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Methodology to measure the transient effect of occlusion on skin penetration and stratum corneum hydration *in vivo*

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SUMMARY

The effect of short duration occlusion on skin penetration and stratum corneum water content was studied *in vivo* in eight human subjects. Percutaneous absorption of hexyl nicotinate was monitored non-invasively by laser Doppler velocimetry (LDV) following each of three randomly assigned pre-treatments: untreated control, 30 min occlusion with a polypropylene chamber and 30 min occlusion followed by exposure to ambient conditions for 1 h. Stratum corneum water content after the same pre-treatments was measured with the dielectric probe technique. The local vasodilatory effect of the nicotinic acid ester was quantified using LDV by the onset of increased blood flow, the time of maximal increase in response, the magnitude of the peak response and the area under the response–time curve. Each of these parameters was significantly different, immediately following occlusion, from the untreated control values. However, if the occluded site was exposed for 1 h prior to hexyl nicotinate application these parameters did not differ significantly from the controls. Stratum corneum water content (expressed as a percentage of a maximal value) showed the same behaviour: the pre-treatment control value was $31{\cdot}8 \pm 4{\cdot}8\%$; after 30 min occlusion, this had risen to $46{\cdot}9 \pm 6{\cdot}2\%$; 1h later, the reading had returned to $32{\cdot}1 \pm 6{\cdot}2\%$. There was a significant correlation between stratum corneum water content and area under the LDV response–time curve. It appears, therefore, that this method may be useful for quantifying the relationship between increased stratum corneum hydration and enhanced percutaneous absorption *in vivo* in man.

Occlusion of the skin and prevention of the insensible loss of body water causes hydration of the stratum corneum.[1] If the occlusive covering is impermeable to water molecules in the vapour phase, the water content of the stratum corneum beneath the occluding device will rise to a maximal value such that the chemical potential of water in this skin layer equals that in the lower aqueous regions of the tissue (i.e. a local equilibrium is established). It has been shown, *in vivo* in

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human subjects, that the increased hydration of the stratum corneum produced by occlusion can lead to an increase in the horny layer 'reservoir' and a promotion of penetrant percutaneous absorption.[2] The observation is considered, on the whole, a general one and is used practically in clinical dermatology to enhance, for example, the therapeutic effect of topically administered corticosteroids.

However, a quantitative relationship between level of hydration and skin penetration promotion has not been elucidated. In addition, the level of enhancement, at a particular degree of hydration, will almost certainly depend upon the physicochemical properties of the penetrant. This relationship, too, has not been quantified. We present here some experimental work in which we have attempted to design a methodology by which the link between skin hydration and promotion of percutaneous penetration can be determined *in vivo* in man.

METHODS

Eight healthy male volunteers (age range 22–35 years), with no history of skin disease, took part in the study. At the time of the experiment they were taking no prescribed medication. Informed consent was obtained in accordance with regulations overseen by the University of California, San Francisco, Committee on Human Research. Strenuous activity, hot drinks, non-prescription medications and alcohol were avoided for 12 h prior to the experimental procedures. For 15 min immediately before the study, the subjects rested quietly and acclimated to the ambient temperature ($23 \pm 2^{\circ}$ C) and relative humidity (50–70%) of the environment in which the experiments were performed.

Measurements were made on the ventral forearm skin surface. Each individual observation required an area of approximately 2·5 cm^2. Experiments were conducted following one of three randomly assigned pre-treatments on different skin areas: untreated control (30 min exposure of the skin to ambient conditions), occlusion for 30 min using a small polypropylene (Hilltop®) chamber, and 30 min occlusion followed by exposure to ambient conditions for 1 h. Assessment of the effects of these treatments on percutaneous absorption and skin hydration were made using laser Doppler velocimetry[3] and the dielectric probe technique,[4] respectively. It should be emphasized that each of the eight subjects underwent each of the three treatment protocols using both investigative techniques. An individual subject provided, therefore, his own control and permitted, as a result, paired comparisons to be made.

Laser Doppler velocimetry

The laser Doppler velocimetry (LDV) technique has been previously described.[3] Following pre-treatment, 10 mM hexyl nicotinate in a 60:40 v/v mixture of propylene glycol and isopropanol was applied topically on a saturated filter paper disc for 15 min. The disc was covered to prevent loss of isopropanol by evaporation. At the end of the application period, the patch was removed, excess solution was wiped away with tissue, and the LDV (Medpacific LD5000) probe, was affixed to the skin in the centre of the pre-treated area with a double-sided adhesive disc. The time-course of the resultant nicotinate-induced vasodilatation was monitored over the next 90 min as previously described.[5] All LDV measurements were corrected for pre-treatment basal flow readings and for occasional small instrumental baseline drift ($\leqslant 30$ mV). The pharmacological response was characterized by the time of onset of action (the time at which a definite and sustained increase in LDV output occurred), the time to peak response (defined as the time after the onset of action, at which d(LDV output)/dt was < 5mV/min), the magnitude of the peak response, and the area under the response–time curve. While

there is no a priori reason for analysis to be performed on each of these four parameters, our present database does not unequivocally show which measurement is most sensitive. Therefore, all characteristics of the response–time curve were evaluated for significance. Statistical analysis of the data employed analysis of variance and paired *t*-tests, and linear regression.

Dielectric probe technique

The microwave probe approach permits dielectric measurements of stratum corneum water content to be made non-invasively *in vivo*.[4,6] The microwave frequency (1 GHz) was determined by the requirement that the radiation interact directly with water molecules while minimizing interactions with salts and proteins. The probe reacts, therefore, to the number of water molecules present and detects their small degree of rotation in response to the applied microwave field. The probe response has been shown to be a linear function of water content.[4,6] Design of the probe is important because microwaves can penetrate several centimetres into biological tissue. Therefore, the probe used in the present work consisted of many interdigitated fingers of gold bonded to a glass substrate. The gaps separating the fingers were 10 μm. This configuration confines the microwave field to within a depth of a few microns and it can be used, in conjunction with inert spacers of different thickness, to measure the water concentration profile across the stratum corneum *in vivo*.[4,6] The probe was calibrated by measuring its response to a drop of pure water and to air. The deflection recorded from water (2·15 V) corresponds to a relative water content of 100%. No deflection is recorded from air and this, therefore, represents the zero baseline. Measurements for skin are then expressed as relative water content between these two extremes. After calibration, relative stratum corneum water contents were evaluated following the three pre-treatments described above. Measurements involved taking dielectric probe readings in direct contact with the skin surface and through a 6 μm thick Teflon sheet. The difference between the probe outputs from these two measurements yielded a voltage deflection which was then converted to a percentage relative water content. The Teflon spacer significantly attenuates the signal picked up by the probe. Measurements were made in this way to minimize day-to-day fluctuations in probe sensitivity and other environmental factors.

RESULTS

The LDV results after topical application of hexyl nicotinate following the three pre-treatments are shown in Figure 1. Time of onset of action, time to peak response, magnitude of peak response and area under the response–time curve are listed in Table 1 which also shows the relative stratum corneum water content levels determined by the dielectric probe technique at the end of the pre-treatment periods.

Statistical analysis showed that a 30-min period of occlusion significantly shortened both the time of onset of the LDV-detected response to hexyl nicotinate and the time to peak response when compared to the untreated controls ($P < 0{\cdot}05$). In addition, the magnitude of the peak LDV response to hexyl nicotinate and the area under the response–time curve were significantly increased ($P < 0{\cdot}05$). If the occluded site was exposed to ambient conditions for 1 h after occlusion before nicotinate application, blood flow measurements were not significantly different from the control values.

The stratum corneum water content values showed the same pattern. Exposure for 1 h after occlusion gave results indistinguishable from the controls, whereas the horny layer water content after 30 min occlusion was significantly elevated ($P < 0{\cdot}001$). There was a significant

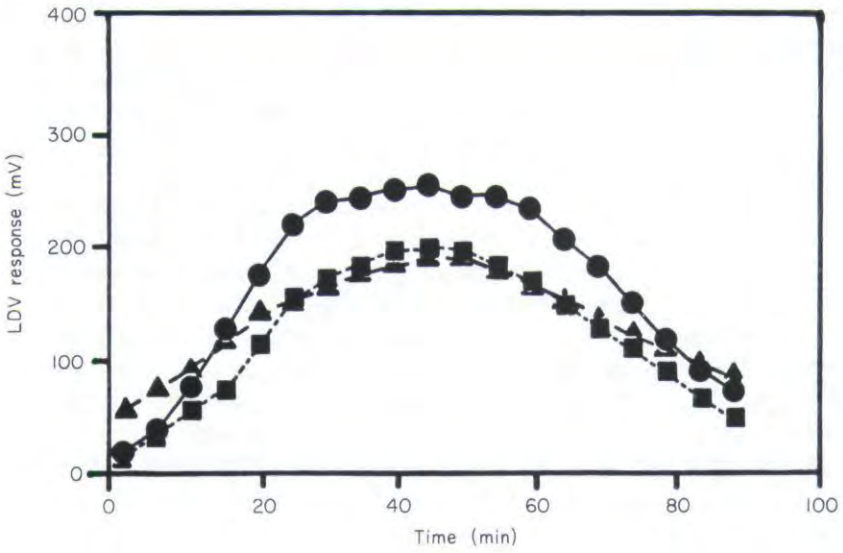

FIGURE 1. LDV responses (expressed as change from baseline) to hexyl nicotinate as a function of time following three pre-treatments of the drug application site: untreated control (■), 30-min occlusion (●), and 30-min occlusion followed by 1 h exposure to ambient conditions (▲). Points are means for eight subjects; the SEM for each point was within the range of 20–40 mV and most were < 30 mV.

correlation between stratum corneum water content and area under the LDV response–time curve after 30 min occlusion ($r = 0{\cdot}8$) ($P < 0{\cdot}05$).

It should be noted that the data obtained after occlusion followed by 1 h exposure generally gave the largest degree of variability. This probably reflects inter-subject variation in skin dehydration rates following a period of occlusion. Extending the period of ambient exposure following occlusion might reduce this variation.

TABLE 1. Effects of pre-treatment on LDV-assessed responses to topical hexyl nicotinate, and dielectric probe measurements of stratum corneum water content values (values are means ± SEM; $n = 8$)

Pre-treatment	Time of onset of action (min)	Time to peak response (min)	Magnitude of peak of response (mV)	AUC* (mV × h)	Stratum corneum water content† (%)
None	12·5 ± 4·0	35·0 ± 3·8	210 ± 28	198 ± 34	31·8 ± 4·8
30-min occlusion	5·6 ± 2·6‡	29·4 ± 2·6‡	254 ± 25‡	267 ± 35‡	46·9 ± 6·2§
30 min occlusion + 1 h ambient exposure	10·6 ± 3·0	37·8 ± 18·5	242 ± 78	213 ± 71	32·1 ± 6·2

* AUC = area under the LDV-detected response–time curve.

† Values expressed as a percentage of the probe response to a drop of pure water.

‡ Significantly different from control ($P < 0{\cdot}05$).

§ Significantly different from control ($P < 0{\cdot}001$).

DISCUSSION

The preliminary results presented here are consistent with the generally accepted role of skin hydration or occlusion on percutaneous absorption, namely, that penetration is greater through hydrated skin. Effects of occlusion on drug delivery across the skin have been studied in detail and extensively reviewed.[1,2,7] Much *in vitro* work has been, and continues to be, conducted with fully hydrated skin tissue.[8] The absorption-enhancing effects of occlusion are employed to good purpose in clinical dermatology.[1,2]

In vivo, however, while the qualitative action of occlusion is clear, the magnitude and duration of the effects induced are less well characterized. The current investigation serves to offer a methodological approach to the better understanding of skin hydration *in vivo*. The combination of non-invasive techniques permits evaluation of both percutaneous penetration and stratum corneum water content and determination of their functional dependence upon each other. Although the LDV test employed here generates a response which is due to a combination of both skin absorption and microvascular stimulation, extensive previous work[9] has shown that the approach is valid when nicotinate levels applied correspond to the linear portion of the dose–response curve. Clearly, however, two levels of biological variability are built into this experiment and these constitute a limitation.

A logical extension of the work would be to include transepidermal water loss measurements as a further non-invasive procedure to evaluate barrier function status. Obviously, as with our recent use of LDV to explore the action of penetration enhancers *in vivo*,[5] there are a number of experimental variables which now need to be studied systematically to investigate the details of the occlusion time–penetration enhancement–water content equation. The simplicity of the experimental system and the ease with which measurements can be made *in vivo* in humans warrant the performance of further investigations.

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