12(S)-Hydroxyheptadeca-5Z,8E,10E-trienoic acid suppresses UV-induced IL-6 synthesis in keratinocytes, exerting an anti-inflammatory activity

Introduction

The UV spectrum is divided by wavelength into UVA (320-400 nm), UVB (290-320 nm) and UVC (200-290 nm) (Matsumoto and Aoyanagihara, 2012). Among them, the acute and chronic exposure of skin to UVB irradiation can cause various inflammatory responses, including sunburn cell formation (erythema), alterations of vascular responses, the production of inflammatory mediators and the inflammation of infiltrating cells, leading to skin disorders (Chen et al., 2011). Upon UVB irradiation in skin epidermis, keratinocytes are a major cell type that contributes to the synthesis of pro-inflammatory cytokines, such as TNF-α, IL-8, IL-6 and IL-8 (Buck et al., 2011; Chong et al., 2016; Gehbardt et al., 2007).

IL-6 is up-regulated in skin inflammatory diseases (e.g., psoriasis and atopic dermatitis) and is involved in the pathogenesis of skin squamous cell carcinoma (SCC) (Greenaway et al., 1990; Shidoko et al., 1994; Yoshimura and Kishimoto, 2004; Federele et al., 2011). In psoriasis, IL-6 potentially induces lymphocyte infiltration and stimulates keratinocyte proliferation (Greenaway et al., 1992). In addition, up-regulated IL-6 exacerbates the symptoms of atopic dermatitis (Shidoko et al., 1994). Also upon UV irradiation, IL-6 is markedly increased and contributes to cutaneous inflammatory responses (Chong et al., 1996; Fornazzari et al., 2002). IL-6-deficient mice show a defective cutaneous immune response following UVB exposure, indicating that IL-6 is a crucial mediator in the inflammatory response of skin (Shidoko et al., 1994; Nishimura et al., 1996). Thus, the regulation of IL-6 expression is expected to be important in understanding inflammatory skin diseases.

12(S)-Hydroxyheptadeca-5Z,8E,10E-trienoic acid (12-HHT) is a cyclooxygenase (COX)-derived arachidonic acid metabolite that is mainly produced by activated human platelets (Hunihara et al., 2012; Shidoko et al., 2008). 12-HHT is an abundant metabolite of the arachidonic acid cascade in tissues and cell types, e.g., vascular tissue, intestinal tissue, alveolar macrophages and bodily fluids (Catzon et al., 1996; Fumagalli et al., 1994; John et al., 1998), but little is known about its physiological roles and pathological relevance.

In this study, we found that 12-HHT inhibits the UVB-induced activation of the p38 MAPK/NF-κB pathway by up-regulating MAPK phosphatase-1 (MKP-1), which leads to the down-regulation of IL-6 synthesis in keratinocytes. These findings provide a novel insight into the function of 12-HHT in UVB-induced skin inflammation and suggest a potential application agent for the treatment of skin inflammatory diseases.

Results

12-HHT down-regulates UVB-induced IL-6 synthesis in HaCaT cells

To investigate the role of 12-HHT in the inflammatory responses of keratinocytes, we initially examined the effect of various UV doses on cell viability. HaCaT cells were irradiated with UVB at 5, 10 and 20 mJ/cm², and the number of viable cells was estimated by a trypan-blue exclusion assay. At 5 mJ/cm², UVB had no effect on cell viability (Figure 1), and thus, a dose of 5 mJ/cm² was chosen for further experiments. To assess the role of 12-HHT in UVB-induced inflammation, we analyzed the effect of 12-HHT on the UVB-induced inflammatory cytokines, IL-6. UVB (5 mJ/cm²) irradiation markedly up-regulated IL-6 synthesis and release (Figure 2A and 2B), which was suppressed by the treatment with 12-HHT in a concentration-dependent manner (Figure 2A and 2B). Taken together, these results suggest that 12-HHT has anti-inflammatory activity by attenuating the UVB-induced IL-6 synthesis in HaCaT cells.

Figure 1

Determination of the optimal UVB irradiation dose that would not damage HaCaT cells. HaCaT cells were starved with serum-free DMEM for 24 h and then irradiated with the indicated doses of UVB. The irradiated cells were further incubated for 24 h.

Figure 2

12-HHT down-regulates UVB-induced IL-6 synthesis in HaCaT cells. (A) HaCaT cells were starved with serum-free DMEM for 24 h and irradiated with UVB (5 mJ/cm²). Total RNA was extracted from the cells at the indicated times (0, 1, 3, 6, 12 and 24 h). (B) 12-HHT reduces UVB-induced IL-6 synthesis via inhibition of the p38 MAPK/NF-κB pathway.