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Research paper

Effects of microemulsions on the stratum corneum and hydrocortisone penetration

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Abstract

We tested a high-water-content hydrophilic microemulsion (ME 1) and a low-water-content lipophilic microemulsion (ME 2) for their suitability for use in dermatology, in general, and as alternative hydrocortisone (HC) vehicles, in particular. The lipophilic component of both study products was isopropyl myristate. The surfactant/cosurfactant system of ME 1 consisted of two sucrose esters and that of ME 2 was a mixture of Tagat® S and Plurololeat®. Both MEs showed no in vitro irritability in the hen's egg test on chorioallantoic membranes.

In 14 subjects, stratum corneum water content was determined by corneometry and transepidermal water loss (TEWL) by the Tewameter before and after 3 days use of ME 1 or ME 2 as well as on two untreated control sites. ME 1 produced dehydration and increased TEWL as evidence of barrier compromise. ME 2 also produced an increase in TEWL but had no dehydrating effect. Subjects then underwent standardized washing with a surfactant solution. Under these conditions, pretreatment with ME 2 also produced dehydration, but to a lesser extent than did pretreatment with ME 1.

In the same subjects, the impact of the two MEs on HC penetration (0.5%, 24 h occlusion) was evaluated in terms of the chromameter-determined blanching effect compared with that on a site treated only with an occlusive film dressing. The comparator was an ambiphilic cream (Basiscreme (BC) Deutscher Arzneimittel Codex (German Formulary)). Irritative skin redness produced by ME 1 was significant and that produced by ME 2 was slight but visible, compared with BC. HC penetration was demonstrable from all the study products via the blanching effect and was significantly greater from ME 1 and slightly greater from ME 2 than from BC. However, neither ME would improve HC therapy because the irritative effects were so great that the blanching effect of HC formulated in ME 1 was significantly smaller and that of HC in ME 2 slightly smaller than that of HC formulated in BC.

Keywords: Microemulsion; Stratum corneum water content; Drug penetration; Stratum corneum barrier; Hydrocortisone

1. Introduction

Microemulsions (MEs) have been described as 'critical solutions'. The ME system combines the properties of emulsions (such as scattered laser light measurability of tiny particles) with those of solutions (drugs show saturation solubility in MEs and, unlike in macroemulsions, no partition coefficient; no measurable interface tension between the oil and water components; thermodynamic stability).

Having low or no interface tension, MEs are thought to rapidly penetrate into the stratum corneum where they will blend into the skin's lipid mantle [1]. As colloid systems or 'swollen micelles', MEs have both hydrophilic and lipophilic areas, but, unlike macroemulsions, MEs are not made up of a continuous and a discontinuous phase. This may explain why the MEs are better at penetrating the stratum corneum and blending into the skin lipids. However, MEs may have the potential to compromise the epidermal barrier as they interact with the stratum corneum lipids. Also, their high-emulsifier-content may cause the MEs to produce irritative effects on living cells.

High water-absorbing capacity may result in ME supersaturation with the drug as a function of drug lipophilicity, which, as demonstrated by the example of diclofenac [1], may lead to an increase in the amount of drug penetrating the stratum corneum. Low ME water-absorbing capacity, on the other hand, may result in the conversion to a macroemulsion in the stratum corneum and probably in slower drug penetration, which, however, because of rapid ME penetration into the stratum corneum, would still be better than that of conventional macroemulsions.

The impact of the MEs on cutaneous drug penetration and permeation has been described in numerous accounts in the

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literature [1–14]. However, research interest has focused on the use of MEs as transdermal drug carriers. Those studies used ex vivo and various animal models to evaluate the impact of a number of ME variables including qualitative and quantitative composition, polarity, partition coefficient, and viscosity.

Although the risk of tissue irritation due to the relatively high surfactant/cosurfactant concentrations of ME is known, few in vivo studies have looked at this issue. Changez and Varshney [14] described histopathologic studies in rabbits and an irritation test in mice for AOT-containing MEs. Delgado-Charro et al. [15] determined transepidermal water loss (TEWL) and subpapillary cutaneous blood flow after the use of MEs in humans. Both studies suggested no significant ME-induced skin irritation.

The objective of the present study was to evaluate the barrier-compromising, dehydrating, and irritative effects of a high-water-content hydrophilic ME and a low-water-content lipophilic ME in healthy volunteers, and to determine whether improved penetration of hydrocortisone (HC) from these MEs, if present, may, despite potentially increased vehicle irritability, confer vasoconstrictor and hence anti-inflammatory benefit.

2. Materials and methods

2.1. Substances

Isopropyl myristate was obtained from Caesar & Loretz GmbH, Hilden, Germany; Tagat® S (hydrophilic/lipophilic balance number, HLB = 16.4) from Goldschmidt AG, Essen, Germany; and Plurololeat® WL 1173 (HLB = 10) from Gattefossé, Saint Priest, France. The sugar surfactants, sucrose ester L 595 (HLB = 5) and L 1695 (HLB = 16), were kindly provided by Syntapharm, Gesellschaft für Pharmachemie mbH, Mühlheim-Ruhr, Germany. Basiscreme (BC) was obtained from Vaselinfabrik E. Wasserfuhr GmbH, Bonn, Germany. The HC met the quality requirements of the European Pharmacopoeia.

2.2. Formulations

Two ME formulations were tested: ME 1 (water-continuous system; [16]) and ME 2 (oil-continuous system; [17]) with the following compositions:

- ME 1 contains isopropyl myristate 4% (w/w), sucrose laurate L 595 6% (w/w) and L 1695 12% (w/w) (these sugar esters have different ester compositions), propylene glycol 30% (w/w), and distilled water 48% (w/w).
- 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).

ME 1 was produced by mixing all the ingredients by agitation for 3 h (magnetic stirrer) and heating (90°C). To produce ME 2, isopropyl myristate, Tagat® S, and Plurololeat® were mixed with gentle heating (30–40°C) and magnetic stirring. Then water was added, and the mixture was stirred until a clear yellow solution was obtained (approximately 4 h).

BC, DAC (Deutscher Arzneimittel Codex (German Formulary)), an ambiphilic cream, was used as comparator. BC has the following composition: glycerol monostearate 4% (w/w), cetyl alcohol 6% (w/w), polyoxyethylene glycerol monostearate 7% (w/w), medium-chain triglycerides 7.5% (w/w), propylene glycol 10% (w/w), white petrolatum 25.5% (w/w), and distilled water 40% (w/w).

2.3. Physicochemical characterization of study MEs

pH was determined using a pH meter (model CG 825, Schott-Geräte GmbH, Hofheim a.Ts., Germany). The refractive index was measured at room temperature using an Abbé refractometer (Carl Zeiss Jena, Germany), density was determined using a pycnometer, and viscosity was measured at 22°C using an Ubbelohyde capillary viscosimeter (Jenaer Glaswerke Schott & Gen., Germany). Particle size characterization was obtained by photon correlation spectroscopy (4 mW helium–neon laser, Coulter model N 4 MD, Krefeld, Germany) at an angle of 90° and a temperature of 37°C. The solutions were filtered through a Minisart SRP 25 0.2 μm membrane filter. To measure surface tension, a Lauda tensiometer (Messgeräte-Werk Lauda, Dr D. Wobser KG, Königshofen, Germany) was used at room temperature.

2.4. ME in vitro tolerance study (test serie 1)

The in vitro study of the physiological tolerance of the two MEs used the HET–CAM method described by Lüpke [18] on six appropriately prepared chorioallantoic membranes of hen's egg at a dosage of 0.2 ml per test membrane. The following variables were evaluated at 30, 120, and 300 s post-dose: vascular injection, hemorrhage, lysis, coagulation, and platelet aggregation. Classification was done by the rating system of Wetering and van Erp [19], a scoring system (maximum score 11) similar to the Draize system. A score of 0–1 indicates practically no irritation; 1.1–2.9, mild irritation; 3.0–4.9, moderate irritation; ≥5, severe irritation. The studies were performed by Confarma GmbH, Geretsried-Gelting, Germany.

2.5. ME in vivo tolerance study by TEWL determination and corneometry

2.5.1. Subjects

TEWL determination and corneometry were carried out on a sample of 10 females and four male volunteers with healthy skin. Mean age was 29.1 years (range, 20–53). All the subjects had signed a written informed consent form. Exclusion criteria were skin conditions, known allergy to

any of the components, pregnancy, and lactation. Also, subjects were instructed not to use any skin cleansing or skin care products on the test sites during the week before and during study.

2.5.2. Study procedure

For TEWL determination and corneometry, two circular test areas 3 cm in diameter were marked using a template on the flexor sides of both forearms of all the subjects. Both variables were determined on the morning of study day 1 in a climate-controlled chamber (ambient temperature 22 ± 2°C, relative humidity 41%). Subjects were allowed a 30 min adaptation period prior to the measurements. After baseline readings were obtained, the subjects themselves applied 0.05 ml of each study formulation (ME 1 and ME 2) to the two test areas on one arm using a tuberculin syringe three times daily for 3 days, while the test areas on the other arm were left untreated. The order of application of the formulations to the test areas was rotated in such a way that the sides treated with the test formulations and the control sides varied from subject to subject. On the morning of study day 4, repeat TEWL and corneometry readings were obtained.

Immediately thereafter, a standardized washing procedure with sodium lauryl sulfate (SDS) solution was carried out. A sponge roller (diameter 3.5 cm, length 5 cm) attached to a handle was soaked in an aqueous solution of 0.01 mol/l SDS (Texapon® K 12, Caesar & Lorentz Co., Hilden, Germany) and rolled over the test area 30 times within 3 min. This was done without exerting pressure other than that from the weight of the wet sponge (approximately 200 g). The sponge was dipped into the washing solution at the end of the first and second minutes. The forearms were kept in a horizontal position during the washing procedure. At the end of the washing procedure, subjects were asked to rinse their forearms in clean water at body temperature for 3 min to remove all the surfactant from their skin. The test areas were then dried by dabbing with non-fraying paper towel. Readings were taken again 45 and 90 min after washing.

2.5.3. TEWL determination and corneometry (test serie 2)

Barrier function was evaluated by measuring the TEWL ($g/cm^2\,h$) using the Tewameter TM 210 (Courage & Khazaka, Cologne, Germany) in accordance with applicable guidelines [20]. TEWL is considered an important measure of epidermal barrier function. Evaporimetry consists of applying a probe with two twin sensors directly to the skin, with one sensor pair measuring humidity and the other temperature. The acquired data are used by an integrated microcomputer to compute the water vapor partial pressures at the two parallel levels of each sensor pair and, via the partial pressure gradient, the rate of evaporation. To minimize outside interference, the measurements were carried out in an open-top Plexiglas chamber with closed sides.

Stratum corneum water content was determined by measuring electrical capacitance using the Corneometer CM 820 (Courage & Khazaka, Cologne, Germany) (arbitrary units, AU) in accordance with applicable guidelines [21]. Corneometry is based on the capacitance determination of the different dielectric constants of water and other substances. A spring presses the membrane of an axially mobile probe to the skin surface with a constant force of 3.5 N. The conductivity of this closed system changes as a function of stratum corneum hydration. The probe is connected to a microcomputer, which processes and displays the current reading. Relative values were obtained.

2.6. Chromametry determination of blanching effect of HC-MEs versus ME vehicles

2.6.1. Subjects

The subjects were the same as those described in Section 2.5.1. The tests were performed concurrently with the in vivo skin irritation study described above (Section 2.5.2).

2.6.2. Study procedure

The blanching effect of the HC-MEs was studied on the subjects' backs. Six $2 \times 2\ cm^2$ test areas were marked using a template at the level of the upper thoracic spine. Chromametry was performed on the test areas on the morning of study day 1. After baseline chromametry, three areas on one side of the back were treated with the following formulations:

0.5% HC in ME 1
0.5% HC in ME 2
0.5% HC in BC

The three areas on the other side of the back were treated with the corresponding vehicles (containing no HC). The side treated with the test formulation and that treated with the vehicle were varied from subject to subject. Using a tuberculin syringe (1 ml), 0.1 ml of each study product was injected into a 1.6 cm diameter Finn Chamber (Hermal Chemie, Kurt Herrmann, Reinbek, Germany). The Finn Chambers were attached with small cellulose discs over the test areas (Zelletten, Lohmann, Neuwied, Germany) using an airtight, waterproof self-adhesive film (Applica OP-Folie, Beiersdorf AG, Hamburg, Germany). After 24 h occlusion, the Finn Chambers were removed, carefully wiping off any residual study product with a lint-free cellulose cloth, and chromametry was performed again.

2.6.3. Chromametry (test serie 3)

Skin color can be measured by tristimulus (blue, red, green) analysis of light reflected from skin using Chromameter CR 200 (Minolta, Osaka, Japan). The color of the reflected light from a pulsed xenon arc lamp was analyzed by three high-sensitivity silicon photocells filtered to match the CIE standard observer curves for the primary colors, blue (450 nm, $b*$-value), green (550 nm, L-value), and red (610 nm, $a*$-value). Higher $a*$-values mean greater

skin redness. The measurements were made in accordance with applicable guidelines [22].

2.7. Statistical analysis

The in vivo tolerance study (see Section 2.5) analyzed the percent changes in the readings after 3 days (before washing and 45 and 90 min after washing) versus baseline. Analysis of the HC blanching effect (see Section 2.6) was based on the percent change in readings after 24 h occlusion versus baseline. As the difference-in-readings data failed to show normal distribution, columnar statistics were limited to the medians, 25 and 75% percentiles and minima and maxima.

As multiple tests were performed, the a priori listed hypotheses procedure of Maurer et al. [23] was used.

In vivo tolerance test hypotheses:

- *Hypothesis #1*: the two MEs differ from the corresponding symmetrical untreated site.
- *Hypothesis #2*: the ME 1 minus symmetrical untreated site difference is different from the ME 2 minus symmetrical untreated site difference.

HC blanching effect hypotheses:

- *Hypothesis #1*: the HC formulations differ from the corresponding vehicles.
- *Hypothesis #2*: the HC formulations minus corresponding vehicle differences are different between the three study formulations.
- *Hypothesis #3*: the HC formulations differ in the change in skin redness.
- *Hypothesis #4*: the vehicles differ in the change in skin redness.

The next higher hypothesis was tested only if the preceding hypothesis was significant at $P < 0.05$ in at least one instance. Except for Hypotheses #2–4 in the HC blanching effect studies, statistical comparisons were made by the Wilcoxon matched-pairs signed-ranks test. In these tests, Hypotheses #2–4, the a priori listed hypothesis involved multiple testing, so the Wilcoxon–Wilcox multiple-comparisons test [24] was used.

3. Results

3.1. Characterization of the MEs

Table 1 lists key physicochemical variables of ME 1 and ME 2. It emerges from Table 1 that ME 1 shows lower viscosity (ideal viscous behavior), whereas ME 2 is, due to its high-lipid content, clearly more viscous but also a Newtonian fluid. The pH values are expected to be dermatologically acceptable. Because of their low surface tension, these formulations spread well on the skin.

Water absorption (%) was determined titrimetrically: the clear systems showed persistent turbidity and, therefore, were transformed into macroemulsion systems. The saturation solubility (c_s) of HC was the same for both MEs ($\approx 0.6\%$). However, 16.5% HC was soluble in the anhydrous base mixture (BM) of ME 1, but only 4.8% in the anhydrous BM of ME 2.

3.2. ME in vitro tolerance study

The hen's egg test on chorioallantoic membranes (HET–CAM) gave a total score of less than 1.0. According to the Confarma GmbH Study Report, both MEs were therefore classified as 'practically non-irritant' to mucous membranes.

3.3. ME in vivo tolerance study by TEWL determination and corneometry

TEWL data are listed in Table 2, the statistical results in Table 4. It emerges from Table 2 that both ME 1 and ME 2 produced significant increases in TEWL versus the untreated control site. Washing caused an increase in TEWL at all sites, as expected. The ME 1 or ME 2 versus untreated control site comparisons showed that TEWL on the ME-treated skin areas was significantly greater than TEWL on the untreated control areas. There was no statistically significant difference between ME 1 and ME 2.

Corneometry results are listed in Table 3, the statistical results in Table 4. It emerges from these results that 3 days' treatment with ME 1 produced significant stratum corneum dehydration, while ME 2 did not. The difference between ME 1 and ME 2 was significant. Washing caused dehydration at all sites, as expected. Post-washing stratum corneum water content was significantly lower on the ME 1- or ME 2-treated side than on the respective untreated control side. 45 min after washing, ME 1 produced significantly greater dehydration than ME 2. 90 min after washing, this difference was still present, but not statistically significant.

3.4. Chromametry determination of HC ME blanching effect

Results are listed in Tables 5 and 6. The HC formulations produced significant reductions in skin redness versus the

Table 1
Physicochemical parameters of the MEs

	ME 1	ME 2
Viscosity (mPa)	26	141
Density (g cm^3)	1.050	0.918
Refractive index	1.398	1.439
Surface tension (mN/m)	26.49	27.78
pH value	6.4	7.75
Mean particle diameter (nm)	2.5	
Polydispersity	0.41	
Saturation solubility of HC (mg/ml)	6.5	6.3
Water absorption (%)	> 100	5.5

Table 2
Percentage change of TEWL after application of ME 1 and ME 2 and on the corresponding control sites (test serie 2)

	Median	25% Percentile	75% Percentile	Minimum	Maximum
After 3 days					
ME 1	37.55	5	65.35	−5.2	140
control	−5.75	−15.75	27.9	−44.7	147.8
ME 2	36.4	8.3	95.65	−2.8	153.8
control	8.05	−12.05	43	−50	78.9
45 min after washing					
ME 1	56.2	29	111.6	4.4	233.3
control	4.75	−1.65	46.45	−14.7	137.5
ME 2	46.25	15.2	122.5	3.3	243.5
control	14.8	−1	52.1	−30	115.8
90 min after washing					
ME 1	49.45	20.2	135.1	4.4	273.3
control	5.4	−14.7	41.25	−39.5	84
ME 2	42.2	27.75	93.05	−4	172.2
control	5.7	−0.7	44.9	−15.6	136.8

corresponding vehicles, so the drug was obviously released (Hypothesis #1). Comparison of the three HC formulations with regard to the difference between HC formulation and the corresponding vehicle showed a significantly greater difference for ME 1 than for BC (Hypothesis #2). Comparison of the changes in skin redness by the three HC formulations in absolute terms revealed that neither HC-ME had a greater blanching effect than HC-BC. In fact, the blanching effect of HC-BC was significantly *greater* than that of HC-ME 1 (Hypothesis #3). Comparison of the change in skin redness produced by the three vehicles (containing no HC) showed a significantly greater increase in skin redness for ME 1 than for BC (Hypothesis #4).

4. Discussion

Drug release and the impact of the stratum corneum barrier on drug penetration are central considerations in prescribing low-potency topical dermatologic corticosteroids and most non-steroidal anti-inflammatory products. Equally important is the formulation's irritability because it counteracts the drug's anti-inflammatory effect. MEs may improve drug penetration and/or produce skin irritation. We, therefore, studied a high-water-content ME (ME 1) and a low-water-content ME (ME 2) for these two activities.

Theoretical considerations suggest that MEs may improve drug penetration. In an in vitro animal skin

Table 3
Percentage change of corneometric values after application of ME 1 and ME 2 and on the corresponding control sites (test serie 2)

	Median	25% Percentile	75% Percentile	Minimum	Maximum
After 3 days					
ME 1	−5.7	−11.5	0.35	−20.7	9.4
control	0.55	−4.15	6.05	−9.1	12
ME 2	3.75	−6.85	7.4	−10.9	46.9
control	1.45	−4.85	4	−10.4	21.7
45 min after washing					
ME 1	−21.95	−25.4	−10.65	−32	−0.3
control	−9.7	−15.55	−4.2	−20.4	3.6
ME 2	−15.8	−21.45	−10.25	−24.9	−3
control	−9.65	−14.35	−3.6	−24.5	0.9
90 min after washing					
ME 1	−19.4	−24.2	−8.65	−33.7	−2.9
control	−6.25	−11.25	1	−23.1	4.5
ME 2	−14.25	−18.7	−7	−37	−5.1
control	−9.2	−14.4	−5.75	−16.4	−1.7

Table 4
Results of statistical evaluation – test serie 2 (corresponding to Tables 2 and 3)[a]

	After 3 days	45 min after washing	90 min after washing
TEWL			
Hypothesis 1			
ME1 vs. control	$P < 0.05$	$P < 0.01$	$P < 0.01$
ME2 vs. control	$P < 0.01$	$P < 0.05$	$P < 0.01$
Hypothesis 2			
ME1 – control vs. ME2 – control	ns	ns	ns
Corneometry			
Hypothesis 1			
ME1 vs. control	$P < 0.01$	$P < 0.01$	$P < 0.01$
ME2 vs. control	ns	$P < 0.01$	$P < 0.05$
Hypothesis 2			
ME1 – control vs. ME2 – control	$P < 0.01$	$P < 0.05$	ns

[a] ns = non-significant.

model, MEs increased methoxsalen penetration 1.9- to 4.5-fold [12]. Also, in an in vitro animal skin model, lidocaine formulated as a ME showed up to fivefold greater penetration rates than lidocaine formulated as an oil-in-water (O/W) emulsion [13]. In the same model, a prilocaine ME showed up to 10 times greater penetration than a prilocaine hydrogel [13]. Substantial increases in penetration were also demonstrated for supersaturated diclofenac MEs compared with a commercial formulation [1]. Consistent with these data, the results of our studies showed improved HC penetration from both MEs. Comparison of the HC-MEs with the corresponding vehicles (containing no HC) demonstrated a significantly greater blanching effect of HC in ME 1 than in BC. While a similar tendency was found for ME 2, the difference was not statistically significant.

The irritability of emulsifiers has been demonstrated in a wide variety of experimental models (for reference, see Refs. [25,26]). Emulsifier irritability is due to a host of different effects. Thus, emulsifiers per se have an irritative effect on living tissues, as demonstrated by us [26] in the Duhring chamber scarification test and by other authors using a large number of different methods [27]. Also, when used as wash solutions, emulsifiers produce stratum corneum dehydration and barrier compromise [28,29].

W/O emulsions produce stratum corneum hydration. O/W emulsions either produce stratum corneum hydration or have no effect on stratum corneum moisture content [30]. Stratum corneum barrier function is improved by W/O emulsions [31]. O/W emulsions also have an occlusive effect, albeit a delayed one, thus improving barrier function [32].

Emulsifier irritability may cause MEs to have an irritative effect on living cells. The interactions between stratum corneum lipids and MEs may also cause dehydration and barrier function compromise. In experimental models, these effects cannot always be neatly separated because barrier compromise will increase irritant penetration and hence the irritative effect. Changez and Varshney [14] performed histopathologic examinations and other studies of potential irritative skin reactions following the use of MEs in laboratory animals but failed to demonstrate significant irritative activity. Delgado-Charro et al. [15] reported a brief increase in TEWL and no increase in subpapillary cutaneous blood flow (laser Doppler) by similar MEs. The brief TEWL increase was explained by evaporation of water following occlusion. It was similar to that produced by water and propylene glycol and clearly smaller than that produced by 5% oleic acid in propylene glycol. Kreilgaard et al. [13] studied prilocaine hydrochloride steady state flux with and without ME pretreatment, comparing these data with the results obtained with water. Their findings were not compatible with general stratum corneum barrier compromise by the ME. Studies by other authors [33,34] documented the absence of an irritative effect of the ME gels, which, however, are not comparable to the MEs tested by us.

The hen's egg test on chorioallantoic membranes in our study showed no significant irritability. On the other hand, the chromametry study clearly demonstrated that the study MEs must have irritative activity, and also showed that the high-water-content ME 1, produced dehydration, while the low-water-content ME 2, did not. In the subsequent wash test with an SLS solution, however, both MEs increased dehydration. Moreover, both MEs increased TEWL as evidence of barrier compromise. The pronounced irritative effect observed in the in vivo model was presumably due to

Table 5
Percentage change of the $a*$-value in chromametry after application of BC, ME 1, and ME 2 with and without 0.5% HC and results of statistical evaluation (test serie 3)

	Median	25% Percentile	75% Percentile	Minimum	Maximum
BC + HC	−23.65	−28.7	−5.25	−44.9	61.9
BC	−10.95	−21.5	−1.45	−31.5	85.9
ME 1 + HC	0.85	−20.45	16.4	−29	64.4
ME 1	27.05	11.2	60.1	−28.2	116.9
ME 2 + HC	−12.05	−24.95	−2.6	−39.8	10.6
ME 2	9.4	−3.5	27	−23.8	62.3

Table 6
Results of statistical evaluation – test serie 3 – chromatometry (corresponding to Table 5)[a]

Hypothesis 1	
BC + HC vs. BC	$P < 0.05$
ME1 + HC vs. ME1	$P < 0.01$
ME2 + HC vs. ME2	$P < 0.01$
Hypothesis 2	
(BC + HC)-BC vs. (ME1 + HC)-ME1	$P < 0.01$
(BC + HC)-BC vs. (ME2 + HC)-ME2	ns
(ME1 + HC)-ME1 vs. (Me2 + HC)-ME2	ns
Hypothesis 3	
BC + HC vs. ME1 + HC	$P < 0.05$
BC + HC vs. ME2 + HC	ns
ME1 + HC vs. ME2 + HC	$P < 0.05$
Hypothesis 4	
BC vs. ME1	$P < 0.01$
BC vs. ME2	ns
ME1 vs. ME2	ns

[a] ns = non significant

the barrier compromise-induced increase in irritant penetration although the MEs per se may have only low irritative activity on living cells.

In conclusion, intrinsic ME irritative activity counteracts increased anti-irritant drug penetration. Evaluation of the change in skin redness by the three HC study products revealed that the formulation of HC in ME 2 does not confer benefit versus BC, and that the formulation of HC in ME 1 is indeed counterproductive in that the vasoconstrictor effect of increased HC penetration is smaller than the hyperemic effect of irritation. The use of MEs as vehicles may be more useful with drugs used in conditions where irritation is negligible (such as in hyperkeratotic plantar and palmar fungal infections), and in transdermal therapeutic systems.

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