β -Hydroxysteroid Dehydrogenase Type 1 Inhibition Attenuates the Adverse Effects of Glucocorticoids on Dermal Papilla Cells

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There are NO conflicts of interest declared in this presentation.

Introduction

A role of chronic stress on hair loss

- ✓ Emotional stress is related with several types of hair loss such as telogen effluvium, androgenetic alopecia, female pattern hair loss^{2,3}
- ✓ Inhibitory effect on hair growth in stress mouse model organ-cultured human scalp hair follicles^{4,5}

Functional hypothalamic-pituitary-adrenal (HPA) axis in normal hair follicles and alopecia

- ✓ Hair follicles have functional peripheral equivalent of HPA axis⁵
- ✓ Increased HPA axis tone is observed centrally and peripherally (hair follicles) in mouse model of alopecia^{6,7}

Glucocorticoids (GC)

- ✓ Major player in adverse consequence of stress
- ✓ Induces hair follicle regression in *in vivo* experiment^{8,9}
- ✓ Inhibit proliferation of dermal papilla cells (DPCs) by inducing cell cycle arrest and suppressing the expression of growth factors important in hair follicle growth in DPCs¹⁰

11 β -hydroxysteroid dehydrogenase (11 β -HSD) : a tissue-specific regulator of glucocorticoid response

- ✓ Catalyze the interconversion between hormonally active cortisol and inactive cortisone in cells
- ✓ 11 β -HSD type 1 (11 β -HSD1)
- ✓ Reductase which activates cortisone from inactive cortisone
- ✓ Keratinocytes, fibroblast, and sebocytes
- ✓ Involved in cell proliferation, wound healing, inflammation, and aging in skin¹⁴
- ✓ However, the expression and function of 11 β -HSD1 in human hair follicles remains unknown.

Objective

- ✓ To investigate the expression and regulation of 11 β -HSD1 in human DPCs *in vitro* and *in vivo*
- ✓ To examine whether modulation of 11 β -HSD1 activity can regulate the negative effects of glucocorticoids on DPCs

Material and Methods

Immunohistochemistry (IHC) for 11 β -HSD1

- ✓ 12 Paraffin-embedded normal human scalp skin samples
- ✓ Primary anti-11 β -HSD1 antibody (rabbit, polyclonal, 1:100 dilution, Abcam, Cambridge, UK)
- ✓ Secondary horseradish peroxidase-conjugated antibody

Cell culture and reagents

- ✓ DPCs of human origin [PromoCell GmbH (Heidelberg, Germany)]
- ✓ Cortisol [Sigma Aldrich (H6909, St Louis, MI, U.S.A.)] and a selective inhibitor of 11 β -HSD1, 385581 [Merck (Darmstadt, Germany)] dissolved in DMSO, a final concentration of 0.1 μ M

Immunofluorescence Analysis of Ki-67 Expression

Western blot analysis for 11 β -HSD1, Wnt5a, alkaline phosphatase (ALP), and vascular endothelial growth factor (VEGF)

Statistic analysis

- ✓ Two-tailed Student t test or one-way ANOVA with Dunnett's post test Graph Pad In Stat version 3.00 software (Graph Pad Software, San Diego, CA, USA)

Results

Human scalp hair follicles express 11 β -HSD1 in ORS, matrix keratinocytes, and dermal papilla

Figure 1. Expression of 11 β -HSD1 by immunohistochemical staining in human scalp hair follicles. 11 β -HSD1 immunoreactivity was found in ORS keratinocytes (a), the matrix keratinocytes (MK) (b and c; arrow, original magnification $\times 200$), and in the DP in situ (b and c, original magnification $\times 200$).

Human DPCs express 11 β -HSD1 protein *in vitro* and GC upregulates 11 β -HSD1 protein expression in cultured human dermal papilla cells (hDPCs)

Figure 2. Western blot analysis of 11 β -HSD1 expression in unstimulated and cortisol-stimulated human DPCs. Bars show the result of densitometric analysis of the 11 β -HSD1 protein band relative to the corresponding GAPDH protein band. Results are presented as the mean $\pm$ SD. (* $p < 0.05$).

Inhibition of 11 β -HSD1 by selective inhibitor partially reverses the GC-induced decrease in the proliferation of hDPCs

Figure 3. The effect of the selective 11 β -HSD1 inhibitor on the proliferation in cortisol-stimulated hDPCs. (a) hDPCs were treated with 10⁻⁷ M cortisol for 48 hours with/without 30 min pretreatment with 385581 (100 nmol L⁻¹), and immunofluorescent staining was performed with anti-Ki-67 antibody. (b) Percentage of Ki-67-positive human DPCs (green in nuclei) under at least 5 high power fields was counted. (* $p < 0.05$, ** $p < 0.01$).

The selective 11 β -HSD1 inhibitor partially reverses the glucocorticoid-induced decrease in the expression of dermal papilla markers and growth factor in DPCs

Figure 4. The effect of a selective 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) inhibitor on the expression of Wnt5a, ALP, and VEGF in cortisol-stimulated human dermal papilla cells. Human DPCs were pre-treated with 100 nmol L⁻¹ 11 β -HSD1 inhibitor for 30 minutes and stimulated with 10⁻⁷ M cortisol for 48 hours. (a) After stimulation, the expression of Wnt5a, ALP, and VEGF was analyzed by Western blot. (b) Bars show the results of densitometric analysis relative to the corresponding GAPDH protein band. Results are presented as mean $\pm$ SD. (* $p < 0.05$, ** $p < 0.01$).

Summary & Conclusions

- ✓ This is the first demonstration of 11 β -HSD1 expression in human DPCs both *in situ* and *in vitro* at protein level and the regulation of 11 β -HSD1 by cortisol in human DPCs.
- ✓ Cortisol suppresses the expression of dermal papilla biomarkers involved in the maintenance of human dermal papilla properties, including Wnt5a and ALP, a ubiquitous dermal papilla marker.
- ✓ Inhibition of 11 β -HSD1 can prevent glucocorticoid-induced suppression of proliferation, growth factor expression, and the expression of dermal papilla markers in human DPCs.

- ✓ We can suggest that DPCs are not only the target cells for GCs but also metabolize and synthesize active form of GC via the presence of 11 β -HSD1.

- ✓ Our data provide insights into the mechanisms underlying chronic stress-related hair loss and suggest that 11 β -HSD1 inhibitors can potentially be used to prevent stress-related hair loss

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