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Biophysical Properties of photobleached porcine and cow RPE melanosomes – an *in vitro* model of aged human RPE melanosomes

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Biophysical properties of human retinal pigment epithelium (RPE) melanosomes undergo substantial age-related changes. It has been postulated that these modifications, presumably resulting from chronic exposure of RPE to significant fluxes of visible light and high oxygen tension, can decrease the melanosomes antioxidant and photoprotective capacity. We studied how photobleaching of cow and porcine RPE melanosomes – an *in vitro* model of melanosomes aging – affects their ability to inhibit peroxidation of liposomal lipids and oxidation of melanin-associated β -actin induced by irradiation with strong blue light or by Rose Bengal photosensitised reaction. We also tested effects of melanosomes photobleaching on the responses of living ARPE-19 cells to photic stress. Purified melanosomes were photobleached with visible light derived from a solar simulator and characterized by EPR spectroscopy. Lipid peroxidation was monitored by EPR oximetry and iodometric assay for lipid hydroperoxides. Protein was re-stripped from melanosomes and analysed for carbonylation by Western blotting. Cells, containing phagocytosed particles, were exposed to intense violet light and analysed for apoptotic blebbing as a dynamic measure of reduced cell survival. Under the conditions used, untreated melanosomes moderately protected unsaturated lipids from photo-induced peroxidation and had no effect on photosensitised oxidation of the associated protein. Untreated melanosomes did not decrease photic injury to ARPE-19 cells compared to controlled particles. On the other hand, photobleached melanosomes stimulated photoperoxidation of lipids and photooxidation of β -actin, and made cells containing them significantly more sensitive to light-induced cytotoxicity. The results suggest that aged RPE melanosomes lose their antioxidant efficiency and may even become phototoxic.

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