

Cosmetic benefit of a biomimetic lamellar cream formulation on barrier function or the appearance of fine lines and wrinkles in randomized proof-of-concept clinical studies

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Abstract

OBJECTIVE: Two studies were designed to evaluate the potential cosmetic benefit of a biomimetic, niacinamide-containing moisturizing cream for the first time in humans.

METHODS: In both studies, healthy women were randomized to use two treatments, one for the left side of the body and one for the right, from three options: the test cream, a positive control or no treatment (use of standard cleanser only). Treatments were applied twice daily for 4 weeks to the face and forearms (Study 1) or the face only (Study 2). Instrumental and clinical skin assessments were performed by trained technicians. Study 1 involved tape stripping and a 5-day no-treatment ('regression') period at the end of the 4 weeks. Independent lay graders were asked to grade the skin texture of subjects in Study 2 from high-resolution photographs.

RESULTS: In Study 1 ($n = 66$), the test cream significantly decreased the transepidermal water loss (TEWL) values on the forearm, and in the cheek area of the face, relative to baseline and compared to no treatment, and increased skin Corneometer values. The improvements were partially retained during a subsequent 5-day period of no treatment. Increases in TEWL values on skin subjected to tape stripping were significantly lower after 4 weeks of using the test cream compared to no treatment. In Study 2 ($n = 72$ subjects with visible signs of ageing), there was a favourable trend in the change from baseline of a skin roughness parameter, R_a , for the test cream compared to no treatment. There were statistically significant improvements in the Fitzpatrick wrinkle score compared to no treatment, decreases in TEWL and increased Corneometer values and Cutometer values ($R5$ elasticity parameter). Grading of high-resolution images failed to detect the improvements in skin texture (defined as pores, smoothness and unevenness) for the test cream vs. no treatment. No treatment-related serious or severe adverse events were reported.

CONCLUSION: Twice daily application of the test cream over 4 weeks had beneficial effects on skin barrier function, moisturization, wrinkle dimensions and elasticity compared to no treatment. These studies provide proof-of-concept evidence and highlight the

cosmetic benefit of the biomimetic lamellar cream formulation. Study registration: NCT03216265, NCT03180645.

Résumé

OBJECTIF: Deux études ont été conçues pour évaluer pour la première fois chez l'être humain l'éventuel bénéfice cosmétique d'une crème hydratante biomimétique contenant de la niacinamide.

MÉTHODES: Dans les deux études, des femmes en bonne santé ont été randomisées pour utiliser deux traitements, un pour le côté gauche du corps et un pour le côté droit, choisis entre trois options : la crème testée, un contrôle positif ou aucun traitement (utilisation d'un nettoyant standard uniquement). Les traitements ont été appliqués deux fois par jour pendant 4 semaines sur le visage et les avant-bras (Étude 1) ou seulement sur le visage (Étude 2). Des évaluations instrumentales et cliniques de la peau ont été effectuées par des techniciens qualifiés. L'étude 1 impliquait un stripping et une période de 5 jours sans traitement (« régression ») à la fin des 4 semaines. Il a été demandé à des évaluateurs profanes indépendants d'évaluer la texture de la peau des participantes dans l'Étude 2 à partir de photographies à haute résolution.

RÉSULTATS: Dans l'Étude 1 ($n = 66$), la crème testée a diminué de manière significative les valeurs de la perte en eau transépidermique (transepidermal water loss, TEWL) au niveau de l'avant-bras, et au niveau de la joue, par rapport à la valeur de base, et par rapport au groupe sans aucun traitement, et a augmenté les valeurs des paramètres cutanés mesurés avec un cornéomètre. Les améliorations ont été partiellement conservées pendant une période ultérieure de 5 jours sans aucun traitement. Des augmentations des valeurs TEWL sur la peau exposée à un décollement d'un ruban adhésif étaient significativement plus faibles après 4 semaines d'utilisation de la crème testée par rapport à l'absence de traitement. Dans l'Étude 2 ($n = 72$ participantes avec des signes visibles de vieillissement), il y avait une tendance favorable au niveau de la variation par rapport à la valeur de base du paramètre relatif à la rugosité de la peau, R_a , pour la crème testée par rapport à l'absence de traitement. Il y a eu des améliorations statistiquement significatives du score de Fitzpatrick pour les rides par rapport à l'absence de traitement, des diminutions des valeurs TEWL et une augmentation des valeurs des paramètres mesurés avec un cornéomètre et des valeurs des paramètres mesurés avec un

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cutomètre (paramètre élasticité R5). L'évaluation des images à haute résolution n'a pas permis de détecter les améliorations de la texture de la peau (définie par les pores, la finesse et les irrégularités) pour la crème testée par rapport à l'absence de traitement. Aucun événement indésirable grave ou sévère lié au traitement n'a été rapporté.

CONCLUSION: Une application deux fois par jour de la crème testée pendant 4 semaines a eu des effets bénéfiques sur la fonction barrière de la peau, l'hydratation, l'aspect des rides et l'élasticité par rapport à l'absence de traitement. Ces études fournissent des éléments de preuve de concept et soulignent les bienfaits cosmétiques de la formule lamellaire biomimétique de la crème. Numéro d'enregistrement de l'étude : NCT03216265, NCT03180645.

Introduction

The stratum corneum (SC) is a highly organized, structural layer of dead skin cells that is essential for the maintenance of the water gradient between the uppermost layers of the skin. Many factors can render the skin's moisture barrier prone to perturbation and potentially induce dryness, irritation or itch having an overall impact on the quality of the skin [1]. Topically applied cosmetic products can help repair and prevent skin barrier disruption, enhance moisturization and reduce the severity of existing skin conditions [2–4].

Wrinkle development is a consequence of skin ageing, both chronological (or intrinsic) ageing and ageing caused by cumulative exposures to environmental (extrinsic) factors, especially ultraviolet light [5, 6]. The intrinsic and extrinsic factors result in changes in the skin, including changes in the amount of major structural molecules collagen, elastin and glycosaminoglycan [6]. Age-related decline in hyaluronic acid content within the skin results in compromised moisturization and firmness [7, 8]. Collectively, the range of age-related changes to the skin's elasticity, firmness and structure contributes to areas of collapse and irregularity and ultimately, manifest as fine lines, wrinkles and texture problems.

The development of cosmetic biomimetic lamellar lipid topical formulations to improve skin health has been a focus of research at our skin health laboratories. The clinical efficacy of our earlier formulations has been reported [9] and other studies have looked at the use of a lamellar moisturizer in skin conditions or after fractional laser administration [10–13]. In previous publications, we have discussed the development and use of biophysical methods to measure, characterize and demonstrate the molecular organization and *in vitro* barrier efficacy of our topical formulations containing biomimetic technology [14, 15]. Modifications to the composition and relative concentration of long-chain mono- and di-acyl lipids based upon learnings described in those publications, in conjunction with insights from *in silico* molecular simulations, have informed the development of additional novel, biomimetic lamellar lipid formulations. The first clinical efficacy studies of these newer formulations are described in this report.

Two proof-of-concept clinical studies were designed to determine whether a novel biomimetic cream formulation, that also contains niacinamide as a key functional ingredient, has cosmetic benefits of skin hydration, barrier repair and anti-ageing endpoints when tested in humans for the first time. Niacinamide has proven effects on the skin, which include protecting

the skin's barrier function, improving moisture content in the horny layer, and, on application to ageing skin, improving surface structure and smoothing out wrinkles [16–19]. The test cream in both studies was almost identical, only the concentration of niacinamide was 1% w/w lower in the cream used in Study 1 than Study 2, and the studies had similar efficacy endpoints.

Objectives

The primary objective in Study 1 was to assess the skin barrier function on the forearm after 28 days of using the test cream twice daily compared to no treatment. The primary objective in Study 2 was to evaluate wrinkle dimensions on the periorcular/crow's feet area after 28 days of using the test cream twice daily compared to no treatment.

Methods

Both studies were randomized, controlled, evaluator-blind clinical studies conducted at the proDERM Institute for Applied Dermatological Research in Hamburg, Germany between February 2017 and April 2017. Both were proof-of-concept studies designed to detect evidence of cosmetic benefit of a moisturizing cream formulated with biomimetic technology and niacinamide. There was a lower concentration of niacinamide in the test cream used in Study 1 than the test cream in Study 2 (both developed by GSK, Brentford, U.K.).

Study 1 was designed to investigate the effects of the cream on the barrier function of the skin. Study 2 was designed to evaluate the effect of the cream on wrinkles in subjects with visible signs of ageing.

Investigators in both studies obtained written informed consent from all subjects before enrolment. Summarized study protocols are available at www.gsk-clinicalstudyregister.com.

Screening

Study 1

Adult females (18 and 65 years inclusive), with self-reported dry, sensitive skin on the face and body and Fitzpatrick skin type I–IV were enrolled. At screening, and throughout, subjects were dermatologically assessed at the same four test areas: the left and right sides of the face, the volar surface of the left forearm and the volar surface of the right forearm. The parameters of dryness assessed were roughness, dull appearance, scaling and feeling of tightness. Each parameter was assessed on a scale of 0 (none) to 4 (very severe) in half-point increments. Subjects were considered eligible to participate if on each side of the face the total dryness score was ≥ 3 (sum across the four dryness parameters); if at each test area the score for roughness was ≥ 1 ; and if there was no more than a 0.5 point difference in the total score between test areas. Subjects with a score of 4 for any individual dryness parameter at any test area were excluded.

Study 2

Adult females (30 and 65 years inclusive), with self-reported sensitive skin, Fitzpatrick skin type I–IV and a visual clinical Fitzpatrick wrinkle score [20] of 3–6 in the periorcular (crow's feet) area of both sides of the face were enrolled.

Washout period

Eligible subjects underwent a 5- to 7-day washout in Study 1 or a 3-day washout in Study 2, during which the test areas were cleansed using a standard cleanser provided by the study (Simple Pure Soap in Study 1 or Simple Kind to Skin Moisturizing Facial Wash in Study 2 [Unilever, London, U.K.]). A bar soap was used in Study 1 and a liquid soap in Study 2. No other skincare products were permitted.

Baseline

After washout, subjects were reassessed for eligibility at baseline. Only subjects who continued to meet the dryness scoring criteria were considered eligible to continue in Study 1. Only subjects who still had a bilateral periocular Fitzpatrick wrinkle score between 3 and 6 could continue in Study 2.

Randomization and treatment period

In both studies, each subject was assigned two treatments: one treatment for the test areas on the left side of the body (both the face and forearm in Study 1, or only the face in Study 2) and a different treatment for the right side. For the purposes of the studies, an assigned 'treatment' could be a cream or no cream (no treatment to act as a negative control).

Subjects were randomized to one of the three treatment groups according to the randomization schedule generated by the sponsor, prior to the start of the study, using validated internal software. In addition to specifying the treatment group, each randomization number specified the side of the body (left/right) to which an assigned treatment was to be applied. The groups were:

- test cream/no treatment
- test cream/Olay ProX Wrinkle Smoothing Cream (Procter & Gamble, Cincinnati, OH, U.S.A.)
- Olay ProX Wrinkle Smoothing Cream/no treatment.

All subjects were given a standard cleanser to use on the test areas as required or prior to application of cream. Subjects assigned to no treatment used only the standard cleanser (no other product permitted) on the respective test area. The test cream or Olay ProX cream (the positive control) was applied topically and twice daily (morning and evening) for 28 days. Subjects were reminded that, as with all skincare products, they should avoid getting the cream into the eyes. If contact with eyes did occur, they were to rinse thoroughly with water.

In Study 1, a period of 5 days with no treatment ('regression' period) was included following the 28-day treatment phase. During this phase no topical cream was used, only the standard soap was used for cleansing as needed. This was designed to evaluate the retention of skin barrier function and moisturization improvements following cessation of test cream or Olay ProX cream use.

The trained technicians performing the study measurements were blinded to the treatment group assignments.

Assessments

Study 1

Instrumental assessments of skin barrier function measured by TEWL using a Tewameter[®] TM 300 (Courage + Khazaka electronic GmbH, Cologne, Germany) and skin moisturization using a

Corneometer[®] CM 825 (Courage + Khazaka electronic GmbH) were performed before the first study product application on Day 1 (baseline) and at each subsequent visit (Days 2, 15, 29, 30, 31, 32, 33 and 34 [final visit]). Two areas on the volar surface of each forearm that were as close together as possible without overlap were chosen for all TEWL and Corneometer assessments – one area closer to the elbow and one closer to the wrist. Similarly, two areas on each side of the face were selected for all assessments, along the cheekbone between the ear and nose. A reduction in TEWL is indicative of a stronger skin barrier function. An increase in the Corneometer value is indicative of a skin-moisturizing effect.

Tape stripping was performed on selected sites of the forearm and face at the end of the 28-day treatment period (Day 29) to evaluate the effect of a physical challenge to the skin barrier; the protein content of D-Squame[®] discs (Clinical and Derm, Dallas, Texas, U.S.A.) was analysed using a SquameScan[™] 850 (Heiland electronic GmbH, Wetzlar, Germany). Adverse events (AEs) were assessed throughout.

Study 2

Clinical and instrumental assessments were performed on Days 1 (baseline), 15 and 29 (final visit), including, in the periocular/crow's feet area, clinical assessments of fine lines and wrinkles by the Fitzpatrick wrinkle score [20] and instrumental measurements of skin topography using the DermaTOP-V3 system (Eotech, Marcoussis, France). Instrumental measurements of TEWL using a Tewameter[®] TM 300, skin moisturization using a Corneometer[®] CM 825 and skin elasticity using a Cutometer[®] MPA 580 (Courage + Khazaka electronic GmbH) were performed in the subocular/cheek area (from the corner of the eye onto the middle of the cheekbone). The Cutometer vacuum was set to 100 mbar, with a suction time of 5 s and a subsequent measuring period of another 5 s after release and Cutometer parameters were reported for each test area once.

High-resolution images of the left and right side of each subject's whole half-face were taken at Day 1 (baseline) and Day 29 (final visit) using a colour 50-megapixel camera (Hasselblad H5D-50c, Hasselblad Group, Göteborg, Sweden) under fixed conditions. At the end of the study, each blinded image pair was randomly displayed on a colour-calibrated screen and assessed for texture (defined as pores, smoothness and unevenness) by a blinded panel of 24 independent lay graders recruited by the study site; they were instructed to rank each image on a scale of 1 (=better texture) or 2 (=worse texture). Adverse events (AEs) were assessed throughout.

Endpoints

Study 1

The primary objective was to assess the skin barrier function (TEWL) on the forearm after 28 days of using the test cream twice daily compared to no treatment. The change from baseline in TEWL measurements (primary endpoint) was summarized by treatment arm (test cream, positive control and no treatment) using descriptive statistics, and treatment arms were compared using analysis of covariance (ANCOVA) with subject as a random effect, treatment arm and side of body (right, left) as main effects and baseline value as a covariate.

Secondary endpoints included assessment of TEWL on the face after the 28-day treatment period, the impact on TEWL of a physical challenge (D-Squame tape stripping) and protein levels on D-Squame discs removed from both forearms and the face. Additional assessments included Corneometer assessments during the treatment period.

TEWL and Corneometer assessments through the 5-day regression period and AEs. The same ANCOVA method used for the primary endpoint was used for secondary efficacy analyses comparing treatment arms. The frequency and severity of AEs were summarized.

Study 2

The primary objective was to evaluate wrinkle dimensions on the periocular/crow's feet area after 28 days of using the test cream twice daily compared to no treatment. The primary endpoint was the change from baseline in the DermaTOP roughness parameter R_a , defined according to the standard DIN EN ISO 4287, at 28 days. A decrease in a roughness parameter corresponds to a decrease in the degree of wrinkles. This was summarized by treatment arm (test cream, positive control and no treatment) using descriptive statistics, and treatment arms were compared using ANCOVA with subject as a random effect, treatment arm and side of body (right, left) as main effects and baseline value as a covariate.

Secondary objectives included evaluating other measures of wrinkle dimensions on the periocular/crow's feet area at Days 15 and 29 (after 2 and 4 weeks of treatment) and evaluating TEWL, skin moisturization and elasticity on the subocular/cheek area at Days 15 and 29. Cutometer parameters R5 (net elasticity) and R7 (portion of elasticity compared to the complete visco-elastic curve) were calculated; the closer the R5 or R7 value is to 1, the more elastic the skin. R5 and R7 have been negatively correlated with age, particularly R7 of the face which researchers found decreased significantly with ageing [21].

Secondary objectives were also to evaluate the appearance of skin texture on the face after using the test cream or the positive control, compared to no treatment, and to evaluate the safety of the study products. The same ANCOVA method used for the primary endpoint was used for primary and secondary efficacy analyses. The frequency and severity of AEs were summarized.

As proof-of-concept, both studies were considered to be successful if at least trends in the primary endpoints in favour of the test cream were found compared to no treatment. All efficacy analyses were performed on the intent-to-treat (ITT) population, defined as all randomized subjects with ≥ 1 post-baseline study assessment. The positive control was included in both studies to support validation of the clinical model and trial results. Based on statistical estimations, approximately 90 subjects were planned to be screened in each study in order to randomize approximately 66 subjects in Study 1 and at least 72 subjects in Study 2 and to ensure at least 20 subjects per treatment group in Study 1 and approximately 22 subjects per treatment group in Study 2.

Results

Sixty-six of 93 screened women met the study criteria and were randomized in Study 1. Seventy-two of 86 screened women met the study criteria and were randomized in Study 2. All randomized subjects received at least one study product application and were therefore included in the safety analysis; however, one randomized subject in Study 1 and two randomized subjects in Study 2 were excluded from the ITT analysis because they lacked post-baseline data (Fig. 1). Sixty subjects completed Study 1 and 69 subjects completed Study 2.

The 66 randomized subjects in Study 1 had a mean age of 48.0 years (standard deviation [SD] 12.69; range 21–65 years) and the 72 randomized subjects in Study 2 had a mean age of 50.3 years (SD 7.65; range 33–65 years). In both studies, all

randomized subjects were white females, most had a Fitzpatrick skin type of II or III (Table I), and the demographic characteristics of the ITT and safety populations were comparable across the three treatment groups.

Study 1

TEWL. TEWL values ($\text{g m}^{-2} \text{h}$) decreased in the test cream and positive control groups over 4 weeks of twice daily application to the forearm and face (Fig. 2). On the forearm, the adjusted mean change from baseline in the test cream group was significantly different from the no-treatment group, in which increases from baseline in TEWL were observed; therefore, the primary endpoint was met (difference = -1.44 , 95% confidence interval [CI] -2.10 , -0.78 ; $P < 0.0001$). The results of TEWL measurements on the face were similar – the difference between test cream and no treatment at 4 weeks was -1.24 (95% CI: -2.30 , -0.18 ; $P = 0.0230$). Similar results were observed for the comparison between positive control cream and no treatment.

At the end of the treatment period (Day 29), TEWL on the forearm was lower than baseline in the test cream and positive control groups, whereas in the no-treatment group, TEWL was greater at Day 29 compared to baseline. Over the 5-day regression period (Day 29–34), there was no obvious change in forearm TEWL values in the test cream group – the values during the regression period were similar to Day 29. The spike in forearm TEWL in the Olay ProX cream group during the regression period (Fig. 2A) was due to one subject who had an extremely high TEWL value ($>50 \text{ g m}^{-2} \text{h}$) on Day 30; this high value was confirmed as valid, but of unknown cause. The forearm TEWL values in the no-treatment group increased slightly during the regression period.

At the end of the treatment period (Day 29), TEWL on the face was lower in all three treatment groups compared to baseline. In the regression period, there was an increase in face TEWL values in the test cream and positive control groups, whereas no obvious change was observed in the no-treatment group. Importantly, all three groups maintained a significantly lower TEWL value on the face compared to baseline (Day 1) through to Day 34.

Corneometer assessments. Forearm Corneometer measurements were generally lower in the no-treatment group than the test group and positive control group during the treatment period (Fig. 3). There were significant increases from baseline in forearm Corneometer values 30 min after the first applications of the test cream and positive control cream. On both the face and forearms, significant differences between the test cream and no treatment, in favour of the test cream, were observed in the adjusted mean changes from baseline, with a difference on the forearms of $+6.81$ at 4 weeks (95% CI: 4.42, 9.21; $P < 0.0001$) and $+11.28$ on the face (95% CI: 8.23, 14.32; $P < 0.0001$). Comparison between positive control cream and no treatment also revealed significant differences in favour of the positive control.

Mean Corneometer values in the test-cream group (forearm and face) declined during the 5-day regression phase, but they continued to be significantly larger at Day 34 compared to baseline, indicating partial retention of the moisturization benefits observed during the treatment period. A similar result was observed in the positive control group for Corneometer assessments of the face. In contrast, the significant mean change from baseline in Corneometer values observed at Day 29 on the forearm in the positive

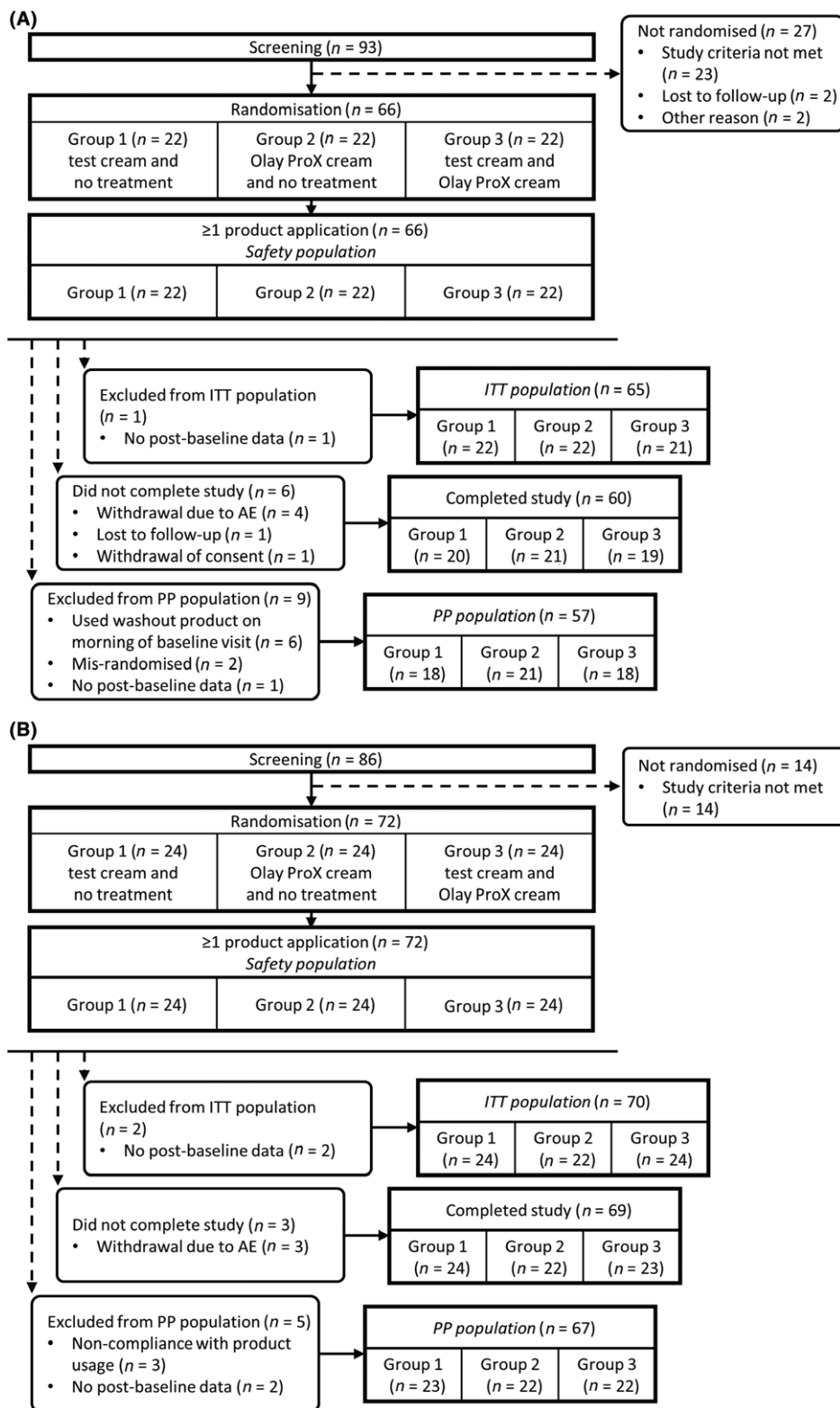


Figure 1 Subject flow. Screening and randomization of study subjects in (A) Study 1 and (B) Study 2. PP, per protocol; ITT, intent-to-treat.

Table 1 Study subjects' demographic characteristics: ITT populations

	Study 1				Study 2			
	Test cream (n = 43)	Olay ProX cream (n = 43)	No treatment (n = 44)	Overall (n = 65)	Test cream (n = 48)	Olay ProX cream (n = 46)	No treatment (n = 46)	Overall (n = 70)
Sex, female (%)	43 (100)	43 (100)	44 (100)	65 (100)	48 (100)	46 (100)	46 (100)	70 (100)
Age, years								
Mean (SD)	47.8 (12.43)	47.1 (13.61)	49.0 (12.36)	48.0 (12.79)	50.3 (7.25)	49.7 (8.12)	50.9 (7.84)	50.3 (7.72)
Range	21–65	21–65	25–65	21–65	36–65	33–65	33–65	33–65
Race, white (%)	43 (100)	43 (100)	44 (100)	65 (100)	48 (100)	46 (100)	46 (100)	70 (100)
Fitzpatrick skin type, n (%)								
I	1 (2.3)	2 (4.7)	1 (2.3)	2 (3.1)	4 (8.3)	8 (17.4)	8 (17.4)	10 (14.3)
II	18 (41.9)	15 (34.9)	15 (34.1)	24 (36.9)	19 (39.6)	16 (34.8)	19 (41.3)	27 (38.6)
III	18 (41.9)	22 (51.2)	24 (54.5)	32 (49.2)	18 (37.5)	16 (34.8)	14 (30.4)	24 (34.3)
IV	6 (14.0)	4 (9.3)	4 (9.1)	7 (10.8)	7 (14.6)	6 (13.0)	5 (10.9)	9 (12.9)

n, number of subjects.

control group was no longer significant compared to baseline (Day 1) at Day 34 (Fig. 3B).

Tape stripping challenge. In all treatment groups, forearm and face TEWL values increased significantly following the removal of the adhesive discs in the tape stripping challenge, consistent with induced disruption to the skin barrier function. Increases in TEWL after the challenge were significantly less pronounced in the test-cream group than in the no-treatment group ($P = 0.0359$ after removal of discs on the forearm; $P = 0.0197$ after removal of discs on the face; Table II). Similar results were observed for the comparison between positive control and no treatment.

The cumulative level of protein extracted from the D-Squame discs was analysed. There was a significant difference in disc protein levels between test cream and no treatment (Table III; $P < 0.0001$ for both forearm and face) with removal of less protein from the skin after using the test cream. Similar results were observed for the comparison between the positive control and no treatment.

Study 2

Instrumental and clinical wrinkle assessments. The adjusted mean change from baseline to 4 weeks in the R_a parameter was greater in the test-cream group than the no-treatment group (Fig. 4) although the between-group comparison was not significant (difference = -1.07 , 95% CI: $-2.21, 0.06$; $P = 0.0638$). This favourable numerical trend for the test cream met the primary efficacy endpoint. No improvement was observed for the positive control over no treatment (difference -0.11 , 95% CI: $-1.27, 1.05$). In contrast, Fitzpatrick wrinkle scores for both the test cream and positive control cream were significantly improved at 4 weeks vs. no treatment (Table IV).

TEWL, Corneometer and Cutometer measurements. Both the test and positive control cream had a beneficial effect on skin barrier function compared to no treatment after 2 and 4 weeks of use, as measured by TEWL (Table IV). Both the test and positive control cream provided a significant skin moisturization benefit

compared to no treatment after 2 and 4 weeks, as measured by Corneometer scores. Analyses of the adjusted mean changes from baseline in Cutometer parameters R5 and R7 showed that the test cream was significantly more effective than no treatment at increasing the elasticity of the skin after 2 and 4 weeks

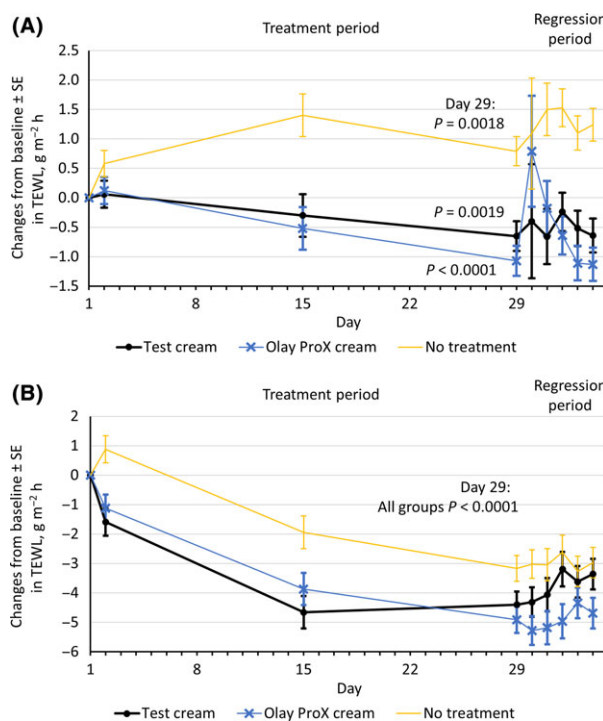


Figure 2 TEWL measures – Study 1. Forearm (A) and face (B) TEWL measurements over time: ITT population in Study 1 ($n = 65$). Least squares mean changes from baseline. Baseline is measured prior to any study product application on Day 1. An increase in TEWL values shows damage to the skin barrier function. The P values shown are for the changes from baseline within a group. SE, standard error.

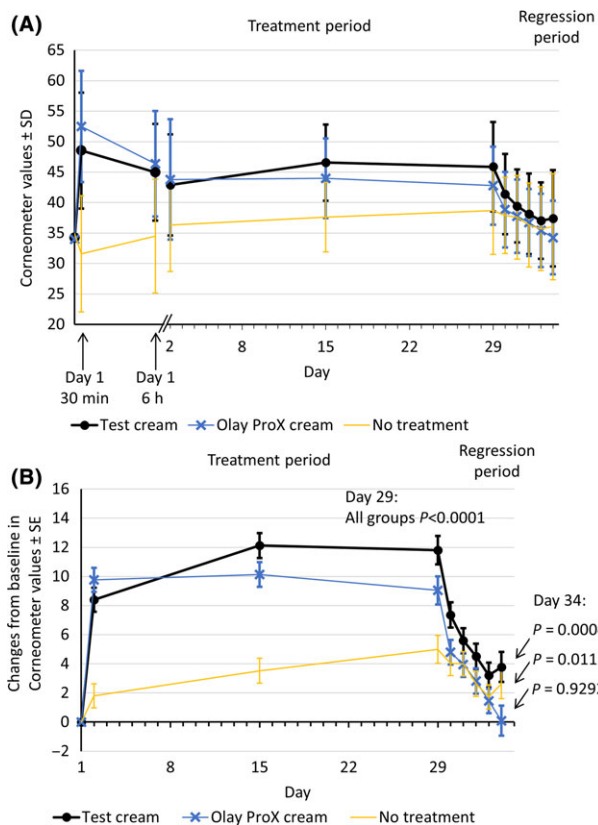


Figure 3 Corneometer values – Study 1. Forearm Corneometer values over time: ITT population in Study 1 ($n = 65$). (A) Raw means are presented. (B) Least squares mean changes from baseline. Baseline is measured prior to any study product application on Day 1. An increase in Corneometer value is indicative of a skin-moisturizing effect. The P values shown are for the changes from baseline within a group. SD, standard deviation; SE, standard error.

Table III Differences between treatment groups in D-Squame protein content (forearm) – ITT population in Study 1

Comparisons between treatments	Difference ^a	95% CI	P value
Test cream vs. No treatment	-32.4	-43.27, -21.61	<0.0001
Olay ProX cream vs. No treatment	-38.2	-49.06, -27.40	<0.0001

^aDifference is the first named treatment adjusted (least squares) mean minus the second named treatment adjusted mean. A negative difference favours the first named treatment.

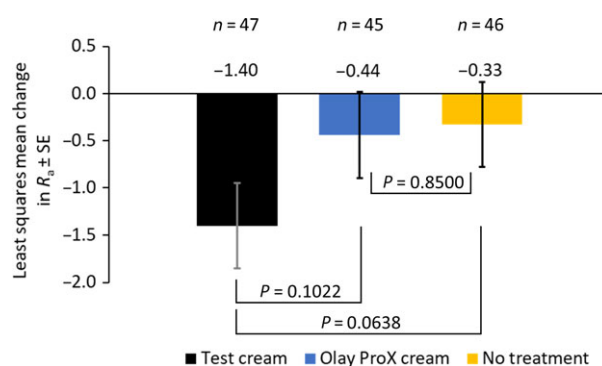


Figure 4 Day 29 change from baseline in roughness parameter R_a – Study 2. Day 29 change from baseline in roughness parameter R_a on the periocular/crow's feet area in ITT Population of Study 2 ($n = 70$). A decrease in a roughness parameter corresponds to a decrease in the degree of wrinkles. SE, standard error.

Table II Differences between treatment groups after tape stripping challenge in change from pre-challenge TEWL – ITT population in Study 1

	Comparisons between treatments	TEWL difference ^a	95% CI	P value	
Forearm	After 4 discs	Test cream vs. No treatment	-0.89	-1.45, -0.33	0.0021
		Olay ProX cream vs. No treatment	-0.95	-1.52, -0.38	0.0013
	After 8 discs	Test cream vs. No treatment	-1.26	-2.24, -0.27	0.0130
		Olay ProX cream vs. No treatment	-1.55	-2.56, -0.54	0.0031
Face	After 12 discs	Test cream vs. No treatment	-1.99	-3.85, -0.13	0.0359
		Olay ProX cream vs. No treatment	-2.36	-4.26, -0.45	0.0162
	After 3 discs	Test cream vs. No treatment	-1.26	-2.53, 0.01	0.0511
		Olay ProX cream vs. No treatment	-1.15	-2.44, 0.15	0.0820
	After 6 discs	Test cream vs. No treatment	-3.98	-6.53, -1.44	0.0026
		Olay ProX cream vs. No treatment	-4.72	-7.33, -2.12	0.0005
	After 9 discs	Test cream vs. No treatment	-4.48	-8.23, -0.74	0.0197
		Olay ProX cream vs. No treatment	-6.95	-10.8, -3.11	0.0006

Analysis model (ANCOVA) included subject as a random effect, treatment arm and side of body (right, left) as fixed effects and pre-challenge value as a covariate. ^aDifference is the mean TEWL change for the first named treatment minus the mean TEWL change for the second named treatment, where mean changes are adjusted (least squares) mean changes in TEWL from the pre-challenge value. A negative difference favours the first named treatment.

Table IV Efficacy endpoints summary in ITT population in Study 2

	Test cream (<i>n</i> = 48)	Olay ProX cream (<i>n</i> = 46)	No treatment (<i>n</i> = 46)
R_a parameter (primary endpoint)			
Baseline, mean (SD)	24.24 (5.743)	22.48 (4.594)	23.35 (4.895)
Day 29 LS mean change from baseline (SE)	-1.40 (0.449)	-0.44 (0.460)	-0.33 (0.452)
Day 29 difference vs. no treatment (95% CI) ^a	-1.07 (-2.21, 0.06; <i>P</i> = 0.0638)	-0.11 (-1.27, 1.05; <i>P</i> = 0.8500)	
Fitzpatrick Wrinkle score			
Baseline, mean (SD)	4.77 (0.951)	4.72 (1.068)	4.72 (0.911)
Day 15 LS mean change from baseline (SE)	-0.33 (0.072)	-0.28 (0.074)	-0.15 (0.074)
Day 15 difference vs. no treatment (95% CI) ^a	-0.18 (-0.38, 0.03; <i>P</i> = 0.0917)	-0.13 (-0.34, 0.08; <i>P</i> = 0.2141)	
Day 29 LS mean change from baseline (SE)	-0.50 (0.090)	-0.58 (0.092)	-0.22 (0.091)
Day 29 difference vs. no treatment (95% CI) ^a	-0.28 (-0.52, -0.04; <i>P</i> = 0.0244)	-0.36 (-0.61, -0.12; <i>P</i> = 0.0041)	
TEWL, g m⁻² h			
Baseline, mean (SD)	23.83 (5.219)	24.20 (5.681)	23.30 (5.320)
Day 15 LS mean change from baseline (SE)	-4.36 (0.622)	-5.43 (0.633)	-2.37 (0.633)
Day 15 difference vs. no treatment (95% CI) ^a	-1.99 (-3.42, -0.56; <i>P</i> = 0.0070)	-3.06 (-4.52, -1.60; <i>P</i> < 0.0001)	
Day 29 LS mean change from baseline (SE)	-5.29 (0.571)	-6.30 (0.580)	-3.19 (0.576)
Day 29 difference vs. no treatment (95% CI) ^a	-2.10 (-3.36, -0.84; <i>P</i> = 0.0014)	-3.11 (-4.40, -1.82; <i>P</i> < 0.0001)	
Corneometer value			
Baseline, mean (SD)	56.56 (10.736)	59.16 (10.988)	58.66 (10.824)
Day 15 LS mean change from baseline (SE)	5.77 (1.199)	7.06 (1.223)	-0.66 (1.221)
Day 15 difference vs. no treatment (95% CI) ^a	6.43 (3.48, 9.38; <i>P</i> < 0.0001)	7.72 (4.72, 10.72; <i>P</i> < 0.0001)	
Day 29 LS mean change from baseline (SE)	7.68 (1.208)	10.54 (1.234)	1.77 (1.219)
Day 29 difference vs. no treatment (95% CI) ^a	5.90 (2.77, 9.04; <i>P</i> = 0.0003)	8.76 (5.58, 11.95; <i>P</i> < 0.0001)	
Cutometer value for parameter R5			
Baseline, mean (SD)	0.43 (0.114)	0.46 (0.119)	0.44 (0.084)
Day 15 LS mean change from baseline (SE)	0.05 (0.014)	0.02 (0.014)	-0.05 (0.014)
Day 15 difference vs. no treatment (95% CI) ^a	0.09 (0.06, 0.13; <i>P</i> < 0.0001)	0.07 (0.03, 0.10; <i>P</i> = 0.0006)	
Day 29 LS mean change from baseline (SE)	0.07 (0.015)	0.06 (0.015)	0.03 (0.015)
Day 29 difference vs. no treatment (95% CI) ^a	0.04 (0.01, 0.08; <i>P</i> = 0.0262)	0.03 (-0.01, 0.07; <i>P</i> = 0.1282)	
Cutometer value for parameter R7			
Baseline, mean (SD)	0.27 (0.069)	0.27 (0.066)	0.26 (0.064)
Day 15 LS mean change from baseline (SE)	0.02 (0.008)	0.01 (0.008)	-0.03 (0.008)
Day 15 difference vs. no treatment (95% CI) ^a	0.05 (0.03, 0.07; <i>P</i> < 0.0001)	0.04 (0.02, 0.06; <i>P</i> = 0.0005)	
Day 29 LS mean change from baseline (SE)	0.03 (0.008)	0.02 (0.009)	0.01 (0.008)
Day 29 difference vs. no treatment (95% CI) ^a	0.02 (-0.00, 0.04; <i>P</i> = 0.0816)	0.01 (-0.01, 0.03; <i>P</i> = 0.4446)	
Texture ranking of high-resolution facial images			
Percentage of subjects considered to have better texture on Day 29 compared to baseline, %	41.49	39.54	40.58
Treatment vs. no treatment log odds ratio of Day 29 image better than baseline (95% CI for the log odds ratio) ^b	0.04 (-0.13, 0.21; <i>P</i> = 0.6612)	-0.04 (-0.21, 0.13; <i>P</i> = 0.6206)	

A decrease in a roughness parameter R_a corresponds to a decrease in the degree of wrinkles. Decreasing Fitzpatrick wrinkle scores indicate a less wrinkled appearance. An increase in TEWL values shows damage to the skin barrier function. An increase in Corneometer value is indicative of a skin-moisturizing effect. The closer the R5 or R7 Cutometer values are to 1, the more elastic the skin. At Day 29, *n* = 47 for test cream and *n* = 45 for Olay ProX cream.

^aDifference vs. no treatment in adjusted mean change from baseline (95% CI for difference).

^bAnalysis model (logistic regression) including treatment arm and side of the face as fixed effects.
n, number of subjects; SE, standard error.

(significant differences in favour of test cream at both time points for R5 and at the 2-week mark for R7), whereas the positive control cream was favoured over no treatment only at the 2-week mark (differences for R5 and R7 were significant only at 2 weeks).

Skin texture. Grading of high-resolution images failed to detect an improvement in skin texture (defined as pores, smoothness and unevenness) for the test and positive control cream over no treatment. There was no difference in the percentage of subjects considered to have a better texture on Day 29 compared to baseline between the test or the positive control group and the no-treatment group.

Safety

Study 1

The number of subjects experiencing at least one treatment-emergent AE (TEAE) was similar for all three treatments: 22.7–27.3% (Table V). One subject who was randomized to the test and positive control group experienced severe nephritis; this was not considered by the investigator to be related to either study treatment, but it led to withdrawal. All other reported TEAEs were mild or moderate in severity. The most commonly reported AEs were headache (five subjects, 7.6%) and nasopharyngitis (four subjects, 6.1%) – none of which were considered related to a study product.

Table V Treatment-emergent AEs: safety populations

	Study 1							Study 2								
	Test cream (n = 44)		Olay ProX cream (n = 44)		No treatment (n = 44)		Overall (n = 66)	Test cream (n = 48)		Olay ProX cream (n = 48)		No treatment (n = 48)		Overall (n = 72)		
	n (%)	nAE	n (%)	nAE	n (%)	nAE	n (%)	nAE	n (%)	nAE	n (%)	nAE	n (%)	nAE	n (%)	nAE
Any treatment-emergent AE	10 (22.7)	26	11 (25.0)	22	12 (27.3)	23	20 (30.3)	41	12 (25.0)	19	10 (20.8)	17	11 (22.9)	14	17 (23.6)	28
Serious ^a	1 (2.3)	1	1 (2.3)	1	0	0	1 (1.5)	1	0	0	0	0	0	0	0	0
Severe	1 (2.3)	1	1 (2.3)	1	0	0	1 (1.5)	1	0	0	0	0	0	0	0	0
Leading to discontinuation	4 (9.1)	13	2 (4.5)	7	2 (4.5)	6	4 (6.1)	13	1 (2.1)	6	3 (6.3)	9	1 (2.1)	1	3 (4.2)	10
Considered related to study treatment	2 (4.5)	5	3 (6.8)	4	3 (6.8)	6	7 (10.6)	15	1 (2.1)	6	4 (8.3)	10	1 (2.1)	1	4 (5.6)	11

^aAccording to regulatory definitions of serious adverse events [26, 27].
n, number of subjects; nAE, number of adverse events.

Seven subjects (10.6% of safety population) experienced at least one TEAE that was considered treatment related. Of these, eyelid oedema, application site erythema, application site papules, application site pruritus and application site scab (reported in one subject each) were related to the test cream; application site papules (two subjects), application site dryness (one subject) and application site pain (one subject) to the positive control cream and application site erythema (two subjects), eyelid oedema, application site reaction, application site pruritus and application site scab (one subject each) to no treatment.

Overall, four subjects (6.1% of safety population) discontinued treatment because of TEAEs. In the test/positive control group, one subject with nasopharyngitis and another with pyrexia, cystitis, ear infection, oropharyngeal pain and nephritis discontinued the study. In the test/no-treatment group, one subject who experienced pyrexia and nasopharyngitis and another subject with nausea, upper abdominal pain, application site erythema and an application site scab discontinued the study (application site AEs were considered causally related to both the test moisturizer and the standard soap [no treatment]).

Study 2

The number of subjects experiencing at least one TEAE was similar for all three treatments: 20.8–25.0% (Table V). All TEAEs were mild or moderate in severity. No serious AEs were reported. Headache was the most frequently reported TEAE in seven subjects overall (9.7% of safety population).

Four subjects (5.6% of safety population) experienced at least one TEAE that was considered treatment related. Of these, dry skin, erythema, skin exfoliation, skin tightness, pruritus and papular rash (reported in one subject each) were related to the test cream; dry skin, skin tightness, pruritus, papular rash (one subject each), burning sensation, erythema and skin exfoliation (two subjects each) to the positive control cream; skin exfoliation (one subject) to no treatment. Three of the four subjects who experienced treatment-related AEs (4.2% of safety population) discontinued treatment because of AEs.

Discussion

Both studies achieved their primary endpoint, providing evidence for proof-of-concept and suggesting that the biomimetic lamellar cream formulation has beneficial cosmetic application.

In Study 1, significant TEWL differences at the forearm in favour of the test cream over no treatment indicated that twice daily application of the test cream for 4 weeks was beneficial to skin barrier function. These results were supported by the outcome of the tape stripping skin challenge, which indicated that skin treated with the test cream helped strengthen the skin barrier function to resist a physical challenge compared to no treatment. Removal of less protein from the skin (i.e. a lower protein content extracted from the discs) during the D-Squame challenge was also indicative of increased cohesion between cells in the SC and therefore a stronger skin barrier [22]. Corneometer results indicated that the test cream had a beneficial effect on skin moisturization both after the first use and with ongoing twice daily application. The regression period showed that benefits to barrier function and moisturization were partially retained for at least 5 days after stopping the use of the test cream.

There is heterogeneity between different areas of the body regarding the presence of hair follicles, the number of sebaceous and sweat glands and prior levels of exposure to sunlight and the resulting effects of ultraviolet radiation. Including test sites on both the volar forearm and the face in Study 1 enabled us to investigate the potential cosmetic benefit of the test cream at two locations on the body. The volar forearm was selected as the primary test area because of the homogenous nature of the skin there and the minimal amount of hair and sebaceous glands that could affect instrumental assessments, compared to facial skin which has a higher level of sebum production and may have been exposed to varying amounts of ultraviolet radiation and other environmental challenges. Bazin and Fanchon reported that the volar forearm is a good representation of the face for studying moisturization and

biomechanical properties, and that results from tests on the volar forearm are relevant for the assessment of the efficacy of a product destined for facial use [23]. Facial application of the test cream led to similar results in Study 1 as forearm application, supporting Bazin and Fanchon.

Study 2 was considered a success because the primary efficacy endpoint, of a favourable trend in the periorbital skin R_a values, was met in subjects using the test cream. The results of secondary assessments support the test cream's potential anti-ageing benefits, including significant reductions in the Fitzpatrick wrinkle score, and significant improvements in elasticity parameters that reflect the skin's ability to maintain and restore its own structure. Grading of high-resolution images failed to detect an improvement in skin texture compared to no treatment for the test cream and positive control cream. This suggests that the assessment was not a sufficiently sensitive method for measurement of change in skin texture and we hypothesized that the interpretation of 'texture' by the panel of graders was too subjective. It may be possible to improve the sensitivity of this procedure by training or calibrating the graders, but the primary goal of this endpoint was to understand whether naïve graders could detect visible improvements in skin texture.

These studies were complementary; skin barrier function and moisturization results from instrumental assessments along the cheekbone in Study 1 were consistent with the results of the same assessments (on the subocular/cheek area) in Study 2. This is true despite the differences between the study protocols (e.g. use of a different standard/wash-out cleanser), the wider age range of the study population in Study 1, which included women under 30 years of age and the lower concentration of niacinamide in the test cream used in Study 1. This suggests wider generalizability of the cosmetic benefits of the lamellar formulation, which can be assessed in future studies. Furthermore, we believe the anti-ageing endpoints in Study 2 support the moisturization assessments performed in both studies, since the hydration state of the SC affects both SC micro- and macro-topography, including wrinkle dimensions, through its influence on biomechanical properties, bulk rheology, and skin turgor. In fact, increased SC hydration over days or weeks improves the specific activity of SC desquamatory enzymes, which supports further incremental smoothing of SC surface topography [24, 25].

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In Study 1, results for the positive control cream overall were consistent with those of the test cream, which supports the validity of the clinical model. The choice of Olay ProX cream as the positive control in both clinical studies was based on manufacturer claims for formulations in the same commercial range. However, in Study 2, the positive control failed – there was no improvement in the primary efficacy endpoint, change in R_a , compared to no treatment. Numerically smaller baseline R_a values for subjects in the positive control group compared to the no-treatment group may have contributed to this outcome (mean 22.48 vs. 23.35, respectively). Clinical Fitzpatrick assessments were successful for both study products over no treatment and this may, therefore, be a more appropriate method to evaluate the visual impact of a cosmetic moisturizer on wrinkle appearance for future work.

AEs were reported in a similar proportion of subjects from each treatment group (test cream, positive control or no treatment) and no treatment-related serious or severe AEs were reported. AEs considered treatment related were generally localized reactions and less than 7% of the study population in either study discontinued because of an AE.

Based on these promising efficacy and safety results, pivotal studies of the niacinamide-containing lamellar formulation are warranted.

Data sharing

Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

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