Effects of a New Emollient-Based Treatment on Skin Microflora Balance and Barrier Function in Children with Mild Atopic Dermatitis

Pascale Bianchi, Ph.D.,*† Jennifer Theunis, M.D.,*† Christiane Casas, Ph.D.,* Cecile Villeneuve, B.Sc.,* Annalisa Patrizi, M.D.,† Chloe Phulpin, Pharm.D.,* Adeline Bacquey, C.R.A.,* Daniel Redoulès, Ph.D.,* Valerie Mengeaud, Ph.D.,* and Anne-Marie Schmitt, Ph.D., M.D.*

*European Center for Skin Research, Pierre Fabre Dermo-Cosmétique, Toulouse, France, †Department of Dermatology, University of Bologna, Bologna, Italy

Abstract

Background/Objectives: The use of emollients is widely recommended for the management of atopic dermatitis (AD), especially between flares. An imbalance of skin microflora is suspected of playing a key role in exacerbations of AD. Our aim was to evaluate the effect of a new emollient balm on clinical parameters (SCORing Atopic Dermatitis [SCORAD], xerosis, pruritus), skin barrier function (transepidermal water loss and loricrin, filaggrin, corneodesmosin, and involucrin expression), skin microflora biodiversity, and Staphylococcus aureus and Staphylococcus epidermidis balance in children with mild AD.

Methods: Fifty-four children (1–4 yrs old) were enrolled in this randomized, controlled study. Subjects applied a hygiene product and the emollient balm (emollient group, n = 28) or the hygiene product only (control group, n = 26) twice a day for 28 days.

Results: We found improvement in favor of the emollient group in SCORAD (p < 0.001), pruritus (p = 0.06), and xerosis (p = 0.06) after 28 days of application. Moreover, transepidermal water loss decreased in the emollient group by 34% (p = 0.06) and involucrin expression by 37% (p = 0.001) at day 28 from baseline in association with improvement in barrier function, whereas other barrier-specific proteins did not vary. S. aureus increased significantly in the control group only (6.5 times, p = 0.01), whereas S. epidermidis remained stable in both groups. The Shannon index (H' = 2.3) did not vary with treatment in either group.

Conclusion: Twice-daily application of a new emollient balm in children with mild AD protected the skin from S. aureus proliferation and preserved microflora biodiversity.
Atopic dermatitis (AD) is a chronic relapsing inflammatory disease characterized by impairment of the skin barrier function and abnormalities in the innate and adaptive immune responses of the skin (1,2).

Lower bacterial diversity in the skin microbiome was recently reported to be of major importance in AD pathogenesis (3). \textit{Staphylococcus aureus} in particular has been shown to be involved in exacerbations of AD, with the density of \textit{S. aureus} colonization in AD lesions correlating with the severity of dermatitis (4,5).

Regardless of AD severity, restoration of skin barrier integrity is one of the main therapeutic goals achieved by using emollients, which are strongly recommended as first-line agents (2,6–9). Although lipid-containing emollients can act by forming an occlusive layer over skin, they may have other mechanisms of action (9).

To improve our knowledge of these mechanisms, we assessed the effects of a new emollient balm on AD clinical parameters (SCORing Atopic Dermatitis [SCORAD], xerosis, pruritus), skin barrier function (transepidermal water loss [TEWL] and loricrin, filaggrin, corneodesmosin, and involucrin expression), skin microflora biodiversity, and \textit{S. aureus} and \textit{Staphylococcus epidermidis} balance in children with mild AD.

PATIENTS AND METHODS

This open, randomized controlled clinical study was conducted in three hospitals in Italy and Romania in accordance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice guidelines (CPMP/ICH/135/95), and national regulations. Local independent ethics committees approved the protocol. Volunteers were included after their parents or guardians had provided written informed consent.

Patients

One- to 4-year-old children with mild AD (SCORAD 5–20) and mild to moderate xerosis on the selected measurement areas (SCORAD xerosis score of 1 or 2) not exhibiting AD flares at inclusion were enrolled. Exclusion criteria were use of topical corticosteroids within 7 days or systemic corticosteroids within 15 days before inclusion, history of allergy to study product components, immunodeficiency or chronic or acute diseases, treatment with a reintroduction diet or immunosuppressants in the month before inclusion, systemic antibiotics, topical or systemic antiinflammatory treatment or probiotics within 2 weeks before inclusion, topical immunosuppressants, antibiotics, antiseptics or antihistamines in the week before inclusion, or use of any emollient cream within 48 hours before inclusion. Breastfed children of mothers receiving immunosuppressants in the month before inclusion; systemic corticosteroids, antibiotics, or antiinflammatory agents or probiotics in the 2 weeks before inclusion; or antihistamines in the week before inclusion were excluded.

Treatments

The study product (Avene Xeracalm balm; Pierre Fabre Dermo Cosmétique, Boulogne, France) was an oil-in-water emulsion to be applied to the dry skin of the children’s face and body after bathing twice a day for 28 days. The associated hygiene product (emollient cleansing gel; Trixera; Pierre Fabre Dermo, Boulogne, France) was indicated for dry and atopic skin and was used for bathing and then rinsed off once a day for 28 days.

Study Design

Children were randomized to receive the study product and the hygiene product (emollient group) or the hygiene product only (control group) and were followed for 28 days with visits at day 0 (D0, baseline) and after 15 (D15) and 28 days (D28). Clinical and barrier function parameters were assessed at each visit and microbiological diversity at D0 and D28.

Clinical Parameters

AD global severity was assessed using the SCORAD index, a validated clinical tool for assessing AD global severity (10–12). The intensity of xerosis and pruritus was measured over the whole body using the respective SCORAD-derived scales (0 = absence, 3 = severe).

Barrier Function Parameters

TEWL was assessed using an Aquaflux AF200 (Biox Systems, London, UK) on an area prone to AD flares. RNA expression of involucrin, loricrin, filaggrin, and corneodesmosin was quantified using real-time reverse transcription polymerase chain reaction (RT-PCR) analysis. Skin samples were taken by scratching the skin using a sterile microabrasive tool (Vitry,
France). Total RNA was isolated using an automated RNA purification kit (RNeasy mini QIAcube kit; Qiagen, Courtaboeuf, France) and complementary DNA (cDNA) was synthesized using a high-capacity cDNA reverse transcription kit (Life Technologies, Saint-Aubin, France), in accordance with the manufacturers’ instructions. DNA amplification was performed as described previously (13) using primers and probes Hs00221623_m1 (corneodesmosin), Hs00856927_g1 (filaggrin), Hs00902520_m1 (involucrin), Hs01894962_s1 (loricrin), and RPLP0 (Hs99999902_m1) (Life Technologies). Results are expressed as fold change $= 2^{\Delta \Delta Ct}$.

**Skin Microbiological Diversity**

Purification of DNA was performed using the QIAamp DNA Investigator Kit automated on the QIAcube apparatus (Qiagen) in accordance with the manufacturer’s directions. The V1–V3 16S ribosomal RNA (rRNA) gene variable regions were amplified by PCR using 454 sequencing methods (Roche, Boulogne-Billancourt, France), as described previously (14). Purified amplicon DNAs were sequenced on a 454 instrument using FLX titanium chemistry (GenoScreen, Lille, France) in accordance with the manufacturer’s instructions. Sequence analysis was performed with GnS-PIPE (Genosol, Dijon, France). The reads were assigned to operational taxonomic units using the SILVA database with USEARCH. Biodiversity was estimated by calculating the Shannon index ($H'$; accounts for abundance and evenness of species present in a community).

To quantify *S. aureus* and *S. epidermidis*, real-time PCR was performed with absolute quantification analysis using a standard curve (ATCC 700699 for *S. aureus*, 12228 for *S. epidermidis*): *S. aureus* sodA: primers forward: GAGCATCAATCACTAGGGA, reverse: ACCGCCATTATCGGACTG, FAM-probe: TGCTAACTTAGACAAGGTACC GG; *S. epidermidis* sodA: primers forward: TTTAGAAGCTAAATCAATCGAAGAAA, reverse: GGTGACCACCGCCATTATTA, FAM-probe: TGCCATCTAATATTCAACAGCTGT. Because sodA is present in a single copy number, the number of copies corresponds to the number of bacteria.

**Statistical Analysis**

Analysis of covariance was performed on changes from baseline using visit, site, and product as fixed factors and baseline as covariate. Results were considered significant at $p < 0.05$.

**RESULTS**

**Flow of Subjects and Demographic Characteristics**

Of the 55 children included, 1 was excluded from the full analysis set (FAS) population (all randomized subjects having received one or more applications of the study product) because of the absence of postbaseline values. The FAS was therefore composed of 54 children ages 2.5–1.0 years (range 1–4 years) with mild AD (mean SCORAD 11.7 ± 3.1): 28 in the emollient group and 26 in the control group. There was no significant difference between groups at baseline for any of the parameters analyzed.

**Effect of the Emollient Balm on Clinical Parameters**

The SCORAD was significantly lower in both groups at D15 and D28 than at baseline, with a significantly greater change in the emollient group than in the control group (36% vs 17%, $p = 0.02$, at D15; 48% vs 20%, $p < 0.001$, at D28) (Table 1). Pruritus intensity was also significantly less in both groups at D15 and D28 than at baseline but more in the emollient group than in the control group at D28 (75% vs 36%, $p = 0.06$). Xerosis intensity was significantly lower in the emollient group than in the control group at D15 (48% vs 20%, $p = 0.03$) and D28 (48% vs 20%, $p = 0.06$) (Fig. 1).

**Effect of the Emollient Balm on Skin Barrier Function**

TEWL values in the emollient group were 32% lower at D15 ($p = 0.04$) and 34% lower at D28 ($p = 0.06$) than at baseline, whereas they were unchanged in the control group, regardless of the duration of application (data not shown).

Of the four barrier-specific genes studied, only involucrin was modified in the emollient group, with 37% lower expression ($p = 0.001$) at D28 than at baseline, whereas no change in expression was observed in the control group (Fig. 2). The intergroup difference was not significant ($p = 0.06$).

**Effect of the Emollient Balm on Skin Microflora Biodiversity and Balance Between *S. aureus* and *S. epidermidis***

At D0, *S. aureus* was found in 28% of the subjects in the emollient (8/28) and control groups (7/26) and *S. epidermidis* was detected in 100% of the subjects in the area studied. At D28, the number of *S. epidermidis* was not different in either of the two groups (Fig. 3A). In contrast, *S. aureus* was 6.5 times as great
TABLE 1. Clinical Parameters Before (D0) and After 15 (D15) and 28 (D28) Days of Treatment with the Emollient or a Hygiene Product (Control) in the Full Analysis Set Population

|                             | D0      | D15     | Change at D15 from D0, % | D28     | Change at D28 from D0, % |
|-----------------------------|---------|---------|--------------------------|---------|--------------------------|
| SCORing Atopic Dermatitis   | Emollient | 11.7 ± 3.1 | 7.5 ± 2.7               | -36.1†  | 6.1 ± 2.4               | -48.0† |
|                             | Control  | 10.2 ± 3.3 | 8.5 ± 5.0               | -16.9*  | 7.8 ± 5.8               | -19.7* |
| p-value‡                    | NS      | 0.02      |                         | <0.001  |                          |        |
| Pruritus intensity          | Emollient | 1.67 ± 1.20 | 0.86 ± 1.11             | -48.5†  | 0.43 ± 0.68             | -74.6† |
|                             | Control  | 1.77 ± 1.52 | 1.02 ± 1.20             | -42.5*  | 1.01 ± 1.73             | -35.9* |
| p-value‡                    | NS      | NS       |                         | 0.06    |                          |        |

Versus D0, *p < 0.01, †p < 0.001.
‡Intergroup comparison.
NS, not significant.

Figure 1. Effect of emollient on xerosis. Clinical assessment of xerosis at baseline and after 15 (D15) and 28 (D28) days of treatment. Data are expressed as the percentage of subjects with different degrees of severity of xerosis (*p < 0.05).

Figure 2. Involucrin gene expression analysis after application of hygiene product and emollient for 28 days. Values are expressed as fold change from baseline (D0). Data are mean ± standard error of the mean (**p < 0.01 vs baseline).

(p = 0.01) in the control group but was unchanged in the emollient group (Fig. 3B), with a significant between-group difference at D28 (p = 0.049).

In terms of the distribution of skin bacterial communities (Fig. 4), the Shannon index (H') remained stable in both groups at D28 from D0.

DISCUSSION

In this study, we demonstrated that daily use of emollient balm for 28 days significantly improved mild AD, with a greater decrease in all clinical parameters, SCORAD, xerosis, and pruritus than with a simple hygiene product. The emollient induced a decrease in TEWL and involucrin gene expression not observed in the control group. Most importantly, we showed that twice-daily application of emollient balm in children with mild AD could protect the skin from *S. aureus* proliferation without affecting microflora biodiversity.
Although AD severity in our cohort was mild according to SCORAD (15), it improved significantly after 15 days of treatment, together with a decrease in TEWL, suggesting that the restoration of barrier function mediated the positive effect of the emollient on clinical status. This result is in line with the significant correlations observed previously between clinical scores, TEWL, and skin hydration in individuals with AD (16–18).

In addition to a decrease in TEWL, the restoration of skin barrier function induced by the emollient might also indicate a decrease in involucrin messenger RNA (mRNA) expression since no other protein involved in barrier function (filaggrin, loricrin, corneodesmosin) was affected. These results are consistent with the data of Grzanka et al (19), who observed overexpression of involucrin mRNA in lesional skin followed by a significant decrease after administration of pimecrolimus, a treatment shown to be effective in restoring the epidermal barrier, whereas filaggrin and loricrin gene expression was not modified.

Impairment of skin barrier function in individuals with AD is also associated with a greater susceptibility to *S. aureus* (2,20), the main bacterial species found to be directly correlated with AD severity (5). In our study population, the low prevalence of *S. aureus* in xerotic areas (28% of patients before treatment in both groups) is consistent with the mild severity of their disease; it can reach up to 90% in inflammatory areas in patients with severe AD (5,16). On the other hand, *S. aureus* levels were unchanged after emollient treatment, whereas they increased significantly in the control group. This suggests that the emollient may protect against an increase in *S. aureus* population by restoring barrier function and modulating involucrin, which is known to play an important role in *S. aureus* regulation.

**Figure 3.** Effect of emollient on the balance between *Staphylococcus aureus* and *Staphylococcus epidermidis*. Quantification of (A) *S. epidermidis* and (B) *S. aureus* using quantitative polymerase chain reaction and absolute quantification. Data are expressed as sodA gene copy number ± standard error of the mean. *S. aureus* copy values for inflammatory and xerotic areas are given as references for AD subjects with a SCORing Atopic Dermatitis score of 20 (*p* < 0.05 vs baseline).

**Figure 4.** Effect of treatments on skin microflora diversity. Genus proportion calculated from 16S ribosomal RNA pyrosequencing data associated with the Shannon index (diversity index) at baseline (D0) and after application of emollient or hygiene product for 28 days (D28).
adhesion via a staphylococcal adhesin receptor (21) and thus maintain the balance between *S. aureus* and *S. epidermidis*, which plays a major role in AD severity and flare frequency.

New insights into skin microbiome composition in individuals with AD have revealed that exacerbation of the disease is strongly associated with lower skin bacterial diversity (3). In our patients with mild AD, the Shannon index ($H' = 2.3$) was intermediate between that observed after a flare in a similar population ($H' = 2$) and that in healthy subjects ($H' = 3$) and much higher than in patients with severe AD ($H' = 1$) (3). This index did not change over the treatment period in either group, showing that skin bacterial diversity was maintained with both treatments. The fact that the Shannon index was not modified in the control group despite an increase in *S. aureus* density could be because the change in density was too small (222 vs 33 copies) and that this was statistically but not clinically significant. The magnitude of increase was much lower than in inflammatory or xerotic areas of patients with severe AD (Fig. 3B).

Because this study lasted only a month, we were unable to prove that our emollient can postpone flares by maintaining the balance between *S. aureus* and *S. epidermidis* and that an increase in the *S. aureus* population can predict disease worsening and flares. Furthermore, the absence of placebo is a limitation but may be explained by the difficulty of designing and developing placebos in cosmetic studies (22). To our knowledge, there are no data available on the evolution of the skin microbiome in mild AD in the absence of emollient treatment. It thus seemed interesting to compare the balance between *S. aureus* and *S. epidermidis* over several weeks in children with mild AD who used the emollient daily with that of children with the same skin condition without any emollient treatment. Our results emphasized the role of the emollient in skin hydration and of microbiota balance for maintenance treatment in AD.

In our study, a pediatric population with mild AD was chosen specifically because it is the target of cosmetic emollients. Despite the mild nature of the condition, we demonstrated in a controlled randomized pilot study that an emollient balm is effective in improving AD severity, restoring skin barrier function, and maintaining a balance between *S. aureus* and *S. epidermidis*. Further long-term controlled studies are needed to evaluate the role of emollients in maintaining a diversified skin microflora and to establish whether an increase in *S. aureus* precedes AD flares.

ACKNOWLEDGMENTS

We would like to thank Dr. Sibieanu and Dr. Cavaropol, dermatologists in Bucharest, Romania, for their involvement as investigators. We are also grateful to Christophe Lauze and Chantal Sovran for their active participation in this trial as biostatistician and data manager, respectively. Finally, we are grateful to Marielle Romet and Françoise Nourrit-Poignet for editorial assistance. The study was funded and conducted by Pierre Fabre Dermo-Cosmétique. P. Bianchi, J. Theunis, C. Casas, C. Villeneuve, C. Phulpin, A. Bacquey, D. Redoulès, V. Mengeaud, and A-M Schmitt are employees of Pierre Fabre Dermo-Cosmétique. The principal investigator, Dr. Patrizi, signed a contract with Pierre Fabre Dermo-Cosmétique for this study.

REFERENCES

1. Cork MJ, Danby SG, Vasilopoulos Y et al. Epidermal barrier dysfunction in atopic dermatitis. J Invest Dermatol 2009;129:1892–1908.
2. Leung DY, Guttmann-Yassky E. Deciphering the complexities of atopic dermatitis: shifting paradigms in treatment approaches. J Allergy Clin Immunol 2014;134:769–779.
3. Kong HH, Oh J, Deming C et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res 2012;22:850–859.
4. Zollner TM, Wichelhaus TA, Hartung A et al. Colonization with superantigen-producing *Staphylococcus aureus* is associated with increased severity of atopic dermatitis. Clin Exp Allergy 2000;30:994–1000.
5. Casas C, Ginisty H, Alvarez-Georges S et al. Molecular characterization of inflammation and *Staphylococcus aureus* colonization of involved skin of atopic dermatitis patients. A non-invasive approach. Skin Pharmacol Physiol 2008;21:260–268.
6. Ring J, Alomar A, Bieber T et al. Guidelines for treatment of atopic eczema (atopic dermatitis) part I. J Eur Acad Dermatol Venereol 2012;26:1045–1060.
7. Schneider L, Tilles S, Lio P et al. Atopic dermatitis: a practice parameter update 2012. J Allergy Clin Immunol 2013;131:295–299. e1–e27.
8. Darsow U, Wollenberg A, Simon D et al. ETFAD/ EADV eczema task force 2009 position paper on diagnosis and treatment of atopic dermatitis. J Eur Acad Dermatol Venereol 2010;24:317–328.
9. Lee HJ, Lee SH. Epidermal permeability barrier defects and barrier repair therapy in atopic dermatitis. Allergy Asthma Immunol Res 2014;6:276–287.
10. Stalder JF, Taieb A. Severity scoring of atopic dermatitis: the SCORAD index. Consensus report of the European Task Force on Atopic Dermatitis. Dermatology 1993;186:23–31.
11. Kunz B, Oranje AP, Labreze L et al. Clinical validation and guidelines for the SCORAD index: consensus
report of the European Task Force on Atopic Dermatitis. Dermatology 1997;195:10–19.
12. Schmitt J, Langan S, Deckert S et al. Assessment of clinical signs of atopic dermatitis: a systematic review and recommendation. J Allergy Clin Immunol 2013;132:1337–1347.
13. Bianchi P, Ribet V, Casas C et al. Analysis of gene expression in atopic dermatitis using a microabrasive method. J Invest Dermatol 2012;132:469–472.
14. Wu GD, Lewis JD, Hoffmann C et al. Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16s sequence tags. BMC Microbiol 2010;10:206.
15. Oranje AP, Glazenburg EJ, Wolkstorfer A et al. Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol 2007;157:645–648.
16. Na SY, Roh JY, Kim JM et al. Analysis of colonization and genotyping of the exotoxins of Staphylococcus aureus in patients with atopic dermatitis. Ann Dermatol 2012;24:413–419.
17. Hon KL, Wong KY, Leung TF et al. Comparison of skin hydration evaluation sites and correlations among skin hydration, transepidermal water loss, SCORAD index, Nottingham Eczema Severity Score, and quality of life in patients with atopic dermatitis. Am J Clin Dermatol 2008;9:45–50.
18. Gupta J, Grube E, Ericksen MB et al. Intrinsically defective skin barrier function in children with atopic dermatitis correlates with disease severity. J Allergy Clin Immunol 2008;121:725–730. e722
19. Grzanka A, Zebracka-Gala J, Rachowska R et al. The effect of pimecrolimus on expression of genes associated with skin barrier dysfunction in atopic dermatitis skin lesions. Exp Dermatol 2012;21:184–188.
20. Baurecht H, Irvine AD, Novak N et al. Toward a major risk factor for atopic eczema: meta-analysis of filaggrin polymorphism data. J Allergy Clin Immunol 2007;120:1406–1412.
21. Clarke SR, Andre G, Walsh EJ et al. Iron-regulated surface determinant protein a mediates adhesion of Staphylococcus aureus to human corneocyte envelope proteins. Infect Immun 2009;77:2408–2416.
22. Loden M. Effect of moisturizers on epidermal barrier function. Clin Dermatol 2012;30:286–296.