

**P008** An *in vitro* investigation of the mechanism of dermatological PPIX-induced photodynamic therapy with a view to enhancing this treatment modality

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Aminolaevulinic acid-induced photodynamic therapy (ALA-PDT) is a basal cell carcinoma treatment involving 3 components: ALA, a pro-drug for the natural photosensitiser protoporphyrin IX (PPIX), light (636 nm) and tissue oxygen.

This study investigated the molecular mechanisms of PPIX-PDT-induced apoptosis, assessing the amount/type of cell death and reactive oxygen species (ROS) production. The clinically relevant ALA methyl ester derivative (MAL) was also investigated, in addition to the effect of the novel iron chelator CP94 (1,2-diethyl-3-hydroxypyridin-4-one).

Human dermal fibroblasts (84BR) were exposed to 0.5 mM ALA/MAL±CP94 for 6 or 3 hours, and then irradiated for 5 min (25 J/cm<sup>2</sup>, 630±10 nm; Aktelite®, CL16). Sixteen hours post-irradiation, cell death was assessed by flow cytometry, using annexin V (for apoptosis) and propidium iodide (for necrosis).

ALA- or MAL-PDT-exposed cells demonstrated a statistically-significant increase in late apoptosis and necrosis compared to non-exposed cells ( $p < 0.05$ ; Student's t-test). Addition of CP94 to either ALA- or MAL-PDT treatment caused a statistically-significant increase in necrosis.

PPIX exposed to light (636 nm) in the presence of the spin trap TMP (2, 2, 6, 6-tetramethyl-4-piperidinol) generated the TEMPOL radical (4-hydroxy-2, 2, 6, 6-tetramethyl-4-piperidine-1-oxyl), suggesting singlet oxygen generation.

Iron metabolism and ROS generation are key factors in PPIX-induced PDT.