Synopsis of Original Research Paper

Non-invasive measurement of radical reactions in skin induced by UV and ionizing radiation and its application to evaluation of antioxidants for protection of skin injury

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There have been little reports about direct evidence of generation of free radicals including oxygen radicals during exposure to ultraviolet (UV) light, although free radicals may be involved in various injuries caused by UV light. In this study, generation mechanism of oxygen radicals during photodynamic reaction was examined precisely with in vitro spin trapping technique, and then induction of radical reaction in skin of living mouse was examined under UV light by using in vivo electron spin resonance (ESR) spectroscopy. ESR signal of hydroxyl radical ($^{\bullet}OH$) adduct of spin trapping agent, DMPO, formed during uroporphyrin photosensitization increased in the presence of NADPH. This increase was suppressed by addition of scavengers of $^{\bullet}OH$ and singlet oxygen ($^1O_2$), and enhanced in deutrated solvent. The appearance of $^1O_2$, as determined by the oxidation of TEMPD, was delayed with an increase in the concentration of NADPH, while the production of $^{\bullet}OH$ was upregulated. Addition of H2O2 did not increase the signal. These results suggest that the $^{\bullet}OH$ was produced $^1O_2$-dependently, and that its production involves neither superoxide anion radical nor H_2O_2. An aqueous solution of carbamoyl-PROXYL was injected intravenously as a redox probe to an anesthetized mouse, and ESR spectrum of the probe was measured at the dorsal region of hair-removed ddY mice and hairless mice using an L-band ESR spectrometer with a surface-coil-type resonator. The rate of signal decay increased during irradiation of UV light. The increase was statistically significant. The increase of signal decay rate was suppressed by pre-administration of spin trapping agent, PBN, while PBN did not change the decay rate of non-irradiated mouse. These observations suggest that the estimation of radical generation under UV-irradiation may be possible by using in vivo ESR spectroscopy with a nitroxyl redox probe./