Blocking local glucocorticoid activation improves skin thinning and impaired wound healing in Cushingoid mice

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Introduction

Cushing's disease presents with multiple symptoms of systemic glucocorticoid (GC) excess including increased skin thinning and poor wound healing (WH).

Local GC concentrations are regulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which activates cortisol or corticosterone from cortisone or 11-dehydrocorticosterone in human or mouse tissues respectively, including skin (Fig. 1). We recently demonstrated elevated 11β-HSD1 activity during early WH (**Tiganescu *et al*. 2014**). Here, we hypothesize that local 11β-HSD1 blockade improves the WH delay caused by systemic GC excess.

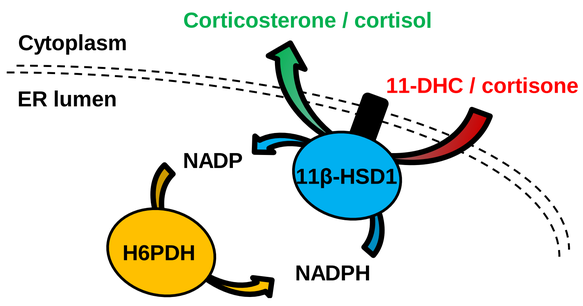

Fig. 1. GC activation by 11β-HSD1 requires NADPH co-factor supplied by hexose-6-phosphate dehydrogenase (H6PDH)

Methods

We examined the effects of systemic GC excess (or vehicle) in female SKH1 mice which developed Cushingoid features and suppressed endogenous serum corticosterone vs. vehicle (5.5 vs. 18.5ng/ml, $p<0.01$) over 5 weeks corticosterone (CORT) therapy (100μg/ml in drinking water). Mice were treated bi-daily with 30mM (200μg) topical carbenoxolone (CBX, 11β-HSD1 inhibitor) or vehicle (VEH), one week prior to and post-wounding (by double 5mm dorsal biopsy).

Results

1. 11β-HSD1 blockade did not affect skin function in young healthy SKH1 female mice

CBX treatment inhibited 11β-HSD1 activity by 70% before wounding (Fig. 2a) and >90% during WH (Fig. 2b).

11β-HSD1 inhibition did not affect epidermal thickness (Fig. 3a).

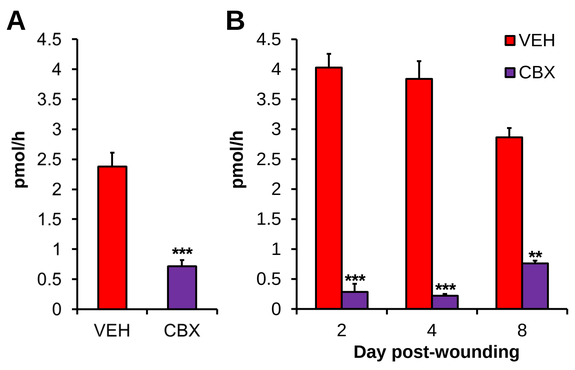

Fig. 2. 11β-HSD1 activity determined by incubation of 5mm skin explants with 100nM 11-DHC (100pmol) labelled with [^{3}H] 11-DHC pre-wounding (**A**) and during healing (**B**), n=4

CBX treatment did not affect transepidermal water loss (TEWL; a measure of epidermal barrier integrity) neither before disruption by tape stripping nor during subsequent recovery (Fig. 3b). The number of tape strips required for disruption was unaffected by CBX treatment (VEH 3.3±0.1 S.E vs. CBX 3.2±0.1). Wound healing rate was also unaffected by CBX (Fig. 3c).

2. Systemic GC excess induces GC activation in skin

11β-HSD1 activity in unwounded CORT skin was 42% higher than VEH and was inhibited >60% by CBX (Fig. 4a), supported by elevated 11β-HSD1 mRNA pre- and post-wounding (Fig. 4b). H6PDH and glucocorticoid receptor mRNA were also upregulated (Fig. 4c, 4d).

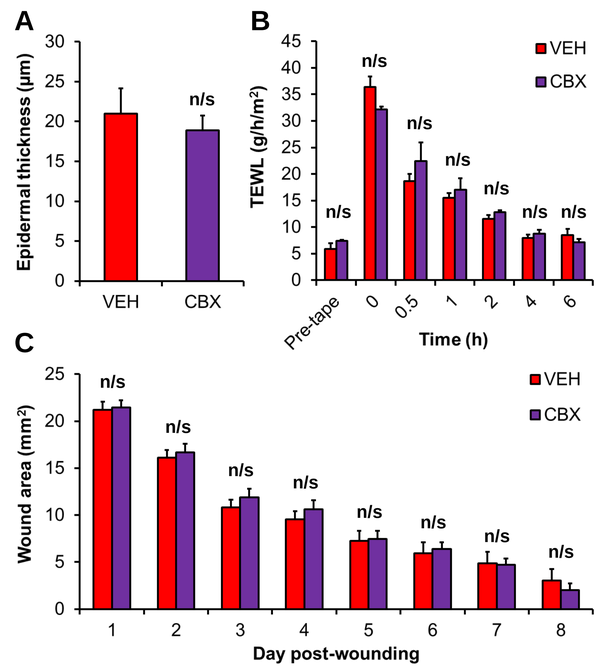

Fig. 3. 11β-HSD1 inhibition did not affect epidermal thickness (**A**, n=4), pre-disruption transepidermal water loss and recovery following tape stripping (**B**, n=4) or wound healing (**C**, n=8-24) in healthy SKH1 mice

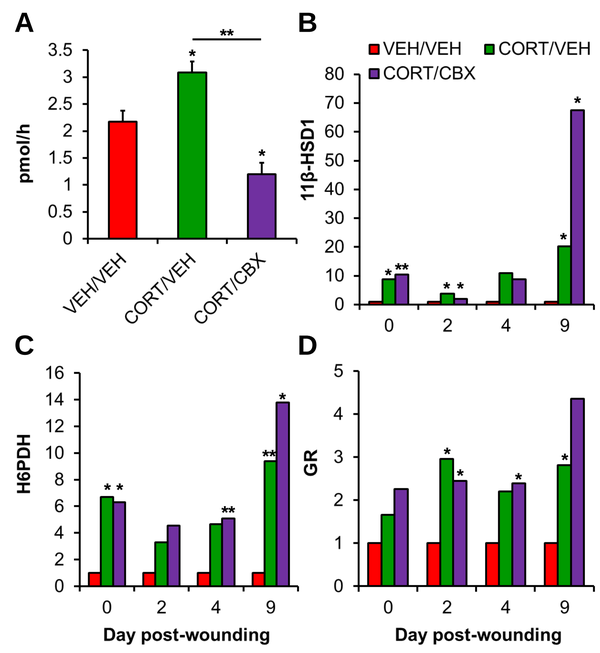

Fig. 4. 11β-HSD1 activity was elevated in unwounded CORT mouse skin (**A**, n=5). 11β-HSD1 (**B**), H6PDH (**C**) and glucocorticoid receptor (GR, **D**) mRNA were also elevated during WH (fold-change vs. VEH, n=3-5)

3. Detrimental consequences of systemic GC excess on epidermis are improved by topical 11β-HSD1 blockade

Epidermal thinning induced by CORT treatment was reduced by 35% with CBX (Fig. 5a, 5b).

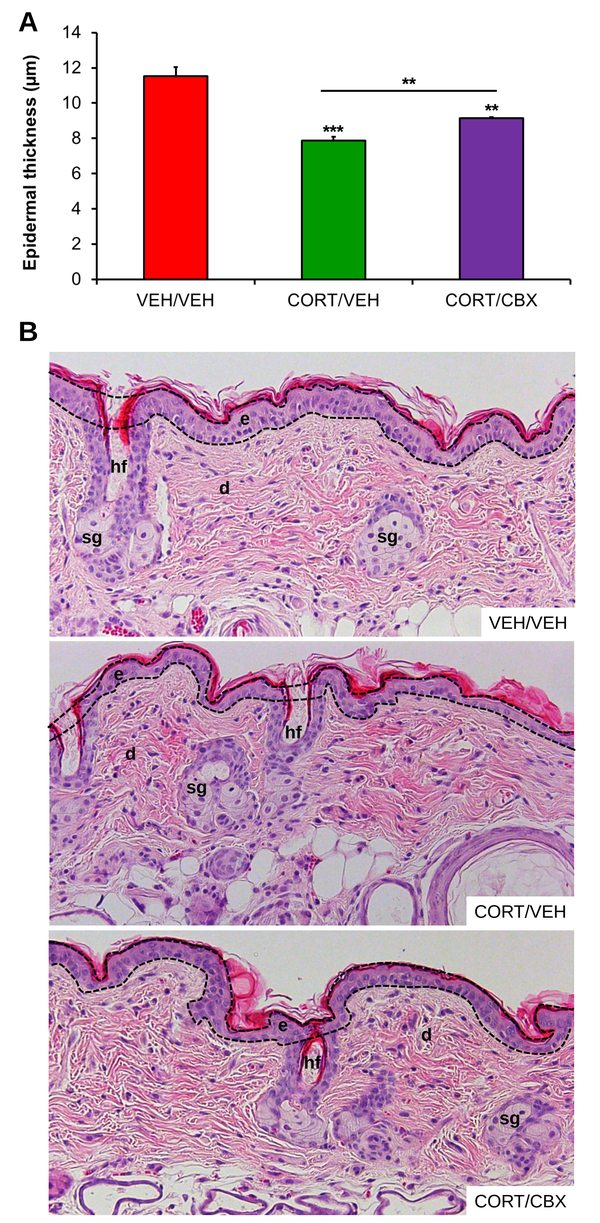

Fig. 5. CORT-induced epidermal thinning was improved by CBX (**A**). Representative H&E sections (**B**), **e**; epidermis, **d**; dermis, **hf**; hair follicle, **sg**; sebaceous gland, 20X magnification n=4

Systemic GC excess did not influence resting TEWL, nor recovery following disruption (Fig. 6a). However, CORT-treated skin was more resistant to tape stripping, requiring 2.7 more tapes to induce TEWL comparable to VEH. This was normalized by CBX (Fig. 6b).

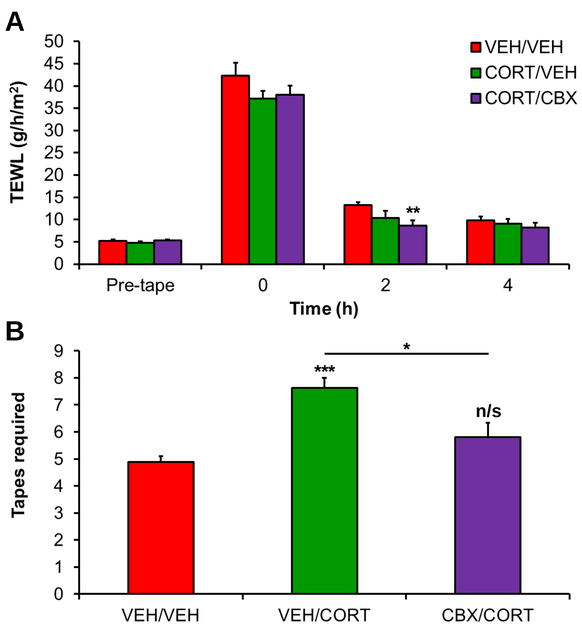

Fig. 6. CORT did not affect pre-disruption transepidermal water loss or recovery following tape stripping (**A**). CORT-treated mice required more tapes to attain a comparable degree of disruption, normalized by 11β-HSD1 inhibition (**B**), n=7-12

4. GCs inhibit early and drive late human keratinocyte differentiation *in vitro*

Primary human keratinocytes (hK) were incubated in 0.07mM calcium 154CF±100nM cortisol for 48h before differentiating in 1.2mM calcium for 6d. Loricrin, and desmoglein-1 proteins (late differentiation) were elevated whereas keratin 10 (early differentiation) was lower in cortisol-treated cells (Fig. 7a, 7b). Conversely, 5μM RU486 (GR inhibitor) treatment reduced loricrin and desmoglein-1 during differentiation (Fig. 7c). 11β-HSD1 activity also increased during differentiation (Fig. 7d).

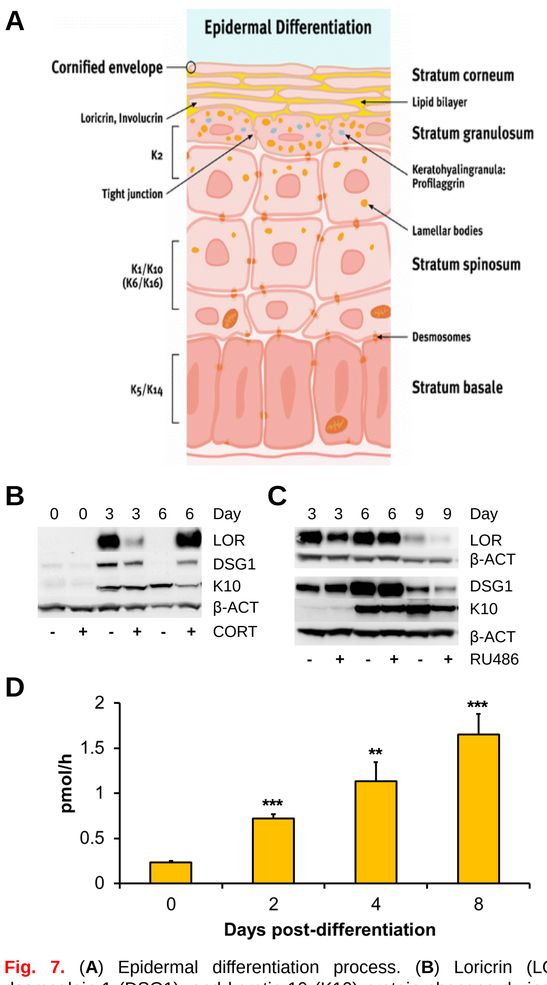

Fig. 7. (**A**) Epidermal differentiation process. (**B**) Loricrin (LOR), desmoglein-1 (DSG1), and keratin-10 (K10) protein changes during hK differentiation ± cortisol or (**C**) ± RU486. (**D**) 11β-HSD1 activity n=3

5. CBX improves WH in CORT-treated mice

WH 2-4d post-wounding was unaffected by CORT or CBX treatment . From this point, no further healing was observed for CORT/VEH mice whereas CBX-treated wounds continued healing to 48% by 9d compared to control wounds which healed 93% by 9d (Fig. 8a, 8b).

CBX also normalized mRNA changes induced by CORT during impaired WH for several genes including interleukin-6, fibroblast growth factor-7, matrix metalloproteinase-9 and collagen-1 (Fig. 9). Expression of filaggrin, tumor necrosis factor, interleukin-1β and transforming growth factor-β1 was not regulated by CORT in unwounded skin or during WH (data not shown).

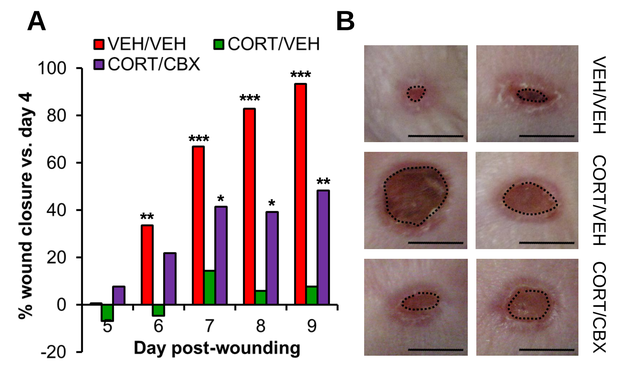

Fig. 8. Topical 11β-HSD1 inhibition improved WH in CORT-treated mice (**A**). Representative images of wounds (**B**). Scale bar 3mm n=8-24

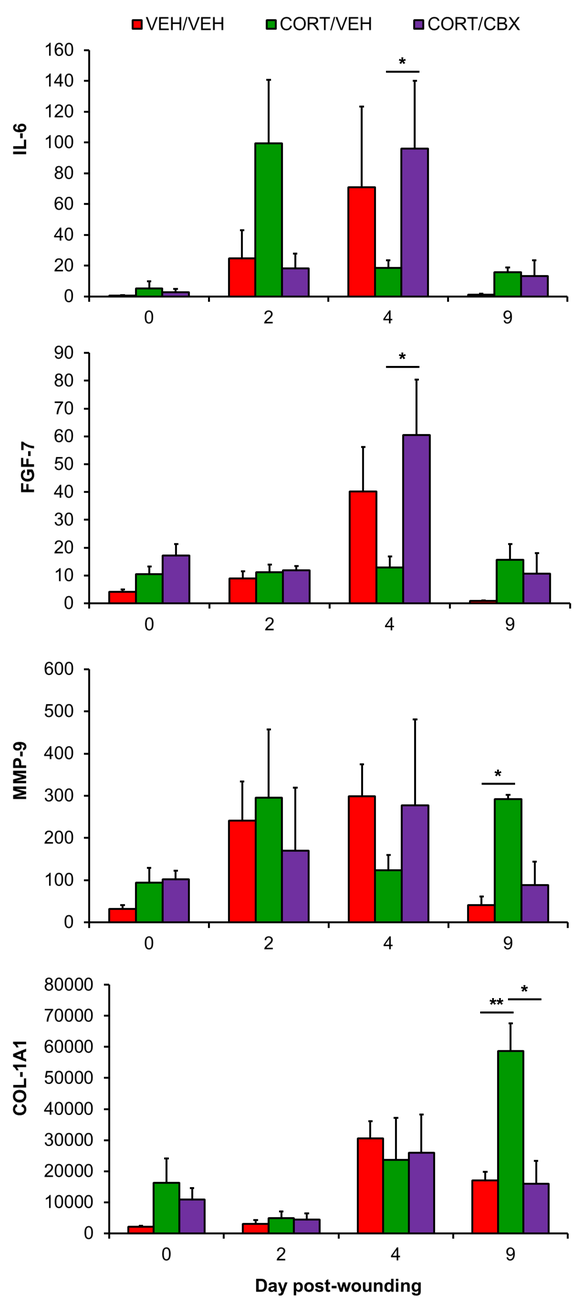

Fig. 9. 11β-HSD1 blockade normalized mRNA expression of interleukin-6 (IL-6), fibroblast growth factor-7 (FGF-7), matrix metalloprotease-9 (MM-9) and collagen-1 (COL-1A1) during WH n=3-6

Conclusion

We recently demonstrated 11β-HSD1 activity increases during mouse skin WH. Although this activity was effectively blocked with the 11β-HSD1 inhibitor CBX, measures of skin function were unaffected in control mice.

However, local CBX therapy partially reversed the effects of systemic corticosterone treatment on epidermal thickness, resistance to barrier disruption and WH suggesting the adverse effects of organismal GC excess (Cushing's syndrome, chronic stress) are mediated by peripheral tissue reactivation of 11-DHC/cortisone by 11β-HSD1.

11β-HSD1 inhibitors may be of particular benefit in older patients where increased 11β-HSD1 activity has been reported in skin **Tiganescu *et al*. 2013, 2011**) and other tissues, potentially amplifying and prolonging the effects of stress in the elderly.

Publications

Tiganescu A *et al*. Increased glucocorticoid activation during mouse skin wound healing. **J Endocrinol.** 2014

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

Tiganescu A *et al*. Localization, age- and site-dependent expression, and regulation of 11β-hydroxysteroid dehydrogenase type 1 in skin. **J Invest Dermatol.** 2011 131: 30-6

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