Endogenous photosensitizers

Between skin sun damage and photodynamic therapeutic intervention

Abstract

Endogenous UVA-chromophores may act as sensitizers of phototoxic stress and formation of reactive oxygen species (ROS) underlying cutaneous photoaging and photocarcinogenesis. Shortly after Raab’s, Jodlbauer’s, Jesionek’s and von Tappeiner’s seminal observations of the oxygen-dependent lethal effects of sunlight and fluorescent dyes on protozoa and skin carcinoma cells that was referred to as ‘photodynamic’, Meyer-Betz in 1913 noticed prolonged severe phototoxicity upon self-injection of sulfuric acid extracted human blood and thereby established the potential photodynamic action of chromophores derived from human tissue, i.e., hematoporphyrin, on human skin. Here we report that a novel endogenous photosensitizer, 6-formylindolo[3,2-b]carbazole (FICZ), a tryptophan photopродuct and endogenous high affinity aryl hydrocarbon receptor agonist, displays activity as a nanomolar photosensitizer potentiating UVA- and visible light-induced oxidative stress and apoptogenicity in human malignant melanoma cells. We have shown earlier that in human HaCaT and primary epidermal keratinocytes, photodynamic induction of apoptosis was elicited by the combined action of solar simulated UVA (6.6 J/cm²) and FICZ (10 nM), whereas exposure to the isolated action of UVA or FICZ did not impact cell viability (J. Invest. Dermatol. 2015 Jun;135(6):1649-58).

Likewise, photodynamic elimination of A375 human malignant melanoma cells was observed upon UVA/FICZ co-exposure at low nanomolar FICZ concentrations. Apoptotic elimination of melanoma cells was also observed upon FICZ photocytotoxicization using a blue light source (LED 460 nm). Taken together, our data suggest that novel tryptophan-derived endogenous photosensitizers may be useful for the photodynamic elimination of cutaneous malignant cells.

FICZ: A nanomolar sensitizer of UVA-induced apoptosis in cultured human HaCaT keratinocytes

(A) Visualization of HaCaT keratinocytes exposed to the isolated or combined action of FICZ (100 nM) and UVA (6.6 J/cm²) (upper panels; TEM ×2,650, lower panels; light microscopy). (B) HaCaT keratinocytes were exposed to the isolated or combined action of FICZ (100 nM) and UVA (6.6 J/cm²) or remained untreated (control). After incubating cells in medium for 24 h, viability was assessed employing flow cytometry (annexin V-FITC/propidium iodide). (C) Flow cytometric detection of caspase 3 activation using an Alexa488-conjugated antibody directed against cleaved procaspase 3. (D) Cell viability as determined using flow cytometry examining cytochrome c (CC) release from mitochondria. (E) FICZ-induced oxidative stress in A375 malignant melanoma cells (DIC microscopy). (F) Dose response relationship of FICZ-induced apoptotic cell death (FICZ: 100 nM; UVA: 6.6 J/cm²).

FICZ photodynamic elimination of malignant skin cells: UVA- and blue light-induced melanoma cell apoptosis

(A-B) A375 malignant melanoma cells were exposed to the isolated or combined action of FICZ (10 and 100 nM) and UVA (6.6 J/cm²) or remained untreated (control). After incubating cells in medium for 24 h, viability was assessed employing flow cytometry (annexin V-FITC/propidium iodide). (C) Likewise, cells were exposed to UVA and visible light and FICZ. Blue light (LED 460 nm; 2.5 J/cm²)-induced apoptosis (FICZ: 100 nM; mean ± S.D., n = 3). (D) After glutathione-depletion (BSO: 1 mM; 24 h pretreatment) FICZ/UVA photodynamic activity was assessed as in panel A.

FICZ is a photodynamic inducer of phototoxic oxidative genotoxic stress in A375 malignant melanoma cells

(A-B) A375 malignant melanoma cells were exposed to UVA and FICZ (100 nM) or remained untreated (control). After incubating cells in medium for 24 h, viability was assessed employing flow cytometry (annexin V-FITC/propidium iodide). (C) Flow cytometric detection of caspase 3 activation using an Alexa488-conjugated antibody directed against cleaved procaspase 3. (D) Cell viability as determined using flow cytometry examining cytochrome c (CC) release from mitochondria. (E) FICZ-induced oxidative stress in A375 malignant melanoma cells (DIC microscopy). (F) Dose response relationship of FICZ-induced apoptotic cell death (FICZ: 100 nM; UVA: 6.6 J/cm²).

Summary scheme: FICZ as an endogenous AhR agonist (pathway 1) and a photosensitizer (pathway 2) with potential photodynamic therapeutic application (scheme adapted from: J. Invest. Dermatol. 2015 Jan;135(1):1649-58).