Increased 11β-hydroxysteroid dehydrogenase type 1 activity in UVB-irradiated mice

Ana Tiganescu, Melanie Hupe, Yoshikazu Uchida, Daniel D. Bikle, Peter M. Elias and Walter M. Holleran

VA Medical Center (NCIRE), University of California San Francisco

Introduction

UVB exposure induces skin damage including dermal atrophy, telangiectasia, fragility and poor wound healing; symptoms also attributable to glucocorticoid (GC) excess (e.g. Cushing's syndrome).

In peripheral tissues including skin, GC availability is regulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which activates cortisol and corticosterone from cortisone and 11-dehydrocorticosterone (11-DHC) in humans and rodents respectively (Fig. 1). We previously demonstrated increased 11β-HSD1 levels in photo-exposed vs. photo-protected human skin (**Tiganescu *et al*. 2013**). Here we investigated UVB regulation of 11β-HSD1 *in vivo*.

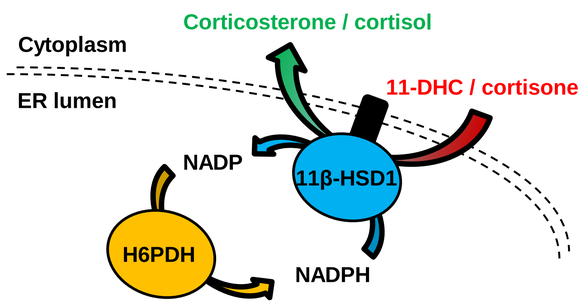

Fig. 1. GC activation by 11β-HSD1 requires NADPH co-factor supplied by hexose-6-phosphate dehydrogenase (H6PDH)

Methods

Female SKH1 mice were irradiated with 400mJ/cm^2 UVB and dorsal skin collected at days 1, 3 and 7 post-exposure. 11β-HSD1 activity was determined by overnight incubation of 5mm biopsies with 100nM 11-DHC in 1ml DMEM containing ~1000cpm [H^3] 11-DHC. 11β-HSD1, H6PDH and glucocorticoid receptor (GR) expression was determined by qPCR. 11β-HSD1 protein was detected by Western blotting and immunofluorescence in paraffin-embedded tissue. Prior to tissue collection barrier integrity was determined by measuring transepidermal water loss (TEWL).

In a second experiment. mice were irradiated with 50, 100 and 200mJ/cm^2 UBV and the above parameters were re-evaluated at day 3 post-exposure.

Results

1. *In vivo* UVB exposure induces 11β-HSD1 expression and activity in mouse skin

Increased 11β-HSD1 mRNA (2.7-fold) was detected 1 day post-exposure to 400mJ/cm^2 UVB and remained elevated at subsequent time points although expression was more variable (normalized to 18S rRNA, Fig. 2a). H6PDH mRNA was also elevated (2.5-fold) 1 day post-exposure (Fig. 2b). Contrastingly, GR mRNA was 31% decreased 3 days after irradiation (Fig 2c).

11β-HSD1 protein was increased by 3 days post-exposure (normalized to β-actin, Fig. 3).

Correspondingly, 11β-HSD1 activity also increased by 76% at day 3 post-exposure but was comparable to non-irradiated skin by 7 days post-exposure (Fig. 4).

2. 11β-HSD1 activity is upregulated by physiologically relevant doses of UVB

At 3 days post-exposure, 11β-HSD1 activity was also induced by 200 and 100 but not 50mJ/cm^2 UVB (Fig. 5). 100mJ/cm^2 is readily attainable by exposure to 60-90min midday summer sun.

3. UVB-mediated increase in 11β-HSD1 protein localizes to hyperproliferative epidermis

Epidermal hyperplasia was observed at UVB doses >100mJ/cm^2 as reported by others (Fig. 6A-D).

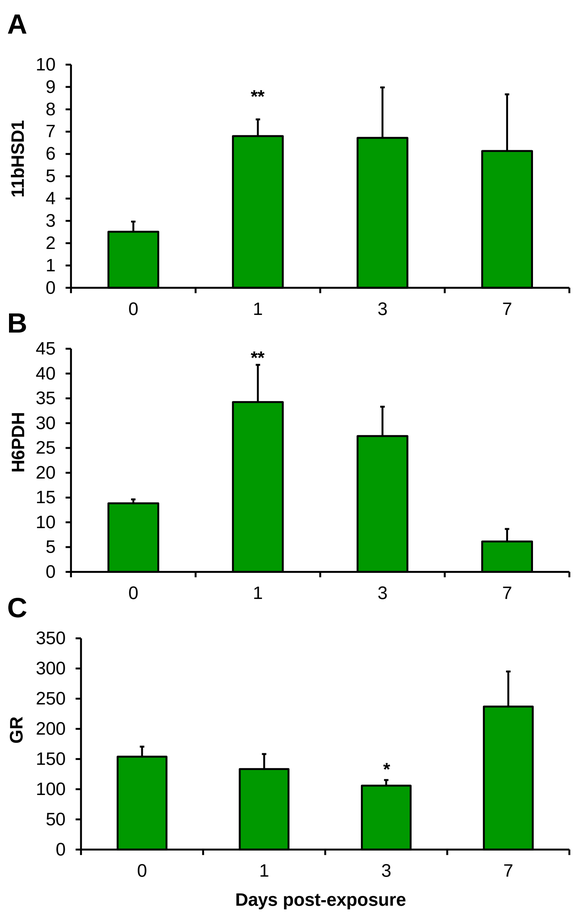

Fig. 2. mRNA expression of 11β-HSD1 (**A**) and H6PDH (**B**) were upregulated at day 1 while that of GR (**C**) was downregulated at day 3 post-exposure to 400mJ/cm^2 UVB. n=4

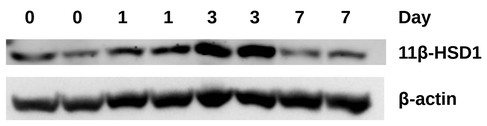

Fig. 3. Protein expression of 11β-HSD1 (34kDa) was elevated at day 3 post-exposure to 400mJ/cm^2 UVB. β-actin (42kDa) was used as a loading control, n=4

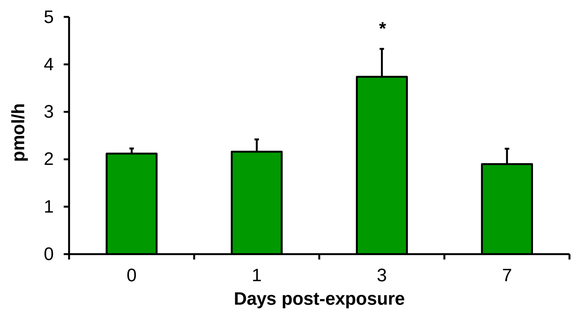

Fig. 4. 11β-HSD1 activity was elevated at day 3 post-exposure to 400mJ/cm^2 UVB. n=4

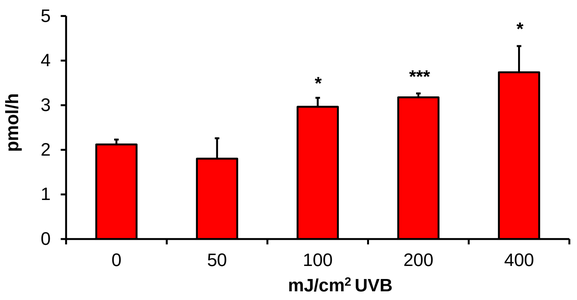

Fig. 5. 11β-HSD1 activity was also increased by 200 and 100mJ/cm^2 UVB at day 3 post-exposure. n=4

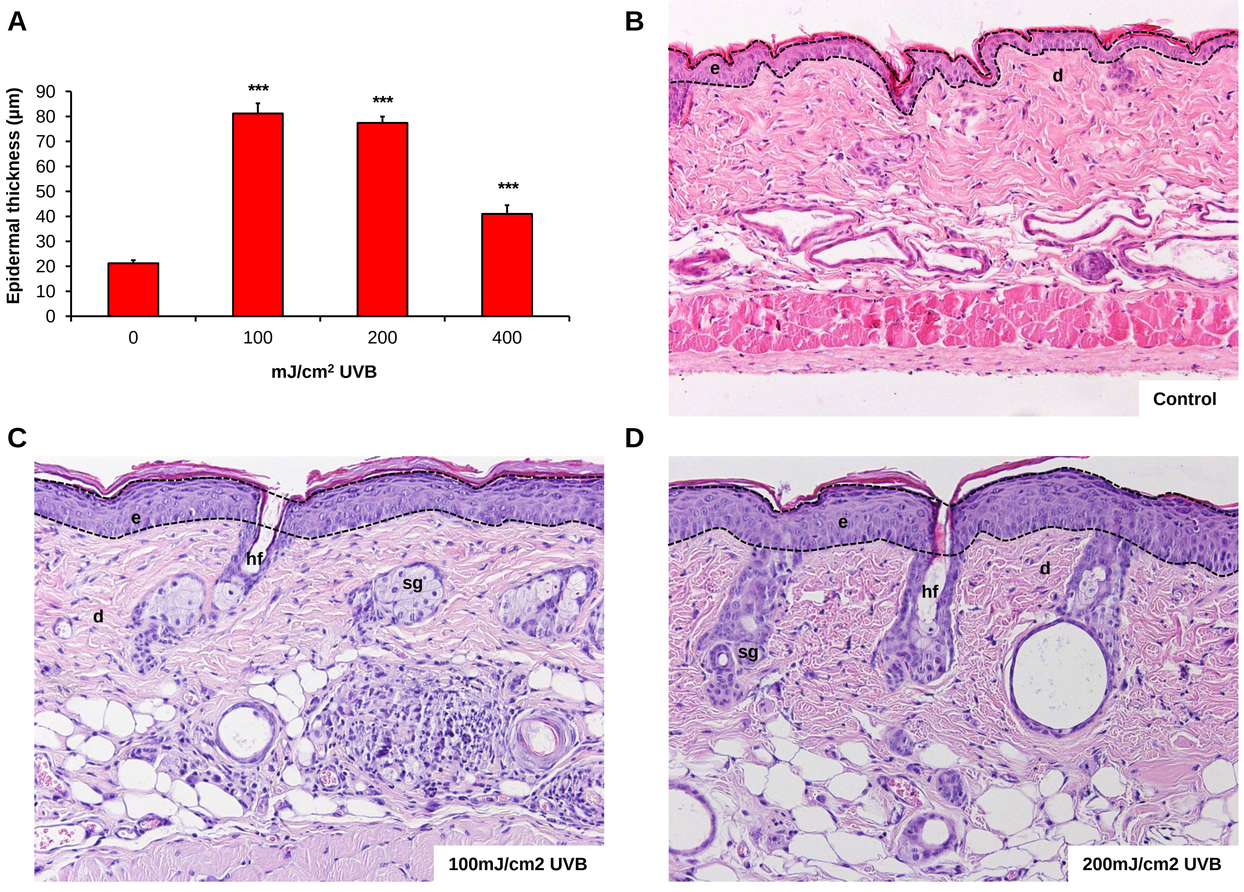

Fig. 6. UVB-induced epidermal hyperplasia (**A**). Representative H&E sections (**B**), **e**; epidermis, **d**; dermis, **hf**; hair follicle, **sg**; sebaceous gland, 20X magnification, n=4

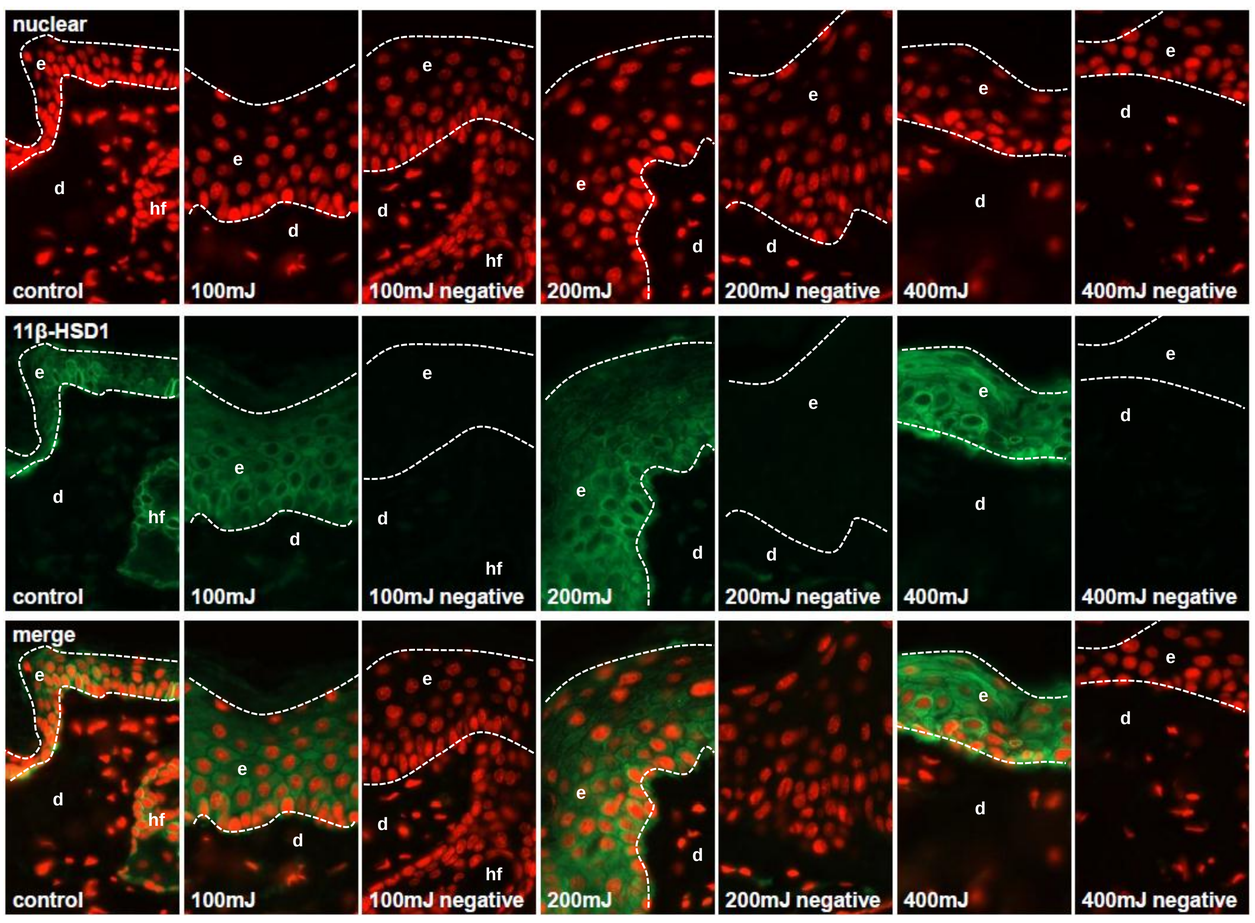

Fig. 7. 11β-HSD1 protein localizes to hyperproliferative epidermis (at 3 days post-UVB exposure). 11β-HSD1 expression in dermal cells was lower than in epidermal keratinocytes and was affected by UVB exposure. Nuclear marker (**red**), 11β-HSD1 marker (**green**), **e**; epidermis, **d**; dermis, **hf**; hair follicle. Anti-11β-HSD1 primary antibody omitted for negative controls. 40X magnification, n=5

11β-HSD1 protein was expressed in the hyperproliferative epidermis, with no changes in dermal expression (Fig. 7).

4. Increased 11β-HSD1 levels correlate with epidermal barrier deficiency following UVB exposure

Trans-epidermal water loss (TEWL) was determined as a measure of epidermal barrier efficiency. 400mJ/cm^2 UVB resulted in a 15-fold increase in TEWL (indicating barrier deficiency) at 3 days post-exposure (Fig. 8a), coinciding with the increase in 11β-HSD1 activity (Fig. 4). A similar association was observed between TEWL induced by lower doses of UVB at 3 days post-exposure (Fig. 8b) and 11β-HSD1 activity (Fig. 5).

Conclusion

Although studies have previously indicated increased 11β-HSD1 activity and expression in photo-exposed human skin (**Tiganescu *et al*. 2013**) and in response to UVB exposure in cultured human keratinocytes (**Skobowiat *et al*. 2013**) the findings presented here are the first to demonstrate elevated 11β-HSD1 levels in skin as a direct consequence of UVB irradiation *in vivo*.

As GC are known to drive keratinocyte differentiation, our data indicating 11β-HSD1

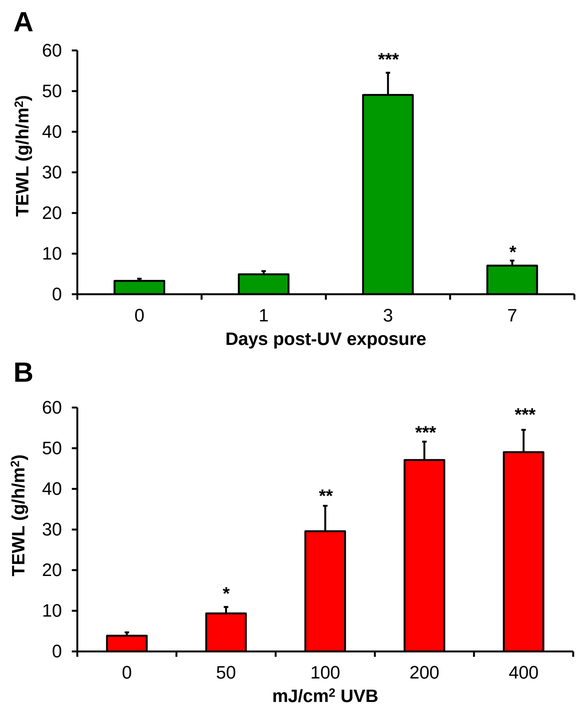

Fig. 8. Transepidermal water loss (TEWL) was increased at 3 days post-exposure with 400mJ/cm2 UVB, (A) and in a UVB dose-responsive manner (B) corresponding with the increase in 11β-HSD1 activity (Fig. 4 and Fig. 5). n=4

localization to the hyperproliferative epidermis post-exposure suggests increased local GC activation following UVB irradiation may function to potentiate epidermal barrier restoration. Alternatively, as GC also induce epidermal thinning, increased 11β-HSD1 activity may function to limit UVB-induced epidermal hyperplasia.

However, repeated exposure to UVB could result in constitutively elevated 11β-HSD1 and subsequently GC levels in skin which may accelerate photodamage. Topical 11β-HSD1 inhibitors may provide a novel means to protect against the adverse outcomes associated with chronic sun exposure (e.g. epidermal thinning, dermal atrophy and telangiectasia).

As GC treatment is also associated with impaired DNA repair following UV irradiation (**Kelly *et al*. 1987**), 11β-HSD1 blockade may also prevent the development of skin cancer.

References

Tiganescu A *et al*. 11β-Hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects. **J Clin Invest.** 2013 123: 3051-60

Skobowiat C *et al*. Ultraviolet radiation regulates cortisol activity in a waveband-dependent manner in human skin ex vivo. **Br J Dermatol.** 2013 168: 595-601

Kelly GE *et al*. Scheduled and unscheduled DNA synthesis in epidermal cells of hairless mice treated with immunosuppressive drugs and UVB-UVA irradiation. **Br J Dermatol.** 1987 117: 429-40.

Contact: ana.tiganescu@gmail.com