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Poster: strategies to reduce animal numbers for testing biologicals

## **ARPE-19 retinal pigment epithelia cells as an *in vitro* model for the examination of light induced cell damage in the eye**

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**Introduction:** Photochemical damage to retinal pigment epithelial (RPE) cells and photoreceptors is involved in the pathogenesis of age-related macular degeneration (AMD). Numerous studies have suggested that photochemical damage includes oxidative events by which retinal cells die of apoptosis. Heme oxygenase (HO-1), a stress-responsive protein is induced by a variety of stimuli. HO-1 and its products are also known to contribute to diverse physiological cytoprotective mechanisms against oxidative injury and cellular stress. Transcriptional activation of the HO-1 gene was observed after light damage which is considered as an adaptive response to oxidative and cellular stress. Because ascorbic acid (AA) is known to prevent or delay retinal degenerative processes we investigated the effects of AA on photochemically damaged retinal pigment epithelial cells especially in respect of affecting HO-1 gene expression.

**Methods:** ARPE-19 cells were plated at a density of 25,000 cells/cm<sup>2</sup> and maintained in culture for 6-8 weeks for differentiation. Light damage was induced by preincubating the cells with the photodynamic active substance Merocyanin 540 (MC540) for 4h with subsequently illuminating the cells with a light dose between 1-4 J/cm<sup>2</sup>. Uptake of AA in ARPE-19 cells was measured by HPLC-analysis. Gene expression profiles of light damaged cells were assessed by microarray analysis. The expression of HO-1 mRNA was measured by quantitative PCR (qPCR). HO-1 protein expression and PARP cleavage were detected by Western blot analysis. Cell viability was determined by LDH-release and MTT-assay.

**Results and Discussion:** Light induced cell damage resulted in an upregulation of the oxidative stress defence enzyme, HO-1 in differentiated ARPE-19 cells. As expected, preincubation with AA attenuated significantly light induced cell damage. Not expected was the increase of HO-1 expression more than 2-fold on the protein level in AA-supplemented cells after light-induced cell damage. AA upregulates HO-1 significantly only under conditions in which the light stress response is provoked. The inhibition of HO-1 activity by ZnPPIX showed that the main cytoprotective effect of AA was caused by the induction of HO-1 and not by its role as an antioxidant. The *in vitro* RPE model of light-induced cell damage and the new detected survival pathway induced by AA may be of interest in the understanding of the pathophysiology of retinal degenerations and in exploration of new therapeutic modalities.

*Keywords: ascorbic acid, HO-1, light-induced cell damage, in vitro RPE model*