Effects of Single Application of a Moisturizer: Evaporation of Emulsion Water, Skin Surface Temperature, Electrical Conductance, Electrical Capacitance, and Skin Surface (Emulsion) Lipids

C. W. BLICHTHANN, 1 J. SERUP2 and A. WINTHEN3

1Department of Dermatology, Gentofte Hospital, Hellemp, 2Department of Dermatology, Rigshospitalet, Copenhagen and 3Medical Department, Frederiksberg Arne Sygehus in Hillerød, Hillerød, Denmark

Effects of single application of an oil in water emulsion were studied on the forearm skin of 12 healthy volunteers. Five different non-invasive methods were used. Values were followed for 360 min after application of the emulsion with the contralateral forearm as untreated control. The evaporation of emulsion water from the skin surface immediately rose to high values, but within 15 min returned to the original level. A parallel initial increase in conductance was observed; however, this was followed by a slightly increased level throughout the 360 min study. Electrical capacitance was also slightly increased throughout the study. Skin surface lipids, dominated by emulsion lipids, were increased, with high values for at least 120 min, followed by a gradual decline toward normal. Single application of emulsion is characterized by an initial evaporation phase, with evaporation of emulsion water, which lasts less than 15 min, followed by a lipidization phase, which lasts at least 360 min, dominated by the oil-constituent of the emulsion undergoing epidermal absorption. During the lipidization phase, epidermal hydration parameters are slightly but consistently improved.

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C. W. Blichtmann, Svanevangen 13 A, DK-2100 Copenhagen, Denmark.

Moisturizing emulsions are frequently used in dermatology in the treatment of a variety of diseases such as atop dermatitis. Emulsions are also popular as vehicles for active substances in local treatments. However, knowledge about the physiological effects of moisturizers and cream bases is still limited.

In the present study, we combined a number of new and non-invasive methods to follow physiological changes in the skin resulting from single application of an oil in water emulsion.

MATERIAL AND METHODS
Twelve healthy females, aged 45-66 years, were studied. They were asked not to use any skin care products for 2 days before testing. They could continue normal personal washing with a bar soap. Physical and mental stress was avoided 30 min before measurements.

The flexor aspects of both forearms was used. A test area measuring 6 x 18 cm was indicated on each side, and the area subdivided into 7 equal areas. Measurements were performed, after randomization, either in the distal or in the proximal direction 3-5, 15, 30, 60, 120, 240 and 360 min. after application of the test substance. Values were recorded from the distal, middle and proximal thirds of the forearm immediately after application.

Test lotion (0.4 ml) was spread over the 6 x 18 cm test area. This gives a calculated lotion thickness of 37 µm. The lotion was applied to only one arm, after randomization, with the contralateral arm serving as control. Measurements were made symmetrically during the experiment. Decubal, an oil in water lotion, was used as test substance. The oil phase consists of cetyl alcohol, lanolin P-95 (Westbrook Lanolin Company, UK), isoproxy myristate and Span 60 (sorbitan stearate). The water phase consists of glycerol, Tween 80 (polyisorbate 80), methyl-β-hydroxybenzoate, propyl-β-hydroxybenzoate, and aqua purificata. The water content of the test emulsion was 80.4%.

Evaporation from the skin surface (transdermal water loss and evaporation of emulsion water) was measured by the Servo Med Evaporimeter EPM1® (Servo Med AB, Stockholm) (1, 2), electrical conductance by the Skicon 100® 3.5 MHz skin surface hydrometer (IBI Ltd., Tokyo) (3, 4), electrical capacitance by the Corneometer CM 420® (Schwarz-haupt GmbH, Cologne) (4), skin surface lipids by the Schumetter® (Schwarz-haupt GmbH, Cologne) (3), and skin surface temperature by the Comar 200® (Comar Electronics Ltd., Rustington, UK) contact thermometer. Determination of skin surface lipids was based on optical transmission of a skin surface imprint obtained with a frosted plastic foil.

Recordings were performed in a laboratory room with temperature 20-23°C and constant humidity. Attempts were made to avoid convection of air. Participants were asked to refrain from walking or talking. The study was performed during December 1986.

Analysis was carried out by the Student’s test for paired observations by A. Merup Jensen MSc (Dumer A/S, Copenhagen). p-values less than 0.05 were considered significant.
RESULTS

Results are given in Table I. The evaporation was significantly increased immediately after application of the test substance. After 15 min, the evaporation was already equal to the control side. The electrical conductance showed a great increase, analogous to the evaporation curve. After 15 min, conductance values dropped to a level which was, however, still significantly increased in comparison with contralateral control throughout the 360 min. experiment. The electrical capacitance was significantly increased throughout the whole experiment with values holding at a plateau. Measurement of skin surface lipids showed a broad peak between 0 and 120 min followed by a gradual decline toward zero during the examination period. Skin surface temperature did not change significantly. Values gradually increased during the day.

Fig. 1 gives a summary (mean values, ranges not indicated) of parameters assessed and their relation. Median temperature tended to decrease immediately after application of substance, followed by a slight and constant increase. The initial decrease probably reflected evaporation of emulsion water.

Statistical analysis of pre-test values showed no right-left differences and no systematic proximal-distal difference of the area of forearm skin studied.

Table 1. Successive measurement of moisturizer

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<th>Median</th>
<th>Ranges</th>
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<tr>
<td>Water evaporation (g/m² h)</td>
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<td>Electrical conductance (1/μs/1m)</td>
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<td>Electrical capacitance (a. u.)</td>
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<td>Skin surface lipids (μg/cm²)</td>
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<td>Skin surface temperature (°C)</td>
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DISCUSSION

This study indicates that following single application, moisturizer can be divided into two phases: an evaporation phase, which is dominated by evaporation, and a lipidization phase, lasting longer and dominated by the oil phase, with minor improvements in skin hydration. The gradual and slow change in skin temperature is probably due to evaporation of lipid molecules, which leads to invisible desquamated keratinocytes. Thus, during the application of moisturizer and this is associated with increased electrical conductance and capacitance, hydration parameters of the skin, and supply from the emulsi...
DISCUSSION

This study indicates that physiological changes follow- ing single application of an oil in water emulsion can be divided into two distinct phases, i.e. an initial evaporation phase, which lasts less than 15 min, dominated by evaporation of emulsion water, and a lipidization phase, lasting at least 360 min and dominated by the oil phase of the emulsion, associated with minor improvements of hydration parameters. The gradual and slow clearing of skin surface lipids or oil during the lipidization phase cannot be explained by evaporation of lipids, and it is not likely to be due to invisible desquamation since lipids bind small particles. Thus, during the lipidization phase, emulsion lipids probably penetrate into the outer epidermis, and this is associated with increments in electrical conductance and capacitance. The alteration of these hydration parameters cannot be explained by external supply from the emulsion water since diffusion equilibrium between the skin surface and the ambient air takes place within a few minutes. The intercellular lipid-rich compartment of the epidermis is of significance for the barrier of the skin. It is likely that emulsion lipids penetrate the outer epidermis and mix up with this compartment with consequences for epidermal hydration and scaling. Thus, in a cream base or lotion the water phase seems only of importance as a vehicle, and the oil phase seems to exert the therapeutic effects.

REFERENCES

Acitretin Induces an Increased Adherence of S. aureus to Epithelial Cells

P. LIANOU,1 H. BASSARIS,2 D. VLACHODIMITROPOULOU1 and D. TSAMBAOS2

1Department of Microbiology, University of Athens, 2Department of Internal Medicine, University of Patras, and 2Department of Dermatology, University of Patras, Greece

Recently, synthetic retinoids have been implicated as causing a rise in the incidence of staphylococcal infections in patients orally treated with these compounds for various disorders of keratinization. Since the adherence of bacteria to epithelia is an important early event in the development of bacterial infections, in the present study we investigated the in vitro effects of acitretin on the adherence of Staphylococcus aureus to epithelial cells of the anterior nares of 15 healthy human subjects. It was found that pre-incubation of nasal epithelial cells with acitretin causes a statistically significant (p < 0.001) increase in the adherence of S. aureus to these cells, as compared to the controls. The growth of S. aureus cultures in the presence of acitretin exerted no effect on the staphylococcal adherence. These results suggest that the oral acitretin-induced increase in S. aureus colonization and in the incidence of cutaneous staphylococcal infections may be related to the enhancement of staphylococcal adherence to epithelia caused by this compound.

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P. Lianou, Department of Microbiology, University of Athens, Greece.

Staphylococcus aureus is a bacterial pathogen of major concern in dermatology since it is the most common cause of cutaneous infections (1). Recently, isotretinoin and etretinate have been implicated as causing an increased colonization of anterior nares by S. aureus and a rise in the incidence of staphylococcal infections in patients orally treated with these compounds for various disorders of keratinization (2, 3, 4).

Adherence of bacteria is an important early event in the bacterial colonization of epithelial surfaces and in the pathogenesis of the subsequent infections (5, 6, 7). Furthermore, selective adherence seems to be responsible for species and tissue bacterial tropisms (1).

Since acitretin, the main metabolite of etretinate, shares very similar side-effects with the parent drug (8) in the present study we investigated the in vitro effects of acitretin on the adherence of S. aureus to epithelial cells of the anterior nares of healthy human subjects.

MATERIAL AND METHODS

Epithelial suspensions
Epithelial cells were obtained from the anterior nares of 15 healthy volunteers by gently scraping with a wooden tongue depressor and suspended in phosphate buffered saline (PBS; 0.07 M phosphate; 0.15 M NaCl, pH 7.4). More than 95% of the cells were viable, as indicated by their ability to exclude 0.4% trypan blue. For the bacterial adherence assays we used epithelial cells pre-incubated with 450 ng/ml acitretin (in 1% DMSO) for 30 min. In preliminary experiments we found no significant differences between the effects of acitretin in concentrations of 450, 800 and 1300 ng/ml. Epithelial cells pre-incubated in 1% DMSO without acitretin served as controls.

Bacterial suspensions
A clinical strain of S. aureus was grown overnight (18 h) at 37°C in nutrient broth containing 450 ng/ml acitretin. Cultures without acitretin were used as controls. All cultures were then centrifuged, washed and suspended in PBS at a final concentration of 10^8 bacteria/ml.

Bacterial adherence assay
The ability of the test organism to bind to epithelial cells was tested as previously described (9). The epithelial cells were

Table I. Influence of acitretin on the adherence of S. aureus to nasal epithelial cells

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<tr>
<th>Treatment of epithelial cells</th>
<th>S. aureus cultures</th>
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<td>Pre-incubation of epithelial cells with 1% DMSO and untreated cultures</td>
<td>Pre-incubation of S. aureus cultures with acitretin</td>
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<td>Pre-incubation of epithelial cells with acitretin</td>
<td>Pre-incubation of S. aureus cultures with acitretin</td>
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*p < 0.001.

washed three times and resuspended in PBS containing 10^5 cells/ml. The bacterial culture was mixed and rotated for 30 min at 37°C. The cultures were prepared in duplicate and in each culture, crystal violet and examined in a different field by two different observers. Adherence was determined by counting the number of S. aureus adherent to each epithelial cell. For each experiment, the Student’s t test was used to analyze the results.

RESULTS

The results of the present study show an increase in the adherence of S. aureus to the controls (p < 0.001).

S. aureus cultures in pre-incubation with acitretin exert any significant effect on the adherence to nasal epithelial cells.

DISCUSSION

In the last few years, acitretin has been shown that acitretin, the main metabolite of etretinate, is equally effective as the parent drug in the treatment of severe forms of keratinization disorders (10, 11).

Acitretin is much safer than isotretinoin, and women of childbearing age can be treated with this drug (12). Two compounds with a side-effect pattern, particularly the various side-effects are contrasted.

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