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Response of the endogenous antioxidant system after administration with anti-oxidants and moderate stress induction in keratinocytes

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The skin is exposed to many stress factors which, in turn, can promote a shift of the antioxidant (AO) network towards the prooxidative side, supporting the development of various skin disorders. A lot of studies have demonstrated that AOs can counteract the development of oxidative stress, although several studies revealed that the AOs do not always yield positive effects but weakened the metabolism, instead.

The AO status of skin cells (secondary keratinocytes, HaCaT cells) after UV exposure at different wavelengths has been successfully investigated by electron paramagnetic resonance (EPR) spectroscopy. An increased radical formation could be counteracted by a targeted supplementation with the endogenous AO coenzyme Q10, which was loaded into nanocarriers. A physiological concentration of Q10 provides an effective protection against radical formation for the UVA and UVB spectral region, respectively. But the process of supplementation was limited; the cell viability was negatively affected after the AO concentration exceeded a specific threshold.

These initial investigations raise the questions, how the endogenous AO system can counteract an excess of exogenously supplied AOs and to what extent such an over- supplementation can influence the efficiency of the endogenous AO system.

To answer these questions HaCaT cells were treated with various ß-carotene concentrations with subsequent stress treatment by moderate irradiation (700-2000nm). To facilitate the uptake of ß-carotene, an innovative nanocrystal formulation was used. By resonant Raman spectroscopy a concentration-dependent uptake of ß-carotene was demonstrated. The redox status was determined before and after supplementation with two selected ß-carotene concentrations (0.02

and 0.1 µg/ml) and moderate irradiation. Significant redox changes were shown by EPR spectroscopy. By a fluorescent-based assay, the endogenous redox status for the AO glutathione was evaluated in parallel. An increased formation of reactive oxygen species (ROS) after irradiation was shown, which could be reduced after supplementation with both ß-carotene concentrations. An active interdependence between the applied ß-carotene concentrations (exogenous AOs), the endogenous AO system glutathione and the radical formation was shown. Nevertheless, a more effective protection against moderate stress could be observed for the lower dose. The high dose turned pro-oxidative.