ASSESSMENT OF DRY SKIN USING DYNAMIC METHODS

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Received: October, 2006

Key words: Xerosis; Laser Doppler velocimetry; Barrier function;

Summary

The quantitative assessment of xerosis has been mainly based on the superficial and instantaneous measurements of skin capacitance and transepidermal water loss, or in subjective scoring systems. Modelling the transepidermal water loss curves obtained after occlusion is a versatile methodology to assess the stratum corneum water holding-capacity. On the other hand, laser Doppler velocimetry has often been employed to study the vasodilatory effect of nicotinates on the cutaneous microcirculation and investigate the penetration enhancing/retarding potential of permeants. Since dry skin presents alterations in the water accumulation dynamics and an impaired barrier function, the previous methodologies can potentially be employed in the study of xerosis. This work aims to test the ability of the two dynamic tests to quantitatively assess differences between normal and dry skin. Twenty volunteers participated in this study. Volunteers were submitted to a plastic occlusion stress test, after which the TEWL curves were recorded. A mathematical model was adjusted to the data points, and the evaporation half-life and dynamic water mass were calculated. In the second part of the study, a solution of methyl nicotinate was applied to the skin and the subsequent increase in the blood flow was measured. Time for onset and maximum response were determined. Results indicate that in normal skin, the decrease in TEWL after occlusion is rapid and pronounced, but when dry skin is occluded, TEWL decreases at a slower pace. These results are translated in a higher \( t_{1/2_{\text{evap}}} \) for dry skin, almost double of that obtained in normal skin. Studies with methyl nicotinate reveal differences between the onset of action observed in the two skin types. These findings confirm that the dynamic methodologies employed can detect differences in the water dynamics and barrier function of normal and dry skin. This suggests their potential application to the area of efficacy testing of products that are used to re-establish skin hydration.

Riassunto

La valutazione quantitativa per verificare il grado di xerosi cutanea si basa principalmente sulla misurazione della capacitanza (idratazione) e della perdita d'acqua attraverso la traspirazione (TEWL) o su un sistema soggettivo basato sul punteggio. Poter portare su un modello le curve di
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TEWL ottenibili attraverso l’occlusione della pelle, rappresenta una metodologia semplice e versatilie per misurare la capacità dello strato corneo nel trattenere e legare l’acqua.
Vista da un’altra angolazione la velocimetria Laser-Doppler è stata impiegata per valutare l’effetto vasodilatatorio provocato dai nicotinati a livello del microcircolo cutaneo controllando così la minore o maggiore potenzialità penetrativa di eventuali permeati.
Dato che la pelle secca presenta una evidente alterazione sia nella dinamica di accumulo dell’acqua che nella funzionalità della barriera cutanea, si pensa che queste metodologie possano essere utili per la valutazione della cute xerotica.
Sono state quindi utilizzate per valutarne l’efficacia su cute normale e cute xerotica, su venti volontari che hanno partecipato allo studio. Sottoposti ad uno stress-test mediante un cerotto occlusivo ne sono stati determinati le curve di TEWL utilizzando modelli matematici che permettono di calcolare sia il tempo di evaporazione che la quantità di acqua dinamica.
Nella seconda parte dello studio è stata applicata sulla pelle una soluzione di nicotildimetile che ha permesso di determinare le variazioni del flusso sanguigno.
I risultati ottenuti dimostrano come, nella cute normale, la diminuzione della TEWL dopo occlusione sia rapida e pronunciata mentre nella cute secca decresce molto lentamente rivelando una minore intensità.
Risultati analoghi si ottengono anche con il test al nicotinato dimetile.
Questi risultati dimostrano come i due test possano essere utilizzati per determinare l’efficacia di prodotti cosmetici quando, in presenza delle alterazioni della barriera cutanea, si voglia dimostrare come sia possibile ricondurla alla norma reidratando al contempo la pelle mediante l’uso di prodotti cosmetici.
INTRODUCTION

Dry skin, or xerosis, is perhaps the most widely occurring skin disorder. It is more prevalent in the geriatric population, but the presence of areas of the body with xerotic skin in children and adults is also common. Despite such high incidence, the characterization and definition of dry skin has only recently been completed (1,2). Dry skin can be described as presenting a rough, scaly and flaky surface, often associated with sensations of loss of elasticity and itch, and that worsens in low humidity conditions (3). It was initially considered to be a consequence of reduced sebum secretion, but it is in fact the result of a range of environmental and pathological factors that affect epidermal proliferation and differentiation (4). The rate of proliferation of epidermal cells is increased, but the desquamation of corneocytes is impaired, which contributes to epidermal thickening (5). The stratum corneum lipid bilayers and the epidermal components are altered, which results in a decrease in the water content and barrier function (6). Alterations also take place in the multiple metabolic processes that normally occur in the upper layers of the epidermis.

The most commonly employed strategy to quantitatively assess xerosis has been mainly measurements of skin capacitance and/or transepidermal water loss (TEWL). These methods are valid and useful in the characterization of dry skin, however, they are at the same time insufficient, since the first technique only detects superficial hydration and both are limited by providing instantaneous data. Additionally, in the clinical practice, xerosis is commonly evaluated by subjective visual scoring systems (7,8). Alternative tests have been developed to overcome such difficulties. These are based on a dynamical assessment of cutaneous bioengineering variables, where the response of the skin to certain stimuli is evaluated and the factors that influence the cutaneous water content in the deeper layers and the barrier function can be investigated.

The mathematical modelling of the transepidermal water loss curves that result from a plastic occlusion stress test (POST) is a versatile dynamic methodology that enables the measurement of the stratum corneum water holding-capacity. Previous studies have indicated that inflicted damage to the skin barrier (such as tape stripping or application of lipid extracting solvents) can be detected by this method (9). The objective of the present study is to extend the test of the discriminative capacity of the model to the quantitative assessment of differences between normal and dry skin.

Laser Doppler velocimetry (LDV) has been extensively employed to study the vasodilatory effects of various nicotinates on the cutaneous microcirculation and, in turn, investigate the penetration enhancing/retarding potential of several substances (10). Topical application of methyl nicotinate induces a rapid local vasodilatation which is clinically expressed as a cutaneous erythema (contact urticaria type). Since xerotic skin is characterized by alterations in the skin barrier function (11), the permeation of methyl nicotinate should be facilitated, and the erythema should appear sooner than in normal skin. It is therefore reasonable to assume that this dynamic methodology can also be applied to the study of xerosis.

MATERIAL AND METHODS

Healthy female volunteers participated in this study, aged between 20 and 55 (mean 30.4±13.4), and were fully informed of the nature of the study and the procedures involved. The work was developed in accordance with the Declaration of Helsinki and its subsequent amendments. The skin type was evaluated by
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self assessment and clinical scoring systems (7). Since xerosis is generally more severe in the frontal aspect of the leg, the entire study was conducted on this anatomical site. The subjects were allowed to rest 15 minutes before the experiments. The skin at the measuring site was left uncovered, the subjects remained in an air-conditioned room, under constant temperature (22 ± 2°C) and humidity (30-50%) and were kept relaxed throughout the measurements. Heat sources and air draughts were avoided.

In the first part of the protocol, 20 volunteers (10 with normal skin and the remaining with dry skin) were submitted to a POST, involving the application of an occlusive patch for 24 hours. After this period the patch was removed and TEWL was recorded for 30 minutes. The patch consisted of a layer of cling film “sandwiched” between Parafilm™ (Pechiney Plastic Packaging, Chicago, IL, USA) and covered with gauze, which was then applied to the skin using surgical adhesive (Leukoplast, Beiersdorf S.A., Hamburg, Germany). TEWL measurements were performed with an evaporimeter (Tewameter TM300, Courage and Khazaka, Germany) following published guidelines (12, 13). Data points were continuously registered for 30 minutes. The mathematical model was adjusted to the data points using a specially modified simplex routine and the software ADAPT written in Fortran. Calculated parameters considered relevant to the study were $t_{1/2\text{cvap}}$ (evaporation half-life) and DWM (dynamic water mass) (14, 15).

In the second part of the work, another panel of 20 volunteers was used, where 10 presented dry skin and the remaining normal skin. Two sites were marked in the frontal aspect of the leg: one was used as the control and in the other a 0.1M aqueous solution of methyl nicotinate (Aldrich, Dorset, UK) was applied for exactly 60 seconds using a saturated filter paper disc. Immediately after removal of the paper disc, the alterations in the blood flow in both sites were measured for 1 hour using a two-probe laser Doppler perfusion monitor (PF5010, Perimed, Järfalla, Sweden), following published guidelines (16). Time for onset ($t_0$) and time for maximum response ($t_{\text{max}}$) were the parameters considered more relevant for data analysis.

Non parametric comparative analysis (Wilcoxon sign rank test) was used in this study. A 0.05 significance level was adopted.

RESULTS

The desorption curves obtained after removal of the occlusive patch applied for 24 hours are represented in figure 1. It can be readily appreciated that dry and normal skin present different profiles. In normal skin, the decrease in TEWL after occlusion is rapid and pronounced. On the other hand, when dry skin is occluded, TEWL decreases at a slower pace. These results are translated in a higher $t_{1/2\text{cvap}}$ for dry skin, almost double of that obtained in normal skin, and in a more elevated DWM (table I). There is a marked statistical difference between the $t_{1/2\text{cvap}}$ results ($p=0.018$), which is less pronounced for DWM ($p=0.059$).

Studies with methyl nicotinate also reveal differences between the two skin types. Two typical outputs provided by volunteers with normal skin and xerosis are presented in figure 2. It can be readily seen that the blood flow does not increase immediately after drug application. Most responses from volunteers with normal skin occur after 150 to 300 seconds. After this onset time a rapid increase is followed by a plateau and subsequent decay. It can be clearly appreciated that the increase in blood perfusion, the vasodilation induced by methyl nicotinate, occurs earlier in volunteers with dry skin. The
profiles provided by the control sites are not included in the figure, since, as expected, there was no significant variation in the blood flow throughout the 60 minutes. The parameters used in this study were onset time $t_0$ and maximum response time $t_{\text{max}}$ (table II). $t_0$ is markedly lower in the group with dry skin and these results are statistically different from the group with normal skin ($p=0.002$). The differences in $t_{\text{max}}$ are less marked ($p=0.07$), however, as can be seen from figure 2, $t_{\text{max}}$ is far more difficult to assess with precision.

<table>
<thead>
<tr>
<th></th>
<th>$t_{1/2_{\text{evap}}}$ (min)</th>
<th>$D_{\text{WM}}$ (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal skin</td>
<td>2.03±0.91 p=0.018</td>
<td>497.46±80.87 p=0.059</td>
</tr>
<tr>
<td>Dry skin</td>
<td>3.95±1.90</td>
<td>613.58±130.99</td>
</tr>
</tbody>
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**Fig. 1** Desorption profiles obtained after removal of the occlusive patch (mean, $n=10$).
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**Tab. II**

Onset time \( (t_0) \) and time for maximum response \( (t_{max}) \) values (mean ± SD, \( n=10 \))

<table>
<thead>
<tr>
<th></th>
<th>( t_0 )</th>
<th>( t_{max} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(seconds)</td>
<td>(seconds)</td>
</tr>
<tr>
<td>Normal skin</td>
<td>309.6±199.7</td>
<td>600.4±243.1</td>
</tr>
<tr>
<td>Dry skin</td>
<td>103.7±13.4</td>
<td>409.0±189.5</td>
</tr>
</tbody>
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**DISCUSSION**

The first attempts to quantify skin dryness were essentially based on the visual observation and grading of parameters such as flake size, roughness and colour (7,8). This approach is evidently subjective, but nevertheless, in the clinical perception of dry skin there is an evident need for the integration of different parameters, which can only be perceived through the human intervention (17). The development of noninvasive bioengineering methods has undoubtedly contributed to a better characterization of xerosis. Multiple devices have been developed over the last three decades with applications in this area.
Some of the most commonly employed are based in the direct measurement of the hydric content of the stratum corneum, using electrical properties (18) or photothermal/acoustic techniques (19,20). Others are indirect, that is, they are based in the evaluation of changes in a certain property of the skin that is dependent of the presence of water in the tissue (21). The assessment of the mechanical properties of the skin (22), the analysis of skin surface appearance (by squametry or profilometry (23,24)); and the assessment of the stratum corneum barrier function by measurements of the TEWL (25) are examples of indirect methodologies.

The previously mentioned static tests are very useful to study stratum corneum function and competence in moisture content and retention. However, dynamic tests are in general more informative, mainly because they are not reliant in single measurements.

Previous studies confirmed that the mathematical modelling of TEWL curves obtained after prolonged occlusion was able to detect inflicted damage to the skin barrier (tape stripping or application of lipid extracting solvents) (9). The present study aimed to extend the test of the discriminative capacity of the model to the quantitative assessment of differences between normal and dry skin.

The marked differences observed in the results confirm the applicability of this methodology. Subjects with dry skin presented a greater water accumulation capacity and different water dynamics. The higher DWM can be explained by two factors. First, the weakened barrier function, since it causes a higher loss of water through the skin. Additionally, dry skin has an increased rate of proliferation of epidermal cells, but the desquamation of corneocytes is impaired, which contributes to epidermal thickening. This leads to a greater capacity to accumulate water and, therefore, to the higher amount of water released after occlusion. The $t_{1/2\text{vap}}$ is extended because the mass of water being released is greater.

Studies with laser Doppler velocimetry provided differences between the onset of action after application of methyl nicotinate, which can be attributed to an impaired barrier function in the group with dry skin. Some authors advise caution when correlating these two variables, because of the multiple factors involved in the response to nicotinates (26), but valid extrapolations have previously been established (27, 28, 29).

These findings confirm that the modelling of the TEWL curves resulting from a POST and the study of the vasodilatory action of methyl nicotinate are sensitive methodologies, able to detect differences in the water dynamics and barrier function of normal and dry skin. This shows that the methods are valid for the objectives proposed and suggests its potential application to the area of efficacy testing of products that are used to re-establish skin hydration.
References


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