Letter to the Editor

Anacardic acid ameliorates ultraviolet irradiation-induced damage to human skin

Ultraviolet (UV) radiation is a leading cause of premature skin aging (photoaging) and skin cancer [1]. UV exposure of the skin induces proinflammatory cytokines such as interleukin-6 (IL-6), mediated by nuclear factor kappa B (NF-κB) activation, which affects the expression levels of multiple target genes, such as matrix metalloproteinases (MMPs) and type I procollagen, leading to the appearance of wrinkles in photoaged skin, as well as to acute inflammatory responses [1]. Epigenetic regulation has been increasingly appreciated to play an important role in aging; in particular, histone acetylation patterns are altered in aged tissues and during aging-related diseases such as cancer and neurodegeneration [2]. However, the effect of age-related acetylation changes may vary in different tissues [2], and there is still little evidence of association between histone acetylation and skin aging. UV irradiation induces acetylation of histone H3, which leads to subsequent chromatin relaxation and activation of gene expression [3,4]. More specifically, UV irradiation causes increased recruitment of acetylated histone H3 (acetyl-H3) and histone acetyltransferase (HAT) p300 to a specific region near the p300 binding site in the MMP-1 promoter, indicating that MMP-1 expression, induced by UV, is mediated by histone modification and p300 HAT activity [4]. We have also demonstrated the role of histone acetylation in UV-induced inflammation and matrix damage *in vitro* and *in vivo*. Suppression of p300 HAT using p300 small-interfering RNA or anacardic acid (AA) inhibited the UV-induced MMP-1 gene transcription and histone modification in human dermal fibroblasts *in vitro* [5]. Additionally, AA could suppress the UV-induced histone modification, as well as MMP-13, MMP-9, cyclooxygenase-2, and tumor necrosis factor-α expression in hairless mouse skin *in vivo* [6]. AA is a bioactive compound isolated from the *Anacardium occidentale* (cashew) nutshell and consisting of salicylic acid substituted with varying saturated or unsaturated alkyl chains [7]. AA inhibits the HAT activities of transcription co-activators, p300 and p300/cyclic AMP response element-binding protein-associated factor (pCAF) [8]. Besides, AA suppresses the NF-κB-regulated pathway, reduces the level of IL-6 and tyrosinase activity, and exerts anti-inflammatory and anticancer effects [7].

Here, we investigated whether and how AA affected the acute skin responses induced by UV in human skin *in vivo*. All experiments were conducted after obtaining written consent in accordance with the Declaration of Helsinki principles and were approved by the institutional review board. A total of 11 human volunteers (ages 29–39 years) were recruited. On their buttock skin, four 1 × 1 cm squares were outlined and assigned to particular treatment groups, i.e., the vehicle, AA-treated, UV-irradiated/vehicle-treated, and UV-irradiated/AA-treated groups (Fig. 1**A**). To determine the photo-protective and anti-inflammatory effects of AA on the human skin, AA (0.1%) or its vehicle (ethanol: polyethylene glycol = 70:30) was topically applied after a single dose of two minimal erythema doses (MEDs) of UV or sham irradiation. The spectral distribution of the UV emission by an F75/85W/UV21 fluorescent lamp (Philips, Eindhoven, Netherlands) was 56.7% UVB, 42.8% UVA, and 0.5% UVC (emission spectrum: 275–380 nm, peak: 310–315 nm). A Kodacel filter (Kodak, Rochester, NY, USA) was used to filter UVC. The skin of the treated subjects was obtained 24 h after UV irradiation.

The topical application of AA significantly prevented a UV-induced increase in the erythema index (Fig. 1**A** and **B**), assessed using a Mexameter MX18^{TM} (Courage–Khazaka Electronic GmbH, Köln, Germany). Histological findings from skin biopsies revealed significantly reduced epidermal thickness in the 0.1% AA-treated group compared with that in the UV-treated group (Fig. 1**C** and **D**). Immunofluorescence staining showed a large increase in the level of thymine dimers in the UV-irradiated group, which was almost abrogated by the AA treatment following UV irradiation (Fig. 1**E, S1A**). While UV irradiation induced apoptotic cells, which were positive in the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, in both epidermis and upper dermis, AA significantly reduced the number of apoptotic cells (Fig. 1**F, S1B**). UV exposure is associated with DNA damage, which includes histone modification and DNA double-strand breaks. Chromatin remodeling occurs due to DNA damage triggering the phosphorylation of histone H2AX (γ-H2AX), a DNA damage marker, and acetyl-H3. Consistent with our previous *in vitro* and *in vivo* findings in mice, UV irradiation induced a substantial increase of γ-H2AX and acetyl-H3, whereas a topical treatment with AA after UV irradiation markedly attenuated the expression of γ-H2AX (Fig. 1**G, S1C**) and acetyl-H3 (Fig. 1**H, S1D,** and **2A**).

Subsequently, we examined the effects of AA on MMP-1 expression, which is mainly responsible for UV-induced matrix impairments seen in photoaged skin [1]. AA inhibited the UV-induced mRNA and protein expression of MMP-1, compared with the levels in the UV-irradiated group (Fig. 2**A** and **B**). Furthermore, the mRNA and protein expression levels of type I procollagen, decreased by UV-irradiation, were restored to a moderate extent in the AA-treated group post-UV irradiation (Fig. 2**A** and **C**). IL-6 is a potent cytokine exerting a wide range of physiological and pathological actions, including responses to skin injury such as chronic dermatitis or ultraviolet irradiation [9]. UV-induced production of IL-6 in keratinocytes is mediated by DNA damage [10]. Consistently, IL-6 significantly increased in the human skin after UV-irradiation and was reduced by the topical application of AA (Fig. 2**D**). The expression of molecular markers of UV-induced skin responses, such as MMP-1, IL-6, and type I procollagen as well as histone modification, were ameliorated by AA treatment, but the change was not significant because of high inter-subject variations. It is also possible that the expression of these molecular markers may vary with time, contributing to reduced alterations in a given timepoint. However, gross and histologic examination indicates that AA significantly restores UV-induced skin responses such as erythema, epidermal thickening, thymine dimer formation, cell apoptosis, and DNA damage. Future larger-scale and time-course studies are warranted to reinforce the molecular efficacy of AA treatment upon UV irradiation.

Collectively, our data suggests that topical application of AA, an inhibitor of p300 HAT, may prevent the UV-induced skin responses via epigenetic regulation, suggesting that targeting epigenetic modifications may potentially be an effective therapeutic modality against photoaging.

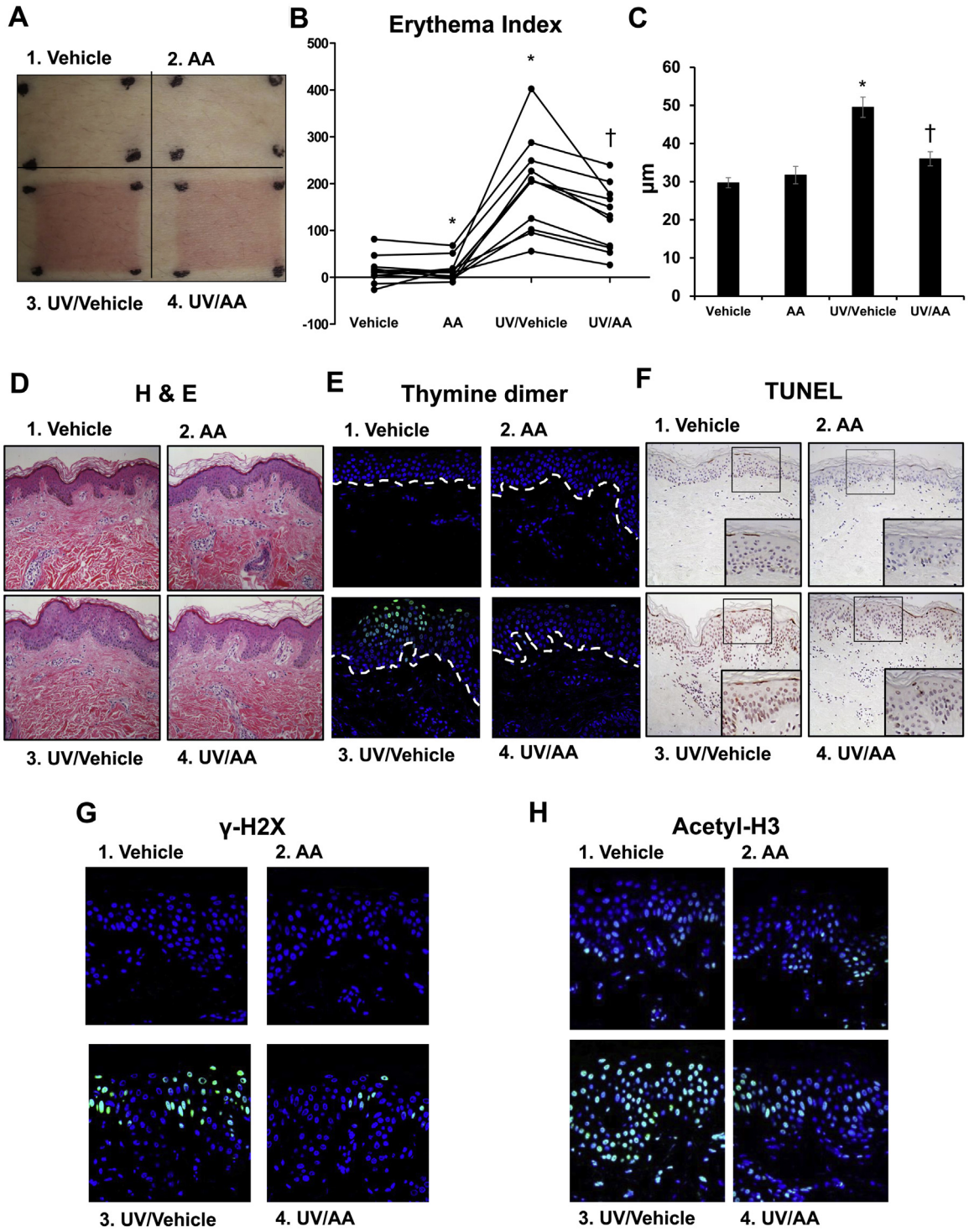

Fig. 1. Anacardic acid decreases the UV-induced erythema index, apoptotic cells, DNA damage, and acetyl-H3 in human skin *in vivo*. Buttock skin of 11 volunteers (mean age: 37.3 years; age range: 29–39 years) was topically treated with 0.1% AA or its vehicle after two MEDs of UV-irradiation or sham irradiation of buttock skin. Treated skin was obtained at 24 h after the irradiation. (A, B) Topical application of AA significantly reduced the UV-induced increase in the erythema index, measured by Mexameter™. (C, D) Epidermal thickness was measured in hematoxylin and eosin-stained sections. Original magnification: × 200. Results are presented as the mean ± standard error of the mean (SEM). $^{*}p < 0.05$ versus the vehicle-treated control group; $^{\dagger}p < 0.05$ versus the UV-irradiated vehicle-treated group. (E) Immunofluorescence staining of thymine dimers. (F) Apoptotic cells detected by the TUNEL assay. (G, H) Immunofluorescence staining of (G) γ-H2AX and (H) acetyl-H3.