UVA/Ketoprofen-Induced Human Methemoglobin Radicals Detected by Immuno-Spin Trapping

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Ketoprofen (3-benzoyl-a-methylbenzeneacetic acid, KP) is a widely used non-steroidal anti-inflammatory drug (NSAID) that causes both phototoxicity and photoallergy. Here we have investigated the formation of radicals in human methemoglobin (metHb) induced by UVA/ketoprofen by utilizing "*Immuno-Spin Trapping*", a novel approach which combines the specificity of spin trapping with the sensitivity of antigen-antibody interactions. The metHb radicals react with DMPO to form nitrone adducts that are specifically recognized by antiserum against DMPO nitrone. We have found that the formation of nitrone adducts in metHb depended on the UVA dose, KP concentration and the presence of DMPO, as determined by an Enzyme-Linked Immuno-Sorbent Assay (ELISA) and Western Blotting. KP in the dark did not generate metHb radical-derived nitrone adducts, while UVA alone resulted in the formation of metHb radical-derived nitrone adducts that increased with UVA dose from 4 to 10 J/cm^2. However, KP (25 and 200 µM) plus UVA (4 and 10 J/cm^2) resulted in a significant increase in the formation of metHb radical-derived nitrone adducts as compared to UVA or KP alone, indicating that KP photosensitized the production of the metHb radicals in the presence of UVA. In contrast, no metHb radical-derived nitrone adduct was detected in the absence of DMPO, even though KP and UVA were present. In addition, we compared the photo-production of nitrone adducts by KP/UVA, with 3-ethylbenzophenone (3-EtBP)/UVA, the main photoproduct of KP which has the same chromophore as KP. However, no significant nitrone adduct was generated by UVA irradiation (10 J/cm^2) in the presence of 3-EtBP (200 µM). Spectrophotometric measurements showed that heme degradation occurred in the presence of KP/UVA, which resulted in a decrease in metHb peroxidase activity. These studies have shown that the "*Immuno-Spin Trapping"* technique can be used to detect radical damage in proteins as a result of photosensitizing reactions.