, increased BM thickness, basal laminar and linear deposits, and accumulation of lipofuscin granules and undigested photoreceptor outer segment-laden phagosomes. In microarray-based analysis, the RPE complex of the aged 5XFAD mice shows differential gene expression profiles consistent with dry AMD in the inflammation response, immune reaction pathway, and decreased retinol metabolism. Taken together, we suggest that aged 5XFAD mice can be used as a mouse model of dry AMD to study A β related pathology and develop a new therapeutic approaches.

PMID: 28467791

Mol Vis. 2017 Apr 14;23:228-241. eCollection 2017.

Early AMD-like defects in the RPE and retinal degeneration in aged mice with RPE-specific deletion of Atg5 or Atg7.

Zhang Y, Cross SD, Stanton JB, Marmorstein AD, Le YZ, Marmorstein LY.

PURPOSE: To examine the effects of autophagy deficiency induced by RPE-specific deletion of Atg5 or Atg7 in mice as a function of age.

METHODS: Conditional knockout mice with a floxed allele of Atg5 or Atg7 were crossed with inducible VMD2-rtTA/Cre transgenic mice. VMD2-directed RPE-specific Cre recombinase expression was induced with doxycycline feeding in the resulting mice. Cre-mediated deletion of floxed Atg5 or Atg7 resulted in RPE-specific inactivation of the Atg5 or Atg7 gene. Plastic and thin retinal sections were analyzed with light and electron microscopy for histological changes. Photoreceptor outer segment (POS) thickness in plastic sections was measured using the Adobe Photoshop CS4 extended ruler tool. Autophagic adaptor p62/SQSTM1 and markers for oxidatively damaged lipids, proteins, and DNA were examined with immunofluorescence staining of cryosections. Fluorescence signals were quantified using Image J

software.

RESULTS: Accumulation of p62/SQSTM1 reflecting autophagy deficiency was observed in the RPE of the Atg5 Δ RPE and Atg7 Δ RPE mice. 3-nitrotyrosine, advanced glycation end products (AGEs), and 8-hydroxy-2'-deoxyguanosine (8-OHdG), markers for oxidatively damaged proteins and DNA, were also found to accumulate in the RPE of these mice. We observed retinal degeneration in 35% of the Atg5 Δ RPE mice and 45% of the Atg7 Δ RPE mice at 8 to 24 months old. Degeneration severity and the number of mice with degeneration increased with age. The mean POS thickness of these mice was 25 μ m at 8-12 months, 15 μ m at 13-18 months, and 3 μ m at 19-24 months, compared to 35 μ m, 30 μ m, and 24 μ m in the wild-type mice, respectively. Early age-related macular degeneration (AMD)-like RPE defects were found in all the Atg5 Δ RPE and Atg7 Δ RPE mice 13 months old or older, including vacuoles, uneven RPE thickness, diminished basal infoldings, RPE hypertrophy/hypotrophy, pigmentary irregularities, and necrosis. The severity of the RPE defects increased with age and in the mice with retinal degeneration. RPE atrophy and choroidal neovascularization (CNV) were occasionally observed in the Atg5 Δ RPE and Atg7 Δ RPE mice with advanced age.

CONCLUSIONS: Autophagy deficiency induced by RPE-specific deletion of Atg5 or Atg7 predisposes but does not necessarily drive the development of AMD-like phenotypes or retinal degeneration.

PMID: 28465655 PMCID: PMC5398883

J Exp Med. 2017 May 2. [Epub ahead of print]

Inflammatory signals from photoreceptor modulate pathological retinal angiogenesis via c-Fos.

Sun Y, Lin Z, Liu CH, Gong Y, Liegl R, Fredrick TW, Meng SS, Burnim SB, Wang Z, Akula JD, Pu WT, Chen J, Smith LEH.

Abstract: Pathological neovessels growing into the normally avascular photoreceptors cause vision loss in many eye diseases, such as age-related macular degeneration and macular telangiectasia. Ocular neovascularization is strongly associated with inflammation, but the source of inflammatory signals and the mechanisms by which these signals regulate the disruption of avascular privilege in photoreceptors are unknown. In this study, we found that c-Fos, a master inflammatory regulator, was increased in photoreceptors in a model of pathological blood vessels invading photoreceptors: the very low-density lipoprotein receptor-deficient (Vldlr $^{-/-}$) mouse. Increased c-Fos induced inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor (TNF), leading to activation of signal transducer and activator of transcription 3 (STAT3) and increased TNF α -induced protein 3 (TNFAIP3) in Vldlr $^{-/-}$ photoreceptors. IL-6 activated the STAT3/vascular endothelial growth factor A (VEGFA) pathway directly, and elevated TNFAIP3 suppressed SOCS3 (suppressor of cytokine signaling 3)-activated STAT3/VEGFA indirectly. Inhibition of c-Fos using photoreceptor-specific AAV (adeno-associated virus)-hRK (human rhodopsin kinase)-sh_c-fos or a chemical inhibitor substantially reduced the pathological neovascularization and rescued visual function in Vldlr $^{-/-}$ mice. These findings suggested that the photoreceptor c-Fos controls blood vessel growth into the normally avascular photoreceptor layer through the inflammatory signal-induced STAT3/VEGFA pathway.

PMID: 28465464

Prog Retin Eye Res. 2017 Apr 29. [Epub ahead of print]

Roles of exosomes in the normal and diseased eye.

Klingeborn M, Dismuke WM, Rickman CB, Stamer WD.

Abstract: Exosomes are nanometer-sized vesicles that are released by cells in a controlled fashion and mediate a plethora of extra- and intercellular activities. Some key functions of exosomes include cell-cell communication, immune modulation, extracellular matrix turnover, stem cell division/differentiation,

neovascularization and cellular waste removal. While much is known about their role in cancer, exosome function in the many specialized tissues of the eye is just beginning to undergo rigorous study. Here we review current knowledge of exosome function in the visual system in the context of larger bodies of data from other fields, in both health and disease. Additionally, we discuss recent advances in the exosome field including use of exosomes as a therapeutic vehicle, exosomes as a source of biomarkers for disease, plus current standards for isolation and validation of exosome populations. Finally, we use this foundational information about exosomes in the eye as a platform to identify areas of opportunity for future research studies.

PMID: 28465248

ACS Nano. 2017 May 5. [Epub ahead of print]

Glycol Chitosan Engineered Autoregenerative Antioxidant Significantly Attenuates Pathological Damages in Models of Age-Related Macular Degeneration.

Mitra RN, Gao R, Zheng M, Wu MJ, Voinov MA, Smirnov AI, Smirnova TI, Wang K, Chavala S, Han Z.

Abstract: Age-related macular degeneration (AMD) is the foremost cause of irreversible blindness in people over the age of 65 especially in developing countries. Therefore, an exploration of effective and alternative therapeutic interventions is an unmet medical need. It has been established that oxidative stress plays a key role in the pathogenesis of AMD, and hence, neutralizing oxidative stress is an effective therapeutic strategy for treatment of this serious disorder. Owing to autoregenerative properties, nanoceria has been widely used as a nonenzymatic antioxidant in the treatment of oxidative stress related disorders. Yet, its potential clinical implementation has been greatly hampered by its poor water solubility and lack of reliable tracking methodologies/processes and hence poor absorption, distribution, and targeted delivery. The water solubility and surface engineering of a drug with biocompatible motifs are fundamental to pharmaceutical products and precision medicine. Here, we report an engineered water-soluble, biocompatible, trackable nanoceria with enriched antioxidant activity to scavenge intracellular reactive oxygen species (ROS). Experimental studies with in vitro and in vivo models demonstrated that this antioxidant is autoregenerative and more active in inhibiting laser-induced choroidal neovascularization by decreasing ROS-induced pro-angiogenic vascular endothelial growth factor (VEGF) expression, cumulative oxidative damage, and recruitment of endothelial precursor cells without exhibiting any toxicity. This advanced formulation may offer a superior therapeutic effect to deal with oxidative stress induced pathogenesises, such as AMD.

PMID: 28463509

Proc Natl Acad Sci U S A. 2017 May 1. [Epub ahead of print]

MEF2D haploinsufficiency downregulates the NRF2 pathway and renders photoreceptors susceptible to light-induced oxidative stress.

Nagar S, Noveral SM, Trudler D, Lopez KM, McKercher SR, Han X, Yates JR 3rd, Piña-Crespo JC, Nakanishi N, Satoh T, Okamoto SI, Lipton SA.

Abstract: Gaining mechanistic insight into interaction between causative factors of complex multifactorial diseases involving photoreceptor damage might aid in devising effective therapies. Oxidative stress is one of the potential unifying mechanisms for interplay between genetic and environmental factors that contribute to photoreceptor pathology. Interestingly, the transcription factor myocyte enhancer factor 2d (MEF2D) is known to be important in photoreceptor survival, as knockout of this transcription factor results in loss of photoreceptors in mice. Here, using a mild light-induced retinal degeneration model, we show that the diminished MEF2D transcriptional activity in Mef2d^{+/-} retina is further reduced under photostimulation-induced oxidative stress. Reactive oxygen species cause an aberrant redox modification on MEF2D, consequently inhibiting transcription of its downstream target, nuclear factor (erythroid-derived 2)-like 2 (NRF2). NRF2 is a master regulator of phase II antiinflammatory and antioxidant gene expression. In the

Mef2d heterozygous mouse retina, NRF2 is not up-regulated to a normal degree in the face of light-induced oxidative stress, contributing to accelerated photoreceptor cell death. Furthermore, to combat this injury, we found that activation of the endogenous NRF2 pathway using proelectrophilic drugs rescues photoreceptors from photo-induced oxidative stress and may therefore represent a viable treatment for oxidative stress-induced photoreceptor degeneration, which is thought to contribute to some forms of retinitis pigmentosa and age-related macular degeneration.

PMID: 28461502

Front Pharmacol. 2017 Apr 18;8:207. eCollection 2017.

CCR3 Is Associated with the Death of a Photoreceptor Cell-line Induced by Light Exposure.

Kuse Y, Tsuruma K, Kanno Y, Shimazawa M, Hara H.

Abstract: The C-C chemokine receptor type 3 (CCR3) is the receptor for eotaxins (CCL-11, 24, 26), RANTES (CCL-5) and MCP-3 (CCL-7). It was reported that an inhibition of CCR3 by antagonists or antibodies reduces the degree of laser-induced choroidal neovascularization in mice, a model for wet age-related macular degeneration (AMD). Although several chemokine receptors have the potential of reducing the degree of the chronic inflammation in experimental dry AMD, the association of CCR3 remains unknown. The purpose of this study was to determine the role played by CCR3 in the death of 661W cells which are cells of a murine photoreceptor-derived cell line as an in vitro model of dry AMD. The expression of CCR3 was increased in the 661W cells after light exposure. Inhibition of CCR3 reduced the rate of cell death induced by light exposure. A blockade of CCR3 signaling by CCR3 silencing and two kinds of CCR3 antagonists, SB 328437 and SB 297006, reduced the rate of light-induced cell death. In addition, CCR3 inhibition decreased the level of reactive oxygen species and the activation of caspase-3/7 induced by light exposure. These findings indicated that the CCR3 blockade should be considered for the treatment of the dry AMD.

PMID: 28458639 PMCID: PMC5394117

Epidemiology

Klin Monbl Augenheilkd. 2017 Apr;234(4):478-482. Epub 2017 May 3.

Complement Component C3 Variant (R102G) and the Risk of Neovascular Age-Related Macular Degeneration in a Tunisian Population.

Habibi I, Sfar I, Kort F, Bouraoui R, Chebil A, Limaiem R, Ayed S, Ben Abdallah T, El Matri L, Gorgi Y.

Purpose: To explore the association between the polymorphism (S/F) p.R102G in the complement component 3 (C3) gene and age-related macular degeneration (AMD) in a Tunisian population.

Methods: The molecular study was performed by polymerase chain reaction using sequence-specific primers (PCR-SSP) in 207 control subjects free of any eye disease (fundus normal) and 145 patients with exudative AMD. The CH50 activity and quantification of C3 and C4 have been made by technical home method and nephelometry, respectively.

Results: The prevalence of C3 GG genotype polymorphism was significantly higher in AMD patients compared to controls (OR: 2.41, IC 95% [1.90-3.05], $p = 0.0007$). However, no correlation was found between this allelic variant and the type of neovascularization. Similarly, there is no association between this polymorphism and the presence of functional and/or quantitative hypocomplementemia.

Conclusions: The C3 GG genotype of the gene could be a susceptibility factor for AMD in the Tunisian population. However, it does not seem to influence the clinical profile of the disease.

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Genetics

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Mendelian Randomization Implicates High-Density Lipoprotein Cholesterol-Associated Mechanisms in Etiology of Age-Related Macular Degeneration.

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PURPOSE: Undertake a systematic investigation into associations between genetic predictors of lipid fractions and age-related macular degeneration (AMD) risk.

DESIGN: Two-sample Mendelian randomization investigation using published data.

PARTICIPANTS: A total of 33 526 individuals (16 144 cases, 17 832 controls) predominantly of European ancestry from the International Age-related Macular Degeneration Genomics Consortium.

METHODS: We consider 185 variants previously demonstrated to be associated with at least 1 of low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, or triglycerides at a genome-wide level of significance, and test their associations with AMD. We particularly focus on variants in gene regions that are proxies for specific pharmacologic agents for lipid therapy. We then conduct a 2-sample Mendelian randomization investigation to assess the causal roles of LDL-cholesterol, HDL-cholesterol, and triglycerides on AMD risk. We also conduct parallel investigations for coronary artery disease (CAD) (viewed as a positive control) and Alzheimer's disease (a negative control) for comparison.

MAIN OUTCOME MEASURES: Diagnosis of AMD.

RESULTS: We find evidence that HDL-cholesterol is a causal risk factor for AMD, with an odds ratio (OR) estimate of 1.22 (95% confidence interval [CI], 1.03-1.44) per 1 standard deviation increase in HDL-cholesterol. No causal effect of LDL-cholesterol or triglycerides was found. Variants in the CETP gene region associated with increased circulating HDL-cholesterol also associate with increased AMD risk, although variants in the LIPC gene region that increase circulating HDL-cholesterol have the opposite direction of association with AMD risk. Parallel analyses suggest that lipids have a greater role for AMD compared with Alzheimer's disease, but a lesser role than for CAD.

CONCLUSIONS: Some genetic evidence suggests that HDL-cholesterol is a causal risk factor for AMD risk and that increasing HDL-cholesterol (particularly via CETP inhibition) will increase AMD risk.

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Diet, lifestyle & low vision

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Dietary intake of α -linolenic acid and risk of age-related macular degeneration.

Wu J, Cho E, Giovannucci EL, Rosner BA, Sastry SM, Schaumberg DA, Willett WC.

Background: The relation between α -linolenic acid (ALA), a plant-derived omega-3 (n-3) fatty acid, and age-related macular degeneration (AMD) is unclear. European researchers reported that $\leq 40\%$ of ALA can be present as trans forms.

Objective: We aimed to evaluate the associations between intake of ALA and intermediate and advanced AMD.

Design: Seventy-five thousand eight hundred eighty-nine women from the Nurses' Health Study and 38,961 men from Health Professionals Follow-Up Study were followed up from 1984 to 2012 and from 1986- to 2010, respectively. We assessed dietary intake by a validated food-frequency questionnaire at baseline and

every 4 y thereafter. One thousand five hundred eighty-nine incident intermediate and 1356 advanced AMD cases (primarily neovascular AMD) were confirmed by medical record review.

Results: The multivariable-adjusted HR for intermediate AMD comparing ALA intake at the top quintile to the bottom quintile was 1.28 (95% CI: 1.05, 1.56; P-trend = 0.01) in the analyses combining 2 cohorts. The HR in each cohort was in the positive direction but reached statistical significance only in the women. However, the positive association was apparent only in the pre-2002 era in each cohort and not afterward (P-time interaction = 0.003). ALA intake was not associated with advanced AMD in either time period. Using gas-liquid chromatography, we identified both cis ALA (mean $\pm$ SD: 0.13% $\pm$ 0.04%) and trans ALA isomers (0.05% $\pm$ 0.01%) in 395 erythrocyte samples collected in 1989-1990. In stepwise regression models, mayonnaise was the leading predictor of erythrocyte concentrations of cis ALA and one isomer of trans ALA. We also found trans ALA in mayonnaise samples.

Conclusions: A high intake of ALA was associated with an increased risk of intermediate AMD before 2002 but not afterward. The period before 2002 coincides with the same time period when trans ALA was found in food and participants' blood; this finding deserves further study.

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One-year daily consumption of buttermilk drink containing lutein-enriched egg-yolks does not affect endothelial function in fasting and postprandial state.

van der Made SM, Berendschot TTJM, Kijlstra A, Plat J.

Abstract: Previous results have shown that one-year daily consumption of a lutein-enriched egg yolk containing dairy drink did not significantly affect fasting serum lipid and lipoprotein concentrations in adults with early signs of macular degeneration. The current study further substantiates these findings with parameters reflecting endothelial function. Additionally, we extend our observations from the fasting to the postprandial situation. Subjects participated in a 1-y randomized placebo-controlled dietary intervention trial. 52 subjects were included in the active (Egg) group and 49 in the control (Con) group. Changes in postprandial biochemistry (triacylglycerol (TAG), glucose and non-esterified fatty acids (NEFA)) following a mixed meal and flow-mediated dilation (FMD) analyses were evaluated at the start and after one year intervention. Postprandial glycemic and lipemic responses before the intervention as well as the differences in postprandial responses after one-year intervention were comparable between the Egg and the Con group. Fasting FMD was comparable between the groups before the intervention started and at the end of intervention. Additionally, the change in FMD following a mixed meal was comparable between the groups. To conclude, one-year consumption of a lutein-enriched egg yolk incorporated in a dairy drink has no effect on postprandial lipid and glucose metabolism or endothelial function.

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Oral fast-dissolving films containing lutein nanocrystals for improved bioavailability: formulation development, in vitro and in vivo evaluation.

Liu C, Chang D, Zhang X, Sui H, Kong Y, Zhu R, Wang W.

Abstract: Lutein is widely used as diet supplement for prevention of age-related macular degeneration. However, the application and efficacy of lutein in food and nutritional products has been hampered due to its poor solubility and low oral bioavailability. This study aimed to develop and evaluate the formulation of oral fast-dissolving film (OFDF) containing lutein nanocrystals for enhanced bioavailability and compliance. Lutein nanocrystals were prepared by anti-solvent precipitation method and then encapsulated into the films by solvent casting method. The formulation of OFDF was optimized by Box-Behnken Design (BBD) as

follows: HPMC 2.05% (w/v), PEG 400 1.03% (w/v), Cremophor EL 0.43% (w/v). The obtained films exhibited uniform thickness of $35.64 \pm 1.64 \mu\text{m}$ and drug content of $0.230 \pm 0.003 \text{ mg/cm}^2$ and disintegrated rapidly in $29 \pm 8 \text{ s}$. The nanocrystal-loaded films with reconstituted particle size of 377.9 nm showed better folding endurance and faster release rate in vitro than the conventional OFDFs with raw lutein. The microscope images, thermograms, and diffractograms indicated that lutein nanocrystals were highly dispersed into the films. After administrated to SD rats, t_{max} was decreased from 3 h for oral solution formulation to less than 0.8 h for OFDF formulations, and C_{max} increased from 150 ng/mL for solution to 350 ng/mL for conventional OFDF or 830 ng/mL for nanocrystal OFDF. The AUC 0-24h of conventional or nanocrystal OFDF was 1.37 or 2.08-fold higher than that of the oral solution, respectively. These results suggested that drug nanocrystal-loaded OFDF can be applied as a promising approach for enhanced bioavailability of poor soluble drugs like lutein.

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Disruption of Angiogenesis by Anthocyanin-Rich Extracts of Hibiscus sabdariffa.

Joshua M, Okere C, Sylvester O, Yahaya M, Precious O, Dluya T, Um JY, Neksumi M, Boyd J, Vincent-Tyndall J, Choo DW, Gutsaeva DR, Jahng WJ.

Abstract: Abnormal vessel formations contribute to the progression of specific angiogenic diseases including age-related macular degeneration. Adequate vessel growth and maintenance represent the coordinated process of endothelial cell proliferation, matrix remodeling, and differentiation. However, the molecular mechanism of the proper balance between angiogenic activators and inhibitors remains elusive. In addition, quantitative analysis of vessel formation has been challenging due to complex angiogenic morphology. We hypothesized that conjugated double bond containing-natural products, including anthocyanin extracts from Hibiscus sabdariffa, may control the proper angiogenesis. The current study was designed to determine whether natural molecules from African plant library modulate angiogenesis. Further, we questioned how the proper balance of anti- or pro-angiogenic signaling can be obtained in the vascular microenvironment by treating anthocyanin or fatty acids using chick chorioallantoic membrane angiogenesis model in ovo. The angiogenic morphology was analyzed systematically by measuring twenty one angiogenic indexes using Angiogenic Analyzer software. Chick chorioallantoic model demonstrated that anthocyanin-rich extracts inhibited angiogenesis in time- and concentration-dependent manner. Molecular modeling analysis proposed that hibiscetin as a component in Hibiscus may bind to the active site of vascular endothelial growth factor receptor 2 (VEGFR2) with $\Delta G = -8.42 \text{ kcal/mol}$ of binding energy. Our results provided the evidence that anthocyanin is an angiogenic modulator that can be used to treat uncontrolled neovascular-related diseases, including age-related macular degeneration.

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