Protective Role of Zeaxanthin and α-tocopherol During Photodynamic Damage of ARPE-19 Cells

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It has been previously demonstrated that zeaxanthin and α-tocopherol provide efficient protection against photosensitized lipid peroxidation in liposomal systems [1] and against photooxidative damage of cultured RPE cells [2]. Here, the effect of both antioxidants on phagocytic activity of ARPE-19 cells, on accumulation of cholesterol hydroperoxides, and on expression of receptor proteins MerTK and integrin αvβ5 was analyzed in ARPE-19 cells subjected to sub-lethal photodynamic (PD)-stress mediated by merocyanine-540 (MC-540) or rose Bengal (RB). In the tested model system, survival of cells, subjected to PD-stress, was determined by the MTT assay. Specific phagocytosis of FITC-labeled photoreceptor outer segments (POS), isolated from cow retinas, was measured by flow cytometry. Peroxidation of membrane lipids, induced by PD-treatment of the cells, was determined by HPLC-EC(Hg) measurements of characteristic cholesterol hydroperoxides, using cholesterol as a mechanistic reporter molecule, and the levels of αv integrin subunit, β5 integrin subunit, MerTK and actin were quantified by Western blot analysis. Supplementation with antioxidants protected ARPE-19 cells against photodynamic killing only when higher doses of photodynamic treatment were used. Moreover, antioxidants administered to the cells prior to PD-stress mediated by both dyes reduced the stress inhibitory effect on phagocytic activity of ARPE-19 cells. Substantial inhibition of the accumulation of 5α-OOH and 7α/β-OOH was also observed when a combination of antioxidants was employed. Quantitative immunoblotting confirmed that antioxidant supplementation prior to PD-treatment mediated by both dyes increased the abundance of MerTK relative to non-supplemented cultures. Antioxidants also increased both integrin subunit proteins at 0.5 hr after PD-stress with both photosensitizers. The synergistic action of zeaxanthin and α-tocopherol indicates the importance of the antioxidant interaction in protection of the human retina against photooxidative stress through efficient protection of cell membranes and other cell components involved in phagocytosis of POS against oxidative damage.

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