Molecular signatures order the potency of topically applied anti-inflammatory drugs in patients with atopic dermatitis

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Background: Atopic dermatitis (AD) presents a large unmet need for treatments with better safety and efficacy. To facilitate development of topical therapeutics, we need an efficient model for assessing different formulations and concentrations. The "plaque model" has been successfully implemented in patients with psoriasis, another common inflammatory disease, to assess the efficacy of topical treatments. This model has not been validated for AD, which has higher placebo responses and less stable lesions than psoriasis.
Objective: We aimed to assess changes in molecular signatures of intrapatient target lesions treated with topical therapeutics.
Methods: We enrolled 30 patients with mild-to-moderate AD in a randomized, double-blind, intraindividual comparison of 3 approved agents applied blindly at the investigator site daily for 14 days: pimecrolimus, betamethasone dipropionate, clobetasol propionate, and a vehicle/emollient control. Changes in total sign scores (TSSs), transepidermal water loss, and tissue biomarkers (determined by using RT-PCR and immunohistochemistry) were evaluated.
Results: TSSs showed improvements of 30%, 40%, 68%, and 76% at 2 weeks with vehicle, pimecrolimus, betamethasone, and clobetasol, respectively, with parallel changes in transepidermal water loss ($P < .05$). Significant differences versus vehicle values were limited to steroids ($P < .0001$). Steroids (particularly clobetasol) restored epidermal hyperplasia and terminal differentiation versus minimal changes with vehicle or pimecrolimus ($P < .001$). Levels of cellular infiltrates and cytokines (IL-13, IL-22, and S100As) were similarly reduced only by steroids ($P < .001$). TSS improvement correlated with changes in hyperplasia, infiltrates, and differentiation markers.
Conclusion: We detected significant clinical and tissue differences between agents, providing a novel approach to study the differential effects of topical formulations using a limited sample size. (J Allergy Clin Immunol 2017;140:1032-42.)

Key words: *Atopic dermatitis, topical treatments, emollients, plaque model, topical calcineurin inhibitor, topical steroids, psoriasis, biomarkers*

Atopic dermatitis (AD) is the most common inflammatory skin disease, affecting 4% to 7% of adults and 20% of children, with a major effect on quality of life.[1,2] Patients with AD experience itchy erythematous patches with frequent skin infections.[3] Topical treatments are widely used in both patients with mild and those with moderate-to-severe AD.[4,5]

Currently, topical treatments for AD are limited, consisting of emollients, corticosteroids, and calcineurin antagonists, which can be associated with side effects.[6] Steroids cause skin thinning, striae, petechiae, and acne, whereas calcineurin antagonists are associated with burning and irritation. Model systems have linked calcineurin antagonists to carcinogenicity, resulting in a US Food and Drug Administration (FDA) black-box warning.[7,8] Thus there is a large unmet need for better and safer topical treatments for patients with AD.

To facilitate the development of topical therapeutics for AD, we need an efficient model for assessing different formulations and concentrations. The "plaque model" has been successfully implemented in psoriasis, another common inflammatory skin disease, to assess the efficacy of topical treatments.[9-12] This model provides reliable dose- and time-response curves, allowing concomitant application of different topical compounds on

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Abbreviations used
AD: Atopic dermatitis
DC: Dendritic cell
LC: Langerhans cell
TAA: Target Area Assessment
TCI: Topical calcineurin inhibitor
TEWL: Transepidermal water loss
TSS: Total sign score

multiple lesions in the same patient, allowing for direct comparison of treatment effect in a small number of subjects.[12] However, this model is difficult to apply to AD because it usually involves application of a self-adhesive tape to delineate the treated area, which could exacerbate or induce an irritant reaction in patients with AD. Furthermore, this approach has not been validated for AD, a disease with high placebo responses and typically less stable lesions compared with psoriasis.[13] Thus we aimed to examine a novel paradigm using intrapatient comparison of 3 active topical compounds and an emollient in adults with mild-to-moderate AD. We performed a randomized, double-blind, intraindividual design trial in 30 patients with AD treated daily for 2 weeks with a calcineurin antagonist (pimecrolimus [Elidel; Valeant Pharmaceuticals, Bridgewater, NJ]), 2 different strengths of topical steroids (betamethasone dipropionate [Diprosone, Merck & Co, Kenilworth, NJ] and the highly potent clobetasol propionate [Dermovate, GlaxoSmithKline, Brentford, United Kingdom]), and the emollient Glaxal Base (WellSpring Pharmaceutical, Oakville, Ontario, Canada) as a placebo control. Clinical efficacy and biomarker changes in biopsy specimens were evaluated over 2 weeks. Although all products showed significant clinical improvements compared with baseline values, only the steroids but not pimecrolimus induced significant clinical and tissue improvements compared with vehicle (particularly clobetasol). Our study provides a valid intrapersonal method to comparatively assess topical formulations for AD that can be incorporated in future trial design with a limited number of patients.

METHODS

Patients and interventions

Thirty adult patients (16 male and 14 female patients; mean age, 24 years; age range, 18-71 years) with mild-to-moderate AD (Investigator Global Assessment of 2 or 3) were enrolled under an institutional review board–approved protocol in a randomized, double-blind, intraindividual comparison trial (NCT02376049, Table I). Four different topical agents were applied daily for 2 weeks to each target lesion (approximately 3 cm in diameter and >2 cm apart, excluding the face and scalp; Fig 1, *B*), including Glaxal Base (vehicle), pimecrolimus 1% (Elidel), betamethasone dipropionate 0.05% (Diprosone), and clobetasol propionate 0.05% (Dermovate) in a 30-μL volume per application (approximately 1.5-2.0 mg/cm^2), with no occlusion. Each application area and surrounding landmarks were drawn on a transparency at baseline. Circular application areas were drawn on the skin with a marker and redrawn at subsequent visits when faded. No adhesives were used to identify the target application areas. We excluded patients with a Fitzpatrick Skin Type score greater than 5, patients treated with systemic immunosuppressants in the last 4 weeks, topical steroids/immunomodulators in the last 2 weeks, and moisturizers in the past 3 days before treatment. Patients participating in other interventional trials within 4 weeks before randomization were excluded. One patient was withdrawn after 1 week because of use of prohibited medications. Treatments were randomly assigned to target lesional areas by using Latin square randomization, so that treatments were blinded to both patients and investigators. No serious adverse events were reported (see Table E1 in this article's Online Repository at www.jacionline.org).

Clinical and tissue measures

The primary clinical measure was the total sign score (TSS),[14-16] which includes 6 signs, erythema, edema/papulation, oozing/crusting, excoriation, lichenification, and dryness, graded according to a 4-point scale (0, absent; 1, mild; 2, moderate; and 3, severe) with a total TSS range of 0 to 18. We also evaluated the 6-point Target Area Assessment (TAA) (0 = clear to 5 = very severe). Transepidermal water loss (TEWL)[17] for functional barrier assessment used a closed condenser chamber measurement system (Aquaflux AF200; Biox, London, United Kingdom). Clinical and barrier evaluations occurred at baseline/1 day, 1 week, and 2 weeks. On day 15, 4.5-mm punch biopsy specimens were taken from lesional areas and nonlesional skin for biomarker assessment. Normal biopsy specimens from healthy control subjects were not included, and assessments evaluated reversal to nonlesional skin levels.

Immunohistochemistry and real-time RT-PCR

Immunohistochemistry was performed on frozen sections by using purified mouse anti-human mAbs, as previously described.[18,19] Antibodies are listed in Table E2 in this article's Online Repository at www.jacionline.org. Epidermal thickness and cells per millimeter were quantified with ImageJ V1.42 software (National Institutes of Health, Bethesda, Md). RNA was extracted with the miRNeasy Mini Kit (Qiagen, Hilden, Germany). One-step quantitative PCR was performed with TaqMan Fast Virus 1-Step Master mix (Applied Biosystems, Foster City, Calif), as previously described.[19,20] TaqMan gene expression assays are listed in Table E3 in this article's Online Repository at www.jacionline.org. Expression values were normalized to human acidified ribosomal protein. Zero on bar graphs represents nonlesional skin expression values.

Statistical analysis

Analysis of primary outcomes. A mixed-effects model approach was used to model the change of TSSs with various treatments. The model included a random intercept for each patient, the fixed categorical effects of treatment and treatment-by-visit interaction, and the fixed covariate of baseline score centered around its sample. Likelihood ratio tests and the Akaike information criterion were used to determine which covariance structure (or unstructured) provided the best fit for the data. If no significant differences were found between models fitted with both structures ($P > .05$), the covariance structure was selected; otherwise, the model with the smaller Akaike information criterion was used. Contrasts were defined in the mixed model to test for treatment differences at different time points. Final results are communicated as the percentage change from baseline calculated from the least square means of the model above.

Analysis of exploratory outcomes. For ordinal outcomes as changes in severity scores (TSSs and TAA scores), the change at 15 days was compared by using the Wilcoxon signed-rank test. A secondary analysis included ordinal logistic models that account for dependency of the repeated measures. mRNA expressions of selected genes were normalized to human acidified ribosomal protein. Values of less than the quantification level were imputed by using 20% of the smallest normalized value observed for that gene across all samples above the detection limit. Imputed data were log-transformed before analysis. Changes in expression levels across treatments were evaluated by using mixed-effects model. Similar analyses were performed for TEWL and immunohistochemistry.

Exploratory analyses. Exploratory analyses aimed to elucidate the relationship between clinical treatment effects, TEWL, and biomarkers. A histologic score was created by coupling epidermal thickness and K16 expression with multivariate U-scores (μScores) by using the R package.[21] The change with treatment at 15 days was calculated for each treatment and patient. Spearman correlation coefficients were used to assess pairwise correlations and unsupervised clustering, assessing cluster structures within variables.