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Keratolytic Activity of Microemulsions

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Key Words
Microemulsion · Keratolysis · Chromametry · Silver nitrate test · Salicylic acid

Abstract
Objective: To compare the keratolytic activities of a drug-free hydrophilic microemulsion (ME) and a drug-free lipophilic ME with water, and with regard to the hydrophilic ME also with a 5% salicylic acid gel on the sole of the foot. ***Methods:*** Twenty healthy volunteers had their plantar forefoot, midfoot, and rearfoot stratum corneum blackened with silver nitrate and a photographic developer, and a chromameter was used to determine the extent of removal of this black dye by a* value and L value measurement at 24 and 48 h. ***Results:*** Both drug-free MEs produced significantly greater increases in a* value and L value than water, and the hydrophilic ME was also more effective than 5% salicylic acid gel. ***Conclusion:*** The irritating effect of MEs is rather negligible on the sole of the foot because of the thick plantar stratum corneum. Both MEs therefore appear suitable for the elimination or prevention of plantar desquamative and hyperkeratotic skin changes.

Introduction

In a narrow sense, keratolysis denotes the dissolution of protein structures, describing the mechanism of action of depilation creams for instance. In a wider sense, keratolysis is used to denote the breaking-up of the intercellular glue that holds the corneocytes together, a typical objective of dermatologic interventions. In this paper, the term keratolysis is used in the latter meaning.

A distinction should be made between direct and indirect keratolytic activity. The prototype of a direct keratolytic agent is salicylic acid, which has been demonstrated to break up the intercellular glue [1]. The prototypes of indirect keratolytic agents are retinoids, which interfere with the keratinization pro-

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cess. Direct keratolytic activity has also been postulated for surfactants [2–4]. Microemulsions (MEs) with a relatively high surfactant content may therefore also have keratolytic activity. The objective of this study was to determine the keratolytic activities of a hydrophilic ME (ME 1) and a lipophilic ME (ME 2).

Materials and Methods

Study Products

(1) ME 1 contains isopropyl myristate 4% (w/w), sucrose laurate L 595 6% (w/w) and L 1695 12% (w/w), propylene glycol 30% (w/w), and distilled water 48% (w/w).

(2) ME 2 consists of isopropyl myristate 65% (w/w), polyoxyethylene glycerol monostearate (Tagat® S) 20% (w/w), polyglyceryl-6-dioleate (Plurololeat® WL 1173) 10% (w/w), and distilled water 5% (w/w).

(3) Ethanolic salicylic acid gel 5% per New German Formulary of the Deutsche Arzneimittel Codex (NRF) 11.54 . This formulation contains 5% salicylic acid (w/w), 16% ethanol 96% (w/w), 4% hydroxypropyl cellulose 400 (w/w), 0.1% monobasic sodium phosphate dihydrate (w/w), 59% propylene glycol (w/w), and 15.9% purified water (w/w).

We have described the supplies and the preparation of the study MEs as well as the physicochemical properties of the two MEs in an earlier paper [5].

Subjects

The study was performed on 20 healthy volunteers (8 men, 12 women; mean age 28.5 years, range 18–40 years). Exclusion criteria were skin conditions, in particular plantar keratosis, pregnancy, lactation, and volunteers under 18 years of age. Subjects were informed about the study design both verbally and in writing, and they all gave their written informed consent. The Ethics Committee (Institutional Review Board) of the University of Freiburg, Germany, approved the study.

Test Areas and Randomization Procedure

Symmetrical test areas of 6 cm^2 on both plantar forefeet, midfeet, and rearfeet were used for the study. The sides treated with a test formulation or control varied from subject to subject, but there was no randomization between forefoot, midfoot, and rearfoot test areas. Comparisons were thus made only between symmetrical sites treated with different products. Study product allocation to the test sites was as follows: (1) forefeet: ME 1 versus purified water (Ph.Eur.); (2) midfeet: ME 1 versus ethanolic salicylic acid gel 5% per NRF 11.54; (3) rearfeet: ME 2 versus purified water (Ph.Eur.).

Study Design

We performed the silver nitrate test as it was described in an earlier paper [6]. Silver nitrate produces a black discoloration of the skin. The disappearance of this black discoloration was measured by chromametry (a* value and L value) in accordance with applicable guidelines [7].

Statistical Analysis

The differences between the 24- or 48-hour and baseline readings were calculated for both study products and controls, using the original experimental data. Then the symmetrical test sites were compared. Statistical analysis used the two-tailed Wilcoxon matched-pair signed rank test because normal distribution of the differences could not be established. Figures 1 and 2 show the medians, 25th and 75th percentiles, and the maxima and minima.

Results

Figure 1 shows the results obtained for the a* value. It emerges that both ME 1 and ME 2 produced significantly greater skin redness than did the control. Moreover, ME 1 produced significantly greater skin redness than ethanolic salicylic acid gel 5% per NRF 11.54 after 48 h.

Figure 2 shows the results obtained for the L value: both ME 1 and ME 2 achieved significantly greater increases in the L value than did the control. Both MEs thus produced greater blanching. Moreover, ME 1 achieved a significantly greater increase in the L value than did ethanolic salicylic acid gel 5% per NRF 11.54.

Both redness and blanching are considered measures of black dye (produced by silver nitrate solution) removal. Both MEs therefore have keratolytic activity (as defined in the

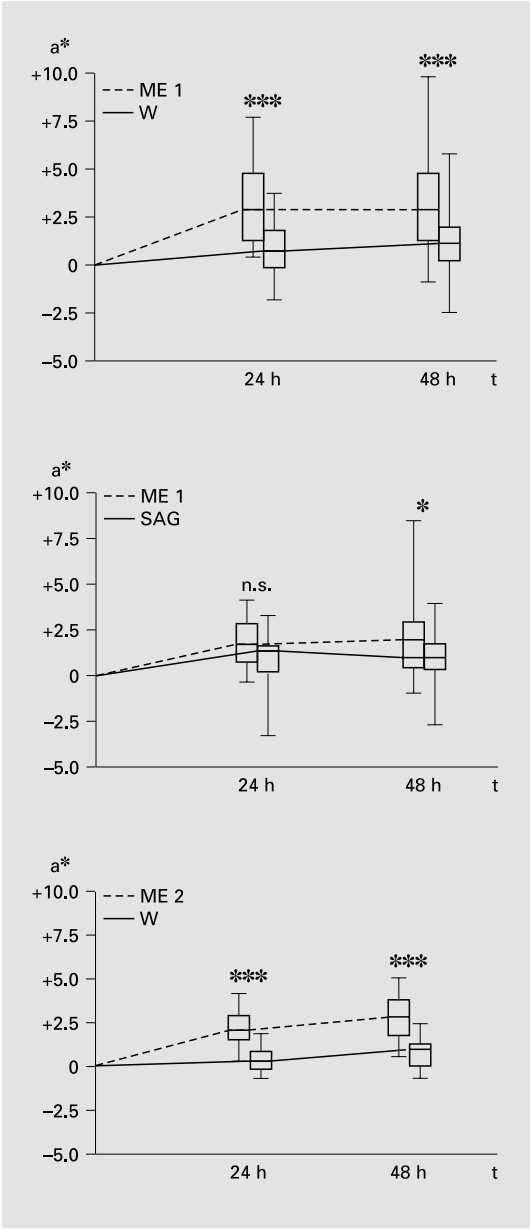

Fig. 1. Changes in the a* value at the three paired test sites versus baseline. The extent of the a* value increase (= skin redness) is a measure of keratolysis. Forefoot test areas: ME 1; W = water. Midfoot test areas: ME 1; SAG = 5% salicylic acid gel. Rearfoot test areas: ME 2; W = water. Statistical differences: n.s. = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

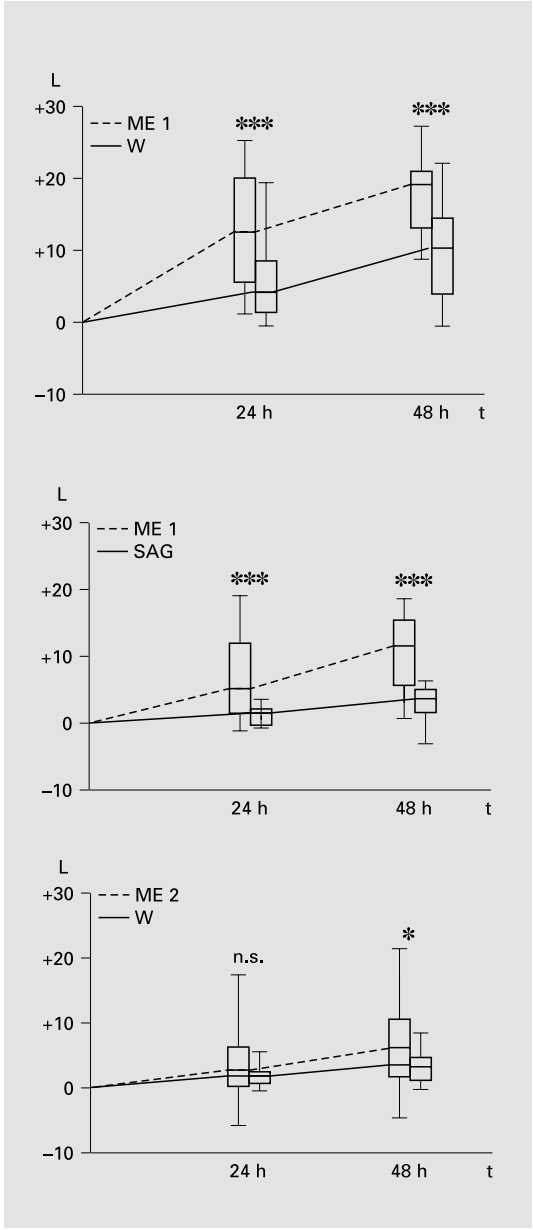

Fig. 2. Changes in the L value at the three paired test sites versus baseline. The extent of the L value increase (= blanching) is a measure of keratolysis. Forefoot test areas: ME 1; W = water. Midfoot test areas: ME 1; SAG = 5% salicylic acid gel. Rearfoot test areas: ME 2; W = water. Statistical differences: n.s. = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Introduction). This activity was greater with ME 1 than with the comparator product, 5% ethanolic salicylic acid gel, NRF 11.54.

Discussion

MEs are clear to faintly opalescent, thermodynamically stable, isotropic multicomponent systems consisting of a lipophilic and a hydrophilic component, a surfactant, and typically also of a cosurfactant [8, 9]. Unlike classical emulsions, MEs are rather like 'colloidal' solutions of minute, highly flexible agglomerates measuring <200 nm across. As there is practically no interface tension between the lipophilic and hydrophilic components, MEs can readily blend with the lipid mantle of the skin and quickly penetrate the stratum corneum.

In the human cantharidin blister model, a surfactant mixture of triethanolamine sulfate (Texapon® 50%) and an ethoxylated partial glyceride mixture of saturated, even-numbered C8–C12 fatty acids (Softigen® 767) (ratio 7:1) produces 'thinning' of the stratum corneum similar to that produced by 6% salicylic acid in 70% isopropanol or by 5% precipitated sulfur in white petrolatum [2]. Studying both healthy volunteers and patients with atopic dermatitis, White et al. [3] found reductions in stratum corneum cell layers and intercellular lipids in patients with atopic dermatitis. In both populations, washing with soap (no details given) twice daily for 7 days produced reductions in stratum corneum cell layers and intercellular lipids. Quantitative and qualitative superficial scalp lipid analysis has demonstrated that an increase in cholesterol content is characteristic of dandruff formation [10]. A similar increase in cholesterol was found when the scalp was washed three times at 1-week intervals using the cited surfactant mixture of Texapon TH/Softigen 767 [4]. These findings support keratolytic activity and suggest that MEs depending on the surfactants they contain may also have keratolytic activity.

The present study used the silver nitrate method which, very much like photographic development, involves the application of a silver salt to the skin. Exposure to light, enhanced by the additional exposure to a photographic developer, results in the precipitation of elemental silver, which binds very strongly to corneocytes and, therefore, is very hard to remove from the skin. In fact, elemental silver does not disappear from the skin until the corneocyte is shed by desquamation. This method is therefore well-suited for detecting keratolytic activity provided that the blackening of the skin is uniform. The interpretive value of this method is essentially identical to that of the dansyl chloride test, where a marker compound, dansyl chloride, is used to produce fluorescence on the skin, and to that of the dihydroacetone test, where dihydroacetone is used to produce orange-brown coloring of the skin [11]. All three test procedures involve an alteration of skin color, and all three tests measure the extent of restitution of the original skin hue over time. In the silver nitrate test, the extent of black dye removal is determined by measuring the a* value (a measure of skin redness) and the L value (a measure of skin lightness). Previous work has demonstrated that the a* value is a particularly useful and reliable measure of black dye removal [6]. Another option would have been to determine the E*ab value ($L^2 + a^{*2} + b^{*2}$) [11]. We decided not to determine this parameter because earlier pilot studies had suggested no increase in interpretive power, nor did the dihydroacetone test produce data of significant additional value [11]. We preferred in vivo studies to ex vivo studies using stratum corneum strippings [11] because the time course of marker compound removal can only be monitored in an in vivo model. The results

presented here demonstrate parallel, highly consistent effects on both the L value and the a* value, which, in earlier studies, had not been the case to the same extent, presumably because of differences in skin color between the sole of the foot and body skin. Bias from measurement-induced changes in skin hue had been ruled out in pilot studies on skin not pretreated with silver nitrate.

In the present study, the black dye produced by silver nitrate in the stratum corneum disappeared clearly faster when ME 1 or ME 2 was applied than when water was the test substance, suggesting faster shedding of corneocytes from the skin surface and demonstrating that the MEs have keratolytic activity. The findings of our study also show that the hydrophilic ME (ME 1) was significantly more effective on plantar skin than was the salicylic acid gel.

Stratum corneum intercellular structure compromise may limit the therapeutic utility of the study MEs. Both MEs used in the present study have been studied earlier for their dehydrating, barrier destabilizing, and irritative hyperemizing effects [5]: both MEs, particularly ME 1, produced significant dehydration of the stratum corneum. Also observed were a significant increase in transepidermal water loss resulting from the barrier destabilizing effect and an increase in skin redness (significant with ME 1, similar tendency but not significant with ME 2) resulting from hyperemia in the subpapillary plexus after ME application. Both study MEs, especially hydrophilic ME 1, therefore, appear little useful for practical use on body skin. However, these effects would not appear relevant to the use of these MEs on the sole of the foot. In fact, the plantar stratum corneum is so thick that irritation appears negligible. Given their potent keratolytic activity, we therefore consider both MEs to be useful options for plantar skin care in people with a tendency for hyperkeratosis and desquamative skin conditions. No specific keratolytic agent, such as salicylic acid for instance, needs to be added.

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References

1 Huber C, Christophers E: Keratolytic effect of salicylic acid. Arch Dermatol Res 1977;257:293–297.
2 Gloor M, Beier B: Untersuchungen zum keratoplastischen Effekt von Salicylsäure, Schwefel und einem Tensidgemisch. Z Hautkr 1984;59:1657–1660.
3 White MI, Jenkinson DM, Lloyd DH: The effect of washing on the thickness of the stratum corneum in normal and atopic individuals. Br J Dermatol 1987;116:525–530.
4 Gloor M, Jäger B, Baldes G: Wirkungseffekt waschaktiver Substanzen in Kopfwaschmitteln. Hautarzt 1977;28:404–406.
5 Lehmann L, Keipert S, Gloor M: Effects of microemulsions on the stratum corneum and hydrocortisone penetration. Eur J Pharm Biopharm 2001;52:129–136.
6 Gloor M, Fluhr J, Wasik B, Gehring W: Klinische Wirkung von Salicylsäure und hochdosiertem Harnstoff bei Applikation in standardisierten Magistralrezepturen aus dem Neuen Rezeptur Formularium (NRF). Pharmazie 2001;56:810–814.
7 Pierard GE: EEMCO guidance for the assessment of skin color. Eur J Dermatol Venereol 1998;10:1–11.
8 Müller BW: Mikroemulsionen als neue Wirkstoff-Trägersysteme; in Müller RH, Hildebrand GE (eds): Pharmazeutische Technologie: Moderne Arzneiformen. Stuttgart, Wissenschaftliche Verlagsgesellschaft, 1998, pp 161–168.
9 Keipert S, Siebenbrodt I, Lüders F, Bornschein M: Mikroemulsionen und ihre potentielle pharmazeutische Nutzung. Pharmazie 1989;44:433–444.
10 Gloor M, Kohler H: A contribution to a new test method for dandruff-inhibiting and 'keratolytic' action of drugs. Eur J Clin Pharmacol 1977;11:377–380.
11 Piérard GE, Piérard-Franchimont C: Dihydroxyacetone test as a substitute for the dansyl chloride test. Dermatology 1993;186:133–137.