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A new topical panthenol-containing emollient: Results from two randomized controlled studies assessing its skin moisturization and barrier restoration potential, and the effect on skin microflora

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ABSTRACT

Purpose: Two randomized, intra-individual comparison studies were performed in healthy subjects to evaluate the skin moisturization and barrier restoration potential of a new topical panthenol-containing emollient (NTP-CE) (Study 1), and its effect on skin microflora (Study 2).

Methods: In Study 1 ($N = 23$), two skin areas, one challenged with 0.5% sodium dodecyl sulfate (SDS) solution and one unchallenged, were treated with NTP-CE for 3 weeks. Transepidermal water loss (TEWL), skin hydration, and intercellular lipid lamellae (ICLL) organization were measured at regular intervals during the study. In Study 2 ($N = 20$), quantitative bacterial cultures were obtained over 6 h from a skin area undergoing wash stress with 10% SDS with subsequent single application of NTP-CE.

Results: In Study 1, mean AUC for TEWL reduction from baseline was more pronounced with NTP-CE compared with control (−168.36 vs. −123.38 g/m^2/h, $p = 0.023$). NTP-CE use was also associated with statistically significant improvements in stratum corneum hydration and an increase in mean ICLL length from baseline (day 22: 120.61 vs. 35.85 nm/1000 nm^2, $p < 0.001$). In Study 2, NTP-CE use had no negative impact on bacterial viability.

Conclusions: NTP-CE use has favorable and lasting effects on barrier function and repair as well as skin hydration without negatively influencing bacterial viability.

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Emollient; skin; moisturization; skin barrier; repair; microbiome; randomized controlled study; panthenol; topical

Introduction

During the past years, considerable progress has been made in the understanding of skin barrier function and the interactions between topically applied substances and epidermal biochemistry (1). In addition, skin microbiome research showed that a balanced resident skin microflora is one of the requirements toward a healthy skin (2,3). It has been suggested that new emollients should be developed to allow the growth of commensal bacteria to recalibrate the diversity of the skin microbiome supporting the beneficial effects and the protective role of the microflora (4).

These points led to the development of a new topical panthenol-containing emollient (NTP-CE, Bepanthen® SensiDaily*). The effort was in large motivated by the need for a tailored proper skin care in subjects with sensitive skin (e.g. basic emollient care for skin barrier and its flora during post-inflammatory atopic dermatitis (AD) maintenance stages (5)) or dry skin conditions in general.

Impaired skin barrier plays a major role in various skin conditions like dry skin, sensitive skin, AD or contact dermatitis (6,7). Moisturization and restoration of the stratum corneum skin barrier are therefore important properties of any skin care product. Emollients and moisturizing creams belong to the most widely used preparations to relieve symptoms of dryness and improve skin barrier function (1). NTP-CE is a water-in-oil emulsion formulation (pH = 5.5) to be used as cosmetic product for daily care. Apart from the well-known panthenol (8), it contains glycerin, different lipids (e.g. ceramide 3), vitamin B_3 which is known to increase skin lipid production (9), and the prebiotic α-glucan oligosaccharide as key elements. In addition, NTP-CE incorporates a lipid lamellar technology which is characterized by a structure similar to the naturally occurring intercellular lipids in the epidermis. The lamellar lipid organization in the stratum corneum is considered crucial for the skin barrier function (10). It was expected that NTP-CE has skin moisturizing potential and provides beneficial effects on skin barrier repair. Moreover, it was hypothesized that the skin microflora may benefit from the presence of α-glucan oligosaccharide, a topical prebiotic (3). *In vitro* studies showed that α-glucan oligosaccharide selectively supports the growth of the desired cutaneous bacterial strains *Corynebacterium xerosis* and *Micrococcus kristinae* leading to a competitive growth inhibition of pathogenic bacteria like *Staphylococcus aureus* (3).

It has been previously emphasized that for selecting emollients/moisturizers, an evidence-based approach should be pursued as not all formulations perform the same (1,11). In addition, according to cosmetic regulations, claims for cosmetic products have to be supported by adequate and verifiable evidence (12).

Taking these recommendations into account, one randomized controlled study was conducted which investigated the effects of NTP-CE on transepidermal water loss (TEWL), hydration of the

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stratum corneum, lipid lamellae structure, and stratum corneum lipid content in healthy adult subjects with dry skin (Study 1). Another randomized controlled study explored the effect of NTP-CE on recolonization of skin microflora after wash stress in healthy adults (Study 2).

*NTP-CE consists of aqua, caprylic/capric triglyceride, glycerin, olea europaea fruit oil, isostearyl isostearate, 1,2-hexanediol, cetearyl alcohol, panthenol, butyrospermum parkii butter, niacinamide (vitamin B_3), glyceryl stearate citrate, α-glucan oligosaccharide, limnanthes alba seed oil, hydrogenated lecithin, hippophae rhamnoides fruit extract, tocopheryl acetate, xanthan gum, sclerotium gum, squalene, tetrasodium glutamate diacetate, ceramide 3, and citric acid.

Methods

The trials were conducted in healthy adult subjects at *proDERM* Institute for Applied Dermatological Research, Schenefeld/Hamburg, Germany between November and December 2014. Both studies were performed according to the requirements of the Declaration of Helsinki with all its amendments. Subjects gave written informed consent to participate after being informed about the study. The new cosmetic emollient Bepanthen® SensiDaily (Bayer Consumer Care AG, Basel, Switzerland) was used in both trials. For both studies, Ethics approval was obtained from an independent German Ethics Committee.

Given the exploratory nature of the two studies, no formal sample size calculation was performed. For the same reason, no primary and secondary variables were defined. Based on historical data, it was expected that scientifically meaningful results can be obtained with the selected sample sizes (8,13–16).

Study 1: moisturization and barrier restoration study

Study design

This was an open-label, randomized, intra-individual comparison study in healthy adult subjects with dry skin. Study visits took place at screening (day 1), baseline (day 2) as well as on study days 3, 5, 8, 10, 15, 22, and 29. The subjects returned to the study site without having applied NTP-CE in the morning. There was no overnight confinement of study subjects.

Two sites (approximately 50 cm^2 each) were selected on each volar forearm (i.e. four test areas in total). At one site on each forearm, skin barrier dysfunction was experimentally induced by application of 0.5% sodium dodecyl sulfate (SDS, 250 µL) solution to the pre-marked test areas under occlusive plastic chambers (Finn Chambers®, SmartPractice, Barsbüttel, Germany) for 24 h. The other two areas (one on each volar forearm) remained unchallenged with SDS. After SDS challenge had been completed (day 2), NTP-CE was applied to two areas of the same volar forearm (one challenged, one unchallenged). On the contralateral arm, one challenged/untreated area and one unchallenged/untreated site served as positive and negative control, respectively. The allocation of areas to treatment (unchallenged/untreated, unchallenged/treated, challenged/untreated, challenged/treated) was done according to a balanced randomization list generated by a data manager prior to study start, with the caveat that NTP-CE treated areas had to be on the same forearm.

A pea-size amount of NTP-CE (2 mg/cm^2) was to be applied to the assigned test areas on a twice-daily schedule (morning and evening). Following baseline measurements (day 2), application of NTP-CE was done by the subjects themselves for 21 days. The assigned sites were marked and numbered which facilitated correct placement of NTP-CE. Compliance was assured by weighing each bottle with NTP-CE before and after the use period.

Subjects and assessments

In total, 23 healthy male and female subjects between 18 and 50 years of age with white skin (type I-III on Fitzpatrick scale (17)) were enrolled. At screening, they had to have dry skin on the volar forearms corresponding to a corneometer value of less than 40 instrumental units (i.u.). Subjects were not allowed to use other topical preparations on the test areas within 7 days prior to and during the study. For inclusion, females had to be non-pregnant and non-breastfeeding. Female subjects of childbearing age were required to use reliable methods of contraception during the study. Subjects were excluded if they had an active skin disease at the test areas, insulin-dependent diabetes, allergies to SDS or any ingredient of NTP-CE, infection requiring treatment with antibiotics within 4 weeks before the study or ongoing, any condition at the test areas influencing assessments (e.g. moles, tattoos, scars, irritated skin, hair), or took any antiphlogistic/analgesic agent (except for low doses of acetylsalicylic acid or paracetamol) within 3 days prior to the study.

Before instrumental measurements of TEWL and skin hydration during study visits, subjects remained in a climatized room (21 ± 1 °C, 50 ± 5% relative humidity) for at least 30 min. Over the study course, the measurements were made on the same challenged and unchallenged skin areas. TEWL (MPA Tewameter® TM300, Courage & Khazaka, Cologne, Germany), a noninvasive and sensitive method to quantify stratum corneum barrier function (18,19), was determined on each of the four test areas at days 2 (baseline), 3, 5, 8, 10, 15, and 22, as well as at 1 week (day 29) after cessation of NTP-CE application. There was one measurement per test area per assessment time. A decrease in TEWL reflects improvement in skin barrier function. At the same time points, skin hydration was determined by corneometry (Corneometer® CM825, Courage & Khazaka, Cologne, Germany) which measures the electrical capacitance of the skin surface. The latter is considered a function of the water content in the stratum corneum (20). Five measurements were performed in the test areas per assessment time. Values less than 30 i.u. represent very dry skin, 30–40 i.u. reflect dry skin, and values ≥40 i.u. are typically associated with normal skin. Thus, an increase in corneometer values mirrors a skin-moisturizing effect (21)).

On days 2 (baseline), 15, 22, and 29, skin probes from SDS-unchallenged test areas were collected in 12 subjects for analysis of stratum corneum lipid lamellae structure and lipid content. For that purpose, a noninvasive skin sampling technique (Lipbarvis®, Microscopy Services Dähnhardt GmbH, Flintbek, Germany) was applied as described previously (22). Sample collection took place from a skin area not previously used and which was different from other measurements. Specifically, the individual test areas were divided into two parts (one for instrumental measurements of TEWL and skin capacitance, and the other one for taking the Lipbarvis® samples). After collection, the skin probes were prepared for a subsequent blinded transmission electron microscopy (TEM) analysis of intercellular lipid lamellae (ICLL) organization in the stratum corneum (23). An increase in ICLL length corresponds to better barrier functions of the skin (22). The skin probes of all 12 subjects were also analyzed for lipid content (ceramide 3, cholesterol, and free fatty acids) using a high-performance thin layer chromatographic method (24) with densitometry. Adverse event (AE) monitoring took place over the entire study course.

Statistical evaluation

All statistical analyses were performed using SAS 9.3 for Windows. The full analysis set (FAS; all enrolled subjects with least one post-

application assessment) and safety population (all enrolled subjects who received at least one application of NTP-CE) were analyzed. The robustness of these results was to be tested by comparison with per protocol (PP) analysis results. For all measures, changes from baseline were calculated. In addition, for changes from baseline in TEWL and skin capacitance, the area under the curve (AUC) over the 3-week treatment period was calculated as a summary measure for each subject using the cumulative trapezoidal rule (25). Bilateral differences in the effect AUC between NTP-CE-treated and non-treated areas were statistically analyzed using the paired *t*-test at the significance level of 0.05. Results from ICLL length analysis and lipid analyses were subject to the same statistical testing. No adjustment for multiple testing was made. AEs were evaluated descriptively.

Study 2: skin microflora study

Study design

This was an open-label, randomized, intra-individual comparison study in healthy adult subjects. Study participants were confined to the study center for 1 day and received one single topical application of NTP-CE.

Four sites (approximately 21 cm^2 each) were selected for sampling on each volar forearm. Specifically, both forearms were washed with 10% SDS for 1 min, followed by rinsing with water. SDS at this concentration exerts transient antimicrobial effects rendering it possible to explore the type of skin microbiome following recolonization (26). After the washing procedure, a baseline dermal swab was performed on both forearms. Then, a pea-size amount of NTP-CE (2 mg/cm^2) was applied to one volar forearm once. The contralateral arm remained untreated. Each skin area (treated or not) was demarcated into four subsections, each for one quantitative assessment of total bacteria. The allocation of areas to treatment was done according to a balanced randomization scheme.

Subjects and assessments

In total, 20 healthy male and female subjects between 18 and 50 years of age with white skin (type I-III on Fitzpatrick scale (17)) were enrolled. Subjects were not allowed to use other topical preparations and any detergents on the test areas within 14 and 3 days, respectively, prior to study start. For inclusion, females had to be non-pregnant and non-breastfeeding. Female subjects of childbearing age were required to use reliable methods of contraception during the study. Subjects were excluded if they had an active skin disease at the test areas, allergies to any ingredient of NTP-CE, infection requiring treatment with antibiotics within 4 weeks before the study, any condition at the test areas influencing assessments (e.g. moles, tattoos, scars, irritated skin, hair), or regular/professional use of detergents on the test areas.

Quantitative bacterial cultures were obtained from the test areas at baseline and at 2, 4, and 6 h after start of NTP-CE application according to the scrub-wash method of Williamson and Kligman (27). In addition, sampling was performed from a non-SDS washed volar forearm skin area. Duplicate cultures were prepared from each bacterial extraction. For each time point, the total number of colony forming units (cfu) was quantified in a blinded fashion and the skin flora characterized for occurrence of commensal *Micrococcus* and *Corynebacterium* species (28). Results from duplicate plates were averaged. Given the high prevalence of *Staphylococcus epidermidis* on the skin of volar forearms (29), the growth of this strain was considered sufficiently reflected by the total number of viable bacteria. AE monitoring took place during the study.

Statistical evaluation

All statistical analyses were performed using SAS 9.3 for Windows. The FAS and safety populations were analyzed. The robustness of the results was to be tested by comparison with PP analysis results. Following logarithmic (log_{10}) transformation of the microbial counts, changes from baseline were calculated. In addition, for changes from baseline in log_{10} cfu counts, the AUC over the 6-h sampling period was calculated for each subject according to the trapezoidal rule (25). Bilateral differences (washed/NTP-CE-treated versus washed/untreated areas) in the change from baseline at each post-application time point and in the effect AUC were statistically analyzed using the Wilcoxon signed-rank test at the significance level of 0.05. No adjustment for multiple testing was made. Occurrence of commensal *Micrococcus* and *Corynebacterium* species was not analyzed statistically. AEs were to be evaluated using descriptive statistics.

Results

Study 1: moisturization and barrier restoration study

In total, 23 healthy subjects (19 females, 4 males) were enrolled. The mean age ± standard deviation (SD) was 36.3 ± 11.0 years; all subjects were included in the FAS. No PP analysis results are presented because the number of subjects in the FAS and PP population was virtually identical (23 vs. 22) as were the results. One study participant prematurely discontinued the study due to AEs (local erythema, itching, and papules).

Transepidermal water loss

The SDS challenge caused a marked skin barrier dysfunction as reflected by an approximately 3-fold increase in TEWL values compared with unchallenged test areas at baseline (Table 1). At the end of the study (day 29), there were no longer discernable TEWL differences to unchallenged areas (data not shown).

Following SDS challenge, a recovery of the skin barrier was observed in both NTP-CE-treated and untreated areas as reflected by negative values for TEWL change from baseline (Table 1). However, the reduction of TEWL was more pronounced and occurred earlier in the skin area treated with NTP-CE. Mean AUC for TEWL change from baseline over the 3-week treatment period, a robust parameter to identify response, was significantly lower

Table 1. Change from baseline in TEWL following twice-daily topical application of NTP-CE over 3 weeks.

	A	B	C	D
Day 2 (baseline)	6.77 ± 2.59	6.19 ± 2.21	20.07 ± 7.01	20.69 ± 6.99
Day 3	3.02 ± 6.55	2.22 ± 3.27	10.00 ± 9.36	11.79 ± 12.88
Day 5	1.50 ± 1.85	1.43 ± 1.49	0.00 ± 4.49	−2.63 ± 5.54
Day 8	2.39 ± 2.93	2.32 ± 2.23	−5.20 ± 4.71	−7.98 ± 5.48
Day 10	3.19 ± 4.98	3.14 ± 5.44	−5.67 ± 7.07	−8.41 ± 7.02
Day 15	−0.10 ± 1.37	0.02 ± 1.49	−10.26 ± 6.62	−13.05 ± 5.71
Day 22	−1.32 ± 1.50	−1.68 ± 1.35	−14.30 ± 6.14	−16.45 ± 5.52
$AUC_{0-22\ d}$	23.25 ± 31.78	19.83 ± 29.24	−123.38 ± 103.46	−168.36 ± 101.54*

$N = 21–23$. Data are given in $g/m^2/h$. All values are presented as mean ± SD.
A = unchallenged and untreated area (negative control); B = unchallenged area and treated with NTP-CE; C = SDS-challenged and untreated (positive control); D = SDS-challenged and treated with NTP-CE. AUC_{0-22d} = change from baseline over entire NTP-CE treatment period; d = day.
Baseline = baseline TEWL value before product application; baseline values were assessed after SDS challenge (C and D) and always before first topical application of NTP-CE.
*$p = 0.023$ if compared with non-treated control (C), paired *t*-test.
Note: A reduction of TEWL reflects improvement in skin barrier function.

when NTP-CE was applied to the SDS-challenged skin area compared with untreated area on the contralateral volar forearm ($p = 0.023$) indicating a greater improvement in skin barrier function. No difference was seen between treated and non-treated areas not undergoing SDS challenge.

Table 2. Change from baseline in skin capacitance following twice-daily topical application of NTP-CE over 3 weeks.

	A	B	C	D
Day 2 (baseline)	34.77 ± 6.17	35.51 ± 5.20	24.51 ± 7.78	23.43 ± 7.47
Day 3	1.55 ± 3.75	7.00 ± 4.08	13.93 ± 6.52	23.12 ± 8.69
Day 5	−0.55 ± 5.82	7.04 ± 7.47	−1.16 ± 11.87	3.14 ± 13.86
Day 8	−2.51 ± 4.86	6.01 ± 6.79	−6.23 ± 12.09	2.01 ± 12.24
Day 10	−2.83 ± 4.86	5.10 ± 5.77	−6.16 ± 10.60	9.41 ± 11.23
Day 15	2.62 ± 4.59	7.27 ± 5.90	5.85 ± 6.16	22.20 ± 11.39
Day 22	−0.15 ± 4.52	6.23 ± 6.27	10.82 ± 7.75	19.23 ± 7.39
$AUC_{0-22\ d}$	−0.95 ± 70.77	129.23 ± 97.30*	49.95 ± 132.46	274.87 ± 156.16†

$N = 22–23$. Data are given in i.u. All values are presented as mean ± SD.
A = unchallenged and untreated area (negative control); B = unchallenged area and treated with NTP-CE; C = SDS-challenged and untreated (positive control); D = SDS-challenged and treated with NTP-CE. AUC_{0-22d} = change from baseline over entire NTP-CE treatment period; d = day.
Baseline = baseline skin capacitance value before product application; baseline values were assessed by corneometry after SDS challenge (C and D) and always before first topical application of NTP-CE.
*$p < 0.001$ if compared with non-treated control (A), paired *t*-test.
†$p < 0.001$ if compared with non-treated control (C), paired *t*-test.
Note: An increase in skin capacitance reflects a skin-moisturizing effect.

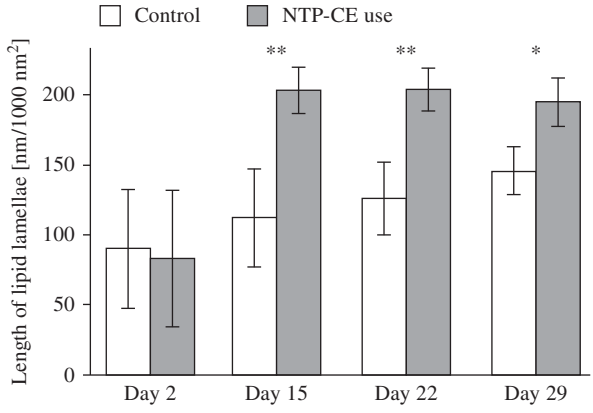

Figure 1. Mean (±95% confidence interval) length of intercellular lipid lamellae following twice-daily topical application of NTP-CE over 3 weeks (day 22) and at 1 week post-use (day 29). $N = 12$. Day 2 = baseline. **$p < 0.001$ for change from baseline, paired *t*-test. *$p = 0.001$ for change from baseline, paired *t*-test.

Stratum corneum hydration

At baseline, the non-SDS-challenged skin areas designated to be treated or untreated had similar values for dryness corresponding to dry skin. In accordance with previous findings (15), both skin areas which underwent SDS challenge were dryer at baseline than the unchallenged skin areas and revealed corneometer values in accordance with the definition of very dry skin (Table 2). At the end of the study (day 29), there were no longer discernable differences in skin dryness to unchallenged areas (data not shown).

In both SDS-unchallenged and SDS-challenged skin areas, the 3-week' use of NTP-CE produced an increase in stratum corneum hydration as reflected by an enhanced electrical capacitance of the skin surface compared with baseline (Table 2). On both SDS-challenged test areas, skin capacitance increased on day 3 which can be attributed to transient inflammatory reactions following SDS patch removal. Mean AUC for skin capacitance change from baseline over the 3-week treatment period was significantly greater when NTP-CE was applied to SDS-challenged and SDS-unchallenged skin areas compared with untreated areas on the contralateral volar forearm ($p < 0.001$ for both comparisons) indicating that NTP-CE application is associated with skin-moisturizing effects.

Stratum corneum lipid lamellae structure and lipid content

The mean length of ICLL increased from 82.93 to 203.53 nm/1000 nm^2 after the 3-week' use of NTP-CE (day 22) whereas in the untreated control area the increase was less pronounced (from 89.59 to 125.45 nm/1000 nm^2). Longer ICLL were even maintained over the 1-week follow-up period (day 29) post-use (Figure 1). For the area treated with NTP-CE, mean change from baseline was significantly greater ($p \leq 0.001$) at all post-baseline assessments than in the untreated control area. Specifically, on days 15, 22, and 29, mean ± SD change from baseline was 120.35 ± 79.17, 120.61 ± 90.13, and 111.50 ± 70.74 nm/1000 nm^2 for the NTP-CE-treated area and 22.46 ± 29.80, 35.85 ± 54.71, and 55.83 ± 80.79 nm/1000 nm^2 for the untreated area, respectively, thereby suggesting that NTP-CE has favorable and sustained effects on barrier functions of the skin.

Figure 2 shows representative TEM images after twice-daily topical application of NTP-CE over 3 weeks illustrating that application of NTP-CE led to a significant increase in ICLL organization in the stratum corneum compared with untreated control.

The NTP-CE-induced structural change of lipid arrangement within the stratum corneum, as assessed by morphometric analysis, was associated with increasing ceramide 3, cholesterol and free fatty acids contents over the study course. For the area treated with NTP-

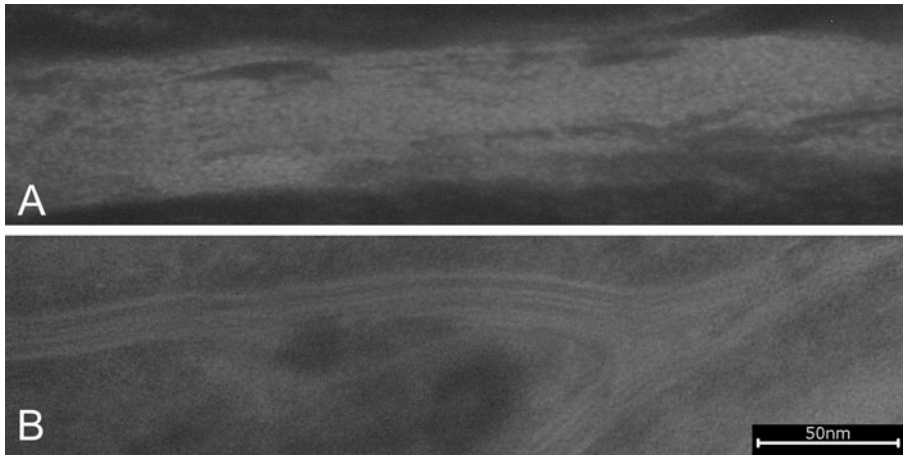

Figure 2. Selected TEM images of untreated skin (A) and skin following twice-daily topical application of NTP-CE over 3 weeks (B).

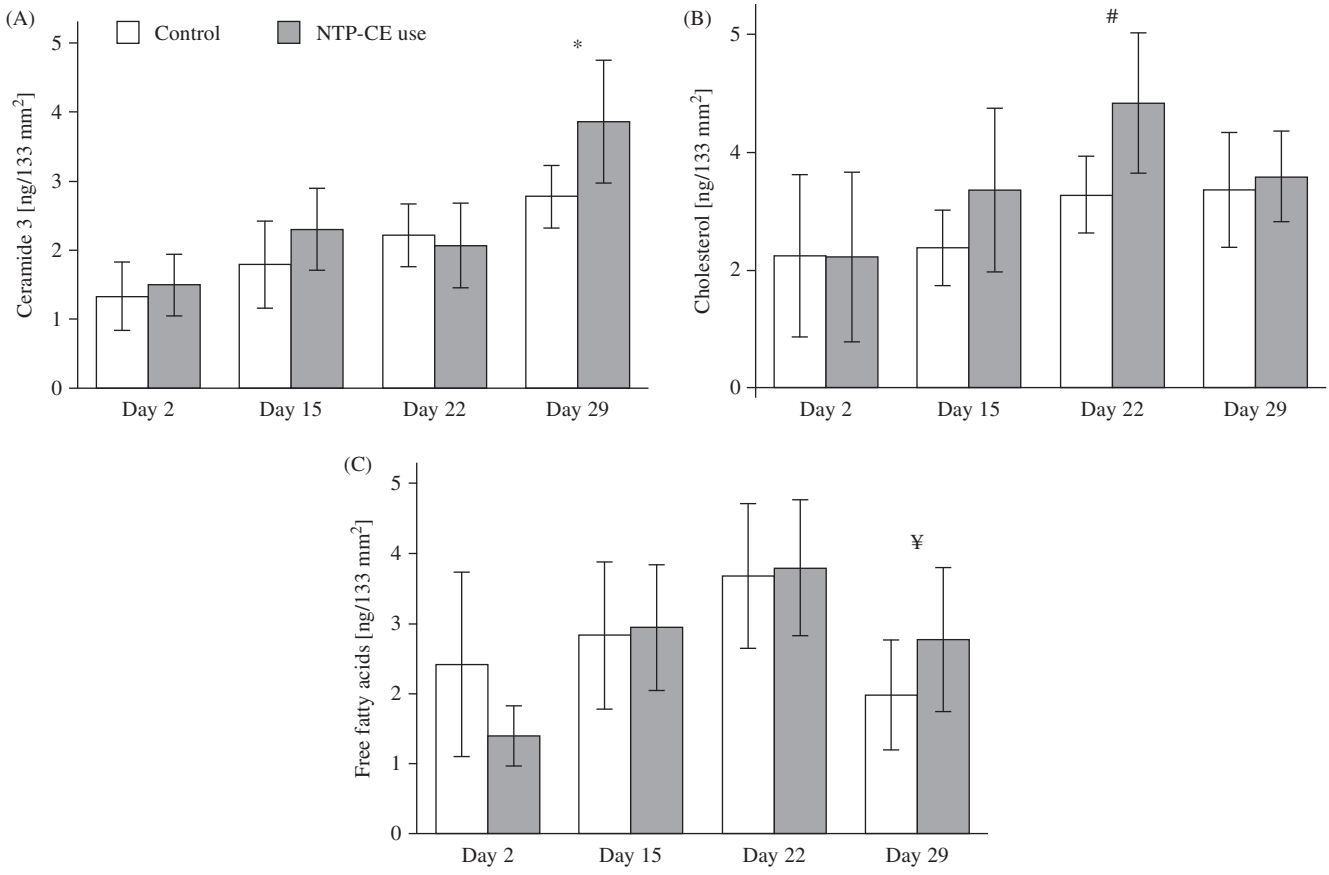

Figure 3. Mean (±95% confidence interval) content of ceramide 3 (A), cholesterol (B), and free fatty acids (C) in stratum corneum following twice-daily topical application of NTP-CE over 3 weeks (day 22) and at 1-week post-use (day 29). $N = 12$. Day 2 = baseline. *$p = 0.045$ for change from baseline, paired t-test. #$p = 0.027$ for change from baseline, paired t-test. ¥$p = 0.009$ for change from baseline, paired t-test.

CE, the mean changes from baseline were significantly greater in comparison with untreated skin at day 22 or day 29 (Figure 3).

Tolerability

One subject reported local AEs (erythema, itching, and papules) of mild to moderate severity at the site NTP-CE was applied. Following discontinuation of NTP-CE, the conditions improved. Otherwise, no AE or serious AE was recorded.

Study 2: skin microflora study

In total, 20 healthy subjects (15 females, 5 males) were enrolled and all completed the study. The mean age ± SD was 38.6 ± 8.9 years; all subjects were included in the FAS. No PP analysis was performed because the number of subjects in the FAS and PP population was identical.

Quantitative assessment of bacteria

Quantitative assessment of total bacteria from the skin area not washed with 10% SDS was consistent with normal skin flora (2.772 $\log_{10}$ cfu). SDS washing reduced the number of viable bacteria by approximately one log unit (Table 3). By the end of the 6-h follow-up period, the total number of bacteria had slightly increased from baseline with no significant difference between NTP-CE-treated and untreated areas at this or any other time point. Similarly, mean AUC for $\log_{10}$ cfu change from baseline over the 6-h follow-up period was not significantly different between NTP-CE-treated and untreated sites indicating that NTP-CE application did not negatively influence the number of viable bacteria (Table 3).

After application of NTP-CE, the number of subjects with *Micrococcus* species on their skin increased by about 70% (from 10 to 17 after 4 and 6 h) while it remained largely unchanged on the untreated skin. The number of subjects with *Corynebacterium* species on their skin increased about 3 times (from 5 to 15 after 6 h) following NTP-CE application, while only a 2-fold increase was observed on the untreated skin (from 5 to 11 after 4 and 6 h) indicating that NTP-CE application may support recolonization with commensal bacteria (Table 4).

Table 3. Change from baseline in total viable bacteria after single topical application of NTP-CE; 6-h follow-up period.

	A	B	p Values*
Baseline	1.595 ± 0.870	1.730 ± 1.013	–
2 h	−0.049 ± 0.860	−0.349 ± 0.958	0.121
4 h	−0.008 ± 0.710	0.208 ± 1.034	0.277
6 h	0.217 ± 0.883	0.202 ± 1.072	0.927
$AUC_{0-6\,h}$	0.104 ± 3.604	−0.079 ± 4.677	0.729

$N = 20$. Data are given in $\log_{10}$ cfu (at 2- to 6-h assessments) or $\log_{10}$ cfu.h (for AUC). All values are presented as mean ± SD.
A = untreated area; B = treated with NTP-CE. $AUC_{0-6\,h}$ = change from baseline over entire follow-up period; h = hour.
Baseline = baseline $\log_{10}$ cfu value before product application; baseline values were assessed after completion of SDS washing procedure.
*For mean change from baseline (B vs. A), Wilcoxon signed-rank test.

Tolerability

No AE or serious AE was reported during the study.

Discussion

In the context of the development of NTP-CE, one study investigated the effects on TEWL, stratum corneum hydration and lipid lamellae structure in healthy subjects with dry skin. Another study

Table 4. Number of subjects with occurrence of *Micrococcus* and *Corynebacterium* species after single topical application of NTP-CE; 6-h follow-up period.

	A*		B†	
	Micrococcus	*Corynebacterium*	*Micrococcus*	*Corynebacterium*
Baseline	13	5	10	5
2 h	14	9	13	8
4 h	12	11	17	12
6 h	14	11	17	15

*$N = 20$.
†$N = 19$. Data show the number of subjects.
A = untreated area; B = treated with NTP-CE.
Baseline = number of subjects before product application; baseline values were assessed after completion of SDS washing procedure.

explored the effect on skin microflora after wash stress in healthy volunteers. If compared with untreated skin areas, the findings of these two studies can be summarized as follows: (1) in a model of experimentally induced skin barrier dysfunction, barrier restoration was significantly more pronounced with NTP-CE; (2) NTP-CE use was associated with significant improvements in skin hydration; (3) application of NTP-CE on dry skin led to a sustained significantly increased ICLL length in the stratum corneum as assessed by ultrastructural morphometric analysis demonstrating a structurally improved skin barrier; (4) the NTP-CE-induced structural change of lipid arrangement within the stratum corneum was associated with an increasing ceramide 3, cholesterol, and free fatty acids content over the study course; and (5) NTP-CE use had no negative influence on bacterial viability and showed the tendency to exert favorable effects on skin microflora as reflected by a numerically higher proportion of subjects showing commensal bacteria which are important for the diversity of the skin microbiome and the protective role of the microflora (4).

The results of our two studies are in accordance with previous findings from investigations in which individual key components of NTP-CE have been studied, with the caveat that direct comparisons were hampered by the use of different methodologies or study designs. Topical panthenol-containing cream (70% lipids, 5% panthenol), enhanced skin barrier repair and stratum corneum hydration in experimentally damaged human skin when applied twice-daily for 7 days (15). In another study, topical panthenol formulated in two different lipophilic vehicles was applied to the skin of healthy subjects. Twice-daily application over 7 days significantly improved stratum corneum hydration and reduced TEWL compared with controls (8). For glycerin, it was shown to stimulate barrier repair in experimentally damaged human skin when applied for 3 days (14). The repeated application (twice-daily for 10 days) of a 20% glycerin-containing cream resulted in a significant increase in stratum corneum hydration compared with placebo in a double-blind study in healthy volunteers. However, glycerin showed no superiority in influencing TEWL (30,31). In a double-blind study, a lipid-rich emollient (containing ceramide 3) promoted barrier recovery in a model of experimentally induced skin barrier dysfunction when applied once-daily to healthy subjects for up to 7 days (16). It has been hypothesized that the percentage of lipids in the formulation is important for the desired effect since speed of recovery from induced skin damage correlated with increasing lipid content (32,33). Furthermore, it could be demonstrated that the lipid chain length (particularly with regard to ceramides and free fatty acids) is positively correlated with both skin barrier function and lipid organization in the stratum corneum (10). For the latter, a lamellar structure appears to be important. In healthy human skin, the intercellular lipid matrix shows a unique lamellar arrangement in the stratum corneum and consists mainly of ceramides, cholesterol, and free fatty acids (34,35).

Considering the results of our moisturization and barrier restoration study, it could be that key ingredients of NTP-CE act in an additive fashion. In fact, it has been previously postulated that more complex barrier formulas containing also ceramides/lamellar lipids appear to be beneficial for barrier repair (5).

The results of our skin microflora study can be interpreted as tentatively supporting previous *in vitro* investigations showing that α-glucan oligosaccharide promotes the growth of commensal cutaneous bacterial strains (3). Commensal bacteria contribute to a healthy skin microflora by inhibiting colonization and biofilm formation of pathogenic bacteria on the skin (36). It has been shown that topical application of gluco-oligosaccharides was successful in controlling *Staphylococcus aureus* colonization of AD skin (37). In cosmetics, prebiotics have the potential to increase selectively the activity and growth of beneficial skin microflora (38); the maintenance of a balanced microbiome does also contribute to an optimal and functional skin barrier (39). Reduction of microbial diversity and increase in *Staphylococcus aureus* have been associated with the occurrence of AD flares (4).

Dry skin is both a condition itself and a symptom of other conditions including AD (6). Clinical studies suggested that the daily use of emollients prevents AD or reduces flare-ups by improving the associated skin barrier defect (5,40,41). This is also mirrored in current guidelines which recommend long-term use of emollients for the maintenance of stable disease (42). Hence, NTP-CE may become a valuable addition to the armamentarium of emollients needed for daily care of dry and sensitive skin. The mechanisms by which NTP-CE exerts beneficial effects on dry skin have not been fully elucidated. It is assumed that better hydration, less evaporation of water, maintenance of a healthy balanced microbiome, and sustained restoration of lamellar lipid structure of the stratum corneum cumulatively keeps dry and sensitive skin at a stage resembling normal skin. NTP-CE was well tolerated in our studies, consistent with historical data gathered with emollients (1).

A limitation of our two studies may be the use of healthy subjects rather than a diseased population (e.g. AD patients). However, the background variability in healthy subjects is generally lower than in patients. Specifically, SDS challenge in healthy subjects is a well-established model which provides a uniform degree of skin barrier dysfunction (19). In contrast, use of lesional atopic skin with different degrees of involvement would provide highly variable baseline und subsequent measurements (43). Another limitation of both studies was that an untreated control was used instead of placebo. However, considering the complex formula of NTP-CE it was not possible to control for all ingredients considered important for the desired effect of an emollient. Finally, NTP-CE was not compared with an active comparator. Therefore, no superiority claims over other emollients can be made.

Conclusions

Recent studies have shown the importance of intercellular skin barrier lipids (10) and the role of the skin microflora in maintaining a healthy skin barrier (4). The findings of our two studies suggest that twice-daily application of NTP-CE is associated with significant and lasting improvements in skin barrier restoration, skin moisturization, and ICLL organization in the stratum corneum. In addition, there is some indication that NTP-CE may exert favorable effects on the skin microflora by supporting the growth of commensal bacteria. The results of the two studies support the

daily use of NTP-CE with favorable effects on skin barrier and skin microflora in the setting of dry and sensitive skin (e.g. basic emollient care for the maintenance of stable AD disease). This suggests that NTP-CE could be a useful adjunct in the management of AD as recent studies have shown that the use of emollients can prolong the time between AD flares (44). It would be helpful if future studies compare the skin moisturization and barrier restoration potential of NTP-CE with other emollients.

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Disclosure statement

Hans Stettler, Peter Kurka, and Holger Lenz are employees of Bayer Consumer Care AG, Basel, Switzerland. The other authors report no conflicts of interest.

Consent

We acknowledge that study participants cannot be identified via this paper; we have fully anonymized them.

Health and safety

It is confirmed that we complied with all mandatory health and safety procedures in the course of conducting the work reported in our paper.

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