**Photoreactivity of natural melanin pigments and their phototoxic potential in model systems and *in vitro* cells**

Protecting human skin cells against the adverse effects of high-energy ultraviolet (UV) and visible light is one of the most important biological functions of melanin pigments. Melanins efficiently absorb solar radiation and dissipate the energy of photons in the form of heat. Up until recently, melanins, especially brown-black eumelanins, were thought to be almost perfect photoprotectors, while pheomelanins were known to exhibit prooxidative behaviour and photogenerate reactive oxygen species. Most of the studies regarding photoreactive and protective properties of melanin pigments were conducted using synthetic models, which allowed one to examine a range of mechanisms related to the response of melanins to light. However, using such an approach provides limited insight into the photoreactivity of melanin that occurs naturally on skin or hair. In this work, natural melanin pigments isolated from hair samples obtained from healthy donors of different phototypes (I, II, III, V) were used as a model of natural melanins. Electron paramagnetic spectroscopy, spectrophotometry, dynamic light scattering, and HPLC analysis of degradation products were used to determine physicochemical properties of isolated pigments. The photoreactivity of natural melanins was examined using EPR oximetry, EPR spin-trapping, and time-resolved singlet oxygen phosphorescence. The rate of interaction of melanins with stable free radical DPPH and singlet oxygen was used to examine the antioxidative properties of natural melanins. The kinetics of experimental photodegradation of natural melanins was examined, and its effects on physicochemical, photoreactive, and antioxidative properties were determined. The photoprotective properties of native and photodegraded melanosomes of phototypes I, II, III, and V were studied in an *in vitro* model of the human keratinocyte cell line HaCaT. To assess biological effects, HaCaT cells containing melanosomes were exposed to solar-simulated light, and their viability, mitochondrial membrane potential, and concentration of lipid hydroperoxides were measured. The photoreactive and antioxidative properties of natural melanins strongly depend on their subunit composition and pigment size, with the highest photoreactivity found in phototype II and phototype III melanins. Prolonged exposure of natural melanins to intense violet-blue light (400 nm) leads to degradation of melanin free radicals, oxidative modification of their structure and physicochemical properties, resulting in increased photoreactivity, especially photogeneration of singlet oxygen, and decreased quenching abilities. The probability of the products of residual photoreactivity of melanin to escape the melanin pigment granules into cell compartments is relatively low for native pigments, especially rich in eumelanin, due to their

effective self-quenching of reactive oxygen species. Photodegradation promotes the prooxidative and phototoxic effect of both pheomelanin- and eumelanin-rich pigments in the *in vitro* model used. Oxidative stress induced by photosensitized reactions of degraded melanins could result in premature skin aging and the induction of skin cancers, including malignant melanoma.