Morphological and functional evaluation of inflammatory processes in eye surface diseases and age-related macular degeneration

PhD Thesis

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Background

Dry eye syndrome and Age-Related Macular Degeneration (AMD) are two different diseases, however recently published results suggest similar pathophysiological features due to chronic inflammatory processes. Calcitonin Gene Related Peptide (CGRP) and Substance P (SP) containing sensory nerve endings were identified in the conjunctiva, in the accessorry lacrimal glands, and in the uveal tract. SP and CGRP are released from peripheral nerve terminals as the principle mediators of neurogenic inflammation, which is a characteristic feature of the activation of capsaicin-sensitive subgroup of afferent nerve fibers. Muscarinic, adrenergic and Vasoactive Intestinal Peptide (VIP) containing nerve terminals were detected around goblet cells also. Direct functional effect of these neural pathways on conjunctival mucin secretion has been obtained only in case of parasympathetic nerves. VIP has been found to stimulate mucin secretion from cultured goblet cells in vitro.

Although the retina has no sensory innervation, SP and CGRP containing nerve endings have been observed in the choroid and in the retina in various species including humans. In general intrinsic SP acts on its specific receptor presumably in a paracrine fashion as an excitatory neurotransmitter raising the spontaneous activity level of both amacrine and ganglion cells, suggesting that substance P influences functions of multiple retinal circuits.

The current pathophysiologic concept on AMD assigns a primary role to the age-related, cumulative oxidative damage to the RPE due to an imbalance between generation and elimination of reactive oxygen species, resulting in impaired mitochondrial metabolism. Epidemiological studies supported this concept showing low antioxidant diet as a risk factor for AMD, while higher antioxidant uptake seemed to attenuate risk for AMD. Based on this concept several single antioxidants or their combinations were suggested for treating AMD. A previous pilot study on the efficacy of these mitotropic compounds (combination of acetyl- *L* -carnitine, n-3 fatty acids, and coenzyme Q10) reported a marked improvement of retinal functions in a small group of patients affected by early AMD.

Purpose

Based on the previous assumptions the aim of our study was:

1. to present morphological and functional evidence to evaluate whether tear secretion and neuropeptide expression in the retina is influenced by stimulation of sensory nerve endings of the eye,
2. to evaluate morphological alterations of retinal pigment epithelial cells in AMD
3. as well as to examine the effect of mitochondriotropic dietary supplements on the clinical course of early AMD in a prospective, controlled clinical trial.

Methods

The effect of sensory nerve stimulation on tear secretion and expression of retinal neurotransmitters in a rat model

Experiments were carried out on male Wistar rats weighing 250-350 g with the previously described technique of electrical stimulation of rat trigeminal ganglion. The left trigeminal ganglion was electrically stimulated with increasing number of electrical pulses (300-2400 pulses) by adjusting the parameters between 0.5 and 2 Hz for 10 to 20 min at 15 V. Effects of inadequate electrode position or incidental lesion of trigeminal ganglion was examined by placing the stimulating electrode in false position or no stimulation at a correct position. At the end of the experiments the animals were killed by overdose of thiopentone-sodium.

Measurement of tear secretion

Tear samples were collected by placing a capillary tube into the tear sac with extreme care not to evoke reflex tearing by touching the eye surface. The amount of collected tear was measured in each capillary tube and recorded in mm.

Goblet cell density

At the end of the experiments upper and lower lids were removed immediately, and fixed in 10% formaldehyde followed by staining with combined hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) staining was performed to visualize the mucus; slides were examined by

standard light microscopy. Goblet cell density (GCD) was determined by quantification of the mucus containing goblet cell distribution in the conjunctiva.

Tear protein analysis

Secreted volume was measured and tear samples were diluted for a standard Bradford protein analysis. Total protein excretion was calculated from dilution, concentration, and volume data. 3 μg of protein were analyzed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein bands were visualized by common Coomassie Brilliant Blue R250 staining combined with a silver intensification method. Polyclonal anti rat immunoglobulin A and anti hen egg white lysozyme antibodies (IgA: goat 1:50, Sigma; lyozyme: rabbit, 1:50, Acris) were used for primary immuno reaction. The secondary antibodies were horseradish peroxidase (HRP) conjugated immunoglobulins. The immune reaction was visualized either by peroxidase-diamino-benzidine staining with silver post intensification or enhanced chemiluminescence.

Pretreatments

Prior to electrical stimulation of the trigeminal ganglion rats received either atropine (1 mg/kg iv. 5 min before the stimulation, n=8) to block parasympathetic pathways; or guanethidine (8 mg/kg ip., 1 hour before stimulation, n=8) to exclude the involvement of adrenergic response; or hexamethonium (10 mg/kg iv., n=8) to block synaptic transmission in ganglia. Capsaicin (5 μl of 1% solution, n=8) applied as an eye drop once a day for 3 days in both eyes to deplete neurotransmitters from sensory nerve endings; or SR140333 (240 nmol/kg iv. 15 min before stimulation, n=8) to antagonize neurokinin 1 (NK1) receptors.

Histological examinations of the retina

The following molecules were investigated: neurotransmitters SP (anti-SP, Chemicon International), CGRP (anti-CGRP, a Santa Cruz), VIP (anti-VIP, Chemicon International), and nNOS (anti-nNOS, Chemicon International). Eyeballs were removed after the sacrifice and processed for immunohistochemistry. Sections of retina obtained from the treated and untreated eyeballs were exposed to the primary/secondary antibodies the developed dark-brown (intense=++), yellow-brown (slight=+) questionable (±) imunostaining or no staining at all (-) was recorded. Immunostaining was evaluated in four localization of the retina. The outermost

layer was the retinal pigment epithelium (RPE) and photoreceptor outer segment (POS) complex. Further localization for evaluating immonostaining were the outer and inner granular layers and ganglion cell layer.

Morphological examainations of human age-related macular degeneration

Sixty-five human eyes, ages 2–87 years, were selected for these electron microscopic studies. Thirty-one of them were affected by early AMD and 31 non-affected eyes were used for age- and sex-matched controls for both qualitative and quantitative morphomertic studies. The selection criteria for early AMD was based on the presence of drusen and/or basement membrane thickening of the RPE, while for controls no drusen or basement membrane thickening of RPE, as observed by electron microscopy. Late form of AMD (geographic atrophy and/or choroidal neovacuslarization) were excluded from these studies. All these human eyes were surgically removed because of malignant tumors or severe ocular trauma, neither of which affected the posterior pole of the eyeball.

Electron microscopy

Small pieces of the retina and choroid were dissected at the posterior pole in less than 2 min after the removal of the eyeball, processed for standard electron microscopical fixation protocol and studied with an electron microscope.

Assesment of macular functions during treatment of age-related macular degeneration with mitochondriotropic compounds

Study Design

The study was designed as a randomized, double-blind, placebo-controlled, single-center protocol. Treatment of patients was carried out in the Department of Ophthalmology, University of Pecs (Pecs, Hungary). A total of 106 patients were enrolled and analyzed. Patients had to have a diagnosis of early bilateral AMD with best corrected visual acuity between 0.8 and 0.4 Snellen decimal chart (in the most affected eyes), be 55–70 years of age at enrollment. Patients were randomly assigned to receive either Phototrop® or placebo for 12 months. Medication consisted

of 2 oral capsules per day, containing either: 100 mg of Acetyl-L-Carnitine, 530 mg of n-3 Fatty Acids, and 10 mg of Coenzyme-Q10 or an equal quantity of soy oil.

Visual Functions

Primary efficacy variable was visual field mean defect (VFMD). Secondary efficacy variables were visual acuity as measured by the Snellen visual acuity chart and by the chart of the Early Treatment Diabetic Retinopathy Study (ETDRS), as well as foveal sensitivity as measured by perimetry

Fundus Alterations

Fundus photographs of both eyes were taken with a retinal camera from the central 35° area centered to the fovea. No image manipulation was used before or during grading. A 6000 μm diameter circle concentric with the center of the macula was superimposed onto the photographs. The number of different size of drusens (diamters of <63, <125, <250, and >250 μm) were counted in all cases, and the total area of drusen-covered area was also dtermined.

Statistical methods

Data were expressed as mean±SEM in the animal research and morphological studies and mean±SD int he clinical study and were analyzed using Statistica 6.0 software (StatSoft Inc. Tulsa, OK, USA). Correlation analysis was measured with regression analysis by determining the 'p' and 'r' value. The normality of the data was checked with the Shapiro-Wilk's W test, Mann-Whitney U test was used to assess the significance between two groups. The effect of pretreatments during the animal research was analyzed with factorial-ANOVA combined with a post-hoc Dunnett test with categorical predictor variables of treatment vs. control and different types of pretreatments. The 2x2 tables were analyzed with Fisher's exact test. In all cases $p<0.05$ was considered statistically significant.

Results

Effect of sensory nerve stimulation on tear secretion

Tear secretion

In animals without any intervention secreted tear volume expressed in mm rise in the capillary tube showed almost identical amounts (3.75±0.37 mm) as observed in animals stimulated at an inadequate electrode position (3.25±0.37mm) or in non-stimulated animals at a proper electrode position (4.25±0.31mm, $p>0.05$). Since the contralateral (unstimulated) side of stimulated animals (4.00±0.38 mm) showed no difference ($p>0.05$) in secreted tear volume compare to animals without any intervention, the control samples were taken from the contralateral side of stimulated animals. Electrical stimulation of the trigeminal ganglion resulted in an increase of tear secretion on the stimulated side, demonstrated by clearly visible accumulation of tear on the eye surface and increased tear volume in capillary tubes. More pronounced effects were observed on lacrimation and goblet cell density after stimulation of the trigeminal ganglion with increasing number of electrical pulses. Secreted tear increased from 10.6±0.93 mm to 17.6±0.93 mm and GCD decreased from 57.4±1.86 to 45±1.81 ($r=0.69$, $p=0.0007$ and $r=-0.72$, $p=0.0003$, respectively, difference between r: $p<0.0001$).

In the absence of pretreatments, tear secretion evoked by electrical stimulation increased to 18.00±0.86 mm on the stimulated side vs. 3.75±0.53 mm at the control side ($p<0.0001$). Atropine, guanethidine and hexamethonium pretreatments had no inhibiting effect on stimulated tear secretion (atropine: 19.50±0.68 mm, guanethidine: 16.63±1.24 mm, hexamethonium: 17.13±1.11 mm, $p>0.05$). Pretreatment with capsaicin or the NK1 receptor antagonist SR140333 inhibited the increase in tear secretion on the stimulated side significantly (4.39±0.65 mm and 8.75±1.09 mm, respectively $p<0.0001$), however tear secretion remained significantly elevated after SR140333 pretreatment compared to the control side.

Goblet cell density (mucus secretion)

In non-pretreated animals goblet cell density was 48.25±0.99 on the stimulated and 68.63±2.15 on the control side ($p<0.001$). Atropine pretreatment had no significant inhibition on GCD decrease (47.38±1.50, $p>0.05$) but capsaicin desensitization almost prevented the electrical stimulation induced reduction of GCD (63.38±1.58, $p<0.001$).*Tear protein concentration*

In non-pretreated rats protein concentration was 10.23±0.89 μg/μl at the stimulated and 18.40±1.52 μg/μl ($p<0.005$) at the non-stimulated side. Since total tear volume on the stimulated side increased (8.13±1.05 μl at the stimulated side vs. 1.76±0.27 μl at the non-stimulated side $p<0.005$) a remarkable increase of total secreted protein (83.17±11.03 μg vs. 32.35±4.28 μg, $p<0.005$) was observed. Without any pretreatment no apparent difference was observed in the pattern of protein bands between the stimulated and non-stimulated sides by SDS gel electrophoresis of the collected tear. Using of immunoblotting technique IgA and lysozyme were detected selectively. Analysis of equal amount of tear protein with luminol chemiluminescence indicated similar volume of these components on both sides (lysozyme: 2711±347 vs. 2802±331 counts, $p>0.05$, and IgA: 2718±369 vs. 2383±281 counts, $p>0.05$).

Effect of antidromic nerve stimulation on expression of neurotransmitters in the retina

In the control eyes questionable or very mild immunostaining for SP, VIP and nNOS was found in the outer and inner granular layers and for SP also in the ganglion cell layer, but for CGRP it was negative in all layers. In the electrically stimulated rats immunostaining for SP, CGRP, VIP and nNOS in all four examined layers were markedly increased in comparison with the control eyes.

Electron microscopic findings in age-related macular degenereation

Age-related changes of the RPE and Bruch'scmembrane

Mitochondria in young RPE were numerous, mostly bacillus-like shaped, and oriented parallel with the apical-basal axis. They were typically rich in well preserved crista. In normal aged eyes, mitochondria of the RPE clearly decreased in number. They were variable in size, usually oval shape or rarely bacillus-like and without any preferential orientation. Most of them had normal appearing cristae and matrices. However, in some instances, there was disorganization of cristae, including focal to complete loss, in association with decreased electron density of matrix. Peroxisomes were usually localized in the basal region of the cytoplasm, and occasionally next to the basal and basolateral cell membrane. In normal aged eyes, peroxisomes were more numerous and more variable in size, electron-density and distribution than in young

eyes. Furthermore, in young eyes they were dispersed randomly in the cytoplasm of the RPE, while in aged eyes they formed small groups containing four to five peroxisomes. Lipofuscin granules and residual bodies were exceptionally rare in early age, but they clearly increased in number and size with time. In aged adults they were abundantly distributed in the cytoplasm of the RPE. Numerous melanolipofuscin granules were also present, formed by fusion of melanosomes and lipofuscin. These organelles were located in the apical half of the RPE in normal aged eyes. With normal aging, there was an increase in electron density of Bruch's membrane. Moreover, both inner and outer collagenous layers contained electron-dense granular and vesicular material.

Changes of the RPE and Bruch's membrane in AMD

Mitochondria of the RPE in AMD eyes appeared to decrease in number and size compared to the controls. They were often round or oval form, and focal or even complete losses of cristae were associated with more extensive decreases in matrix density. In some instances, either bleb formation or interruption of the mitochondrial internal and external membranes was observed. Although, no abnormalities of mitochondria specific for AMD were found, all these mitochondrial alterations were apparently more marked and more extensive in AMD compared to normal aging. The distribution of peroxisomes in AMD eyes was highly variable within each RPEcell. Occasionally they were located in the apical cytoplasm among the lipofuscin granules. Rarely, peroxisomes were in close topographic correlation with mitochondria and the nucleus of the RPE cells. Lipofuscin granules and residual bodies in AMD specimens showed similar morphology and distribution as in control eyes. Bruch's membrane showed characteristic differences in AMD compared to normal aged eyes. In addition to the age-related increase in thickness and electron-density of collagenous layers, AMD specimens usually showed multiplex, focal thickenings of the inner collagenous layer known as hard or soft drusen. Both of these elevated the RPE, but the hard dusen were more circumscribed and structurally more dense and homogeneous. However, some hard drusen had membrane fragments that were similar to, but less evident than, fragments in soft drusen. Besides drusen, thickenings of the RPE basement membrane due to basal laminar deposits were also present. In most cases they were focal, wart-like depositions of filamentous material in which some electron-dense areas, possibly composed of lipids, were present.

Morphometric studies

The total number of mitochondria decreased significantly in both aged (r^2=0.455; p<0.001) and AMD (r^2=0.778; p<0.001) groups. The decrease in AMD group was more severe, and the difference was statistically significant (p=0.038). The area of mitochondria also decreased significantly with age in both control (r^2=0.743; p<0.001) and AMD (r^2=0.919; p<0.001) groups. The decrease in AMD group was again more severe, and the difference between control and AMD groups was statistically significant (p=0.019). Comparison of the regression lines showed that the area of mitochondria was similar in both ages at 40–49, but it decreased more rapidly in AMD compared to normal aging. The number of well-defined mitochondrial cristae was also counted, and it showed a significant decrease in both control (r^2=0.861; p<0.001) and AMD (r^2=0.918; p<0.001) groups. However, the difference between controls and AMD was not significant (p=0.28). Morphometrie analysis showed a significant increase in the number of peroxisomes in both control (r^2=0.207; p<0.01) and AMD (r^2=0.608; p<0.001) groups. Moreover, the difference between eontrols and AMD was statistically significant (p=0.044). There was a significant increase of lipofuscin granules in both control (r^2=0.432; p<0.001) and AMD (r^2=0.535; p<0.001) groups. However, the difference between the two groups was not statistically significant (p=0.61).

Effect of mitochondriotropic compounds on the course of age-related macula degeneration

Functional results

When VFMD of the *most affected eyes* was considered in the treated group there was an improvement after 12 months from baseline (0.77±2.57 dB). In the placebo group, there was a deterioration after 12 months (–0.31±3.70 dB), however, none of these changes proved to be significant between the treated and the placebo group. We also performed the same set of analyses for the *less affected (fellow) eyes*. In the treated group tbere was an improvement after 12 months (0.53±2.36 dB) as compared to the baseline. In the placebo group there was a worsening at the end of the study (–0.39±1.52 dB), with a statistically significant difference between the two groups at the end of the study (p=0.004) in favor of the treated group. During the examination ±2.0 dB was applied as a range for long-term fluctuation (i.e. 'unchanged'). When the *most affected eyes* were considered, in the treated group 47 out of 48 cases (98%) were

'improved' or 'unchanged', and 1 (2%) 'deteriorated'. In the placebo group 44 cases out of 53 (83%) were 'improved' or 'unchanged' and 9 (17%) 'deteriorated'. The difference between treated and placebo groups was significant (p=0.006, odds ratio:10.93). Comparison of changes in VFMD of the *less affected eyes* showed similar results. In the treated group all eyes were 'improved' or 'unchanged' (100%), and no eyes 'deteriorated'. In the placebo group 40 cases from 45 (89%) were 'improved' or 'unchanged' and 5 (11%) 'deteriorated'. The difference between treated and placebo groups was significant (p=0.031, odds ratio:11.81).

Foveal sensitivity of the *most affected eyes* in the treated group were 'improved' or 'unchanged' in 33 (69%) eyes and 'deteriorated' in 15 (31%) eyes. In the placebo group 26 (49%) cases were 'improved' or 'unchanged' and 27 (51%) 'deteriorated'. The difference between treated and placebo groups was statistically significant (p=0.035, odds ratio:2.29).

Comparing mean change in the visual acuity by Snellen chart between baseline and 12 months, in the treated group 37 (77%) cases were 'improved' or 'unchanged' and 11 (23%) cases were 'deteriorated'. In the placebo group 29 (55%) eyes out of 53 were 'improved' or 'unchanged' and 24 (44%) 'deteriorated'. The difference between the treated and placebo groups was statistically significant (p=0.015, odds ratio:2.78). Change in visual acuity expressed in ETDRS also resulted in a statistically significant difference. In the treated group 36 (75%) eyes were 'improved' or 'unchanged', and 12 (25%) eyes 'deteriorated'. In the placebo group 29 (55%) were 'improved' or 'unchanged' and 24 (45%) 'deteriorated' (p=0.027, odds ratio:2.48).

Morphological changes

Comparisons of changes in the drusen-covered area of *most affected eyes* (ratio of drusen area at 12 months to drusen area at screening) showed a statistically significant difference between treated and placebo groups (0.85±0.39 vs. 1.11±0.65, p=0.045). When the *less affected eyes* were compared, the difference in the drusen-covered area between screening and 12 months was statistically significant (p=0.0003) in the treated group, but not in the placebo group (p=0.587), and comparisons of changes in drusen-covered area showed a statistically significant difference between treated and placebo groups (0.77±0.43 vs. 1.13±0.77, p=0.017).

Conclusions

The present results revealed that electrical stimulation of trigeminal ganglion elicits tear secretion by activation of conjunctival goblet cell, exorbital and accessory lacrimal gland. These data suggest that the observed effects are due to antidromic stimulation of trigeminal sensory nerve endings in the eye. Ganglionic transmission blockade by hexamethonium pretreatment didn't influence the increased tear secretion following stimulation, providing direct evidence for the exclusive involvement of trigeminal sensory pathways. Either atropine or guanethidine pretreatment didn't show statistically significant impact on tear flow proposing that neither muscarinic nor noradrenergic mediation plays role in this process. SDS gel electrophoresis showed similar protein patterns on both sides suggesting a proportional increase in tear protein secretion, which is confirmed by luminol chemiluminescence in cases of IgA and lysozyme. The enhancement of aqueous phase suggests the role of the exorbital and accessory conjunctival lacrimal glands, which are known to receive extensive sensory innervation. Moreover results of luminol chemiluminescence indicate that secretion of IgA and lysozyme which are produced mainly in the exorbital lacrimal gland can be influenced by activation of sensory nerve endings. The significantly reduced number of mucus-containing goblet cells on the stimulated side was due to the release of the mucus-containing granules providing indirect evidence for enhanced mucus secretion by antidromic trigeminal stimulation. On the other hand tear secretion was not influenced by contralateral nerve stimulation or stimulation at a false electrode position it is suggested that neither orthodromic spread of stimulation nor involvement of other neural pathways influence the response. The absence of response following topical capsaicin desensitization suggests a cardinal role of the released sensory neuropeptides (such as substance P, CGRP) from the capsaicin-sensitive sensory nerve endings of the eye. The selective NK1 receptor antagonist SR140333 significantly inhibited tear secretion enhancement by antagonizing the effect of substance P on NK1 receptors. Since the blockade was not complete following SR140333 pretreatment, other transmitters are supposed to participate in mediation of neurogenic tear secretion. Present data suggest an important local role of sensory nerve endings in regulation of tear secretion and maintenance of the integrity of ocular surface. This local effector fuction of the capsaicin-sensitive sensory nerve fibres controls the watery and protein phases of tear secretory mechanism and influences mucin excretion by controlling conjunctival goblet cells.

Disruption of this neural regulation presumably contributes to pathogenesis of diseases associated with disturbed mucin and tear production. An irritative stimulus of the ocular surface activates sensory nerve endings and results in the release of sensory neuropeptides; released substances presumably stimulate goblet cell secretion by a paracrine mechanism. Further studies are needed for overall comprehension of these processes in inflammatory eye surface diseases.

Our findings showed that immunostainng for SP, CGRP, VIP and nNOS were markedly increased in four localization of the retina: in the RPE/POS, in the inner and outer granular layers and in the ganglion cells, in the electrically stimulated rats in eomparison to the control eyes. Previous studies described immunoreactive for neuropeptides only in amacrine and ganglion cells of the rat retina. After electric stimulation of the trigeminal ganglion immunoreactivity increased in these cells and marked immunoreactivity was also found in the outer granular layer and RPE/POS. Previous studies showed that SP and CGRP act as excitatory neurotransmitter in the retina. These results indicate that CGRP may play a functional (excitatory) role in modulating retinal responses to light stimulation. Moreover SP mediates positive effects on dopamine release, which is related to light intensity indicating that the functional role of SP is likely to be related to light adaptation. According to this, the enhanced expression of these neuropeptides due to neurogenic inflammation might alter retinal responses to light, and may have a role in some form of macular diseases.

Our electron microscopic studies demonstrated that mitochondria of the RPE undergo significant morphological changes with age. These alterations are characterized by marked decreases in the number and area of mitochondria that were significantly more severe in AMD compared to age-matched controls. These data give a morphological support to the concept that mitochondrial membrane and subsequent mitochondrial dysfunetions may play a crucial role in the development of retinal degeneration, in particular of AMD. Mitochondrial abnormalities were also accompanied by proliferation of peroxisomes. Morphometry showed that the age-related increase in peroxisome number in AMD specimens was significantly greater than in age-matched controls. The increased number of peroxisomes in aging and AMDmay be a morphologic manifestation for activation of an alternative pathway for lipid degradation. Alterations of mitochondrial membranes were accompanied by significant accumulation of lipofuscin in both AMD and aged eyes, although there was no difference between groups.

In our clinical study improvement was found in each of the four parameters of *visual*

functions in the most affected eyes of early AMD patients taking Phototrop®. It is particularly important that VFMD, visual acuity and foveolar sensitivity showed statistically significant differences in changes comparing treated with placebo groups. Comparison of the most and less affected eyes showed another important finding. According to the results less affected eyes respond better to Photorop treatment than the most affected eyes. These findings seem reasonable taking into consideration that the less affected eyes had better baseline visual function, and that they responded better to this treatment. This clinical trial also showed that improvement in *fundus alterations* can be achieved after 1 year of treatment with Phototrop®. The drusen-covered area decreased in the treated group while it increased in the placebo group, and the difference between the treated and the placebo groups became statistically significant in favor of the treated group by the end of the study. Comparison of treatment efficacy in fundus alterations of subgroups showed a more marked improvement if the less affected eyes were evaluated. In conclusion, the results of this clinical trial suggest that treatment of early AMD with a combination of ALC, n-3 FA, and CoQ10 may improve both visual functions and fundus alterations likely by improving the metabolism of the photoreceptor/RPE/Bruch's membrane complex. Although our results have immediate clinical significance for treating early AMD, further studies are certainly needed to support this hypothesis, but first of all, to learn more on the pathophysiology of AMD.

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3. **I Kovacs**, Zs Recsan, G Salacz. Combined Cataract Surgery and PPV: IOL Calculation Error from Resolved Macular Edema. Congress of the European Society of Cataract and Refractive Surgeons (ESCRS), Lissbon, 2005
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1. **I Kovacs**, M Scwoeller, B Kovacs B, J Feher. Correlation between fundus alterations and visual functions in age-related macular degeneration. *Congress of the Hungarian Society of Ophthalmology*, Sopron, 2006.

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3. **I Kovacs.** Ophthalmological complications of pediatric diabetes. *Educational course at the Department of Ophthalmology, Semmelweis University,* 2006. [Hungarian language]
4. **I Kovacs.** Dry eye: anatomy, physiology, pathophysiology. *Dry eye course*, Balatonalmádi, Hungary, 2005. [Hungarian language]
5. **I Kovacs.** Contact lens wear and dry eye syndrome. *Dry eye course*, Balatonalmádi, Hungary, 2005. [Hungarian language]
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