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LETTER

# Evaluation of 11-β hydroxysteroid dehydrogenase type 1 in cutaneous fibroblasts cultures of psoriatic lesional skin before and after narrow band-UVB phototherapy

Dear Editor,

The endogenous glucocorticoid (eGC) group include those molecules, similar to exogenous synthetic ones in terms of chemical structure, which are synthesized in the human body: the largest amount is produced by the adrenal cortex and the rest by the skin, with a slower process. The known eCG effects in the skin include epidermal thinning, melanogenesis impairment, inflammation suppression and erythema reduction.[1,2]

Cortisol is the most important among eGCs, and its availability depends on the pre-receptorial regulation of the 11β-hydroxysteroid dehydrogenase (HSD) enzymes, that is 11β-HSD1 (converts cortisone to the active form, cortisol) and 11β-HSD2 (catalyses the opposite reaction).[1-3] Over the last years, the role of 11β-HSD1 as a key regulator of eGC activity was investigated in several tissues (eg ovary, placenta, lung, central nervous system, adipose tissue, liver, colon and kidney).[1,4] Of note, it was recently identified in the epidermis and the dermis,[3-6] particularly in keratinocytes,[6] fibroblasts[4] and endothelial cells.[5]

Psoriasis is an inflammatory disease characterized by a chronic-relapsing course and a rapid response to topical application of exogenous glucocorticoids. The nbUVB phototherapy 311 nm is also effective in mild-to-moderate psoriasis, but its exact mechanism of action on psoriatic skin is yet to be fully elucidated.

On these premises, we aimed to investigate the modifications in 11β-HSD1 expression in psoriatic lesions of patients after treatment with nbUVB phototherapy.

A total of 10 psoriatic patients with average PASI (Psoriasis Area and Severity Index) = 9.6 (Table 1) were recruited in the Phototherapy Unit of the Dermatology Department of Siena University Hospital: they gave signed, informed consent after receiving written and oral information on the study. The study was carried out between September 2015 and December 2019, approved by the local ethics committee and conducted according to Helsinki Declaration ethical conditions. Inclusion criteria were as follows: age >18 years; mild (PASI<7) and moderate (PASI 7-17) plaque psoriasis[7]; presence of a psoriatic plaque of at least 5 cm in diameter within a photo-protected area (ie the medial surfaces of the forearm/arm/tights/bottom or side) which can be selected for skin biopsy; and having patient's commitment not to expose to the sun/not to apply topical corticosteroid preparations on the selected plaque for the whole duration of the study (Table 1). At baseline, a 6-mm punch biopsy was performed from the lesional margin of a selected psoriatic plaque, for histopathological examination and human cell cultures set up; 2 weeks after the last phototherapy session, a second biopsy was done 3-5 mm distant from the scar point (Figure 1A). All patients performed standard protocol of nbUVB doses (from 500 to 1300 mj/cm$^2$) of 3 sessions/week (total 24 consecutive sessions) from October to April. A whole-body irradiation was performed with GP24H medical bed, Cosmedico®, Medizintechnik; patients wore protective sunglasses during all sessions. Patients were followed up every 3 months for 1 year. Control healthy skin samples were procured from 20 healthy patients aged 25-65 undergoing dermatologic surgery for benign lesions, matched for age, sex and body site.

Human lesional fibroblasts were isolated from a skin biopsy and cultured in DMEM (*Dulbecco's modified Eagle's medium*, *Sigma-Aldrich*), 10% FBS (*fetal bovine serum*, *Euroclone*), 5 mM penicillin/streptomycin (*Euroclone*) and 2 mM L-glutamine (*Euroclone*) in an incubator at 37°C. From each sample, total RNA was extracted using NucleoSpin RNA kit (*Macherey-Nagel*), according to the manufacturers' instructions. cDNA was prepared from 500 ng RNA using the *SuperScript III First-Strand Synthesis SuperMix* for qRT-PCR kit (*Invitrogen by Life Technologies*). Experiments were done with the RT-PCR protocol of the manufacturer of *TransStart Top Green qPCR SuperMix* (*Transgen Biotech*, China). Target gene *11β-HSD1* expression was normalized using *GAPDH*, and the expression in each sample was calculated relative to the average of control samples, using the $2^{-\Delta\Delta ct}$ method analysis. qRT-PCR analysis was performed on *MJ Research PTC-200 (Bio-Rad)*. For proteins analysis, fibroblasts were lysed by a Lysis Buffer (LB) (20 mM Tris-HCl, 135 mM NaCl, 10% glycerol, 1% Nonidet P-40, 10 mM EDTA, pH 8, with proteases inhibitor cocktail (*Thermo Scientific*). Protein concentration was quantified by using *QuantumMicroProtein* (EuroClone). Samples were separated by SDS-PAGE (Precast gel Bolt 4%-12% Bis-Tris Plus, *Invitrogen by Thermo Fisher Scientific*) and then transferred to a nitrocellulose membrane (*GE Healthcare by Life Science*) in order to detect 11β-HSD1 protein. Membranes were incubated overnight at 4°C with anti-11β-HSD1

**TABLE 1** Patients' characteristics at baseline and follow-up times

| Patient | Age | Sex | BMI | Phototype | Disease duration (months) | Biopsied plaque area (cm2) | PASI | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *baseline* | *time 3* | *time 7* |
| 1 | 51 | F | 23 | III | 24 | 140 | 10 | 6 | 4 |
| 2 | 47 | M | 24 | III | 36 | 200 | 12 | 8 | 6 |
| 3 | 36 | M | 22 | II | 8 | 90 | 7 | 4 | 2 |
| 4 | 41 | F | 24 | III | 18 | 130 | 8 | 5 | 2 |
| 5 | 57 | M | 25 | III | 21 | 240 | 9 | 6 | 3 |
| 6 | 25 | F | 22 | III | 10 | 80 | 9 | 5 | 2 |
| 7 | 43 | M | 24 | II | 30 | 110 | 11 | 8 | 4 |
| 8 | 33 | M | 22 | III | 6 | 90 | 9 | 7 | 4 |
| 9 | 45 | F | 24 | III | 28 | 160 | 10 | 10 | 6 |
| 10 | 65 | M | 25 | III | 32 | 180 | 11 | 9 | 5 |

*Note: time 3 = 1 month after last phototherapy session; time 7 = 3 months after last phototherapy session*

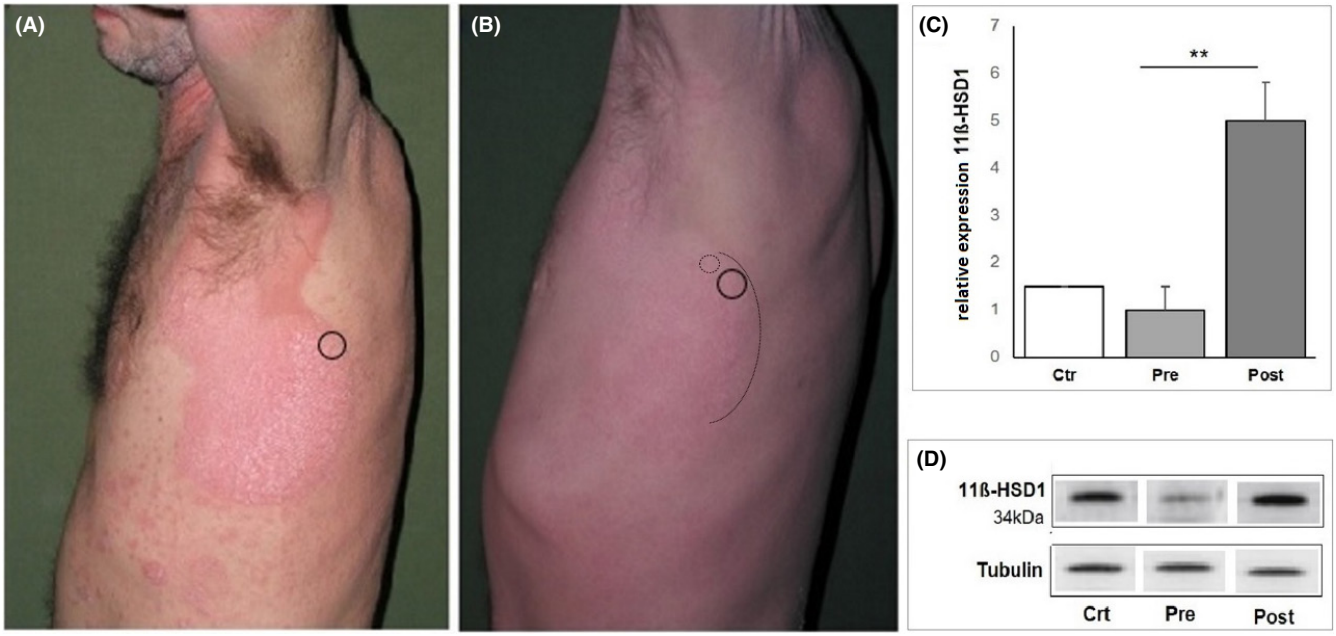


**FIGURE 1** A 41-year-old man with moderate plaque psoriasis: clinical appearance at baseline (A) with a PASI of 13, 4 and (B) 1 mo after the last phototherapy session, with a PASI of 3. Black circles with continue line, correspond to the body site biopsied for histological analysis and setting of the skin cultures. Dotted line circle identifies the baseline biopsy site and dotted line the margin of the psoriatic plaque. Levels of mRNA relative expression of 11β-HSD1 detected in human cultures of healthy fibroblasts (*Ctr*), of lesional fibroblasts isolated from a psoriatic plaque before (*Pre*) and after treatment (*Post*) (C). Western blot analysis of 11β-HSD1 showing a 34 kDa protein (D), along with tubulin, was immunodetection for loading control

antibody 1:200 (*Polyclonal Antibody, Cayman Chemical*) and monoclonal anti-α tubulin antibody (*GeneTex*), used as a quantification control. Bands were detected by 1-hour incubation with secondary antibodies 1:1000 (*Anti-rabbit IgG HRP-linked antibody, Cell Signaling*), and they were finally analysed by *ChemiDoc (Bio-Rad)*.

One month after last phototherapy session (time 7), average PASI was 3.8, with relevant improvement on the biopsy site (Figure 1B, Table S1). The average 11β-HSD1 mRNA expression values obtained from all analysed samples are reported in Figure 1C: in psoriatic patients, 11β-HSD1 levels were lower at baseline compared with control subjects, but significantly increased r ($P = .0015$) after nbUVB treatment. Western blot analysis (Figure 1D) confirmed the mRNA results showing a 34-KDa immunoreactive band evident in all samples, with decreasing levels of intensity from post to Ctrl to pre. Normalization

through α-tubulin is also shown. No statistically significant difference was observed between PASI<7 and PASI 7-12 patients.

Psoriasis is a frequent skin disease with reported prevalence ranges between 0.09% and 11.43%, with at least 100 million individuals affected worldwide.[7] Its aetiology is multifactorial, and the molecular alterations at tissue levels are complex and still not fully understood.[8] In particular, few data are available about the specific role of the skin cutaneous glucocorticoid homeostasis in the development of psoriasis.[1,2,8] Nevertheless, investigating the steroids skin metabolism represents a crucial step to understand the physiopathology of the disease and the therapeutic response of psoriatic plaque to both topical CG treatments and nbUVB irradiation. Indeed, previous investigations of Slomisnki et al on the regulatory networks of skin steroidogenesis suggested that some key pathways can be crucial to understand the physiopathological tissue mechanisms underlying several inflammatory skin diseases.[1,9,10] In particular, UVB rays appeared to be able to elicit, more efficiently than UVA, the tissue production of multiple molecules (eg cytokines, corticotropin-releasing hormone and proopiomelanocortin-peptides), that can further regulate neuroendocrine central hypothalamic-pituitary-adrenal axis axes after released into circulation.[10] To date, a higher expression of 11β-HSD1 was detected in UVB and UVA exposed skin in murine models,[11,12] while an reduced eCGs synthesis was found skin.[8]

Here, in this study, we demonstrated the key role of 11β-HSD1 enzyme in psoriatic human skin treated with nbUVB phototherapy. Firstly, we observed that 11β-HSD1 is constitutionally down-expressed in lesional psoriatic skin, causing a reduced availability of the cortisol active form and suggesting that a relevant impairment in local steroidogenesis occurs in these patients. This finding is in line with a reduced expression of the steroidogenesis promoter *CCHR1* gene detected in human keratinocyte cultures, derived from untreated psoriatic plaques, reported by Tiala et al[8] Secondly, we demonstrated that the nbUVB phototherapy is able to induce either a normalization and a significant increase in the 11β-HSD1 levels (ie 4-time fold) compared with baseline cells and healthy controls cells, respectively. This direct effect of nbUVB on psoriatic keratinocytes and fibroblasts is likely to determine a larger availability of endogenous cortisol at tissue level that can explain, at least partially, the beneficial effects of the nbUVB at clinical level, in line with the overmentioned in vitro experiments.[9-12] Moreover, the present findings can explain the higher durability of nbUVB effects in time compared with the anti-inflammatory effects exerted by the topical application of exogenous CG ointments.

In this context, the possibility to enhance the eGC production is of paramount importance in the management of chronic-relapsing psoriasis and spare the collateral effects of exogenous CGs. Further studies are, however, needed to confirm the present findings, adding parallel investigations on the glucocorticoid receptor expression before and after phototherapy on psoriatic and normal keratinocytes.

## KEYWORDS

nbUVB phototherapy, plaque psoriasis, human skin cultures, 11β-HSD1

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## CONFLICT OF INTEREST

None.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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