Inflammatory cytokines decrease viability and alter ganglioside profile in retinal pigment epithelium cells

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ABSTRACT BOOK

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• F001

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Purpose Early stages of Age related Macular Degeneration (AMD) are characterized by dysfunction and degeneration of the retinal pigment epithelium (RPE) cells, which participate in the death of the overlying photoreceptors ultimately leading to loss of vision.Gangliosides (GG) make a wide and heterogeneous family of sialic-acid-containing glycosphingolipids, composed of a sugar chain branched on a ceramide. They are major components of cellular membranes, particularly abundant in the brain and nervous tissue, including retina. While their developmental and neuroprotective actions have been demonstrated, their precise role in retina's function and its pathologies is still poorly understood.The present study aimed to investigate the role of GG in the response of RPE cells to inflammation, which is known as one of the pathophysiological features of AMD.

Methods Cultured human RPE cells (ARPE19) were exposed to an inflammatory cytokine mixture (ICM): TNF-α, IL-1β and IFN-g for 72 hours. Cell viability was assessed and GG were analyzed by Liquid Chromatography coupled with tandem mass spectrometry.

Results ICM had deleterious effects on ARPE19 viability: cell number decreased by half between control and treated conditions. GM3 appeared to be the main GG class present in ARPE19 cells. Interestingly, ICM exposure was associated with modifications in the GM3 profile: relative amounts d18:1/16:0 species increased whereas those of d18:1/24:1 species decreased.

Conclusion Our observations suggest that GG might be implicated in ARPE19 cell response to inflammatory cytokines, although the precise biological role of the change in fatty acid profile of GM3 still needs to be clarified.

• F002

Blue light toxic action spectrum on A2E-loaded RPE cells in sunlight normalized conditions

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Purpose Sunlight exposure is supposed to induce cumulative damage to the retina in retinal pathologies, such as AMD. The high energy visible spectrum between 380 nm and 500 nm (blue light) is incriminated. The goal of this study was to identify the spectrum of retinal toxicity induced by sunlight in physiological irradiance conditions.

Methods RPE cells incubated for 6 hours with 0, 12.5, 20 and 40 µM of A2E were exposed for 18 hours to 10 nm illumination bands with the first band centered at 390 nm and going up to 520 nm. Light irradiances were normalized with respect to the natural sunlight reaching the retina after filtering by the eye ocular media. Six hours after light exposure, cell viability, necrosis and caspase-3/7 activity were assessed using the Apotox-Glo Triplex Assay.

Results A2E-loaded RPE cells presented fluorescent bodies within the cytoplasm with a similar spectrum to that of A2E. Exposure to the 10 nm illumination bands induced morphological changes associated to a loss in cell viability. Light toxicity was dependant of A2E concentration and was higher in the blue spectral range with maxima in a specific 30 to 40 nm bandwidth. In addition, caspase-3/7 activity, indicative of cell apoptosis, was highly induced by the same narrow range whereas necrosis was not significantly different to that of cells maintained in darkness.

Conclusion We described for the first time the precise spectrum of light toxicity in physiological irradiance conditions on an in vitro model of AMD. The 415-455 nm narrow spectral range generated the greatest phototoxic risk to RPE cells. This provides new information for designing selective protective filters, without disrupting visual and non-visual functions.

• F003

Chicken peptidylarginine deiminase type I and III are constitutively expressed in the retinal neuron

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Purpose Peptidylarginine deiminase (PAD) is a post-translational modification enzyme that catalyzes the conversion of protein-bound arginine residues to citrulline residues in the presence of calcium ion. PAD genes are distributed generally throughout vertebrates. In chickens, three PAD genes have been identified and are orthologous to the mammalian genes encoding PAD1, PAD2 and PAD3, respectively. The expression levels and tissue/cellar location of each PAD under normal physiological conditions have not been elucidated as yet.

Methods cPAD1 or cPAD3 specific antibodies were prepared from rabbits immunized with a synthetic peptide and used for western blot analyses and immunohistochemistry assays. To determine the intracellular localization of chicken PADs in retina cells, immunoelectron microscopy was also carried out. Whether or not the deiminated proteins were produced in retinal tissues was also examined by immunohistochemistry using an anti-modified citrulline detection method.

Results We found that both cPAD1 and cPAD3 are expressed in the chicken retina. cPAD1 was localized in the inner nuclear layer (INL) whereas cPAD3 was localized in the outer photoreceptor (OP). cPAD3 was present at especially high levels of detection in the lamella structure. Deiminated proteins were also detected in the INL and OP, suggesting that cPAD1 and cPAD3 function in the chicken retina under normal physiological conditions.

Conclusion Our findings suggest that chicken PAD1 and PAD3 play a homeostatic role in the chicken retina in governing sight.

• F004

Does cyclodextrin affect penetration of diclofenac sodium through amniotic membrane?

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Purpose To investigate permeability of amniotic membrane (AM) to diclofenac sodium containing eye drops with or without cyclodextrin (CD)

Methods Cryopreserved AM pieces on cellulose acetate filter membranes were mounted in the previously established vertical Franz-diffusion cell system equipped with autosampler. In vitro penetration of two commercially available eyedrops containing 0.1% diclofenac sodium was examined. Voltaren Ophtha CD (VO, with CD) and Uniclophen (UN, without CD) were compared. Drug release was determined by quantitative absorbance measurement carried out with a high performance liquid chromatography (HPLC).

Results The initial two hours, release of diclofenac from VO was lower than penetration of diclofenac from UN. At 30 minutes only 6.6% of diclofenac penetrated AM from VO vs 11.5% from UN. At 2 hours difference between two eyedrops was not siginificant (25.69% for VO and 27.39% for UN; $p>0.05$). After two hours, greater concentration of VO could be measured in the acceptor phase than that of UN and this difference remained significant over the study period. Seven and half hour after instillation we detected 56.26% for VO versus 35.62% for UN of baseline concentrations

Conclusion Drug penetration of diclofenac sodium was affected by CD. Until 120 minutes following instillation, CD decreased drug release from eyedrop containing CD compared to eyedrop without CD. After 2 hours, however drug penetration became significantly greater from solution with CD compared to solution without CD.