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International Journal of Pharmaceutics 271 (2004) 251–256
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Non-invasive assessment of the effect of formulation excipients on stratum corneum barrier function in vivo

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Received 9 November 2003; accepted 18 November 2003

Abstract

The objective of the present work was to investigate the effect of formulation excipients on human stratum corneum (SC) barrier function in vivo. Two formulations, an ointment and an oil-in-water cream, were applied to the skin of human volunteers under both occlusive and non-occlusive conditions. The effects of each treatment were then evaluated using three non-invasive biophysical techniques: transepidermal water loss (TEWL), impedance spectroscopy (IS) and attenuated-total-reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. These measurements were combined with a simple tape-stripping protocol to allow information to be derived across the entire SC. IS and TEWL provided basic information on the effect of each formulation on skin barrier function, while ATR-FTIR enabled (i) the tracking of formulation excipients and evaluation of their concentration profiles within the SC, and (ii) deduction of mechanistic detail with which to explain the TEWL and IS results. It was found that occlusion of the skin either in the presence or absence of the cream caused TEWL to be increased when the treatment was terminated at 6 h. Uptake of ointment into the SC, on the other hand, inhibited the post-application TEWL rate. In parallel, treatment with the ointment caused an increase in relative low-frequency skin impedance, consistent with the entry of additional lipophilic constituents into the SC. The latter was confirmed by ATR-FTIR spectroscopic measurements. Overall, the combined use of the three biophysical measurements allowed formulation effects on, and uptake into, the SC to be deduced and evaluated, and the approach may prove useful for the future selection and optimization of topical drug delivery vehicles.

Keywords: Skin; Transepidermal water loss; Impedance spectroscopy; Infrared spectroscopy; Topical drug delivery; Percutaneous absorption

1. Introduction

The stratum corneum (SC), the outermost layer of the skin, acts as a dual barrier by limiting both outward water loss from the body (Blank et al., 1984) and the inward percutaneous absorption of external agents. In addition, the SC binds water efficiently and thereby maintains smoothness and flexibility of the skin surface. The level of hydration of the SC is not uniform; rather, there exists a decreasing concentration gradient from the inner zone facing the living epidermis towards the outermost layers; the wetter the surface layer, the smaller the concentration gradient and the lower the rate of transepidermal water loss (TEWL) (Blank et al., 1984). The dependence of percutaneous absorption (for both lipophilic

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doi:10.1016/j.ijpharm.2003.11.016

and hydrophilic drugs) upon skin hydration has been demonstrated (Rougier et al., 1989), and a linear relationship between percutaneous penetration and the baseline TEWL at the site of absorption has been revealed. Formulation excipients may enhance drug penetration into the SC by decreasing the membrane resistance (as in the case of penetration enhancers) or by increasing skin hydration. Modification of skin barrier function by formulations has been demonstrated in several studies using TEWL measurements (Loden, 1992; Kompaore et al., 1991). Objective evaluation of product occlusivity has been achieved by monitoring the decrease in TEWL in vivo in humans (Baker, 1969); the same technology can also be used to examine the moisture enhancing properties of various humectants.

The water content in, and its concentration gradient across, the SC may also be assessed by other techniques. For example, impedance spectroscopy (IS), measures variations in skin electrical properties, which are, in turn, sensitive to the degree of skin hydration (Lévêque and De Rigal, 1983). Physically speaking, the SC represents a dielectric medium, which is resistant to ion flow and poorly conductive. Hydration of the skin causes marked changes (i.e. decreases) in its impedance, reflecting the fact that ion movement is now more easy across the SC due to the enhanced presence of water (De Nuzzio and Berner, 1990). The method has therefore found application in the evaluation of the moisturizing properties of cosmetic formulations, in particular via changes in the SC low-frequency impedance which is most closely related to the membrane's electrical resistance (Burnette and De Nuzzio, 1997; Clar et al., 1975; Lévêque and De Rigal, 1983). Infrared spectroscopy is another biophysical technique which has been used to assess SC barrier function in vivo (Naik and Guy, 1997). ATR-FTIR has ascertained the extent of skin hydration both in pathological skin conditions and following different moisturizing treatments. Different regions of the IR spectrum have been used to determine the concentration of water in the SC, with the absorbance band near 2100 cm^{-1} providing perhaps the most direct and quantitative measurement (Bommannan et al., 1990; Potts et al., 1985). However, one of the difficulties with this in vivo assessment of SC hydration is that the measurement itself can alter the water content of the test site due to its occlusion while in contact with the internal reflectance element (Naik and Guy, 1997).

The aim of the present work was to compare the effects of hydrophilic (oil-in-water cream) and hydrophobic (ointment) formulations on the barrier function of human SC in vivo using TEWL, IS, and ATR-FTIR. The effect of occlusion on the penetration of certain formulation excipients into the SC was also investigated.

2. Materials and methods

The ointment and hydrophilic cream were provided by Novartis AG, Basel, Switzerland. The cream contained peanut oil (25%), isopropyl myristate (15%), propylene glycol (5%), ascorbyl palmitate (0.05%), sorbic acid (0.10%), cetyl alcohol (4%), stearyl alcohol (4%), polysorbate 60 (6.10%), sorbitan monostearate 60 (1.9%), cetyl palmitate (2%) and water (36.5%); in contrast, the components of the ointment were lanolin alcohol (73%), caprylic triglyceride (3.45%), a fatty alcohol (2.25%), a glycol (9%), and microcrystalline wax (5%).

Healthy human volunteers (aged 24–35 years), with no history of dermatological disease, participated in the study. They maintained the sites under investigation (on the ventral forearm) free from application of all topical products prior to, and during, the study. The experimental protocol was approved by the Commission d'éthique, Département des Neurosciences Cliniques et Dermatologie, Hôpitaux Universitaires de Genève.

Transepidermal water loss was measured with an Evaporimeter EP1 (Servomed, Sweden). Measurements were recorded over a 3-min period, to allow fully stabilized values to be obtained. Impedance spectroscopy in vivo was performed as previously described using an identical experimental set-up (Kalia and Guy, 1995). Infrared spectra were recorded with a Nicolet 730 FTIR spectrophotometer (Madison, WI) equipped with a liquid N_2-cooled mercury–cadmium–telluride detector. An ATR-crystal (ZnSe, dimensions: 7 cm × 1 cm, incident angle: 45°) enabled horizontal positioning of the subject's forearm. Spectra obtained represented an average of 64 scans, collected at a resolution of 2 cm^{-1}. Subsequent spectral analysis was performed using Spectra Calc™

software (Galactic Industries Corporation, Salem, NH, USA).

TEWL impedance (over the frequency range 1–100 Hz) and ATR-FTIR measurements were recorded sequentially at the site of application before treatment. These measurements served as pre-treatment control values. Subsequently, 45 mg of formulation (cream or ointment) were spread over a 22.5 cm^2 area of the ventral forearm (9 cm × 2.5 cm; ∼2 mg/cm^2). The formulation was maintained in contact with the skin for 6 h either with or without occlusion. In the non-occlusive experiments, a cellulose pad (Tela®, Balsthal, Switzerland) was applied to the treated skin area and the entire site was covered with a transparent dressing (Tegaderm®, 3M, St. Paul, MN, USA). For the experiments under occlusion, the treated area was covered with an impermeable polyester film (Scotchpak®, type 1006, 3M, St. Paul) and affixed using a dressing retention sheet (Hypafix®, Fisch-Smith & Nephew Laboratories, France) and Tegaderm® film. At the end of the treatment period, the dressings were removed and the skin was gently wiped with a cotton pad before consecutive measurements of TEWL, impedance spectroscopy and IR were made. The SC at the treatment site was stripped up to a maximum of 20 times with the biophysical measurements being repeated after every other tape-strip. Each series of measurements (i.e. TEWL, then IR, and finally impedance spectroscopy) required a total of no longer than 10 min. Control experiments (in which no formulation was applied) were also performed with and without occlusion.

3. Results and discussion

The relative TEWL values, i.e. post-treatment measurement divided by pre-treatment control, are in Table 1. Statistical analysis (two-way ANOVA followed by the Student–Newman–Keuls test, $P < 0.05$) revealed a significant increase in water loss after treatment with the cream under occlusion compared to non-occlusive conditions. The control data demonstrated the same trend. In contrast, with the ointment, occlusion did not increase TEWL relative to the open application. By the same token, TEWL following occlusive application of the cream was significantly higher than that following administration of the ointment, but was not different from the control measurement. Occlusion of untreated skin is well-known, of course, to lead to increased TEWL subsequent to removal of the impermeable dressing. The application of formulations may impact this phenomenon in two ways: either the presence of the formulation in the SC may contribute an additional diffusional resistance to water efflux; or the formulation or excipients therein, may alter the level of hydration of the barrier. Both situations may modify the TEWL post-treatment. Here, application of an oil-in-water cream did not influence TEWL relative to the corresponding control; this is a reasonable result for an essentially aqueous formulation which offers no resistance to water diffusion, nor contains excipients (e.g. humectants) likely to alter the SC hydration level. The elevated TEWL immediately after occlusion, both in the absence and presence of cream, is consistent with this hypothesis. On the other hand, treatment with a hydrophobic ointment impedes the transport of water (thus decreasing TEWL) across the SC which now possesses a supplementary lipophilic coating, an observation which is most pronounced in the case of occlusion.

Table 1
Relative TEWL values (i.e. TEWL post-6 h treatment divided by the pre-treatment control; mean ± S.D.) following application of cream and ointment formulations with and without occlusion

Treatment	Relative TEWL	
	Occluded	Non-occluded
Control ($n = 4$)	3.3 ± 1.0[a]	1.3 ± 0.4[b,c]
Ointment ($n = 4$)	1.7 ± 0.7[d]	1.0 ± 0.2[b,e]
Cream ($n = 4$)	4.4 ± 1.6[a]	1.0 ± 0.3[b,c]

[a] Values not significantly different ($P < 0.05$).
[b] Values not significantly different.
[c] Values significantly different from the corresponding occluded measurements.
[d] Value significantly less than those for the control and cream.
[e] Values not significantly different from the occluded measurements.

Representative skin impedance spectra, over the frequency range 1–100 Hz, as a function of tape strip number, following occlusive and non-occlusive applications of the ointment and cream are compared to the corresponding controls in Figs. 1–3. Data are expressed as relative impedance, i.e. all impedance values have been normalized by the measured pre-treatment impedance at 1 Hz. Application of the ointment (particularly under occlusion) increased skin impedance compared to the cream and

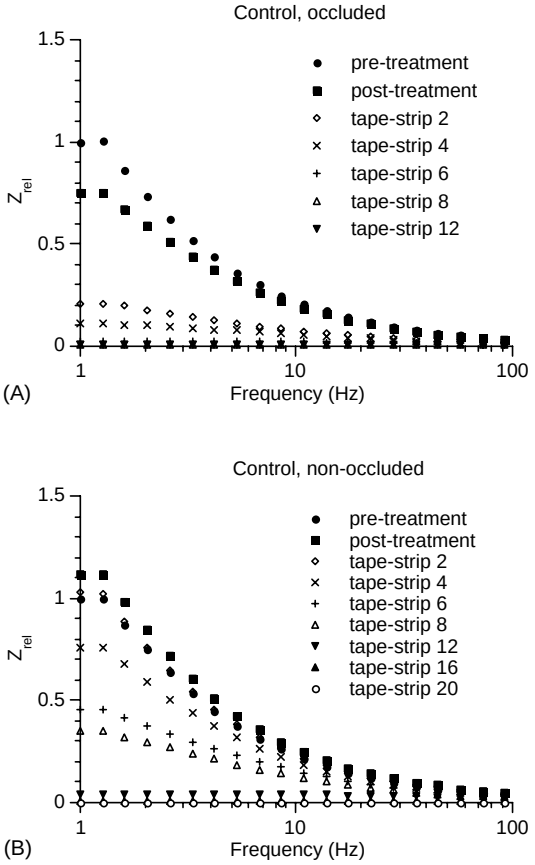

Fig. 1. Representative impedance spectra (normalized by the pre-treatment impedance measured at 1 Hz) before and after 6-h of a "control" treatment under (A) occluded and (B) non-occluded conditions, and then as a function of tape-stripping. Note that, under the occluded conditions, no further changes in the relative impedance spectrum was observable after 12 tape-strips.

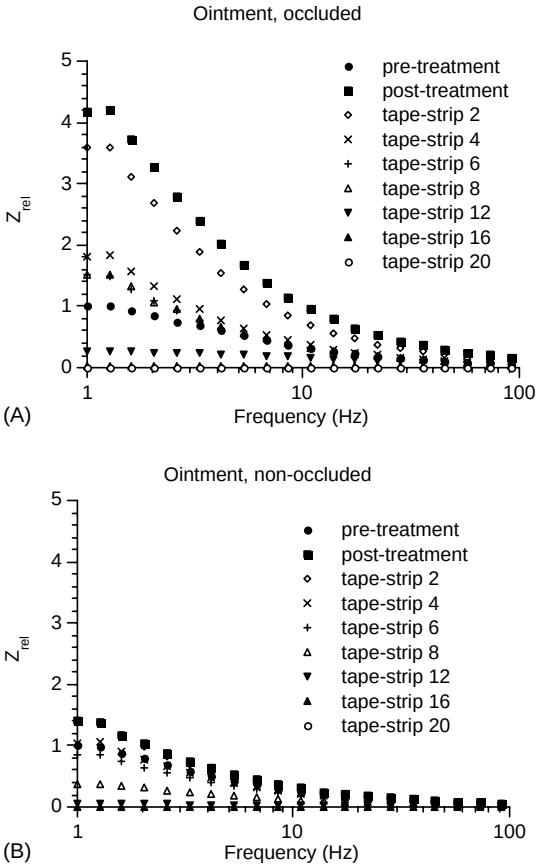

Fig. 2. Representative impedance spectra (normalized by the pre-treatment impedance measured at 1 Hz) before and after 6-h treatment with an ointment under (A) occluded and (B) non-occluded conditions, and then as a function of tape-stripping.

the control, the difference being particularly notable in the outermost layers of the SC. After the removal of the initial tape-strips, the subsequent changes in impedance were not formulation-dependent.

Normalized, low-frequency skin impedance values (i.e. impedance at 1 Hz post-treatment divided by the corresponding pre-treatment measurement) are presented in Table 2. Statistical comparison (two-way ANOVA) of the data did not reveal any significant differences between the non-occlusive protocols (control, ointment, cream). However, the measured impedance ratio subsequent to a 6-h application of the ointment under occlusion was significantly elevated with respect

Table 2
Relative low-frequency skin impedance values (i.e. impedance at 1 Hz post-6 h treatment divided by the corresponding pre-treatment measurement; mean ± S.D.) following application of cream and ointment formulations with and without occlusion

Treatment	Relative impedance	
	Occluded	Non-occluded
Control ($n = 4$)	0.3 ± 0.3[a]	0.8 ± 0.5[b,c]
Ointment ($n = 4$)	1.3 ± 0.6[d]	0.9 ± 0.2[b,c]
Cream ($n = 4$)	0.4 ± 0.3[a]	0.7 ± 0.2[b,c]

[a] Values not significantly different ($P < 0.05$).
[b] Values not significantly different.
[c] Value not significantly different from the corresponding occluded measurement.
[d] Value significantly greater than those for the control and cream.

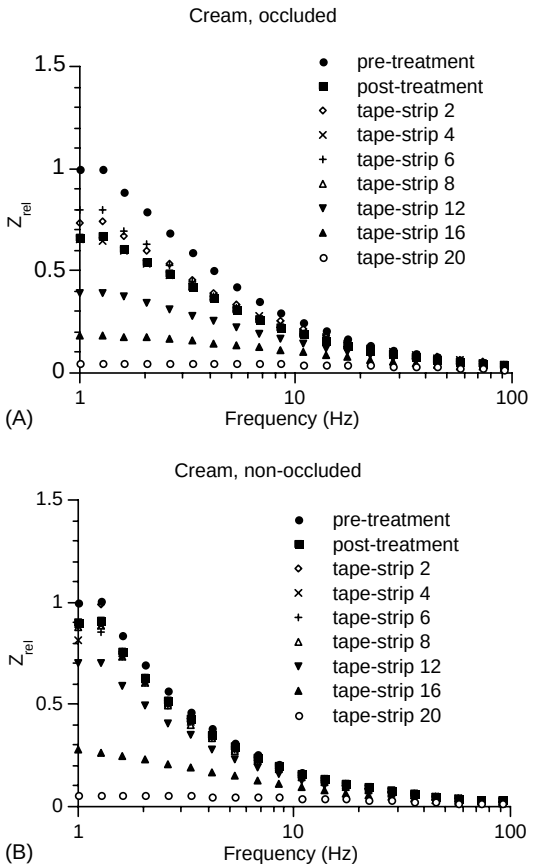

Fig. 3. Representative impedance spectra (normalized by the pre-treatment impedance measured at 1 Hz) before and after 6-h treatment with a cream under (A) occluded and (B) non-occluded conditions, and then as a function of tape-stripping.

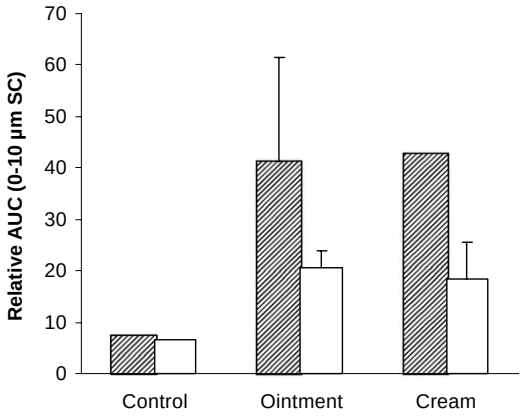

Fig. 4. Integrated areas under the relative methylene group stretching absorbances (2850–2920 cm^{-1}) from the outer 10 μm of the SC following application of cream and ointment formulations with (hatched bars) and without (opened bars) occlusion. The control and cream with occlusion values are the averages from two subjects. For the ointment with ($n = 4$) and without ($n = 4$) occlusion and the cream without occlusion ($n = 3$), mean ± S.D. are shown.

to the corresponding control, though indifferent relative to the non-occlusive treatment with the same formulation. These results are consistent with the TEWL measurements (Table 1) and suggest that the ointment provides an effective barrier to ion and water transport which is not further modified by the addition of a water-impermeable covering.

The SC uptake of the hydrocarbon (H/C) base components in each formulation was monitored by ATR-FTIR. The entry of these lipophilic constituents into the SC elicits significant increases in the methylene (CH_2) group vibrations located in the 2850–2920 cm^{-1} region of the IR spectrum (i.e. over and above those already observed which originate from the intercellular SC lipids). The integrated intensity of these absorbances, relative to the pre-treatment situation, reflects, therefore, the quantity of H/C base constituents taken up into the SC. Fig. 4 summarizes the relative presence of H/C in the outer 10 μm of the SC following application of the ointment and cream formulations, with and without occlusion, and compares these results to the corresponding no-treatment controls. The *overall* uptake of the H/C base constituents (notwithstanding variations in extinction coefficients) was clearly evident for both the ointment and the cream. Integrated over 10 μm of the SC, no effect of occlusion could be discerned for either the ointment or cream; however, occlusion did lead to apparently greater H/C levels in the very outermost layers of the barrier (data not shown). These spectroscopic observations nicely substantiate data from the TEWL and impedance studies for the ointment, treatment with which both decreases TEWL and increases impedance across the skin immediately after removal of the excess formulation and prior to tape-stripping. Once tape-stripping commences and layers of SC (together with permeated formulation) are removed, TEWL and impedance values return to normal. However, ATR-FTIR is not sufficiently sensitive, at least when considering the CH_2-group vibrations, to distinguish the ointment from the cream. The latter, while containing significantly less hydrophobic excipients

than the ointment, nevertheless includes other components which contribute to the CH_2-group absorbances.

In conclusion, this study has shown that occlusion is able to increase skin hydration, but that this effect is highly dependent upon the nature of the excipients present in the formulation (hydrophilic versus hydrophobic). Moreover, the effects of semisolid H/C formulation excipients on the stratum corneum barrier function are limited to the outermost layers of this membrane. TEWL measurements in combination with impedance and infrared spectroscopy appear to be useful complementary techniques to rapidly evaluate the effect of different formulations and treatment protocols (occlusion versus non-occlusion) on SC barrier function.

Acknowledgements

We thank the Fonds National Suisse de la Recherche Scientifique for financial support, Novartis for provision of the formulations studied, and our colleagues at Archamps for their participation as volunteers in these experiments.

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