Accepted: 20 February 2018

DOI: 10.1111/jocd.12535

ORIGINAL CONTRIBUTION

WILEY JCD Journal of Cosmetic Dermatology

Absence of human skin irritation and allergenic potential after repeated patch applications of a lamellar moisturizer

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Funding information
GlaxoSmithKline, Grant/Award Number: RH01864

Summary

Background: New cosmetic products should undergo clinical evaluation for skin sensitization potential.

Objectives: To assess the irritation and sensitization potential of a moisturizer containing lamellar structured lipids after repeated patch application in humans, using human repeated insult patch test methodology.

Methods: This 6-week, single-center, open-label study compared a lamellar moisturizer with negative saline control in human subjects aged 18-70 years and skin phototype (Fitzpatrick) classification I–IV. During an initial induction phase, semi-occlusive multi-test patches were applied to the skin of participants' backs three times per week for 3 consecutive weeks; clinical assessments were performed per International Contact Dermatitis Research Group criteria. Participants subsequently underwent a challenge phase, where a new patch was applied to a contact-naïve area of the skin to assess sensitization to the moisturizer.

Results: The study commenced with 233 voluntary participants, 214 of whom completed the study and underwent the final dermatological assessment. Most participants (232/233; 99.6%) demonstrated negative patch test results. One participant had a positive reaction at the lamellar moisturizer application site, with visible erythema and edema (classified as an adverse event [AE]); however, this reaction was observed 24 hours after a reaction to another product in the patch test panel (a prototype cleanser). Importantly, no skin reactions were detected during the challenge phase. Two participants had AEs of mild contact dermatitis in the area of patch adhesive application during the induction phase. No serious AEs occurred during the study.

Conclusions: These findings suggest that the lamellar moisturizer has low irritant and allergenic potential.

KEYWORDS
allergenic, human repeated insult patch test, irritation, moisturization, sensitization

1 | INTRODUCTION

Topical exposure to components of cosmetic products can result in a range of skin reactions, including irritation and allergic responses. Such dermatitis is characterized by redness, edema, oozing, crusting, scaling, and occasionally vesicles.[1] Approximately 80% of cases of contact dermatitis are accounted for by irritant contact dermatitis, which occurs in response to contact between a substance and the

skin.[1] Allergic contact dermatitis is a type IV hypersensitivity reaction that has two phases: sensitization to an allergen and an allergic response to that allergen following re-exposure.[1] During the development of a novel cosmetic product, it is important to identify any skin reactions indicative of potential sensitization, given that cosmetics are not expected to induce allergic skin reactions.

The human repeated insult patch test (HRIPT) methodology[2] is commonly used to investigate the risk potential for a possible irritant and/or sensitizing agent that triggers a reaction when in contact with human skin, under exacerbated conditions of product exposure. HRIPT compatibility protocols are of particular benefit to validate a no-effect level of sensitization to a product or its components, in comparison with a control substance.[3] The application of test products under occlusion favors a high level of skin contact, thus maximizing the detection of any irritant or allergenic potential of the product. The initial application of the product to the skin enables assessment of any primary irritability and/or preexisting sensitization that may exist. Further detection of the irritant potential of the product is facilitated by repeated applications of the product to the same skin site; typically, the irritant action of the tested substance will induce test site reactions that subside within 24 h of patch removal. Sensitization to the product is further assessed by the subsequent application of the product to a different skin site.[4]

HRIPT-induced skin sensitization reactions are typically characterized by erythema associated with other dermal manifestations, such as edema, papules, vesicles, blisters, and pruritus.[2] Any response that occurs and persists at both the induction site (the site of primary and repeated product application) and a challenge site (subsequent testing at a different location) indicates skin sensitization and may be confirmed by repetition of the test.[4] Products observed to be associated with extreme responses during such induction and challenge testing may be considered to present a risk for development of allergy in humans and therefore would not be recommended for cosmetic use.

The present study was conducted to assess the irritation and sensitization potential of a moisturizer containing lamellar structured lipids after repeated patch application in humans.

2 | METHODS

2.1 | Study design

This was a 6-week, open-label study comparing a lamellar moisturizer with a negative saline control in human subjects. The overall objective was to assess the irritation and sensitization potential of the moisturizer after repeated patch applications, following the HRIPT methodology. The study was conducted between April 28 and June 5, 2014, at a single center in Vila Martina, Valinhos, Brazil.

The study was approved by the Research Ethics Committee of Faculdade de Medicina de Jundiai and conducted in accordance with Good Clinical Practice guidelines,[5] the Declaration of Helsinki,[6] and Conselho Nacional de Saúde[7] Administrative Rule 466/12. All participants provided written informed consent prior to inclusion in the study.

2.2 | Participants

Male or female subjects aged 18-70 years and with skin phototype (Fitzpatrick) classification I–IV[8] were eligible for inclusion. Participants were required to agree to comply with study procedures and requirements, including prespecified attendance for assessments, and to provide informed consent. Exclusion criteria included: pregnant and nursing women, or their partners; active dermatoses or skin marks in the experimental area that could interfere with study results; a history of severe allergic reactions to topical products, cosmetics, or drugs; immunodeficiency; intensive sun exposure or tanning session exposure up to 15 days before initial assessment or during the study; esthetic or dermatological body treatment up to 3 weeks before screening or during the study; topical or systemic use of immunosuppressants, antihistamines, nonsteroidal anti-inflammatory drugs, and corticosteroids up to 2 weeks prior to screening; vaccination up to 3 weeks before or during the study; participation in another clinical study; any history of allergy to materials used in the study; dermatographism; participation during the study in any activity leading to intensive sweating; or a history of noncompliance with a study protocol. Individuals who did not meet the study eligibility criteria or who decided not to participate in the study at screening were considered to be screening failures.

During the study, participants were asked not to apply any product at the experimental region that could interfere with study assessments, or to change other cosmetic habits, including hygiene products. In addition, participants were requested not to: have facials, body exfoliation, or other esthetic treatments performed at the product application area; expose themselves to excessive sunlight or use artificial tanning beds; change dietary habits; change hormonal treatment; wet the patches during baths, or by means of pool or sea bathing, or through use of saunas or excessive sweating; remove the patches, or wear tight clothes that may remove the patch by friction or cause skin redness; miss one of the scheduled clinic visits; use any of the following restricted medications, including nonsteroidal anti-inflammatory drugs continuously for more than 3 days, corticoids, antihistamines, immunosuppressants, vitamin A and its derivatives, or any esthetic, cosmetic, or dermatological treatment at the product application site.

2.3 | Study procedures and assessments

The lamellar moisturizer evaluated in this study contained the following ingredients: aqua, butyrospermum parkii butter, caprylic/capric triglyceride, carbomer, ceramide NP, cocos nucifera oil, glycerin, hydrogenated lecithin, hydroxyethylcellulose, pentylene glycol, sodium carbomer, squalane, and xanthan gum.

Semi-occlusive patches made of a hypoallergenic material (transparent adhesive plaster [CREMER S.A], paper filter disks [FILLTRUS Ind. e Com. Ltda], Silicone Paper BR M2 [Adere Prod. Auto Adesivos

Ltda]), with 16 × 1.2 cm diameter round cells containing absorbent material, were applied to the skin of participants' backs. Patches were secured using microporous tape (CREMER S.A). Each patch was used to assess the irritation and sensitization potential of 15 products; however, this manuscript reports the results pertaining to the lamellar moisturizer only. To achieve this, 20 μL of undiluted lamellar moisturizer was applied in patch cell 02, with cell 16 of the patch filled with 20 μL of saline to act as a control.

Following screening, participants entered a 3-week induction phase where patches were applied three times per week for 3 consecutive weeks (nine applications in total). During the induction phase, participants returned to the clinic every 48-72 hours for patch removal, assessment, and reapplication; clinic visits were scheduled on Mondays, Wednesdays, and Fridays.

Clinical assessments were performed using the criteria recommended by the International Contact Dermatitis Research Group (ICDRG),[9] including measurement of the minimal erythemal dose (MED) according to the Fitzpatrick classification[8] and using the Dermatone Skin Analyzer™ device (Youabian, Inc., Los Angeles, CA, USA). MED assessments were performed using ICDRG criteria, rated from "no skin changes at the test area" (negative test result) to erythema and edema with a variable presence of vesicles (positive test results, graded as +, ++, or +++) and irritant reactions of different types. The products tested would be considered as nonirritant if ≤3% of + reactions were triggered in relation to the total number of applications or ≤2% of ++ reactions.[10] Any reaction graded as +++ or above was considered as presenting a risk for development of allergy in humans. Adverse events (AEs), including abnormal laboratory findings, symptoms, or diseases temporally associated with the use of the study product, were also recorded as appropriate. Participant reports of discomfort, such as itching, were noted qualitatively.

After the induction phase, there was a 2-week rest period, with no patch application. Subsequently, participants entered the challenge phase, where a new patch was applied to a contact-naïve area. During the challenge phase, participants returned to the clinic 48 hours after patch application for patch removal and medical assessment, performed after 30 minutes of rest; the final assessment was carried out 24 hours later (72 hours after patch application).

Participants could be withdrawn from the study under the following circumstances: at their own request; if they or their partner became pregnant; or at the discretion of the principal investigator if there was a failure to comply with the research protocol, in the event of any reaction considered to pose a risk to the participant's integrity, in the event of any complication that could interfere with data analysis, or if AEs made it impossible to continue to use the study product. All participants meeting these criteria were reassessed at all follow-up visits.

2.4 | Statistical considerations

A total of 233 participants were planned for inclusion to ensure that at least 200 evaluable participants would complete the study. All results obtained until study completion, discontinuation of study product use, or participant withdrawal from the study were included in the final analysis, with descriptive statistics used to summarize the data.

3 | RESULTS AND DISCUSSION

3.1 | Participants

Participant flow through the study is presented as a CONSORT diagram in Figure 1. Overall, 234 participants were screened, with one participant considered a failure of selection. Therefore, the study commenced with 233 voluntary participants, 214 of whom completed the study and had the final dermatological assessment performed.

Participant characteristics are shown in Table 1. The study population was predominantly female (83.7%), with a mean age of 44.8 years (range: 18-70 years). The majority of participants had skin phototype classification II or III.

3.2 | Patch tests

Skin reactions detected during the study are summarized in Table 2. The majority of study participants (232/233; 99.6%) demonstrated negative patch test results. Only one skin reaction was detected

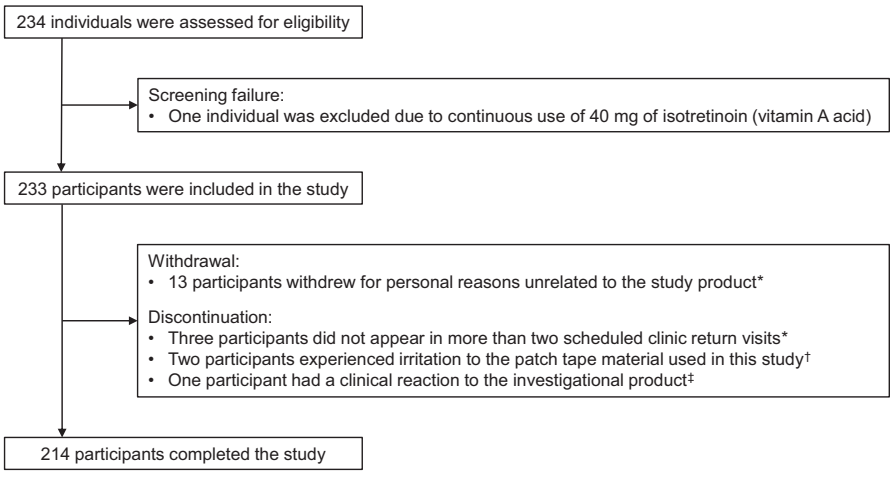

FIGURE 1 CONSORT diagram. *Participants were contacted by telephone and reported no adverse reactions or discomfort; they did not attend the clinic for the final dermatological evaluation. †Participants were followed until resolution of the irritation to the patch tape material, and application of the product was discontinued; no participants showed signs of irritation or sensitization to the applied products. ‡The case description is described in the safety section of the results

TABLE 1 Participant characteristics

	Participants enrolled n = 233
Female, n (%)	195 (83.69)
Mean age, years (SD)	44.8 (13.37)
Range	18-70
Phototype classification, n (%)	
I (always burns, never tans [MED[a] 15-30 mJ/cm^2 eff; PS[b] 35-50])	12 (5.15)
II (always burns, tans minimally [MED[a] 25-35 mJ/cm^2 eff; PS[b] 51-60])	106 (45.49)
III (burns moderately, tans gradually [MED[a] 30-50 mJ/cm^2 eff; PS[b] 61-75])	79 (33.91)
IV (burns minimally, tans well [MED[a] 45-60 mJ/cm^2 eff; PS[b] 76-85])	36 (15.45)

MED, minimal erythemal dose; PS, pigmentation scale; SD, standard deviation.
[a]Typical MED, according to the Fitzpatrick classification.[8]
[b]Measured using the Dermatone Skin Analyzer™ device.

during the study. During the induction phase, one participant had a positive reaction at the site of the lamellar moisturizer application, with visible erythema and edema; however, this reaction occurred 24 hours after a reaction was observed to another product in cell 01 of the patch test panel (a prototype cleanser). Importantly, no skin reactions were detected during the challenge phase of the study.

3.3 | Safety

AEs and participant reports of discomfort are presented in Table 3. No serious AEs occurred during the study. Two participants developed mild contact dermatitis in the area of patch adhesive application during the induction phase of the study; both events were considered to be unrelated to the lamellar moisturizer. A third participant, who had reported no prior cases of allergy at screening, experienced a skin reaction during the induction phase; this participant's reaction has also been described above in the patch test section. An equivocal reaction (classification +?, as per Table 2) to a prototype cleanser in cell 01 of the patch test panel (the lamellar moisturizer was in cell 02 and the saline control in cell 16) was recorded at clinic visit 6. At clinic visit 7, a strong positive skin reaction (classification ++, as per Table 2) was recorded, comprising erythema with strong infiltrate and microvesicles (no papules), which was visible at the lamellar moisturizer test site; the participant also reported itching at the patch site and removed the patch at home to alleviate the itching. The investigator discontinued study product application, and subsequent assessments 72 hours and 96 hours after patch removal indicated that the condition had regressed; total regression was recorded 192 hours after patch removal. Given that this participant experienced clinical signs of mild-to-moderate intensity in response to more than one product, the investigator related the causality of the reaction to the test product and defined the event as a strong allergic reaction to an ingredient in the test products in the patch test panel. However, this patch test did not include the individual ingredients in the test product, so the investigator was unable to determine which ingredient had caused the reaction.

4 | DISCUSSION

Our findings indicate that the lamellar moisturizer has low irritant and allergenic potential, as only one positive skin reaction was

TABLE 2 Irritant and allergic reactions to the test product during the study (study population, n = 233)

Reaction grade[a] (interpretation)	Reaction description	Participants with reactions, *n* (%)
– or absent (negative test)	No skin changes at the test area	232 (99.6)
+? (equivocal reaction)[b]	Weak macular erythema, not palpable	0
+/++ (weak/strong reaction)	Erythema and edema (± vesicles): • Palpable erythema • Presence of edema/infiltrate • Absence of papules or vesicles-many papules, vesicles, and/or microvesicles	1 (0.4)[c]
+++ (extreme reaction)	Coalescent vesicles and/or blisters ulceration	0
IR (irritant reaction)	Irritant reaction of different types: • No infiltration • Small petechiae • Pustules • Efflorescences different from papules or vesicles • Inflammation limited to the exposed area	0

ICDRG, International Contact Dermatitis Research Group.
[a]ICDRG criteria for irritant and allergic reactions classification.[9]
[b]Considered as a negative test; participants would not be withdrawn from the study, patch application continued.
[c]Participant withdrawn from the study, patch application discontinued.

TABLE 3 Adverse events and feelings of discomfort, per participant

Reaction intensity[a] (severity)	Event	Start-end date of reaction (duration, h)	Considered related to test product?
Mild[a] (not severe)	Contact dermatitis	May 12-14, 2014 (48)	No
Mild[a] (not severe)	Contact dermatitis acneiform	May 14-19, 2014 (120)	No
Moderate[b] (not severe)	Itching	May 11-12, 2014 (24)	Yes, cell 01
++[c] (not severe)	Strong reaction—positive test	May 12-14, 2014 (48)	Yes, cell 02
+[c] (not severe)	Weak reaction—positive test	May 14-19, 2014 (120)	Yes, cell 02

ICDRG, International Contact Dermatitis Research Group.
[a]Not graded according to ICDRG criteria, as the reaction occurred in the area of adhesive application and not at the site of study product application.
[b]Graded according to the participant report, rather than ICDRG criteria.
[c]ICDRG criteria for irritant and allergic reactions classification.[9]

recorded during the study, and this participant experienced a reaction to more than one of the test products in the patch test panel. Given that this subject presented a positive reaction to multiple products, it is possible that they were presensitised to a common ingredient within these formulations. This one strong positive skin reaction was substantially below the cutoff of ≤3% of weak reactions or ≤2% of strong reactions above which the product would be considered an irritant, and was not classified as an extreme reaction, for which the product would be considered as presenting a risk for development of allergy in humans.[10] Strengths of this study include that these data from human subjects are directly applicable to risk assessments. Moreover, due to the number of study product applications in the induction phase, this study allows assessment of primary irritability and irritant potential after repeated applications (accumulated irritability), enabling two cases of irritation caused by the study patch adhesive to be detected.

The 6-week total duration of this study is sufficient time for development of a humoral immune response, meaning that this study was also able to evaluate the sensitizing potential of this formula, in addition to any preexisting sensitization for the test product.[4] Given that no sensitization to the lamellar moisturizer was detected when applied to the skin under occlusion in all but one participant in this study, this suggests that this product has low allergenic potential. A limitation of the study is that while there was considerable variability between the individuals tested, including a wide age range, no participants had skin pigmentation levels (Fitzpatrick phototype classification) above IV, limiting generalizability of the results to populations with deep skin pigmentation. Considerable differences in skin properties and the ability to develop responses against chemical agents exist in individuals with skin pigmentation of I–IV compared with those above IV, which may be due to a more impenetrable barrier in the latter group.[11] The irritant and sensitization potential of the lamellar moisturizer remains to be evaluated in populations with skin pigmentation levels of V and VI.

5 | CONCLUSION

Our findings suggest that the investigated lamellar moisturizer has low irritant or allergenic potential.

ACKNOWLEDGMENTS

I thank all of the subjects for their participation and Dr Regina Mayumi Doi, the principal investigator, for her assistance during this clinical study. This study, RH01864, was funded by GSK Consumer Healthcare. Editorial support in the form of development of a draft outline, editorial suggestions to draft versions of this paper, assembling tables and figures, collating author comments, copy editing, fact checking, referencing, and graphic services was provided by Jennifer Lawton, PhD, of Gardiner-Caldwell Communications, Macclesfield, UK, and was funded by GSK Consumer Healthcare.

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How to cite this article: Nisbet SJ. Absence of human skin irritation and allergenic potential after repeated patch applications of a lamellar moisturizer. *J Cosmet Dermatol*. 2019;18:377–382. https://doi.org/10.1111/jocd.12535