Apremilast for moderate hidradenitis suppurativa: no significant change in lesional skin inflammatory biomarkers

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ABSTRACT

Background

Treatment with apremilast has demonstrated clinically meaningful improvement in moderate hidradenitis suppurativa (HS).

Objective

To evaluate the change in expression of inflammatory markers in lesional skin of HS patients receiving apremilast 30 mg twice daily (n = 15) for 16 weeks compared with placebo (n = 5).

Methods

At baseline 5-mm punch biopsies were obtained from an index lesion (HSL) and non-lesional skin (HSN) as control in the same anatomical area. Subsequent HSL samples were taken as close as possible to the previously biopsied site at week 4 and week 16. After sampling, biopsies were split; one half was processed for *in vivo* mRNA analysis using real-time quantitative PCR; the other half was cultured for *ex vivo* protein analysis using a proximity extension assay (Olink). Linear mixed effects models were calculated to compare the levels of inflammatory markers in HSL skin between apremilast and placebo over time.

Results

At baseline, 17 proteins with a fold change >2 in HSL versus HSN skin were identified in 20 patients. The top 5 were IL-17A (5), S100A12, CST5, IL-12/23p40, and CD6 (1) with respective fold changes ranging from 6.6 to 1638 (FDR < 0.044). Linear mixed effects models for 75 assays were calculated. Protein levels of S100A12 decreased during treatment in the apremilast group compared with the placebo group (p = 0.014, FDR = 0.186). None of the 14 genes exhibited significant changes in expression over time. However, an evident downward trend in relative mRNA expression of IL-17A and IL-17F was demonstrated in patients receiving apremilast.

Conclusion

We did not detect statistically significant changes in inflammatory markers in lesional skin of HS patients receiving apremilast compared with placebo, despite clinical improvement in the apremilast group. Nonetheless, S100A12 and IL-17A were significantly elevated in HS lesional skin and showed a decrease in response to apremilast. The translational model in clinical trials involving HS clearly needs further improvement.

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic, recurrent, auto-inflammatory skin disorder with limited effective treatment options.[1] Recently, treatment with apremilast for 16 weeks has demonstrated clinically meaningful improvement in moderate HS. In this study (NCT03238469; EudraCT 2016-000859-27) twenty patients with moderate HS (HS-PGA score of 3) were randomised in a 3 : 1 ratio to receive blinded treatment of apremilast 30 mg twice daily or placebo, respectively. Demographic and disease characteristics were similar between treatment arms. The mean age of the study population was 35 years (range 21-64), most patients were female (17/20) and current smokers (16/20), the mean (± SD) BMI was 32.7 ± 6.2, and the mean abscess and inflammatory nodule (AN) count was 6.0 ± 1.8. The objective of this translational study was to evaluate the change in expression of inflammatory markers in lesional skin of HS patients receiving apremilast 30 mg twice daily for 16 weeks compared with placebo.

MATERIALS AND METHODS

Ethical statement

This investigator-initiated trial was approved by the medical ethics committee of the Erasmus University Medical Center Rotterdam, The Netherlands (NL.57003.078.16). All patients provided written informed consent for study participation and use of their skin biopsies.

Biopsy procedure

At baseline, two 5-mm punch biopsies were taken: one was obtained from an erythematous, indurated, inflamed lesion, i.e. the index lesion (HSL), and one from the normal-appearing, unaffected non-lesional skin (HSN) in the same anatomical area (Figure 1). Subsequent HSL skin samples were taken as close as possible to the previously biopsied index site at week 4 (early response) and week 16 (end of study). After sampling, biopsies were split whereby one half was processed for mRNA analysis of the biopsy, representing the *in vivo* gene expression, and the other half was cultured for *ex vivo* protein analysis. The supernatants and the biopsies in 250 µL lysis buffer containing 1% ß-mercaptoethanol were transferred to a polypropylene tube and stored at –20°C until further analysis.

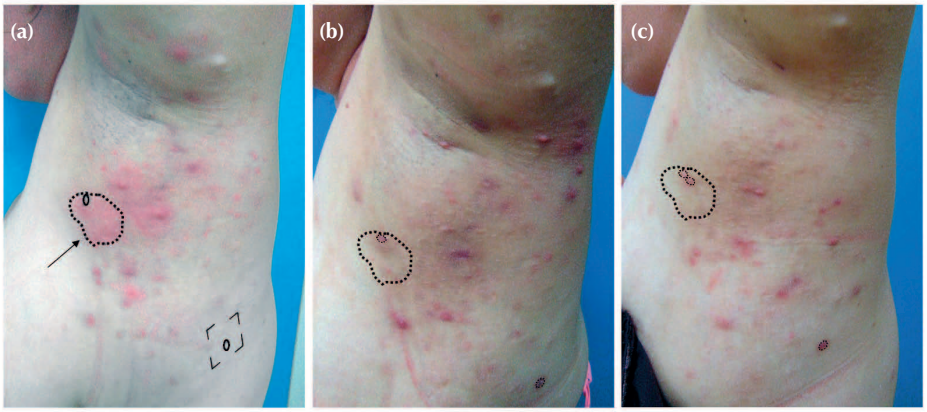

Figure 1. Biopsy procedure in a patient with a good clinical response to apremilast. Note that all deep-seated lesions have resolved. Erythematous lesions seen at week 16 are scars and superficial folliculitis. **(a)** Baseline. A 5-mm punch biopsy was obtained from an erythematous, indurated, inflamed lesion (arrow pointing to index lesion). An additional 5-mm punch biopsy of the non-lesional skin was taken from the same anatomical area to serve as control (circle within the square). **(b)** Week 4 and **(c)** Week 16. Subsequent skin samples were taken as close as possible to the previously biopsied index site (small circles within dashed line).

Ex vivo skin culture and protein quantification

After sampling, half of the 5-mm biopsy was cultured for 24h at 37°C in an atmosphere of 5% CO2 and 98% humidity in a transwell system (Netwell; Costar, Cambridge, MA) as previously described.[2,3] In short, samples were placed in a 12-well plate with the epidermis exposed to the air and the dermis immersed in 1 mL Iscove's modified Dulbecco's medium (Gibco, Paisley, U.K.) containing 0.5% human AB serum, penicillin (100 U/mL) and streptomycin (100 U/mL). A broad spectrum of 92 inflammatory markers was measured in the supernatant using the Olink Proseek Multiplex Inflammation panel (Olink Proteomics, Uppsala, Sweden).

In vivo gene expression

Total RNA was isolated using the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MA), treated with 0.1U/µL DNAse (Invitrogen), and cDNA was synthesised with SuperScript II reverse transcriptase, random hexamer primers (Invitrogen) and oligo(dT)15 (Promega). Primers and probes were designed and chosen using ProbeFinder Software and the Universal Probe Library (Roche Applied Science, Indianapolis, IN). *ABL1* was used as housekeeping gene. Real-time quantitative PCR for 14 genes (*IFN-γ*, *TNF-α*, *IL-1ß*, *IL-6*, *IL-8*, *IL-10*, *IL-23p19*, *IL-12/23p40*, *IL-17A*, *IL-17F*, *IL-31*, *CXCL-10*, *CCL-5*, and *MPO*) was performed with the ViiA7 System and using the QuantStudio Real-Time PCR Software version 1.3 (Applied Biosystems, Waltham, MA).

Statistical analysis

Protein data are presented as normalised protein expression (NPX), which are log^2-transformed arbitrary units. Relative mRNA expression levels for week 4 and week 16 were calculated using the $2^{-\Delta\Delta CT}$ method using a baseline value of 1 for each individual sample.[4] First, differentially expressed proteins at baseline were explored by calculating a fold change with the median NPX of HSL skin divided by the median NPX of HSN skin. If the fold change was more than 2, a Wilcoxon signed rank test was subsequently used to pairwise compare protein levels between HSL and HSN skin. Second, linear mixed effects models and associated ANOVA for repeated measures were calculated to compare inflammatory protein and mRNA expression levels between apremilast and placebo over time. Fixed effects were treatment, time and treatment*time. Third, the Spearman's rho was used to assess translational linkage, i.e. correlation between the levels of inflammation and the AN count. A two-sided *p*-value below 0.05 was considered significant. *P*-values were adjusted for multiple testing within each test using the Benjamini-Hochberg approach. Statistical analyses were conducted in R Statistical Software version 3.5 (R Foundation for Statistical Computing, Vienna, Austria) using the lmerTest package.

RESULTS

At baseline, 17 proteins with a fold change >2 were identified (Table 1). The top-5 were IL-17A (5), S100A12, CST5, IL-12p40, and CD6 (1) with respective fold changes ranging from 6.6 to 1638 (FDR < 0.044). Out of 92 Olink assays, 75 were included in the linear mixed effects models. The removed assays had less than 25% detectability for among others TNF-α and IFN-γ. Only the protein levels of S100A12 decreased over time in the apremilast versus placebo group. However, this was not statistically significant after correction for multiple testing ($p = 0.014$, FDR = 0.186; Figure 2).

None of the 14 genes analysed by linear mixed effects models yielded significant differences. Only the relative mRNA expression of *IL-17A* and *IL-17F* demonstrated an evident but nonsignificant downward trend in patients receiving apremilast versus placebo ($p > 0.05$; Figure 3). In addition, there was no correlation between S100A12 protein levels or *IL-17A/F* mRNA expression and the favourable clinical response in the apremilast group as measured by the AN count (data not shown).

Table 1. Proteins with a fold change >2 in HSL versus HSN skin at baseline.

#	Protein	Fold change HSL/HSN	NPX HSL N = 20 median	NPX HSL N = 20 IQR	NPX HSN N = 20 median	NPX HSN N = 20 IQR	Unadj. *p*-value	Adj. *p*-value FDR	Missing data %
1	CD6	1638	3.5	2.0 - 4.1	0.002	0.0 - 0.2	3.4 e-07	0.018	None
2	IL-12p40	38	0.9	0.5 - 1.8	0.024	0.0 - 0.1	7.2 e-06	0.038	7.5
3	CST5	23	0.9	0.6 - 1.1	0.041	0.0 - 0.1	6.1 e-07	0.024	1.1
4	S100A12	11	4.5	3.1 - 6.3	0.4	0.4 - 0.8	2.7 e-05	0.041	20.0
5	IL-17A	6.6	3.1	1.8 - 3.8	0.5	0.5 - 0.6	2.9 e-05	0.044	26.3
6	TNFSF14	4.5	3.4	2.5 - 4.1	0.8	0.4 - 1.0	1.0 e-06	0.029	6.3
7	AXIN1	4.4	1.9	1.7 - 2.1	0.4	0.4 - 0.5	8.8 e-07	0.026	8.8
8	β-NGF	3.2	1.1	0.6 - 1.4	0.3	0.3 - 0.6	5.8 e-04	0.047	15.0
9	TNFSF11	3.1	2.2	1.2 - 3.3	0.7	0.7 - 0.7	4.6 e-07	0.021	28.8
10	CD244	3.0	2.7	2.3 - 3.1	0.9	0.9 - 0.9	1.1 e-07	0.012	23.8
11	CD5	3.0	7.3	5.9 - 7.6	2.5	2.2 - 2.6	1.3 e-08	0.003	None
12	TNFRSF9	2.9	6.4	5.2 - 7.6	2.2	1.8 - 2.7	1.3 e-08	0.006	1.3
13	OSM	2.9	6.4	6.0 - 7.8	2.2	1.3 - 3.4	4.0 e-06	0.035	3.8
14	SIRT2	2.8	5.0	4.5 - 5.4	1.8	1.5 - 1.9	1.9 e-07	0.015	5.0
15	GDNF	2.7	2.9	2.0 - 3.8	1.1	0.8 - 1.2	1.6 e-06	0.032	5.0
16	IL-18R1	2.3	4.2	3.6 - 5.2	1.8	1.7 - 2.0	1.3 e-08	0.009	None
17	ST1A1	2.1	1.5	0.8 - 1.8	0.7	0.5 - 1.2	1.3 e-02	0.050	10.0

Missing data: samples with NPX-values below background level throughout all samples, i.e. HSN and HSL baseline, HSL week 4 and HSL week 16. Unadj. *p*-value: unadjusted *p*-value by Wilcoxon signed rank, NPX HSL vs. NPX HSN. Adj. *p*-value (FDR): adjusted *p*-value Benjamini Hochberg test, NPX HSL vs. NPX HSN. FDR: false discovery rate. HSL: HS lesional. HSN: HS nonlesional. IQR: interquartile range. NPX: normalised protein expression, a high NPX value corresponds with a high protein concentration.

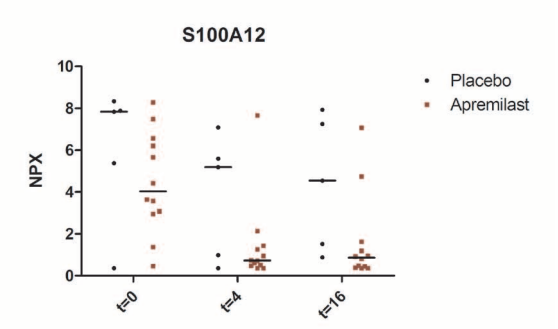

Figure 2. Protein levels of S100A12 in patients receiving apremilast (n = 15) and placebo (n = 5), linear mixed effect model: $p = 0.014$, FDR = 0.186. Relative expression: expression on t = 4 and t = 16 relative to baseline using the $2^{-}\Delta\Delta^{CT}$ method with a baseline value of 1 for each individual sample. Data at week 16 for two patients receiving apremilast are missing as these two patients discontinued after respectively week 4 and week 8. NPX, Normalized Protein eXpression; t, week.

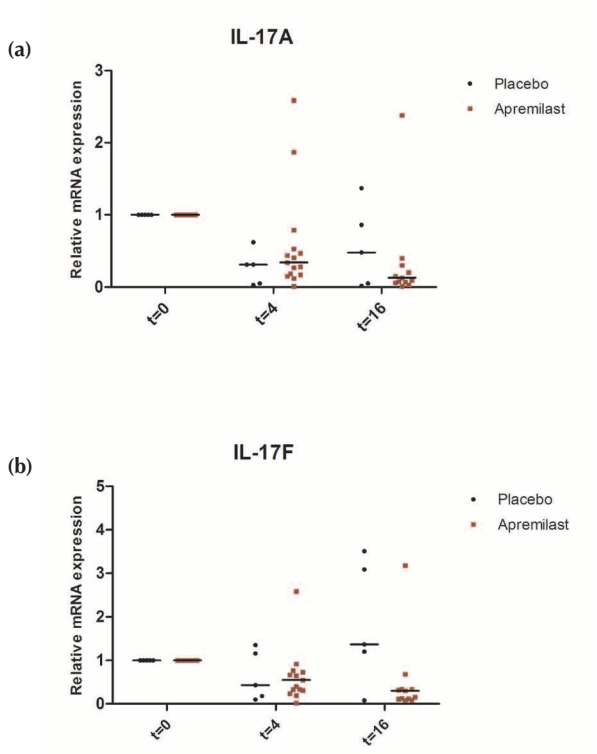

Figure 3. Relative mRNA expression levels of IL-17A and IL-17F in patients receiving apremilast (n = 15) and placebo (n = 5), linear mixed effect model: $p > 0.05$. **(a)** IL-17A. At t = 16 in the apremilast group: one data point is out of the y-axis range. **(b)** IL-17F. At both t = 4 and t = 16 in the apremilast group one data point is out of the y-axis range. Data at week 16 for two patients receiving apremilast are missing as these two patients discontinued after respectively week 4 and week 8. IL, interleukin; t, week.

DISCUSSION

This translational study aimed to identify inflammatory skin markers in response to apremilast 30 mg twice daily for 16 weeks in patients with moderate HS, which showed positive clinical results in a previously published study. Only S100A12 protein levels and *IL-17A* and *IL-17F* mRNA expression showed a clear but nonsignificant decrease in patients receiving apremilast compared with placebo.

S100A12 (calgranulin C, EN-RAGE) is a pro-inflammatory, calcium-binding antimicrobial protein that interacts with the receptor for advanced glycation end products (RAGE) and is overexpressed in various inflammatory skin diseases, e.g. psoriasis.[5,6] Previously, S100A8 and S100A9 (calprotectin or calgranulin A/B) have been demonstrated to be elevated in the serum and lesional skin of HS patients.[7,8] It is known

that IL-17 is a potent inducer of S100A8 and S100A9 in keratinocytes.[9,10] The other way around, S100A12 stimulates T cells to produce IL-17A.[11] Thus, elevated levels of IL-17A and S100A12 in HSL skin at baseline and the responsiveness of these inflammatory markers to apremilast treatment is not surprising. Moreover, apremilast's impact on the Th17 pathway was recently demonstrated by the significant reduction of IL-17A and IL-17F protein levels in the plasma of psoriasis patients.[12]

The nonsignificant findings using a broad panel of inflammatory markers may be explained by the regression to the mean phenomenon, particularly at the individual level.[13] The highly inflammatory index lesions could have spontaneously improved over time in the context of the fluctuating nature of the disease, especially in the placebo group. The effect of regression to the mean increases with larger measurement variability.[13] Expanding the number of biopsies from index lesions, representing several degrees of inflammation, would theoretically be a solution. However, taking more biopsies in the same patients would not be feasible for ethical reasons.

Another explanation for the decreased levels of inflammation over time in both groups could be that the inflammatory infiltrate of the index lesion was gradually reduced by successively taking biopsies from the same nodule. Moreover, the substrate for the foreign body inflammation such as hair fragments and keratin fibres, may have also been (partially) removed by the biopsy procedure.[14] Furthermore, this exploratory study might be underpowered which may also have resulted in nonsignificant results.

Previously, two prospective uncontrolled studies investigating infliximab and ustekinumab in HS were also unable to link translational data to the clinical response.[15,16] However, in these studies only inflammatory markers in serum were investigated. Strengths of our study include the placebo-controlled trial design, the use of skin (target organ) instead of serum or plasma, and the standardised biopsy procedure of an index lesion. A limitation is the small study population.

In conclusion, this translational study investigating apremilast in moderate HS did not detect statistically significant changes in important inflammatory markers in lesional skin compared to placebo over 16 weeks of treatment, despite positive clinical findings. Nonetheless, related S100A12 and IL-17A were significantly elevated in HSL skin and showed a decline only in the apremilast group. In addition, our findings highlight the challenge of assessing pharmacodynamics in the skin in a highly fluctuating inflammatory disease. A better translational model in clinical trials involving HS has yet to be developed.

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