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SUPPVB induces epidermal 11 β -hydroxysteroid dehydrogenase type 1 activity *in vivo*

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Abstract

Detrimental consequences of ultraviolet radiation (UVR) in skin include photoageing, immunosuppression and photocarcinogenesis, processes also significantly regulated by local glucocorticoid (GC) availability. In man, the enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) generates the active GC cortisol from cortisone (or corticosterone from 11-dehydrocorticosterone in rodents). 11 β -HSD1 oxo-reductase activity requires the cofactor NADPH, generated by hexose-6-phosphate dehydrogenase. We previously demonstrated increased 11 β -HSD1 levels in skin obtained from photoexposed versus photoprotected anatomical regions. However, the direct effect of UVR on 11 β -HSD1 expression remains to be elucidated. To investigate the cutaneous regulation of 11 β -HSD1 following UVR *in vivo*, the dorsal skin of female SKH1 mice was irradiated with 50, 100, 200 and 400 mJ/cm² UVB. Measurement of transepidermal water loss, 11 β -HSD1 activity, mRNA/protein expression and histological studies was taken at 1, 3 and 7 days postexposure. 11 β -HSD1 and hexose-6-phosphate dehydrogenase mRNA expression peaked 1 day postexposure to 400 mJ/cm² UVB before subsequently declining (days 3 and 7). Corresponding increases in 11 β -HSD1 protein and enzyme activity were observed 3 days postexposure coinciding with reduced GC receptor mRNA expression. Immunofluorescence studies revealed 11 β -HSD1 localization to hyperproliferative epidermal keratinocytes in UVB-exposed skin. 11 β -HSD1 expression and activity were also induced by 200 and 100 (but not 50) mJ/cm² UVB and correlated with increased transepidermal water loss (indicative of barrier disruption). UVB-induced 11 β -HSD1 activation represents a novel mechanism that may contribute to the regulation of cutaneous responses to UVR exposure.

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Author contributions

AT performed the research; AT, YU and WMH designed the research study; MH prepared tissue sections for immunohistochemistry; AT and YJJ performed irradiation experiments; AT and AC analysed the immunofluorescence data; TM, DD and PME contributed essential equipment; and AT analysed the data and wrote the manuscript. All authors evaluated and approved the final manuscript.

Ethical approval

All work was approved by the Institutional Animal Care and Use Committee (11-027) at the San Francisco VA Medical Center.

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Conflict of interest

The authors have declared no conflicting interests.

Keywords

11 β -hydroxysteroid dehydrogenase type 1; glucocorticoid; skin; transepidermal water loss; UVB

Introduction

Exposure to excessive ultraviolet radiation induces a wide range of effects detrimental to skin structure and function. Short-term consequences of acute exposure include cutaneous inflammatory erythema (skin redness), pigmentation, epidermal hyperplasia and immunosuppression, while chronic repeated exposures lead to photoageing and increased photocarcinogenesis risk (1).

Glucocorticoids (GCs) are steroid hormones which also modulate multiple elements of these processes including suppressing erythema (2), impairing melanogenesis (3,4), inducing epidermal thinning (5,6) and driving immunosuppression (7). GCs also regulate skin carcinogenesis with both pro-carcinogenic (8–12) and anticarcinogenic (13–15) effects reported.

Local GC levels are largely mediated by 11 β -hydroxysteroid dehydrogenase (11 β -HSD) isozyme expression in a diverse range of tissues, including skin. In intact tissue and cells, 11 β -HSD1 oxoreductase activity converts cortisone to the active human GC cortisol and 11-dehydrocorticosterone (11-DHC) to corticosterone in rodents and is ubiquitously expressed (16,17). This reaction requires adequate NADPH cofactor availability supplied by the enzyme hexose-6-phosphate dehydrogenase (18) (H6PDH). Conversely, 11 β -HSD2 dehydrogenase activity catalyses the opposing reaction, converting cortisol and corticosterone to their inactive precursors. 11 β -HSD2 expression predominates in mineralocorticoid-responsive tissues protecting the mineralocorticoid receptor from inappropriate activation by GC (19). GC signalling is mediated through the GC receptor (GR α) which translocates to the nucleus following ligand binding and regulates target gene expression through transactivation, transrepression and non-genomic mechanisms (20).

It is important to note that 11 β -HSD-mediated steroid metabolism is distinct from de novo corticosteroidogenesis (from cholesterol) which has also been reported to contribute to GC availability in human skin under local hypothalamic–pituitary–adrenal (HPA) axis regulation (21–23).

In human skin, 11 β -HSD1 is expressed by epidermal keratinocytes, dermal fibroblasts, outer hair root sheath keratinocytes, seb-ocytes, sweat gland cells and endothelial cells (24,25), whereas 11 β -HSD2 is primarily expressed by sweat glands and to a lesser degree, keratinocytes and endothelial cells (26,27). Similar expression is observed in murine skin apart from the notable lack of 11 β -HSD2 expression (24,28) owing to the absence of sweat glands.

UVR exposure is known to induce GC activation in skin, with cutaneous HPA axis stimulation and subsequent corticosteroidogenesis identified as possible mechanisms (21,29). We previously reported elevated 11 β -HSD1 activity and protein expression in skin

biopsied from photoexposed compared to donor-matched photoprotected anatomical locations, in addition to greater 11 β -HSD1 activity in site-matched samples from older donors (25), suggesting 11 β -HSD1 may also contribute to increased local GC availability following UVR exposure.

In this report, we examined the novel regulation of 11 β -HSD1 in mouse skin following an acute dose of UVB. We demonstrate upregulation of 11 β -HSD1 mRNA and protein expression and enzyme activity in a UVB time- and dose-dependent manner with immunofluorescence studies localizing 11 β -HSD1 protein to the hyperproliferative epidermis. Interestingly, we also report that 11 β -HSD1 activity correlates with increased transepidermal water loss (TEWL), a measure of impaired skin barrier function. Our findings indicate that increased 11 β -HSD1 expression and activity by epidermal keratinocytes following UVB exposure may regulate the cutaneous response to UVR *in vivo*.

Methods

Study approval

Studies presented in this study were approved by the Institutional Animal Care and Use Committee and San Francisco Veterans Affairs Medical Center Veterinary Medical Unit. Materials were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

UVB exposure studies

Female SKH1 hairless mice (8–10 weeks) were obtained from Charles River Labs (Wilmington, MA) and acclimatized in-house for 2 weeks prior to initiation of experiments. Mice were group-housed (4 per cage), fed on a basal chow diet *ad libitum* and exposed to standard 12-h inverted light/dark cycle. Dorsal skin was exposed to UVB irradiation from an UVX Radiometer (UVP, LLP, Upland, CA) equipped with a sensor to measure the 254–365 nm wavelength output from low-pressure fluorescence lamps. Mice were irradiated initially with 400 mJ/cm². Following euthanasia, full-thickness dorsal skin was collected at day 1, 3 and 7 postexposure to determine optimal time point for dose–response studies. Mice were subsequently irradiated with 50, 100 and 200 mJ/cm² and samples analysed at day 3 postexposure. Tissue collection for all subsequent analyses was standardized by 5-mm punch biopsy (Acuderm, Fort Lauderdale, FL, USA). Data represent biological replicates (e.g. *n* = 4 represent samples from four separate mice).

Transepidermal water loss measurement

Prior to euthanasia, *in vivo* TEWL readings were obtained following exposure to UVB and in non-irradiated skin, at the time points specified above, using a Tewameter TM 300 probe connected to a Multiprobe Adaptor 5 device (Courage and Khazaka, Cologne, Germany).

Quantitative PCR

Freshly isolated tissue (20–40 mg skin) was immediately snap-frozen and stored at –80°C. Tissues were homogenized in 1 ml Tri-zol reagent and RNA extracted using Purelink RNA Mini Kit (Life Technologies, Grand Island, NY, USA). A total of 1.2 μ g RNA was used for RT-PCR by Tetro cDNA Synthesis Kit (Bioline, Taunton, MA, USA). qPCR was conducted

in 10 μ l reactions containing SensiFAST Probe Kit mastermix (Bioline), TaqMan target cDNA primers (900 nM)/FAM probe (250 nM) mix, TaqMan 18S rRNA primers (50 nM) and VIC probe (200 nM) mix (Applied Biosystems, Carlsbad, CA, USA) and 10 ng cDNA. Duplicate PCRs were performed on a 384-well plate format using a 7900 HT instrument (Applied Biosystems) with the following parameters: 5 min 95°C followed by 40 cycles of 95°C for 10 s and 60°C for 50 s. Raw Ct values were normalized to internal control 18S rRNA expression ($-C_t$) and averaged. Statistical analysis was performed on $-C_t$ values of biological replicates although these values were transformed to arbitrary units (2^{-C_t}) for graphical purposes.

Protein isolation and Western Blot

A total of 20–40 mg snap-frozen tissue was homogenized in 300 μ l RIPA buffer (Pierce, Rockford, IL, USA) supplemented with protease inhibitor cocktail (Roche, Mannheim, Germany). Proteins were quantified using a BCA Protein Assay Kit (Pierce) and denatured in 25 μ l reactions as follows: 25 μ g total protein lysate, NuPAGE LDS buffer (Life Technologies) and 5% β -mercaptoethanol heated to 95°C for 5 min. A total of 20 μ g denatured protein was loaded into 1 mm, 12-well NuPAGE 4–12% Bis-Tris Gels and run in NuPAGE MES SDS buffer at 100 V using the XCell SureLock Mini platform (Life Technologies). Separated proteins were transferred to 0.2 μ m Immobilon-P nitrocellulose membrane (BioRad, Hercules, CA, USA) in NuPAGE transfer buffer at 30 V for 2 h using XCell II Blot Module (Life Technologies).

Membranes were blocked for 1 h at room temperature (RT) under agitation in 5% milk in phosphate-buffered saline (PBS) 0.05% Tween-20 (PBST), rinsed briefly in PBST and incubated under agitation overnight at 5°C in blocking buffer with 1 μ g/ml rabbit anti-11 β -HSD1 (ab83522; Abcam, Cambridge, MA, USA) or 0.4 μ g/ml mouse anti- β -actin- peroxidase conjugated primary antibody (A3854; Sigma-Aldrich), as previously described (28). Membranes were washed 3 $\times$ 15 min and incubated under agitation in blocking buffer with 1:5000 sheep anti-mouse IgG-peroxidase antibody (RPN4201; GE Healthcare, Pittsburgh, PA, USA) for 1 h at RT (omitted for anti- β -actin-peroxidase-treated membranes). Following a further 3 $\times$ 15 min PBST washes, membranes were incubated with ECL Plus Western Blotting Substrate for anti-human 11 β -HSD1-treated membranes (Pierce) according to manufacturer's guidelines. Proteins were visualized using a las-3000 luminescent image analyser (FujiFilm, Stamford, CT, USA).

11 β -HSD activity assays

11 β -HSD1 (oxoreductase): Freshly isolated tissue (20–40 mg) was incubated immediately in 1 ml high glucose, pyruvate, DMEM (Life Technologies) containing 100 nM 11-DHC (Steraloids, Newport, RI, USA) with ~1500 cpm of [3H] 11-DHC generated in-house as follows: 5 μ Ci [3H] corticosterone, specific activity 70–100 Ci/mmol (Perkin Elmer, Waltham, MA, USA), 450 μ g homogenized mouse placenta protein (exhibiting high 11 β -HSD2 activity) and 500 μ M NAD⁺ cofactor in 500 μ l (total volume) 0.1 M potassium phosphate buffer pH 7.4 (Bio-World, Dublin, OH, USA) incubated under agitation at 37°C for 3 h. [3H] 11-DHC was extracted by vortexing in 5 ml dichloromethane, removal of aqueous/protein phase by aspiration and concentrating under air at 55°C for 20 min. [3H]

11-DHC was resuspended in 100 μ l dichloromethane, spotted onto foil-backed silica plates and separated by TLC in a 186:14 ml chloroform: ethanol mobile phase for 90 min with 10 mM 11-DHC run in adjacent lanes. Plates were visualized under UV to localize 11-DHC, and this area also containing [3H] 11-DHC was excised and eluted overnight in 500 μ l ethanol at 4°C. Ethanol concentration was adjusted to 1500 cpm/ μ l by liquid scintillation.

11 β -HSD1 assays were incubated at 37°C for 16 h (predetermined by time course to generate ~25% conversion in non-irradiated control samples). Subsequently, tissues were weighed and discarded and steroids extracted and separated as above (co-migrating with 10 mM 11-DHC/corticosterone standards). Plate regions containing 11-DHC and corticosterone were excised and % conversion determined by liquid scintillation.

Immunohistochemistry

Freshly isolated tissue was stored in Formalde-Fresh (Fisher Scientific, Pittsburgh, PA, USA) and processed into paraffin blocks. A total of 5- μ m sections were dewaxed (30 min 56°C, 2 $\times$ 10 min xylene), rehydrated through graded washes of ethanol in water (2 $\times$ 5 min 100% ethanol, 5 min 70% ethanol, deionized water) and stained by H&E according to manufacturer's protocol (Leica, Buffalo Grove, IL, USA and Thermo Scientific, Kalamazoo, MI, USA). Epidermal thickness was recorded by measuring epidermal area using ImageJ software (NIH, Bethesda, MD, USA). For 11 β -HSD1 staining, following rehydration, antigen unmasking was performed by boiling for 10 min in citrate buffer pH6 (Vector Labs., Burlingame, CA, USA) before gradual cooling to RT. Following washing for 5 min in PBS, sections were blocked in 4% bovine serum albumin + 0.5% cold water fish gelatin in PBS for 1 hr at RT. Blocking solution was then replaced with anti-11 β -HSD1 primary antibody (ab83522; Abcam) at 1 μ g/ml in blocking solution for 30 min at RT. Primary antibody neutralized with immunizing peptide was used for negative control sections. Slides were rinsed 3 $\times$ 5 min in PBS and incubated with Alexa Fluor® 488 goat anti-rabbit IgG (H+L) secondary antibody (A11008; Life Technologies) diluted 1:2000 in blocking solution for 30 min at RT. Following 3 $\times$ 5 min washes in PBS, sections were counter-stained for cell nuclei with propidium iodide solution in deionized water for 15 s at RT. Slides were rinsed 3 $\times$ 5 min in PBS, cover-slipped in Fluoro-Gel aqueous mounting media (Electron Microscopy Sciences, Hatfield, PA, USA) and visualized using an epifluorescence Zeiss Observer D1 microscope (Carl Zeiss International, Thornwood, NY, USA) connected to a Lambda XL mercury lamp (Sutter Instruments, Novato, CA, USA). Excitation filters for propidium iodide and Alexa Fluor® 488 were BP 550/25 nm and 470/40 nm, dichroics were FT 570 nm and 495 nm, and emission filters were 605/70 nm and 525/50 nm, respectively.

Statistical analysis

Following confirmation normal distribution, significance levels were determined by Student's *t*-test or Pearson correlation using GraphPad Prism (La Jolla, CA, USA).

Results

UVB induces 11 β -HSD1 expression and activity *in vivo*

To examine the regulation of 11 β -HSD1 by UVR, mice were exposed to 400 mJ/cm² UVB. 11 β -HSD1 mRNA expression increased 2.7-fold 1 day postexposure compared to non-irradiated control skin (Ct $\pm$ SEM 20.5 $\pm$ 0.2 vs 22.0 $\pm$ 0.3, respectively, $P < 0.01$), although 11 β -HSD1 mRNA expression remained elevated at 3 and 7 days postexposure, this was not statistically significant (Fig. 1a). Increased 11 β -HSD1 protein expression was detectable by day 3 postexposure (Fig. 1b). Supporting these expression data, we observed an 1.8-fold increase in 11 β -HSD1 activity compared to control skin at day 3 (pmol/h $\pm$ SEM 3.7 $\pm$ 0.6 vs 2.1 $\pm$ 0.1, respectively, $P < 0.05$, Fig. 1c). Both 11 β -HSD1 protein expression and enzyme activity subsequently declined to non-irradiated control levels by day 7 (Fig. 1b,c). These findings demonstrate that a single dose of UVB induces 11 β -HSD1 expression and activity *in vivo*, which could drive increased local GC activation and modulate postexposure recovery mechanisms.

H6PDH (11 β -HSD1 co-factor supplying enzyme) mRNA expression was 2.5-fold greater 1 day postexposure (Ct $\pm$ SEM 18.3 $\pm$ 0.4 vs 19.5 $\pm$ 0.1, $P < 0.01$), declining to control levels by day 7 (Fig. 1d). In contrast, GR α mRNA expression was 30% lower than control skin at day 3 (Ct $\pm$ SEM 16.5 $\pm$ 0.1 vs 16.0 $\pm$ 0.2, respectively, $P < 0.05$), suggesting a possible negative feedback mechanism (Fig. 1e). Consistent with previous studies (28), 11 β -HSD2 mRNA expression was undetectable in control skin and was also unaffected by UVB exposure.

As 11 β -HSD1 activity was greatest at day 3 postexposure (Fig. 1c), this time point was used to further examine UVB dose–response effects. 11 β -HSD1 activity was upregulated by 200 mJ/cm² and 100 mJ/cm² but not 50 mJ/cm² UVB (1.5-fold, $P < 0.001$ and 1.4-fold, $P < 0.05$, respectively), indicating regulation also occurs at doses of UVB relevant to human exposure levels (Fig. 1f).

11 β -HSD1 expression localizes to hyperproliferative epidermis postexposure to UVB

Compared to non-irradiated control skin, histological H&E analysis of skin at day 3 following UVB exposure revealed a hyperproliferative epidermis (Fig. 2a). Epidermal thickness was 3.8-fold ($P < 0.001$), 3.7-fold ($P < 0.001$) and 1.9-fold ($P < 0.001$) greater in skin irradiated with 100 mJ/cm², 200 mJ/cm² and 400 mJ/cm² UVB, respectively (Fig. 2b).

11 β -HSD1 immunofluorescence was observed in the epidermis, hair follicle outer root sheath cells (continuous with basal epidermal keratinocytes) and to a lesser extent in dermal fibroblasts (Fig. 3a). Epidermal 11 β -HSD1 immunofluorescence was concentrated in the (basal) *stratum basale*, more diffuse in the suprabasal layers and absent in the (cornified) *stratum corneum*. Irradiation with 100 mJ/cm² UVB increased total 11 β -HSD1 expression in the epidermis, largely due to the increased epidermal thickness rather than increased keratinocyte expression (Fig. 3b). A similar response in 11 β -HSD1 localization was observed following irradiation with 200 mJ/cm² UVB (Fig 3c); however, irradiation with 400 mJ/cm² UVB resulted in immunofluorescence of increased intensity extending to the more distal (granular) *stratum granulosum* (Fig. 3d). These findings are in agreement with

the comparable inductions in 11 β -HSD1 activity at all three UVB doses (Fig. 1f), despite a decreased epidermal thickness with 400 mJ/cm² UVB compared to 200 mJ/cm² or 100 mJ/cm² UVB (Fig. 2b). We also noted the presence of dorsal lesions 3 days postexposure in animals exposed to 400 mJ/cm² UVB (Tiganescu A, unpublished observation). Negligible fluorescence was observed following pre-incubation of anti-11 β -HSD1 antibody with immunizing peptide as negative control (Fig. 3d inset).

Dermal 11 β -HSD1 immunofluorescence was found to be unaffected by UVB exposure at 100 mJ/cm² or 200 mJ/cm² but displayed a modest increase in fluorescence at 400 mJ/cm² UVB (Figure S1). However, relative to epidermal expression, the dermal contribution to overall 11 β -HSD1 activity at the maximal dose of UVB remains limited. We did not observe any discernible differences in 11 β -HSD1 protein localization in other skin appendages (e.g. sebaceous glands) following UVB irradiation.

Increased TEWL correlates with 11 β -HSD1 activity postexposure to UVB

To determine whether increased local GC activation following UVR (due to increased 11 β -HSD1) may modulate repair mechanisms, we analysed TEWL as measure of skin barrier integrity. Indicative of barrier disruption, TEWL was 12.7-fold greater in skin irradiated with 400 mJ/cm² UVB 3 days postexposure compared to controls (g/h/m² $\pm$ SEM 49.1 $\pm$ 5.5 vs 3.9 $\pm$ 0.8, $P < 0.001$), decreasing to control levels by day 7 postexposure (Fig. 4a). TEWL was also increased by 50 mJ/cm² (2.4-fold, $P < 0.05$), 100 mJ/cm² (7.6-fold, $P < 0.01$) and 200 mJ/cm² UVB (12.2-fold, $P < 0.001$) 3 days postexposure (Fig. 4b).

Interestingly, TEWL was positively correlated with 11 β -HSD1 activity ($r = 0.95$, $P < 0.01$, Fig. 4c). Although a coincidental association cannot be excluded, our findings indicate that increased 11 β -HSD1 activity following UVB irradiation is concomitant with increased barrier disruption, suggesting local GC availability may regulate UVR damage or repair processes.

Discussion

Although the atrophogenic effects of GC on skin structure and function are well documented (30), regulation of local GC metabolism in this organ remains largely unexplored compared to the extensively documented roles of 11 β -HSDs in liver, adipose, muscle, pancreas, kidney, vasculature, heart, bone, brain and other tissues (16,17). Furthermore, the direct effects of UVR on 11 β -HSD1 expression *in vivo* were previously unknown. In this study, we investigated the regulation of the GC-activating isozyme 11 β -HSD1 following acute UVB irradiation in mice.

We previously reported increased expression of 11 β -HSD1 during skin ageing and wound healing (24,25,28), where 11 β -HSD1 blockade accelerated lesion closure and improved age-associated impairments in skin structure demonstrating the ability of local GC activation by 11 β -HSD1 to regulate these processes (25). This may be of particular relevance in conditions of systemic GC excess, for example following physical restraint stress (31), where not only active GC secretion is increased but also 11 β -HSD1 substrate availability following deactivation of cortisol/corticosterone to cortisone/11-DHC by 11 β -HSD2 in the

kidneys (32). Indeed, a recent report by Morgan et al. (33) demonstrated, strikingly, that 11 β -HSD1 KO mice with circulating GC excess are protected from the glucose intolerance, hyperinsulinemia, hepatic steatosis, adiposity, hypertension, myopathy and dermal atrophy.

Here, we report the direct stimulation of 11 β -HSD1 mRNA and protein expression and enzymatic activity following a single dose of UVB *in vivo*, supporting our previous observations of increased 11 β -HSD1 expression and activity in human skin derived from UVR exposed sites (25). Although the increase in 11 β -HSD1 levels may be considered modest, it is of a similar magnitude to that previously demonstrated to be functionally relevant in mouse skin (25). Importantly, a recent study also demonstrated increased circulating corticosterone levels following *in vivo* UVB exposure (34). It is likely that these translate to elevated skin corticosterone levels postexposure *indirectly* by increasing 11 β -HSD1 substrate (11-dehydrocorticosterone) availability (33).

Studies have also demonstrated that steroidogenesis contributes to UVB-induced local GC generation (21); however, we recently reported that 11 β -HSD1 activates considerably more corticosterone (the active rodent GC) than synthesized by steroidogenesis from progesterone in unwounded mouse skin and during wound healing (due to the absence of CYP11B1, the final enzyme required for the conversion of 11-deoxycorticosterone to corticosterone) (28).

Our results indicate that increased 11 β -HSD1 activity in UVB-irradiated skin results from keratinocyte hyperproliferation (at doses 100 mJ/cm²) and increased keratinocyte 11 β -HSD1 expression (at 400 mJ/cm²). The greatest increase in 11 β -HSD1 activity was noted at the highest dose of UVB, supported by increased 11 β -HSD1 immunofluorescence despite a less pronounced increase in epidermal thickness – likely due to sunburn-induced keratinocyte apoptosis which has been previously reported (35) and supported by our observations of dorsal lesions at this dose. Our results are in agreement with a recent report of increased 11 β -HSD1 mRNA and protein expression following UVB (but not UVA) irradiation of neonatal human epidermal keratinocyte/melanocyte cocultures and human foreskin explants (36). However, these *in vitro* studies were limited by the absence of a fully differentiated epidermis. These authors also observed a decrease in GR α expression supporting our finding of reduced GR α mRNA expression 3 days postexposure. This is also in agreement with our earlier studies demonstrating negative feedback regulation of the GR α by GC in dermal fibroblasts (24). Interestingly, Skobowiat et al. (36) further reported a differential increase in 11 β -HSD2 (GC deactivating) expression following UVA irradiation. While this finding does not impact on the current study (as mice lack skin 11 β -HSD2 expression), it identifies a necessity for future studies using human material to evaluate the effect of combined UVA/UVB exposure on overall 11 β -HSD levels.

Although the data presented here demonstrate that UVB induces 11 β -HSD1 expression predominantly in the epidermis, regulation of GC metabolism by fibroblasts in the underlying dermis may also contribute to 11 β -HSD activity following UVR exposure. Indeed, our previous studies using sections obtained from photoexposed (lower outer arm) compared to photoprotected (upper inner arm) human skin revealed increased 11 β -HSD1 protein expression in both keratinocytes and dermal fibroblasts (25). This may be of particular relevance to UVA irradiation which can penetrate to the dermal compartment,

while UVB is largely absorbed by the epidermis (37) and may explain the lack of dermal 11 β -HSD1 upregulation reported here.

Our results also demonstrate a positive correlation between UVB-induced 11 β -HSD1 activity and TEWL. Although a coincidental association cannot be excluded, GCs are known to induce TEWL (38), suggesting the increase in 11 β -HSD1 following UVR may drive epidermal barrier disruption. Conversely, increased local GC availability through 11 β -HSD1 postexposure may assist barrier recovery by promoting keratinocyte terminal differentiation (39,40), an effect also mediated by UVB exposure (41). Interestingly, improved epidermal barrier function has been reported in murine inflammatory skin disease models following restraint stress (42). While promoting differentiation, GCs concomitantly inhibit keratinocyte proliferation (30,43) therefore increased local GC availability following acute UVR exposure may function to normalize epidermal hyperproliferation. However, persistent exposure could result in constitutive 11 β -HSD1 activation and contribute to the epidermal thinning associated with photodamage (44). Moreover, a GC-mediated bias favouring keratinocyte differentiation may limit the necessary proliferative capacity required for effective wound healing, supported by observations of impaired healing in photoexposed skin (45).

UVR-induced keratinocyte apoptosis is also impaired by GC treatment (40). As apoptosis is a crucial pathway involved in protection against photocarcinogenesis (46), UVB-mediated induction of 11 β -HSD1 may contribute to increased melanoma risk and resistance to subsequent therapy. Indeed, anti-apoptotic effects of GC have been reported for several cancers including brain, liver, ovarian and breast (9–12). GC treatment was also shown to impair normal repair of UVR-induced DNA lesions, further pre-disposing skin to mutagenic events (8).

However, anticarcinogenic effects of GC following UVR irradiation have also been reported (13,14). Further, a decrease in local GC availability resulting from decreased 11 β -HSD1 and/or increased 11 β -HSD2 expression has been associated with increased tumor size and proliferative capacity and poorer prognosis in several cancers including adrenal cortical carcinoma and adenoma (47), pituitary adenoma (48), colorectal cancer (49), leukaemic cells (50) and osteosarcoma (51). Interestingly, recent studies report chemical-induced carcinogenesis is more pronounced in epidermal GR-deficient mice (15); however, the role of 11 β -HSDs in regulating skin carcinogenesis remains to be investigated.

Although the exact factors driving local GC activation following UVB exposure are currently unknown, pro-inflammatory cytokines including TNF- α and IL-1 that are upregulated during erythema (52) are also potent inducers of 11 β -HSD1 in skin and other tissues (17). Through this mechanism, 11 β -HSD1 may contribute to resolution of inflammation following UVR exposure. However, growing evidence suggests that the inflammation-modulating functions of GC are more complex than previously anticipated. For example, although 11 β -HSD1-deficient mice exhibit an earlier onset of inflammation during serum-induced experimental arthritis and more pronounced inflammatory cell recruitment following myocardial infarction compared to wild-type mice (53,54), they also display decreased inflammatory cell infiltration of mesenteric adipose tissue following high-

fat diet feeding (55). 11β -HSD1 inhibition also reduces inflammation in atherosclerotic lesions in *ApoE*^{-/-} mice (56). Therefore, under specific conditions that require further clarification, GC can potentiate inflammation, challenging traditional views that regard them primarily as anti-inflammatory molecules (57). These contradictory effects may result from tissue-specific responses, inflammatory stimulus type and duration (e.g. acute or chronic) or most likely, a combination of these elements (58). It is possible that TNF- α and IL-1 may also contribute to increased 11β -HSD1 expression during wound healing and ageing, where pro-inflammatory stimuli are strongly implicated (59,60). Moreover, GCs regulate 11β -HSD1 levels in a forward feedback manner (while H6PDH expression is largely unaffected by GC treatment) (16,24) also acting in synergy with pro-inflammatory cytokines to further amplify local GC availability (61). Finally, it is noteworthy to speculate that the findings presented here may contribute to the underlying mechanism of phototherapy in psoriasis, a condition also effectively treated with topical GC (62).

In summary, our findings demonstrate that 11β -HSD1 activity increases following UVB irradiation *in vivo* and correlates with postexposure barrier disruption. Further studies are warranted to delineate beneficial/adverse effects of local GC metabolism on UVR response processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Regulation of local glucocorticoid metabolism following UVB exposure. (a) 11 β -HSD1 mRNA expression (normalized to 18S rRNA) at 1, 3 and 7 days postexposure to 400 mJ/cm² UVB compared to non-irradiated controls ($n = 4$). (b) Corresponding 34 kDa 11 β -HSD1 protein expression (with 42 kDa β -actin expression presented as a loading control). (c) Corresponding 11 β -HSD1 activity [rate of corticosterone activation from 100 nM 11-dehydrocorticosterone (11-DHC), $n = 4$]. (d) H6PDH mRNA expression (normalized to 18S rRNA) at 1, 3 and 7 days postexposure to 400 mJ/cm² UVB compared to non-irradiated controls ($n = 4$). (e) Corresponding GR α mRNA expression ($n = 4$). (f) 11 β -HSD1 activity (rate of corticosterone activation from 100nM 11-DHC) at day 3 following exposure to 50, 100, 200 and 400 mJ/cm² UVB ($n = 4$). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Figure 2.

Increased epidermal thickness following UVB irradiation. (a) Representative H&E stained sections of non-irradiated control skin compared to skin irradiated with 100, 200 and 400 mJ/cm² UVB at day 3 postexposure. d, dermis; e, epidermis; sg, sebaceous gland; pc, *panniculus carnosus* muscle. Scale bar 50 μm. (b) Fold change in epidermal thickness at day 3 postexposure to 100, 200 and 400 mJ/cm² UVB compared to non-irradiated controls ($n = 5$). *** $P < 0.001$.

Figure 3.

11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) localizes to epidermal keratinocytes following UVB irradiation. Skin irradiated with 100, 200 and 400 mJ/cm² UVB at day 3 postexposure compared to non-irradiated skin immunostained for 11 β -HSD1 (green) and counterstained with red nuclear stain ($n = 4$). Negative control sections were treated with anti-11 β -HSD1 antibody pre-incubated with immunizing peptide. d, dermis; e, epidermis; hf, hair follicle. Magnification 40 $\times$.

Figure 4.

Increased transepidermal water loss (TEWL) following UVB irradiation correlates with 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) activity. (a) TEWL (increased TEWL denotes impaired barrier integrity) at 1, 3 and 7 days postexposure to 400 mJ/cm² UVB compared to non-irradiated controls ($n = 4$). (b) TEWL at day 3 following exposure to 50, 100, 200 and 400 mJ/cm² UVB ($n = 4$). (c) Correlation of TEWL with 11 β -HSD1 activity. Points represent the means for all dose and time points measured ($n = 4$). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.