The Effect of Iontophoresis on Skin Barrier Integrity: Non-invasive Evaluation by Impedance Spectroscopy and Transepidermal Water Loss

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INTRODUCTION

The primary function of skin is to serve as a barrier which prevents excessive water loss and precludes entry by harmful agents that are present in the external environment. The "brick and mortar" mosaic structure of the stratum corneum is the rate-limiting barrier for chemical transport across skin (1,2). To utilize iontophoresis as a *controlled* method to enhance transdermal drug transfer, it must first be established that there are no concomitant deleterious side-effects that compromise skin barrier function. To date, transepidermal water loss (TEWL) has been the primary means to investigate development of barrier function integrity *in vivo* (3), in addition to the effects of acute and chronic barrier disruption (4–9). In providing an independent means to corroborate TEWL data, impedance spectroscopy (IS) is a robust technique that can quantitatively evaluate the electrical properties of the barrier. It has been used primarily to develop circuit models of the skin and to rationalize the circuit elements in terms of the biological structures present (10,11). However, impedance analysis can also be used as a non-invasive diagnostic tool.

The impedance of skin is determined by the ability of ions to flow through it. If the skin is damaged, then barrier function is impaired and ion transport becomes easier: thus, the impedance decreases. Impedance measurements can therefore be used to probe the integrity of barrier function. Also, since ionic species prefer aqueous conditions, the greater the degree of skin hydration, the more facile ion transport becomes and the lower the impedance (12,13).

We have previously used impedance spectroscopy to determine the electrical characteristics of human skin *in vivo* and to investigate the effect of iontophoresis on skin impedance (12). The objectives of this study were: (i) to establish the extent to which iontophoresis compromises skin barrier function and (ii) to evaluate whether transepidermal water loss, the classical determinant of barrier integrity, is capable of detecting changes caused by iontophoresis. We have used IS and TEWL measurements to investigate the effect of iontophoresis on the permeability of human skin *in vivo,* and to monitor the rate of recovery to a basal permeability level after iontophoretic current flow. The effect of increasing the iontophoretic current density on skin barrier function and the relative rate of recovery were also explored using both techniques.

MATERIALS AND METHODS

Chemicals

N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) buffer and NaCl were obtained from Sigma Chemical Company (St. Louis, MO). De-ionized water (resistivity $\geq$ 18 MΩ cm^{-1}) that had been purified by a Millipore System (Milli-Q UFplus; Bedford, MA) was used to prepare all solutions.

Electrodes

Both the alternating current, required for the impedance measurements, and the iontophoretic current, were applied using Ag/AgCl electrodes. These were prepared by chloridizing silver wire (1mm diameter, 99.99% pure; Aldrich Chemical Company Inc., Milwaukee, WI) immersed in 133 mM NaCl solution (Pt-cathode) for approximately 3 hours at an applied current of 0.5 mA.

Subjects

There were five human volunteers (4 male, 1 female), aged from 25–31 years. All subjects were in good health and had no history of dermatological disease. Informed consent was obtained from all participants. The study was approved by the UCSF Committee on Human Research.

Experimental Apparatus

A Macintosh Quadra 800 (Apple Computers Inc., Cupertino, CA) equipped with LabVIEW 3.0.1 (National Instruments Inc., Austin, TX) was used to control a Pulse/Function Generator (Hewlett-Packard Co., Model HP8116A, North Hollywood, CA). At an applied voltage of 1.0 V (peak-to-peak), this produced a sinusoidal alternating current whose frequency was raised from 1 Hz to 1 kHz incrementally with 10 frequency points being sampled per decade. The electrical circuit for the impedance measurements included a 2 MΩ resistor in series with the skin. Thus, at the applied voltage of 1.0 V (peak-to-peak), this ensured that the sinusoidal current remained approximately constant ($\approx$0.25 μA). The potential difference across the skin was measured with a Stanford Research Systems Lock-in amplifier (Stanford Research Instruments Inc., Model SR850 DSP Sunnyvale, CA).

TEWL measurements were made using a Servo Med Evaporimeter EP1 (Servomed AB, Stockholm, Sweden).

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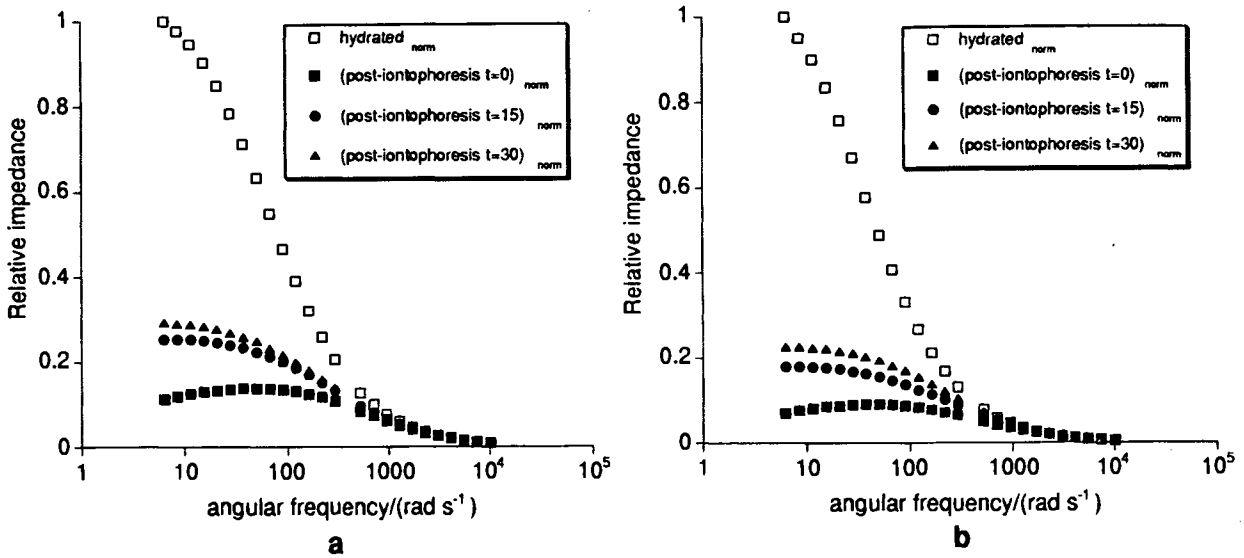

Fig. 1. (**a**) Effect of iontophoresis (0.1mA cm^{-2} applied for 5 minutes) on skin impedance (Subject B). The raw data are normalized by dividing the impedance values by the impedance measured at 1Hz (6.28 rad s^{-1}) of hydrated skin pre-iontophoresis. (**b**) Effect of iontophoresis (0.1mA cm^{-2} applied for 15 minutes) on skin impedance (Subject C).

Experimental Procedure

After recording the basal TEWL levels on both forearms of the subject, two electrode chambers were placed on the individual's right ventral forearm surface and attached securely to the skin with adhesive tape (TimeMed Labeling Systems Inc., Burr Ridge, IL). The chambers were filled with an electrolyte solution comprising 133 mM NaCl and 25 mM HEPES at pH 7.4. Each chamber contained two electrodes. The surface area of the skin exposed to the electrodes in each chamber was 3.14 cm^2. An initial impedance spectrum was recorded to provide a control measurement. Since an impedance spectrum requires about 7 minutes to complete, skin hydration occurs and a second pre-iontophoresis measurement was made to examine the extent of this effect. Then, an iontophoretic current (delivered using a Kepco Power Supply APH 1000M, Flushing, NY) of 0.1 mA cm^{-2} or 0.3 mA cm^{-2}, was applied for either 5 or 15 minutes. The first post-iontophoresis impedance spectrum was recorded immediately upon termination of the iontophoretic current flow. Further spectra were recorded 15 and 30 minutes after completion of iontophoresis in order to monitor the rate of recovery of skin impedance. The impedance data are presented as plots showing the variation of the impedance as a function of the frequency of the applied signal. The data have been normalized by dividing the impedance values by the impedance at 1 Hz (6.2834 rad s^{-1}) of hydrated skin pre-iontophoresis.

The electrode chambers were then placed on the subject's left forearm and an identical iontophoretic current applied for the same length of time. After iontophoresis, the skin was allowed to aerate for 15 minutes, at which point an initial TEWL measurement was made. Further measurements were made at 15 minute intervals over the next hour.

RESULTS & DISCUSSION

The impedance of the skin has been shown to be substantially reduced after tape-stripping (10,12). At the same time, removal of the stratum corneum compromises the primary barrier to water loss and TEWL shows a marked increase (12). Application of an iontophoretic current (0.1 mA cm^{-2} for 5 minutes) caused a reduction in skin impedance as compared to the value for hydrated skin (Figure 1a). However, there was no concomitant change in TEWL (Table I). Only the first TEWL measurement at the anodal site showed an appreciable increase of ~3.5 g/m^2h, but this was due to residual hydration (Table 1). Increasing the duration of iontophoresis to 15 minutes, caused the skin impedance to again decrease from the basal value; but once more, TEWL remained relatively constant (Figure 1b and Table I). The iontophoretic current density was then increased to 0.3 mA cm^{-2}, again applied for periods of 5 and 15 minutes (Figure 2a and 2b, respectively). The decrease in skin impedance shown after applying an iontophoretic current of 0.3 mA cm^{-2} for 5 minutes is comparable to that found following iontophoresis using 0.1 mA cm^{-2} for 15 minutes; this is to be expected since the same quantity of charge passed into the skin in both cases. The data in Figure 2b and in Table I clearly show that, although the impedance shows a significantly greater reduction after iontophoresis with a current density of 0.3 mA cm^{-2} applied for 15 minutes (as compared to 0.1 mA cm^{-2}, note the use of a logarithmic scale), TEWL was not affected significantly. We note that in order to eliminate variation in the results caused by inter-individual differences, the experiments would need to be repeated on the same subject a number of times, and with strict control of ambient conditions which can induce changes in the response. In general, the majority of subjects experienced greater sensation beneath the anodic chamber and visual inspection revealed that this site also displayed greater erythema (12).

Our results show that an increase in TEWL is accompanied by a corresponding decrease in skin impedance (12). However, reducing skin impedance does not necessarily produce an increase in TEWL. The rate of TEWL is determined by the integrity of the bulk stratum corneum, specifically the intercellular lipid lamellae which fill the spaces between the keratinocytes. The aspect ratio of the corneocytes, the bricks

Table I. TEWL Values Measured After Iontophoresis

Time after iontophoresis[a] (minutes)	Post iontophoresis TEWL rate (g/m^2h)									
	0.1 mA/cm^2[b] (5 minutes)[c] B[d]		0.1 mA/cm^2 (15 minutes) C[e]		0.3 mA/cm^2 (5 minutes) C[f]		0.3 mA/cm^2 (15 minutes) E[g]		Control E[h,i]	
	cathode	anode	cathode	anode	cathode	anode	cathode	anode	site A	site B
15	7.5	6.0	7.7	9.0	5.2	6.8	9.2	10.6	7.3	7.0
30	5.8	6.0	5.0	6.0	5.2	6.5	6.3	6.9	5.0	7.0
45	5.9	2.7	4.3	5.0	5.2	5.6	5.4	6.2	4.1	4.6
60	6.3	5.1	4.8	5.2	5.0	5.1	5.2	5.4	4.0	5.8

[a] Measurements were made 15, 30, 45, and 60 minutes following iontophoresis.
[b] Current density.
[c] Duration of iontophoresis.
[d] Subject B. TEWL prior to iontophoresis = 6.0 g/m^2h.
[e] Subject C. TEWL prior to iontophoresis = 5.6 g/m^2h.
[f] Subject C. TEWL prior to iontophoresis = 5.7 g/m^2h.
[g] Subject E. TEWL prior to iontophoresis = 6.9 g/m^2h.
[h] Subject E. TEWL prior to occlusion = 5.5 g/m^2h.
[i] TEWL measurements recorded following the identical exposure of the skin sites for 15 minutes to the electrolyte solutions but without application of the iontophoretic current.

in this lipid matrix, is such as to maximize the diffusion pathlength, the increased tortuosity further decreasing water loss (14). In order to produce a significant increase in TEWL, the lipid barrier must be compromised. This is effectively achieved by serial tape-stripping and there is a resulting increase in TEWL (4–6) and corresponding decrease in skin impedance (10,12).

In contrast, although iontophoresis significantly reduces skin impedance, there is negligible effect on TEWL. The insensitivity of TEWL to the effects of iontophoresis may provide insight into the mechanism of iontophoresis and the nature of the current pathways. Since iontophoresis appears to proceed without compromising the intercellular lipid lamellae, it may be suggested that iontophoresis involves a different route of permeation, e.g., appendageal pathways (consistent with earlier *in vitro* studies, (15,16)). Although a decrease in impedance may be due to increased local ion concentrations following iontophoresis, the elevated ion levels may have been facilitated by transport through current-induced structural changes in the appendageal routes (12,17). It is also possible that iontophoresis punctures the stratum corneum and creates microscopic pores, the transient nature of which inhibits their ability to change the longer timescale measurements of TEWL. Whether such transport pathways represent amplification of putative pore structures suggested to be present in mammalian SC (18) remains to be seen.

We conclude that measurement of TEWL alone is insufficient to fully characterize the effect of iontophoresis on the integrity of skin barrier function. While TEWL and IS are well-correlated when the stratum corneum is physically damaged

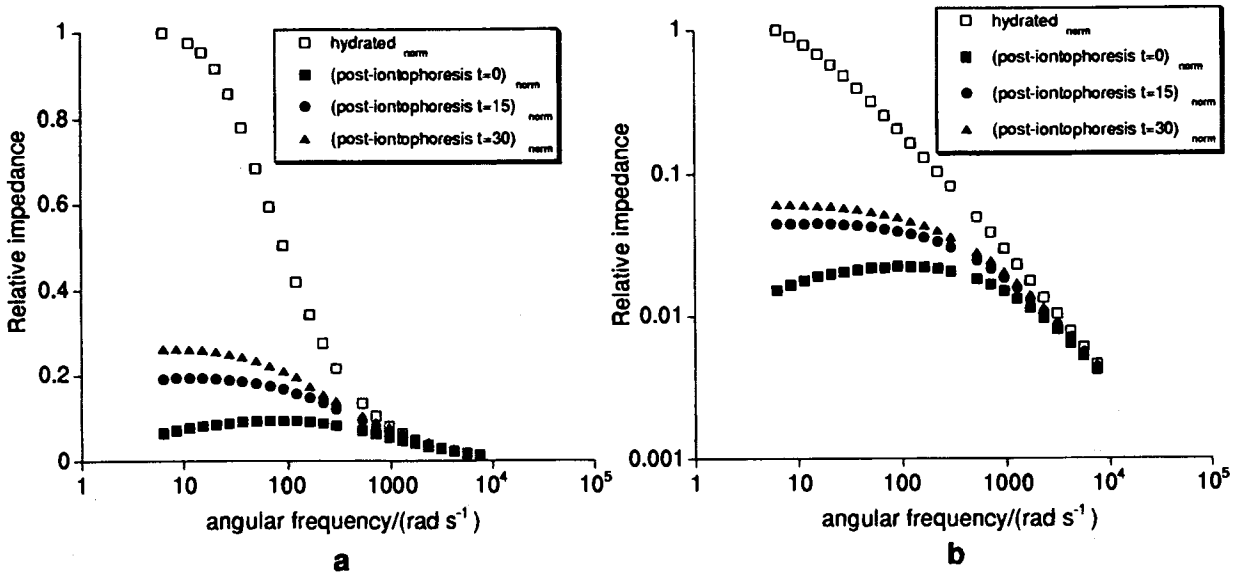

Fig. 2. (**a**) Effect of iontophoresis (0.3mA cm^{-2} applied for 5 minutes) on skin impedance (Subject C). (**b**) Effect of iontophoresis (0.3mA cm^{-2} applied for 15 minutes) on skin impedance; note the use of a logarithmic scale (Subject E).

(or physiologically compromised, e.g., as in premature neonates), we have shown that iontophoresis alters the electrical properties of the skin, as a function of current density and the time of application, whereas TEWL is insensitive to its effects. It follows, therefore, that iontophoresis elicits changes in the barrier distinct from macroscopic perturbation of the stratum corneum intercellular lipids, structures which are generally acknowledged to be responsible for controlling the passive transport of water across the skin.

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