Suppression of 11β-hydroxysteroid dehydrogenase type 1 target gene regulation by hypoxia

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Introduction

Delayed wound healing (WH), characterized by ischemia, is exacerbated by glucocorticoid (GC) excess. Local GC availability is regulated by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which converts cortisol and corticosterone from inert cortisone and 11-dehydrocorticosterone (11-DHC) in humans and rodents respectively (**Fig. 1**).

We previously reported increased 11β-HSD1 activity during WH (**Tiganescu et al. 2014**) and improved WH in 11β-HSD1-null mice (**Tiganescu et al. 2013**) but regulation of 11β-HSD1 by hypoxia in human skin remains unknown.

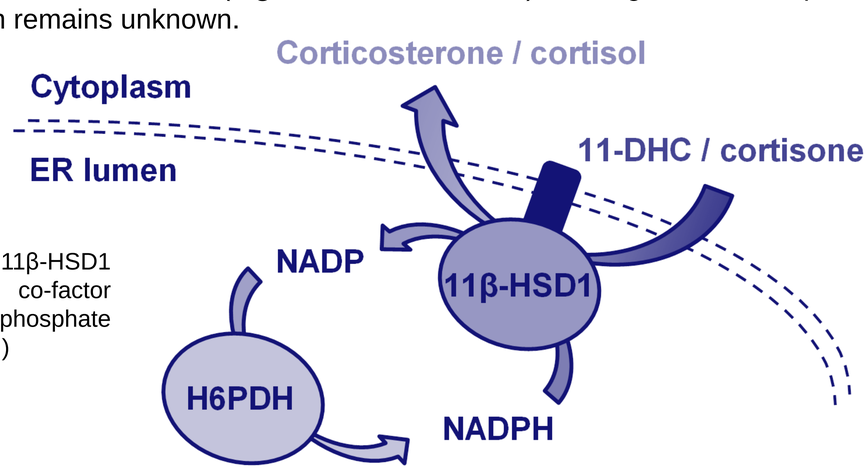

Fig. 1 GC activation by 11β-HSD1 requires NADPH co-factor supplied by hexose-6-phosphate dehydrogenase (H6PDH)

Methods

Primary human dermal fibroblasts (HDF, biological n=3), were treated with vehicle, IL-1β (10ng/ml), cortisol (100nM), IL-1β + cortisol, IL-1β + cortisone (200nM) or IL-1β + cortisone + 11β-HSD1 inhibitor (1μM, **Fig. 2**). Cells were incubated for 96 hours in normoxic (21% O_2) or hypoxic (1% O_2) conditions using a climate-controlled Don Whitley Scientific H35 Hypoxystation (**Fig. 3**). Gene expression was analysed by qPCR after normalizing to 18S rRNA.

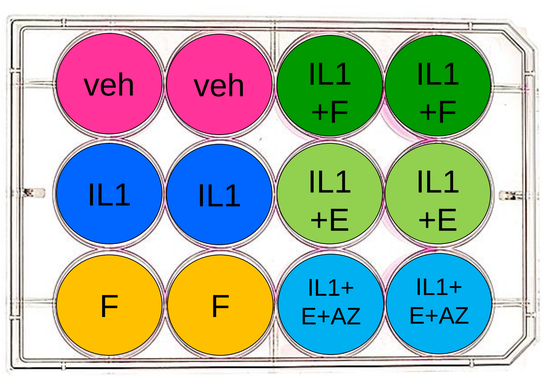

Fig. 2 HDF were treated for 96h with vehicle (veh, 0.05% ethanol), IL-1β (IL1), cortisol (F), IL1 + F, IL1 + cortisone (E) or IL1 + E + the selective11β-HSD1 inhibitor AZD4017 (AZ) in the presence of absence of hypoxia (treatments were conducted in duplicate and each replicate was analysed by qPCR in duplicate with a biological n=3)

Fig. 3 Treated cells were incubated for 96h in a H35 Hypoxystation (Don Whitley Scientific) at 5% CO_2 and 37° C. After incubation, RNA was extracted, cDNA was generated by reverse transcription PCR and gene expression was analysed by Taqman qPCR

Results

1. Suppression of IL-1β-induced 11β-HSD1 mRNA by hypoxia is GC-dependent

IL-1β increased 11β-HSD1 mRNA by 107-fold and 183-fold ($p<0.05$) in normoxia and hypoxia, respectively (**Fig 4**). Hypoxia (vs. normoxia) supressed 11β-HSD1 expression with IL-1β + cortisol 57% ($p<0.05$), with a similar trend for IL-1β + cortisone (32%, $p=0.14$) but not with IL-1β alone or IL-1β + cortisone + AZD4017 (**Fig 4**).

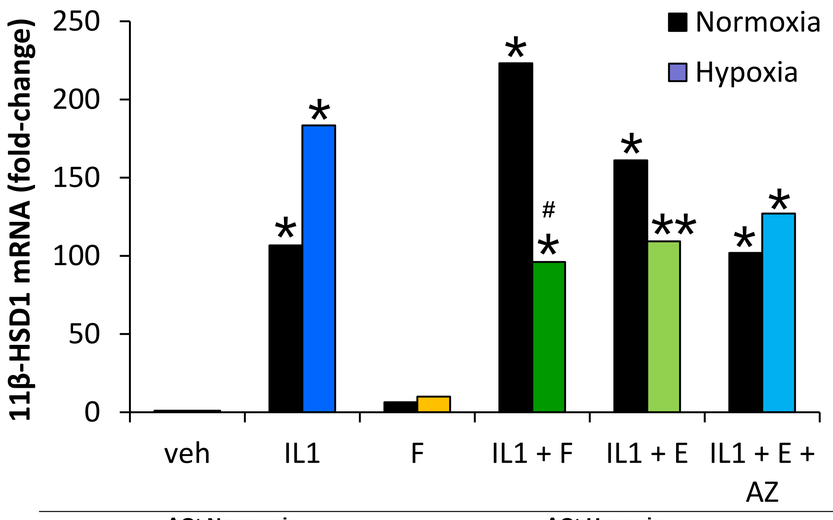

Fig. 4 11β-HSD1 mRNA was induced by IL-1β (IL1) vs. vehicle (veh) in all treatments (*). This was suppressed by hypoxia in the presence of cortisol (F, #), with a similar trend for cortisone (E) but not E + 11β-HSD1 inhibitor AZD4017 (AZ). N=3, * = $p<0.05$, ** = $p<0.01$.

	ΔCt Normoxia			ΔCt Hypoxia			p vs. Normoxia
	n1	n2	n3	n1	n2	n3	
veh	17.9	17.7	20.6	22.1	17.7	20.8	0.406
IL1	14.5	11.1	12.9	13.4	14.3	13.8	0.509
F	19.1	18.7	16.4	22.3	15.1	16.3	0.933
IL1 + F	11.6	11.5	11.6	15.2	13.2	13.6	**0.055**
IL1 + E	13.7	12.1	11.9	15.0	12.3	13.6	**0.147**
IL1 + E + AZ	13.6	12.9	12.6	15.1	13.1	12.9	0.234

2. MMP1 and TIMP4 are less sensitive to GC and unaffected by hypoxia

Matrix metalloproteinase 1 (MMP1) and tissue inhibitor of matrix metalloproteinase 4 (TIMP4) differentially modulate matrix remodelling during WH. Cortisol suppressed IL-1β-induced MMP1 by 71% ($p=0.07$) and 94% ($p<0.05$, **Fig. 5**) and increased TIMP4 mRNA (further increased by IL-1β in hypoxia) by 3.6-fold ($p<0.05$) and 1.9-fold ($p<0.01$) in normoxia and hypoxia, respectively (**Fig. 6**). Cortisone did not significantly reproduce the effects of cortisol for these genes.

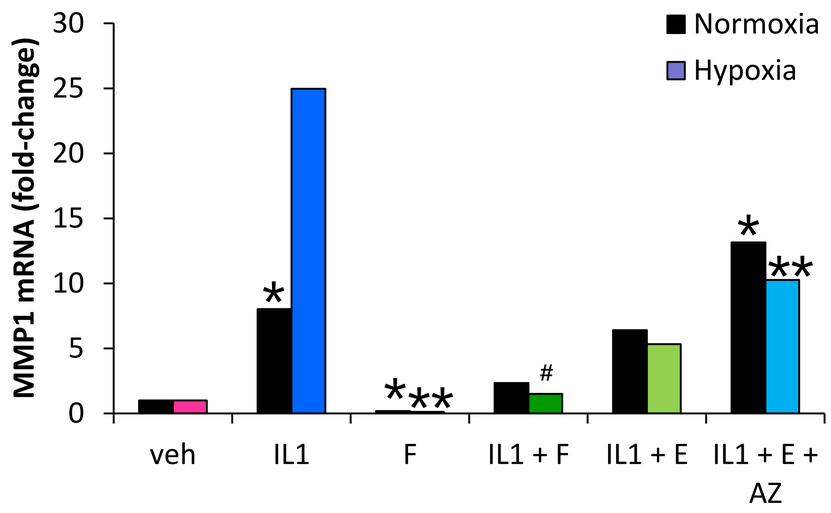

Fig. 5 MMP1 mRNA was induced by IL-1β (IL1) and suppressed by cortisol (F) vs. vehicle (veh, *). F, but not cortisone (E), suppressed IL-1β-induced MMP1 vs. IL-1β (#). These effects were independent of hypoxia. N=3, * = $p<0.05$, ** = $p<0.01$.

	ΔCt Normoxia			ΔCt Hypoxia			p vs. Normoxia
	n1	n2	n3	n1	n2	n3	
veh	9.9	11.1	11.6	14.4	11.0	10.8	0.554
IL1	8.4	8.0	8.0	8.5	9.4	7.5	0.644
F	13.3	13.7	13.4	17.4	13.8	14.0	0.346
IL1 + F	9.3	10.6	9.6	12.7	11.6	11.4	**0.105**
IL1 + E	8.7	8.7	8.1	10.8	10.5	9.4	**0.020**
IL1 + E + AZ	7.7	8.2	6.9	10.5	8.0	7.9	0.299

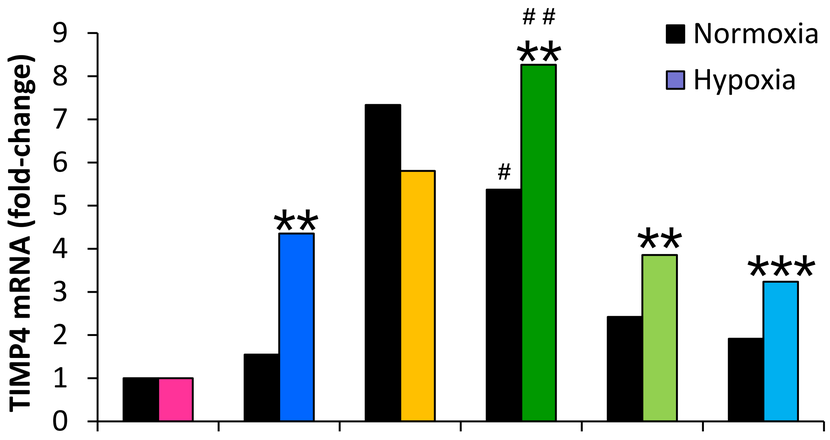

Fig. 6 TIMP4 mRNA was induced by cortisol (F) vs. vehicle (veh, *) and vs. IL-1β (IL1, #) but not by cortisone (E). IL-1β induced TIMP4 expression in hypoxia (but not normoxia) vs. veh (*). N=3, * = $p<0.05$, ** = $p<0.01$.

	ΔCt Normoxia			ΔCt Hypoxia			p vs. Normoxia
	n1	n2	n3	n1	n2	n3	
veh	21.5	23.0	22.7	23.8	23.1	22.6	0.446
IL1	21.3	22.3	21.8	21.5	21.0	20.7	0.237
F	18.5	20.6	19.6	22.8	20.3	19.5	0.485
IL1 + F	19.2	21.4	19.7	20.5	20.0	19.9	0.977
IL1 + E	20.7	22.2	20.9	21.8	21.1	20.8	0.988
IL1 + E + AZ	21.0	23.1	21.0	22.1	21.3	21.0	0.815

3. COX2 is more sensitive to GC and is regulated by 11β-HSD1 and hypoxia

Cyclooxygenase 2 (COX2) is integral to inflammation and WH. IL-1β (vs. vehicle) increased COX2 expression by 91-fold ($p<0.05$) and 241-fold ($p<0.01$) in normoxia and hypoxia, respectively (**Fig. 7**). In contrast to MMP1 and TIMP4, both cortisol and cortisone supressed IL-1β-induced COX2 mRNA by 96% and 93% ($p<0.05$) respectively in normoxia with suppression by cortisone reversed by 11β-HSD1 inhibitor co-incubation ($p<0.05$, **Fig. 7**). A similar effect was seen in hypoxia, although there was a trend towards greater COX expression with cortisone, possibly caused by 11β-HSD1 suppression by hypoxia.

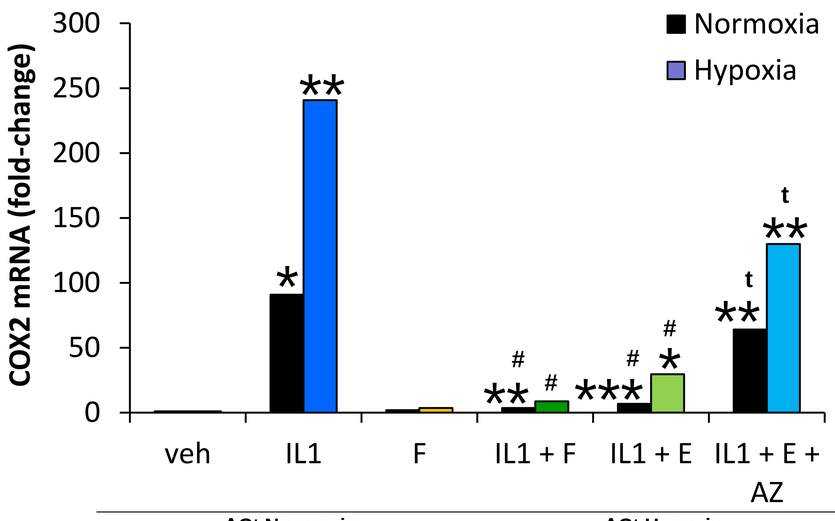

Fig. 7 COX2 mRNA was induced by IL-1β (IL1) vs. vehicle (veh) in all treatments (*). This was suppressed by cortisol (F) and cortisone (E) vs. IL1 (#). Cortisone-mediated suppression was reversed by AZD4017 (AZ) vs. IL1 + E (t). N=3, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

	ΔCt Normoxia			ΔCt Hypoxia			p vs. Normoxia
	n1	n2	n3	n1	n2	n3	
veh	16.6	19.0	18.3	19.3	17.7	16.3	0.903
IL1	11.6	12.1	11.4	10.8	9.8	9.3	**0.075**
F	18.9	17.3	17.0	23.8	15.1	14.1	0.968
IL1 + F	14.7	17.5	16.2	17.7	13.6	13.6	0.643
IL1 + E	13.8	16.2	15.6	13.3	13.7	13.0	**0.111**
IL1 + E + AZ	11.1	13.7	11.6	11.9	10.3	10.5	0.428

Conclusion

We demonstrate a previously unreported cortisol-dependent decrease in 11β-HSD1 expression in hypoxia which may represent a protective mechanism to limit GC exposure in ischemia. Further, we report gene-specific sensitivity to 11β-HSD1-derived cortisol which may regulate responses to inflammation and hypoxia in chronic wounds.

Our findings indicate that hypoxia regulates responses to pro-inflammatory stimuli and GC in a gene-dependent manner (e.g. MMP1 was differentially regulated by IL-1β and GC independently of hypoxia, regulation of TIMP4 by GC was potentiated by IL-1β in hypoxia, induction of 11β-HSD1 by IL-1β was suppressed by GC in hypoxia and COX2 was differentially regulated by IL-1β and GC with increased hypoxia-mediated induction in cortisone-treated cells). Future studies will investigate these effects in hypoxia-regulated pathways e.g. angiogenesis (**see poster P35**).

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*. Increased glucocorticoid activation during mouse skin wound healing. **J Endocrinol.** 2014 221: 51-61

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